Australian Government

Australian Centre for International Agricultural Research

Final report

project

Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea

project number	HORT/2014/083
date published	28/07/2022

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approved by	Ms Irene Kernot, Research Program Manager for Horticulture
final report number	FR2022-006
ISBN	978-1-922787-97-2

published by

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1 Acknowledgments

The work reported in this report was funded by cash investment by ACIAR, Charles Sturt University and the University to Southern Queensland. Additional in-kind support was provided by the National Agricultural Research Institute, Fresh Produce Development Agency and PNG University of Technology.

The project team acknowledges and thanks the farmers that hosted field trials. Thanks also extends to the family of these farmers and their surrounding community for their hospitality.

The project team acknowledges and thanks the Australian growers that allowed access to their farms and for the many conversations about sweetpotato production, pests and diseases. The project team acknowledges and is thankful for the engagement with various sweetpotato researchers from the Queensland Department of Agriculture and Fisheries (QDAF), Central Queensland University (CQU), University of Queensland (UQ) and Henderson RDE.

We are grateful for the engagement with the Brian Bell Group for conversations regarding importing the pheromone lure for monitoring the sweetpotato weevil.

We acknowledge that the advances made in this project are founded on earlier ACIAR investment in PNG sweetpotato projects, especially work on development of pathogen tested plant material.

2 Executive summary

The project has developed improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea, analysed the performance of the new crop protection methods, and communicated these options to end users. On-farm trials of the efficacy of an integrated pest and disease management strategy (comparing this with conventional agronomic practice) extended over the three provinces in which sweetpotato is most important (Eastern Highlands Province, Western Highlands Province and Jiwaka Province), and took place on multiple farms in each province over three production seasons. This was complemented by assessment of crop yields and economic performance in two seasons. This evaluation provided a rich data set from which the robustness of the performance metrics (pest and disease incidence, yield, and economics) for new options can be inferred. The new integrated pest and disease management strategy comprised: (i) the use of pathogen tested planting material, (ii) sanitation, removal of sweetpotato crop residues and weeds, (iii) isolation, separating new crops from existing crops by at least four meters, and (iv) pheromone traps for sweetpotato weevil. Compared with conventional practice, this led to measurable reductions in biotic threats to the crop, especially the two major weevil species, gall mite and scab. This, in turn, translated to increased yields. Over all trials, total yield of storage roots when crops were protected by the integrated pest and disease management strategy averaged 20.16 t/ha. This was almost double the yield in the control treatment consisting of conventional farm practice. An additional benefit was improved quality of the storage roots from the integrated pest and disease management strategy. Illustrating this, unmarketable yields in that treatment were lower (1.46t/ha) than in the control (2.25t/ha) whilst the all-important marketable yield was greatly increased from 7.99t/ha to 18.70t/ha. Importantly, these benefits were robust over sites and provinces rather than resulting from highly levels of performance in a few locations. For example, marketable yields in the control treatment never *exceeded* an average of 10.28 for any province yet were never *below* 17.44 in the integrated pest and disease management strategy treatment. This, in turn, led to a major economic advantage (i.e., comparing the net income from the new strategy, after cost of the additional labour and materials, with the net income from the conventional practice) that averaged across provinces 6,284 Kina/ha in the first year and 10,567 Kina/ha in the second year. Complementary studies identified optimal forms of mulches, species of barrier plants to reduce pest ingress, and entomopathogenic fungi isolates that could be added to the pest and disease management strategy to enhance crop protection. Trials in Eastern Highlands Province and Jiwaka Province compared the expanded integrated pest and disease management strategy with the original best bet strategy. Changes in net income in Jiwaka Province trials from use of mulches were more attractive than the original strategy (giving an average advantage of 1,883 Kina per hectare extra compared with conventional practice) but highly variable across sites and on average negative in the Eastern Highlands. In contrast, adding barrier plants to the best bet strategy gave robust economic benefits in both provinces with an average of up to 12,23 Kina per ha in Jiwaka Province. Laboratory bioassays with a still wider range of entomopathogenic fungi have identified isolates with activity against both weevil species. Two strains of the *Metarhizium* that were isolated from PNG soils (Western Highlands Province and Unitech Agri. Farm in Morobe Province) were produced on a larger scale to evaluate in an additional trial on the Unitech farm and Poahom village (Morobe Province). These have scope to enhance pest suppression in future work. An additional series of field trials was conducted to partition the respective effects of pheromone traps for weevils, of crop sanitation, and crop isolation, each when paired with the use of pathogen tested planting material. Weevil control, yields and economic performance all benefitted in treatments where pathogen tested planting material was complemented by one or more other method, compared with conventional practice. Overall, multiple different combinations of methods from the ‘toolbox’ of plant protection options can be used with confidence to complement pathogen tested planting materials. Crop isolation, sanitation, mulching and barrier plants are all methods that are likely to have direct or indirect

negative effects on virus vectors so contribute to the durability of this germplasm. A range of communication initiatives have been made to deliver impact. An illustrated manual, method-specific information sheets, and animated presentations for social media have been developed to complement a series of farmer training sessions that were held in multiple districts in the Highlands. Overall, the project has addressed the need identified in the preceding Small Research Activity: that pests and diseases were considered damaging by growers but that use of plant protection interventions was uncommon. Growers now have a much more comprehensive ‘toolbox’ of validated methods, that have proven economic benefit, and considerable training and outreach to growers has occurred. The project leaves a legacy of communication materials that will support further adoption.

3 Background

Sweetpotato is the major staple food crop of Papua New Guinea (PNG) and is becoming increasingly commercialised, especially in the Highlands, where it is beginning to rival coffee as a preferred source of cash income. The fresh roots are sold along roadsides and in local markets but, increasingly, value chains are being developed to supply sweetpotato from the highlands to urban centres in the lowlands, such as Lae and, via Lae, to Port Moresby. Small quantities of sweetpotato are also marketed for processing into flour, baked goods and other products.

Reflecting the cultural and food security significance of the crop, ACIAR has supported research on sweetpotato since the inception of its collaborative program in PNG in the 1980s. Much of this work has been directed towards understanding, and if possible, reversing, a perceived decline in the productivity of sweetpotato, linked in general terms with a combination of declining soil fertility and increasing pest and disease problems (linked in turn to increasing population pressure in the highlands and reduced fallow periods). Within the plant protection area, considerable progress has been made in understanding the viruses affecting sweetpotato in PNG (and Australia) and advances have been made in addressing the problem – mainly through thermotherapy to eliminate viruses and the distribution of virus-free planting material (often described as 'PT' material, alluding to its 'pathogen-tested' status). Sweetpotato weevils are widespread in PNG and sometimes abundant, and so are believed to contribute to the decline in productivity. However, work on these pests in PNG has, for various reasons, been fragmentary and inconclusive, to the extent that their pest status is poorly defined and pest management strategies ill defined. Meanwhile in Australia, sweetpotato weevil is recognised as a major cause of loss of quality loss (visibly damaged roots being largely unmarketable) and farmers are heavily dependent on the use of pesticides (including active ingredients that are likely to be withdrawn in the near future).

In 2013-14, in response to a request from the National Agricultural Research Institute (NARI) for a more integrated and better coordinated approach to sweetpotato research, ACIAR commissioned a Small Research Activity (SRA), SMCN/2012/016 entitled *Review of research needs on natural resource management and crop protection for sweetpotato based cropping systems in PNG*, led by Gunnar Kirchhof (to focus on soil fertility issues) and Geoff Gurr (on plant protection issues). The aim of the SRA was to review what had been done, assess current needs and to make recommendations for future investment. This current project was designed to address the priority crop protection issues identified in the SRA.

The SRA involved a survey of smallholder farmers in the PNG Highlands. Two of the key findings of the survey were that (i) 'weevils' are the biggest concern to smallholders and (ii) the vast majority of growers do not practice any form of pest management intervention. It seemed they do not feel they have viable options and sufficient knowledge. Accordingly, the development of a commercial sweetpotato sector that produces greater volumes of high-quality roots on a more consistent basis required the development of a comprehensive pest and disease management strategy based on an adequate understanding of the biotic threats and the efficacy of appropriate management tools.

The SRA also involved a review of previous research in the field, including ACIAR-funded work in PNG. The most significant finding here was from an ACIAR project (PC/2011/053, led by M Hughes) which found that the West Indian sweetpotato weevil *Euscepes postfasciatus* (Coleoptera: Curculionidae) is much more common and widely distributed than previously suspected and may indeed be a bigger problem in PNG than the well-known sweetpotato weevil *Cylas formicarius* (Coleoptera: Curculionidae). Since little is known about the biology of *Euscepes* it is important to establish its significance as a pest and how it differs from *Cylas* because this will affect possible management options. For example, unlike the adults of *Cylas*, those of *Euscepes* have not been reported to fly and

this opens possibilities for exclusion from a given garden by simple physical barriers of locally available borders of non-host or repellent plants.

The headline result of the SRA was that sweetpotato growers in the Highlands of PNG consider pests and diseases to be a major biotic constraint to production. Despite this, only a very small range of plant protection methods were being sued by growers and many reported taking no action to mitigate crop loss. Essentially then, the overarching aim of the present project was to equip growers with a more comprehensive 'toolbox' of methods to combat pests and diseases. The focus of the project was on commercial and semi commercial production rather than smallholder subsistence farming.

More generally, the project addressed relevant components of the NARI Strategic Program Implementation Plan 2012-2020:

- NARI Program 1.2.1 Sweetpotato marketing systems is addressed by increasing the amount and quality of marketable sweetpotato available for sale and processing.
- NARI Program 1.4.1 Soil Health in SP Systems is addressed by determining the effects of rotations and other management practices on crop protection issues including weevils, nematodes and black rot, and by training local staff to address the identified capacity gap of human talent skills and competencies.
- NARI Program 1.5.1 Sweetpotato pest and disease management is addressed by increasing the numbers of crop protection technologies and practices available to farmers, and by training local staff to address the identified capacity gap of human talent skills and competencies.

The project aimed also to deliver benefits to Australia. Sweetpotato is a small but significant crop in Australia and sweetpotato weevil (*C. formicarius*) is considered one of the most important pests in this industry. Although previous ACIAR-funded work has illustrated the benefits of using grids of pheromone baited traps to detect 'hotspots' of high pest density where control efforts can be concentrated and of 'area-wide management' to reduce carry-over populations in infested crop residues, farmers currently remain heavily dependent on a narrow range of pesticides (which are increasingly likely to be withdrawn from use). Accordingly, new crop protection approaches developed in PNG, such as biological control agents, could be adapted for, and adopted in, Australia. Opportunities for the biological management of other soil borne pests such as root knot nematode and wireworms were also to be assessed through this project.

Improved suppression of PNG sweetpotato pests and diseases that are not currently present in Australia (e.g., West Indian sweetpotato weevil, *E. postfasciatus*) will reduce the magnitude of the biosecurity risk of an incursion to the Australian industry. Furthermore, knowledge gained in identification and biology of these pests will increase Australia's biosecurity preparedness.

4 Objectives

The central aim of this project was to develop improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea; with secondary benefits to flow to Australian growers as outlined in the preceding section.

Specific objectives were:

1. To evaluate the impact of soil management interventions on the incidence of pests and diseases, including plant-parasitic nematodes.
2. To develop and evaluate the effectiveness of novel pest management options.
3. To evaluate and promote the adoption of ‘best-bet’ combinations of integrated pest and disease management (IPDM) options including sanitation/area-wide management in a semi-commercial setting.
4. To evaluate the social and economic impacts of promising IPDM combinations.
5. To build the capacity of individuals and organisations in PNG to conduct IPDM research.

5 Methodology

Objective 1: To evaluate the impact of soil management interventions on the incidence of pests and diseases – including that of plant-parasitic nematodes – in collaboration with SMCN/2012/105

1.1 Finalise location of non-TEAM sites (TEAM sites are already defined)

This work involved NARI personnel, aided during a visit to PNG by Drs Liu and Wilson, surveying field trials set up by the Soil Project (SMCN/2012/105) team. Trials took place at the Aiyura Research Station and on-farm sites at Meteyufa, Nipuka and Kuka. Detail of methods is provided in Appendices 1 and 2).

Each of the trials was established using pathogen tested planting material and conventional planting material and a split-plot, factorial design then imposed in which soil management and plant nutrition treatments can be compared. These included the use of coffee pulp, animal manure and organic matter treatments based on 'cut and place' of locally available plant materials to enhance soil fertility and structure, as well as rotational treatments. The randomised, replicated experiment at Aiyura station included treatments with 'nutrient accumulator' plants that also had potential pesticidal properties such as *Tephrosia* spp., legumes and purchased inputs such as waste materials as well as planted or 'bush' (i.e., spontaneous vegetation) fallows.

1.2 Establish monitoring protocol at all sites.

Prior to trials being established, a comprehensive set of protocols was established by the full project team to efficiently capture data on pest and disease numbers/severity. See Appendix 1 "HORT2014083-Field survey protocol 2017-11 revised.docx", and Appendix 2 "HORT2014083-Field survey protocol 2018-06 revised.docx". Surveying for weevil species used a method common to that used in the ACIAR project PC/2010/065 led by M Furlong. Foliar symptoms were used to assess effects of experimental treatments on scab (fungal disease) and gall mites (minute arthropods that cause distinct foliar symptoms. End of season assessment of storage roots allowed below-ground symptoms to be similarly quantified as well as root yields to be measured.

1.3 Survey of soilborne pests and diseases in Qld and NSW

The originally planned baseline survey of soilborne insects and diseases (especially root knot nematode) in the two major sweetpotato regions in Australia (around Bundaberg in Queensland and Cudgen in northern New South Wales) was not undertaken. After consultation with members of the Australian Sweetpotato Growers Inc (ASPG), key researchers at QLD Government's Department of Agriculture and Fisheries (QDAF) and Craig Henderson (Henderson RDE) in 2016, the survey of soilborne pests and diseases of sweetpotato in QLD and NSW was primarily undertaken as a desktop exercise, owing to the available field guide, previous reports of soilborne pests and disease available online, newly funded projects on nematodes and extension services offered by QDAF to the growers (especially focused on viruses). When field visits were made (limited by COVID-19 in the final project stages), any potentially useful pests and disease information (from the field or via conversations with individual growers) was recorded. The survey was updated over the life of the project to reflect any new information and personal observations in the field. Preliminary diagnostic protocols using loop mediated isothermal amplification (LAMP) have been established for some of the pests of sweetpotato and beneficial microbes. The Hort Innovation funded project 'PW17001: Integrated pest management of nematodes in sweetpotatoes', led by The Queensland Department of Agriculture and Fisheries (QDAF) (started in 2018) conducted soil surveys, which have

identified root-knot nematode species present in sweetpotato growing areas in Qld and NSW (see Appendix 3a for the survey of soilborne pests and diseases and see Appendix 3b for LAMP protocols).

Early in the project (2016-2017) colonies of sweetpotato weevil were established for laboratory, glasshouse and field studies at USQ and CSU. Several visits were made to Bundaberg, the Lockyer Valley and Cudgen to collect weevils for experimentation at USQ and CSU. This involved both pheromone trapping and collection of male weevils as well as collection of infested storage roots from the field to obtain female (and male) weevils. Rarely were infested storage roots present as most growers adhere to strict hygiene practices to limit infestations (i.e., roots are disced into the ground or deeply buried). A cohort of mixed weevils was obtained from infested storage roots from a farm in the Bundaberg region. Pheromone traps were constructed from modified yellow fly traps (Envirosafe, Bunnings) or bucket traps (PHEROCON® Unitraps, Trécé Inc.). For the fly traps, the bait was removed, and holes were drilled into the plastic under the lid to secure the lid (and trap) to a post using a cable tie. This allowed for easy access and removal of live weevils. The female *Cylas formicarius* sex-pheromone *Z*3-dodecenyl-*E*2-butenoate was acquired in bulk (Sapphire Bioscience, NSW, Australia). Lures were made by pipetting 20 µL (equivalent to 1 mg per lure) of pheromone into the inside of 2 cm lengths of rubber tubing (orange natural rubber tubing, 5 mm internal diameter). One lure per trap was suspended inside the trap with metal wire (1 mm diameter). For the bucket traps, the lure was positioned within the lid in the designated compartment for lures. Pheromone traps were installed on wooden stakes or metal star pickets 50 cm from the ground, on the outer edge of a crop. Typically, pheromone traps were installed for 24 hr before collecting live male weevils and transporting them back to Toowoomba to be reared in the laboratory (and sent to CSU for rearing).

Collection of wireworms was more challenging and did not occur in high enough numbers for experimentation. An attempt to rear a small cohort (ca. 12) sugarcane wireworms (ex-Sugar Research Australia, unsprayed plots in Bundaberg) was unsuccessful. Collections were also attempted in Cudgen and in Lockyer Valley using standard baiting methods in sweetpotato fields; however, these were also unsuccessful (owing to the cryptic nature of wireworms and lengthy life cycles). In the last year of the project, various larvae of weevil (white fringed) and curl grubs (African black beetle) were reared in the laboratory to confirm identity as adults. This was performed because there is a lack of knowledge on specific damage caused by various larvae to storage roots, and this allowed us to better understand damage patterns by allowing larvae to feed until they pupated and became adults.

There was a localised incursion of scurf caused by the fungus *Monilochaetes infuscans* (endemic in Australia) (and possibly other fungi causing similar symptoms) at two sweetpotato farms in NSW in 2018/2019, only present in paddocks that had been irrigated by the same water source. More than 20 roots (and >100 sub-cultures of root periderm) of varieties Orleans and WSPF were processed before one culture of *M. infuscans* was eventually obtained and identity confirmed with sequencing. This culture was stored for future work (see Appendix 3c).

A small study was conducted on an existing experiment established by a Hort Innovation funded project (VG13004 “Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry”). Storage roots showing breakdown or wilt (i.e., rots or wilts from bacteria and fungi) were collected from plant beds at two sweetpotato farms in Bundaberg. Other opportunistic collection of storage roots/shoots occurred from other sites too. Briefly, root pieces were surface sterilised in 1% sodium hypochlorite, further sectioned, then plated on microbiological media to encourage bacterial or fungal growth. Outgrowths of bacteria or fungi were sub-cultured until a pure culture was obtained and stored in sterile water and frozen at -80 °C for future DNA extraction and identification, e.g., for Mr Wilfred Wau, NARI for his JAF Research Masters at USQ (See Training section, see Appendix 3d). A handful of the isolates were identified

to species level using PCR and sequencing. These included the bacterial rot causative agent *Pectobacterium carotovorum*, as well as several *Bacillus* spp. *Pseudomonas* spp., *Pantoea* spp. The pathogenicity of these isolated bacteria and fungi will be further explored with Mr Wilfred Wau in 2022, to provide him with the skills required to test Koch's postulates and better understand plant pathogens infecting sweetpotato production in PNG.

Soil was initially collected from Bundaberg for the isolation of entomopathogenic fungi (EPF) before being extended to collections from sweetpotato fields from Rockhampton, the Atherton Tablelands, Esk and Cudgen (see text on Objective 2.1 below).

1.4 Develop and implement monitoring protocols for root knot nematodes, wireworms and sweetpotato weevil.

From an Australian perspective, standardised monitoring protocols for key soilborne pests were not performed on any formal level owing to conversations with APSG, QDAF as detailed in Objective 1.3 (i.e., the task was deemed unnecessary for the Australian industry). More detail on the rationale of not developing/implementing protocols is found below.

Root-knot nematodes (RKN) are the most important pest for industry and testing for RKN differs among farmers depending on damage seen in the previous year. Existing services (including sampling methodology) are available through state government agencies (e.g., QDAF, SARDI Predicta® DNA based soil diagnostics) as well as private agencies (e.g., Biological Crop Protection P/L). In addition, the Central Queensland University lead (CQU) Advance Queensland/ASPG funded project 'Novel approaches for root-knot nematode control' (2017) and the QDAF lead Hort Innovation funded project 'PW17001: Integrated pest management of nematodes in sweetpotatoes' (2018) have performed extensive soil surveys to ascertain the identity and abundance of root-knot nematodes.

Wireworms (true and false) are sporadically a problem for growers, and this was reflected in opportunistic, casual conversations (not formal surveys) with selected growers in Bundaberg, Cudgen and the Lockyer Valley. This made the development of (informal) monitoring protocols difficult when standard methods (e.g., buried baits of germinated wheat seed or cut sweetpotato) yielded no wireworms (see Objective 1.3). For one grower in Bundaberg, wireworms were only an issue when the sweetpotato crop followed sugarcane, but when baiting was attempted, not one wireworm was retrieved (Grower, pers. comm). However, monitoring for wireworm is not typical based on conversations with several growers, but similarly to assessing for RKN, it likely is dependent on issues/damage encountered in previous years.

Many growers in both QLD and NSW use or have used pheromones to locate (and monitor) for high sweetpotato weevil populations (i.e., trapping of male weevils) to complement existing pesticide regimes for weevil control. Lures have typically been purchased from Bugs for Bugs (https://bugsforbugs.com.au/product/sweet-potato-weevil-lure/) using modifications of the described monitoring protocol.

Monitoring efforts, primarily for sampling live weevils were concentrated in Cudgen (NSW) and the Lockyer Valley. In Cudgen, conventional growers were supplied with one or two new traps/lures to monitor (and collect for research purposes) but very few (male) weevils were collected (usually <5 per trap). At an organic sweetpotato farm in Cudgen in 2019, a more extensive trapping regime was adopted. Owing to the undulating geography of this property, separation of paddocks and progression of planting of sweetpotato through the season, 21 traps were installed over a period of 5 months (total farm area about 33 ha, mixed farming). Green Bucket traps (PHEROCON® Unitraps, Trécé Inc.) and modified fly traps (Envirosafe, Bunnings) were fitted with a pheromone lure as described in Objective 1.3. Over a period of 9 months, approximately 82,000 male weevils were collected. A report with weevil counts and recommendations was made to the grower. At another

organic farm in QLD, where weevils previously have not been a problem, a small trapping exercise to assess population (not efficiency of trap) was performed using bucket traps (sprayed red with paint) and modified yellow fly traps. Two paddocks were used: paddock 1 was 0.5 ha (sweetpotatoes ready for harvest) and had 4 traps and paddock 2 was 3 ha (sweetpotato being progressively harvested, volunteer sweetpotato and other weeds). Weevil counts were collected weekly for 4 weeks, with a total of approximately 2300 males trapped.

In Papua New Guinea, training on the use of pheromone traps for the sweetpotato weevil (*Cylas formicarius*) and their deployment was promulgated to farmers in a series of workshops by NARI/FPDA and a brochure detailing their manufacture was produced. For field experiment assessments, protocols for monitoring for *C. formicarius* were developed (see Objective 1.3 and associated appendices). Based on lack of pest pressure from wireworm, protocols were not developed for assessing damage caused by this insect; however, the above-ground survey sheet (see Objective 1.3) allowed for the scoring of wireworm adults (beetles) if they were present. Based on lack of pest pressure from root-knot nematodes (RKN), the presence/absence of RKN damage was recorded only for the final trial (best-bet minus).

1.5 Analyse, interpret and disseminate.

This involved an intermediate milestone of submitting a manuscript to a refereed journal in year 3 month 12. This paper (Rehman et al., 2019), focused on mulches rather than incorporated soil amendments or rotations because this form of soil management intervention showed the greatest promise. Rehman was a Charles Sturt University student recruited to the project and who conducted his laboratory and field studies in Australia. Dr Liu (his co supervisor at CSU) then transferred the methods to NARI staff during a visit in 2019. Given that soil management interventions were observed in early results to have effects, a laboratory study was conducted in NSW to establish whether silicon levels in plant tissue were being affected. This was in accordance with the overall objective aim because silicon is known to affect plant tolerance to biotic and abiotic challenge.

1.6 Information on potential biosecurity pests present in PNG developed.

Work for this activity took the form of desktop research and was undertaken by Dr Wilson of the University of Southern Queensland (USQ). Dr Wilson was invited to be a member of the Sweetpotato Biosecurity Reference Panel (established in 2018). The panel was made up of growers from Australian Sweetpotato Growers Incorporated, principal horticulturalist Craig Henderson RDE, scientists from QDAF, scientists from the Northern Australia Quarantine Strategy (NAQS), a scientist from Hort Innovation and a scientist from USQ. The panel convened annually, with more regular communications regarding high priority pests as well as established pests, diseases and weeds. To maximise the impact and reach of this work, it took the form of a contribution to the development of the sweetpotato biosecurity plan for Australia (Plant Health Australia led). A copy of the plan can be accessed online at https://www.planthealthaustralia.com.au/industries/sweet-potatoes/.

Briefly, there are only a few pests/diseases in Papua New Guinea that are considered high priority pests (HPPs) and these are: *Euscepes postfasciatus* (West Indian Sweetpotato weevil and the Giant African land snail (*Achatina fulica* syn. *Lissachatina fulica*) restricted distribution in PNG e.g., Bismarck Archipelago, New Britain, New Ireland. The presence of a few viruses is queried e.g., Sweet potato mild speckling virus (SPMSV) (with SPFMV and SPCSV), Mild mottle of sweet potato (SPMMV) (with SPFMV and SPCSV) because SPCSV or sweetpotato chlorotic stunt virus has not been detected in PNG, despite previous suggestions that it had been. Whilst global databases do not show the presence of the devastating Guava root knot nematode (*Meloidogyne enterolobii* syn. *Meloidogyne mayaguensis*), it is not clear if surveys have been done in PNG to detect its

presence. This should be a priority in future research in susceptible crops such as sweetpotato, cabbage, potato, corn, cassava.

Objective 2. To develop and evaluate the effectiveness of novel pest management options.

2.1 Screen isolates of weevil entomopathogenic fungi. AND 2.3 Field experiments of entomopathogen efficacy.

Laboratory work on entomopathogenic fungi took place at Unitech in Lae and at Aiyura with studies extending to field trials in which several isolates of *Metarhizium* spp. were added to the best bet strategy, making it the best bet plus strategy (that also included mulches and barrier plants (see below). The field experimentation phase used commercial farms in the TEAM zone sites as well as the Aiyura Station field trial area (Hagga-anantu, just outside of Kainantu).

Because PNG has a longer history of sweetpotato production than Australia and more diverse soils and regional climates, there was good reason to believe that diversity of entomopathogenic fungi would be at least as high as that in Australia. Isolation, screening and assessment of pathogenicity of fungi was overseen by Dr Dotaona of Unitech, assisted by a junior scientist, following methods used in his earlier PhD studies at CSU.

Soil was collected from many locations across the lowlands and highlands of PNG for the isolation of entomopathogen fungi by Unitech staff, NARI staff and Dr Wilson from USQ. Briefly, about 200 g of soil was collected from various plots (see Appendix 5 for details Unitech laboratory bioassays including details of sites and soil), placed into bags and transported back to the laboratory in an esky where possible. Once in tubs, larvae of the palm weevil or cocoa moth were added to the soil to bait entomopathogenic fungi from the soil. Pure cultures eventually obtained from sporulating cadavers were stored securely as agar cubes in sterile water at -80 °C. Molecular methods were used to identify the isolates to species level (see Appendix 5).

The entomopathogen work in Australia complemented that undertaken in PNG. Only new isolates collected from field surveys in Objective 1.3 were evaluated in the laboratory, glasshouse and field experiments. Existing entomopathogenic fungi isolated by Dr Wilson from a previous project at CSU were not obtained, owing to the inability of GRDC and CSU (not project staff) to reach agreement on IP and their transfer to USQ. Prof Ash and his team at USQ attempted negotiations for 18 months. In addition to the soil samples retrieved from Bundaberg for the isolation of EPF, further collections were made from sweetpotato farms in the Atherton Tablelands, Rockhampton, Esk and Cudgen. Accepted methods to isolate EPF from soil were used. Briefly, collected soil was placed into individual 70 ml containers, moistened with sterile water and baited with larval mealworms (*Tenebrio molitor).* Entomopathogenic fungi was then isolated from dead sporulating larvae (*Metarhizium* spp. or *Beauveria* spp.) and processed until a pure culture was obtained (single-spore cultured).

2.2 AND 2.4 Investigate the use of biological control for other pests such as root knot nematode and wireworms.

Isolates within an Australian collection of *Metarhizium* at USQ had previously been screened against Tenebrionidae so were tested further against wireworms in the laboratory. The bulk of this work was undertaken within a PhD project supervised by Prof Ash and Dr Wilson. Additional laboratory and glasshouse studies at USQ explored the interaction with root knot nematode and the ability of the fungi to form endophytic relationships with sweetpotato plants. The endophyte research is presented in Appendix 12b. The work on root-knot nematodes/EPF is not reported.

2.5 Lab/trial station experiments with barrier crops. AND 2.6 On-farm experiments with barrier crops.

Work on pest barriers was initiated at CSU, led by Prof Gurr and Dr Jian Liu. This involved three masters students (Mudassir Rehman, Esther Dada, Grace Malabo) who developed bioassay methods and then conducted screens of multiple potential plants that were selected on the basis of the literature (those that had demonstrated repellence to other insect pests) and the possible utility of the plants to constitute a dual income crop for PNG growers. Subsequently, Dr Liu travelled to PNG and spent a period at the Aiyura Station to deliver specialised minor equipment and conduct training of local NARI staff in the methods. The work based at NARI progressed to field evaluation using field sites as above. The identity of biological active plant compounds was determined by GC-MS analyses at CSU.

Objective 3. To evaluate and promote the adoption of 'best-bet' combinations of integrated pest and disease management (IPDM) options

An overview of the best bet and related methods (flowing from objective 2) is provided in Fig 1.

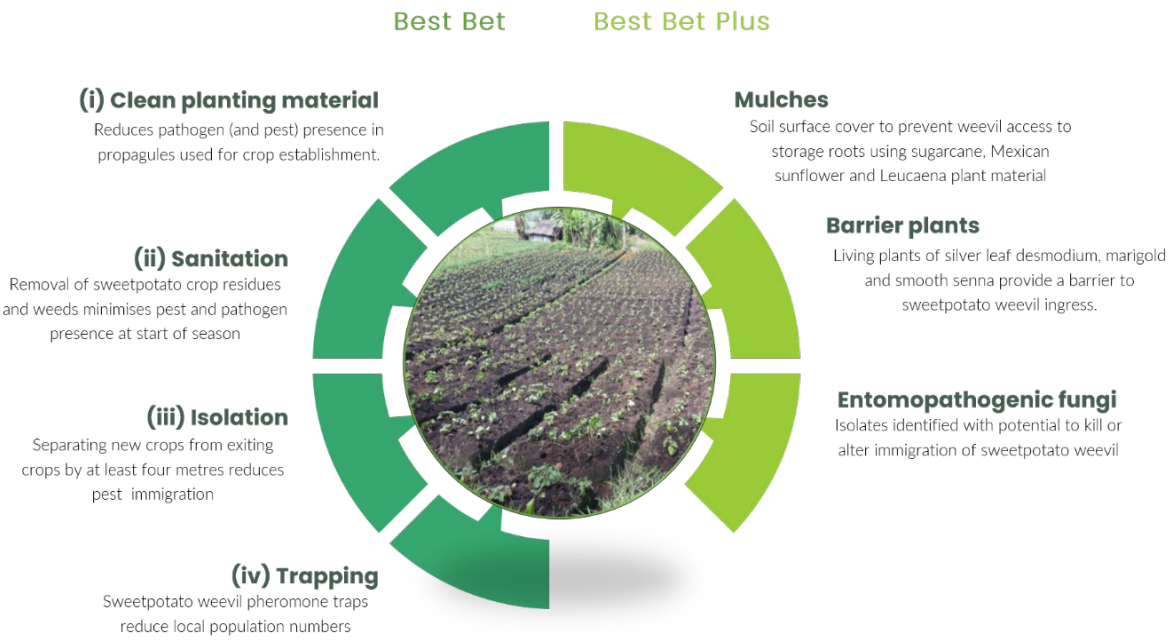


Fig 1. An overview of the pest and disease management tactics studied in this project.

3.1 Review technical data and workshop with stakeholders to identify 'best-bet' IPDM tactics and combinations for further testing. PC and A

An Inception Workshop in February 2016 at NARI headquarters in Lae brought together members of the project team with counterparts in the soil project (SMCN/2012/105) on soil management and crop nutrition and a third project, HORT/2014/097, focusing on sweetpotato commercialisation, within the Transformative Agriculture and Enterprise Development program (TADEP) as well as a range of stakeholders. This provided an opportunity to discuss activities planned for each project, earlier work conducted in PNG sweetpotato systems and plan for cross-project liaison. Thereafter, the CSU based team

led a process to update the review article generated by the preceding Small Research Activity on sweet potato pest and disease management (Johnson, A & Gurr, G. (2016). Invertebrate pests and diseases of sweetpotato (*Ipomoea batatas*): A review and identification of research priorities for smallholder production. *Annals of Applied Biology*. 168. n/a-n/a. 10.1111/aab.12265.). This exercise led to a provisional series of pest and disease management strategies that was refined and 'reality tested' by liaison with the project team. The resulting 'best bet strategy' combined pathogen tested (PT) planting material, crop isolation, sex pheromone trapping of *Cylas*, and sanitation.

3.2 On-farm evaluation of best-bet combinations of IPDM tactics at TEAM sites.

Best Bet

Between December 2017 and December 2020, the 'best bet strategy' was field tested on a total of 24 sites, each a commercial or semi-commercial farm. Four of the 13 host farmers were female. Best bet methods justification was generated, see Appendix 6 "Best bet IPDM methods" .Trials were (numerically) evenly distributed across Jiwaka Province, Eastern Highlands Province and Western Highlands Province. In order to achieve realistic assessment of key variables including plant health, yields and costs, the experimental design comprised a single plot of each treatment (best bet and conventional practice) on each farm. Replication was achieved by having four such farms in each province, these comprising a single large-scale experiment. Results were analysed for each province and also in an overall, aggregated manner across all sites for that year and for all sites plus all years.

Best Bet Plus

In 2020, evaluations progressed to evaluation of the best bet strategy in a form supplemented by a number of additional, methods that had been developed via separate, laboratory and small-scale field studies in Australian and PNG. Four, fully randomised, replicated, on farm trials were run on highlands sites in each of Eastern Highlands Province and Western Highlands Province. An additional trial was conducted in at the lower elevation site of Kainantu in the Eastern Highlands Province. COVID-19 disruption prevented data collection from an additional series of sites that had been established in the Western Highlands sites. A range of mulch treatments and a range of living plant barrier treatments were added as a complement to the (previously evaluated) best bet strategy and the best bet strategy (without complementary interventions) served as the baseline control treatment. Mulch treatments were foliar tissues of sugarcane, Mexican sunflower and leucaena. Plant barriers were Silver leaf desmodium, marigold and smooth senna. Limited supply of entomopathogen inoculum meant that this (*Metarhizium anisopliae* isolate MC01 (originating from farm soil at Meteyufa in the Asaro Valley) was added only to the sugarcane mulch treatment plots in the eight highlands trials.

Best Bet Minus

In 2021 a trial was conducted across three farms in the Asaro area of Eastern Highlands District in which the original Best Bet strategy was partitioned into subsets of treatments. As for the Best Bet trials, each farm has a single replicate of each treatment, with replication achieved by aggregating data from all three sites. A detailed methods protocol was generated in late 2020 via online zoom meeting (Appendix 7 "Individual best-bet method trial-4.docx).

Trials were led by NARI staff, supported by FPDA and by online discussions with CSU and USQ staff. An end-of-project stakeholder workshop was held in August 2021 to interpret results and formulate recommendations. Staff from Unitech were involved in the Best Bet Plus trials since they were leading the PNG-based work on entomopathogens.

3.3 Demonstration, evaluation and training days for sharing progress of best-bet IPDM trials with wider farmer groups.

In accordance with the plans made during the Inception Workshop in 2016, trial work in this project made extensive use of privately owned commercial and semi-commercial farms. These had been established within the Technology Evaluation and Marketing (TEAM) zone initiative for participatory research and experiential learning. In total 12 farms across three Provinces had best-bet strategies implemented on a proportion of the farm and this geographically dispersed, farmer participatory model has helped drive significant interest in the novel plant protection methods. To reach wider farmer groups, between October 2019 and February 2020, 12 farmer training days were conducted by NARI and FPDA staff (see below). An advantage of sharing TEAM zone sites was extensive interaction across project teams and consistency of messaging. For example, Yapo Jeffery of the Soil Project included in his training activities the key recommendations from the present project such as use of pheromone traps and crop sanitation.

An intermediate milestone, a conference presentation, was delivered by NARI staff members W Wau and R Geno, and J Liu of CSU presenting results in a total of three papers at the 49th Australian Entomological Society AGM and Scientific Conference, Alice Springs, Northern Territory, 23rd to 26th September, 2018.

3.4 Preparation and use of IPDM training materials

This activity took place in the second half of the project so that it was empirically supported by results from studies that had commenced earlier in the program of work. Technical information from laboratory and field studies was reviewed by the project team and relevant stakeholders. The practicability of certain promising methods was assessed by appropriate networking. For example, a competitively priced and reliable source of effective sex pheromone lures for trapping sweetpotato weevil was located in China by Dr J Liu. Dr B Wilson led liaison with Australian farmers to determine the best approach to incorporate new technologies such as biopesticides into Australian cropping systems. This is discussed in Appendix 10. “Can biopesticides be incorporated into integrated pest management programs for Australian sweetpotato”

Preparation of IPDM training materials has extended from illustrated print materials for specific technologies such a weevil trapping, and they were complemented by 1-day workshops, bringing together male and female farmers from multiple villages and highland districts. In the final year of the project, a wider range of IPDM training materials was developed including short, video-based productions that were deliverable across a range of electronic platforms including Facebook and YouTube. An illustrated manual was drafted for the end of project stakeholder workshop and thereafter refined in accordance with feedback from stakeholders. Manuals of specific methods were also produced and provided to PNG partners to enhance technical capacity (e.g., Appendices 1, 2, 3a, 6, 7).

Objective 4. To evaluate the social and economic impacts of promising IPDM combinations

4.1 Finalise methodologies for assessment of socio-economic impact AND 4.2 Assess impacts

This work was led by Dr Richard Culas of CSU and involved him visiting PNG for the Inception Workshop and subsequently to work with Mr Alex Agiwa and other project personnel to visit field sites and trial and refine data capture. Work involved a CSU masters student (himself a PNG national), Coleman Pombre

An intermediate milestone was addressed by a conference paper presented from the results of Coleman Pombre's dissertation in the Australasian Agricultural & Resource Economics Society 2021 meeting in Sydney (See Appendix 8).

4.2 Impact assessment.

Benefit: cost analyses were conducted as desktop research by Dr Culas using data collected from the farmers that hosted trials and by NARI and FPDA staff in the second and third years of the TEAM zone trials comparing best bet versus conventional practice , as well as for the trials of best bet plus and best bet minus. Results were presented in the 2021 Annual Report and at the End of Project Stakeholder Workshop.

4.3 The constraints to the practical use of biological control in Australian SP production identified.

A discussion paper on the use of biopesticides as an adjunct or replacement of synthetic pesticides in Australia has been produced by Dr B Wilson and Prof G Ash (see Appendix 10).

Objective 5. To build the capacity of individuals and organizations in PNG to conduct IPDM research.

5.1 Fill all technical positions (2 at NARI and one each at FPDA and Unitech*). PC*

All positions were filled very early in the project in accordance with the standard recruitment processes of NARI, FPDA and Unitech.

5.2 Appointees participate in formal and informal (learn-by-doing) training opportunities in entomopathology, design and conduct of formal field trials, farmer participatory processes and action research… etc

The junior research scientists employed at NARI worked with a senior NARI staff and the other project personnel. This pair of junior research scientists – specifically requested by NARI as a strategic need to address recent staff departures – were trained and mentored over the course of the project such that they obtained experience in field trial design, establishment and data capture, entomology, and plant pathology as well as generic research approaches, including participatory action research with farmers and data management. Both these NARI staff, Mr Wilfred Wau and Robert Geno were funded to attend and present at the Australian Entomological Society conference in 2019. Subsequently Mr Wau was awarded a John Allwright Fellowship to undertake a master degree at USQ in plant pathology though COVID has forced a delayed commencement. Dr Liu and Dr Wilson spend periods in PNG providing hands-on training at NARI and Unitech.

5.3 Production of a manual of techniques

See 3.4 above.

6 Achievements against activities and outputs/milestones

Objective 1: To evaluate the impact of soil management interventions on the incidence of pests and diseases – including that of plant-parasitic nematodes – in collaboration with SMCN/2012/105

no.	activity	outputs/ milestones	completion date	comments
1.1	Finalise location of non-TEAM sites (TEAM sites are already defined) PC	Site list	Site locations were finalised in liaison with soils project (SMCN/2012/105) staff in the first year of the project.	Reported in Year 1 Annual Report.
1.2	Establish monitoring protocol at all sites PC	Data on pest and disease response to SMCN practices	Protocols were developed, refined and agreed in year one.	Copy of protocols provided as Appendix 1 & 2 in order that these can be followed in future projects to give comparable data.
	Intermediate milestone	*Manuscript submitted to journal.*	*Year 3 mo 12.*	Rehman, M., Liu, J., Johnson, A.C. *et al.* Organic mulches reduce crop attack by sweetpotato weevil (*Cylas formicarius*). *Sci Rep* **9,** 14860 (2019). https://doi.org/10.1038/s41598-019-50521-5.
1.3	Survey of soil borne pests and diseases in Qld and NSW, A	Data on pest and disease incidences	Year 3 mo 6	Reported in Year 3 and updated in Year 5 (see Appendix 3a).
1.4	Develop and implement monitoring protocols for root knot nematodes, wireworms and SPW.	Standardised protocols for pest and disease assessments	Protocols were developed, refined and agreed in year one.	These taxa were included in the broader protocol developed for activity 1.2. The assessment for RKN was only done in the final year, where there it was scored as absence/presence for RKN.
1.5	Analyse, interpret and disseminate. PC and A	Annual reports, scientific papers.	Ongoing over course of project.	Updates provided in Annual Reports. Results on the effects of soil management interventions on the incidence of pests and diseases demonstrated that mulches (rather than incorporated soil amendments or rotations) had the greatest effect. Mulches were the focus of an open access scientific paper (Rehman, M. et al. 2019) and were added to the 'best bet' strategy (see below) for later on-farm evaluation.

1.6	Information on potential biosecurity pests present in PNG developed.	Fact sheets on potential quarantine pest produced	2019	Dr Wilson (USQ) was involved in the development of the sweetpotato biosecurity plan for Australia (Plant Health Australia led). https://www.planthealthaustralia.com.au/industries/sweet-potatoes/. This identified several high priority pests; that of greatest relevance to this project is the West Indian sweetpotato weevil (WISW) since it is common in PNG. Production of additional factsheets, with the exception of a short fact sheet on the WISW with our team produced photos (Appendix 11), was deemed redundant due the existence of others in Lucid (https://keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html/TheProblems/Pest-Root&StemInsects/WestIndianSPWeevil/WestIndianWeevil.htm) and PaDIL (https://www.padil.gov.au/pests-and-diseases/pest/main/142363/44217).

PC = partner country, A = Australia

Objective 2: To develop and evaluate the effectiveness of novel pest management options (entomopathogens and plant barriers).

no.	activity	outputs/ milestones	completion date	comments
2.1	Screen isolates of weevil entomopathogenic fungi from PNG and Australia PC and A.	Short list of entomopathogenic fungi for field evaluation	Year 3.	In Australia, seven strains of entomopathogenic fungus were screened against a variety of pest and model insects. From these sweetpotato field-isolated entomopathogenic fungi, one *Metarhizium anisopliae* (Rockhampton, QLD) and one *Beauveria bassiana* (Cudgen, NSW) have been used more extensively in laboratory, glasshouse and field experiments owing to their pathogenicity and rhizosphere competence (see Appendix 12a and 12b. In PNG, ca.15 isolates of *Metarhizium* sp. and 10 isolates of *Beauveria* sp. were screened against weevils in the laboratory (see Appendix 5). Of these, *Metarhizium* sp. Isolate MCO1 (from Meteyufa Conventional Plot #1) was used in the TEAM zone trials of the expanded pest and disease management strategy (Highlands). A Highlands strain (*Metarhizium pinghaense*) and a Lowlands strain (*Metarhizium anisopliae*) were tested at Unitech Ag. Farm and Poahom village (see Appendix 13a and 13b).

	Intermediate milestone	*Manuscript submitted to journal.*	*Year 2 mo 12.*	Entomopathogens conference presentation: TropAg19 (Shah, S., Ash. G., Wilson, B. Investigating the Sporulation of *Metarhizium anisopliae* Formulated in Calcium Alginate in Soil) at TropAg19 in Brisbane (costs covered by USQ and The Crawford Fund). Barrier plant paper: Dada TE, Liu J, Johnson AC, Rehman M, Gurr GM. Screening barrier plants to reduce crop attack by sweet potato weevil (Cylas formicarius). Pest Manag Sci. 2020 Mar;76(3):894-900. doi: 10.1002/ps.5594. Epub 2019 Sep 30. PMID: 31441202.
2.2	Investigate the use of biological control for other pests such as root knot nematode and wireworms	Shortlisting and feasibility of other biocontrol agents	Year 3	USQ isolates formulated on rice or as granules showed excellent control of mealworms in laboratory and glasshouse studies. Other species of *Metarhizium* have been used successfully to control wireworms in other crops such as Irish potato and is likely to be a good option for wireworm in sweetpotato when pest pressure is high. In this project, pilot experiments with the root knot nematode (*Meloidogyne javanica*) and entomopathogenic fungi showed little evidence of control.
2.3	Field experiments of entomopathogen efficacy. PC/A	Aim for two agents to be identified for use in biocontrol.	Year 5.	Two *Metarhizium* sp. isolates (one from the Highlands and one from the Lowlands) were used in the Unitech and Situm trials (Appendix 13a,13b); however, only one isolate of *Metarhizium* was used in the Asaro and Aiyura trials of the expanded pest and disease management strategy. In Australia, one field trial only was completed, owing to COVID19 restrictions (the other planned field trial on an organic farm was interstate, restricting travel). In the Lockyer Valley trial, two of the shortlisted isolates were applied to the field for the management of possible targets such as wireworm, sweetpotato weevil, white fringed weevil and other scarabs like the African black beetle. Insect pressure was low; however, there was only significant difference between entomopathogenic fungus treated rows and the untreated rows for some insect damage measurements. Similar to the PNG trials, soil collected after the conclusion of the experiment had sufficient levels of the applied USQ isolates of *Metarhizium* or *Beauveria* spp. in the experimentally treated soil or indigenous *Metarhizium* sp.in the control rows (as revealed by baiting using mealworms in a laboratory tub experiment. Pest pressure was low at the site, likely due to the aggressive use of light and water traps over the property, trapping 1000s of insects (Appendix 14).

2.4	Evaluation of trap and release approaches, endophytic establishment of biocontrol agents and deterrent effects of agents evaluated.	Ranking of best-bet deployment options	Year 4.	In PNG and Australia, we observed entomopathogenic fungi growing on the inoculated rice and nearby substrates (like sugarcane mulch) as well as on the soil, suggesting that there is a degree of saprophytic growth by our isolates, likely to play a crucial role in the success of entomopathogenic fungi in pest insect management. Several approaches were used to establish the endophytic nature of key USQ isolates. Laboratory trials investigated using shoots growing in an entomopathogenic fungus suspension although no entomopathogenic fungi (*Metarhizium anisopliae*) was reisolated from the shoots or leaves. In a glasshouse set-up, non-sterile potting mix was inoculated with low and high concentrations of entomopathogenic fungi, as well as a mix of the two shortlisted entomopathogenic fungi (KS1 and ECS1). Storage roots were planted and allowed to shoot and develop lateral roots for about 5 months. The current hypothesis is the prevalence and dominance of endophytic *Trichoderma* spp. in all plant parts outcompetes entomopathogenic fungi, not allowing it to enter. In a final experiment, shoots of sweetpotato were planted in sterile potting mix containing KS1 or ECS1. No EPF were isolated from the shoots, leave or lateral roots. The presence of *Trichoderma* spp. growing on the soil surface although away from the entomopathogenic fungi inoculation zone may decrease the chance of entomopathogenic fungi becoming endophytic. Dylan Male awarded first class honours by CSU for his thesis work conducted at Aiyura Research Station on the response of sweetpotato weevil species to volatiles from entomopathogenic fungi.
2.5	Lab/trial station experiments with barrier crops. PC	Data on efficacy	Year 3.	Initial trialling of candidate treatments and method development work in Australia published as: Dada et al. (2020). In PNG, plant species were tested at the Aiyura Research Station.
2.6	On-farm experiments with barrier crops. PC	Data on efficacy	Year 5	Optimal mulch materials tested in on-farm trials in the last year.

PC = partner country, A = Australia

Objective 3: To evaluate and promote the adoption of 'best-bet' combinations of integrated pest and disease management (IPDM) options

no.	activity	outputs/ milestones	completion date	comments

3.1	Review technical data and workshop with stakeholders to identify 'best-bet' IPDM tactics and combinations for further testing. PC and A	IPDM strategy	Year 1.	Technical data workshopped with stakeholders to formulate an initial IPDM strategy in year 1. Termed 'best bet strategy', this combined pathogen tested (PT) planting material, crop isolation, sex pheromone trapping of *Cylas*, and sanitation. This was field tested on multiple sites over three years. End-of-project stakeholder workshop held in August 2021 to interpret results and formulate recommendations.
3.2	On-farm evaluation of best-bet combinations of IPDM tactics ('PT' planting material, mulches and barriers, sanitation…) at TEAM sites; trials planted and routinely managed by lead farmers, with technical support and data collection by farmers	Data on efficacy of IPDM combinations in different situations	Year 5	In years 3 onwards, the best bet strategy trials were complemented by additional trials of a 'best bet plus' strategy. This added to the best bet strategy, optimal barrier plant treatments, optimal mulch treatments and an entomopathogenic fungus. In year 5, a 'best bet minus' trial compared subsets of the treatments used in best bet strategy.
3.3	Demonstration, evaluation and training days for sharing progress of best-bet IPDM trials with wider farmer groups.	Farmers' under-standing of IPDM options enhanced	Year 5	In total 12 farms across three Provinces have had best-bet strategies implemented on a proportion of the farm and this geographically dispersed, farmer participatory model has helped drive significant interest in the novel plant protection methods. Between October 2019 and February 2020, 12 farmer training days were conducted to reach wider audiences (see below).
	Intermediate milestone	*Conference presentation*	*Year 3 mo 9.*	NARI staff members W Wau and R Geno, and J Liu of CSU presented results in a total of three papers at the 49th Australian Entomological Society AGM and Scientific Conference, Alice Springs, Northern Territory, 23rd to 26th September 2018.
3.4	Preparation and use of IPDM training materials	Farm-level IPDM manuals, apps, etc	Year 5.	Fact sheets, manual, demonstration videos produced. Manual provided as Appendix 15.

Objective 4: To evaluate the social and economic impacts of promising IPDM combinations

no.	**activity**	**outputs/ milestones**	**completion date**	**comments**
4.1	Finalise methodologies for assessment of socio-economic impact. PC and A	Written protocol agreed amongst the three sweetpotato projects.	Year 1.	Complete and reported in year one annual report.
	Intermediate milestone	Manuscript submitted to journal.	Year 4.	Conference paper presented from the early results in Australasian Agricultural & Resource Economics Society 2021 meeting in Sydney (See Appendix 8 listing).

4.2	Assess impacts. PC	Impact assessment report		Benefit: cost analyses presented in the 2021 Annual Report and at the End of Project Stakeholder Workshop.
4.3	The constraints to the practical use of biological control in Australian SP production identified.	A discussion paper produced on the use of biopesticides as an adjunct or replacement of synthetic pesticides in Australia will be produced (including legislative and economic aspects).	Year 5 mo12	Discussion paper prepared (Appendix 10)

Objective 5: To build the capacity of individuals and organizations in PNG to conduct such research.

no.	activity	outputs/ milestones	completion date	comments
5.1	Fill all technical positions (2 at NARI and one each at FPDA and Unitech). PC	Personnel appointed to new positions	Year 1.	Previously reported in Annual Report. Some subsequent staff changes reported in later Annual Reports.
5.2	Appointees participate in formal and informal (learn-by-doing) training opportunities in entomopathology, design and conduct of formal field trials, farmer participatory processes and action research… etc	Capacity and skills of personnel enhanced	Year 5.	Mr Wilfred Wau (NARI project scientist), has been awarded a John Allwright Fellowship to undertake a master degree at USQ in plant pathology. Wau and Geno travelled to Australia to present at the Australian Entomological Society conference in 2019. Dr Liu and Dr Wilson spend periods in PNG providing hands-on training at NARI and Unitech. Robert Geno, Gwendolyn Ban and Ronnie Dotaona visited Australia in 2018 as part of an Institutional John Dillon Fellowship (iJDF). Dr Wilson mentored the Unitech staff involved in the iJDF to produce a manual for HDR research supervision. Drs Wilson and Dotaona are supervising an additional research masters on biological control.
5.3	Production of a manual of techniques	Manuals and techniques available to the industry		See 3.4 (above).

7 Key results and discussion

7.1 Impact of soil management interventions on the incidence of pests and diseases

7.1.1 Direct effects of soil management interventions.

A field experiment established in SMCN/2012/10 was sampled to assess the impact of soil management and crop nutrition practices. Results suggest significant reduction in the severity of scab disease, caused by the fungus (*Elsinoe batatas*), by use of pathogen tested planting material compared with non-PT material (Fig 2). Scab severity varied considerably among treatments within planting material type (i.e., PT or non-PT) but not to a statistically significant extent.

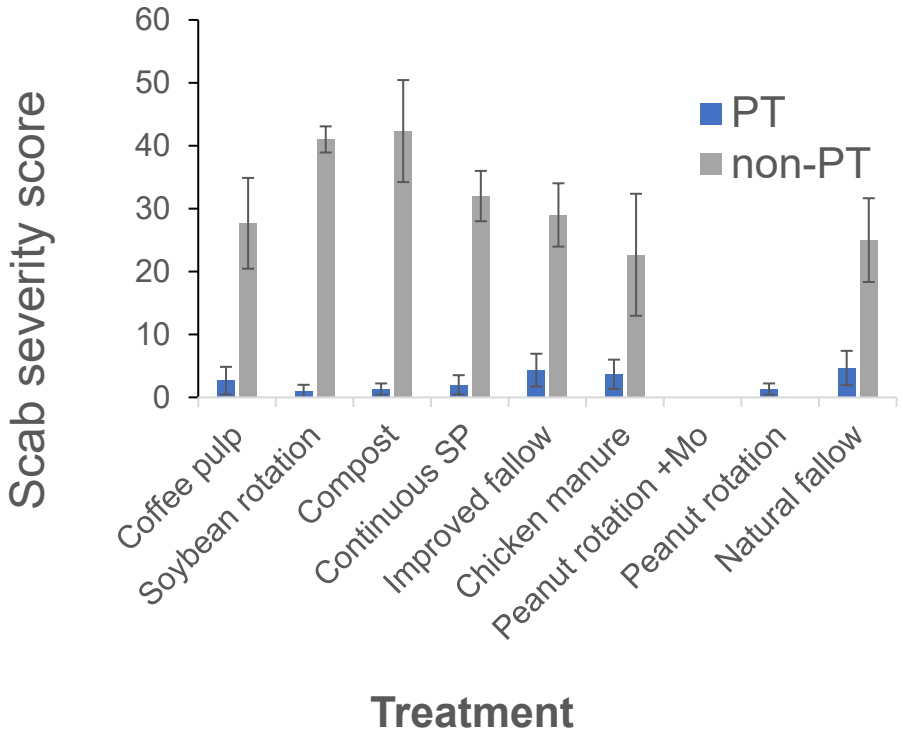


Fig 2. Scab disease severity on sweetpotato plants at Aiyura Research Station is significantly (F = 137.971, df = 1, 47, P < 0.001) by affected by use of pathogen-tested planting material compared with non-pathogen tested planting material. (No significant main effect of use of other treatments or planting material x treatment interaction)

Spiders are an important group of predators of pest insects and are known to be active in Papua New Guinea sweetpotato crops. Densities of spiders in the Aiyura experiment were significantly higher in plots where fallow or some mulch treatments had been applied (irrespective of whether they were established with pathogen tested planting material) (Fig 3). This suggests the possibility of enhancing levels of biological control by use of such methods.

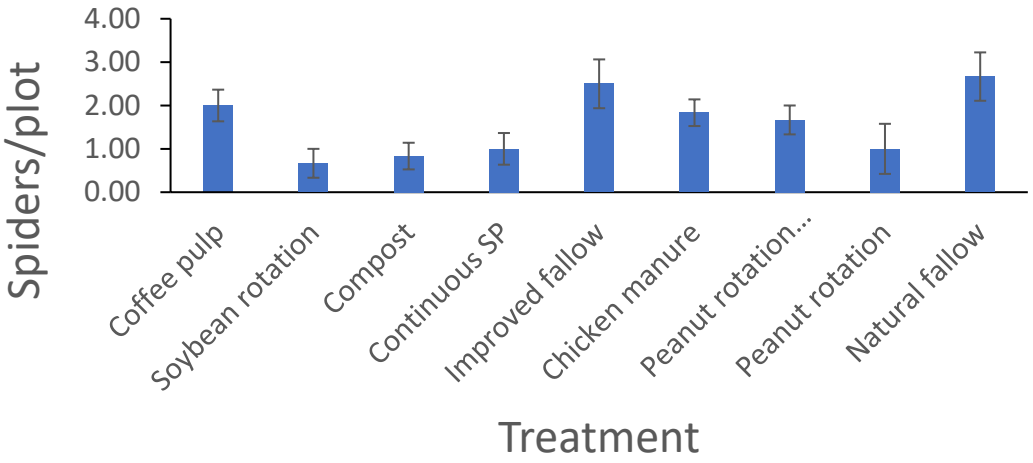


Fig 3. Spider density on sweetpotato plants at Aiyura Research Station is significantly (F = 3.122, df = 8, 47, P = 0.007) by affected by treatment. (No significant main effect of use of pathogen tested planting material or planting material x treatment interaction.)

7.1.2. Indirect, silicon-mediated, effects of soil management interventions.

Silicon (Si) is important in plant defences. Evidence has mounted in the last decade that Si plays important roles in plant defence against biotic and abiotic stress including against insect herbivores and pathogens in rice and cucumber, species known to be active assimilators of Si. No literature exists reporting Si uptake in sweetpotato but there is clear scope for the soil management and crop nutrition treatments to affect Si status of sweetpotato and provide indirect benefits to plant health because of the Si present in materials such as soil amendments. To explore the potential use of Si in integrated pest management in sweetpotato, laboratory studies in Orange NSW developed a Si supplementation method to determine whether the concentration of this element in sweetpotato tissue was affected by availability of Si in the growing medium, indicating the extent to which it is taken up and accumulated. The study incorporated cucumber in addition to sweetpotato because this cucurbit is known to accumulate Si so served as a positive control plant species (Fig 4). Sweetpotato plants were treated using the same method and medium. Results showed that whilst sweetpotato took up less Si than did cucumber, Si supplementation did enhance tissue levels (Figure 5 & 6). The feeder roots especially reached a higher level of silicon (Figure 7) under laboratory conditions.

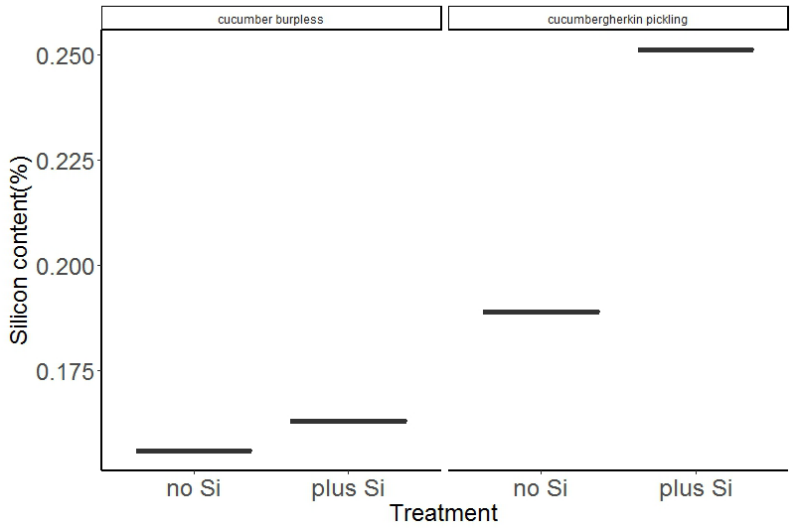


Fig 4. Silicon content in cucumber plants using the Si supplement method

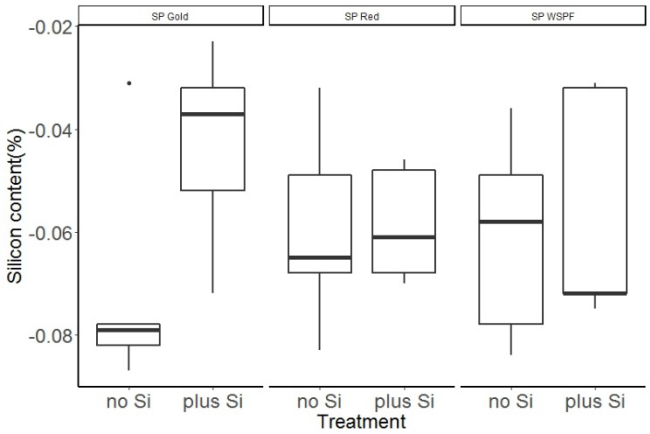


Fig 5. Silicon content in sweetpotato foliage after 4 weeks of Si supplement method

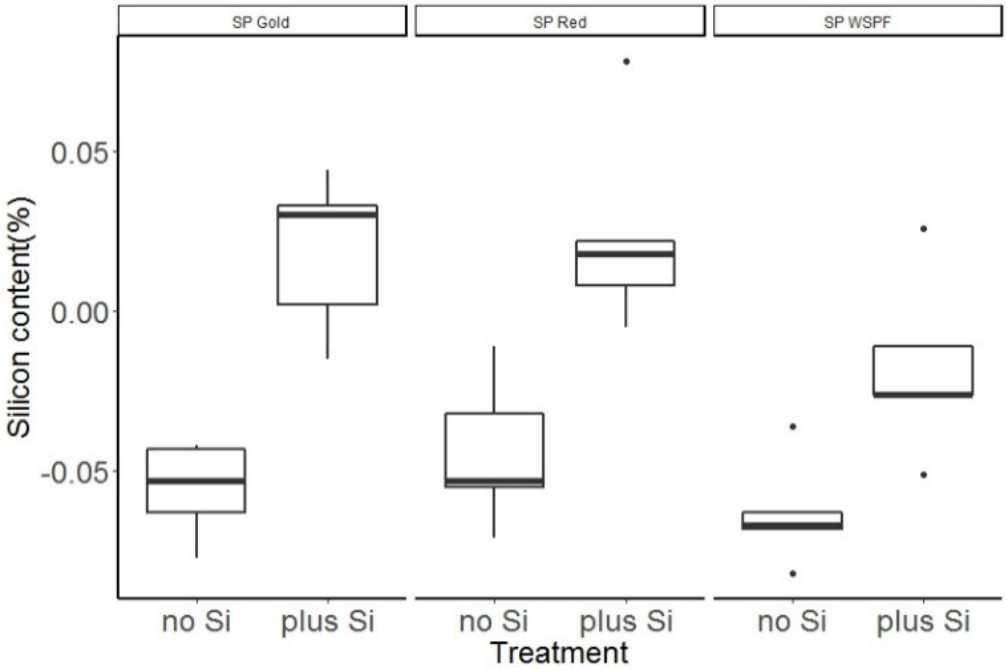


Fig 6. Silicon content in sweetpotato foliage after 8 weeks of Si supplement method.

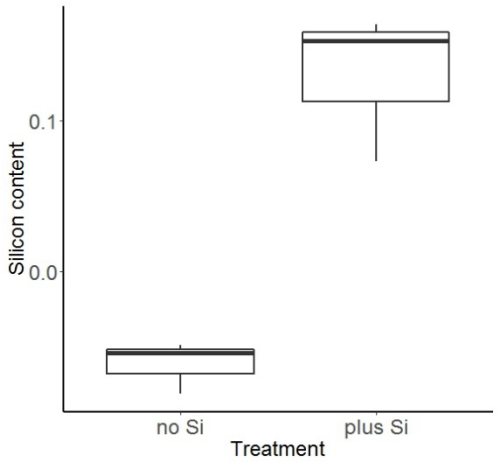


Fig 7. Silicon content in sweetpotato roots after 8 weeks of Si supplement method.

Follow-up work in PNG showed plant samples from Aiyura NARI station had still higher silicon levels in sweetpotato (Fig 8). However, the soil treatments did not have a statistically significant effect on silicon uptake and there was no consistent effect on Si level of planting material type (PT or non-PT). In contrast, Si levels in the tissues of nutrient accumulator plants grown in Aiyura, species that may be used in mulches, showed higher levels of Si, especially wild daka (Fig 9). Overall, the Si study suggested that sweetpotato is not a strong accumulator of Si, even under laboratory conditions and field effects resulting from soil treatments are modest. Accordingly, even Si-rich mulches of plants such as wild daka are unlikely to significantly boost Si levels in sweetpotato

crops. Whilst further studies of mulch materials took place, Si was dropped as a likely causal mechanism for any resulting effects.

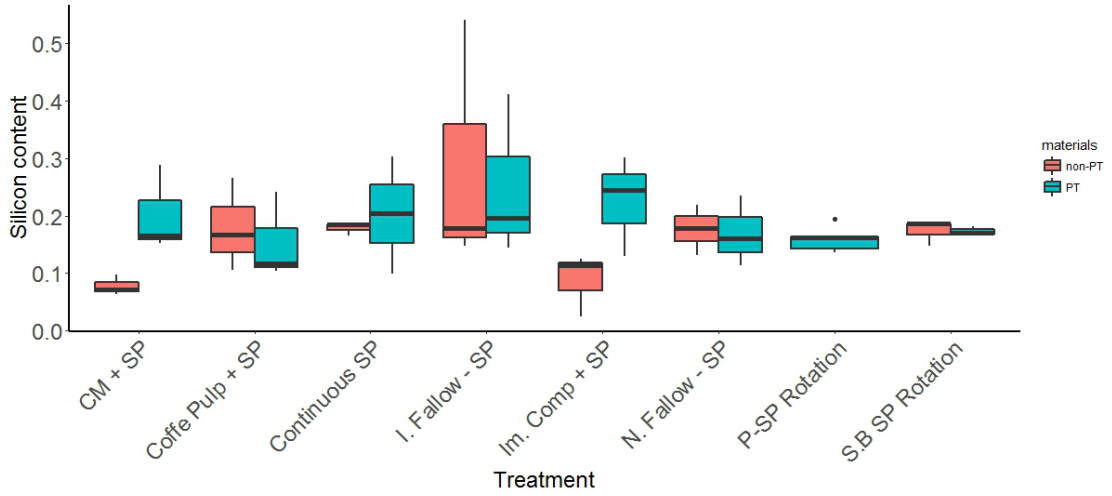


Fig 8. Silicon content in sweetpotato foliage under soil treatment (Soil trial by sister project SMCN/2012/105).

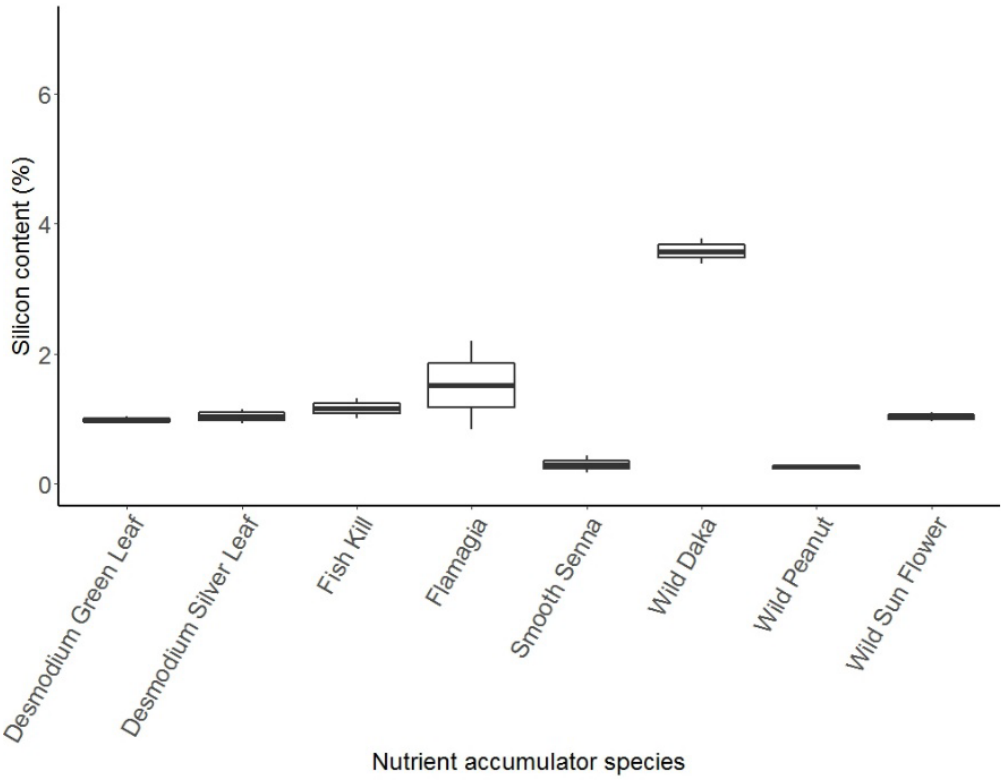


Fig 9. Silicon content in nutrient accumulators growing in Aiyura PNG.

7.1.3 Mulches as barriers to weevils: method development and proof of concept.

Initial work in Orange, NSW, developed bioassay methods for screening candidate plant mulches as potential barriers to weevil immigration. Potentially these could be used as a barrier strip around a crop, or as a cover for the soil surface within the crop. A detailed method has been published, see Appendix 16 “Rehman-2019-Organic-mulches-reduce-crop-attack-.pdf”. A similar design was employed in field studies. Numbers of weevils on the foliage and storage roots within each mulch treatment, as well as feeding holes on the storage roots were subsequently monitored.

In replicated laboratory studies, fresh chopped basil, catnip, and basil lime as well as dry mulches of eucalyptus wood chip, cypress pine bark, lucerne, wheat straw and sugarcane were compared with mulch-free controls. An example of the results is provided in Table 1. In that study, sugar cane, spring onion and lucerne mulches reduced numbers of weevils and storage root damage to a statistically significant extent. Full results are provided as Appendix 16 and published in the open access journal *Scientific Reports* (Rehman, et al.(2019).

Table 1. Mean ± SE for number of *C. formicarius* adult females and feeding holes on sweetpotato storage root during 24 hours’ assessment where mulch treatments were compared with exposed control

	Feeding holes	**Sweetpotato weevils**
Control	369±105.01 A	16±4.97 A
Spring onion	24±12.25 B	2±1.108 B
Sugarcane	37±24.14 B	2±1.22 B
Lucerne	15±5.95 B	0±0 B
Statistical values		
F	10.018	8.16
Df	3, 12	3, 12
P	<0.05	<0.05

Means sharing different letters differ significantly from each other within columns by LSD (<0.05)

Following laboratory screening of multiple mulch treatments, a field study tested a short list of the most promising treatments. An example of the results from this work is provided in Fig 10. In these field studies, two control treatments were used, a storage root covered by soil (reflecting ideal growing conditions) and an uncovered storage root representing non-ideal growing conditions in which the soil had cracked, or the root had become exposed by the roots expanding during growth, or soil being washed away by storm activity. Results showed clearly that uncovered storage roots were much more susceptible to feeding damage than were soil-covered storage roots. Importantly, several of the mulch treatments were able to provide significant levels of reduction to damage for exposed roots.

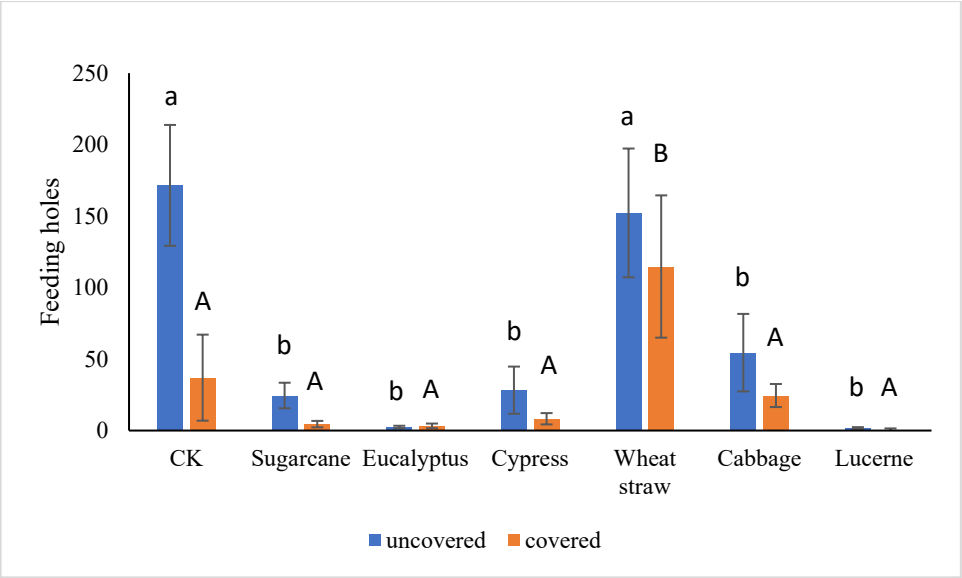


Fig 10. Number of feeding holes (means ± SE) on the sweetpotato storage root by *Cylas formicarius* under covered and uncovered mulching (sugarcane, eucalyptus, cypress, wheat straw and cabbage) compared to control. Means followed by different letters (lowercase) are significantly different under uncovered conditions. Means followed by different letters (uppercase) are significantly different under covered conditions (LSD: P<0.05)

The potential impact of these results is two-fold. The bioassay methods developed in NSW (methods detailed in Appendix 16 and Rehman, et al., 2019) have direct utility for further screening of locally appropriate treatments in PNG including waste materials such as coffee pulp and 'cut and place' vegetation from uncultivated areas adjacent to fields. Second, the results serve as proof of concept that mulches can reduce weevil attack to uncovered storage roots. Applying mulches is likely to be much less labour intensive than cultivating the soil to cover storage roots, particularly during high-risk drought periods when the soil cracks but is so dry that it is difficult to cultivate.

The bioassay methods developed for assessing mulches were communicated to NARI so that equivalent testing could be conducted in-country using plant species and materials of local relevance. In-country work was important also because it allowed studies with *E. postfasciatus* which, not being present in Australia, had not been the subject of work in earlier phases of the project. Prof Gurr delivered minor equipment and demonstrated methods during a visit in February 2018 and Dr Liu made a follow up visit in May 2018 to provide further guidance.

7.1.4 Mulches as barriers to weevils: in-country evaluation.

The protocol developed and validated by the Australian team was transferred to the NARI team. The multiple-choice method was used to screen fifteen potential pest suppressive mulches against both *C. formicarius* and *E. postfasciatus*. This series of studies revealed very strong effects on the numbers of weevils that penetrated the mulch to occupy the surface of the root and the numbers of feeding holes caused (Fig 11, 12). Importantly, the identity of the mulch had very strong effects with some favouring the pest, presumably because they were either attractive and/or offered a favourable microhabitat. For both weevil species, however, there were multiple mulch types that reduced weevil arrival on the root surface and reduced feeding damage. For *C. formicarius,* the plant *Tithonia diversifolia* (wild Mexican sunflower) provided most robust benefits. This is a very common weed on uncultivated areas in the highlands of PNG so readily available for free collection. This plant also was strongly beneficial against *E. postfasciatus,* as was *Senna septemtrionalis*

(smooth senna). These results informed the choice of mulch materials used in later best bet plus field trials.

Fig 11. Response of *C. formicarius* to candidate mulch treatments. Top, uncovered control treatment; bottom, covered control treatment.

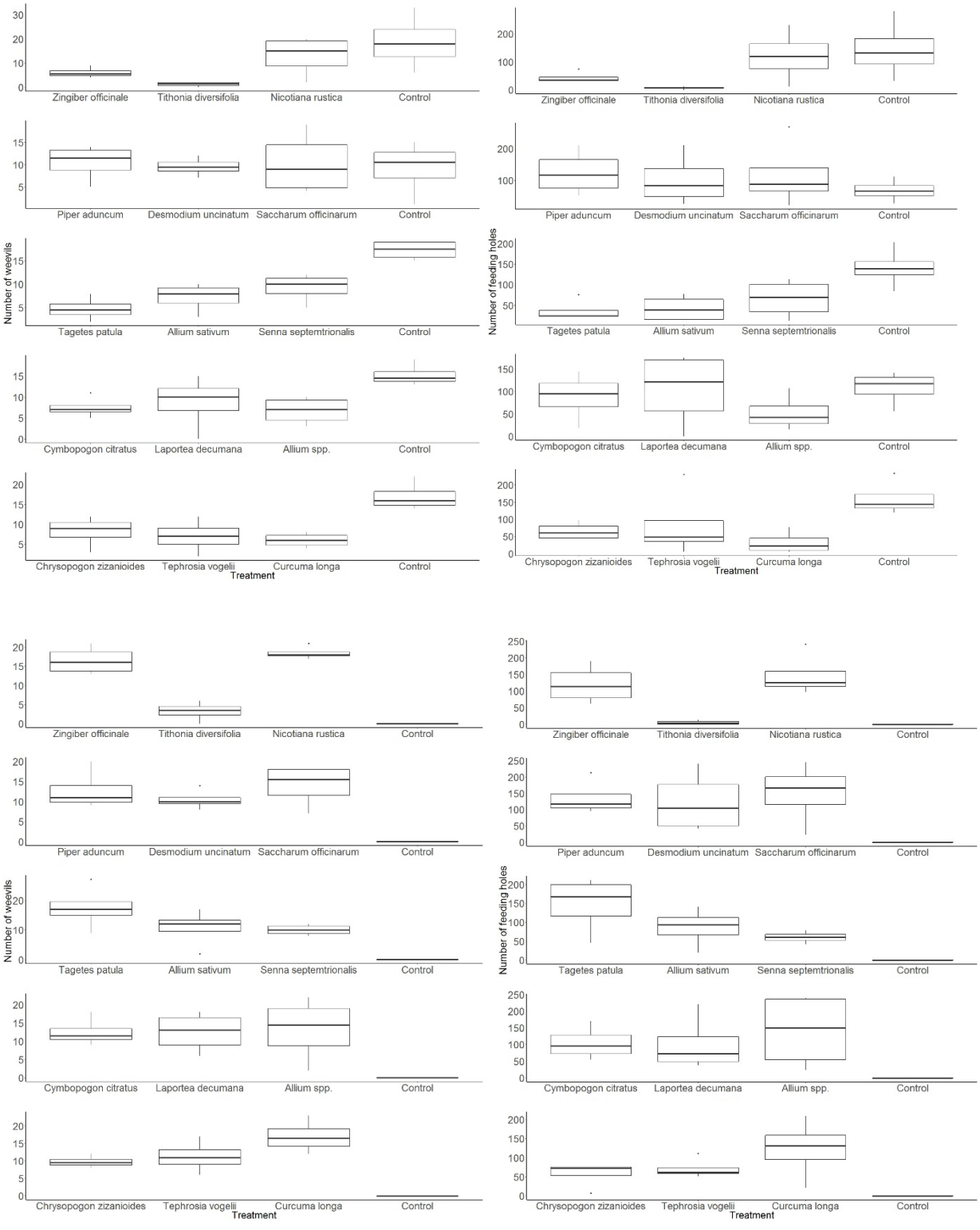
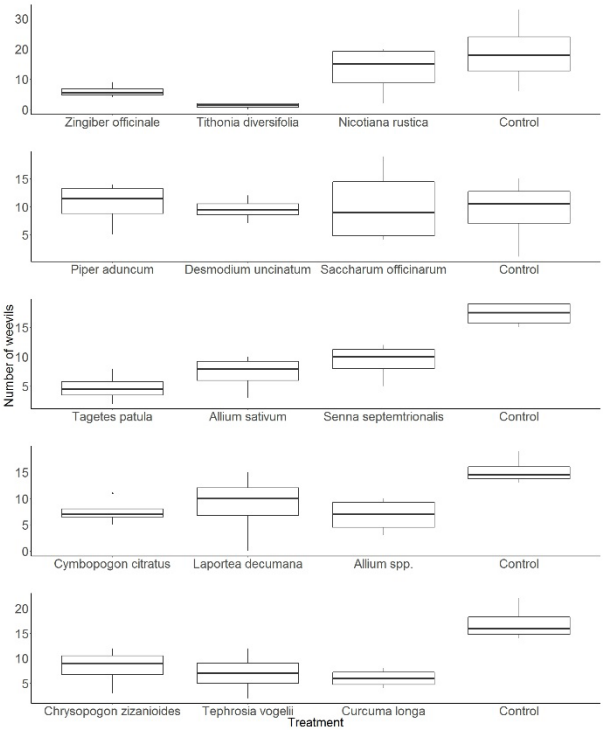
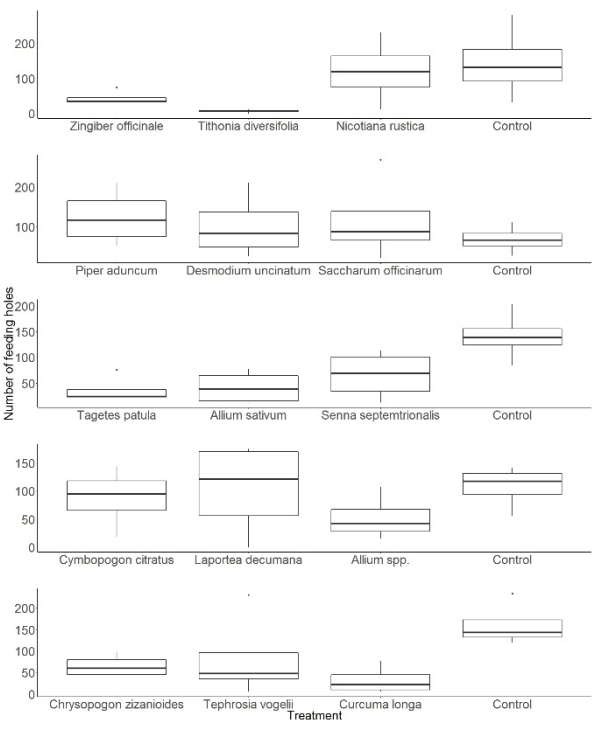
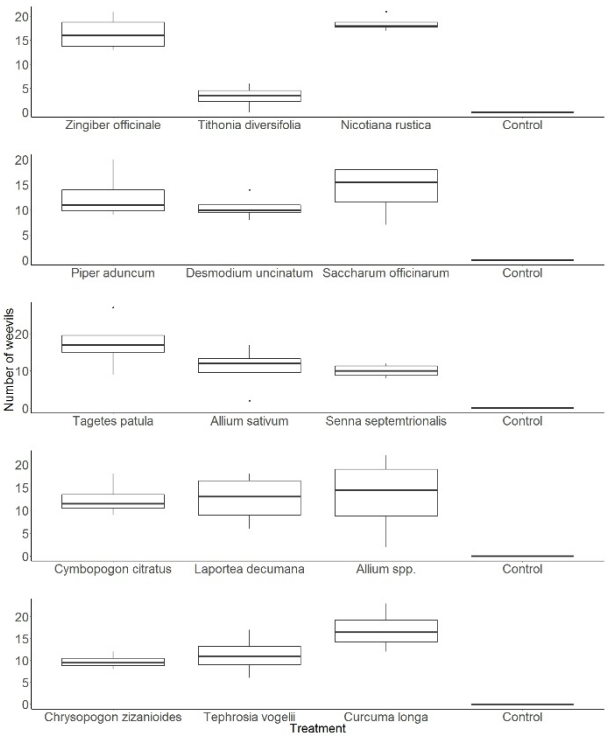


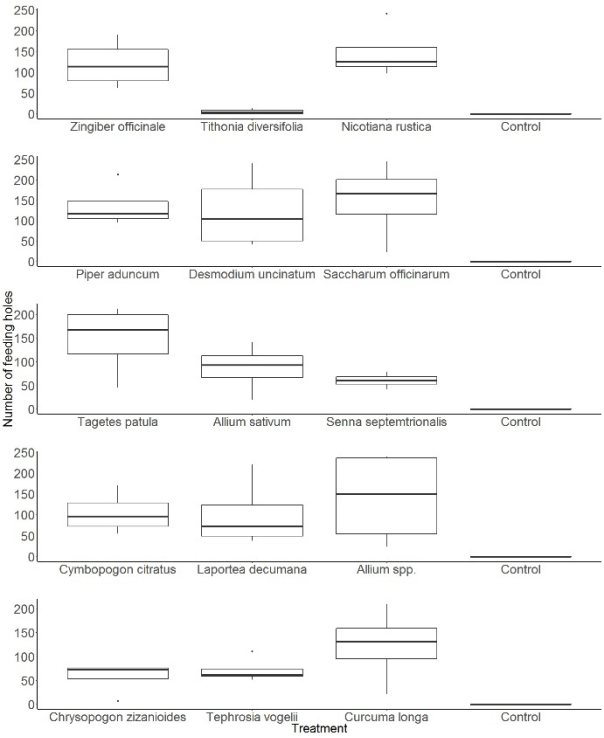
Fig 12. Response of *E. postfasciatus* to candidate mulch treatments. Top, uncovered control treatment; bottom, covered control treatment.

7.2 Novel pest management options









7.2.1 Entomopathogenic fungi

This work corresponds to project activities 2.1, 2.2, 2.3, 2.4, as well as the intermediate milestone of journal publication. The broad pattern of studies began with laboratory work in Australia during which relatively large number of isolates were screened and methods developed followed progressively by transfer of materials, skills, and methods to partners in PNG where on-station and on-farm studies were completed.

7.2.1.1 Isolation of entomopathogenic fungi (EPF) and efficacy testing of EPF in Australia & PNG

A manual of methods for EPF was provided to Unitech in 2017 to keep laboratory methods consistent between Australia and PNG where possible (see Appendix 17). Briefly, in Australia, the large number of isolates was rapidly narrowed down to one *Beauveria bassiana* and five *Metarhizium* spp. initially using a model insect (mealworm) then the sweetpotato weevil (SPW) for screening. This short-list was further narrowed to one *B. bassiana* and one *M. anisopliae* isolate and this also the minimised potential for cross-contamination when using many isolates. In Australia, simple liquid formulations (spraying) were relatively ineffective against SPW (Fig 13); however, conidiated rice (rice as a substrate for growth of the fungi) was highly effective probably because the relative humidity was higher and there was repeated contact between the SPW and the conidiated rice, especially as it was it was found to be actively growing on the soil substrate (Fig 14, 15). By 30 days, the highest doses (and still economically feasible) of the two EPF (10^7 and 10^8 conidia per gram sand/potting mix) caused between 75 and 90% weevil mortality. This finding informed the method used in glasshouse and the field trial in Australia and field trials in PNG. It was hypothesised that if the EPF was able to colonise the soil and form conidia capable of causing infection (and subsequent death) of pest insects, then this was a more-long lasting, cost-effective, and sustainable way in which to apply a biocontrol product. By applying the conidiated rice at planting, the EPF had months to establish and colonise the soil the ideal conditions of soil moisture and temperature and protection from UV being afforded under the developing canopy.

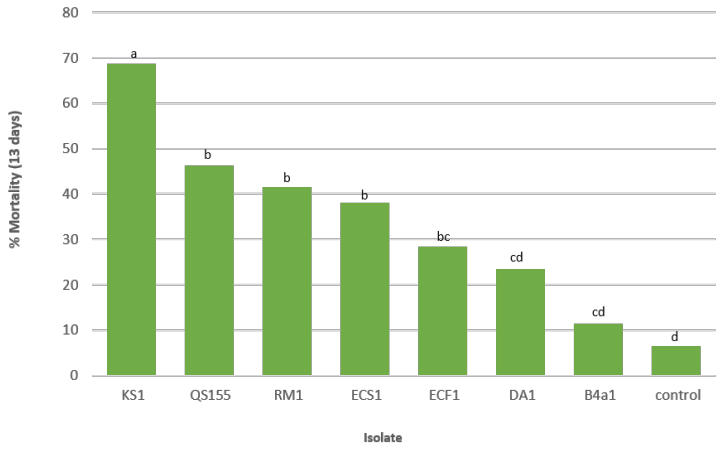


Fig 13. Mortality of weevils 13 days after inoculation. Weevils were exposed to a 10^7 conidia per ml solution via spraying (n = 15, 4 replicates per treatment). Control is 0.05% Tween® 80 solution. Treatments with a different letter are significantly different (P<0.001).

Fig 14. Sweetpotato weevil challenged with inoculated rice with *M. anisopliae*. (a), *B. bassiana*, (b) and sporulation of *B. bassiana* on rice (c).

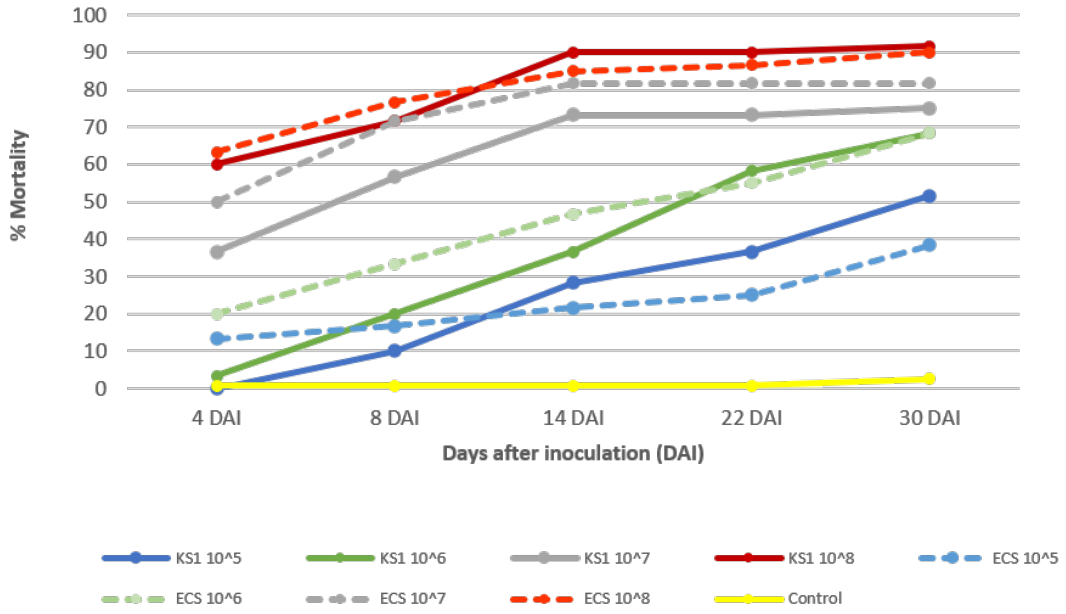


Fig 15. Mortality of weevils over time when inoculated with ECS1 or KS1 (concentrations ranging from 10^5 to 10^8 conidia per gram of sand/potting mix) as conidiated rice in a dose-response bioassay (n = 20, 6 replicates per treatment). Control is sterilised sand/potting mix alone.

In glasshouse studies, the conidiated rice inoculum was trialled against mealworm to establish limitations for control under more realistic conditions in preparation for weevil glasshouse experiments (not presented here). These same experiments examined the endophytic capacity of *B. bassiana* and *M. anisopliae*. Using a relatively low dose of EPF (10^6 conidia per g 'soil') 70% mealworm mortality was achieved. There was no statistically significant advantage of combining the two EPF in terms of insect mortality; however, multiple species in one biocontrol product may be useful to target different pest insects in the field (Fig 16).

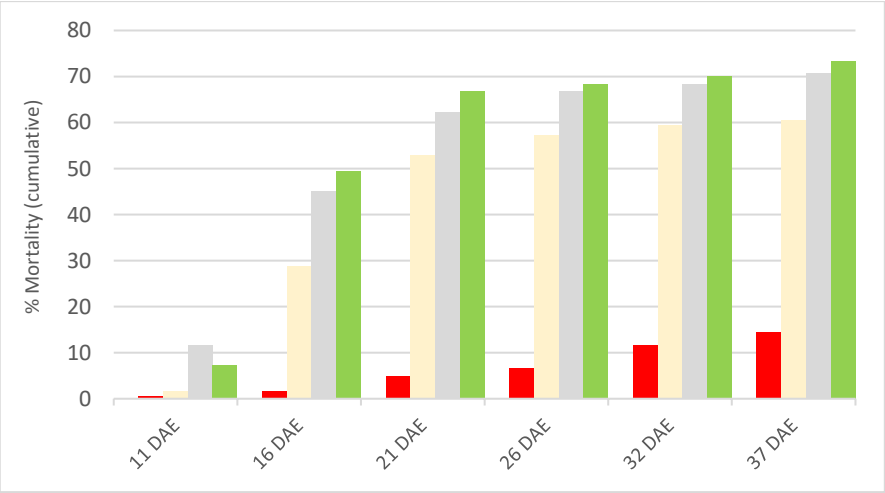


Fig 16. Glasshouse experiment 2. Effect of treatment on the mortality of mealworms over time. Values are the mean of 5 replicates. Red (control), green (*M. anisopliae*), cream (*B. bassiana*), light grey (mix of *M. anisopliae* and *B. bassiana*). At each harvest: 21/5/21 (LSD = 7.29, P=0.013), 26/5/21 (LSD = 15.83, P<0.001), 31/5/21 (LSD = 13.94, P<0.001), 5/6/21 (LSD = 12.34, P<0.001), 11/6/21 (LSD = 12.76, P<0.001) and 16/6/21 (LSD = 12.13, P<0.001).

There was no evidence that the *B. bassiana* and *M. anisopliae* isolates tested in this study were capable of colonising any sweetpotato tissues. Potentially, more than 40 days of growth is required in the presence of EPF. DNA sequencing of the fungi isolated into pure culture from various tissues revealed that >30 fungal species were found to growing endophytically. There was no obvious presence of disease in any of the tissues examined. The beneficial fungi *Trichoderma* spp. were present in >70% of the sections examined and its presence may have prevented successful colonisation by *B. bassiana* and *M. anisopliae.* Importantly, many plant pathogenic fungi (specifically *Fusarium* spp.) were isolated from healthy tissues, suggesting that these fungi may lie 'dormant' until conditions (e.g., heat, moisture) are suitable for it to cause disease (e.g., Fusarium wilts and rots) (see Appendix 12b).

PhD candidate, Sudhan Shah, supervised by Prof. Gavin Ash and Dr Bree Wilson examined the biological control of mealworms as a proxy for wireworms using nutrient fortified-encapsulated *M. anisopliae* ('granules'). Briefly, using an isolate previously identified to kill SPW, *M. anisopliae* QS155 was formulated in granules containing a nutrient source for the EPF and an attractant for mealworms/wireworms. Laboratory studies showed high mortality of mealworms (90%) through ingestion and cuticle contact with the granules. Laboratory studies also showed that significantly greater resporulation of granules (important for the persistence of EPF in the soil and ongoing infection of insects) was achieved in sterile soils. In non-sterile soils, indigenous microbes likely outcompeted with the *M. anisopliae* for nutrients, affecting its ability to resporulate. This result was confirmed in a glasshouse study where significantly greater resporulation and mealworm mortality (76%) was achieved compared to granules inoculated into non-sterile soils. This has important ramifications for the persistence of *M. anisopliae* in the soil when applied as a biocontrol agent: there is high competition with other soil microbes that needs to be overcome for EPF to be highly successful.

Fig 17. Mealworms challenged with unformulated *Metarhizium* sp. (a), formulated granules of *Metarhizium* sp. (b) and pot trial examining effect of soil type (non-sterile and sterile) and formulated granules on mortality of mealworms and storage root damage.

A collaboration was formed between USQ and a local company, Biological Ag, to explore the effectiveness of sweetpotato project-derived isolates on pests of citrus (longicorn beetle larvae, citrus gall wasp), fruit fly, elephant weevil on blueberry and fall armyworm. Whilst results are preliminary, several isolates show promising activity (see Appendix 18).

In PNG, laboratory and field studies were conducted to isolate, screen, and examine the pathogenicity of PNG derived isolates on *C. formicarius* and *E. postfasciatus* (see Appendices for details). Briefly, laboratory bioassays used a very low concentration of conidia (10^5 conidia per ml) of various isolates of *Metarhizium* spp. and *B. bassiana* and *Beauveria* spp. Various application methods were used: immersion of weevils, immersion of the food source (sweetpotato) or the weevils were sprayed. Due to the large number of isolates, separation of sexes and two weevil species, many bioassays were performed separately making it difficult to compare statistically. Generally speaking, across all experiments dipping the sweetpotato in the suspension of conidia was the least effective application method and immersion or spraying of the weevils was the most effective. However, there were often no differences between the control and treatment type. Moreover, there were few instances of differences between isolates and the control, probably because such a low dose of conidia was applied. Despite this low dose, conidia were found to be sporulating on *Metarhizium* spp. or *Beauveria* spp. exposed cadavers, but never on control weevil cadavers. At the time of this report, the species of *Beauveria* had not be confirmed using molecular methods. Broadly speaking, across both sexes for *C. formicarius* the better performing isolates *Beauveria* spp. took between 8.6 and 11 days to cause mortality. Again, broadly speaking, *Metarhizium* spp. treated *C. formicarius* were slower to cause death compared to *Beauveria* spp. and ranged from ca. 13 to 17 days. For *E. postfasciatus* treated with *Beauveria* spp., there was no significant effect of isolate and no difference between control and dipped or sprayed weevils. Immersed weevils took on average 13.5 days to die. For *Metarhizium* spp. treated *E. postfasciatus*, only application type was significant, with immersed weevils taking the least time until death (ca. 13.5 days to death).

A CSU honours student (Dylan Male) supervised by Prof Gurr, Dr Wilson and Dr Liu examined the effect of entomopathogenic fungi combined with mulch on weevil behaviour at PNG Unitech. That work sets the scene for two potential scientific impacts: (i) the odours from entomopathogenic fungi appear to be repel weevil adults (both species) and later there could be scope to use the particular compounds to design repellent strategies points to a synergistic effect of these two tactics that had previously been tested separately; (ii) synergies may be possible in the interactions between mulches and entomopathogenic fungi such that the fungus adds to the protective repellency effect of the mulch and also is benefitted by the mulch serving as a growing substrate and desiccant- and UV-protectant to prolong the field life of inoculum. *Metarhizium, Beauveria* and mulch were all found to have a significant effect ($P < 0.05$) on the number of weevils reaching the sweetpotato storage root in the olfactometer bioassay for both weevil species. There was a high positive correlation (correlation value = 0.748) between the

number of weevils reaching the storage root and number of feeding holes. *Metarhizium, Beauveria* and mulch were all found to have a significant effect on the number of feeding holes on the storage root caused by *C. formicarius,* however only *Beauveria* was found to have a significant effect on the number of feeding holes caused by *E. postfasciatus*. Following the olfactometer bioassay, both weevil species recorded lower survival probabilities for weevils that reached storage roots in the presence of EPF. Emissions of volatile organic compounds (VOCs) from EPF and mulch identified included kessane, pyrazine, oxime- and bicyclo [3.1.1] heptan-3-one. This study concluded that EPF and mulch has the potential to be effective in influencing the behaviour of sweet potato weevil adults so serve as a springboard, justifying future work on the potential of EPF and mulches to be used in pest management infestation in PNG.

7.2.2 Barrier Plants

This work corresponds to project activities 2.5 and 2.6 (Section 6). The broad pattern of studies began with laboratory work in Australia during which relatively large number of plant treatments were screened and methods developed followed progressively by transfer of materials, skills, and methods to partners in PNG where on-station and on-farm studies were completed.

Work in Orange, NSW, developed bioassay methods for screening candidate living plants as potential barriers to weevil immigration. Key features of this include the use of a multi-arm olfactometer (Fig 18) to simultaneously compare the response of adult weevils to candidate barrier plants. Preliminary work determined that weevils needed to be kept under non-crowded conditions for a period after emergence from the (crowded) rearing containers for them to exhibit discrimination between experimental treatments. At the ends of each olfactometer arm, the odour source needed to be sweetpotato foliage and storage root rather than either of these plant parts alone. The intermediate vessel in each arm held live, growing candidate plants. Candidate plants tested were: *Daucus carota, Allium cepa, Thymus vulgaris, Origanum vulgare, Allium fistulosum, Capsicum annuum, Zea mays, Ocimum basilicum, Foeniculum vulgare, Tulbaghia violacea, Mentha piperita, Cymbopogon citratus, Allium schoenoprasum, Tanacetum cinerariaefolium,* and *Rosmarinus officinalis*,

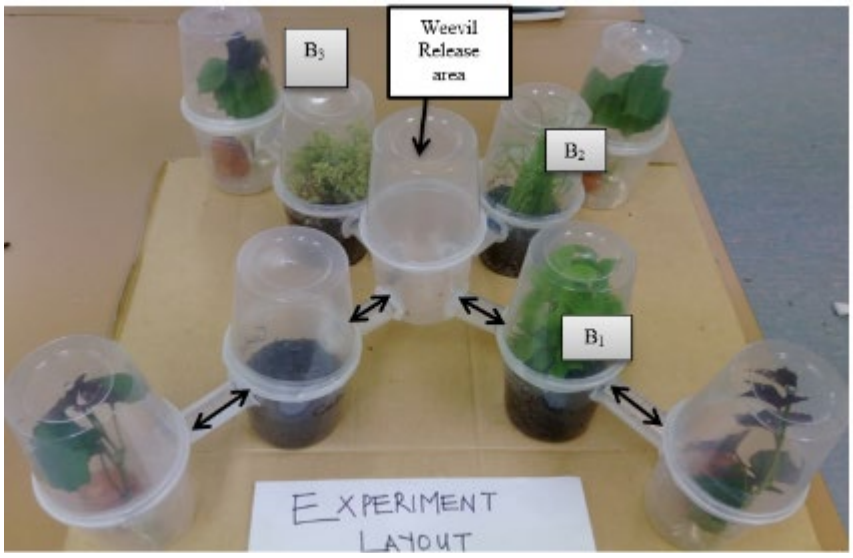


Fig 18. Multi-arm olfactometer to simultaneously compare the response of adult weevils to candidate barrier plants. Arrows indicate the potential movement of weevils in response to the sweetpotato foliage and storage root in the ends of each arm and the differing barrier plant treatments in the intermediate unlabelled control treatment arm with no barrier plant in the intermediate container. B_1- chili, B_2- spring onion, B_3- oregano and Thyme, oregano, sweetcorn, basil, lemon grass, spring onion, fennel, and

mint identified as the most promising living barrier plants. An example of the results is provided in Fig 19.

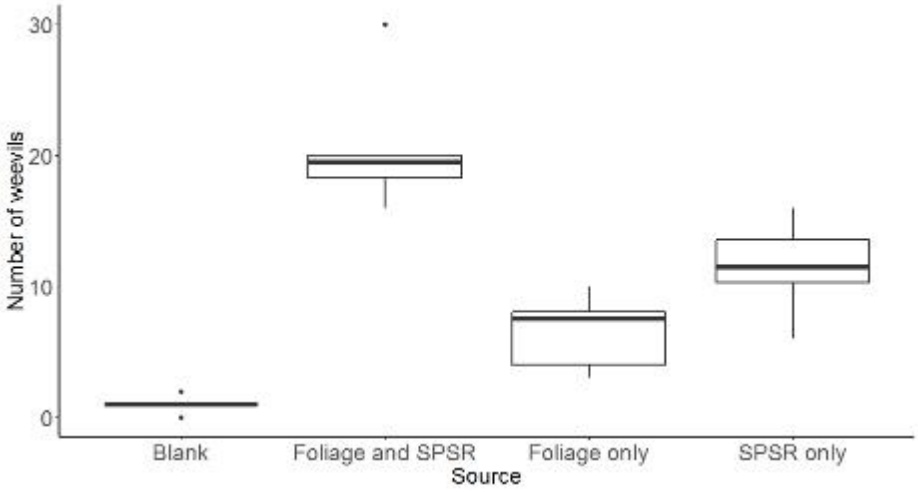


Fig 19. Means ± S.E of sweetpotato weevils found on sweetpotato storage root at each endpoint of the four - arm choice olfactometer after 48 hours.

A subsequent field study used a choice test design to assess the numbers of weevils that penetrated strips of candidate barrier plants from a release area to reach an inner area where sweetpotato was growing. Basil and chives allowed significantly fewer weevils to penetrate (Fig 20). The potential impact of these results is two-fold. The bioassay methods developed in NSW have direct utility for further screening of locally appropriate treatments in PNG. Second, the results serve as tentative proof of concept that living barrier plants can discourage weevil movement into adjacent sweetpotato and reduce levels of damage.

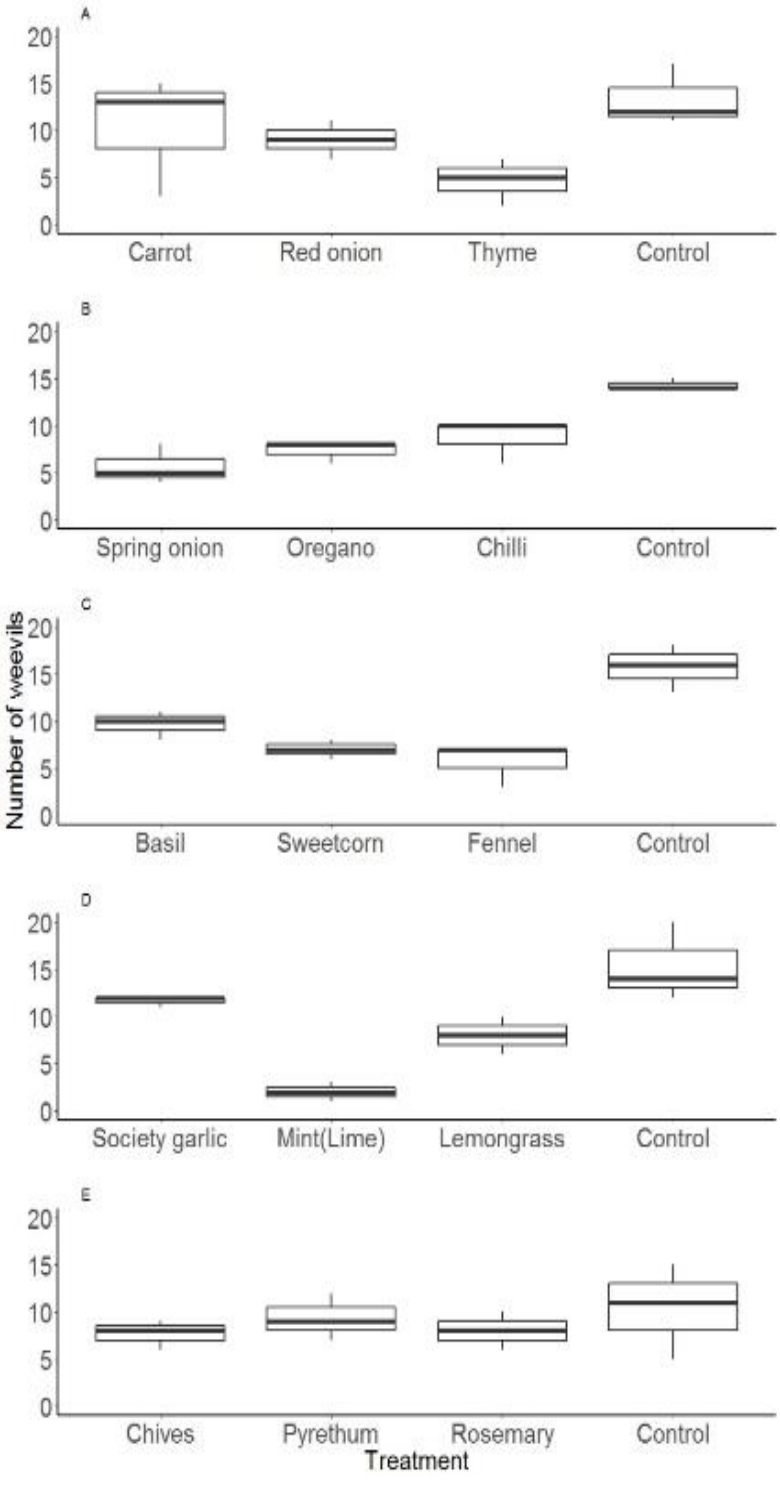


Fig 20. Number of weevils reached sweetpotato material in the terminal container of the olfactometer arm with living barrier plant treatment

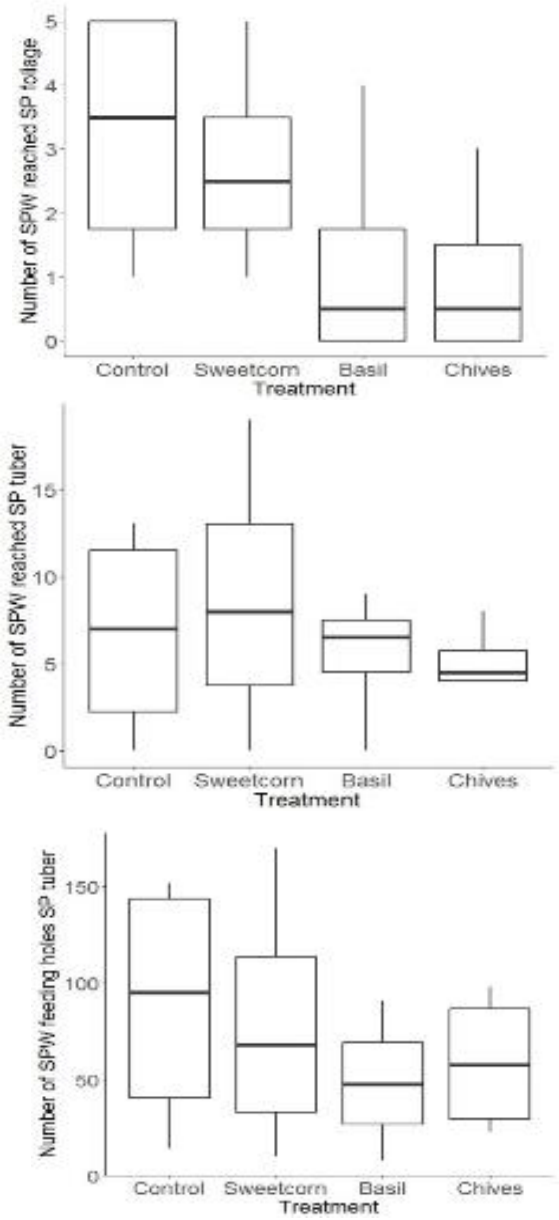


Fig 21. Australian field test results of barrier plant on sweetpotato weevil

Whilst the foregoing work on barrier plants was principally aimed to be a steppingstone to in-country evaluation of locally relevant plant species, a small study was undertaken to develop a basic understanding of some of the plant volatile compounds that may underpin insect responses. Plant volatile chemical analysis identified citronellal and citronellol from geranium; and carvone from mint. This work was presented at the Australian Entomological Society Annual Scientific Conference by Dr J Liu in 2019.

Subsequent work in PNG involved use of the multi-armed olfactometer developed in Australia to screen local plant materials against weevils and expand the scope of work from sweetpotato weevil alone (present in Australia) to encompass the West Indian sweetpotato weevil (a second pest species of great importance in PNG). This successfully identified optimal plant species from a larger range of candidates (18), with these most promising plants used in field studies. Work with *C. formicarius* found several species of plants that

can interfere with adult movement toward sweetpotato under lab conditions, especially: *Plectanthrus, Centratherum, Desmodium, Tithonia, Tagetes* and *Tephrosia* (Fig 22). These same species were also among those that led to the most dramatic reductions in the numbers of feeding holes in the storage roots at the distal ends of the olfactometer arms (Fig 23).

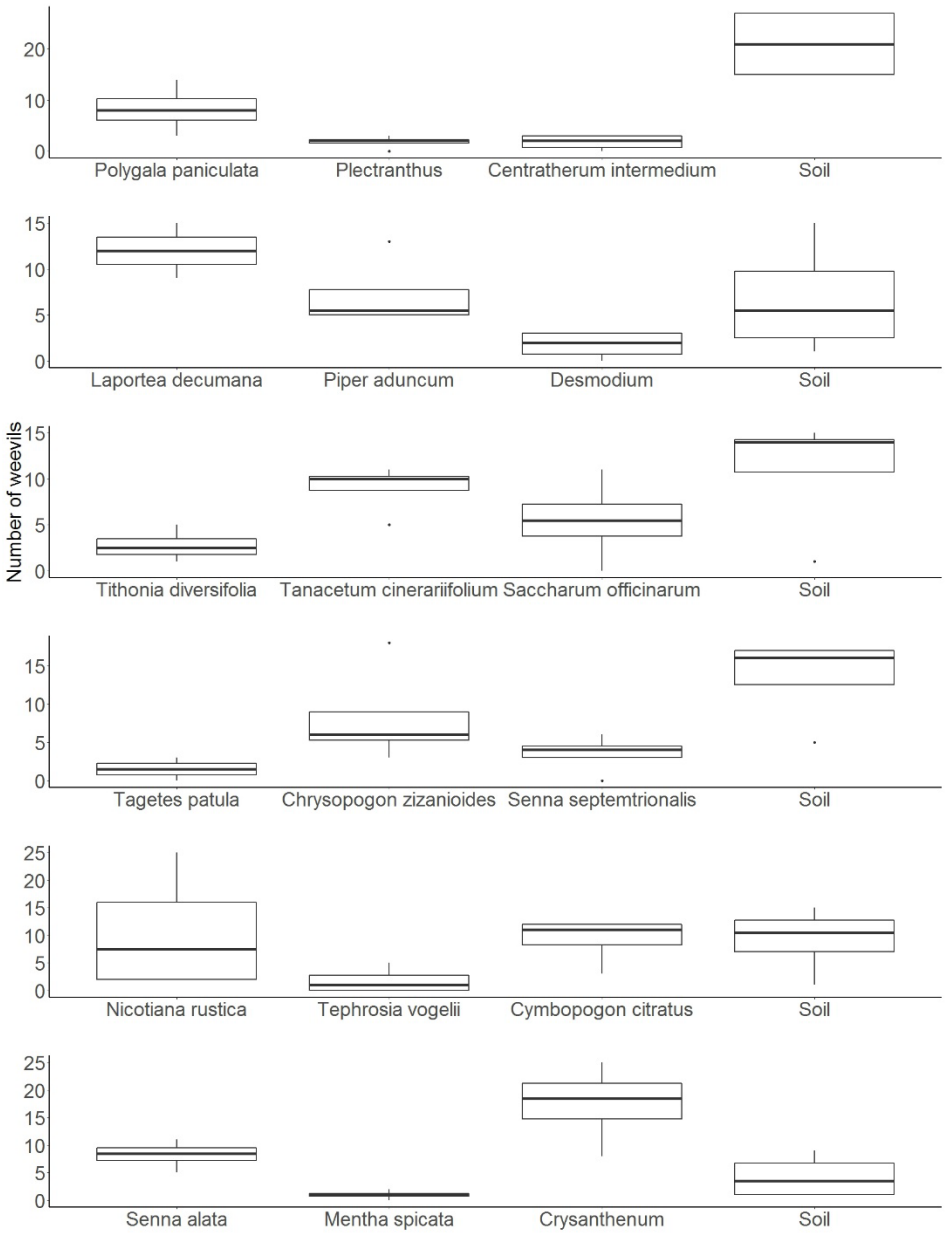


Fig 22. PNG laboratory test results for sweetpotato weevil movement in response to barrier plants.

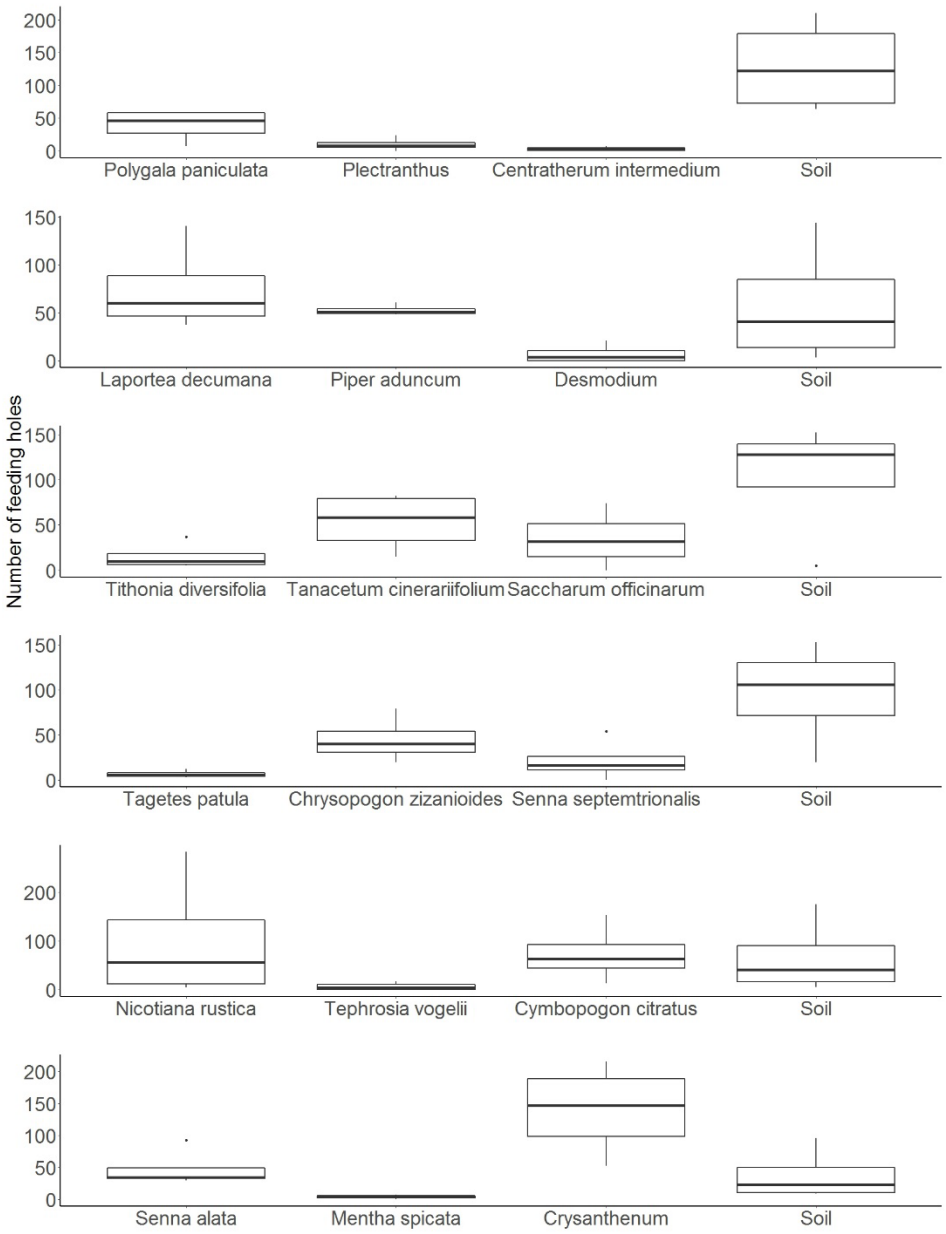


Fig 23. PNG laboratory test results for sweetpotato weevil damage in response to barrier plants.

For *E. postfasciatus,* there were again major differences in the effects of plant. *Desmodium, Tithonia, Saccharum, Tagetes, Senna, Tephrosia* and *Mentha* most strongly impaired movement of weevil adults (Fig 24) and reduced feeding damage to storage roots (Fig 25).

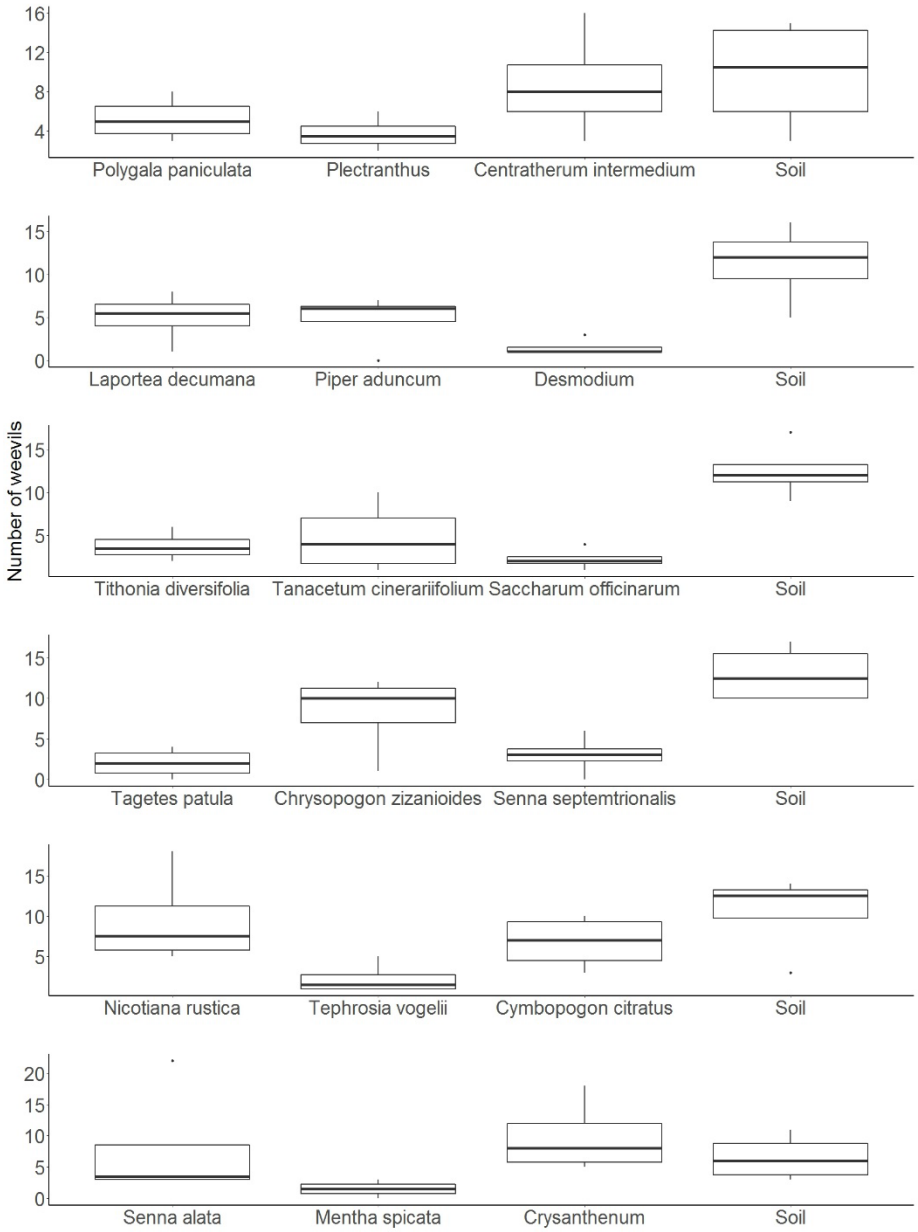


Fig 24. PNG laboratory test results for West Indian sweetpotato weevil movement in response to barrier plants.

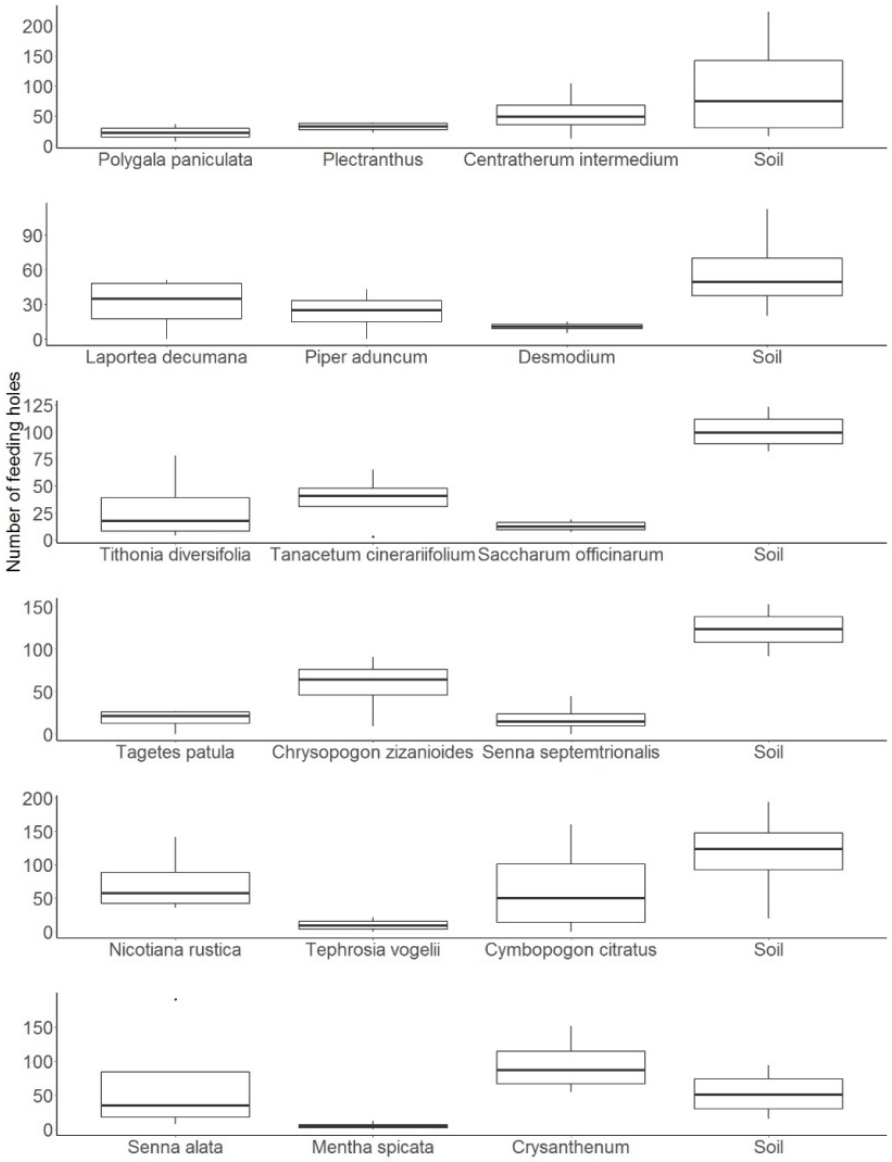


Fig 25. PNG laboratory test results for West Indian sweetpotato weevil damage in response to barrier plants.

Clearly there are major differences in the impact of differing living plants as barrier to weevil ingress, so exploitation of this mechanism depends on choosing the right material. Whilst the plant species named above showed good potential to control one or both weevil species, most other species appeared to offer no promise. Chrysanthemum appeared to promote movement of both weevil species and exacerbate damage and should be avoided.

7.3 'Best-bet' combinations of integrated pest and disease management (IPDM) options

7.3.1 Best bet strategy comparison with conventional practice

Biotic effects

Visual assessment results for the first year of trials in Eastern Highlands Province showed higher numbers of *C. formicarius* in the conventional practice treatment at end of season assessment date (P=0.07) though the cryptically coloured adults of *E. postfasciatus* were not detected (Fig 26). A destructive survey method was also used at harvest time to inspect for weevils inside the plant tissues and this revealed small numbers of *E. postfasciatus* and larger number of *C. formicarius*. For both weevil species there was a strong treatment effect with a lower number in the best bet strategy treatment (P<0.05) (Fig 27).

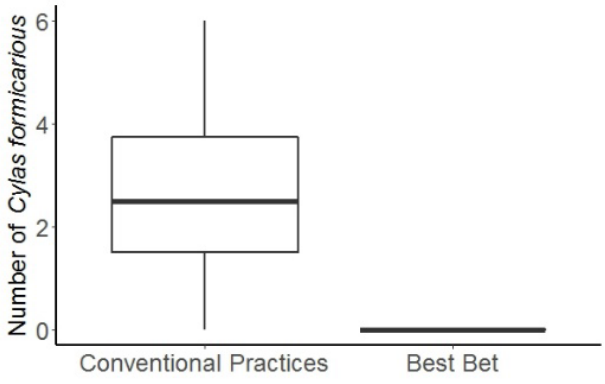


Fig 26. Above ground field survey for best-bet strategy implementation in Eastern Highlands Province sweetpotato farms.

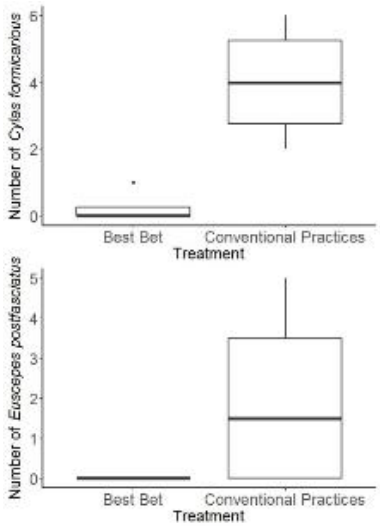


Fig 27. Destructive survey of weevils inside sweetpotato plant at harvest time for best-bet strategy implementation in Eastern Highlands Province sweetpotato farms

For scab disease, mid-season severity scores in the Jiwaka field trial were lower in the best bet treatment, but not to statistically significant extent. Gall mite severity was very low at this growth stage in both experimental treatments (Fig 28). At the time of the harvest assessment, scab severity had declined in both treatments, most likely reflecting the growth of healthy plant tissue under conditions not conducive to symptom spread. For gall mite, however, severity has increased markedly, especially in the control treatment (Fig 29). Numbers of weevils were low in this season but in the following year *E. postfasciatus* was relatively common and found to be emerging in higher numbers from the incubated storage roots harvested form the conventional practice treatment compared to best bet.

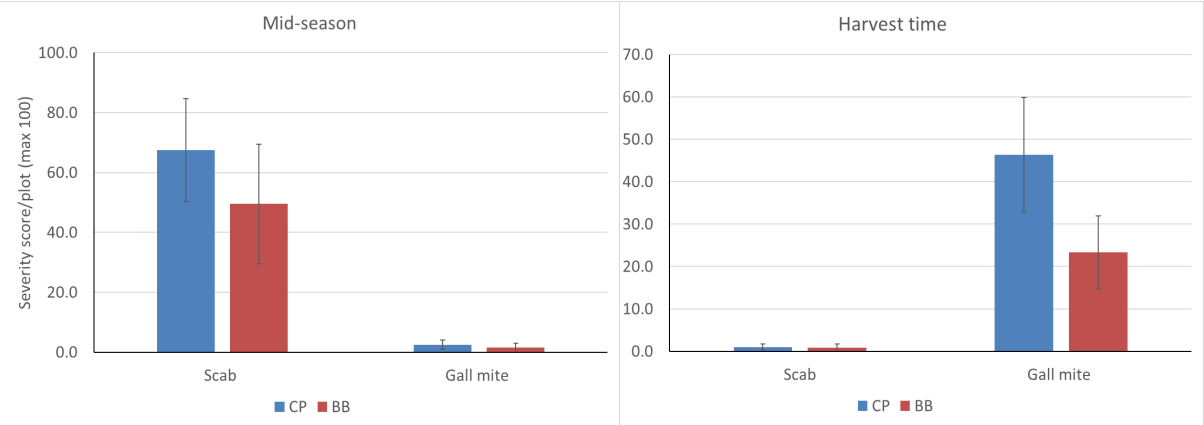


Fig 28. Scab and gall mite severity scores for Jiwaka Province. Left: mid-season assessment. Right: End of season assessment.

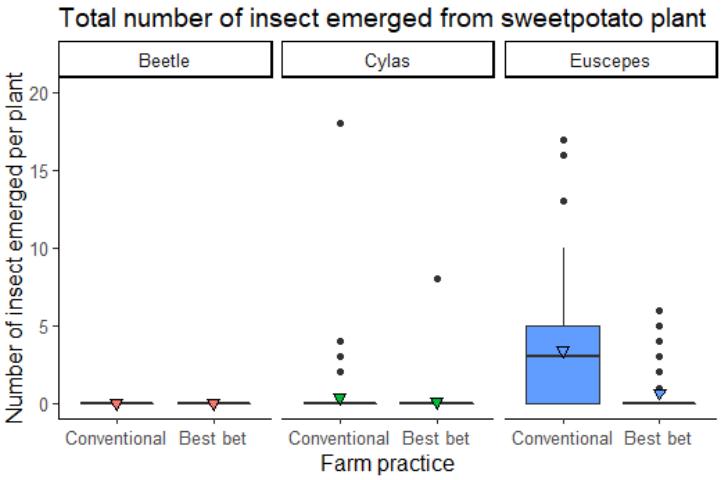


Fig 29. Effect of best bet strategy on reducing numbers of *E. postfasciatus* from storage roots in the best bet treatment.

The FPDA noted that PNG growers were excited about pheromone traps. Work with farmers across multiple districts has revealed strong effects. Fortnightly records of pheromone trap catches show large numbers were initially attracted on all sites but declined markedly on most sites over time (Fig 30). This suggests that pheromone traps may be depleting local populations of this pest because declines in catches in the first two weeks after deployment of traps is too rapid to be attributed to exhaustion of the pheromone within lures. This is supported by the fact that, in other districts, catches

remained high over multiple weeks. Manufacturer's directions state that traps last 6-weeks and this is evident in prolonged catches on other sites.

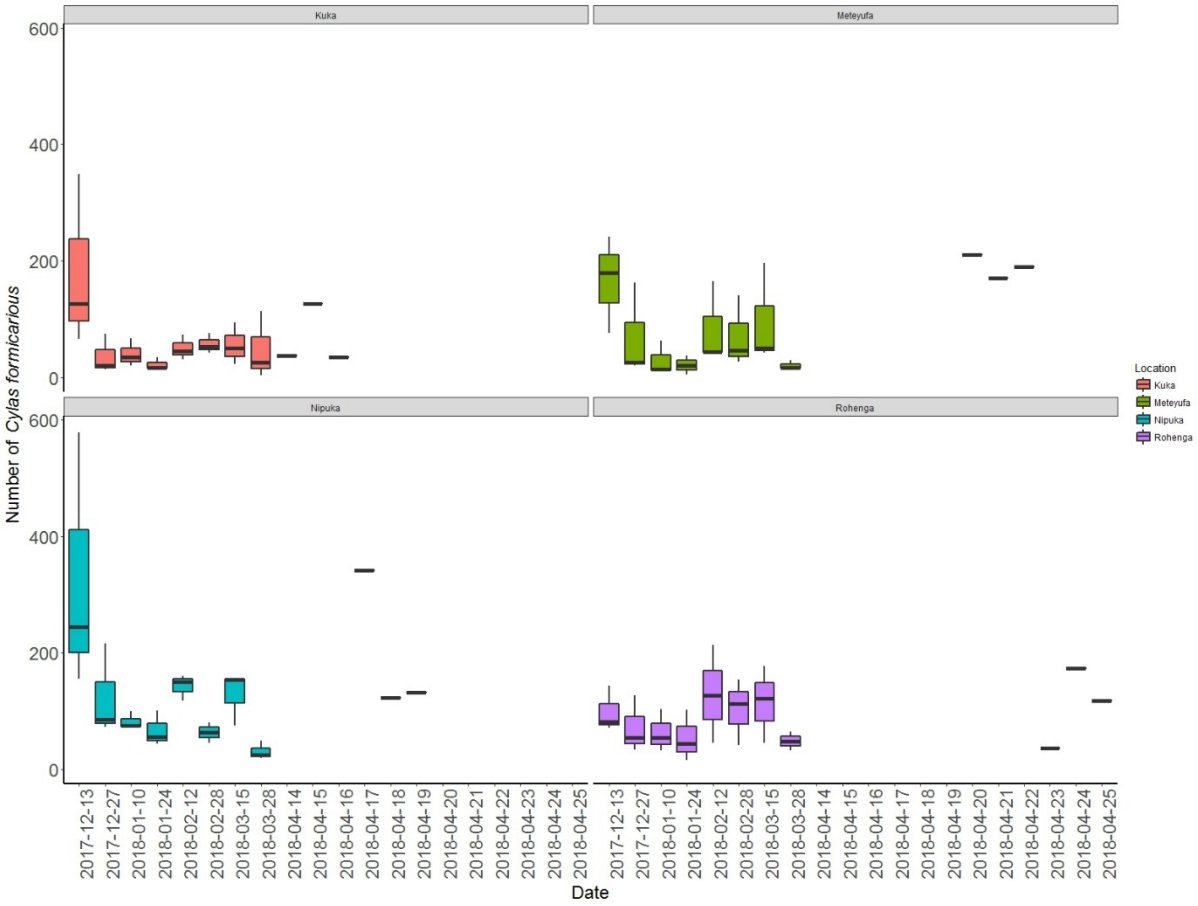


Fig 30. Sweetpotato weevil *Cylas formicarius* male adults caught under pheromone lures traps in 2017-18, Asaro.

The foregoing section provides key results for biotic effects of best bet treatments. Where pests and diseases are not mentioned for a given Province or year, levels of symptoms or numbers of insects were low.

Effects on yield

Over all trials, total yield of storage roots when crops were protected by the integrated pest and disease management strategy averaged 20.16 t/ha. This was almost double the yield in the control treatment consisting of conventional farm practice (Fig 30). An additional benefit was improved quality of the storage roots from the integrated pest and disease management strategy. Illustrating this, unmarketable yields in that treatment were lower (1.46t/ha) than in the control (2.25t/ha) whilst the all-important marketable yield was more than doubled from 7.99t/ha to 18.70t/ha. Importantly, these benefits were robust over sites and provinces rather than resulting from highly levels of performance in a few locations (Fig 32). For example, marketable yields in the control treatment never *exceeded* an average of 10.28 for any province yet were never *below* 17.44 in the best bet integrated pest and disease management strategy treatment.

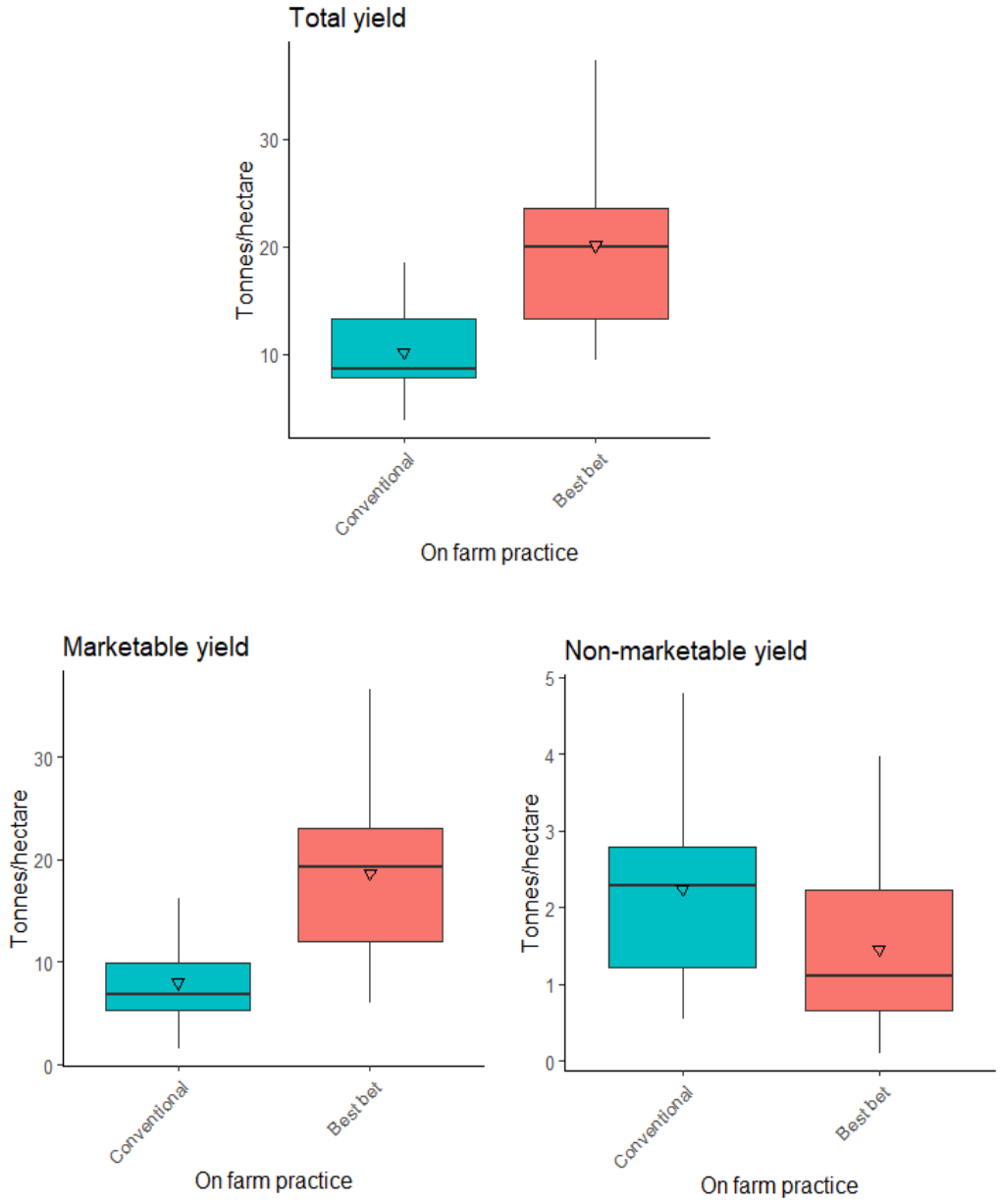


Fig 31. Overall yield benefit of the best bet strategy compared with conventional farmer practice. (Mean for all years and seasons)

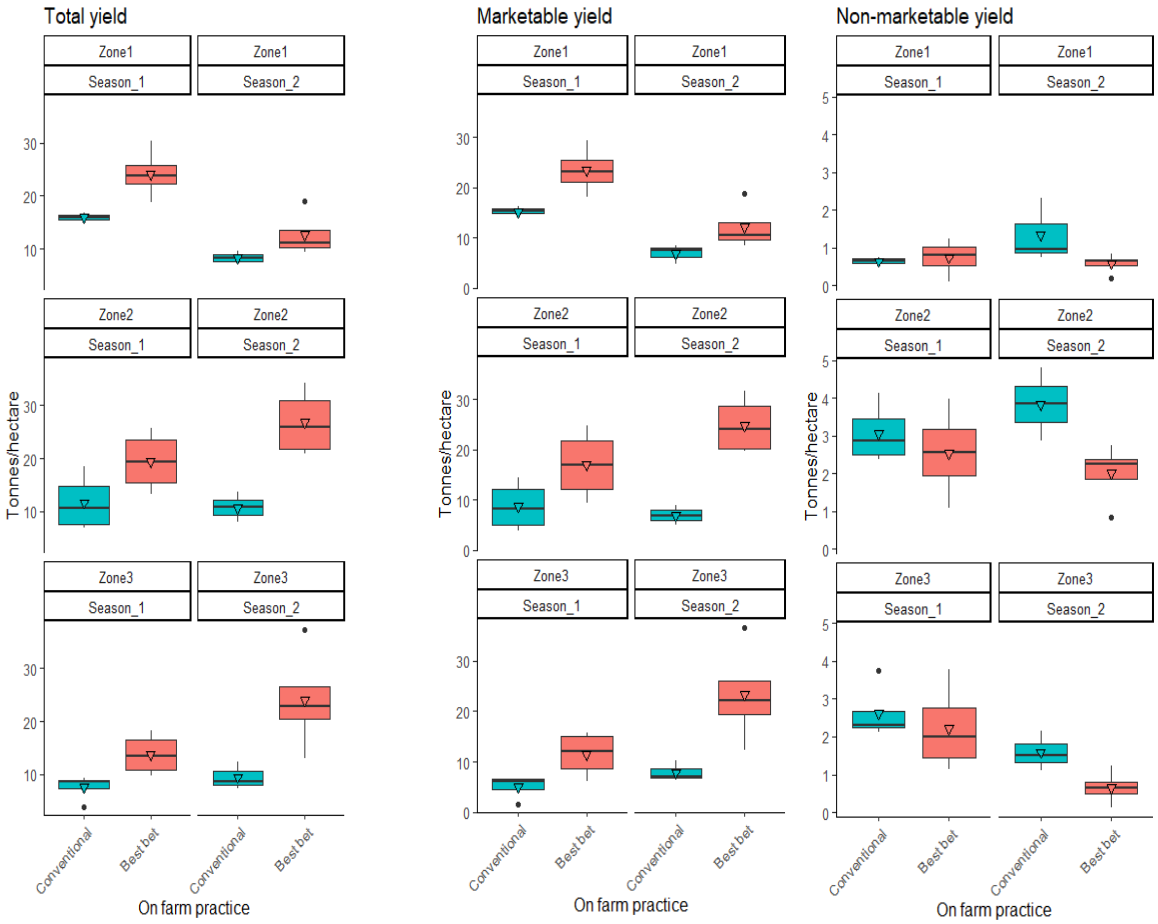


Fig 32. Consistency across provinces and seasons of the yield benefit of the best bet strategy compared with conventional farmer practice. (Mean for all years and sites)

7.3.1 Best bet plus strategies

This series of nine on-farm trial took place in 2020 and involved supplementing the best bet strategy with optimal forms of mulch and barrier plant (entomopathogen was added to one of the mulch treatments).

Mulches

Aggregating data from all nine sites, each of the mulch treatments significantly reduced the number of *E. post*fasciatus emerging from end of season samples of incubated storage roots but numbers of *C. formicarius* were lower (and treatment effects were not significant) (Fig 33). Partitioning results into trials conducted in Eastern Highlands Province (Zone 1) and Jiwaka Province (Zone 2), effects were consistent across both provinces (Fig 34).

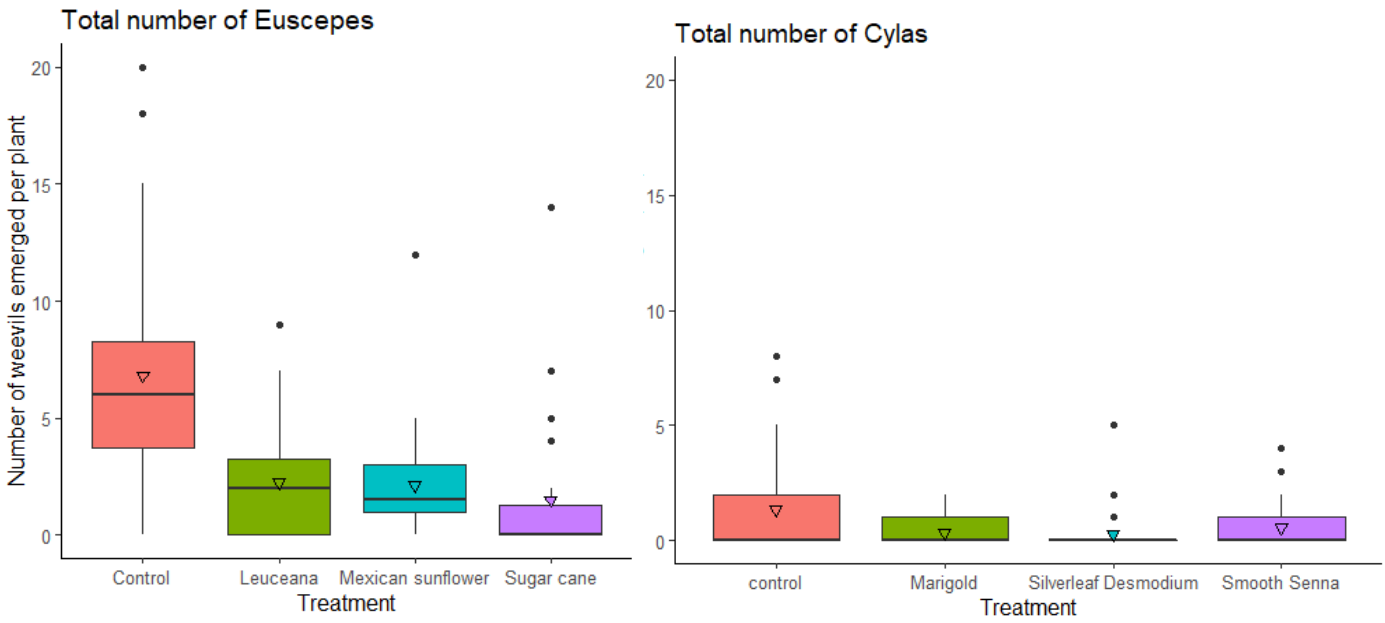


Fig 33. Effect of mulch treatments on *Euscepes postfasciatus* and *Cylas formicarius* (overall analysis).

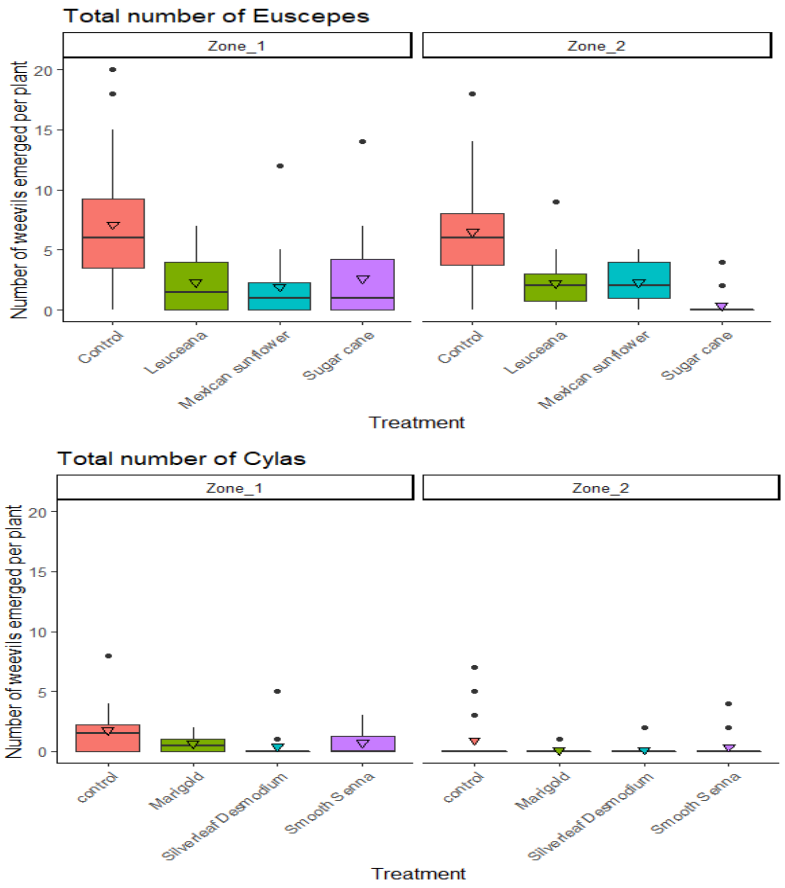


Fig 34. Effect of mulch treatments on *Euscepes postfasciatus* and *Cylas formicarius* (showing consistency of effects across Eastern Highlands Province (Zone 1) and Jiwaka Province (Zone 2), effects consistent across both provinces (Fig 35).

Aggregating data from all nine sites, marketable yield in the best bet plus mulch treatments were not significantly higher than in the best bet treatment (in which there was no mulch). This was the case even for the sugar cane mulch which was supplemented with the entomopathogenic fungus (Fig 35). The lack of significant treatment effects is explained, at least in part, by the high level of variability in yields among sites.

Partitioning data into individual sites revealed marked variability in the response of marketable yield to treatments (Fig 36). On only one site (Kongabil) was there a statistically significant effect of treatments with the Mexican Sunflower and leucaena treatments exhibiting lower yields than the Sugarcane plus entomopathogen and the control (best bet with no mulch) treatment. Data for total yield of storage roots showed equivalent results including statistical separation of means for the Kongabil site. This indicates that the mulch treatments tested do not offer strong scope to provide benefits to yield above the use of the best bet strategy under conditions of low pest and diseases pressure.

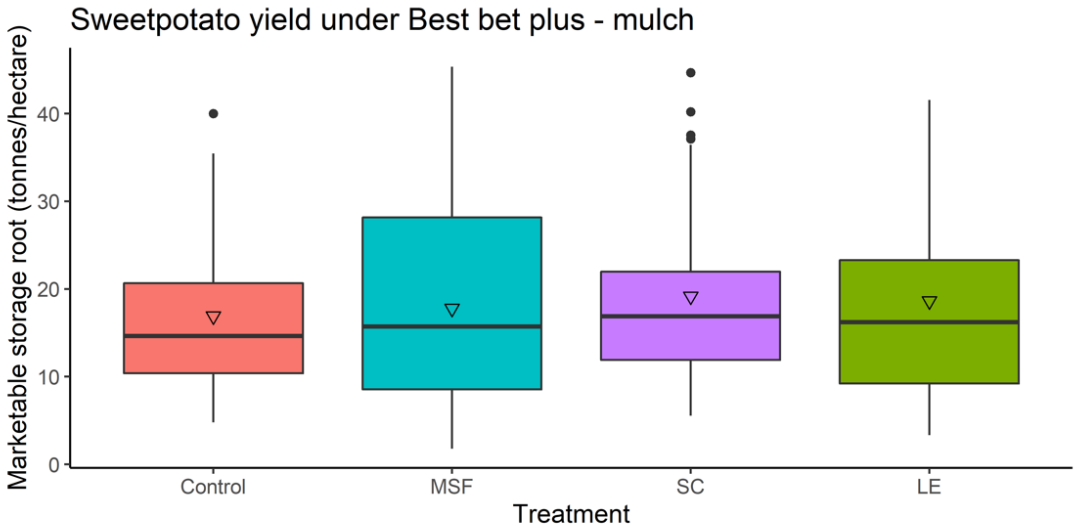


Fig 35. ANOVA shows no significant difference between treatments (MSF: Mexican Sunflower, SC: Sugar cane, LE: Leucaena) ($F_{(treatment, 3)} = 0.356$, $p = 0.785$)

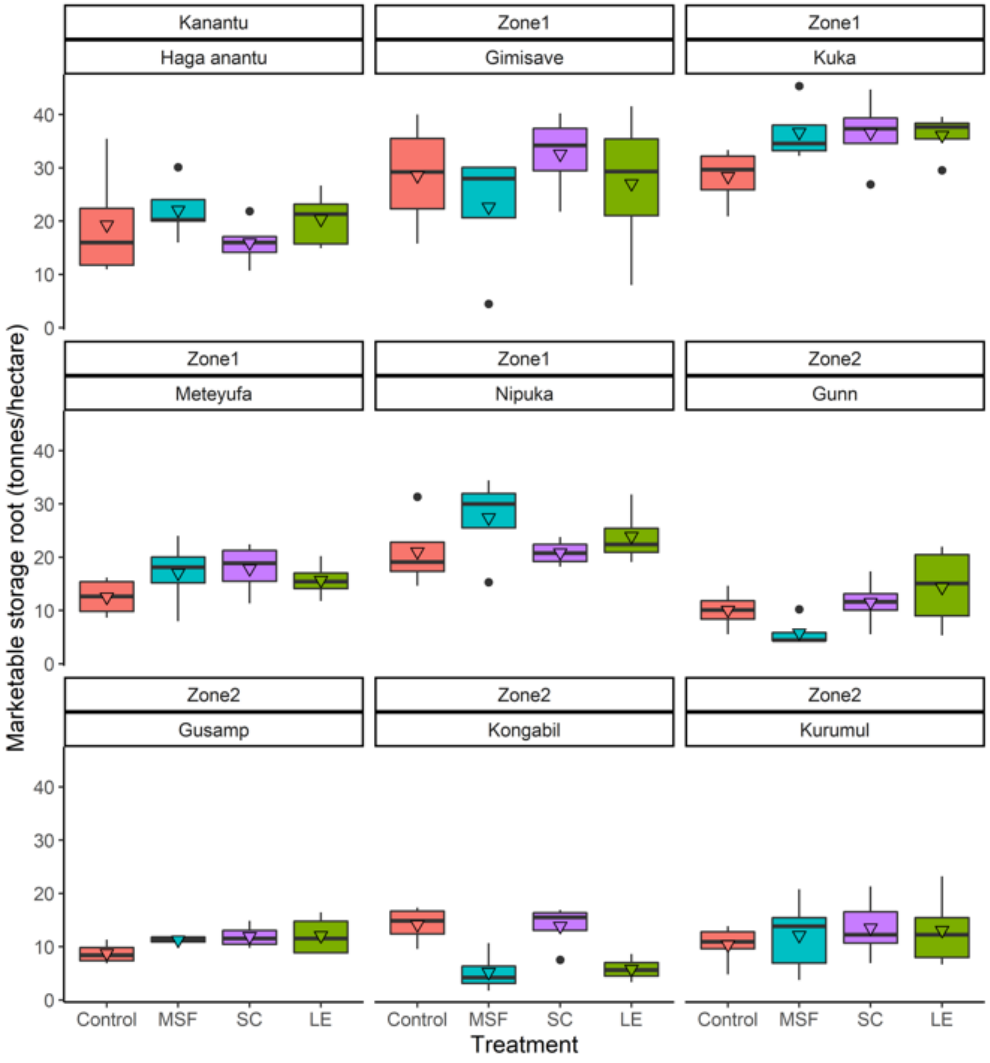


Fig 36. Comparison among farm sites on Sweetpotato marketable yield under mulch treatment (mean ± sd). (MSF: Mexican Sunflower, SC: Sugar cane (with entomopathogenic fungus), LE: Leucaena).

Living barrier plants

Aggregating data from all nine sites, each of the barrier plant treatments significantly reduced the number of *E. post*fasciatus emerging from end of season samples of incubated storage roots and vines. Numbers of *C. formicarius* were lower but treatment effects were again significant when comparing the control (best bet) with each of the best bet plus (barrier plant) treatments (Fig 37). Partitioning results into trials conducted in Eastern Highlands Province (Zone 1) and Jiwaka Province (Zone 2), effects were broadly consistent across both provinces though with higher absolute numbers in the latter province (Fig 38).

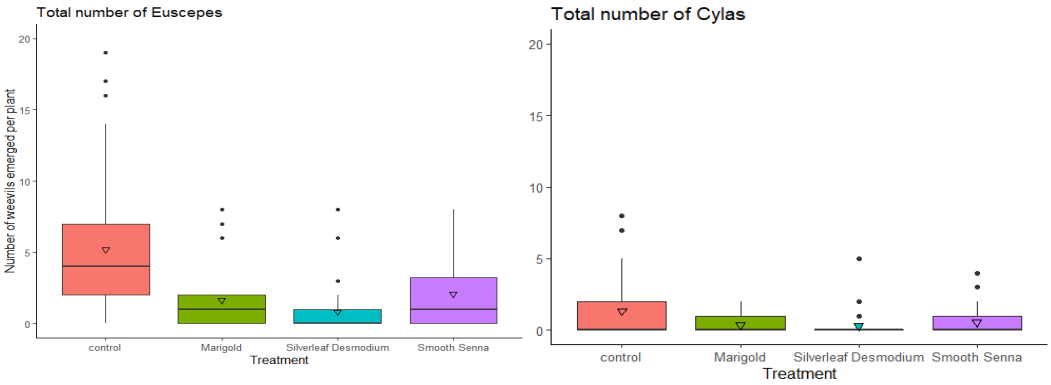


Fig 37. Effect of barrier treatments on *Euscepes postfasciatus* and *Cylas formicarius* (overall analysis).

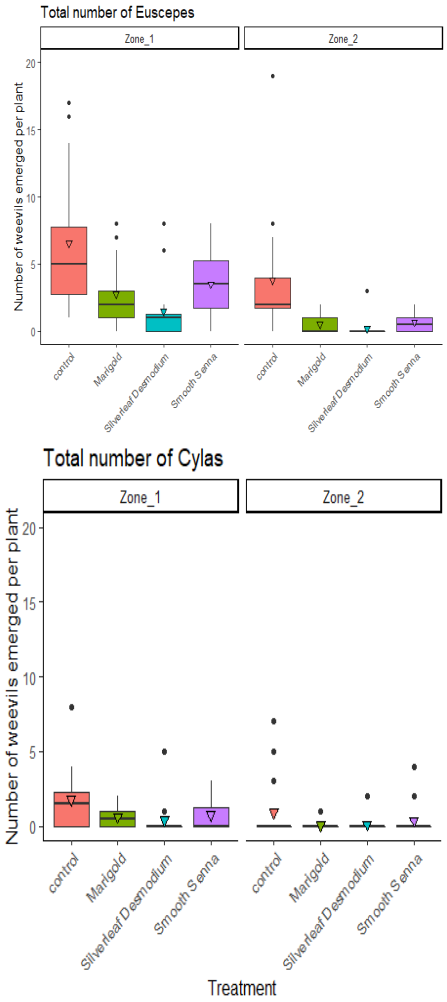


Fig 38. Effect of barrier plant treatments on *Euscepes postfasciatus* and *Cylas formicarius* (showing consistency of effects across Eastern Highlands Province (Zone 1) and Jiwaka Province (Zone 2), effects consistent across both provinces.

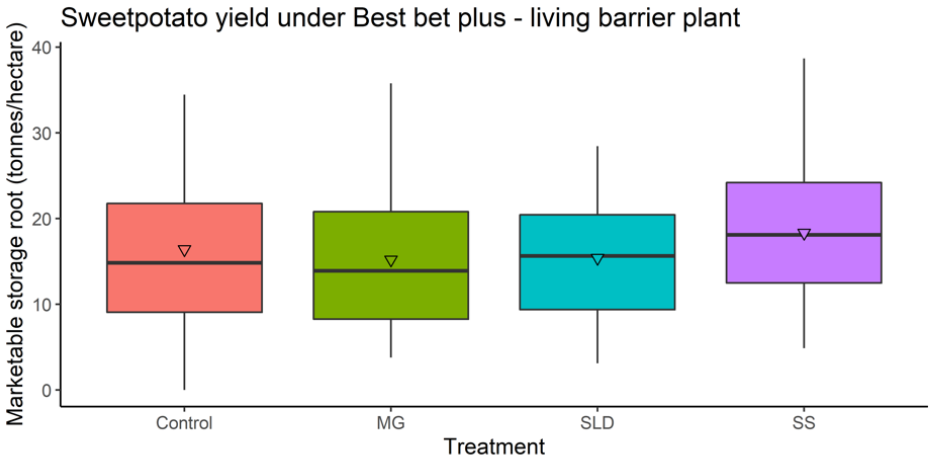


Fig 39. Yields under best bet plus treatments with barrier plants (MG: Marigold, SLD: Silver leaf Desmodium, SS: Smooth Senna) ($F_{(treatment, 3)} = 1.08$, $p = 0.359$)

Aggregating marketable yield data from all nine sites, barrier plant treatments were not significantly higher than in the best bet treatment. The lack of significant treatment effects is explained, at least in part, by the high level of variability in yields among sites (Fig 40).

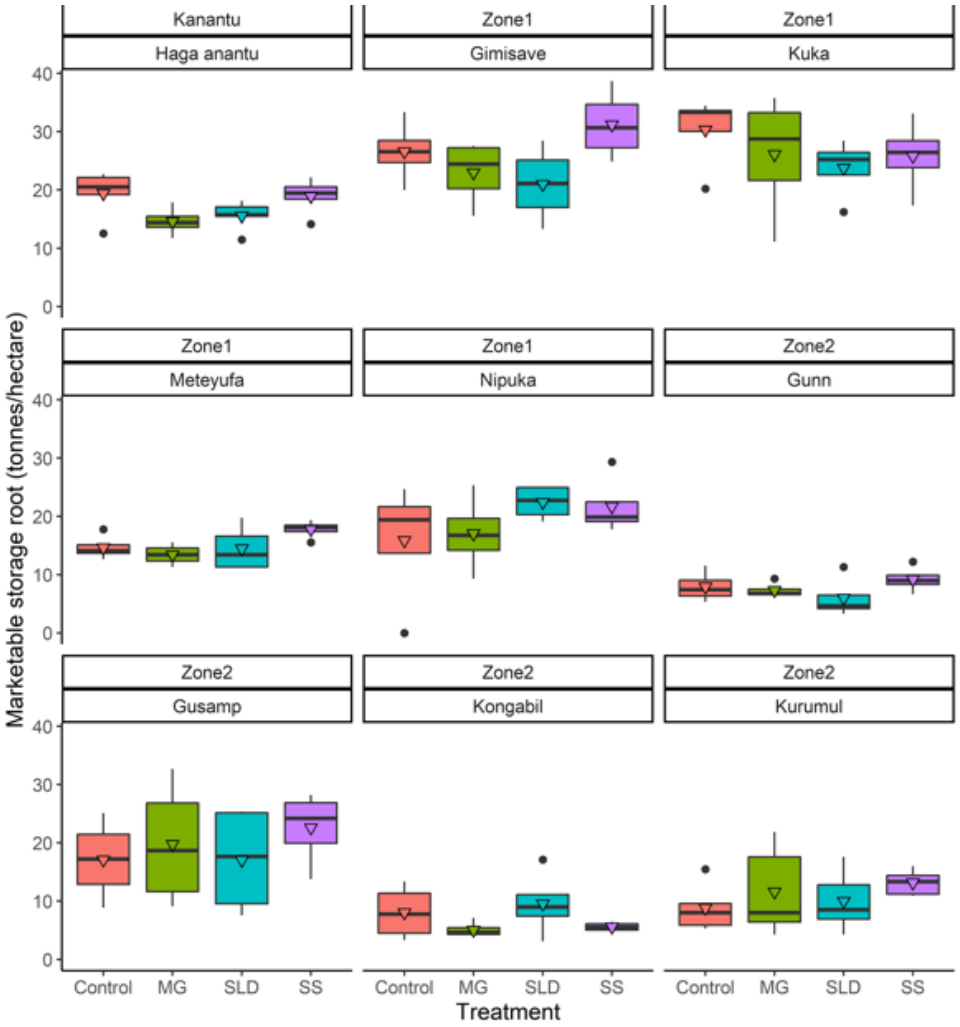


Fig 40. Comparison among farm sites on Sweetpotato total yield under living barrier plant treatment (mean ± sd). (MG: Marigold, SLD: Silver leaf Desmodium, SS: Smooth Senna).

Results from the best bet trials overall indicate that the mulch and barrier plant treatments tested offer good scope to provide reduce weevil infestation but effects on yield might be apparent only under conditions of greater pest pressure. Competition by the barrier plants for water or nutrients, and possible draw-down of nitrogen as mulches decomposed (reducing supply of this nutrient for plant growth) could offset any potential advantage in pest suppression when pest pressure is light.

7.3.1 Best bet minus trial

Aggregating data across all three farms revealed treatment effects only for numbers of *E. postfasciatus* emerging from the vines following destructive, end-of-season sampling. Numbers were relatively high in the conventional farmer practice treatment but zero emerged from vines in all other treatments. No *C. formicarius* emerged from vines or other plant parts that were incubated. Yields of storage roots were highly variable among sites and this precluded statistical separation of the best bet minus intervention treatment means though conventional practice yields were significantly lower than in the other treatments. This suggests that all the tested combinations of methods from the original Best Bet strategy (PT planting material with either isolation or sanitation or pheromone traps or all three of these) were equivalent in terms of utility, all superior to conventional practice. However, this work was conducted only in a single season and across three sites in a single region (replication afforded using three sites). COVID-19 restrictions to work precluded a wider series of similar trials to more fully understand the respective effects of each tactic in the best bet strategy.

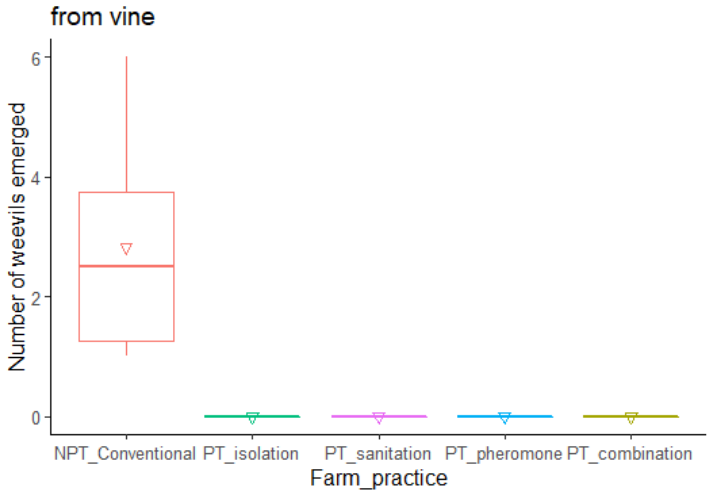


Fig 41. *Euscepes postfasciatus* emergence from sweetpotato samples (no *Cylas formicarius*). Mean of three farm sites. (Zero emergence of both weevil species from the storage roots).

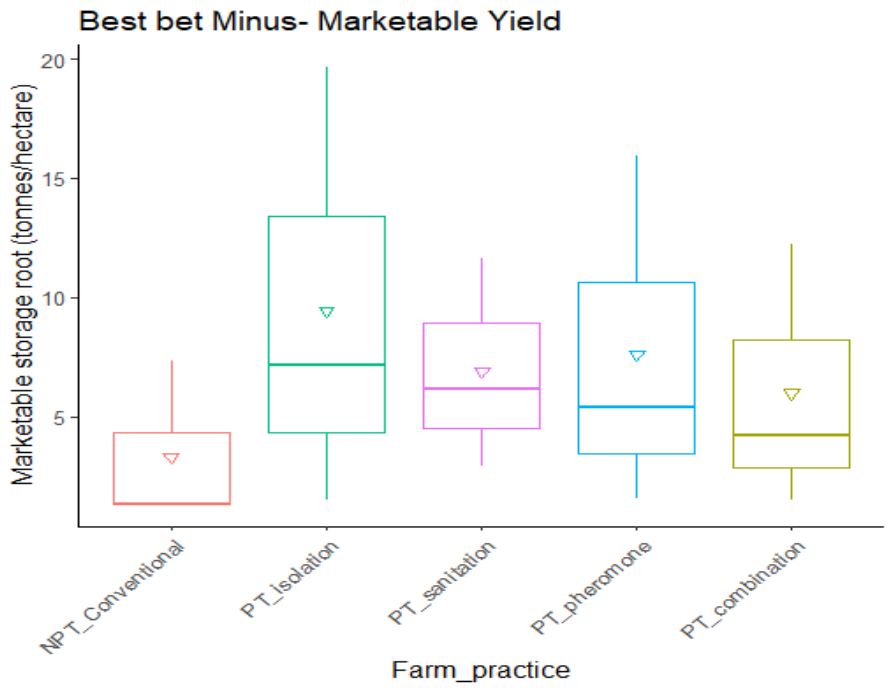


Fig 42. Marketable yields under best bet minus treatments.

7.4 Social and economic impacts of promising IPDM combinations

7.4.1 Farmer socio economics characteristics and perceptions towards adapting IPDM

Household Characteristics

Among the 12 farmer households involved in field trials, farmer age ranged from 30-74 years. More than 60% of farmers have low levels of experience in farming and education level ranged from primary level up to secondary level only. Most of the farming land was customary owned with average farm size ranging from 3ha to 14ha. Mainly land is mixed cropped with vegetable and other food crops. Almost 90% of the farmers responded they have some access to extension officers from FPDA and NARI. However, about only 25% reported having access to financial credits service from the banks, while 75% reported having no access to financial loan for helping their farm business (Table 2).

Table 2. Farm household characteristics

Variable	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12
Respondent age (Years)	30	58	58	56	32	42	41	34	54	43	74	45
Farming experience	Low	Low	Low	High	High	high	High	Low	Low	Low	low	low
Education (Primary = 1 Secondary/ College = 2)	1	2	2	2	1	1	1	2	1	1	1	2
Family size (No)s	3	8	12	7	6	7	6	3	7	6	6	4
Operational land holdings (ha)	6	4.5	20	5.5	2.6	10	6	9	4	4	8	10
Sweet potato farming area (ha)	4.8	3.6	14	4.4	2.6	4	3	0	3	3	6	10
Access to extension services (Yes=1, No=0)	1	1	1	0	1	1	1	1	1	1	1	1
Access to credit service (Yes=1, No= 0)	0	0	0	0	0	0	1	0	1	0	1	1
Access to market sweet potato (Yes=1, No=0)	1	1	1	1	1	1	1	1	1	1	1	1
Family labour in farming (No)	3	3	8	6	6	5	6	3	2	4	6	4

Farmer perception response for clean seed sweetpotato IPDM

Data collected on farmers' perceptions were also compiled. The farmers' responses were scored using perception rating scale from, 0-2, representing 0 being not effective, 1 for less effective and 2 being effective. Summary of these scores is given in percentages out of the four farmers from each zone (Table 3).

All 12 farmers (100%) considered PT material as effective in improving yield compared to conventional planting material. The increase in yield was considered to improved income due to good quality tubers attracting good prices. Awareness of IPDM and clean seed sweetpotato was reported by 80-90% of the farmers. It was reported there were good extension services provided by FPDA and NARI with introduction of clean seed sweet potato. More than 80% of the farmers reported lack of subsidies or other forms of support from the government (Table 3).

Table 3. Farmer perception response for clean sweet potato (IPDM)

Attributes	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12
Reduction in labour cost	0	0	0	0	0	0	0	0	0	0	0	0
Reduction in pesticides use	0	0	0	0	0	0	0	0	0	0	0	0
Increase in yield	3	3	3	3	3	3	3	3	3	3	2	3
High quality market yield	3	3	3	3	3	3	3	3	3	3	2	3
Improves incomes	3	3	3	3	3	3	3	3	3	3	0	3
Extension service and support service	3	3	3	3	3	3	3	2	3	3	2	3
Awareness in IPDM pest and disease management	3	3	3	3	3	3	3	2	3	3	3	3
Reduction in insect pests and disease incidences	3	3	3	3	3	3	3	2	3	3	3	3
Will other farmers willing to accept and use clean seed (IPDM)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are there extension support provided	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Are there any subsidies or support from government	No	No	No	No	No	No	Yes	No	No	No	Yes	Yes

Notes:

- Ratings: 0=ineffective/not relevant; 1=less effective; 2= effective; 3=very effective
- Question response Yes/No

The survey also indicated that sweetpotato remains the preferred major crop cultivated among the farmers, followed by carrot and cabbage. Coffee is seen as major cash crop but only 30% of the farmers cultivated coffee in small parcels of land/ blocks. Rice and livestock are less used (Fig 43).

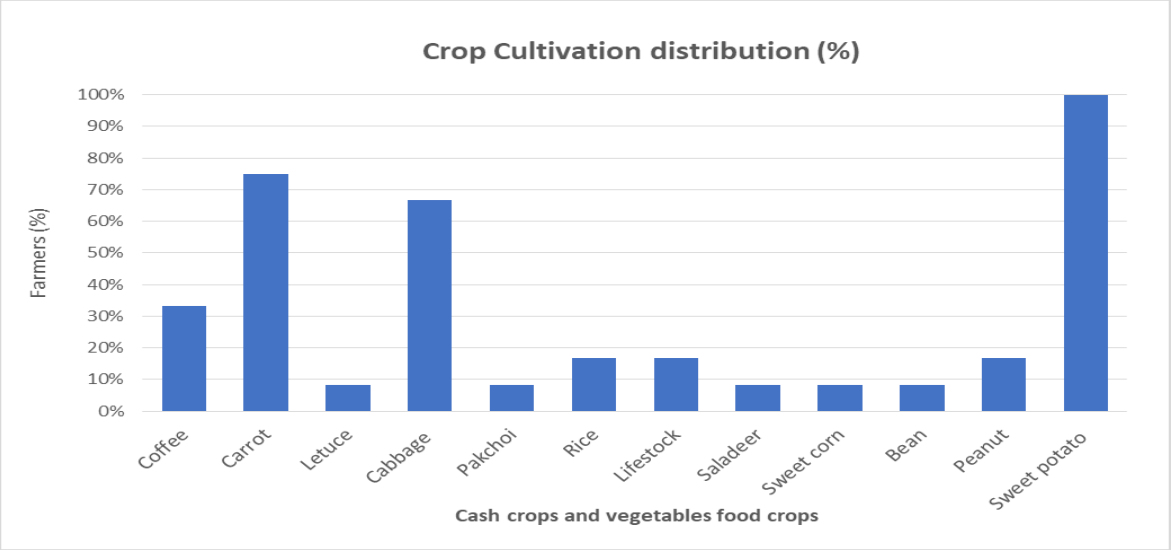


Figure 43. Common food crops and cash crops cultivation by farmers

Farm practices and inputs

The farming practice survey indicated, all 12 farmers reported no use of modern farming practices and inputs, such as fertiliser for maintaining soil fertility and herbicides and insecticides application for pests and disease control. There is also no IPDM input and practice across the farmers surveyed. Basic farming tools, such as spades, manual labour are the main farm inputs in upkeep and maintaining the farm. Some farmers occasionally applied manure or mulch (Table 4).

Table 4. The sweet potato farmers' inputs and practices

Farm Inputs/Activities	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Fertiliser	No	No	No	No	No	No	No	No	No	No	No	No
Chicken Manure	No	Yes	No	No	No	No	No	No	No	No	No	No
Mulching	No	No	Yes	No	No	No	No	No	No	No	No	No
Labour	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Herbicides	No	No	No	No	No	No	No	No	No	No	No	No
Insecticides	No	No	No	No	No	No	No	No	No	No	No	No
Farming tools	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Digging drains	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Making mounds	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Planting vines	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Weeding	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Marketing	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pest & disease management	No	No	No	No	No	No	No	No	No	No	No	No
IPDM practice	No	No	No	No	No	No	No	No	No	No	No	No
Packaging/Bagging	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Post-harvest handling	No	No	No	No	No	No	No	No	No	No	No	No

7.4.1 Best bet strategy

Measuring economic impact of PT methods

The economic impact of famers changing from conventional practice (CP) to Best Bet (BB) methods was measured by BCR and by changes in net incomes.

- BCR (Benefit Cost Ratio) – This can be measured as ratio of total income over total cost (BCR = Total income / Total cost).
- Net income - This is the difference between total income and total cost (Net income = Total income – Total cost).

Both measures can be used to evaluate economic impact of a project, but net income is the more reliable, since a project with different sizes can provide the same BCR. In this project we measure the economic impact of BB as a change (difference) in net income when farmers can replace the conventional practice of control (CP) (see Table 5, 6).

Table 5. Yield, net income, BCR and economic impact from Best Bet and CP methods (2018-2019, Season 1)

Province	Farm Site	Production Practice	Total Yield (t/ha)	Total Income (K/ha)	Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from CP to BB) (K/ha)
Jiwaka	Gunn	BB	16.03	7285.16	2045.30	5239.86	3.56	
	Gusamp	BB	13.34	8116.77	2717.24	5399.53	2.99	5064.11
	Kongabil	BB	25.82	11734.58	3313.64	8420.93	3.54	6255.24
	Kurumul	BB	22.86	6858.40	1591.56	5266.83	4.31	
	Average		**19.51**	**8498.73**	**2416.94**	**6081.79**	**3.60**	**5659.68**
	Gunn	CP						
	Gusamp	CP	7.80	2035.84	1700.43	335.41	1.20	
	Kongabil	CP	18.55	4216.52	2050.83	2165.69	2.06	
	Kurumul	CP						
	Average		**13.18**	**3126.18**	**1875.63**	**1250.55**	**1.63**	
Eastern H'lands	Kuka	BB	30.32	26353.65	2146.29	24207.36	12.28	17722.03
	Meteyufa	BB	23.34	31106.67	1545.26	29561.41	20.13	
	Nipuka	BB	18.69	8488.74	1791.04	6697.70	4.74	531.03
	Rohenga	BB	24.25	12129.76	1619.62	10510.14	7.49	
	Average		**24.15**	**19519.70**	**1775.55**	**17744.15**	**11.16**	**9126.53**
	Kuka	CP	16.72	7598.17	1112.84	6485.33	6.83	
	Meteyufa	CP						
	Nipuka	CP	15.10	6864.64	697.96	6166.68	9.84	
	Rohenga	CP						
	Average		**15.91**	**7231.40**	**905.40**	**6326.00**	**8.33**	
Western H'lands	Baiyer-Koge	BB	15.89	8661.46	2260.00	6401.46	3.83	2708.19
	Bomri	BB	18.24	4885.71	1504.67	3381.04	3.25	
	Tonga Nebilyer	BB	11.04	2007.52	1389.75	617.77	1.44	-340.34
	Kunt Mul	BB	9.83	19665.37	2878.81	16786.56	6.83	12545.93
	Average		**13.75**	**8805.02**	**2008.31**	**6796.71**	**3.84**	**4068.53**
	Baiyer-Koge	CP	8.73	4760.39	1067.13	3693.27	4.46	

	Bomri	CP						
	Tonga Nebilyer	CP	9.30	1688.73	730.63	958.11	2.31	
	Kunt Mul	CP	3.89	5450.00	1209.38	4240.63	4.51	
	Average		**7.31**	**3966.38**	**1002.38**	**2964.00**	**3.76**	
	Economic impact as an overall average from CP to BB (K/ha)							**6284.91**

1. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygiene / Sanitation)
2. CP = Conventional Practice sweet potato
3. Economic Impact is measured by difference in net income from CP to BB (K/ha)
4. Blank spaces indicate data are incomplete to make estimates

The economic impact of adopting novel methods (BB) to control pests and diseases can increase yields and improve net farm income. The results demonstrated that the use of IPDM technology (BB) almost doubled the average marketable yields across all three zones compared to CP methods used (see Table 5 and Table 6).

Table 6. Yield, net income, BCR and economic impact from Best Bet and CP methods (2019-2020, Season 2)

Zones	Farm Site	Production Practice	Total Yield (t/ha)	Total Income (K/ha)	Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from CP to BB) (K/ha)
Jiwaka	Gunn	BB	22.00	9999.93	2045.30	7954.63	4.89	
	Gusamp	BB	20.99	12777.40	2717.24	10060.16	4.70	8210.77
	Kongabil	BB	34.33	15597.75	3313.64	12284.11	4.71	12532.76
	Kurumul	BB	29.93	8978.97	1591.56	7387.41	5.64	
	Average		**26.81**	**11838.51**	**2416.94**	**9421.58**	**4.99**	**10371.77**
	Gunn	CP						
	Gusamp	CP	13.60	3549.82	1700.43	1849.39	2.09	
	Kongabil	CP	7.93	1802.17	2050.83	-248.65	0.88	
	Kurumul	CP						
	Average		**10.77**	**2675.99**	**1875.63**	**800.37**	**1.48**	
Goroka	Kuka	BB	11.79	10246.34	2302.54	7943.80	4.45	5852.45
	Meteyufa	BB	9.40	12526.45	1639.01	10887.44	7.64	
	Nipuka	BB	19.04	8650.56	1978.54	6672.02	4.37	3089.40

	Gimisave	BB	10.64	5334.65	1775.87	3558.78	3.00	
	Average		**12.72**	**9189.50**	**1923.99**	**7265.51**	**4.87**	**4470.93**
	Kuka	CP	7.13	3235.44	1144.09	2091.35	2.83	
	Meteyufa	CP						
	Nipuka	CP	9.52	4327.46	744.84	3582.62	5.81	
	Gimisave	CP						
	Average		**8.33**	**3781.45**	**944.46**	**2836.98**	**4.32**	
Mt Hagen	Baiyer-Koge	BB	22.89	12477.49	2260.00	10217.49	5.52	6572.20
	Bomri	BB	22.80	6107.63	1504.67	4602.96	4.06	
	Kagamuga	BB	13.03	2369.19	1389.75	979.44	1.70	356.99
	Kunt Mul	BB	37.31	74575.64	2878.81	71696.83	25.91	50226.20
	Average		**24.01**	**23882.49**	**2008.31**	**21874.18**	**9.30**	**16861.06**
	Baiyer-Koge	CP	8.64	4712.41	1067.13	3645.28	4.42	
	Bomri	CP						
	Kagamuga	CP	7.45	1353.08	730.63	622.45	1.85	
	Kunt Mul	CP	12.41	22680.00	1209.38	21470.63	18.75	
	Average		**9.50**	**9581.83**	**1002.38**	**8579.45**	**8.34**	
	Economic impact as an overall average from CP to BB (K/ha)							**10567.92**

1. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygiene / Sanitation)
2. CP = Conventional Practice sweet potato
3. Economic Impact is measured by difference in net income from CP to BB (K/ha)
4. Blank spaces indicate data are incomplete to make estimates

The yield improvement was seen on both storage root number as well as fresh weight increase. The yield improvement in BB was attributed to low level of damage by pests and diseases on the storage roots as compared to CP which had high non-marketable storage roots because of pests and diseases damage. The improved quality of sweetpotato and increased weight have subsequently enhanced income for the farmers as an average for the study area by 6000 K/ha (Table 5) and 10000 K/ha (Table 6) years one and two, respectively. The success of BB use is clearly seen in the yield quality (marketable yield versus non-marketable yield) and the net-income difference between the BB and CP.

The average marketable yields compared showed highly significant ($p < 0.01$) difference between BB and CP methods. The significance difference was attributed to BB marketable yields doubled as compared to CP. The increase in yields resulted in significant ($p < 0.05$) increase in incomes for BB method. However, the costs for labour, and transport and marketing costs were significantly ($p < 0.05$) high for BB method than CP. Despite that, overall net return (net income) from BB method was higher than CP method.

7.3.1 Best bet plus strategies

In this project we also measured the economic impact of BB plus strategies as a change (difference) in net income when farmers can replace the conventional practice of control by the BB plus strategies (Table 7).

For the BB plus strategies, as implemented with the introduction of barrier plants and mulches to the BB methods, their economic impacts were evident as improved net income when farmers replace the BB methods by BB plus (see Table 7 and Table 8). This reflects the previously reported benefits in terms of weevil control and yield increase.

Table 7. Yield, net income, BCR and economic impact from Best Bet plus (barrier plants) and BB methods (2019-2020)

Zones	Farm Site	Production Practice	Total Yield (t/ha)	Total Income (K/ha)	Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from BB to BB+) (K/ha)
Goroka	Kuka	BB+	26.44	22982.31	2474.41	20507.90	9.29	**-4948.38**
	Meteyufa	BB+	15.84	21116.46	1876.51	19239.94	11.25	**261.03**
	Nipuka	BB+	21.08	9576.36	3134.79	6441.57	3.05	**863.22**
	Gimisave	BB+	26.24	13147.15	2111.81	11035.34	6.23	**-845.69**
	Average		**22.40**	**16705.57**	**2399.38**	**14306.19**	**6.96**	**-1167.46**
	Kuka	BB (control)	31.94	27758.82	2302.54	25456.28	31.94	
	Meteyufa	BB (control)	15.47	20617.93	1639.01	18978.92	15.47	
	Nipuka	BB (control)	16.64	7556.89	1978.54	5578.35	16.64	
	Gimisave	BB (control)	27.28	13656.90	1775.87	11881.03	27.28	
	Average		**22.83**	**17397.64**	**1923.99**	**15473.65**	**9.04**	
Jiwaka	Gunn	BB+	9.28	4217.40	2701.55	1515.85	1.56	**-757.72**
	Gusamp	BB+	22.26	13546.79	3537.56	10009.23	3.83	**7173.44**
	Kongabil	BB+	7.72	3507.82	4133.96	-626.14	0.85	**666.39**
	Kurumul	BB+	12.11	3631.68	1919.69	1711.99	1.89	**452.34**
	Average		**12.84**	**6225.92**	**3073.19**	**3152.74**	**2.03**	**1883.61**
	Gunn	BB (control)	9.50	4318.87	2045.30	2273.57	2.11	
	Gusamp	BB (control)	21.28	5553.03	2717.24	2835.79	2.04	
	Kongabil	BB (control)	8.89	2021.12	3313.64	-1292.52	0.61	
	Kurumul	BB (control)	11.41	2851.22	1591.56	1259.66	1.79	
	Average		**12.77**	**3686.06**	**2416.94**	**1269.12**	**1.53**	
	Economic impact as an overall average from BB to BB+ (K/ha)							**358.08**

1. BB+ = BB plus- living barrier plant treatments (Marigold, Silver leaf Desmodium, Smooth Senna)
2. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, hygiene / Sanitation)
3. Economic Impact is measured by difference in net income from BB to BB+ (K/ha)
4. Blank spaces indicate data are incomplete to make estimates

The economic impacts are reported for each site within the zones which can be compared for choosing the best strategy between BB and BB plus methods.

Table 8. Yield, net income, BCR and economic impact from Best Bet plus (mulch) and BB methods (2019-2020)

Zones	Farm Site	Production Practice	Total Yield (t/ha)	Total Income (K/ha)	Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from BB to BB+) (K/ha)
Goroka	Kuka	BB+	37.46	32560.28	2684.81	29875.47	12.13	**6780.10**
	Meteyufa	BB+	17.61	23475.33	2086.91	21388.42	11.25	**4650.85**
	Nipuka	BB+	24.92	11317.34	3345.19	7972.15	3.38	**-27.22**
	Gimisave	BB+	28.36	14202.20	2322.21	11880.00	6.12	**-945.21**
	Average		**27.09**	**20388.79**	**2609.78**	**17779.01**	**7.81**	**3376.44**
	Kuka	BB (control)	29.22	25397.90	2302.54	23095.36	11.03	
	Meteyufa	BB (control)	13.79	18376.59	1639.01	16737.57	11.21	
	Nipuka	BB (control)	21.97	9977.91	1978.54	7999.37	5.04	
	Gimisave	BB (control)	29.17	14601.08	1775.87	12825.21	8.22	
	Average		**23.54**	**17088.37**	**1923.99**	**15164.38**	**8.88**	
Jiwaka	Gunn	BB+	12.32	5601.04	2911.95	2689.09	1.92	**-621.15**
	Gusamp	BB+	14.65	8915.50	3747.96	5167.54	2.38	**4802.66**
	Kongabil	BB+	9.63	4371.97	4344.36	27.62	1.01	**-200.83**
	Kurumul	BB+	15.98	4796.44	2130.09	2666.35	2.25	**911.90**
	Average		**13.15**	**5921.24**	**3283.59**	**2637.65**	**1.80**	**1223.15**
	Gunn	BB (control)	11.78	5355.54	2045.30	3310.24	2.62	
	Gusamp	BB (control)	11.81	3082.13	2717.24	364.88	1.13	
	Kongabil	BB (control)	15.58	3542.09	3313.64	228.44	1.07	
	Kurumul	BB (control)	13.39	3346.01	1591.56	1754.45	2.10	
	Average		**13.14**	**3831.44**	**2416.94**	**1414.50**	**1.59**	
	Economic impact as an overall average from BB to BB+ (K/ha)							**2299.79**

1. BB+ = BB plus- mulch treatments (leucaena, Mexican Sunflower, Sugar cane)
2. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygiene / Sanitation)
3. Economic Impact is measured by difference in net income from BB to BB+ (K/ha)
4. Blank spaces indicate data are incomplete to make estimates

Best bet minus trial

In addition to the above, we also measured the economic impact of BB minus trials as a change (difference) in net income when farmers can replace the non-pathogen tested practice of control by the different BB minus trials assessed (Table 9). The BB minus trial showed that each of the permutations of methods had a higher level of economic benefit comparted with conventional practice (Table 9). This reflects the previously reported benefits of reduced weevil infestation and higher yields than offset the higher cost of using PT and implementing the other best bet tactics.

Table 9. Yield, net income, BCR and economic impact from Best Bet minus trials (2020-2021)

Zones	Production Practice	Farm site	Total Yield (t/ha)	Total Income (K/ha)	Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from NPT to other methods) (K/ha)
Goroka	NPT Conventional	Kuka	1.92	1663.44	1437.79	225.65	1.16	
		Nipuka	2.38	1073.90	770.04	303.86	1.39	
		Meteyufa	8.33	11103.60	758.64	10344.96	14.64	
		Average	**4.21**	**4613.65**	**988.82**	**3624.83**	**5.73**	
	PT Combination	Kuka	5.08	4415.00	2302.54	2112.46	1.92	1886.81
		Nipuka	2.14	969.69	1978.54	-1008.85	0.49	-1312.72
		Meteyufa	13.25	17659.49	1639.01	16020.47	10.77	5675.51
		Average	**6.83**	**7681.39**	**1973.36**	**5708.03**	**4.39**	**2083.20**
	PT Isolation	Kuka	1.87	1628.19	1789.35	-161.16	0.91	-386.81
		Nipuka	8.07	3660.26	1121.60	2538.66	3.26	2234.79
		Meteyufa	22.00	29314.87	1110.20	28204.67	26.41	17859.71
		Average	**10.65**	**11534.44**	**1340.38**	**10194.06**	**10.19**	**6569.23**
	PT Pheromone	Kuka	6.23	5414.70	2271.29	3143.41	2.38	2917.76
		Nipuka	2.40	1087.94	1955.10	-867.16	0.56	-1171.02
		Meteyufa	16.82	22412.10	1615.58	20796.53	13.87	10451.57
		Average	**8.48**	**9638.25**	**1947.32**	**7690.93**	**5.60**	**4066.10**
	PT Sanitation	Kuka	3.63	3155.20	1820.60	1334.60	1.73	1108.95

		Nipuka	7.20	3265.51	1145.04	2120.47	2.85	1816.61
		Meteyufa	14.00	18651.15	1133.64	17517.51	2.85	7172.55
		Average	**8.28**	**8357.29**	**1366.43**	**6990.86**	**2.48**	**3366.04**
	Economic impact as an overall average from NPT to Other methods (K/ha)							**4021.14**

1. Economic impact is measured by difference in net income from NPT to other strategies (K/ha)
2. For Kuka farm site changing from NPT to 'PT pheromone' provides the highest net income
3. For Nipuka farm site changing from NPT to 'PT isolation' provides the highest net income
4. For Meteyufa farm site changing from NPT to 'PT isolation' provides the highest net income
5. Economic impact as an overall average from NPT to Other methods is around 4021 Kina per hectare

Summary of methods assessed and economic impacts

- The comprehensive series of best bet strategy developed in this project gave marked economic benefit compared to conventional practice.
- The comprehensive series of best bet plus trials showed economic benefit from using barrier plants and mulches as a complement to the four strategies that made up the best bet strategy.
- The multi-site best bet minus trial showed economic benefit from using subsets of the best bet methods compared with conventional practice.
- There was strong willingness on the uptake of the novel IPDM methods by the farmers hence adaption of the IPDM methods can be promoted in PNG.

8 Impacts

8.1 Scientific impacts – now and in 5 years

In line with the title of this project, "Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea", the main impacts of this work are in the Community domain (see below) rather than being scientific. Notwithstanding this emphasis, the extensive series of on-farm field trials extending over multiple years and sites is noteworthy in terms of its scale. Most studies have lesser temporal or spatial extent. Moreover, the headline result, that large and economically profitable advantages are possible from a relatively simple strategy of pest and disease management methods, will be very publishable. Most importantly in terms of Sustainable Development Goals, the findings demonstrate that enhancing crop yield and improving livelihoods is not dependent on an increase in non-renewable inputs and the associated sophisticated supply chain and marketing infrastructure that had become prevalent in some other developing countries, especially in east Asia. The methods developed and tested in this project are based on locally available inputs of materials and labour or are inexpensive and benign to human health and the environment (clean seed and pheromone lures). This will send a strong signal to the scientific community that is relevant to producers in many developing countries around the world.

Two scientific papers have already been published from the project. Rehman et al. (2019) addressed the fact that mulching with organic materials is a management practice with long history for weed suppression, soil water conservation and erosion control yet its potential impact on crop pests is less well explored. This study conducted in the laboratory and field in NSW assessed the use of mulches for reducing crop damage by sweetpotato weevil. Laboratory bioassays measured the response of adult female weevils to sweetpotato storage roots beneath mulches of fresh or dried plant materials. Weevils were found to be significantly repelled by fresh basil, catnip, basil lime and dry eucalyptus, cypress, lucerne and sugarcane. A subsequent field study focusing on the superior treatments from the lab work found that mulches of dry cypress, eucalyptus and lucerne reduced movement of weevils from a release point to reach sweetpotato plants, and lowered level of damage to storage roots. This work was important to the wider project in demonstrating that mulching with organic materials merits further testing as part of the integrated management of sweetpotato weevil, particularly to protect developing storage roots during dry periods when soil cracking can facilitate access by pests. The methods and supplies of minor equipment used in these bioassays developed at CSU were transferred to NARI personnel at Aiyura Research Station and used successfully to screen and evaluate a wide range of locally available plant materials. The wider scientific impact of the study is evident that the Altmetrics of this article – published in Springer Nature's open access journal *Scientific Reports* - show that it is in the 84th percentile (ranked 43,407th) of the 284,669 tracked articles of a similar age in all journals.

The second published paper, Dada et al. (2019) assessed scope for a use of a barrier plant approach. It developed a novel, two-stage, multiple choice olfactometer method to screen candidate barrier plant species and then a field study of the optimal barrier plants to quantify weevil movement and plant damage. Among 15 candidate barrier plant species, spring onion, oregano, chili, basil, sweetcorn, fennel, lime mint and lemongrass significantly reduced passage of weevil adults and the sweetcorn and lime mint treatments significantly reduced the numbers of weevil oviposition holes in sweetpotato storage roots. This was an important finding because it illustrated that barrier plants could be a secondary crop of value as a dual income source. The subsequent field study showed that basil and chives were effective barrier plants for reducing weevil damage to sweet potato storage roots. Though this article has an official publication date of 2019 it was not actually published until March 2020 and in the 18 months since then it has already been cited in three subsequently published papers.

Three papers were presented by project team members at the Australian Entomological Society Annual Scientific Conference held in Allice Springs in 2018 and abstracts of those papers appear in the proceedings volume but citation and Altmetric data are not available:

- Efficacy of a novel integrated pest management strategy for sweetpotato weevil (*Cylas formicarius*) and West Indian Sweetpotato Weevil (*Euscepes postfasciatus*) in Papua New Guinea. R.K. Geno, W. Wau, L. Enopa, A. Agiwa, G. Gurr, J. Liu, T. Guaf, B. Wilson.

- Integrated Pest and Disease Management (IPDM) Strategies: Field assessment and evaluation of scab (*Elsinoe batatas*) and gall mites (*Eriophyes gastrotrichus*) infestation during a sweetpotato (*Ipomoea batatas*) cropping season in Papua New Guinea. Wilfred Wau, Robert Kei Geno, Lindsay Enopa, Geoff Gurr and Jian Liu.

- Barrier plants – developing novel methods to prevent colonisation of crops by sweetpotato weevil, *Cylas formicarius.* Jian Liu, Mudassir Rehman, Taiwo Esther Dada & Geoff Gurr

8.2 Capacity impacts – now and in 5 years

Several students have been involved in this project, either directly or indirectly. Moreover, two staff involved in the project are enrolled for (Higher degree by research) HDR programs in either Australia or the USA (Table 10).

Mr Wilfred Wau (NARI project scientist), has been awarded a John Allwright Fellowship to undertake a Master degree at USQ in plant pathology. That project will focus on the bacteria and fungi associated with breakdown of bedding roots, a significant practical problem for Australian sweetpotato growers and likely to become a problem in PNG with the upscaling of production and use of plant beds. Commencement was delayed because of the COVID-19 pandemic; Mr Wau plans to arrive in Australia in 2022. Miss Melanie Pitiki, who worked at Unitech during the project, has indicated an interest (and demonstrated the capacity) to undertake a higher degree by research with Prof Gurr's group. Dr Wilson mentored and supported Melanie Pitiki's application for a PhD scholarship application to the University of Hawaii, for which she has been successful. She intends to commence study on 'Sweetpotato IPM and Soil Health Management' in August 2022.

There have been notable impacts at the level of partner organisation interactions. FPDA and NARI worked closely together on pheromone and weevil trapping and training of farmers. NARI staff member W. Wau helped Unitech set up trials and Unitech staff M. Pitiki helped NARI harvest and setup trials on many occasions. Rather than duplicate effort, W. Wau played an integral role in organising planting materials, lures etc and helped communicate with the farmers to ensure that there was consistency across the trials. Unitech has forged a better collaborative bond with NARI resulting in great exchange of skills and knowledge.

Dylan Male (CSU Honours student) received training from Australian and PNG partners (NARI and Unitech) in methods of experiment design, culturing of fungi, maintenance of insect colonies, olfactometer set-up, preparation of plant material, chemical analysis, and data analysis. Dylan received a first-class Honours.

Grace Malabo (CSU Master of Sustainable Agriculture student) successfully completed her dissertation subject under the supervision of Prof Gurr and Dr Liu studying barrier plants and received a Dean's Award in 2019 for her overall academic performance.

Coleman Pombre (CSU Master of Sustainable Agriculture student) successfully completed his dissertation subject under the supervision of Dr R. Culas studying socioeconomics impacts of sweetpotato pests and diseases.

Gerega Maiga completed his Master of Research at PNG Unitech under the guidance of Dr Ronnie Dotaona and Dr Bree Wilson, examining entomopathogenic fungi on sweetpotato weevils.

Dr Bree Wilson has continued to provide training in biological control methods to PhD candidates at USQ and a University of Queensland Master candidate. Dr Kim Khuy Khun examined many entomopathogenic fungi to manage macadamia seed weevil (and published 4 scientific papers that used project derived isolates (see Appendix 19). Mr Sudhan Shah use *Metarhizium anisopliae* to manage mealworms as a proxy for wireworm and Ms Teodora Agostinho examined the pathogenicity of various entomopathogenic fungi to control fall armyworm. Dr Bree Wilson has also provided face to face and distance training to external collaborators (private industry and NSW Department of Primary Industries) for insect bioassays using project derived entomopathogenic fungi.

Drs Wilson and Dotaona are supervising a research Master's project (by Roberta Sio) on the efficacy of entomopathogenic fungi on Coconut Rhinoceros Beetle. This project would not have come about without the association of Unitech and USQ and other associations with Ramu Agri Industries Ltd etc.

The professional capacity of junior scientists has been enhanced by involvement in training and academic presentations. From NARI, R. Geno, T. Guaf and W. Wau; from FPDA, A. Agiwa and L. Enopa have authored papers.

Table 10. Higher degree by research students involved in the project or enrolled for further study in 2022.

Student name	Degree & Topic	Supervisors	Institution	Completion date
Mr Wilfred Wau	***Masters of Research,*** 'Bacteria and fungi associated with breakdown of bedding roots'	Dr Bree Wilson Dr Elaine Gough	USQ	2024
Miss Melanie Pitiki	***Doctor of Philosophy***, 'Sweetpotato IPM and Soil Health Management'	Currently unknown	University of Hawaii	2026
Mr Dylan Male	***Honours***, 'Biological control of weevils in sweetpotato'	Prof Geoff Gurr Dr Bree Wilson Dr Jian Liu	CSU	2020
Ms Grace Malabo	***Master of Sustainable Agriculture***	Dr Jian Liu Prof Geoff Gurr	CSU	2019
Mr Coleman Pombre	***Master of Sustainable Agriculture***, 'Socioeconomics impacts of sweetpotato pests and diseases'	Dr Richard Culas	CSU	2019
Mr Gerega Maiga	***Master of Research,*** 'Entomopathogenic fungi on sweetpotato weevils'	Dr Ronnie Dotaona Dr Bree Wilson	PNG Unitech	2018
Dr Kim Khuy Khun	***Doctor of Philosophy, '***The use of EPF to manage macadamia seed weevil'	Dr Bree Wilson Prof Gavin Ash Dr Mark Stevens Dr Ruth Huwer	USQ	2021
Dr Sudhan Shah	***Doctor of Philosophy*** *'Metarhizium anisopliae* to manage wireworm in sweetpotato'	Prof Gavin Ash Dr Bree Wilson	USQ	2022
Ms Teodora Agostinho	***Masters by coursework*** 'Entomopathogenic fungi to control fall armyworm'.	Dr Bree Wilson Dr Anthony Young Mr Keith Danckwerts	UQ	2022
Ms Roberta Sio	***Masters by Research*** 'Entomopathogenic fungi to control coconut rhinoceros beetle	Dr Ronnie Dotaona Dr Bree Wilson	PNG Unitech	2022

8.3 Community impacts – now and in 5 years

NARI and FPDA personnel ran multiple training courses over the last year focusing on pest and disease management strategies (see Training activities section). Farmers expressed particular interest in using pheromone lure traps for monitoring and locally suppressing sweetpotato weevil. The team is exploring scope to have Brian Bell (a nationwide chain of stores) as the supplier of pheromone lures as a legacy of the project. To date, Dr Liu of CSU has been purchasing batches of the lures from a manufacturer,

Yinggeer (Enjoy) Agricultural Science and Technology Company, in China via Taobao (equivalent to eBay) and having them sent directly to in-country partners. FPDA has being distributing lures in PNG to farmers in the TEAM zones. This aspect of the project has featured in the PNG Newspaper *The National* (12th May 2021 https://www.thenational.com.pg/insect-killing-fungi-an-option-to-manage-sweet-potato-weevils/)

8.3.1 Economic impacts

Trial results provide robust evidence across multiple provinces that sweetpotato yields are strongly increased by the new integrated pest and disease management strategy, comprising the use of pathogen tested planting material, removal of sweetpotato crop residues and weeds, separating new plantings from existing plantings by at least four meters, and pheromone traps for sweetpotato weevil. Compared with conventional practice, this led to measurable reductions in biotic threats to the crop, especially weevils, gall mite and scab. This translated to increased yields. Over all comparisons of the best bet strategy compared with conventional practice, total yield of storage roots when crops were protected by the best bet strategy averaged 20.16 t/ha. This was almost double the yield in the control treatment consisting of conventional farm practice. An additional benefit was improved quality of the storage roots from the best bet strategy. Illustrating this, unmarketable yields in that treatment were lower (1.46t/ha) than in the control (2.25t/ha) whilst the all-important marketable yield was greatly increased from 7.99t/ha to 18.70t/ha. Importantly, these benefits were robust over sites and provinces rather than resulting from highly levels of performance in a few locations. For example, marketable yields in the control treatment never *exceeded* an average of 10.28 for any province yet were never *below* 17.44 in the integrated pest and disease management strategy treatment. This, in turn, led to a major economic advantage (i.e., comparing the net income from the new strategy, after cost of the additional labour and materials, with the net income from the conventional practice) that averaged across provinces 6,284 Kina/ha in the first year and 10,567 Kina/ha in the second year.

For each farm site, the integrated pest and disease management strategy treatment, which combined pathogen tested (PT) vines, sanitation practices, crop isolation and implementation of pheromones traps, included the following costs components for the labour use: labour cost for managing the plot (which included the use of PT vines), the labour cost associated with sanitation practices and the labour costs associated with the implementation of pheromones traps and their management. When this strategy extended to include mulches, the costs of labour required for their collection and use in the field added to the total costs. Similarly, when this strategy extended to include barrier plants, the costs of labour required for their collection and use in the field added to the total costs. All farmers across the three provinces had family labour working on the farm and all farming activities were mainly by manual labour provided by the family members. The costs for the labour use were estimated based of the hourly rate and the hours spend.

8.3.2 Social impacts

The main social impact of this workflows from the very large increases in sweetpotato yield that result from the novel integrated pest and disease management strategies developed. These, in turn, lead to marked economic benefit for growers. In the next few years, as the communication and extension efforts of the project lead to uptake, we realistically envisage major societal impact through livelihoods and value chains. Forty per cent of the growers that attended farmer training events were female. Four of the thirteen farmers that hosted trials were female.

8.3.3 Environmental impacts

The project as a whole is focusing on non-chemical approaches and this will have contrasting effects in PNG and Australia. In PNG, the trajectory of commercialisation of sweetpotato will be supported with a greatly lower risk of growers embracing indiscriminate use of pesticides.Yields are approximately doubled by the use of the integrated pest and disease management strategy we developed and tested on a wide scale and – crucially – this does not include the use of pesticides. This, in turn will avoid downstream problems with insecticide resistance, human poisoning, and environmental impacts.

Since pesticides are routinely used (with the exception of a small number of organically registered growers) the effects of the project will be to lessen this depenence on insecticides and thereby slow the development of resitance in pests such as sweetpotato weevil. Refleting the importance of sweetpotato weevil, more growers, especially organic producers, have become interested in the use of pheromone traps for monitoring this pest; mirroring interest by PNG growers in this technology.

8.4 Communication and dissemination activities

FPDA, with support from Wilfred Wau (NARI), organised a series of training days for farmers. These were of two types. The first focused on new crop protection methods in the ‘Best-Bet’ strategy, especially the use of sex pheromone traps against sweetpotato weevil (Fig 44). These took place in two major phases at twelve locations (Table 10).

Table 10. Date and location of farmer field day pheromone trapping training events held in 2019-20.

Location	Date	Location	Date	Location	Date
Meteyufa	9/10/19	Kuka	17/02/20	Gusamp	19/02/20
Asaro	15/10/19	Gimisave	17/02/20	Kurumul	20/02/20
Jiwaka	16/10/19	Nipuka	18/02/20	Gunn	20/02/20
Mt Hagen	29/01/20	Meteyufa	18/02/20	Kongabil	21/02/20

Fig 44. Wilfred Wau explained the process involved in constructing a pheromone trap using soft drink container (1L). (left) A group photo of trainees (right).

The second type of event involved a series of 'Farmer's Field Day' training events for farmers that covered more generally the whole ACIAR TADEP suite of projects including soils work especially on station trials (organic and inorganic trials), sweetpotato commercialization between FPDA and NARI, and the present pest and disease project including new crop protection methods. These events were initially restricted by COVID-19 but resumed after the lock-down in early 2021. These took place on 16th -21st August at TEAM zone1, on 28th February to 5th March 2021 at TEAM zone 2, and 21st-25th June 2021 at TEAM zone 3 (Table 11).

Pheromone lure traps have become well recognised by farmers and there is great demand for the lures.

A detailed report of this activity is provided in Appendix 20 "Jiwaka Farmers Field Day Report-WWau-fpda", Appendix 21 "WHP Farmers Field Day-Report – Wwau" and Appendix 22 "Asaro Farmers Field Day Report-WWau"

Table 11. Summary of location and participation at training events.

Location TEAM zone 1 (Eastern Highlands Province-Asaro)	No. Participants	Location TEAM zone 2 (Jiwaka Province)	No. Participants	Location TEAM zone 3 (WHP-Western Highlands Province)	No. Participants
Nipuka	56	Gusamp	32	Kelua	32
Gimisave	117	Gunn	97	Tonga	15
Kuka	86	Kurumul	70	Mul	56
Meteyufa	62	Kongabil, Banz	108		

Dr Wilson has presented proposed research and current research and practical demonstration of LAMP molecular diagnostics at industry updates, grower days and to the broader sweetpotato research team throughout 2016-201 (See article in Appendix 23). She also presented recent LAMP and metagenomics data at a PT production course (PNG researchers) in Bundaberg. Dr Wilson has engaged regularly (physical visits and phone/SMS contact) with individual sweetpotato growers in Bundaberg, Cudgen and the Lockyer Valley. She has presented her research informally to 4 school groups, at the opening of USQ's Agricultural Sciences and Engineering Precinct (ASE) and to many tour groups (private industry, R & D corporations, government and university staff and researchers).

Training activities are detailed in the following section. The information sheet on pheromone traps is provided as Appendix 24, as are posters on scab and viruses also used for training (Appendix 25 and 26).

Other project trip reports can be found in Appendix 27.

9 Conclusions and recommendations

9.1 Conclusions

This project has succeeded in meeting its overarching objective as articulated in the project title “Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea”. The preceding SRA established that sweetpotato growers in the PNG Highlands perceived pests and diseases as a major threat to production but that most took no action and only a limited range of management methods was perceived to be available to them. Essentially, the ‘toolbox’ of available methods had been significantly expanded by this project. A core pest and disease management strategy (‘best bet’) distilled from the international literature has been widely tested across multiple farms in three Provinces and over three years. This strongly farmer participatory work constituted a rigorous evaluation of the strategy and gave the work wide grass roots-level reach into farming communities of the Highlands. The strategy had significant impact on pest and disease signs, and symptoms, most notably on gall mite, scab and weevil species (all biotic threats of high priority according to the SRA survey of growers). This alleviation of biotic threats to the crop translated into yields of storage roots that were typically doubled, with quality of the roots also reportedly increased. These results were met with great enthusiasm by growers who also expressed what amounted to empowerment that methods were being made available to them that were technologically appropriate and economically accessible. Aside from the tangible benefits from higher yields, farmers valued the agency provided by inexpensive pheromone lures that they could use to bait simple sweetpotato weevil traps that caught large numbers of adult weevils, a very tangible signal of effect. Economic analyses of the best bet strategy, that accounted for the costs involved in implementation, established that there were very strong economic advantages of adoption.

The best bet strategy was complemented in the second half of the project by addition of several additional methods involving mulches, barrier plants and entomopathogenic fungus. Each of these strategies was the result of rigorous research that involved method development and screening of candidate treatments in Australia, transfer to partners in PNG and evaluation in on-farm trials. The efficacy and economic advantages of these were determined and shown to be worthwhile in terms of providing additional weevil control yield and economic advantage compared with the original best bet strategy. In the final year of the project, the original best bet strategy was partitioned in a field study extending over three sites comparing differing combinations of individual methods that would complement and make more durable the use of pathogen tested planting materials. Weevil control, yields and economic performance all benefitted in treatments where pathogen tested planting material was complemented by one or more other method, compared with conventional practice. COVID-19 impacts most severely affected capacity to conduct field studies in the last 18 months of the study precluding a more robust understanding of the individual effects of all methods. Overall, however, it is clear that multiple different combinations of methods from the ‘toolbox’ of plant protection options can be used with confidence to complement pathogen tested planting materials and contribute to the durability of this germplasm.

Extension materials have been developed by the project and complemented by grower field day events. The materials and enhanced capacity of the project, as well as logistical arrangement (such as to ensure supply of pheromone lures) will leave a legacy from the project.

Throughout the project, junior scientists at NARI, FPDA and Unitech have engaged with more experienced project staff for skill development and wider capacity building that has included travel to Australia for professional development, conference presentations, support with report writing, and joint publication. A NARI junior scientist has been awarded

a John Allwright Fellowship to commence a masters by research in Australia as soon as COVID restrictions will allow.

Recommendations

1. The pest and disease management manual produced by the team in English should be translated into 'tok pisin' to maximise suitability for rural communities across PNG. This is underway and being led by NARI using NARI funds.
2. Complementary extension and communication initiatives recommend by stakeholders in the end-of-project workshop were: (a) make use of smart phones for delivery, (b) further grower field days, (c) centrally located information centres. Accordingly, it is recommended that the project partners with a mandate for communication to growers, FPDA and NARI, incorporate project results and communication materials developed to date in their existing extension initiatives and emphasise the stakeholder recommended communication channels.
3. There is very clear evidence that pathogen tested planting material is a valuable technology and should be recommended as the cornerstone of commercial and semi commercial production in PNG. To facilitate this, the range of cultivars and the geographical availability of planting material should be further expanded. The use of PT planting material needs to be complemented using additional pest and disease management methods such as those evaluated in the present project. This is important to minimise infection of the crop with pathogens and infestation with pests during the growing season and maximise storage root yield and quality. Further, combining the use of PT material with additional methods and thereby minimising infection and infestation will allow the collection from these crops of relatively 'clean' vines for propagation of new crops. The use of crop isolation, sanitation, mulching and barrier plants are likely to have benefits of virus vectors (Hemiptera) in addition to the reported effects on other, non-vector pests such as weevil species. Future studies could extend to the use of some of these methods (especially barrier plants that with aphid-repellent properties or that attract parasitoids and predators of virus vectors) in the vicinity of screenhouses in which PT material is grown. Whilst collecting planting material from non-PT crops is less optimal than the use of PT material for every crop, it is preferable over the use of cuttings from less well protected crops. It may be especially useful in areas with poor access to PT material of for semi-commercial growers to use cuttings from their PT crops to establish home gardens. Among the methods that can complement the use of PT plating material are several that are inexpensive and effective and can be recommended: (a) sanitation (clearing a garden of volunteer sweetpotato plants and weeds prior to planting); (b) isolation (positioning a new planting away from older plantings (at least 4m)); (c) pheromone trapping of sweetpotato weevil; (d) mulches of locally available plant materials (especially in dry years to minimise and cover soil cracks that otherwise allow access to storage roots by weevils); (e) barrier plants.
4. Entomopathogenic fungi require further study to reach the point of products ready for delivery. The optimal isolates identified in Australian and PNG work are a strong foundation for this future work and links with a commercial partner have been established. As outlined in the discussion paper, commercial partners in Australia are more likely to invest in a product (e.g., field trials for registration) that shows promise to control a range of insects. Unfortunately, this is an expensive process and is often a drawn-out process. From the outset, all conversations involving commercialisation of ACIAR funded/university derived isolates have detailed the need for vigorous IP agreements and all future conversations will detail IP too. Future options for use of entomopathogenic fungi should focus on organic producers in Australia as well as commercial growers in PNG. Investment in product development by major multinational companies is unlikely because of market size so small enterprises, even 'cottage industry' start-ups, are more likely to play a role.

5. The earlier proposal to ACIAR to undertake additional work to deliver benefits to smallholder subsistence growers (in contrast to the commercial and semi-commercial growers who were the targeted beneficiaries of the original project) remains a clear opportunity. This would constitute 'low hanging fruit' in terms of delivering benefit to the poorest members of the communities – including women and girls that are central to smallholder production. The body of knowledge established by the current project is a strong foundation for rapid progress in such future work. A key priority of that work would be integrated pest and disease management strategies that did not use PT planting material.
6. It would be valuable to hold a workshop in early 2022 that drew together the teams of the present project, the soils project ((SMCN/2012/105) and the TADEP project (HORT/2014/097), along with a wide range of stakeholders. This would capitalise on the findings from the concluding projects (the present one and HORT/2014/097) and the findings to date of the continuing soils project that does has a focus on smallholder growers. Such a workshop would provide an important opportunity to explore in detail the linkages and potential synergies among the projects and to ensure that future investment by ACIAR in sweetpotato is optimally directed. As an example: the pest and disease management tools developed in the present project are shown to increase crop yield, but this cannot be sustained unless parallel developments are made in soil management and crop nutrition and the expansion of profitable markets for the additional produce.
7. Whilst the project has delivered improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea (a best-bet strategy using pathogen tested planting material, isolation, sanitation plus pheromone trapping of sweetpotato weevil; and a best bet plus strategy that additionally uses mulches and barrier plants) future work would be worthwhile to understand more fully each of these individual methods. It is noted above that future work on non-commercial (smallholder) production systems would need to consider circumstances under which only non-PT planting material is available (to make the best of this situation) though use of PT material is clearly preferable. In the case of pheromone trapping, this was shown to capture large numbers of sweetpotato weevil adults, but a dedicated effort is required to understand the effects of trap density and renewal frequency on damage alleviation and ultimate benefit cost. Similarly, issues like the distance between new and old sweetpotato crops for isolation, timing and depth of different forms of mulch, economics of using crop species as barrier plants for a dual income all merit attention. These investigations could be undertaken as student projects at Unitech and other PNG universities.

10 References

10.1 List of publications produced by project

Rehman, M., Liu, J., Johnson, A. C., Dada, T. E., & Gurr, G. M. (2019). Organic mulches reduce crop attack by sweetpotato weevil (Cylas formicarius). *Scientific Reports, 9*(1), 14860. https://doi.org/10.1038/s41598-019-50521-5

Geno, R.K., Wau, W., Enopa, L., Agiwa, A., Gurr, G., Liu, J., Guaf, T. and Wilson B. Efficacy of a novel integrated pest management strategy for sweetpotato weevil (*Cylas formicarius*) and West Indian Sweetpotato Weevil (*Euscepes postfasciatus*) in Papua New Guinea. 49th Australian Entomological Society AGM and Scientific Conference, Alice Springs, Northern Territory, 23rd to 26th September 2018.

Wilfred, W., Kei, R., Geno, Lindsay Enopa, Gurr, G., and Liu, J. Integrated Pest and Disease Management (IPDM) Strategies: Field assessment and evaluation of scab (*Elsinoe batatas*) and gall mites (*Eriophyes gastrotrichus*) infestation during a sweetpotato (*Ipomoea batatas*) cropping season in Papua New Guinea. 49th Australian Entomological Society AGM and Scientific Conference, Alice Springs, Northern Territory, 23rd to 26th September, 2018.

Jian Liu, Mudassir Rehman, Taiwo Esther Dada & Geoff Gurr Barrier plants – developing novel methods to prevent colonisation of crops by sweetpotato weevil, *Cylas formicarius. 49th Australian Entomological Society AGM and Scientific Conference, Alice Springs, Northern Territory, 23rd to 26th September, 2018.*

10.2 From preceding SRA

Fujinuma, R., Kirchhof, G., Ramakrishna, A., Sirabis, W., Yapo, J., Woruba, D., Gurr, G., & Menzies, N. (2018, 2018/02/15/). Intensified sweetpotato production in Papua New Guinea drives plant nutrient decline over the last decade. Agriculture, Ecosystems & Environment, 254, 10-19. https://doi.org/10.1016/j.agee.2017.11.012

Liu, J., Wilson, B., Guaf, T., Wau, W., Komolong, B., Sar, S., ... & Gurr, G. (2017). The rich tapestry of biological control targets and agents in sweet potato production systems of Papua New Guinea. In 5th International Symposium on Biological Control of Arthropods: ISBCA 2017.

Johnson, A. C., & Gurr, G. M. (2016). Invertebrate pests and diseases of sweetpotato (Ipomoea batatas): a review and identification of research priorities for smallholder production. Annals of Applied Biology, 168(3), 291-320. https://doi.org/10.1111/aab.12265

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Insect pest and disease field survey protocols 2017

For ACIAR HORT/2014/083- Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea

For all the field experiments and trials, insect pests and diseases are surveyed once in the middle of the growing season and again at harvest time.

This is a compilation of TWO separate documents:

Protocol I. Instructions for assessment/sampling on TEAM zone sites.

Protocol II. Instructions for assessment/sampling on designed experiments sites at Aiyura

Each of the two sets of documents is arranged by (i) mid-season assessment and (ii) harvest time assessment and each has an appended recording sheet.

The sampling protocols are similar (but not identical) for the TEAM zones where fields or gardens are the sampling unit, versus designed experiments where small plots are the sampling unit. Weevil-related methods are designed to be compatible with those being used in the ACIAR Pacific Root Crops project led by Prof Mike Furlong.

During sampling and assessing regularly take photographs of the target e.g., insects, plants with scab/gall mite symptoms within quadrats. When taking a photo, make a note of the file number (e.g., 0014.jpg) on the data recording form.

For all the samples, make sure the labels are securely in place. The best way to write a clear label is using a lead pencil on sticky paper label. If sticky labels are unavailable, write using a lead pencil on a piece of paper and stick it inside the sample bag. For labelling tubes, please use high quality fine tipped permanent markers.

Contents

Protocol I: Instructions for assessment/sampling on TEAM zone sites.

In TEAM zone sites, whole fields/ gardens will be used as 'plots'. There will be multiple (i.e., replicated) fields of each treatment. In year 1 at least, there will be tow treatments only: (1) conventional farming practice (the control) and (2) a best-bet integrated pest and disease management strategy based on the use of pathogen tested planting material, crop hygiene and crop isolation.

i. Mid-season assessment

For mid-season assessment, the following assessment/sampling will be conducted:

1) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity

ii. Harvest time assessment

For harvest time assessment, three sets of assessment/sampling will be conducted:

1) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity

2) Sampling for DNA analysis

3) Weevils inside sweetpotato plants survey

1 Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity (on TEAM zone sites)

Because activity of some insects (especially sweetpotato weevils) varies according to time of day, **DO NOT** survey all of the fields/gardens in one treatment type before moving on to the other treatment type (e.g., all the best bet fields, then all the control fields). Rather, sample the fields/gardens in a non-systematic ('random') manner so that no bias is introduced. That is, sample one or two fields/gardens of a given treatment, then one or two fields/gardens of the other treatment, and so on.

In each field, do ALL of the following sampling tasks.

1.1) Active arthropod count

1.2) Foliar scab symptoms assessment

1.3) Gall mite symptoms assessment

1.1 Active arthropod count

Using bamboo canes, make a square quadrat (50 cm x 50 cm). Use a strong tape like gaffer tape or wire to connect the four corners.

Use the quadrat to mark areas of the crop in which detailed observations will be made. In each field/garden, gently place the quadrat sequentially in each of the **10 portions of the field as shown**

in Fig 1. Avoid the outer 1.0m borders of the field. Ensure the quadrat is positioned gently to avoid disturbance of insects.

Observe the whole area within the quadrat frame and count the numbers of each type of arthropod that you see. Sweetpotato weevil adults are obviously the insect of most importance here but you may also see some West Indian sweetpotato weevil adults, grasshoppers, caterpillars, beetles, spiders etc. Record data in the form (Appendix 1).

Before you move the quadrat to the next sample position, record scab and gall mite symptoms as described in the following sections.

When you have assessed active arthropods, scab and gall mites for a given quadrat position, move the quadrat to the next position.

When you have recorded data from all 10 positions in each field/garden (Fig. 1). Move to the next plot (i.e., field/garden).

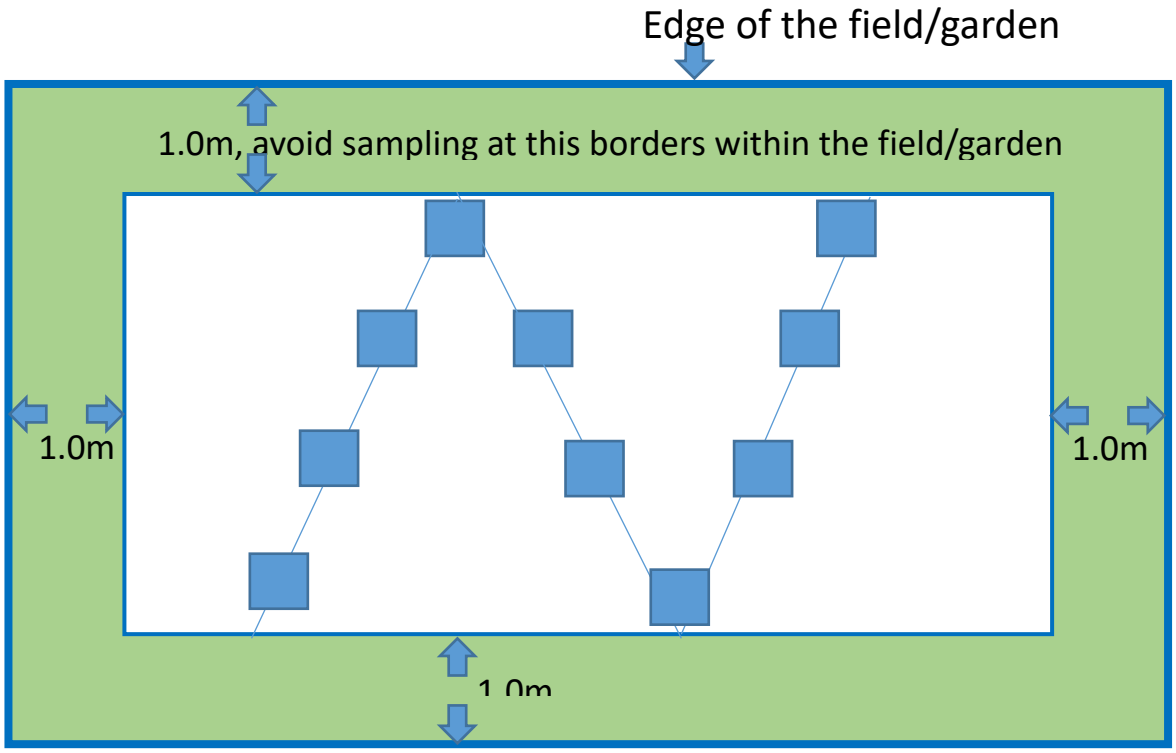


Fig. 1. Sample pattern for visual survey in TEAM zone sites, blue boxes show the position of the 10 quadrat samples

1.2 Foliar scab symptoms assessment

After assessing active arthropod numbers, leave the quadrat in place and select a single vine nearest to one of the corners of the quadrat.

On this vine, carefully observe the leaves, the petioles and the vine stem itself and judge the severity of scab symptoms against the pictures and descriptions (Appendix 2), record score (i.e., a number between 0 and 5) on the recording form (Appendix 3). Now, repeat this for the vine at diagonally opposite corner of the quadrat so that you have a total of 2 severity estimates for the vines at two corners of the quadrat. Now record gall mite severity for the same two vines.

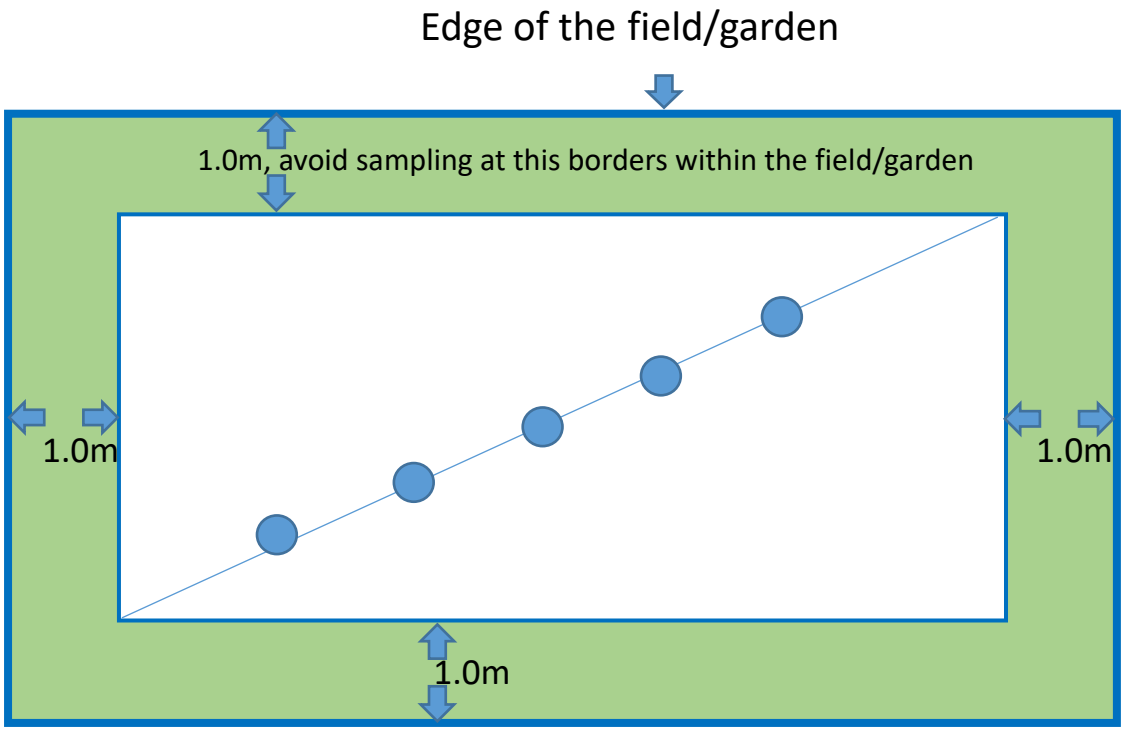
1.3 Gall mite symptoms assessment

Carefully observe the leaves, the petioles and the vine stem itself and judge the severity of gall mite symptoms against the pictures and descriptions (Appendix 4), record data in the recording form (Appendix 3). Write the symptom score (i.e., a number between 0 and 5 on the form). Now, repeat this for the vine at diagonal corner of the quadrat so that you have a total of 2 severity estimates for the vines at two corners of the quadrat.

Now move the quadrat to the next position.

When you have recorded data from all 10 positions in each field/garden (Fig 1). Move to the next field/garden.

2 Sampling for DNA analysis (on TEAM zone sites)



To assess the presence of various organisms and microorganisms, soil and plant leaves samples are taken from 5 randomly chosen plants. Plants need to be selected along a transect that approximates a diagonal across the field/ garden but avoiding the edges. The remaining 4 plants are then collected at approximately equidistant spacing along transect to the far side of the plots (Fig. 2). After taking the samples, keep these plants for the next survey activity (section 3, Weevils inside sweetpotato plants survey).

Fig. 2. Sample pattern for weevils inside plants in TEAM zone sites, blue circles show the position of the 5 plants samples

2.1 Soil sampling

For each of the five plants, use a 15 ml centrifuge tube to scoop up some soil to fill the tube. With a permanent marker, label the tube appropriately with the date, experiment name, plot no., rep no. and plant no. If the label looks like it is coming off, back at the lab, write out the label, place on the tube and cover with clear sticky tape. Store filled tubes in a large zip-lock bag in the freezer.

2.2 Plant leaves sampling

For each of the five plants, remove 3 leaves (a mix of diseased, un-diseased) and place in a zip-lock bag (separate bag per plant). Use a small piece of paper and a pencil (grey 'lead') to record all details as above, then place inside the bag and zip lock the bag.

Take back to the lab on ice in the esky and freeze immediately.

These samples will be processed for DNA analysis (PNG/Australia), where we will test for the presence of various organisms and microorganisms using specific markers.

3 Weevils inside sweetpotato plants survey (harvest time only) (on TEAM zone sites)

-- Same protocol as Mike Furlong for HORT 2010 065

To assess relative abundance of sweetpotato weevil and West Indian Sweetpotato weevil, 5 plants are randomly chosen as above in section 2. Plants need to be selected along a transect that approximates a diagonal across the field/ garden but avoiding the edges. The remaining 4 plants are then collected at approximately equidistant spacing along transect to the far side of the plots (Fig. 2). Same plants sampled in activity 2 (Sampling for DNA analysis) can be used again here.

3.1 Collection of selected plants

For each of the selected plants, randomly select two vines. Cut these off close to the crown of the plant and place them into a large, labelled plastic bag. The other vines can be discarded. Dig up the plant and place the crown of the plant into the same plastic bag as the vines. Finally, dig up the storage roots and randomly select two (without bias) and place these into the bag.

3.2 Separation of plant parts, incubation and destructive sampling.

Assess weevil infestation in the collected plant parts in two ways:

a) <u>Incubation of plant parts</u>

- **For each plant sample, randomly select one of the two vines, one of the storage roots and one half of the crown** (crowns cut in to 2 approximately equal halves). Place these together into a labelled plastic container lined with damp tissue paper and covered with a ventilated lid (e.g., ice cream containers approx. 20 cm x 20 cm x 10 cm). Use mesh to cover the ventilation to avoid escape of adult weevils.
- Incubate the sample (ambient temps, on lab benches) for 4-5 weeks. Ensure they are in a rodent free area. Checked 2-3 times per week to ensure that they had not dried out. Adult weevils are collected weekly then identified and counted. Record data in the form (Appendix 5).
- As weevils emerge from each sample and they are identified and counted, collect them into a **clean**, labelled container with a secure lid. A centrifuge tube of 1.5ml is ideal but the exact type is not critical but it MUST be absolutely clean, i.e., used direct from the manufacturer's packaging, so there is no possibility of DNA contamination. Ensure label can withstand the freezer. Weevils can be progressively added to each container over time but be very careful to avoid mistakes (e.g., putting the weevils from a sample into the incorrect tube. We will use DNA analysis at a later date in PNG or Australia to confirm species and examine genetic diversity if time and resources permit.

b) Destructive sampling of plant parts

- **Remaining vine:** carefully split with a scalpel to collect and count weevil adults, pupae and larvae. (See attached guide for identifying larval weevils).
- **Remaining half of crown:** carefully break apart (scalpel/ scissors used when necessary) to collect and record adults, pupae and larvae. Identify larvae using guide.
- **Remaining storage root:** cut roots through regions were weevil feeding damage can be seen. If no damage is evident following careful inspection of the root surface, the root should be carefully split lengthways and then cut in half again to check for damage/ weevils. When present adults, larvae and pupae were then excavated, identified and recorded. Record data in the form (Appendix 6).

Protocol II. Instructions for assessment/sampling on designed experiments sites at Aiyura

i. Mid-season assessment

For mid-season assessment, the following assessment/sampling will be conducted:

> ***4) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity for designed experiments***

ii. Harvest time assessment

For harvest time assessment, two sets of assessment/sampling will be conducted:

> ***4) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity for designed experiments***
>
> ***5) Weevils inside sweetpotato plants survey***

4 Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity (on designed experiments sites)

Because activity of some insects (especially sweetpotato weevils) varies according to time of day, **DO NOT** survey all of the plots in one treatment/trial type before moving on to the other treatment/trial type (e.g., all the accumulator trial plots, then all the control plots). Rather, sample the plots in a non-systematic ('random') manner so that no bias is introduced. That is, sample one or two plots of a given treatment/trial, then one or two plots of the other treatment/trial, and so on.

In each plot, do ALL of the following sampling tasks.

> **4.1) Active arthropod count**
>
> **4.2) Foliar scab symptoms assessment**
>
> **4.3) Gall mite symptoms assessment**

4.1 Active arthropod count

Using bamboo canes, make a square quadrat (50 cm x 50 cm). Use a strong tape like gaffer tape or wire to connect the four corners.

Use the quadrat to mark areas of the crop in which detailed observations will be made. In each plot, gently place the quadrat sequentially in each of the **5 portions of the field as shown in Fig 3. Avoid the outer 1.0m borders of the field.** Ensure the quadrat is positioned gently to avoid disturbance of insects.

Observe the whole area within the quadrat frame and count the numbers of each type of arthropods that you see. Sweetpotato weevil adults are obviously the insect of most importance here but you may also see some West Indian sweetpotato weevil adults, grasshoppers, caterpillars, beetles, spiders etc. Record data in the form (Appendix 7).

Before you move the quadrat to the next sample position, record scab and gall mite symptoms as described in the following sections.

When you have assessed active arthropods, scab and gall mites for a given quadrat position, move the quadrat to the next position. When you have recorded data from all 5 positions in each plot as shown in Fig. 3. Move to the next plot.

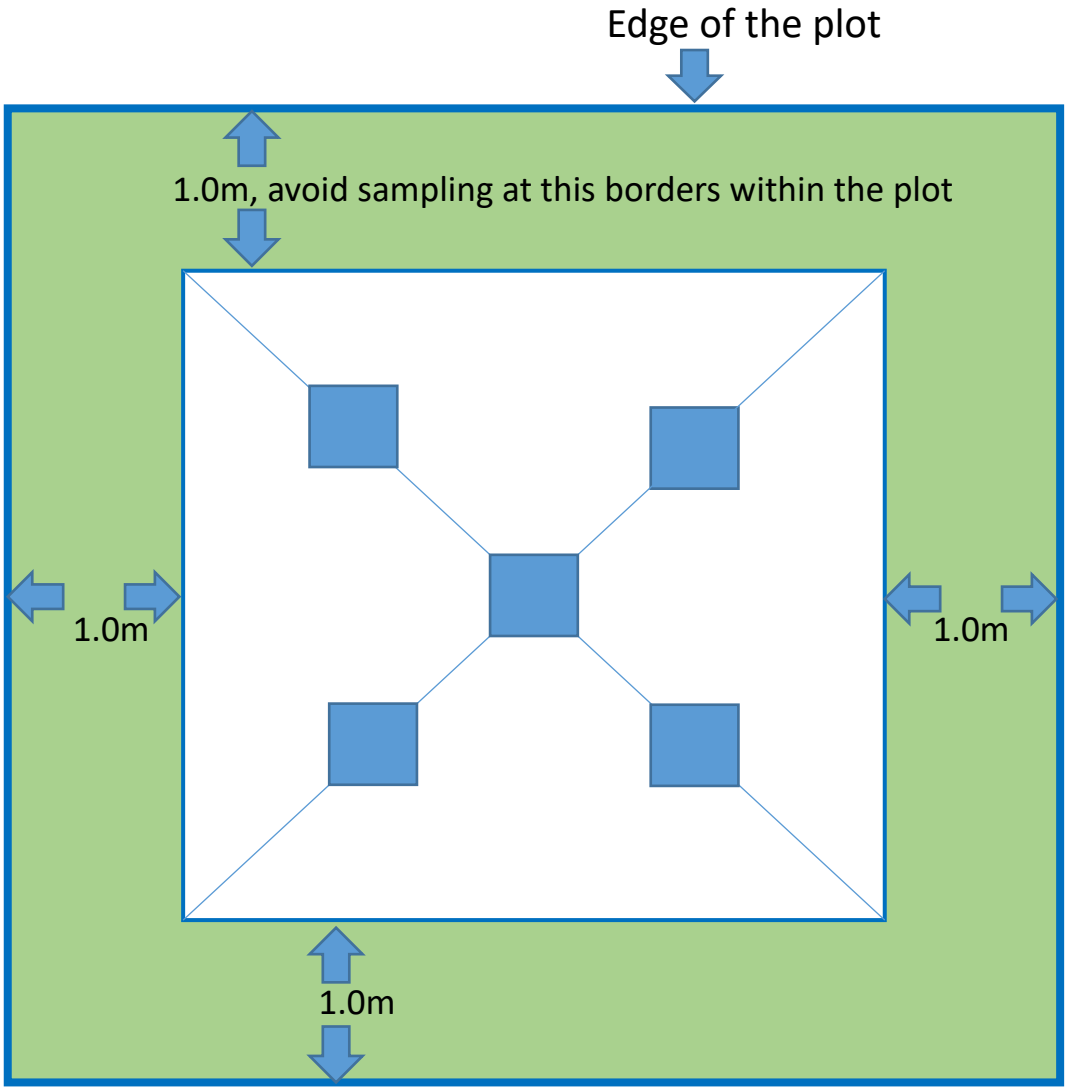


Fig. 3. Sample pattern for visual survey in designed experiment, blue boxes show the position of the 5 quadrat samples.

4.2 Foliar scab symptoms assessment

After assessing active arthropod numbers, leave the quadrat in place and select a single vine nearest to one of the corners of the quadrat.

On this vine, carefully observe the leaves, the petioles and the vine stem itself and judge the severity of scab symptoms against the pictures and descriptions (Appendix 2), record score(i.e., a number between 0 and 5 on the form) on the recording form (Appendix 8). Now, repeat this for vines at all the other corners of the quadrat so that you have a total of 4 severity estimates for the vines at all corners of the quadrat.

Now record gall mite severity for the same four vines.

4.3 Gall mite symptoms assessment

Carefully observe the leaves, the petioles and the vine stem itself and judge the severity of gall mite symptoms against the pictures and descriptions (Appendix 4), record score (i.e., a number between 0 and 5 on the form) on the recording form (Appendix 8). Now, repeat this for vines at all the other corners of the quadrat so that you have a total of 4 severity estimates for the vines at all corner of the quadrat.

Now move the quadrat to the next position.

When you have recorded data from all 5 positions in each plot as shown in Fig. 3. Move to the next plot.

5 Weevils inside sweetpotato plants survey (harvest time only) (on designed experiments sites)

-- Same protocol as Mike Furlong's for HORT 2010 065

To assess relative abundance of sweetpotato weevil and West Indian Sweetpotato weevil, 5 plants are randomly chosen as above in section 4. The initial plant was selected from the middle of the plot. The remaining 4 plants were then collected at approximately equidistant spacing along transects to the corners of the plots as shown in Fig. 3. **Avoid the outer 1.0m borders of the field.** Same plants sampled in activity 2 (Sampling for DNA analysis) can be used again here.

5.1 Collection of selected plants

For each of the selected plants, randomly select two vines. Cut these off close to the crown of the plant and place them into a large, labelled plastic bag. The other vines can be discarded. Dig up the plant and place the crown of the plant into the same paper bag as the vines. Finally, dig up the storage roots and randomly select two (without bias) and place these into the bag.

5.2 Separation of plant parts, incubation and destructive sampling.

Assess weevil infestation in the collected plant parts in two ways:

a) Incubation of plant parts
   - **For each plant sample, randomly select one of the two vines, one of the storage roots and one half of the crown** (crowns cut in to 2 approximately equal halves). Place these together into a plastic container lined with damp tissue paper and covered with a ventilated lid (e.g., ice cream containers approx. 20 cm x 20 cm x 10 cm). Use mesh to cover the ventilation to avoid escape of adult weevils.
   - Incubate the sample (ambient temps, on lab benches) for 4-5 weeks. Ensure they are in a rodent free area. Checked 2-3 times per week to ensure that they had not dried out. Adult weevils are collected weekly then identified and counted. Record data in the form (Appendix 9).

- As weevils emerge from each sample and they are identified and counted, collect them into a **clean**, labelled container with a secure lid. A centrifuge tube of 15ml is ideal but the exact type is not critical but it MUST be absolutely clean, i.e., used direct from the manufacturer's packaging, so there is no possibility of DNA contamination. Ensure label can withstand the freezer. Pencil on a slip of paper placed INSIDE the container is best. Weevils can be progressively added to each container over time but be very careful to avoid mistakes (e.g., putting the weevils from a sample into the incorrect tube. We will use DNA analysis at a later date in PNG or Australia to confirm species and examine genetic diversity if time and resources permit.

b) Destructive sampling of plant parts

- **Remaining vine:** carefully split with a scalpel to collect and count weevil adults, pupae and larvae. (See attached guide for identifying larval weevils).
- **Remaining half of crown:** carefully break apart (scalpel/ scissors used when necessary) to collect and record adults, pupae and larvae. Identify larvae using guide.
- **Remaining storage root:** cut roots through regions were weevil feeding damage can be seen. If no damage is evident following careful inspection of the root surface, the root should be carefully split lengthways and then cut in half again to check for damage/ weevils. When present adults, larvae and pupae were then excavated, identified and recorded. Record data in the form (Appendix 10).

Appendix

Appendix 1. Data recording form for above ground visual survey for active arthropod (mainly sweetpotato weevils) on TEAM zone sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Arthropod species	Number of arthropod present with sample position quadrat									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sweetpotato weevils *Cylas formicarius*										
West Indian sweetpotato weevils *Euscepes postfasciatus*										
Beetles										
Grasshoppers										
Caterpillars										
Spiders										
Others										

Appendix 2. Graphical key for assessing scab severity

Scale of disease	
0 = no disease	
1 = less than 5 scab lesions per 10 cm on stems and petioles, lesions on veins causing slight leaf distortion, no upright/erect presentation terminals	
2 =5-50 scab lesions per 10 cm on stems and petioles, lesions on veins causing slight leaf distortion (cupping, shrivelling), slight upright/erect presentation of terminals	
3 = >50 lesions per 10 cm scab lesions merging over large areas of stem and petiole, leaves distorted, upright presentation of terminals	
4 = scab lesions merging all large areas of the stem and petiole, severe leaf and terminal or apical area severely distorted, upright presentation/erect	
5 = leaves dead, apical meristem severely distorted and dead.	

Images from
http://keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html/TheProblems/DiseasesFungal/Scab/Scab.htm, modified with disease severity key from Ramsey et al. 1988 http://www.publish.csiro.au/an/pdf/EA9880137

Appendix 3. Data recording form for Scab and gall mite severity (score for each sample vine) on TEAM zone sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:

Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant no.	Vine no.	Scab severity (Keys, 0-5)	Gall mite severity (Keys, 0-5)
1	1		
	2		
2	3		
	4		
3	5		
	6		
4	7		
	8		
5	9		
	10		
6	11		
	12		
7	13		
	14		
8	15		
	16		
9	17		
	18		
10	19		
	20		

Appendix 4. Graphical key for assessing gall mite severity

Graphical key	
0 = no symptoms	
1 = less than 5 galls per 10 cm on stems, each petioles and leaf	
2 =5-10 galls lesions per 10cm on stems, petioles and each leaf	 

3 = >10 galls per 10cm on stems, petioles and each leaf	
4 = galls merging all large areas of the stem, petiole and leaves	 
5 = leaves dead, apical meristem severely distorted and dead.	

*Pictures of gall mite are taken by Yapo Jeffery & Geoff Gurr.

Appendix 5. Data recording form for weevils inside sweetpotato plants (incubation results) on TEAM zone sites

a) Data recording form for incubation of plant parts

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Weevil species	Number of weevils emerged in week					
		1	2	3	4	5	Total
1	*C. formicarius*						
	E. postfasciatus						
	Beetle						
2	*C. formicarius*						
	E. postfasciatus						
	Beetle						
3	*C. formicarius*						
	E. postfasciatus						
	Beetle						
4	*C. formicarius*						
	E. postfasciatus						
	Beetle?						
5	*C. formicarius*						
	E. postfasciatus						
	Beetle						

Appendix 6. Data recording form for weevils inside sweetpotato plants (destructive sampling results) on TEAM zone sites

b) Data recording form for destructive sampling of plant parts

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Parts of plant	Number of *C. formicarius*			Number of *E. postfasciatus*		
		Adult	pupa	larvae	Adult	pupa	larvae
1	Single vines						
	Half of crown						
	One root						
2	Single vines						
	Half of crown						
	One root						
3	Single vines						
	Half of crown						
	One root						
4	Single vines						
	Half of crown						
	One root						
5	Single vines						
	Half of crown						
	One root						

Appendix 7. Data recording form for above ground visual survey for active arthropod (mainly sweetpotato weevils) on designed experiment sites

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Arthropod species	Number of arthropod present within sample position quadrat				
	1	**2**	**3**	**4**	**5**
Sweetpotato weevils *Cylas formicarius*					
West India sweetpotato weevils *Euscepes postfasciatus*					
Beetles					
Grasshoppers,					
Caterpillars					
Spiders					
Others					

Appendix 8. Data recording form for Scab and gall mite severity (score for each sample vine) on designed experiment sites

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant no.	Vine no.	Scab severity (Keys, 0-5)	Gall mite severity (Keys, 0-5)
1	1		
	2		
	3		
	4		
2	5		
	6		
	7		
	8		
3	9		
	10		
	11		
	12		
4	13		
	14		
	15		
	16		
5	17		
	18		
	19		
	20		

Appendix 9. Data recording form for weevils inside sweetpotato plants (<u>incubation results</u>) on designed experiment sites

a) <u>Data recording form for incubation of plant parts</u>

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Weevil species	Number of weevils emerged in week					
		1	2	3	4	5	Total
1	*C. formicarius*						
	E. postfasciatus						
	Beetle						
2	*C. formicarius*						
	E. postfasciatus						
	Beetle						
3	*C. formicarius*						
	E. postfasciatus						
	Beetle						
4	*C. formicarius*						
	E. postfasciatus						
	Beetle						
5	*C. formicarius*						
	E. postfasciatus						
	Beetle						

Appendix 10. Data recording form for weevils inside sweetpotato plants (destructive sampling results) on designed experiment sites

b) Data recording form for destructive sampling of plant parts

Date:	Trial:
Time of the season: (mid-season or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Parts of plant	Number of *C. formicarius*			Number of *E. postfasciatus*		
		Adult	pupa	larvae	Adult	pupa	larvae
1	Single vines						
	Half of crown						
	One root						
2	Single vines						
	Half of crown						
	One root						
3	Single vines						
	Half of crown						
	One root						
4	Single vines						
	Half of crown						
	One root						
5	Single vines						
	Half of crown						
	One root						

Appendix 11 Materials and bench space to prepare

Items needed in the field:

- Camera
- Bamboo canes (> 50 cm in length)
- Gaffer tape or wire
- Scissors
- Knife
- Multiple permanent markers, thin tip
- Pencils
- Note books
- Labels for tubes for the freezer
- Large plastic bags
- Snap-lock bags (large and small)
- DNA free 1.5-2 ml centrifuge tubes
- Clean centrifuge tubes (15 ml) or container
- Printed data recording sheets as required
- Clipboards
- Esky with ice

Extra items needed in the lab:

- Paper towel/tissue
- Freezer
- Bench space
- Chopping board
- Materials for DIY mini insect rearing cages:
  Lots of medium sized containers (e.g., large ice cream pots) or storage boxes
  Mesh to keep weevils inside
  Scalpel/blade to cut out lid to place mesh
  Strong glue to glue mesh in place

Insect pest and disease field survey protocols 2018

For ACIAR HORT/2014/083- Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea

For all the field experiments and trials, insect pests and diseases are surveyed once in the middle of the growing season and again at harvest time.

This is a compilation of TWO separate documents:

Protocol I. Instructions for assessment/sampling on TEAM zone sites.

Protocol II. Instructions for assessment/sampling on designed experiments sites at Aiyura

Each of the two sets of documents is arranged by (i) mid-season assessment and (ii) harvest time assessment and each has an appended recording sheets.

The sampling protocols are similar (but not identical) for the TEAM zones where fields or gardens are the sampling unit, versus designed experiments where small plots are the sampling unit. Weevil-related methods are designed to be compatible with those being used in the ACIAR Pacific Root Crops project led by Prof Mike Furlong.

During sampling and assessing regularly take photographs of the target e.g. insects, plants with scab/gall mite symptoms within quadrats. When taking a photo, make a note of the file number (e.g., 0014.jpg) on the data recording form.

For all the samples, make sure the labels are securely in place. The best way to write a clear label is using a lead pencil on sticky paper label. If sticky labels are unavailable, write using a lead pencil on a piece of paper and stick it inside the sample bag. For labelling tubes, please use high quality fine tipped permanent markers.

Contents

Protocol I: Instructions for assessment/sampling on TEAM zone sites.

In TEAM zone sites, whole fields/ gardens will be used as 'plots'. There will be multiple (i.e. replicated) fields of each treatment. In year 1 at least, there will be tow treatments only: (1) conventional farming practice (the control) and (2) a best-bet integrated pest and disease management strategy based on the use of pathogen tested planting material, crop hygiene and crop isolation.

i. Mid-season assessment

For mid-season assessment, the following assessment/sampling will be conducted:

1) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity

ii. Harvest time assessment

For harvest time assessment, three sets of assessment/sampling will be conducted:

1) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity

2) Sampling for DNA analysis

3) Weevils inside sweetpotato plants survey

1 Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity (on TEAM zone sites)

Because activity of some insects (especially sweetpotato weevils) varies according to time of day, **DO NOT** survey all of the fields/gardens in one treatment type before moving on to the other treatment type (e.g., all the best bet fields, then all the control fields). Rather, sample the fields/gardens in a non-systematic ('random') manner so that no bias is introduced. That is, sample one or two fields/gardens of a given treatment, then one or two fields/gardens of the other treatment, and so on.

In each field, do ALL of the following sampling tasks.

1.1) Active arthropod count

1.2) Foliar scab symptoms assessment

1.3) Gall mite symptoms assessment

1.1 Active arthropod count

Using bamboo canes, make a square quadrat (50 cm x 50 cm). Use a strong tape like gaffer tape or wire to connect the four corners.

Use the quadrat to mark areas of the crop in which detailed observations will be made. In each field/garden, gently place the quadrat sequentially in each of the **10 portions of the field as shown**

in Fig 1. Avoid the outer 1.0m borders of the field. Ensure the quadrat is positioned gently to avoid disturbance of insects.

Observe the whole area within the quadrat frame and count the numbers of each type of arthropod that you see. Sweetpotato weevil adults are obviously the insect of most importance here but you may also see some West Indian sweetpotato weevil adults, grasshoppers, caterpillars, beetles, spiders etc. Record data in the form (Appendix 1).

Before you move the quadrat to the next sample position, record scab and gall mite symptoms as described in the following sections.

When you have assessed active arthropods, scab and gall mites for a given quadrat position, move the quadrat to the next position. When you have recorded data from all 10 positions in each field/garden (Fig. 1). Move to the next plot (i.e., field/garden).

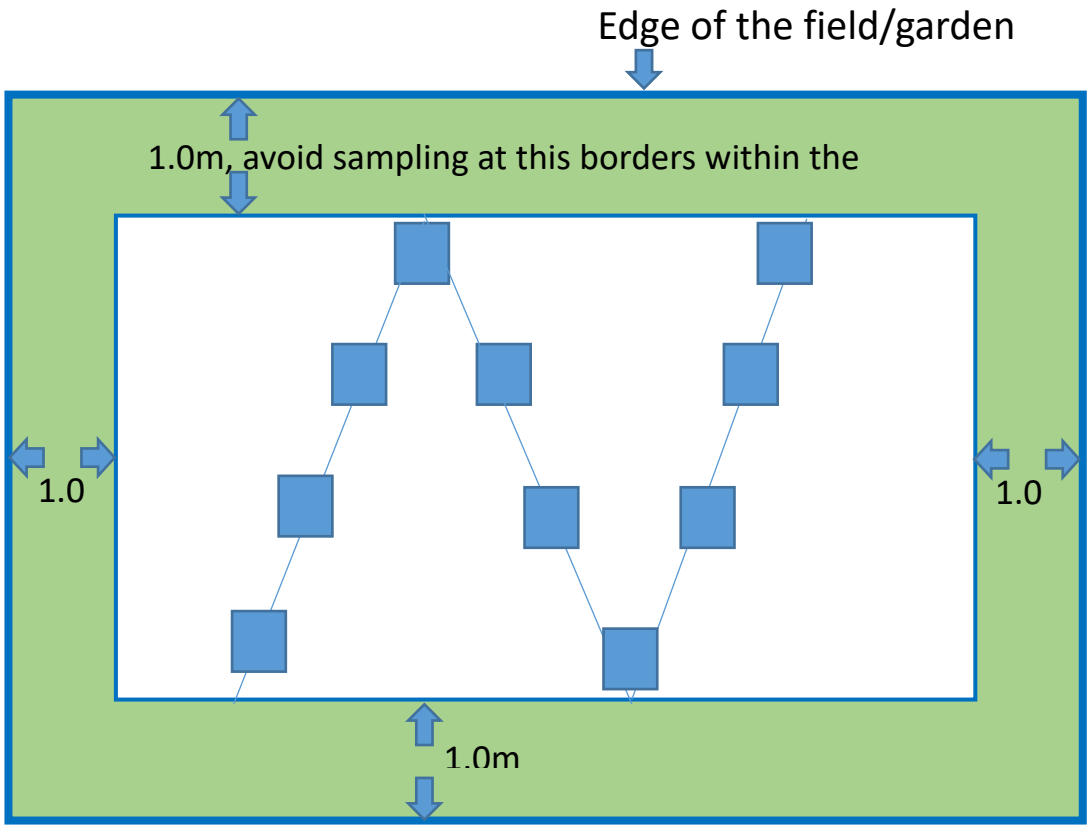


Fig. 1. Sample pattern for visual survey in TEAM zone sites, blue boxes show the position of the 10 quadrat samples

1.2 Foliar scab symptoms assessment

After assessing active arthropod numbers, leave the quadrat in place and select a single vine nearest to one of the corners of the quadrat.

On this vine, carefully observe the leaves, the petioles and the vine stem itself and judge the severity of scab symptoms against the pictures and descriptions (Appendix 2), record score (i.e., a number between 0 and 5) on the recording form (Appendix 3). Now, repeat this for the vine at diagonally opposite corner of the quadrat so that you have a total of 2 severity estimates for the vines at two corner of the quadrat. Now record gall mite severity for the same two vines.

1.3 Gall mite symptoms assessment

Carefully observe the leaves, the petioles and the vine stem itself and judge the severity of gall mite symptoms against the pictures and descriptions (Appendix 4), record data in the recording form (Appendix 3). Write the symptom score (i.e., a number between 0 and 5 on the form). Now, repeat this for the vine at diagonal corner of the quadrat so that you have a total of 2 severity estimates for the vines at two corners of the quadrat.

Now move the quadrat to the next position.

When you have recorded data from all 10 positions in each field/garden (Fig 1). Move to the next field/garden. To assess the presence of various organisms and microorganisms, soil and plant leaves samples are taken from 5 randomly chosen plants. Plants need to be selected along a transect that approximates a diagonal across the field/ garden but avoiding the edges. The remaining 4 plants are then collected at approximately equidistant spacing along transect to the far side of the plots (Fig. 2). After taking the samples, keep these plants for the next survey activity (section 3, Weevils inside sweetpotato plants survey).

2 Sampling for DNA analysis (on TEAM zone sites)

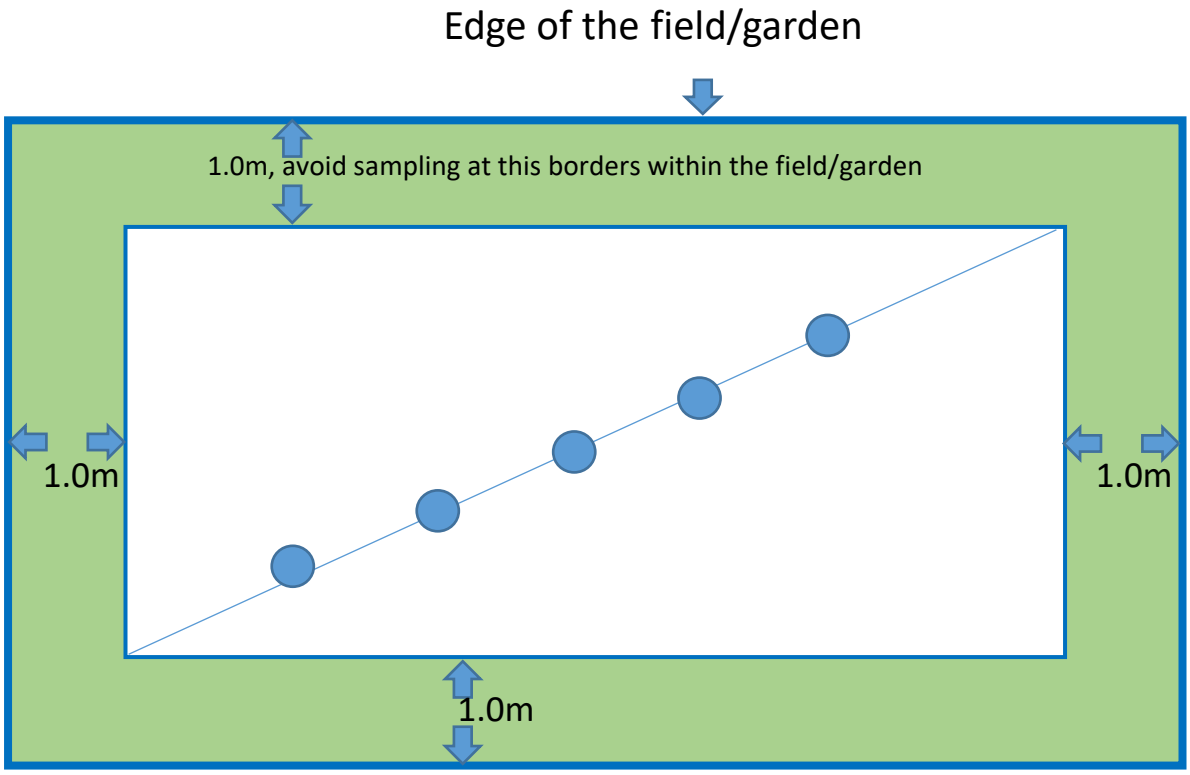


Fig. 2. Sample pattern for weevils inside plants in TEAM zone sites, blue circles show the position of the 5 plants samples

2.1 Soil sampling

For each of the five plants, use a 15 ml centrifuge tube to scoop up some soil to fill the tube. With a permanent marker, label the tube appropriately with the date, experiment name, plot no., rep no. and plant no. If the label looks like it is coming off, back at the lab, write out the label, place on the tube and cover with clear sticky tape. Store filled tubes in a large zip-lock bag in the freezer.

2.2 Plant leaves sampling

For each of the five plants, remove 3 leaves (a mix of diseased, un-diseased) and place in a zip-lock bag (separate bag per plant). Use a small piece of paper and a pencil (grey 'lead') to record all details as above, then place inside the bag and zip lock the bag.

Take back to the lab on ice in the esky and freeze immediately.

These samples will be processed for DNA analysis (PNG/Australia), where we will test for the presence of various organisms and microorganisms using specific markers.

3 Weevils inside sweetpotato plants survey (harvest time only) (on TEAM zone sites)

-- Same protocol as Mike Furlong for HORT 2010 065

To assess relative abundance of sweetpotato weevil and West Indian Sweetpotato weevil, 5 plants are randomly chosen as above in section 2. Plants need to be selected along a transect that approximates a diagonal across the field/ garden but avoiding the edges. The remaining 4 plants are then collected at approximately equidistant spacing along transect to the far side of the plots (Fig. 2). Same plants sampled in activity 2 (Sampling for DNA analysis) can be used again here.

3.1 Collection of selected plants

For each of the selected plants, randomly select two vines. Cut these off close to the crown of the plant and place them into a large, labelled plastic bag. The other vines can be discarded. Dig up the plant and place the crown of the plant into the same plastic bag as the vines. Finally, dig up the storage roots and randomly select two (without bias) and place these into the bag.

3.2 Separation of plant parts, incubation and destructive sampling.

Assess weevil infestation in the collected plant parts in two ways:

a) <u>Incubation of plant parts</u>
   - **For each plant sample, randomly select one of the two vines, one of the storage roots and one half of the crown** (crowns cut in to 2 approximately equal halves). Place these together into a labelled plastic container lined with damp tissue paper and covered with a ventilated lid (e.g., ice cream containers approx. 20 cm x 20 cm x 10 cm). Use mesh to cover the ventilation to avoid escape of adult weevils.

- Incubate the sample (ambient temps, on lab benches) for 4-5 weeks. Ensure they are in a rodent free area. Checked 2-3 times per week to ensure that they had not dried out. Adult weevils are collected weekly then identified and counted. Record data in the form (Appendix 5).
- As weevils emerge from each sample and they are identified and counted, collect them into a **clean**, labelled container with a secure lid. A centrifuge tube of 1.5ml is ideal but the exact type is not critical but it MUST be absolutely clean, i.e., used direct from the manufacturer's packaging, so there is no possibility of DNA contamination. Ensure label can withstand the freezer. Weevils can be progressively added to each container over time but be very careful to avoid mistakes (e.g. putting the weevils from a sample into the incorrect tube. We will use DNA analysis at a later date in PNG or Australia to confirm species and examine genetic diversity if time and resources permit.

b) Destructive sampling of plant parts

- **Remaining vine:** carefully split with a scalpel to collect and count weevil adults, pupae and larvae. (See attached guide for identifying larval weevils).
- **Remaining half of crown:** carefully break apart (scalpel/ scissors used when necessary) to collect and record adults, pupae and larvae. Identify larvae using guide.
- **Remaining storage root:** cut roots through regions were weevil feeding damage can be seen. If no damage is evident following careful inspection of the root surface, the root should be carefully split lengthways and then cut in half again to check for damage/ weevils. When present adults, larvae and pupae were then excavated, identified and recorded. Record data in the form (Appendix 6).

4 Sweetpotato yield survey (harvest time only) (on TEAM zone sites)

To assess sweetpotato yield under different treatment, 5 plants are randomly chosen as above in section 2. Plants need to be selected along a transect that approximates a diagonal across the field/ garden but avoiding the edges. The remaining 4 plants are then collected at approximately equidistant spacing along transect to the far side of the plots (Fig. 2). Same plants sampled in activity 2 (Sampling for DNA analysis) can be used again here. Count the number of marketable tuber number, non-marketable tuber.

Protocol II. Instructions for assessment/sampling on designed experiments sites at Aiyura

i. Mid-season assessment

For mid-season assessment, the following assessment/sampling will be conducted:

4) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity for designed experiments

ii. Harvest time assessment

For harvest time assessment, two sets of assessment/sampling will be conducted:

4) Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity for designed experiments

5) Weevils inside sweetpotato plants survey

4 Above ground visual survey for active arthropods (mainly sweetpotato weevil), scab and gall mite severity (on designed experiments sites)

Because activity of some insects (especially sweetpotato weevils) varies according to time of day, **DO NOT** survey all of the plots in one treatment/trial type before moving on to the other treatment/trial type (e.g., all the accumulator trial plots, then all the control plots). Rather, sample the plots in a non-systematic ('random') manner so that no bias is introduced. That is, sample one or two plots of a given treatment/trial, then one or two plots of the other treatment/trial, and so on.

In each plot, do ALL of the following sampling tasks.

4.1) Active arthropod count

4.2) Foliar scab symptoms assessment

4.3) Gall mite symptoms assessment

4.1 Active arthropod count

Using bamboo canes, make a square quadrat (50 cm x 50 cm). Use a strong tape like gaffer tape or wire to connect the four corners.

Use the quadrat to mark areas of the crop in which detailed observations will be made. In each plot, gently place the quadrat sequentially in each of the **5 portions of the field as shown in Fig 3. Avoid the outer 1.0m borders of the field.** Ensure the quadrat is positioned gently to avoid disturbance of insects.

Observe the whole area within the quadrat frame and count the numbers of each type of arthropods that you see. Sweetpotato weevil adults are obviously the insect of most importance here but you may also see some West Indian sweetpotato weevil adults, grasshoppers, caterpillars, beetles, spiders etc. Record data in the form (Appendix 7).

Before you move the quadrat to the next sample position, record scab and gall mite symptoms as described in the following sections.

When you have assessed active arthropods, scab and gall mites for a given quadrat position, move the quadrat to the next position. When you have recorded data from all 5 positions in each plot as shown in Fig. 3. Move to the next plot.

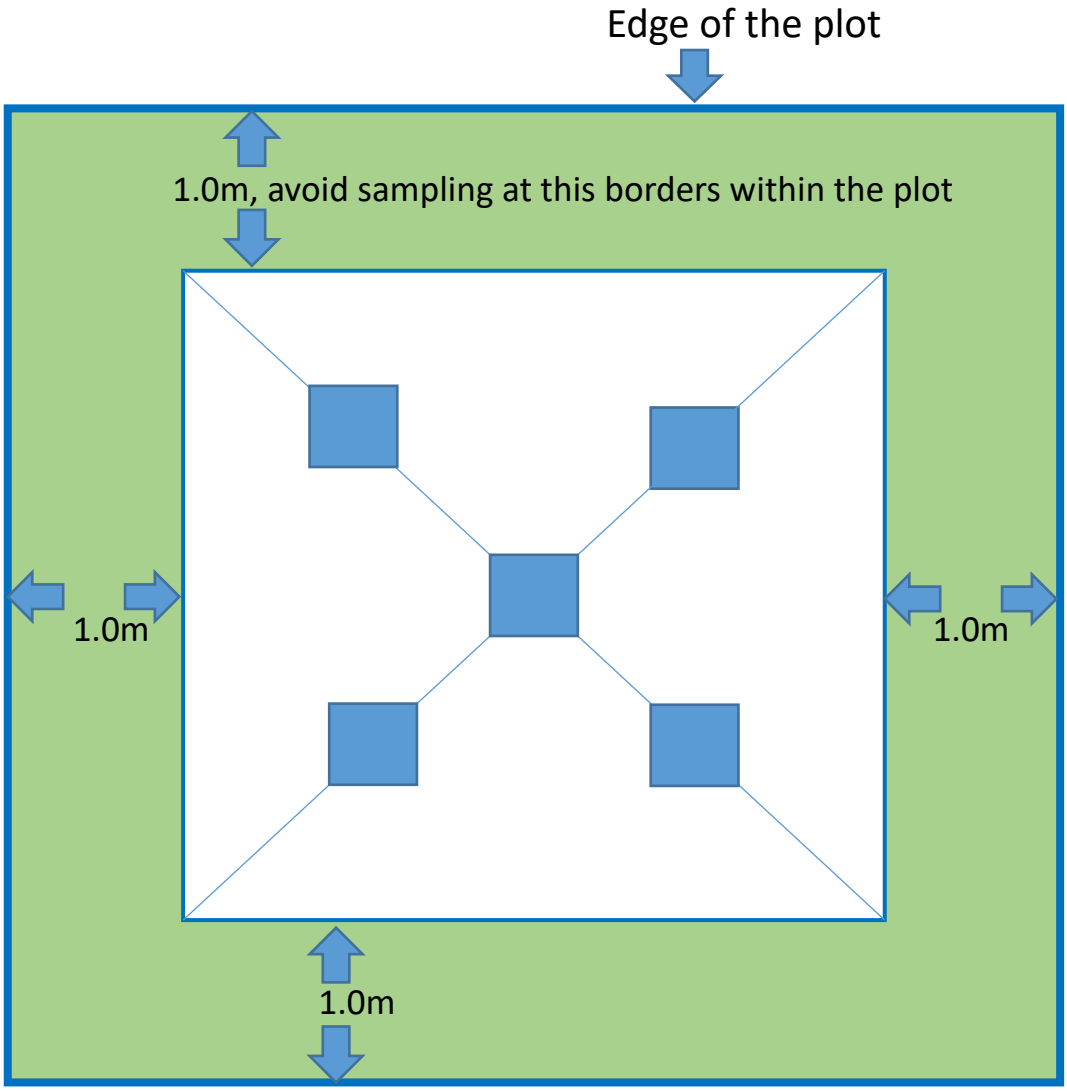


Fig. 3. Sample pattern for visual survey in designed experiment, blue boxes show the position of the 5 quadrat samples.

4.2 Foliar scab symptoms assessment

After assessing active arthropod numbers, leave the quadrat in place and select a single vine nearest to one of the corners of the quadrat.

On this vine, carefully observe the leaves, the petioles and the vine stem itself and judge the severity of scab symptoms against the pictures and descriptions (Appendix 2), record score(i.e., a number between 0 and 5 on the form) on the recording form (Appendix 8). Now, repeat this for vines at all the other corners of the quadrat so that you have a total of 4 severity estimates for the vines at all corners of the quadrat.

Now record gall mite severity for the same four vines.

4.3 Gall mite symptoms assessment

Carefully observe the leaves, the petioles and the vine stem itself and judge the severity of gall mite symptoms against the pictures and descriptions (Appendix 4), record score(i.e., a number between 0 and 5 on the form) on the recording form (Appendix 8). Now, repeat this for vines at all the other corners of the quadrat so that you have a total of 4 severity estimates for the vines at all corner of the quadrat.

Now move the quadrat to the next position.

When you have recorded data from all 5 positons in each plot as shown in Fig. 3. Move to the next plot.

5 Weevils inside sweetpotato plants survey (harvest time only) (on designed experiments sites)

-- Same protocol as Mike Furlong's for HORT 2010 065

To assess relative abundance of sweetpotato weevil and West Indian Sweetpotato weevil, 5 plants are randomly chosen as above in section 4. The initial plant was selected from the middle of the plot. The remaining 4 plants were then collected at approximately equidistant spacing along transects to the corners of the plots as shown in Fig. 3. **Avoid the outer 1.0m borders of the field.** Same plants sampled in activity 2 (Sampling for DNA analysis) can be used again here.

5.1 Collection of selected plants

For each of the selected plants, randomly select two vines. Cut these off close to the crown of the plant and place them into a large, labelled plastic bag. The other vines can be discarded. Dig up the plant and place the crown of the plant into the same paper bag as the vines. Finally, dig up the storage roots and randomly select two (without bias) and place these into the bag.

5.2 Separation of plant parts, incubation and destructive sampling.

Assess weevil infestation in the collected plant parts in two ways:

a) <u>Incubation of plant parts</u>
   - **For each plant sample, randomly select one of the two vines, one of the storage roots and one half of the crown** (crowns cut in to 2 approximately equal halves). Place these together into a plastic container lined with damp tissue paper and covered with a ventilated lid (e.g., ice cream containers approx. 20 cm x 20 cm x 10 cm). Use mesh to cover the ventilation to avoid escape of adult weevils.
   - Incubate the sample (ambient temps, on lab benches) for 4-5 weeks. Ensure they are in a rodent free area. Checked 2-3 times per week to ensure that they had not dried out. Adult weevils are collected weekly then identified and counted. Record data in the form (Appendix 9).

- As weevils emerge from each sample and they are identified and counted, collect them into a **clean**, labelled container with a secure lid. A centrifuge tube of 15ml is ideal but the exact type is not critical but it MUST be absolutely clean, i.e., used direct from the manufacturer's packaging, so there is no possibility of DNA contamination. Ensure label can withstand the freezer. Pencil on a slip of paper placed INSIDE the container is best. Weevils can be progressively added to each container over time but be very careful to avoid mistakes (e.g. putting the weevils from a sample into the incorrect tube. We will use DNA analysis at a later date in PNG or Australia to confirm species and examine genetic diversity if time and resources permit.

b) Destructive sampling of plant parts

- **Remaining vine:** carefully split with a scalpel to collect and count weevil adults, pupae and larvae. (See attached guide for identifying larval weevils).
- **Remaining half of crown:** carefully break apart (scalpel/ scissors used when necessary) to collect and record adults, pupae and larvae. Identify larvae using guide.
- **Remaining storage root:** cut roots through regions were weevil feeding damage can be seen. If no damage is evident following careful inspection of the root surface, the root should be carefully split lengthways and then cut in half again to check for damage/ weevils. When present adults, larvae and pupae were then excavated, identified and recorded. Record data in the form (Appendix 10).

Appendix

Appendix 1. Data recording form for above ground visual survey for active arthropod (mainly sweetpotato weevils) on TEAM zone sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Arthropod species	Number of arthropod present with sample position quadrat									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sweetpotato weevils *Cylas formicarius*										
West Indian sweetpotato weevils *Euscepes postfasciatus*										
Beetles										
Grasshoppers										
Caterpillars										
Spiders										
Others?										

Appendix 2. Graphical key for assessing scab severity

Scale of disease	
0 = no disease	
1 = less than 5 scab lesions per 10 cm on stems and petioles, lesions on veins causing slight leaf distortion, no upright/erect presentation terminals	
2 =5-50 scab lesions per 10 cm on stems and petioles, lesions on veins causing slight leaf distortion (cupping, shrivelling), slight upright/erect presentation of terminals	
3 = >50 lesions per 10 cm scab lesions merging over large areas of stem and petiole, leaves distorted, upright presentation of terminals	
4 = scab lesions merging all large areas of the stem and petiole, severe leaf and terminal or apical area severely distorted, upright presentation/erect	
5 = leaves dead, apical meristem severely distorted and dead.	

Images from
http://keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html/TheProblems/DiseasesFungal/Scab/Scab.htm, modified with disease severity key from Ramsey et al. 1988 http://www.publish.csiro.au/an/pdf/EA9880137

Appendix 3. Data recording form for Scab and gall mite severity (score for each sample vine) on TEAM zone sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:

Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant no.	Vine no.	Scab severity (Keys, 0-5)	Gall mite severity (Keys, 0-5)
1	1		
	2		
2	3		
	4		
3	5		
	6		
4	7		
	8		
5	9		
	10		
6	11		
	12		
7	13		
	14		
8	15		
	16		
9	17		
	18		
10	19		
	20		

Appendix 4. Graphical key for assessing gall mite severity

0 = no symptoms	
1 = less than 5 galls per 10 cm on stems, each petioles and leaf	
2 =5-10 galls lesions per 10cm on stems, petioles and each leaf	 

3 = >10 galls per 10cm on stems, petioles and each leaf	
4 = galls merging all large areas of the stem, petiole and leaves	 
5 = leaves dead, apical meristem severely distorted and dead.	

*Pictures of gall mite are taken by Yapo Jeffery & Geoff Gurr.

Appendix 5. Data recording form for weevils inside sweetpotato plants (incubation results) on TEAM zone sites

a) Data recording form for incubation of plant parts

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Weevil species	Number of weevils emerged in week					
		1	2	3	4	5	Total
1	*C. formicarius*						
	E. postfasciatus						
	Beetle						
2	*C. formicarius*						
	E. postfasciatus						
	Beetle						
3	*C. formicarius*						
	E. postfasciatus						
	Beetle						
4	*C. formicarius*						
	E. postfasciatus						
	Beetle						
5	*C. formicarius*						
	E. postfasciatus						
	Beetle						

Appendix 6. Data recording form for weevils inside sweetpotato plants (destructive sampling results) on TEAM zone sites

b) Data recording form for destructive sampling of plant parts

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Parts of plant	Number of *C. formicarius*			Number of *E. postfasciatus*		
		Adult	pupa	larvae	Adult	pupa	larvae
1	Single vines						
	Half of crown						
	One root						
2	Single vines						
	Half of crown						
	One root						
3	Single vines						
	Half of crown						
	One root						
4	Single vines						
	Half of crown						
	One root						
5	Single vines						
	Half of crown						
	One root						

Appendix 7. Data recording form for above ground visual survey for active arthropod (mainly sweetpotato weevils) on designed experiment sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Arthropod species	Number of arthropod present within sample position quadrat				
	1	**2**	**3**	**4**	**5**
Sweetpotato weevils *Cylas formicarius*					
West India sweetpotato weevils *Euscepes postfasciatus*					
Beetles					
Grasshoppers,					
Caterpillars					
Spiders					
Others					

Appendix 8. Data recording form for Scab and gall mite severity (score for each sample vine) on designed experiment sites

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant no.	Vine no.	Scab severity (Keys, 0-5)	Gall mite severity (Keys, 0-5)
1	1		
	2		
	3		
	4		
2	5		
	6		
	7		
	8		
3	9		
	10		
	11		
	12		
4	13		
	14		
	15		
	16		
5	17		
	18		
	19		
	20		

Appendix 9. Data recording form for weevils inside sweetpotato plants (<u>incubation results</u>) on designed experiment sites

a) <u>Data recording form for incubation of plant parts</u>

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Weevil species	Number of weevils emerged in week					
		1	2	3	4	5	Total
1	*C. formicarius*						
	E. postfasciatus						
	Beetle						
2	*C. formicarius*						
	E. postfasciatus						
	Beetle						
3	*C. formicarius*						
	E. postfasciatus						
	Beetle						
4	*C. formicarius*						
	E. postfasciatus						
	Beetle						
5	*C. formicarius*						
	E. postfasciatus						
	Beetle						

Appendix 10. Data recording form for weevils inside sweetpotato plants (destructive sampling results) on designed experiment sites

b) Data recording form for destructive sampling of plant parts

Date:	Trial:
Time of the season: (mid or harvest)	Treatment:
Location:	Treatment replicate:
Sweetpotato variety: Or PT/non-PT material:	Alternative host around:

Plant sample no.	Parts of plant	Number of *C. formicarius*			Number of *E. postfasciatus*		
		Adult	pupa	larvae	Adult	pupa	larvae
1	Single vines						
	Half of crown						
	One root						
2	Single vines						
	Half of crown						
	One root						
3	Single vines						
	Half of crown						
	One root						
4	Single vines						
	Half of crown						
	One root						
5	Single vines						
	Half of crown						
	One root						

Appendix 11 Materials and bench space to prepare

Items needed in the field:

- Camera
- Bamboo canes (> 50 cm in length)
- Gaffer tape or wire
- Scissors
- Knife
- Multiple permanent markers, thin tip
- Pencils
- Note books
- Labels for tubes for the freezer
- Large plastic bags
- Snap-lock bags (large and small)
- DNA free 1.5-2 ml centrifuge tubes
- Clean centrifuge tubes (15 ml) or container
- Printed data recording sheets as required
- Clipboards
- Esky with ice

Extra items needed in the lab:

- Paper towel/tissue
- Freezer
- Bench space
- Chopping board
- Materials for DIY mini insect rearing cages:
  Lots of medium sized containers (e.g. large ice cream pots) or storage boxes
  Mesh to keep weevils inside
  Scalpel/blade to cut out lid to place mesh
  Strong glue to glue mesh in place

Appendix 3a. Objective 1.3. Survey of soilborne pests and diseases in QLD and NSW sweetpotato

1. Background

After consultation with members Australian Sweetpotato Growers Inc (ASPG), key researchers at the QLD Government's Department of Agriculture and Fisheries (QDAF) and Craig Henderson (Henderson RDE), the survey of soilborne pests and diseases of sweetpotato in QLD and NSW was primarily undertaken as a desktop exercise, owing to the available field guide and previous reports of soilborne pests available online. The survey was updated over the life of the project to reflect any new information and personal observations in the field. Pests and diseases of biosecurity concern to the Australian Industry are also identified.

2. Pests of sweetpotato

2.1. Virus vectors and foliage pests

There are many sweetpotato viruses in Australia that infect both sweetpotato and related weeds (other Convolvulaceae) and the vectors that transmit these are a major concern (Wolfenden, Henderson et al. 2018) particularly in the generation and multiplication of PT plant material and on-farm nurseries (plant beds). Important vectors include aphids (for example cotton or melon aphid *Aphis gossypii* and green peach aphid *Myzus persicae*), silverleaf whitefly (*Bemisia tabaci*) and leafhoppers or jassids for example *Austroasca* spp. (Ekman and Lovatt 2015). At planting material multiplication sites, these vectors are controlled with intensive spray programs, although in the main field, these potential virus (and phytoplasma) vectors are less controlled (C. Henderson, pers. communication).

Because of its rapid growth rate, foliage pests are rarely a problem in sweetpotato. Insects such as cluster caterpillar/tropical armyworm (*Spodoptera litura*), Convolvulus hawk moth (*Agrius convolvuli*), flea beetle (*Xenidia* spp.), green vegetable bug (*Nezara viridula*), mealybug (*Phenacoccus solenopsis*), Rutherglen bug (*Nysius vinitor*), sweetpotato tortoise beetle (*Aspidimorpha* spp.) and sweetpotato leafminer (*Bedellia somnulentella*) occasionally occur; however, this is usually a result of adverse conditions affecting a standard insect control program. Mites (for example bean spider and two-spotted) (*Teranychus* spp.) can also occasionally be a problem (Ekman and Lovatt 2015). Fall armyworm (*Spodoptera frugiperda)* has been observed to consume sweetpotato shoots, leaves and storage roots in the laboratory (B. Wilson, pers. observation), but sweetpotato is unlikely to be preferred host in the field when grasses (for example crops like corn are abundant). Provided foliage pest numbers are managed, and particularly while the canopy is initially developing, they are not usually a problem for industry. Closer attention is usually paid to developing plant beds, as foliage pests can decimate planting material, and disrupt planting programs while the sprouts recover (C. Henderson, pers. communication).

2.2. Soilborne pests

Root-knot nematodes (most commonly *Meloidognyne incognita* and *M. javanica*) are the most important pests for the Australian sweetpotato industry (McCrystal 2010, McCrystal 2014, Uknown

2014, Ekman and Lovatt 2015). Unpublished survey results of root-knot nematodes in Australian sweetpotato soil in 2018 indicate that *Meloidognyne javanica* is mostly commonly found but *M. incognita, M. arenaria* and *M. hapla* are present (Stirling, Cobon et al. 2018). Root knot nematodes induce both morphological and physiological changes in sweetpotato. Typical symptoms are blistered, cracked and malformed storage/lateral roots and infected plants have reduced vigour and can permanently wilt (Ekman and Lovatt, 2015). Other species, for example *Pratylenchus* spp. (root lesion nematode) and *Rotylechulus* spp. (reniform nematode) could be problematic under conditions where root knot nematode is less prevalent (C. Henderson, pers. communication).

The sweetpotato weevil (*Cylas formicarius*) can be a major pest in Australian sweetpotato (McCrystal, 2010, McCrystal, 2014, Unknown, 2014, Ekman and Lovatt, 2015). Although sweetpotato weevil adults can feed on the crowns and shoots weakening the plant, it is the feeding on storage roots and subsequent egg laying on storage roots that is most problematic. Developing larvae tunnel through the storage root causing cavities: this feeding injury causes necrosis and stimulates the plant to produce ethylene, leading to the release of terpenoids (Uritani, Saito et al. 1975), which make the sweetpotato unpalatable to humans or livestock.

There are other chewing pest insects that can cause significant damage to storage roots; however, feeding injury does not induce terpenoid production. The larval stage of these insects feed on the roots, causing holes in roots, making them unmarketable. Observed feeding damage can range from shallow to deep holes and from shallow to deep tracks along the storage root. Rarely is a single storage root completely damaged with feeding marks, more commonly, 1-3 feeding holes (sometimes quite elaborate) are observed on all the roots within a heavily infested area of the paddock (B. Wilson, pers. observation). In conventional sweetpotato farming, these pests are usually well managed with pesticides; however, surface damage leads to product downgrading (sold for processing) or rejection at the market.

The larvae of the whitefringed weevil (*Naupactus leucoloma* formerly *Graphognathus leucoloma*) causes shallow feeding holes and tracks along the storage root can be a problem, but usually reflects a breakdown in management practices, either via crop rotation (whitefringed weevils are polyphagous although legumes are a preferred host) (Barnes and De Barro 2009), crop hygiene, or timely pesticide application (C. Henderson, pers. communication).

True (Elateridae) and false (Tenebrionidae) wireworms can be significant pests of Australian sweetpotatoes causing 'shothole' marks on the storage roots (Ekman and Lovatt, 2015); however, the species infesting sweetpotato in Australia are not well defined (McCrystal, 2010). Due to the proximity of sugarcane fields to sweetpotato production areas (e.g. Bundaberg), the sugarcane wireworm (*Agrypnus variabilis)* and other true wireworms of sugarcane (species belonging to genera such as *Conoderus* or *Heteroderes*) are considered of agronomic importance (McCrystal, 2010). Based on their presence in Queensland field crops, false wireworms that may be of agronomic importance in sweetpotato include *Gonocephalum macleaya, Pterohelaeus alternatus* and *P. darlingensis* (Robertson 1993, McCrystal 2010).

Curl grubs or the larval stage of cockchafers or other scarabs (white grubs, cane grubs, peanut scarabs e.g. *Heteronyx* spp. and the African Black beetle *Heteronychus arato*) (Ekman and Lovatt, 2015) can be problematic. Curl grubs feed on lateral and storage roots in the field causing both shallow and

deep holes and tracks, but again, this usually reflects a breakdown in management practices (poor crop rotation or crop hygiene), or timely pesticide application (C. Henderson, pers. communication).

Black field crickets (*Teleogryllus commodus*) and mole crickets (*Gryllotalpa* spp.) (Ekman and Lovatt, 2015) can cause significant damage and affect the marketability of sweetpotato (for example almost every storage root at one particular farm in Queensland had at least one chewing mark from mole crickets, Wilson pers. observation) and but can also be very problematic in chewing holes in drip irrigation tape (Ekman and Lovatt, 2015, C. Henderson, pers. communication).

3. Diseases of sweetpotato

Australia has a sophisticated clean planting material program that supplies the majority of the industry with pathogen tested (PT) storage roots that are virus free. There are many sweetpotato viruses in Australia that infect both sweetpotato and related weeds (other Convolvulaceae) (Henderson and Dennien, 2018). Sweetpotato fields in Bundaberg and Cudgen are regularly surveyed for the presence of viruses by the Queensland Department of Agriculture and Fisheries (QDAF). The two main viruses currently in Australia are sweetpotato feathery mottle virus (SPFMV; *Potyvirus*) and sweetpotato leaf curl virus (SPLCV; *Begomovirus*), which are endemic in all Australian growing regions (Ekman and Lovatt 2015, Wolfenden, Henderson et al. 2018). Surveying for these viruses is not about area freedom, but about assuring that the PT system is effective for commercial cropping (C. Henderson, pers. communication).). Only one severe infection (100 % incidence in one paddock) by SPFMV was recorded by the author, when sampling the site for weevils; in this case the grower was unable to remove an old crop, allowing for virus spread and infestation by sweetpotato weevils (Wilson, pers. observation). Sweetpotato little leaf, a phytoplasma (*Candidatus* Phytoplasma australasiae), occurs sporadically but is not considered a major production issue (Ekman and Lovatt, 2015). Although no systematic surveys of phytoplasma were undertaken, only one suspected incidence of little leaf was recorded in a plant bed in Cudgen in 2019 (Wilson, pers. observation).

Sweetpotato chlorotic fleck (SPCFV; *Carlavirus*) and sweetpotato collusive virus (SPCV; *Cavemovirus*) have also been reported in Australia, although are very rarely found in commercial cropping (Wolfenden, Henderson et al. 2018). They also have no implications for movement of sweetpotatoes in Australia, or export currently (C. Henderson, pers. communication).

The are several fungal and bacterial diseases that can sporadically affect sweetpotato; causing plant wilting, shoot necrosis, storage root lesions and storage root rot. These diseases can occur in plant beds (affecting sprouts used for planting material), in the main field and in post-harvest storage.

In plant beds and the main field, Southern blight, caused by the fungus *Sclerotium rolfsii* can be problematic when the canopy is dense, after considerable rainfall and in high temperature conditions (>28 °C) (Ekman and Lovatt, 2015). In plant beds, S*. rolfsii* causes sprouts to wilt and collapse, eventually rotting the storage root. Infection on storage roots appear as brown, circular and depressed lesions (Ekman and Lovatt, 2015) making these roots unsaleable. In one QLD farm in 2021, about 3.5% of roots sampled had at least one lesion (Wilson, unpublished data). Because of its wide host range and production of sclerotia ensuring long-term survival, Southern blight is potentially a major concern for industry.

Root diseases such as scurf, caused by the fungus *Monilochaetes infuscans* can be problematic on certain farms, under conditions that favour their growth (very moist soil, high organic matter) (Ekman and Lovatt, 2015) or where there is a long history of incidence. The fungus is slow growing, infection begins at the crown and progresses to the developing storage root, where shallow brown lesions can cover the entire periderm (Ekman and Lovatt, 2015; Wilson, pers. observation). Despite being superficial, this fungal infection makes the sweetpotato unsaleable. *Monilochaetes infuscans* infection on white skin purple flesh sweetpotatoes in the field resulted in a moderate yield loss (30%) to one grower in 2018 (anonymous pers. communication). Some growers in Cudgen commented that they see scurf 'every so often'.

Pox, caused by the bacteria *Streptomyces ipomoea* can be problematic on certain farms, under conditions that favour their growth (dry sandy soil, neutral to alkaline pH) (Ekman and Lovatt, 2015) or where there is a long history of incidence. Both scurf and pox are usually only of moderate concern to industry with current practices.

Postharvest disorders such as bacterial soft rots (caused by *Dickeya* spp. formerly *Erwinia* spp., *Pectobacterium* spp.), dry rots (*Phomopsis phaseoli*), Fusarium root rot (*Fusarium* spp.) and storage rot (*Rhizopus* spp.) are sporadically important and generally associated with adverse conditions close to harvest, or injury/handling issues with harvest or post-harvest procedures (Ekman and Lovatt, 2015; C Henderson, pers. comm). These rotting/wilting fungi and bacteria can also be a problem with breakdown of roots in plant beds, although it is unclear whether they are primary, or secondary infective agents (Wolfenden, Henderson et al. 2018).

4. Biosecurity threats to the Australian sweetpotato industry (exotic pests and diseases)

High priority pests (HPP) identified for the sweetpotato industry include viruses Sweet potato chlorotic stunt virus (SPCSV) (*Sweet potato chlorotic stunt virus* (*Crinivirus*), sweetpotato mild mottle virus (*Ipomovirus*) (with sweetpotato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV), sweetpotato mild speckling virus (SPMSV) (*Potyvirus*) (with SPFMV and SPCSV), Guava root-knot nematode (*Meloidogyne enterolobii*), potato tuber nematode (*Ditylenchus destructor*), sting nematode (*Belonolaimus longicaudatus*), Citrus weevil (*Diaprepes abbreviatus*), West Indian sweetpotato weevil (*Euscepes postfasciatus* syn. *Euscepes batatae*), Turnip moth (*Agrotis segetum*) and Lesser corn stalk borer (*Elasmopalpus lignosellus*), Cuban slug (*Veronicella cubensis*), Giant African land snail (*Achatina fulica*), and the Giant African snail (*Achatina achatina*) (PHA 2021).

Of these, a refined list of HPP is being ratified by the Australian sweetpotato industry, Plant Health Australia (PHA) and Australian Government. This refined list of pests and diseases include all three nematodes listed above, the West Indian sweetpotato weevil and sweetpotato chlorotic stunt virus and of these, only the West Indian sweetpotato weevil is present in Papua New Guinea.

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Appendix 3b. Loop-mediated isothermal amplification (LAMP) for sweetpotato pest and disease diagnostics

Background

Building on the diagnostic skills gained in ACIAR funded project HORT/2012/087 on Bogia Coconut Syndrome, several Loop-mediated isothermal amplification (LAMP) diagnostic protocols were developed for several pests and diseases of sweetpotato. The LAMP method rapidly detects specific microorganisms from the soil. With simple and transportable laboratory equipment, DNA can be isolated from a soil sample and tested for the presence of a specific organism on-site. In the LAMP machine, the extracted DNA is heated and markers attach to the target organism DNA. If that target organism DNA is present, it fluoresces and the machine detects the glow and records it. LAMP is semi-quantitative and has the potential to define threshold limits e.g. nematode numbers in soil. The more DNA in a sample, the faster it is amplified. Much of this work is in the preliminary phase and only been tested on a few targets from laboratory derived specimens. Whilst many sets of LAMP primers were developed, more extensive testing is required to ensure that they do not amplify off target (micro)organisms. LAMP primers developed have not been presented here to prevent issues with publication.

Materials and methods

Identification of macro and microorganisms for LAMP

Several LAMP primer sets were designed to identify various target macro and microorganisms (both beneficial and deleterious) in the soil. The purpose of targeting these organisms is to 1) determine the compatibility of an introduced entomopathogenic inoculum with other soil microorganisms and to track how they change in response to different soil management practices including amendments to the soil (e.g. barrier plants, compost, nutrients etc). The LAMP primers can also play a role in determining the loading of populations of both beneficial and deleterious microorganisms in PT plant beds at Aiyura (NARI) and at the screen houses growing PT material around the highlands (identification of phytoplasma, viruses and insect vectors)

Table 1. LAMP primers designed for various organisms associated with sweetpotato

Species name	**Common name**
*Meloidogyn*e sp.	Root knot nematodes (various)
Pratylenchus sp.	Root lesion nematodes (various)
Cylas formicarius	Sweetpotato weevil
Euscepes postfasciatus	West Indian Sweetpotato weevil
	True wireworm
	False wireworm
Pasteuria penetrans	Nematode infecting bacteria
Metarhizium anisopliae	-
Beauveria bassiana	-
Trichoderma sp.	-
	Phytoplasma (general for sweetpotato)
	Sweet potato little leaf phytoplasma
	Sweet potato feathery mottle virus
	Sweet potato chlorotic fleck virus

LAMP primer development

Depending on the micro(organism), primers were either developed from online sequences (GenBank) or by using DNA extractions from locally acquired samples (e.g. *Cylas formicarius*, mealworms as a proxy for false wireworms, entomopathogenic fungi or *Trichoderma* spp., root lesion and root knot nematodes (RKN) and nematode infecting bacteria *Pasteuria penetrans*). Root knot nematodes and *Pasteuria penetrans* were acquired from Graham Stirling (Biological Crop Protection) or Dr Jady Li (RKN) at Central Queensland University. For the West Indian Sweetpotato weevil (*Euscepes postfasciatus*), DNA was extracted from adults and larvae in Bubia, Papua New Guinea by National Agricultural Research Institute (NARI) colleagues Ms Gou Rauka and project partner Mr Wilfred Wau and sent to Australia for LAMP development. Developing a LAMP bioassay to distinguish sweetpotato weevils in the larval stage (inside a storage root or stem) would be useful to better identify species present.

Depending on the sample, various gene regions were targeted. Primers were designed with LAMP Designer 1.15, PREMIER Biosoft, California, USA. Not all genes are suitable for LAMP primer design (length and sequence- for example COI genes in various insects failed to return sequences). For example, the 18S or 28S ribosomal RNA genes were targeted to separate nematode species e.g. to exclude root lesion nematode amplification by root knot nematode primers and vice versa, or alpha elongation factor for fungi to ensure primers designed for *Trichoderma* did not amplify *Metarhizium* or *Beauveria* spp.

Presentations of methods

The results of some of this LAMP work were presented as a practical demonstration of LAMP molecular diagnostics at industry updates, grower days and to the broader sweetpotato research team throughout 2016-2017. She also presented recent LAMP and metagenomics data at a PT production course (PNG researchers) in Bundaberg.

Preliminary results and discussion

The LAMP output for an example root knot nematode bioassay is presented in Figure 1. The figure below is a dilution series of nematode DNA. The red line represents the DNA from 100 nematodes, the orange line DNA from 10 nematodes, the yellow line is DNA from 1 nematode and the dark green line is DNA from 1/10 of a nematode. Because there is more DNA in the sample represented by the red line, the time taken for its detection is fast (8 min). The amount of DNA in the sample represented by the dark green line is very low, so it takes 22 min to detect.

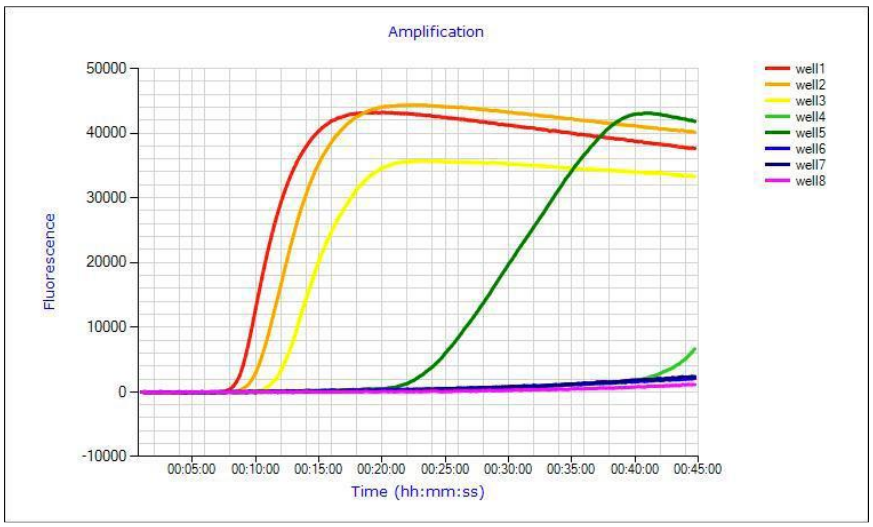

Figure 1. The root knot nematode LAMP assay can detect 1/10 of a single nematode (RKN) (dark green line).

LAMP diagnostics are useful and rapid method for the detection of various microorganisms of sweetpotato. More work is required to ensure detection of off-target specimens does not occur and to ensure that the test is suitable for field-based samples.

Appendix 3c. USQ sweetpotato scurf investigation in Cudgen, NSW

Background

Scurf of sweetpotato (*Ipomoea batatas*), caused by the fungus *Monilochaetes infuscans* has a worldwide distribution. *Monilochaetes infuscans* has a narrow host-range, limited to *I. batatas* and morning glory (*Ipomoea* spp.) (Jackson, 2015). The colour of the lesions caused by *Monilochaetes infuscans* can vary depending on the cultivar and can be grey, brown, purple-brown and black (Jackson, 2015) (Figures 1- Figure 3). Scurf is more problematic in soils with high organic matter and high soil moisture during excessively rainy seasons (Jackson, 2015). Spores from the fungi can survive for 3 years in the soil or on infected storage roots/stems (Jackson, 2015). Management options include long crop rotations (which is not always possible), not adding organic matter to scurfy paddocks and removal of infected planting material (storage roots and shoots). In Australia, the fungicide Thiabendazole (Tecto) is available to sweetpotato growers by permit to manage scurf and field rots (PER12047 Version 4).

Scurf is an occasional issue for Australian growers and is growers noted it more in wet years. In 2018, a two neighbouring sweetpotato growers in Cudgen, NSW recorded a significant scurf outbreak in paddocks that had been irrigated with water from one particular dam. Adjacent paddocks that were irrigated with a different water supply were unaffected. One grower had scurf in Orleans (gold) and the other in the white skin purple flesh (WSPF) cultivar. Although the fungal infection is restricted to the periderm (skin) (i.e. it does not enter the starchy flesh and removed with peeling for example), the damage makes the roots unsaleable or roots are downgraded. One grower estimated 30% loss to scurf in 2018 due to this cosmetic damage.

One of the growers had the dam water tested water although the testing did not reveal the presence of a particular pathogen that could cause disease. A banana plantation was located on the upper side of the dam. Although symptoms of plant disease were not recorded, it is possible that plant or soil associated pathogens may have been washed into the dam used as a water source for the scurfy sweetpotato.

There has been limited research performed on scurf in Australian sweetpotato and the aim of this work was to determine if the fungus *Monilochaetes infuscans* was responsible for all the lesions found on scurfy sweetpotato roots in Cudgen.

Figure 1. Examples of scurfy roots from cv. WSPF possibly showing *Monilochaetes infuscans* at different infection stages (lighter and darker regions) or the presence of multiple fungi occupying the sweetpotato periderm

Figure 2. Examples of scurfy roots from cv. Orleans and WSPF possibly showing *Monilochaetes infuscans* at different infection stages (lighter and darker regions) or the presence of multiple fungi occupying the sweetpotato periderm.

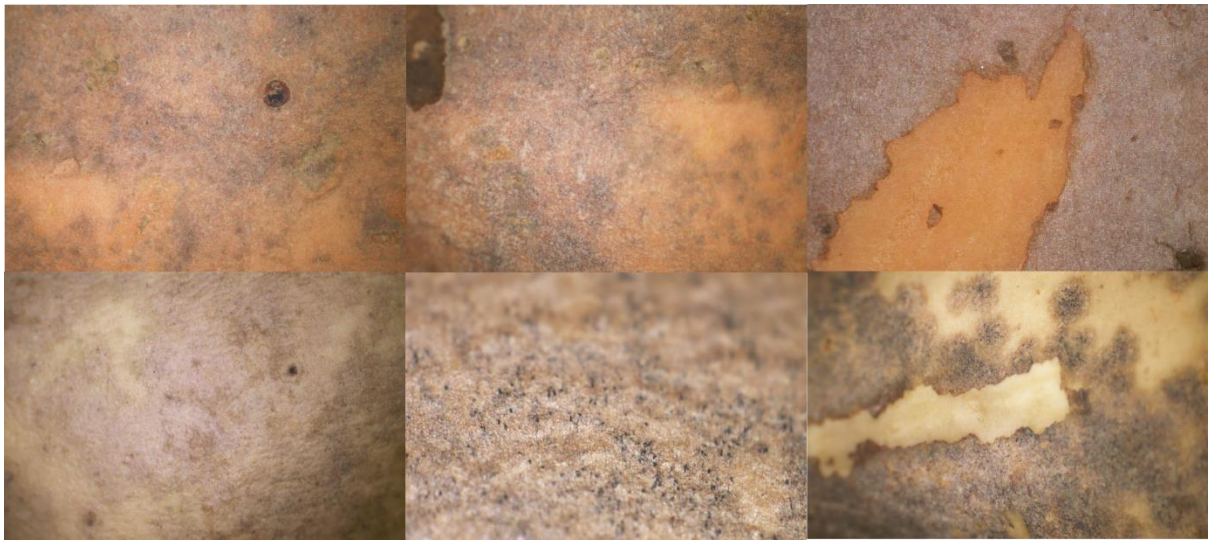

Figure 3. Examples of scurfy roots from cv. Orleans (top) and WSPF (bottom) possibly showing *Monilochaetes infuscans* at different infection stages (lighter and darker regions) or the presence of multiple fungi occupying the sweetpotato periderm.

Materials and Methods

Scurfy storage roots (8 of each cv. Orleans and WSPF) were collected from two separate farms in Cudgen, NSW in 2018 and taken back to the laboratory for processing. Half of the scurfy storage roots were placed in a humid chamber to encourage sporulation of *Monilochaetes infuscans* on the surface. Initially, 1-1.5 cm^2 sections of sweetpotato skin with healthy and diseased tissue were surface sterilised by placing sections in 70% ethanol for 1 min, 4% sodium hypochlorite (1% available NaOCl) for 3 mins, before being rinsed twice in sterile distilled water. Sections were blotted on sterile paper towel before being transferred to potato dextrose agar (PDA) (Bacto Laboratories P/L, NSW) or laboratory made sweetpotato agar (cv. Orleans roots cooked and blended in tap water with 15 g agar per L) in 90 mm petri plates. For each cultivar, 30 plates were used with 4 sections per plate. Plates were incubated at 27 °C with a 12:12 photoperiod. Plates were checked daily for growth and fungal colonies were sub-cultured onto fresh plates as they emerged from the sweetpotato sections. Incubation ceased when the plates were overgrown. Fungal cultures were sub-cultured until a clean contaminant free culture was obtained.

An alternative method to isolate scurf from sweetpotato roots was used after conversations with sweetpotato plant pathologist Chris Clark (Louisiana State University) in 2019. Fresh scurfy roots were obtained from the farms described previously and a nearby farm in Cudgen. Storage roots were washed gently with a Wypall® paper towel under running water before further processing in a biohazard cabinet. A sterile Wypall® paper towel was soaked in 10% bleach (5-6% NaOCl) and used to gently rub a section of the sweetpotato with scurf continuously for 1 min. A small piece (2 mm^2) from the edge of the lesion was excised with a scalpel, removing only the periderm, before placing it on PDA. Three excised pieces of periderm were transferred per plate. About 150 sections were excised from storage roots (75 from five cv. Orleans storage roots and 75 from five cv. WSPF storage roots). Plates were incubated as described above. Fast growing fungi was discarded and slow growing fungi was immediately sub-cultured onto fresh PDA plates to obtain pure cultures. Cubes of mycelium/spore covered agar was transferred to sterile 2 ml tubes containing 500 µl of sterile water and stored at -80 °C for future DNA extraction.


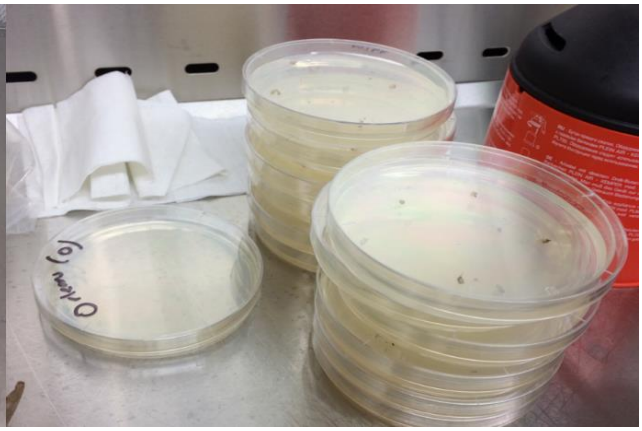

Figure 4. Initially large sections of sweetpotato skin (and flesh) were used for scurf isolations (left) before a refined protocol from Chris Clark (LSU) was adopted (right).

The DNeasy® PowerSoil® kit (Qiagen, Australia) was used to extract genomic DNA from all samples following the manufacturer's protocol with the exception that 3 x 0.5 cm^2 squares of pure fungal culture on agar was used rather than soil. ITS1 and ITS4 primers were used amplify the highly variable ITS1 and ITS2 sequences surrounding the 5.8S-coding sequence and situated between the small sub unit-coding sequence (SSU) and the large sub unit-coding sequence (LSU) of the ribosomal operon. Each PCR reaction was 25 µl and contained 12.5 µl GoTaq®2x GreenMaster Mix (Promega, Alexandria, NSW, Australia), 1 µl of each forward and reverse primers (10 mmol), 9.5 µl of nuclease-free water and 1 µl of fungal DNA (at 25–30 ng/µl). The PCR conditions were an initial denaturation at 94 °C for 1 min, then 30 cycles of 30 s at 94 °C, 30 s at 51 °C and 60 s at 72 °C, with a final extension of 8 min at 72 °C. PCR products were sent to Macrogen Inc. (Seoul, South Korea) for PCR purification and DNA sequencing.

Results and discussion

Only black, grey or slow growing fungi were sequenced. Early sequence results from the initial set of isolations frequently revealed the presence of the fast-growing fungus *Ceratocystis paradoxa* in many of the samples. *Ceratocystis* spp. is known to cause the post-harvest disease 'black rot' in sweetpotato and disease in many other crops, including banana, which was located near the dam. Whilst black rot can be common in sweetpotato, none of the excised tissue had lesions associated with black rot (brown to black sunken round lesions), but it is possible the spores of the fungus attached to the sweetpotato (e.g. from the soil) survived the surface sterilisation process. Scurf seemed to be extremely difficult to isolate from sweetpotato roots. From the 390 excised sections of sweetpotato

(both large and small) only one promising looking culture was identified as *Monilochaetes infuscans* (ex. Orleans) and one other promising looking culture was identified as *Idriella lunata* (Figure 5) (recorded to cause strawberry root rot). Future work is required to test Koch's postulates to see if the fungus *Idriella lunata* causes lesions on sweetpotato similar to that seen above or if it was an endophyte of sweetpotato. If other fungi are responsible for contributing to the grey/black/brown lesions on the storage root then methods of control can be identified, for example examining the efficacy of the fungicide Thiabendazole.

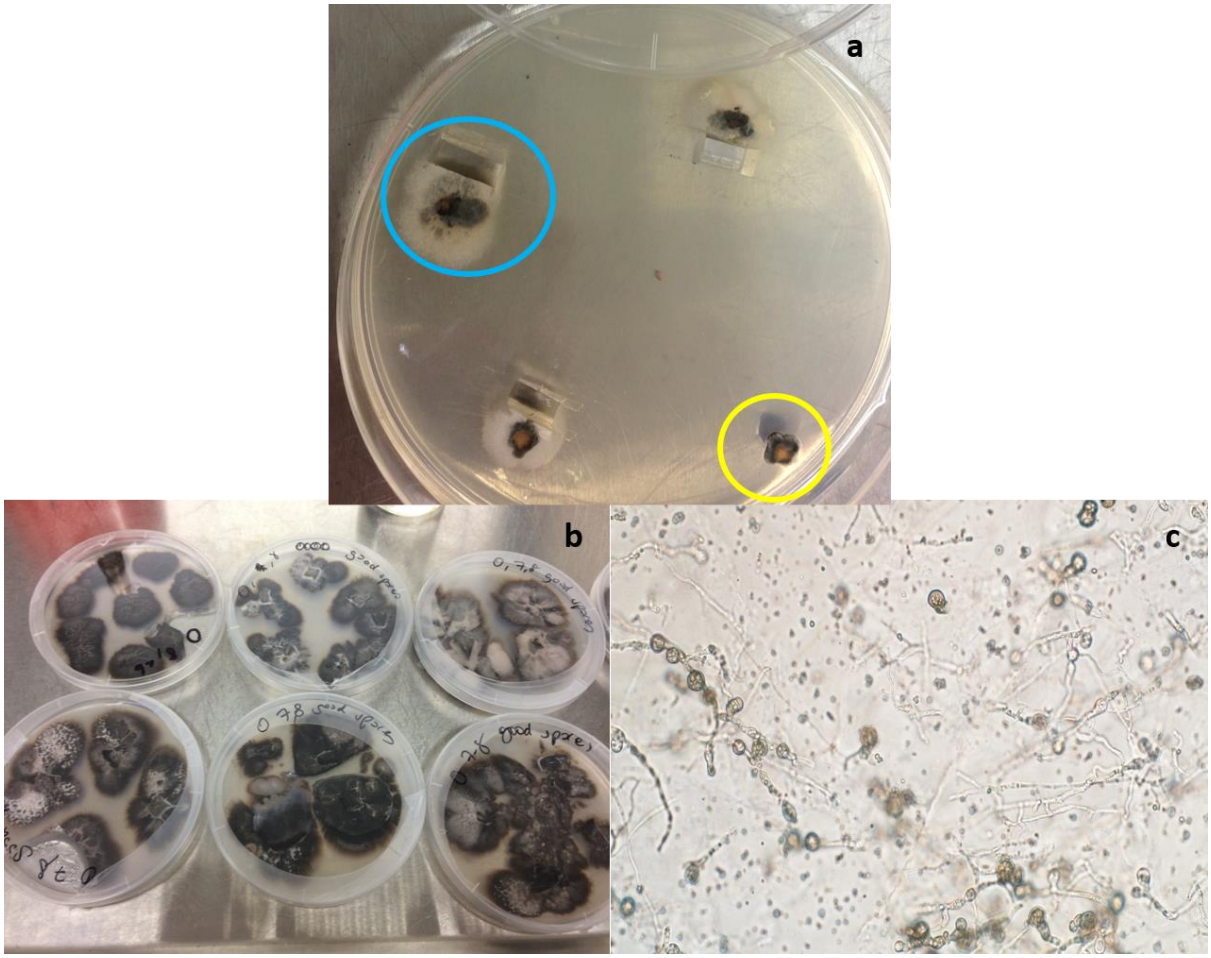

Figure 5. *Idriella lunata* colony (a) (blue circle), *Monilochaetes infuscans* colony (a) (yellow circle), established colonies of *Idriella lunata* (b) spores and mycelium of *Idriella lunata* (c)

Reference

Jackson G, 2015. Sweetpotato scurf (260). In. *Pacific Pests, Pathogens & Weeds.* PestNet. (2021.)

Appendix 3d. Storage root breakdown in Bellevue and other sweetpotato varieties

Update as of May 2022

- *Investigations into storage root breakdown and disease-causing microorganisms in Bellevue, Orleans, Bonita, WSPF, Eclipse, Murasaki and Northern Star from sweetpotato farms in Bundaberg, QLD has recommenced under the direction of Dr Bree Wilson from USQ. The research forms a part of Mr Wilfred Wau's MSc at USQ (ACIAR John Allright Fellow), which began in February 2022 in Toowoomba.*

- *Three farms were extensively sampled- both diseased and healthy sweetpotato material was obtained and has been processed in the laboratory to obtain a collection of pathogenic/endophytic bacteria/fungi that may be contribution to breakdown and disease.*

- *Representative microorganisms will be subject to PCR and sequencing, before being examined for pathogenicity in glasshouse studies to test Koch's postulates.*

- *Results obtained will provide the Australian sweetpotato industry with more knowledge about sweetpotato diseases, which may inform new disease management practices.*

- *The information obtained will also serve as a reference for Australian sweetpotato biosecurity*

- *Skills and knowledge obtained by Wilfred Wau will be invaluable for disease management in sweetpotato production in Papua New Guinea*

Part 1.

Introduction

This preliminary plant pathogen collecting study used material from two experimental sites set-up in Bundaberg, QLD as a part of the Hort Innovation project VG133004 'Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry' where plant bed management (watering regimes) and their impact on storage root breakdown was being investigated. The intention of the study by USQ was to collect material to isolate microorganisms that may be involved in breakdown.

Methodology

Material collection and isolation of fungi and bacteria

Roots showing disintegration/rot and wilting shoots were collected from each site and transported back to the laboratory in an esky with ice. Potato dextrose agar (PDA) and nutrient agar (NA) were

used to support the growth of fungi and bacteria respectively. The collected samples of storage roots differed in their degree of breakdown and consequently were treated differently prior to plating out on agar. For example, some roots were 'mushy' and were subsequently surface sterilised in 1% sodium hypochorite for a shorter time (1 min) than intact storage roots pieces with some healthy tissue (3 min) to prevent complete disintegration in the preparation steps. Shoot sections (0.5 cm) were surface sterilised for 3 min. All samples were rinsed in sterile distilled water, blotted dry, then plated onto duplicate plates of PDA and NA. Plates were sealed with Parafilm^{M} and incubated at 25 °C with a 12:12 photoperiod. Resulting fungi or bacteria growing from the storage roots or shoots were sub-cultured onto fresh PDA or NA plates until a pure culture was obtained. For fungi, agar covered with spores and mycelium was sectioned into 0.5 mm^2 pieces and stored in duplicate 1.5 ml tubes with sterile water. For bacteria, a single colony was picked from the plate and suspended in sterile water in sterile 1.5 ml tubes. All cultures were stored at -20 °C for future DNA extraction, PCR and sequencing.

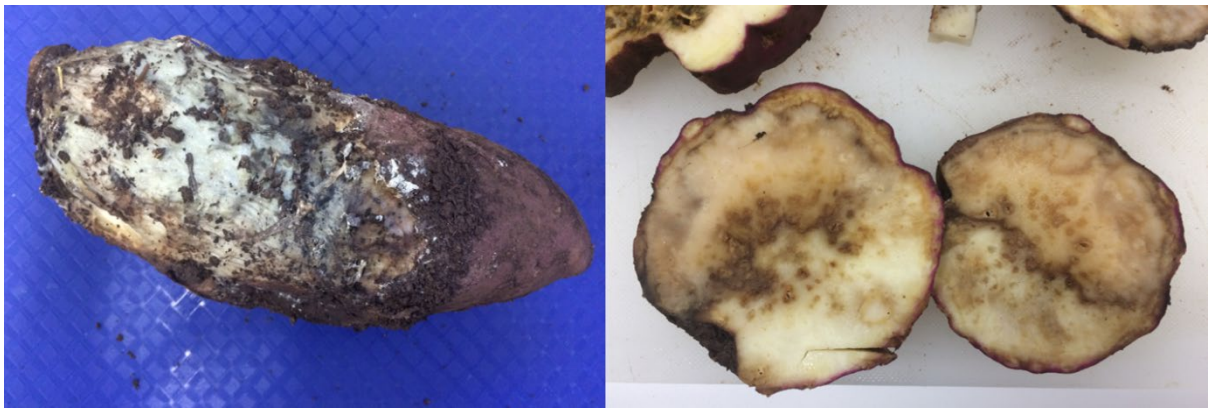

Figure 1. Example of a sweetpotato storage root with breakdown, with evidence of fungi and bacteria

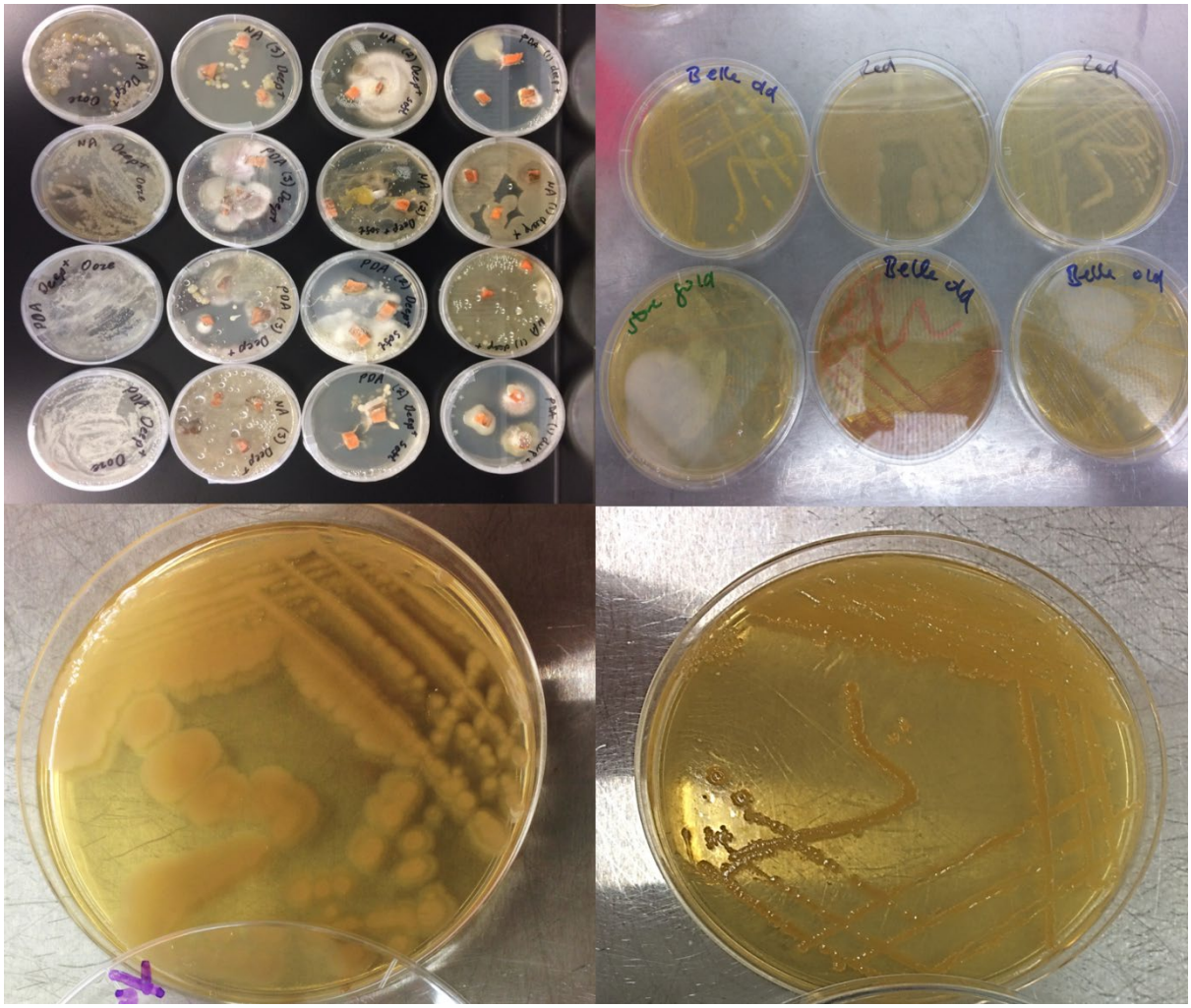

Figure 2. Bacterial and fungal colonies growing from sweetpotato storage roots and pure cultures of bacteria from various samples

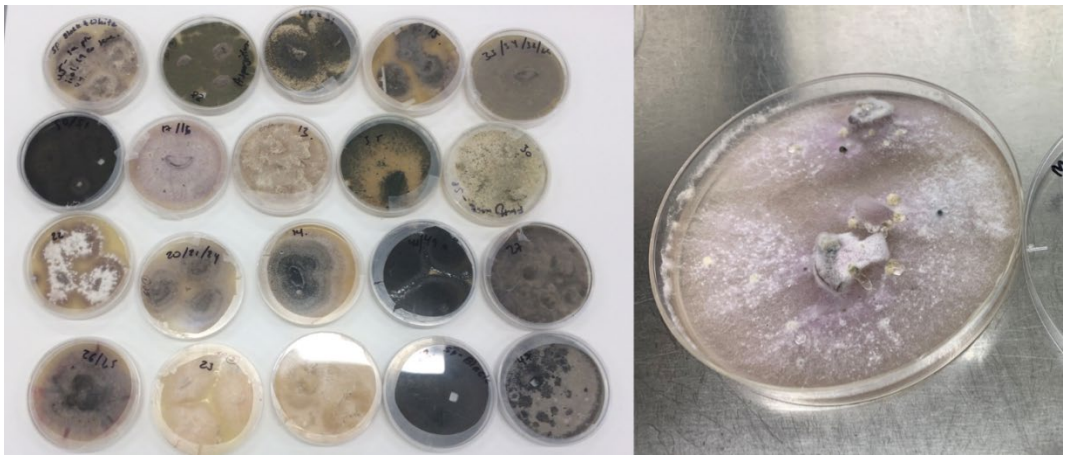

Figure 3. Different fungi isolated from sweetpotato storage roots and shoots

DNA extraction from bacterial and fungal cultures

For bacteria, 50 ul aliquot of stored suspension was placed into a sterile 1.5 ml tube and heated for 1 min at 95 °C to release DNA, before being stored on ice for PCR. For PCR, each reaction contained 12.5 µl 2 x GoTaq® Green Master Mix (Promega, Australia), 2 µl of each primer (27F AGAGTTTGATCCTGGCTCAG, 1492R GGTTACCTTGTTACGACTT), 3 µl of bacterial suspension and 5.5 µl of nuclease free water. The following conditions were used to perform the PCR on a Kyratec thermal cycler 95 °C for 3 min, 30 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C 60 s, followed by a 10 min final extension at 72 °C. For fungi, the DNeasy® PowerSoil® kit was used following the manufacturer's recommendation with the exception that sporulating cultures from agar were used (3 x squares 0.5 cm^2) per sample. For PCR, each reaction contained 12.5 µl 2 x GoTaq® Green Master Mix (Promega, Australia), 1 µl of each primer (EFT1 and EFT2), 2 µl of DNA and 8.5 µl of nuclease free water. The following conditions were used to perform the PCR on a Kyratec thermal cycler 94 °C for 3 min, 34 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C 60 s, followed by a 10 min final extension at 72 °C. PCR products for bacteria were sent to Macrogen Inc. (Seoul, South Korea) for PCR purification and DNA sequencing.

Results and discussion

Whilst most of the bacteria were sequenced, none of the fungal samples were sequenced at the time of this report, although several fungi were morphologically identified as *Fusarium* spp. and *Aspergillus* spp.

The bacteria isolated from disease storage roots showed high sequence similarity to *Arthrobacter creatinolyticus, A. defluvii, Bacillus* sp., *several strains of B. cereus, B. licheniformis, B. stratosphericus, Brevundimonas naejangsanensis, Curtobacterium oceanosedimentum*, *Enterobacter sp., Lysinibacillus sphaericus, Pantoea* sp., *Pantoea agglomerans, Pectobacterium carotovorum* subsp*. carotovorum, Planococcus* sp., several strains of *Pseudomonas* sp., *P. putida, Rahnella aquatilis*, *Solibacillus* sp*. strain silvestris, Sporosarcina* sp., *S. contaminans, S. ginsengisoli, S. luteola.*

Because Koch's postulates were not tested, we cannot determine if these bacterial species caused disease in sweetpotato, if some of these species are beneficial endophytes or insect pathogens like *Lysinibacillus sphaericus* (Berry, 2012). Some of the bacteria here are known to cause disease in humans (e.g. *Bacillus cereus*). *Pectobacterium* spp. (*Pectobacterium carotovorum* subsp*. carotovorum* isolated here) is a known plant pathogen and associated with sweetpotato soft rots; however, until now it was unclear which species caused disease in Australian sweetpotato.

In their work, Khan and Doty (2009) demonstrated several genera of bacterial endophytes capable of plant growth promotion (and able to produce phytohormones such as IAA), some of which were found in this study: *Rahnella, Pseudomonas, Enterobacteria.* However, some *Pseudomonas* spp. are known to be plant pathogenic and further resolution of the species present in this study is required. Another study demonstrated that *Pantoea dispersa* (in this study a *Pantoea* sp and ***P. agglomerans*** was isolated) was an effective biocontrol agent of *Ceratocystis fimbriata* causing black rot of sweetpotato (Jiang et al., 2019).

Understanding the bacterial and fungal species that contribute to the breakdown of sweetpotato bedding roots is an important area that deserves further research and is relevant to commercial sweetpotato production in both Australia and Papua New Guinea, where sweetpotato plant bed nurseries are becoming more common.

References

Berry C, 2012. The bacterium, *Lysinibacillus sphaericus*, as an insect pathogen. *Journal of Invertebrate Pathology* **109**, 1-10.

Jiang L, Jeong JC, Lee J-S*, et al.*, 2019. Potential of *Pantoea dispersa* as an effective biocontrol agent for black rot in sweet potato. *Scientific Reports* **9**, 16354.

Khan Z, Doty SL, 2009. Characterization of bacterial endophytes of sweet potato plants. *Plant and Soil* **322**, 197-207.

Wolfenden R, Henderson C, Dennien S, 2018. Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry. In. *Hort Innovation Project Final Reports.* Sydney, Australia, 159. (Innovation H, ed.)

The information below has been derived and modified from "Appendix 7: University of Southern Queensland Bellevue breakdown pilot study, January/February 2018" written by Mr Craig Henderson (Henderson RDE) with input from Dr Bree Wilson (USQ) for Hort Innovation project VG133004 'Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry' (Wolfenden et al., 2018). ***Supplied with approval from Craig Henderson.***

One addition to the information detailed below is that where possible, pure cultures of fungi and bacteria isolated from the storage roots in this study were stored securely at -80 °C at USQ's Centre for Crop Health for future molecular identification (sequencing).

Appendix 7: University of Southern Queensland Bellevue breakdown pilot study, January/February 2018

Introduction

During the latter years of project VG13004, it was apparent that the commonly grown Gold cultivar *Bellevue* was the most susceptible to premature breakdown in plant beds. The project team and a few sweetpotato growers recognised that there appeared to be both physiological and disease aspects to this breakdown. However, the actual initial causes of premature breakdown were hard to elucidate, and particularly in highly variable field situations.

Craig Henderson and Dr Bree Wilson (Research Fellow, University of Southern Queensland), initiated a pilot study to:

- Explore the potential for using regulated growth cabinets to monitor breakdown of bedding roots under controlled conditions
- Discover what physiological changes occurred in bedding roots after installation, and what organisms could potentially be involved in root breakdown

Although this work was very preliminary, and the diagnostics of disease organisms is still underway, we felt it was useful to report what occurred in this project final report.

Methodology

We purchased 5 kg of sweetpotatoes from the supermarket, relatively confident they were *Bellevue* cultivar, from their shape and colour. We sorted and selected twelve medium roots of a similar size to use in the study. We measured their length, diameter and weight (Table 9).

Table 9 Uniformity of sweetpotato bedding roots for growth cabinet study

Size attribute	**Median**	**Minimum**	**Maximum**	**Standard deviation**	**Coefficient of variation (%)**
Length (mm)	193	160	220	18	9
Diameter (mm)	75	55	80	7	10

Weight (g)	461	370	559	60	13

On 16 January 2018, we prepared three plastic tubs to place in the USQ growth chambers. The tubs were drilled to provide drainage holes for excess water and lined with geotextile, to prevent sand falling through the drainage holes. We used unsterilised, coarse, builder's sand as the growing medium.

Plate 26. Selecting experimental bedding roots and Plate 27. Placing bedding roots in Shallow tub.

We mixed 25 kg of dry sand with 25 g of RICHGRO All Purpose Complete Garden Fertiliser (8-1-6-9). We added a 2 cm layer to the bottom of the tub, and then placed 4 randomly selected sweetpotato bedding roots on the sand. We added the remainder of the soil, until the roots were covered with 2 cm of sand above their upper surfaces.

Tub 2

We mixed 38 kg of dry sand with 25 g of RICHGRO All Purpose Complete Garden Fertiliser (8-1-6-9). We added a 6 cm layer to the bottom of the tub, and then placed 4 randomly selected sweetpotato bedding roots on the sand. We added the remainder of the soil, until the roots were covered with 2 cm of sand above their upper surfaces.

Tub 3

This was the same as Tub 2, however no fertiliser was added to the sand mix.

We buried Chameleon soil moisture and temperature sensors 5 cm below the sand surface in each of the three tubs. The sensors logged soil moisture and temperature every two hours for the duration of the experiment.

We added sufficient water to each of the tubs to moisten them to field capacity. We installed the tubs in the growth chamber, with the lighter one (Tub 1) on the upper shelf.

We set the growth chambers for 13 hr light at 30°C, and 11 hr dark at 24°C, with the relative humidity constant at 60%.

Dr Wilson checked on the tubs regularly and applied sufficient water to maintain the sand at field capacity, for the duration of the study. She took regular photographs of the sprouts as they emerged.

On the 26 February 2018, we processed Tub 1, by removing the shoots, and then washing out the sand from around the bedding roots. Some of the roots had already broken down, however, we tried to keep these as intact as possible. We photographed the root systems, and the conditions of the remaining bedding roots.

Plate 28. Tubs installed in USQ growth cabinet.

Dr Wilson took samples from healthy and diseased plant tissues, including sprouts, root pieces and bedding root mass (Plates 29-32). She then prepared these materials for plating and diagnostics, using standard surface sterilising and plating media.

We repeated this process for Tub 2 and Tub 3, on 28 February 2018.

After one week, Dr Wilson did a preliminary assessment on the organisms isolated from the various plant tissues. Further diagnostics on these organisms is ongoing.

Plate 29. Internal sweetpotato bedding root.

Plate 30. Diseased sweetpotato bedding root.

Plate 31. Infected bedding root skin.

Plate 32. Sweetpotato bedding root ooze.

Results

There was no obvious difference between the two deep tubs (with or without fertiliser), so for simplicity, the study will simply refer to Shallow and Deep tubs.

According to the moisture sensors, all tubs were maintained in a wet state (5-15 kPa soil suction) for the 43 days of the study.

Growth cabinet temperatures

For the first ten days after installation, we had major issues maintaining the correct temperatures in the growth cabinet. This was primarily due to a previously undetected coolant leak. During the first lighting period, the sand temperature in both Shallow and Deep tubs climbed to around 40°C, and then fluctuated irregularly between 20-30°C for the next seven days. To fix the growth cabinets, they were turned off for a 24 hr period, in which time the minimum temperature fell to 15°C. For the remainder of the study, the temperature actually performed as expected, ranging between 23-30°C (Fig. 32). Note that the temperatures in the Shallow tubs were regularly 2-3°C higher than the Deep tubs.

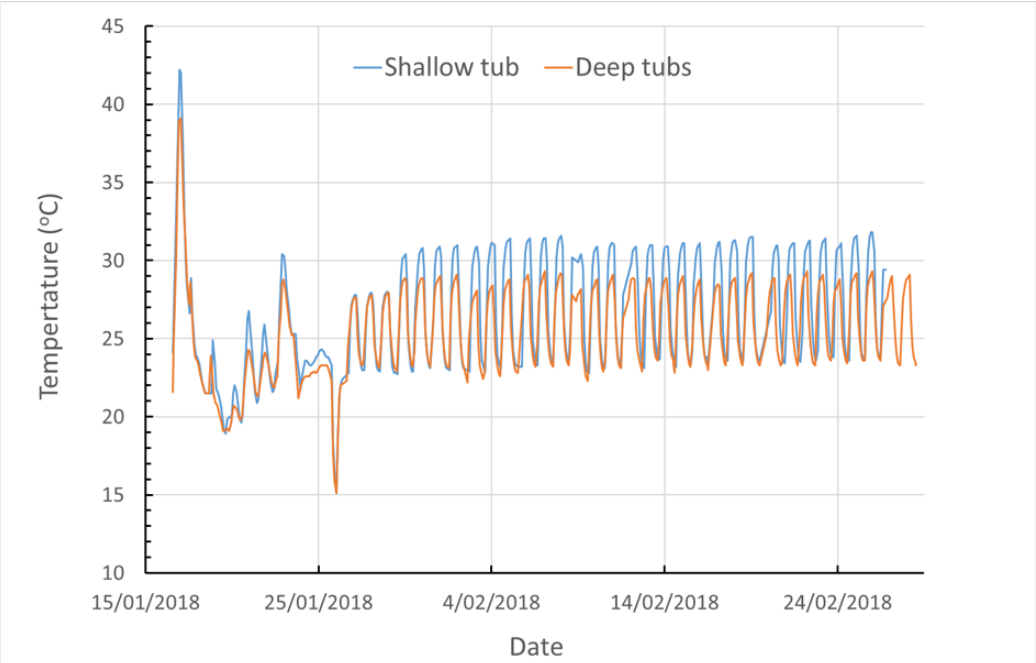

Fig. 32. Soil temperature fluctuations in sweetpotato bedding root tubs.

Sprout emergence and establishment

The first sprouts emerged in both the Shallow and Deep tubs around 23 days after installation (DAI).

Shallow tub

As can be seen in Plates 33-36, only one of the four bedding roots produced sprouts, At 34 DAI, the sprouts from this root were looking reasonably healthy, however by 41 DAI, these sprouts were wilted and obviously dying.

Plate 33. Sprout development in Shallow tub at 23 days after installation.

Plate 34. Sprout development in Shallow tub at 26 days after installation.

Plate 35. Sprout development in Shallow tub at 34 days after installation.

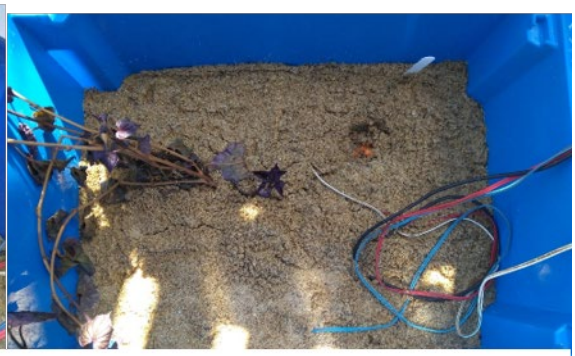

Plate 36. Sprout development in Shallow tub at 41 days after installation.

Deep tubs

In contrast, the Deep tubs produced sprouts from more installed roots, and retained the health of those sprouts through to the final assessment date, 43 DAI.

Plate 37. Sprout development in Deep tub at 23 days after installation.

Plate 38. Sprout development in Deep tub at 26 days after installation.

Plate 39. Sprout development in Deep tub at 34 days after installation.

Plate 40. Sprout development in Deep tub at 43 days after installation.

Plate 41. Healthy sprouts harvested from Deep tub at 43 days after installation.

Bedding root condition 6 weeks after installation

Bedding roots with healthy sprouts

When we looked at the bedding roots supporting healthy sprouts, they had the following characteristics:

- The bedding roots themselves were still firm, and relatively dense (Plates 42, 43).
- They had developed and maintained an established root system directly from the distal end of the bedding root (Plate 44, 45).
- Each of the emerged sprouts had its own, well-developed root system (Plates 45, 46).

Plate 42. Dense, healthy bedding root at six weeks after installation.

Plate 43. Dense, healthy bedding root at six weeks after installation.

Plate 45. Healthy sweetpotato bedding root, supporting good sprout production.

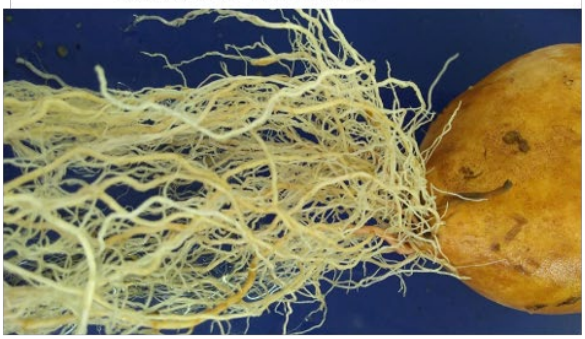

Plate 44. Excellent root production from healthy, sweetpotato bedding root.

Plate 46. Excellent root production from healthy, sweetpotato sprout.

Poor or absent sprouts

When we observed bedding roots from areas without healthy sprout production, we observed various levels of breakdown.

In some instances, we saw evacuation and vacuole development in the distal end of the bedding root (Plates 47, 48). This potentially indicated use of the starch and sugars in respiration, and/or development of roots and sprouts. We also noted some necrosis around those evacuated areas (Plates 49, 50). These changes were often, but not always, associated with browning and necrosis of the proximal end of the root.

We also saw browning and necrosis of the internal sweetpotato tissue without evacuation, often associated with a diseased proximal end. This was probably a fungal pathogen; the exact organism is yet to be determined (Plate 51).

Plate 47. Moderate physiological evacuation of bedding root at six weeks after installation.

Plate 48. Severe physiological evacuation of bedding root at six weeks after installation.

Plate 49. Initial internal browning of bedding root at six weeks after installation.

Plate 50. Internal browning of bedding root at six weeks after installation.

In several instances we also encountered complete internal breakdown of the bedding root (Plates 52, 53), associated with bacterial infection. Certainly *Erwinia* spp. were involved, however the diagnostics suggests other bacteria as well. Again, the exact organisms are still being classified.

Diagnostics

The plating out of the plant materials has demonstrated a range of fungi and bacteria species associated with the breakdown. Because of the range of organisms involved, separating them out, classifying, and then determining their actual pathogenic potential, is beyond the scope of this current project.

Plate 52. **Bacterial infection contained within shell of sweetpotato bedding root at six weeks after installation.**

Plate 53. **Bacterial infection pouring from shell of sweetpotato bedding root at six weeks after installation.**

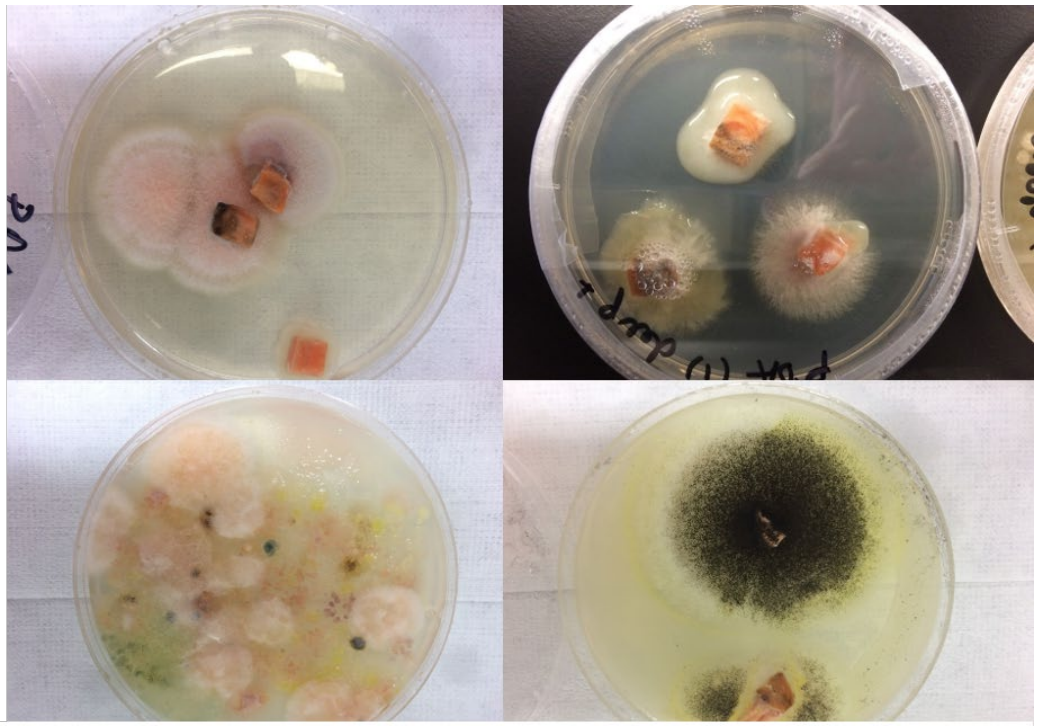

Plate 54. **Fungal species extracted from diseased sweetpotato bedding root materials.**

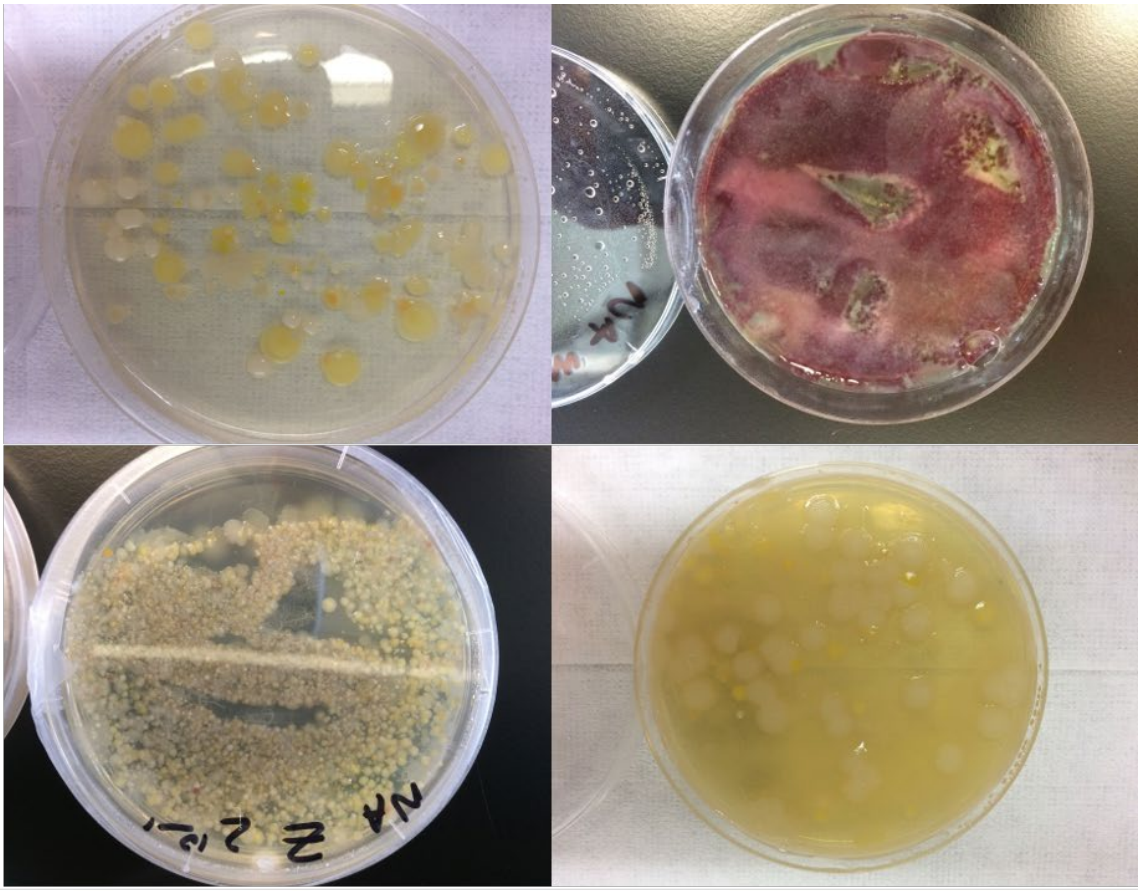

Plate 55. Bacterial species extracted from diseased sweetpotato bedding root materials.

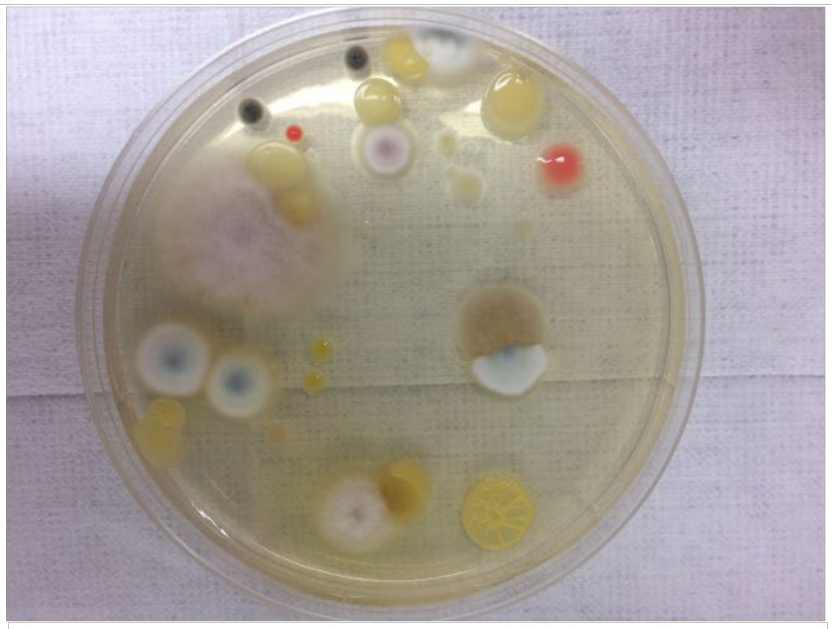

Plate 56. Disease complexes extracted from diseased sweetpotato bedding root materials.

Discussion

Efficacy of growth cabinet

The early issues with the growth cabinet operation outlined the importance of ensuring they

are functioning properly, before commencing experimentation. In hindsight, and in discussions with Prof. Villordon from LSU, even our consistent operational temperatures were probably too high, although they do reflect reality for Australian plant bed conditions.

The weight capacity of the growth cabinet shelves limited us to one tub off the floor of the cabinet. For future work, potentially a supportive frame would need to be installed to increase the load capacity of the upper shelf. Particularly as it appears that there is a benefit for having more media below the bedding roots; i.e. the Deep tub arrangement.

For physiological studies, it may be beneficial to go with a lighter, potting mix type media, rather than a pure sand culture. This may allow full use of the upper shelf. Also, for physiological studies, it would be preferred to sterilise both the bedding root surfaces, as well as the growing media, before installation.

The geotextile did prevent sand egress; however, it also formed a penetrable matting for the sweetpotato roots, making extraction of the roots difficult. Rather than a contiguous mat, perhaps small discs of geotextile or a fine sieve material over the drainage holes would be better.

Effective development of first sprouts

Our observations of the extracted bedding roots showed substantial root development from the roots themselves. So, although sweetpotatoes stored ex-soil do develop sprouts (without any obvious root development), bedding roots buried in moist soil do develop substantial root networks. We are not aware of the role of this root development in sprout initiation and development, which would be an interesting future study. In the interim, we are recommending that growers provide a small amount of soil moisture at installation, to encourage this early root development. However, we know that excessive soil moisture and waterlogging can be associated with catastrophic plant bed breakdown, so we continue to recommend good drainage and careful water management early on.

The healthy sprouts also developed very extensive root systems from their buried stems (between the bedding root and the soil surface). Our field studies show that these root systems can support the sprouts, even if the bedding root has disappeared. However, it is important to note that this probably depends on the timing and cause of the bedding root breakdown. If the bedding root breakdown is associated with pathogenic organisms that can also attack the stems and roots, then it is unlikely the independent sprouts will be maintained.

Physiological breakdown of bedding roots

In several bedding roots that appeared otherwise unaffected by diseases, we did notice evacuated areas, and even some necrosis, within their tissues. This observation appears to support our contention that there are physiological processes going on within the buried roots that are breaking down the internal tissues. This is very likely to be depletion of starches and sugars, simply associated with respiration, as well as provision of sugars for root and shoot development. The initial elevated temperatures in the growth cabinet would certainly have supported enhanced respiration and enzymatic breakdown of those internal storages.

It is unclear whether that physiological breakdown in itself is detrimental to sprout production, or whether its negative influence is via enhanced attacks by pathogens.

It is interesting to note that not all roots appeared to suffer the physiological evacuation of tissues. Whether this was related to the age of the bedding roots, or their agronomic treatment and post-harvest storage is unclear. For future experimentation, it would be important to source root lots from a uniform sample of known growth and storage history.

Pathogenic breakdown of bedding roots

In this study, we clearly encountered a wide range of organisms associated with breakdown of the bedding roots. The bacteria were the most destructive organisms, and no sprouts emerged or survived where the bedding roots were subject to bacterial attack.

The fungal organisms tended to be more localised, although the roots with substantial infections looked like they were on the route to rapid deterioration, which may have spread to the sprouts.

Apart from *Erwinia* and a *Rhizopus spp*., the other pathogens have yet to be determined. However, at this time it is unknown to what extent they are causal, or opportunistic pathogens, taking advantage of the weakened state of bedding roots impacted by physiological breakdown.

Management impacts

This was a very early pilot study; however, it does lead to the following interim suggestions for growers in managing premature breakdown of *Bellevue* in plant beds.

- Where possible, use small-medium bedding roots, preferably no more than a few months old, and kept in consistently cool storage conditions (16^{o}C) prior to installation.
- Don't use roots with unhealed wounds or apparent disease.
- If using plastic to heat beds, make sure the plastic structure is well ventilated, and monitor soil temperatures. Ideally keep soil temperatures below 30^{o}C, and perhaps even around 25-26^{o}C. If temperatures are likely to rise above that level, take the plastic off. Similarly for row covers.
- Avoid installing *Bellevue* into plant beds in circumstances likely to experience hot temperatures. Established plant beds can potentially survive, but it's possible new beds are more vulnerable.
- Ensure any irrigations at installation, and before sprouts are established, are even and light.
- Good drainage is essential.
- Avoid installing plant beds in ground with a known history of diseases, particularly bacterial.

Acknowledgements

Although not officially an original member of the VG13004 project team, Dr Wilson's endeavours in this study, and the support of USQ via provision of their facilities, were invaluable. Her diagnostic expertise, and commitment were very welcome and effective. Hopefully this collaboration in sweetpotato research, development and extension can continue for the Australian and international sweetpotato industries for many years.

Table 1: Income summary for each farm sites from the 3 - zones

Zones	Farm Site	Practice	Marketable Yield (t/ha) Ym	Price for Markatable (K/t)	Marketable Yield Income (K/ha) Im	Non- Marketable Yield (t/ha) Ynm	Price for Non markatable (K/t)	Non Marketable Income (K/ha) Inm	Total Income (K/ha) Im + Inm
Jiwaka	Gunn	BB	13.14	454.64	5973.95	2.89	453.71	1311.21	7285.16
	Gusamp	BB	9.36	608.78	5698.21	3.98	607.68	2418.56	8116.77
	Kongabal	BB	24.74	454.62	11247.27	1.08	451.21	487.30	11734.58
	Kurumul	BB	20.64	299.94	6190.80	2.22	300.72	667.60	6858.40
	Average		**16.97**	**454.50**	**7277.56**	**2.54**	**453.33**	**1221.17**	**8498.73**
	Gunn	CP							
	Gusamp	CP	5.26	260.87	1372.17	2.54	261.28	663.66	2035.84
	Kongabal	CP	14.44	227.38	3283.34	4.11	227.05	933.18	4216.52
	Kurumul	CP							
	Average		**9.85**	**244.12**	**2327.76**	**3.33**	**244.17**	**798.42**	**3126.18**
Goroka	Kuka	BB	29.38	869.33	25540.88	0.94	864.64	812.77	26353.65
	Meteyufa	BB	22.10	1333.00	29459.26	1.24	1328.56	1647.41	31106.67
	Nipuka	BB	18.00	454.40	8179.13	0.69	448.72	309.62	8488.74
	Rohenga	BB	24.15	500.11	12077.67	0.10	520.83	52.08	12129.76
	Average		**23.41**	**789.21**	**18814.23**	**0.74**	**790.69**	**705.47**	**19519.70**
	Kuka	CP	16.18	454.51	7353.96	0.54	452.24	244.21	7598.17
	Meteyufa	CP	NA						
	Nipuka	CP	14.34	454.66	6519.79	0.76	453.74	344.85	6864.64
	Rohenga	CP	NA						
	Average		**15.26**	**454.58**	**6936.88**	**0.65**	**452.99**	**294.53**	**7231.40**
Mt. Hagen	Baiyer-Koge	BB	14.76	545.16	8046.55	1.13	544.17	614.91	8661.46
	Bomri	BB	15.79	267.88	4229.82	2.45	267.71	655.90	4885.71
	Tonga Nebilyer	BB	9.49	181.82	1725.45	1.55	181.98	282.07	2007.52
	Kunt Mul	BB	6.06	1998.68	12112.01	3.77	2003.54	7553.36	19665.37
	Average		**11.53**	**748.38**	**6528.46**	**2.23**	**749.35**	**2276.56**	**8805.02**
	Baiyer-Koge	CP	6.47	545.68	3530.55	2.26	544.18	1229.84	4760.39
	Bomri	CP							
	Tonga Nebilyer	CP	5.55	181.64	1008.12	3.75	181.50	680.62	1688.73
	Kunt Mul	CP	1.56	2000.00	3120.00	2.33	1000.00	2330.00	5450.00

	Average		**4.53**	**909.11**	**2552.89**	**2.78**	**575.22**	**1413.48**	**3966.38**

1. Sweet potato tubers graded as marketable yield (Ym1) and non-marketable yield (Ynm2), based on quality
2. Tubers packed in sacks with weights ranged from 25kg to 112kg and priced according to sack weights
3. Price paid per sack varied among the project zones local markets
4. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygine/Sanitation)
5. CP = Conventional Practice sweet potato
6. Prices for sweet potato are the estimates from survey data collected in 2019
7. Blank spaces indicate data are incomplete to make estimates

Total yield (t/ha) Ym + Ynm	mean total yield BB	mean total yield CP	mean markatable yield BB	mean markatble yield CP	mean non-markatable yield BB	mean non-markatable yield CP
16.03	16.03		13.14		2.89	
13.34	13.34		9.36		3.98	
25.82	25.82		24.74		1.08	
22.86	22.86		20.64		2.22	
19.51						
7.8		7.80		5.26		2.54
18.55		18.55		14.44		4.11
13.18						
30.32	30.32		29.38		0.65	
23.34	23.34		22.10		0.84	
18.69	18.69		18.00		0.20	
24.25	24.25		24.15		0.65	
24.15						
16.72		16.72		16.18		0.54
NA						
15.1		15.10		14.34		0.76
NA						
15.91						
15.89	15.89		14.76		1.22	
18.24	18.24		15.79		0.13	
11.04	11.04		9.49		0.63	
9.83	9.83		6.06		1.00	
13.75						
8.73		8.73		6.47		2.26
9.30		9.30		5.55		3.75
3.89		3.89		1.56		2.33

7.31						
Sum	229.65	80.09	207.61	63.80	15.49	16.29
Mean	19.14	11.44	17.30	9.11	2.21	2.33
Ratio		1.67		1.90		0.95

Table 1: Income summary for each farm sites from the 3 - zones										
Zones	**Farm Site**	**Practice**	**Marketable Yield (t/ha) Ym**	**Price for Markatable (K/t)**	**Marketable Yield Income (K/ha) Im**	**Non- Marketable Yield (t/ha) Ynm**	**Price for Non markatable (K/t)**	**Non Marketable Income (K/ha) Inm**	**Total Income (K/ha) Im + Inm**	**Total yield (t/ha) Ym + Ynm**
Kanantu	**Average**	BB+	16.37			0.86				**17.23**
		BB (control)	19.41			0.66				20.07
Goroka	Kuka	BB+	25.24	869.33	21941.86	1.20	864.64	1040.46	22982.31	**26.44**
	Meteyufa	BB+	15.24	1333.00	20314.89	0.60	1328.56	801.56	21116.46	**15.84**
	Nipuka	BB+	20.41	454.40	9274.22	0.67	448.72	302.14	9576.36	**21.08**
	Gimisave	BB+	25.07	500.11	12537.77	1.17	520.83	609.37	13147.15	**26.24**
	Average		**21.49**	**789.21**	**16017.19**	**0.91**	**790.69**	**688.38**	**16705.57**	**22.40**
	Kuka	BB (control)	30.33	869.33	26366.74	1.61	864.64	1392.08	27758.82	31.94
	Meteyufa	BB (control)	14.67	1333.00	19555.08	0.80	1328.56	1062.85	20617.93	15.47
	Nipuka	BB (control)	15.89	454.40	7220.35	0.75	448.72	336.54	7556.89	16.64
	Gimisave	BB (control)	26.61	500.11	13307.94	0.67	520.83	348.96	13656.90	27.28
	Average		**21.88**	**789.21**	**16612.53**	**0.96**	**790.69**	**785.11**	**17397.64**	**22.83**
Jiwaka	Gunn	BB+	7.52	454.64	3418.88	1.76	453.71	798.52	4217.40	**9.28**
	Gusamp	BB+	19.82	608.78	12066.08	2.44	607.68	1480.71	13546.79	**22.26**
	Kongabal	BB+	6.74	454.62	3064.13	0.98	451.21	443.69	3507.82	**7.72**
	Kurumul	BB+	11.61	299.94	3482.33	0.50	300.72	149.36	3631.68	**12.11**
	Average		**11.42**	**454.50**	**5507.85**	**1.42**	**453.33**	**718.07**	**6225.92**	**12.84**
	Gunn	BB (control)	7.94	454.71	3610.37	1.56	454.17	708.50	4318.87	9.5
	Gusamp	BB (control)	17.11	260.87	4463.47	4.17	261.28	1089.56	5553.03	21.28
	Kongabal	BB (control)	8.06	227.38	1832.67	0.83	227.05	188.45	2021.12	8.89
	Kurumul	BB (control)	8.85	250.09	2213.30	2.56	249.19	637.92	2851.22	11.41
	Average		**10.49**	**298.26**	**3029.95**	**2.28**	**297.92**	**656.11**	**3686.06**	**12.77**

1. Sweet potato tubers graded as marketable yield (Ym1) and non-marketable yield (Ynm2), based on quality
2. Tubers packed in sacks with weights ranged from 25kg to 112kg and priced according to sack weights
3. Price paid per sack varied among the project zones local markets
4. BB+ = BB plus- living barrier plant treatments (Marigold, Silver leaf Desmodium, Smooth Senna)
5. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygine/Sanitation)
6. Prices for sweet potato are the estimates from survey data collected in 2019

7. Blank spaces indicate data are incomplete to make estimates

Yield impact by barrier plants			
Yield by Marigold	Yield by Silver leaf Desmodium	Yield by Smooth Senna	Total yield (average)
15.55	16.37	19.76	**17.23**
27.17	25.05	27.11	**26.44**
14.28	14.92	18.33	**15.84**
17.81	23.19	22.25	**21.08**
24.11	21.78	32.83	**26.24**
9.00	7.67	11.17	**9.28**
22.72	18.61	25.44	**22.26**
5.67	10.81	6.69	**7.72**
11.41	12.91	12.00	**12.11**

Table 2: Cost summary for e

Zones	Farm sites	Practice	Planting Material (vines) Cost					Pheromone Usage (Number of lures/plot)	Cost of Pheromones (K/ha)
			Planting Material QTY	Unit Cost (K)	Total Planting Material Cost (K/plot)	Converstion of area (plot to ha)	Planting Material Cost (K/ha) Cpm		
Kanantu	Average	BB+							
		BB (control)							
Goroka	Kuka	BB+	10	1	10	31.25	312.5	6.00	354.75
	Meteyufa	BB+	10	1	10	31.25	312.5	6.00	354.75
	Nipuka	BB+	10	1	10	31.25	312.5	6.00	354.75
	Gimisave	BB+	10	1	10	31.25	312.5	6.00	354.75
	Average		**10.00**	**1.00**	**10.00**	**31.25**	**312.50**	**6.00**	**354.75**
	Kuka	BB (control)	15.00	1.00	15.00	31.25	468.75	6.00	354.75
	Meteyufa	BB (control)	15.00	1.00	15.00	31.25	468.75	6.00	354.75
	Nipuka	BB (control)	15.00	1.00	15.00	31.25	468.75	6.00	354.75
	Gimisave	BB (control)	15.00	1.00	15.00	31.25	468.75	6.00	354.75
	Average		**15**	**1**	**15**	**31**	**469**	**6**	**355**
Jiwaka	Gunn	BB+	10	1	10	31.25	312.5	6.00	354.75
	Gusamp	BB+	10	1	10	31.25	312.5	6.00	354.75
	Kongabal	BB+	10	1	10	31.25	312.5	6.00	354.75
	Kurumul	BB+	10	1	10	31.25	312.5	6.00	354.75
	Average		**10.00**	**1.00**	**10.00**	**31.25**	**312.50**	**6.00**	**354.75**
	Gunn	BB (control)	10	1	10	31.25	312.5	6.00	354.75
	Gusamp	BB (control)	10	1	10	31.25	312.5	6.00	354.75
	Kongabal	BB (control)	10	1	10	31.25	312.5	6.00	354.75
	Kurumul	BB (control)	10	1	10	31.25	312.5	6.00	354.75
	Average		**10.00**	**1.00**	**10.00**	**31.25**	**312.50**	**6.00**	**354.75**

1. Cpm = planting material cost, Cph = Cost of pheromones, Cs = Cost of implementing sanitation, Ce = Cost of using endomopathogen, Cl = labour cost, Cmt = marketin
2. Transport and market cost are from public transport charges on sacks of sweet potato transported to local market of main urban centres market
3. BB+ = BB plus- multch treatments
4. BB = Best Bet method sweet potato (Pathogen Tested, Pheromones, Crop Isolation, Hygine/Sanitation)
5. Planting material cost includes the cost of pathogen testing
6. Crop isoloation is assumed at no cost

7. Costs for managing pheromones include materials, installation and operational costs
8. Pheromone trap density (numbr/plot/site) is 3 and pheromone lures were replaced every 2 monts (total number of lure/plot/site/session is 6)
9. Cost of sanitaion is considered by 5% of the labour cost involved
10. Mulches are assumed at no cost but Sugar cane multch is **added with entomopathogens**
11. Mulches as materials are assumed at no cost as they naturally avaibale and labour required for their collection and use in the field considered as 4 hours per plot mor
12. Transport and marketing cost, labour cost are the estimates from survey data collected in 2019
13. Cost of pheromones lures and endomopathogen are calculated based on the excahnge rate of 1 AU$ equal to 2.63 Kina
14. Plot size is in each site is closed to 16m x 20m (320 sqm) converted into one hectare as 31.25 times
15. Blank spaces indicate data are incomplete to make estimates

ach farm in the 3 - zones

		Labour Cost							
Cost of managing Pheromones (K/ha)	Pheromones Usage Cost (K/ha) Cph	Labour Cost (K/hr)	Labour Use (hrs/plot)	Labour Cost (K/plot)	Cost of implementing Sanitaion (K/ha) Cs	Cost of using entomopathogens (K/ha) Ce	Labour Cost (K/ha) Cl	Marketing and Transport Cost (K/ha) Cmt	Total Cost (K/ha) Cpm + Cph + Cs + Ce + Cl + Cmt
127.19	481.94	2.5	12.0	30	46.88	210.40	937.50	695.6	2684.81
150.63	505.38	3	9.0	27	42.19	210.40	843.75	172.7	2086.91
478.75	833.50	10	5.5	55	85.94	210.40	1718.75	184.1	3345.19
185.78	540.53	3.75	8.0	30	46.88	210.40	937.50	274.4	2322.21
235.59	**590.34**	**4.81**	**8.63**	**35.50**	**55.47**	**210.40**	**1109.38**	**331.70**	**2609.78**
127.19	481.94	2.50	8.0	20.00	31.25		625.00	695.60	2302.54
150.63	505.38	3.00	5.0	15.00	23.44		468.75	172.70	1639.01
478.75	833.50	10.00	1.5	15.00	23.44		468.75	184.10	1978.54
185.78	540.53	3.75	4.0	15.00	23.44		468.75	274.40	1775.87
236	**590**	**5**	**5**	**16**	**25**		**508**	**332**	**1924**
244.38	599.13	5	10.0	50	78.13	210.40	1562.50	149.3	2911.95
302.97	657.72	6.25	12.0	75	117.19	210.40	2343.75	106.4	3747.96
302.97	657.72	6.25	12.0	75	117.19	210.40	2343.75	702.8	4344.36
127.19	481.94	2.5	8.0	20	31.25	210.40	625.00	469	2130.09
244.38	**599.13**	**5.00**	**10.50**	**55.00**	**85.94**	**210.40**	**1718.75**	**356.88**	**3283.59**
244.38	599.13	5	6.0	30	46.88		937.50	149.3	2045.30
302.97	657.72	6.25	8.0	50	78.13		1562.50	106.4	2717.24
302.97	657.72	6.25	8.0	50	78.13		1562.50	702.8	3313.64
127.19	481.94	2.5	4.0	10	15.63		312.50	469	1591.56
244.38	**599.13**	**5.00**	**6.50**	**35.00**	**54.69**		**1093.75**	**356.88**	**2416.94**

g and transport cost

e than BB

Table 3: Net Income & Benefit-Cost Ratio (B

Zones	Production Practice	Farm site	Total Yield (t/ha)	Total Income (K/ha)
Goroka	NPT Conventional	Kuka	1.92	1663.44
		Nipuka	2.38	1073.90
		Meteyufa	8.33	11103.60
		Average	**4.21**	**4613.65**
	PT combination	Kuka	5.08	4415.00
		Nipuka	2.14	969.69
		Meteyufa	13.25	17659.49
		Average	**6.83**	**7681.39**
	PT isolation	Kuka	1.87	1628.19
		Nipuka	8.07	3660.26
		Meteyufa	22.00	29314.87
		Average	**10.65**	**11534.44**
	PT pheromone	Kuka	6.23	5414.70
		Nipuka	2.40	1087.94
		Meteyufa	16.82	22412.10
		Average	**8.48**	**9638.25**
	PT sanitation	Kuka	3.63	3155.20
		Nipuka	7.20	3265.51
		Meteyufa	14.00	18651.15
		Average	**8.28**	**8357.29**
Economic impact as an overall average from NPT to Other methods (K/ha)				

1. Economic impact is maesured by difference in net income from NPT to other stratergies (K/ha)
2. For Kuka farm site changing from NPT to 'PT pheromone' provides the highest net income
3. For Nipuka farm site changing from NPT to 'PT isolation' provides the highest net income
4. For Meteyufa farm site changing from NPT to 'PT isolation' provides the highest net income
5. Economic impact as an overall average from NPT to Other methods is around 4021 Kina per h

CR)

Total Cost (K/ha)	Net Income (K/ha)	BCR	Economic Impact (difference in net income from NPT to other methods) (K/ha)
1437.79	225.65	1.16	
770.04	303.86	1.39	
758.64	10344.96	14.64	
988.82	**3624.83**	**5.73**	
1973.36	**5708.03**	**4.39**	**2083.20**
1789.35	-161.16	0.91	-386.81
1121.60	2538.66	3.26	
1340.38	**10194.06**	**10.19**	**6569.23**
2271.29	3143.41	2.38	
1947.32	**7690.93**	**5.60**	**4066.10**
1820.60	1334.60	1.73	1108.95
1145.04	2120.47	2.85	1816.61
1133.64	17517.51	2.85	7172.55
1366.43	**6990.86**	**2.48**	**3366.04**

ectare

Appendix 5. Laboratory bioassays investigating efficacy of EPF isolates on *Cylas formicarius and Euscepes postfasciatus* in PNG

1. Introduction

A series of laboratory bioassays were performed to evaluate the virulence of many strains of entomopathogenic fungi isolated from the Lowlands and Highlands of Papua New Guinea (PNG) on two species of weevils: *Cylas formicarius* and *Euscepes postfasciatus*

2. Materials and methods

2.1. Insect cultures

Cylas formicarius individuals were collected from the field from infested storage roots and from pheromone traps placed in the field (males only, Figure 1). A culture of *Euscepes postfasciatus* was supplied to Unitech from NARI in Airuya. For both species, the weevils were reared in large tubs with mesh preventing weevil escape at room temperature in the Agriculture Dept laboratories at Unitech. The weevils were provided with fresh storage roots for feeding and oviposition on a weekly basis. The appropriate number of weevils were starved overnight before use the next day in bioassays.

Figure 1. Trapping of *Cylas formicarius* in the field (A, B, C) and maintaining cultures through weevil emergence from storage roots in the laboratory (Melanie Pitiki) (D).

2.2. Soil collection for baiting of entomopathogenic fungi (EPF) and EPF culture maintenance

Soil samples were collected from various locations in PNG for the purpose of baiting for entomopathogenic fungi. Collected soil was placed into plastic bags and transported back to the laboratory (Figure 2). Soil samples were placed into tubs and several larvae of the sago grub (*Rhynchophorus* sp. palm weevil) or the larvae of the cocoa moth (*Conopomorpha cramerella* or cocoa pod borer*)* were added in an attempt to bait EPF (Figure 3). The surface of the soil was moistened with distilled water, mesh was used to prevent escape of the larvae and tubes were incubated at room temperature until EPF was observed.

Figure 2. Soil collection from the Asaro Valley and preparation for insect baiting at Unitech with Masters student Gerega and Dr Ronnie Dotaona from the Department of Agriculture.

Figure 3. Sago grubs (red palm weevil larvae) were used to bait EPF from the soil (photo credit Gerega).

Infected cadavers were then used to initiate pure cultures of EPF (*Metarhizium* spp. or *Beauveria* spp.) (Figure 4). In a laminar flow cabinet, sporulated cadavers were surface sterilised in 1% NaOCl, rinsed in water, then placed onto Petri dishes (90 mm) with Potato Dextrose Agar (PDA). Petri dishes were sealed with Parafilm and

incubated at room temperature. Resulting colonies were sub-cultured onto PDA until a pure culture was obtained. The details of the isolated EPFs can be found in Table 1.

Figure 4. Infected larvae used to make pure cultures of EPF (e.g. *Metarhizium, Beauveria, Isaria* spp.) (photo credit Gerega).

To prepare inoculum for experiments, each EPF isolate was culture onto multiple PDA plates. The conidia from 2-week old cultures was scraped off and added to 10 ml sterile of 0.05% Tween and serially diluted seven (7) times, resulting in a concentration of 10^5 conidia per ml. Four treatments were used across all the bioassays: Control (C): Immersion in 0.05% Tween, Treatment 1 (T1): Immersion in the conidia suspension, Treatment 2 (T2): Spraying of the conidia suspension (using a spray bottle, fully open and spraying approximately 0.5 ml at one press) Treatment 3 (T3): Substrate contamination (a piece of diced sweet potato root was immersed in the conidia suspension).

2.3. Experimental set-up

Bioassay set-up and measurements: a series of laboratory bioassays were run over time due to low collections of insects, especially *Euscepes postfasciatus.* For each insect species and fungal genera, two separate bioassays were run. There were 10 replicates per treatment and each experimental unit consisted of one male and one female placed into a small tube (75 mm diameter) after being treated. All replicates were treated separately. Mesh (voile type fabric) was secured to each tub with an elastic band after a 2 cm^2 piece of sweetpotato was added for food and this was replaced every 5 days. The assessment for mortality continued for 25 days after inoculation.

Figure 6. Example of tub used in laboratory bioassays

2.4. Statistics

Genstat 21st Edition (VSN International Ltd) was used to perform all ANOVA analyses for the laboratory bioassays after testing for normality.

3. Results

For all the bioassay data that is described below (for *Beauveria* sp., *Metarhizium* spp. and both weevils) when insects were placed in a humid chamber (Figure 6), none of the weevils in the control treatments had signs of infection/death caused by entomopathogenic fungi. A handful of control weevils had other, saprophytic fungal growth, which was unlikely to have caused weevil death.

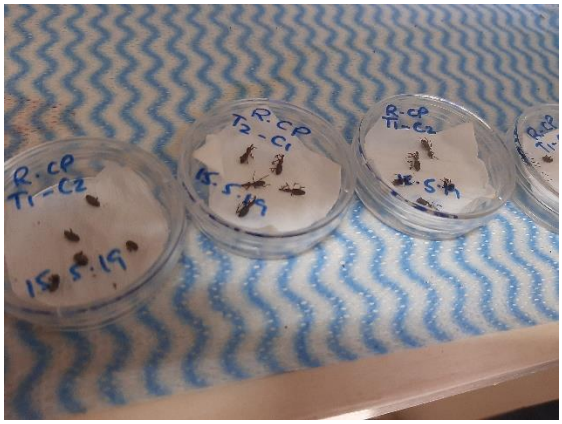

Figure 6. Humid chambers to check for *Beauveria* sp. /*Metarhizium* sp. resporulation from weevil cadavers

3.1. *Bioassay 1: Beauveria sp. virulence on* Cylas formicarius

In the first *Beauveria* sp. bioassay for females, the interaction between treatment and isolate was significant (P = 0.04) (Figure 7). Overall, with all treatments, isolate AVKC01 caused females to die the fastest. The effects of

treatment type and isolate on weevil death were inconsistent, in most cases, the death in the control was not different to other treatments like immersion or spray. However, generally speaking, dipping the sweetpotato before supplying to the weevils was the least effective treatment with more rapid death observed in the control. For male weevils, only treatment type was significant (P <0.001) (Figure 8). Weevils immersed in the conidial suspension died the fastest; however, this was not significantly different to the control. For both sexes, the interaction between treatment and isolate was significant (P = 0.025) and similar trends were observed as for the female only data (Figure 9).

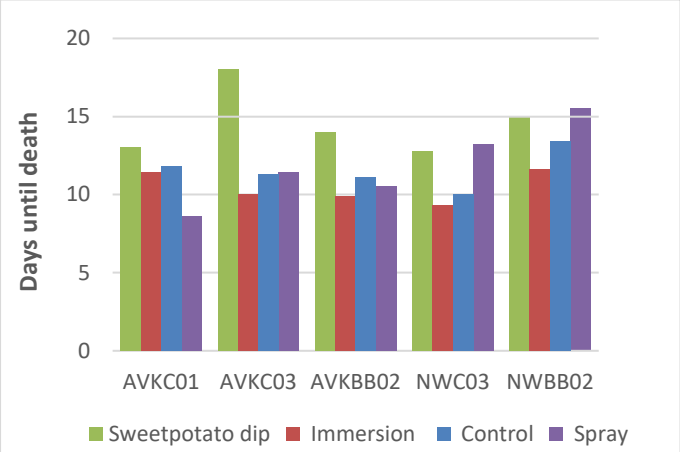

Figure 7. Effect of the treatment x isolate interaction on *Cylas formicarius* females (LSD=3.44 at P<0.05)

Table 1. Details of the Entomopathogenic Fungi Isolates collected from PNG

Sample #	Sample Name	Sample Code	Sample Locality	Date Isolated	Species	Closest GenBank accession no.	Primer pair used in PCR
1 (1)	Asaro Valley Kuka Conventional Plot 1	KC01	Kuka- Asaro, EHP	24/08/2018	*Metarhizium anisopliae*	F- KU593553.1 R - KR998523.1	ITS 1_ ITS 4
		KC01			*M. anisopliae*	F- KR706492.1 R- KX342701.1	EF1T_EF2T
2	Asaro Valley Kuka Conventional Plot 3	AVKCO3	Kuka – Asaro, EHP	24/08/2018	Awaiting sequence: *M. anisopliae?*	-	-
3 (2)	Meteyufa Conventional Plot 1	MCO1	Meteyufa – Asaro, EHP	24/08/2018	*M. anisopliae*	F- KF766520.1 R- KR998523.1	ITS 1_ ITS 4
	******Used in the Best Bet + Trial in Asaro and in Aiyura**	MCO1			*M. anisopliae*	F- KR706492.1 R - KX342701.1	EF1T_EF2T
4 (3)	Meteyufa Best Bet Plot 1	MBBO1	Meteyufa – Asaro, EHP	24/08/2018	*M. anisopliae*	F- JX912940.1 R- KR998523.1	ITS 1_ ITS 4
		MBBO1			*M. anisopliae*	F- KR706492.1 R- KX342701.1	EF1T_EF2T
5 (4)	Markham Old Cocoa Block	MOCB	Markham, Morobe Prov.	10/8/2018	*Metarhizium* sp.	F- KX451122.1 R- KR998523.1	ITS 1_ ITS 4
		MOCB			*M. anisopliae*	F- KX342701.1 R- KX342701.1	EF1T_EF2T
6	Trukai Sorghum Plot	TSPF	Erap, Morobe Prov.	10/08/2018	Awaiting sequence	-	-
7 (5)	**Unitech Ag. Farm- Corn Plot**	**UAFC**	**Lae, Morobe Prov.**	**03/09/2018**	***M. anisopliae***	**F- FJ545314.1 R - KR998523.1**	**ITS 1_ ITS 4**

	**Isolate used in the Unitech and Situm Trial "Lowlands"	UFC			*M. pinghaense*	F- KM091888.1 R- KP178548.1	EF1T_EF2T
8	Unitech Ag. Farm – Sweetpotato Plot	UAFSP	Lae, Morobe Prov.	03/09/2018	Not Sequenced	-	-
9 (6)	Gusamp Best Bet Plot	GpBB	Gusamp – Jiwaka Prov.	26/02/2019	*M. anisopliae*	F- KX057377.1 R- KR998523.1	ITS 1_ ITS 4
		GpBB			*M. robertsi*	F- XM_007823080.1 R- XM_007823080.1	EF1T_EF2T
11 (8)	Kangabil Conventional Plot	KaCP (Changed to KbCP)	Kangabil – Jiwaka Prov.	28/02/2019	*M. anisopliae*	F- FJ545306.1 R- DQ177432.1	ITS 1_ ITS 4
		KbCP			*M. lepidiotae*	F - EU248864.1 R- KX342777.1	EF1T_EF2T
13 (9)	Gunn Best Bet Plot	GBB	Gunn - Mt. Hagen, Western Highlands Prov.	28/02/2019	*M. pinghaense*	F- JF827149.1 R- JF827149.1	ITS 1_ ITS 4
		GBB			*M. pinghaense*	F- KC870072.1 R- KP178544.1	EF1T_EF2T
14 (10)	Rohenga Conventional Plot	RCP	Rohenga – Jiwaka Prov.	26/02/2019	*M. anisopliae*	F- KX806659.1 R - KX806659.1	ITS 1_ ITS 4
		RCP			*M. pinghaense*	F- KM091888.1 R- KP178548.1	EF1T_EF2T
15 (11)	**Hagen Conventional Plot**	**HGNCP**	**Mt. Hagen – Western Highlands Prov.**	**26/02/2019**	***M. anisopliae***	**F- KX057378.1 R- KR998523.1**	**ITS 1_ ITS 4**

	Isolate used in the Unitech and Situm Trial "Highlands"	**HGNCP**			***M. pinghaense***	**F- KM091888.1** **R- KP178548.1**	**EF1T_EF2T**
1	Asaro Valley Kuka Conventional Plot 1	AVKCO1	Kuka- Asaro, EHP	22/08/2018	Not sequenced	-	-
2	Asaro Valley Kuka Conventional Plot 3	AVKCO3	Kuka – Asaro, EHP	21/08/2018	Not sequenced	-	-
3	Asaro Valley Kuka Best Bet Plot 3	AVKBB02	Kuka – Asaro, EHP	21/08/2018	Not sequenced	-	-
5	Nipuka Wani Best Bet Plot 2	NWBB02	Nipuka Wani – Asaro, EHP	22/08/2018	Not sequenced	-	-
6	Meteyufa Conventional Plot 1	MC01	Meteyufa – Asaro, EHP	22/08/2018	Not sequenced	-	-
7	Gunn Best Bet Plot	GBB	Gunn - Mt. Hagen, West. Highlands Prov.	28/02/2019	Not sequenced	-	-
8	Gunn Conventional Plot	GCP	Kangabil – Jiwaka Prov.	28/02/2019	Not sequenced	-	-
9 (13)	Kurumul Best Bet Plot	KUBB	Kurumul – Jiwaka Prov.	26/02/2019	*B. bassiana*	F- KX553851.1 R - R- LN886699.1	ITS 1_ ITS 4
10 (14)	Bomri	BMRI	Mt. Hagen – Western Highlands Prov.	26/02/2019	*B. bassiana*	F- KY640637.1 R- KR998515.1	ITS 1_ ITS 4
11 (15)	Markham Old Cocoa Block - 1	MOCB1	Markham – Morobe Province	17/5/2018	*B. bassiana*	F- KU170584.1 R- HE605256.1	ITS 1_ ITS 4

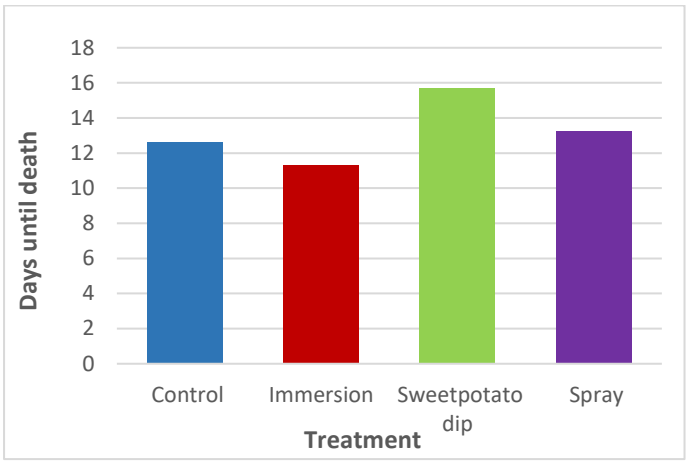

Figure 8. Effect of treatment type of *Beauveria* spp. on *Cylas formicarius* males (LSD=3.43 at P<0.05)

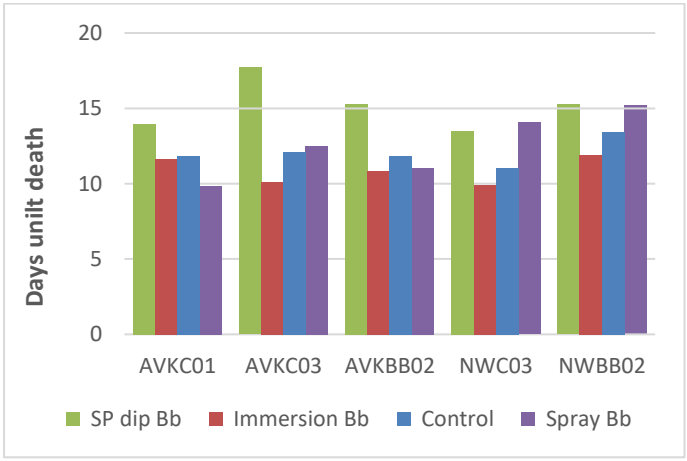

Figure 9. Effect of isolate x treatment on days until death for all *Cylas formicarius* adults (LSD=2.76 at P<0.05)

Table 2. Treatment ranking for *Beauveria* sp. treated *Cylas formicarius* based on sex or total for bioassay 1

	Female	**Male**	**Both**
1	Immersion	Immersion	Immersion
2	Control/sweetpotato dip/spraying	Control/sweetpotato dip/spraying	Control/sweetpotato dip/spraying

Table 3. Isolate ranking for *Beauveria* sp. treated *Cylas formicarius*

	Female	Male	Both
1	AVKC01	-	AVKC01
2	NWC03	-	NWC03
3	AVKBB02	-	AVKBB02

3.2. *Bioassay 2: Beauveria sp. virulence on* Cylas formicarius

For the second group of *Beauveria sp.* isolates, the individual effects of treatment and isolate was significant for female *Cylas formicarius*. For treatment, immersion in the fungal suspension resulted in the least days until death, and this was significant compared to all other treatments (P = 0.003) (Figure 10). The isolate KaCP had the least days until death, but this was not significantly different to that recorded for isolates GCP and BMRI (P = 0.006) (Figure 10).

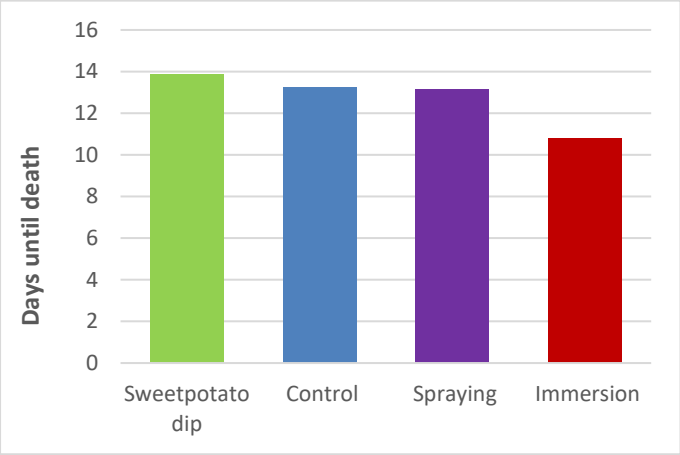

Figure 9. Effect of treatment type of *Beauveria* spp. on *Cylas formicarius* females

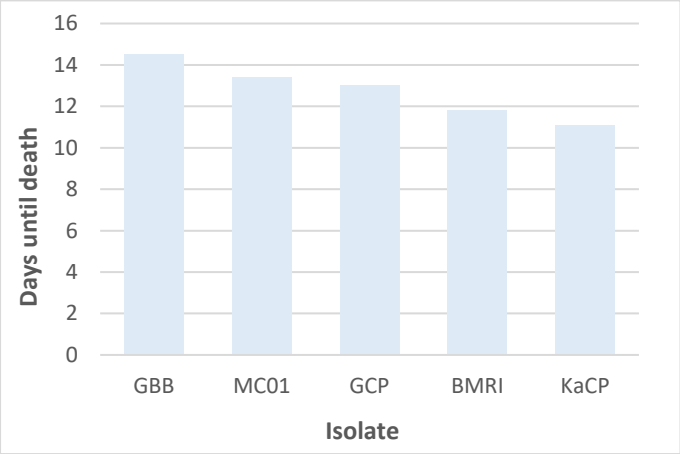

Figure 10. Effect of isolate on the death of *Cylas formicarius* females

For the second group of *Beauveria sp.* isolates, the effect of treatment and isolate was significant for male *Cylas formicarius*. For treatment, immersion of insects in the *Beauveria* sp. suspension resulted in the least days until death, but this was only significant to the control and sweetpotato dip treatments (P = 0.041) (Figure 11). The isolate BMRI had the least days until death, but this was only significantly different to that recorded for isolate MC01 (P = 0.008) (Figure 12).

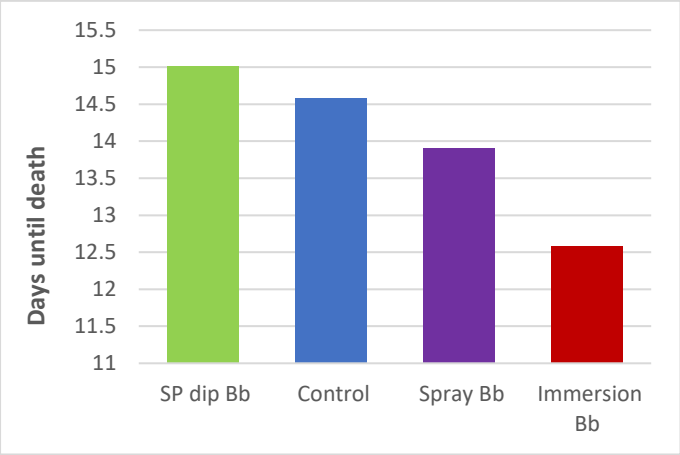

Figure 11. Effect of treatment type on *Cylas formicarius* males

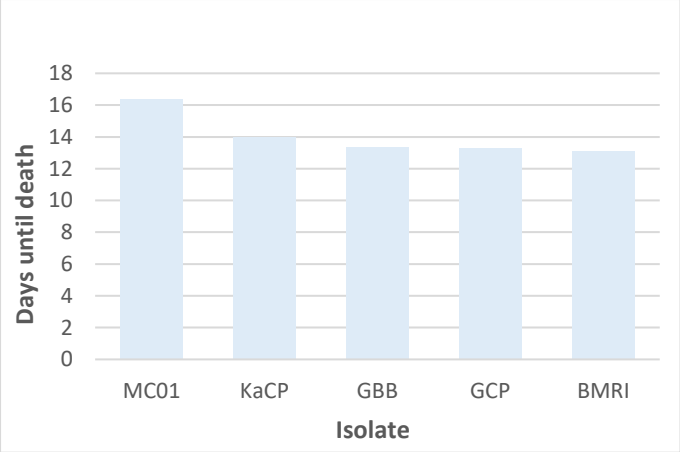

Figure 12. Effect of isolate on *Cylas formicarius* males

For all adults, the effect of treatment alone was significant; immersion of weevils into the *Beauveria* sp. suspension resulted in the least days until death (P = 0.001); however, all other treatments were not significantly different from one another (Figure 13). The effect of isolate was also significant (P <0.001). Isolate BMRI was the most effective isolate, but was only significantly different to isolate MC01 (Figure 14).

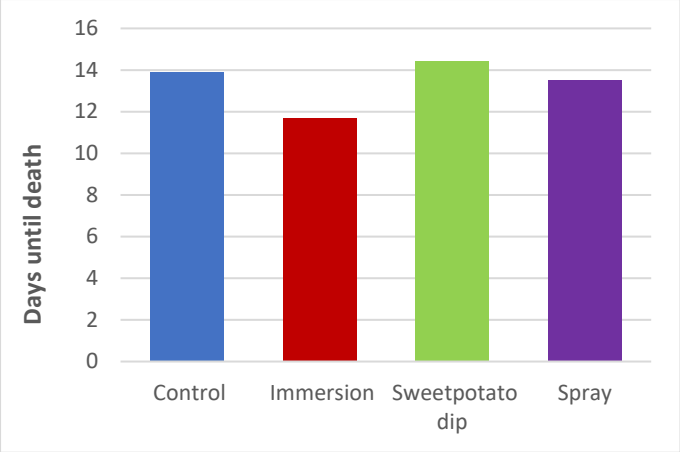

Figure 13. Effect of treatment type on *Cylas formicarius* adults

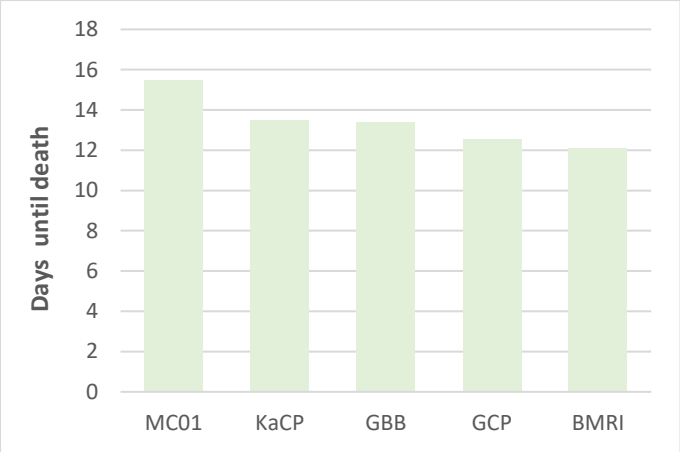

Figure 14. Effect of isolate on *Cylas formicarius* adults of both sexes

Table 4. Treatment ranking for *Beauveria* sp. treated *Cylas formicarius* based on sex or total for bioassay 2

	Female	**Male**	**Both**
1	-	-	Immersion
2	-	-	Control/sweetpotato dip/spraying

Table 5. Isolate ranking for *Beauveria* sp. treated *Cylas formicarius*

	Female	**Male**	**Both**
1	BMRI	-	BMRI
2	CGP	-	CGP
3	GBB	-	GBB

3.3. *Bioassay 1: Metarhizium sp. virulence on Cylas formicarius*

For female weevils, only treatment type was significant (P = 0.001). Weevils inoculated by immersion were significantly faster to die than all other treatments. The control did not differ significantly to roots being dipped in the conidia suspension before being exposed to weevils or weevils that were sprayed with conidia. For male *Cylas formicarius*, the effect of treatment and isolate was significant (P=0.014 and P=0.031 respectively). For treatment, immersing the weevils in *Metarhizium* sp. resulted in the most rapid death, but this did not differ significantly to the spraying treatment. Isolate AVKCO3 caused the most rapid death and was significantly different to all other isolates (Figure 15, 16, 17).

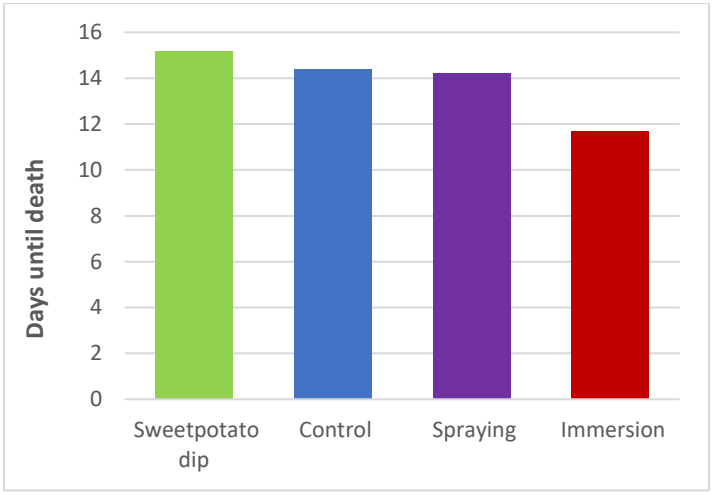

Figure 15. Effect of treatment on death of male *Cylas formicarius*

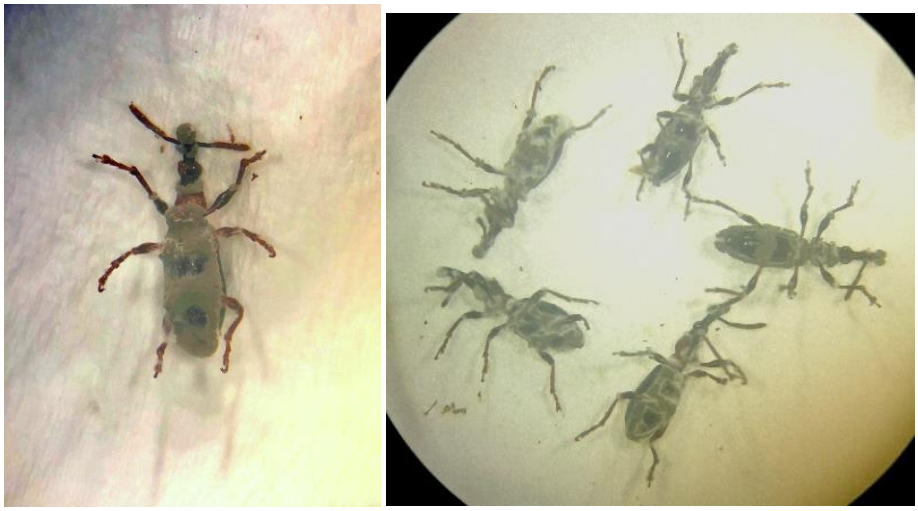

Figure 16. Male *Cylas formicarius* infected with *Metarhizium* sp.

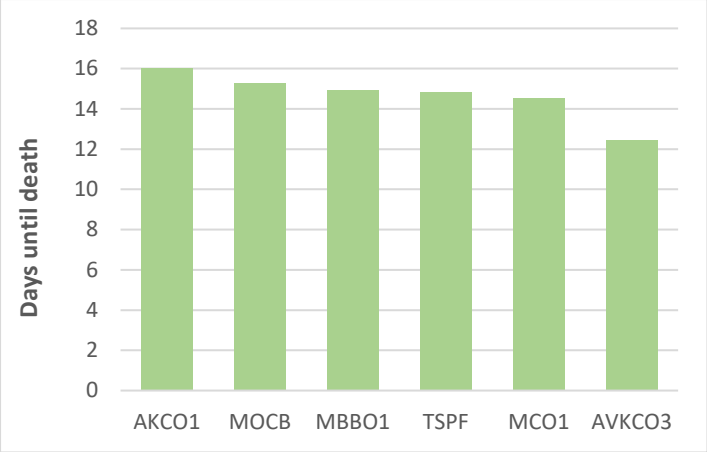

Figure 17. Effect of isolate on *Cylas formicarius* males

For all adults the only the effect of treatment was significant (P<0.001), where immersion caused the most death and was different to all other treatments.

Table 6. Treatment ranking for *Metarhizium* sp. treated *Cylas formicarius* based on sex or total for bioassay 1

	Female	**Male**	**Both**
1	Immersion	Immersion/spraying	Immersion
2	Control/sweetpotato dip/spraying	Control/sweetpotato dip/	Control/sweetpotato dip/spraying

Table 7. Isolate ranking for *Metarhizium* sp. treated *Cylas formicarius*

	Female	**Male**
1	-	AVKC03
2	-	MC01
3	-	TSPF

3.4. *Bioassay 2: Metarhizium sp. virulence on Cylas formicarius*

For Bioassay 2, the effect of treatment and isolate was significant (both P <0.001). Weevils inoculated by immersion were significantly faster to die than all other treatments except the sweetpotato dip treatment (Figure 18). The control did not differ significantly to roots being immersed before being exposed to weevils or

weevils that were sprayed. Isolate GCP caused the most rapid death, but was only significantly different to half of the other isolates (Figure 19).

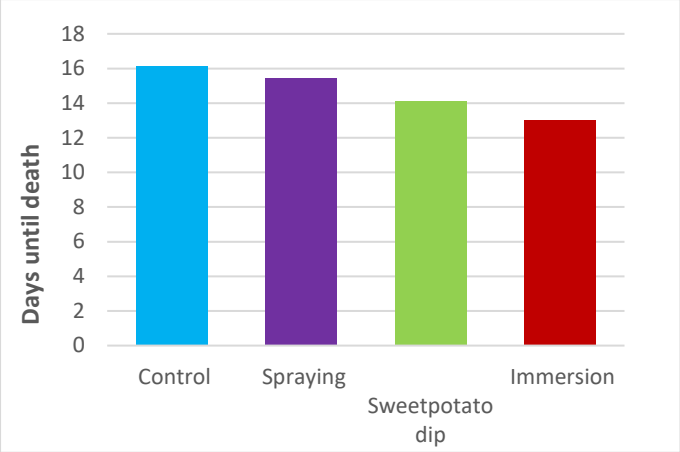

Figure 18. Effect of treatment on female *Cylas formicarius*

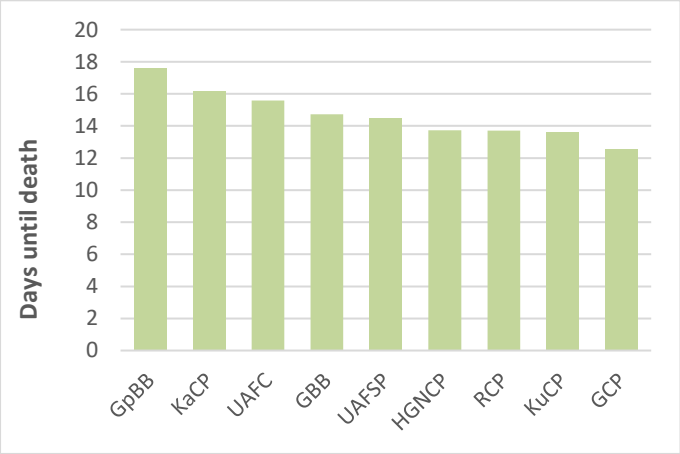

Figure 19. Effect of isolate on female *Cylas formicarius*

For male weevils in the 2nd *Metarhizium* sp. bioassay only treatment type was significant (P <0.001). Weevils inoculated by immersion were significantly faster to die than all other treatments except the spraying treatment. The control did not differ significantly to roots being immersed before being exposed to weevils (Figure 20).

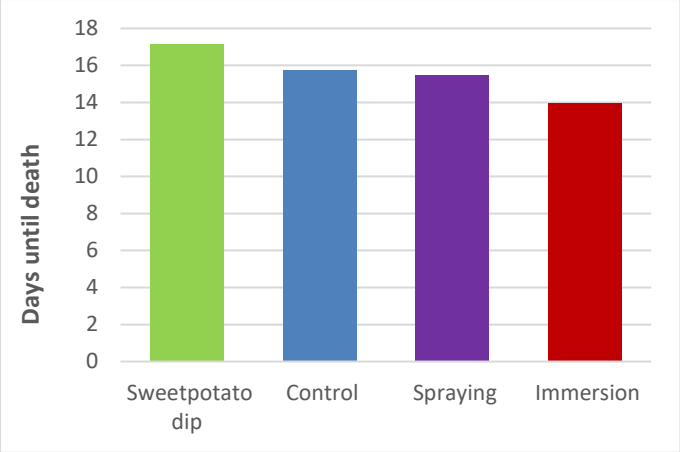

Figure 20. Effect of treatment on male *Cylas formicarius*

For all weevils, the effect of treatment and isolate was significant (both P <0.001). Weevils inoculated by immersion were significantly faster to die compared to all other treatments (Figure 21). The control did not differ significantly to roots being immersed before being exposed to weevils or sprayed weevils. Isolate GCP caused the most rapid death, although this was only significantly different to half of the other isolates (Figure 22).

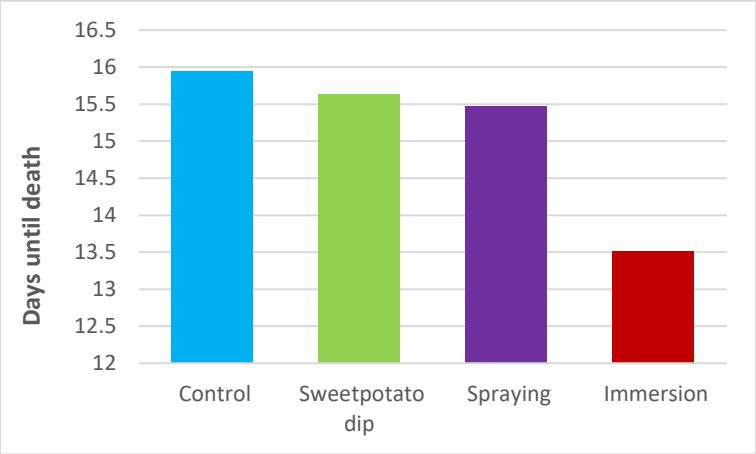

Figure 21. Effect of treatment on all adults of *Cylas formicarius*

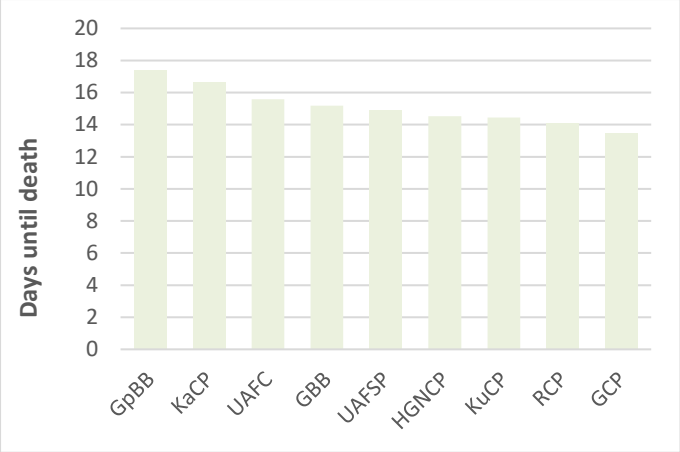

Figure 22. Effect of isolate on all *Cylas formicarius*

Table 8. Treatment ranking for *Metarhizium* sp. treated *Cylas formicarius* based on sex or total for bioassay 2

	Female	**Male**	**Both**
1	Immersion/sweetpotato dip	Immersion/spraying/control	Immersion
2	Control/spraying	Sweetpotato dip	Control/sweetpotato dip/spraying

Table 9. Isolate ranking for *Metarhizium* sp. treated *Cylas formicarius*

	Female	**Male**	**Both**
1	GCP	-	CGP
2	RCP	-	RCP
3	KuCP	-	KuCP

3.5. Bioassay 1: Beauveria sp. virulence on Euscepes postfasciatus

There were no significant effects of treatment or isolate on the time to death for female weevils alone. However, when the sexes were combined, the effect of treatment was significant (P=0.034). Immersion resulted in the most rapid death, but this only differed significantly to the sweetpotato dip treatment (Figure 23).

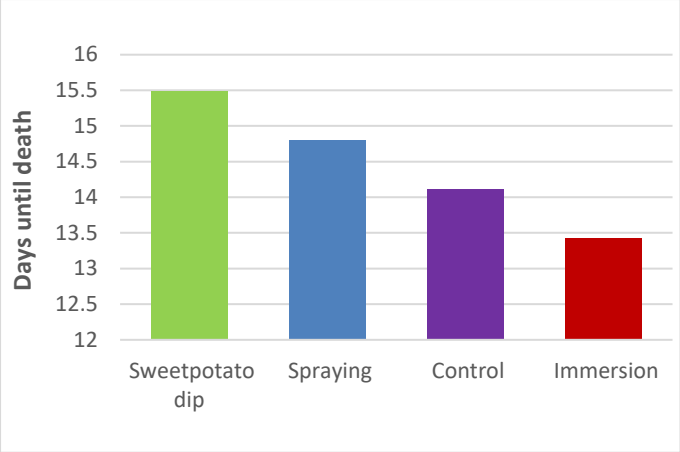

Figure 23. The effect of treatment on all *Euscepes postfasciatus*

3.6. Bioassay 2 Beauveria v Euscepes postfasciatus

There were no significant effects of treatment or isolate on the time to death for female weevils. For males, only the effect of treatment was significant (P=0.021), where the immersion of the weevils resulted in the most rapid death compared to all other treatments. When the sexes were combined, the effect of treatment was also significant (P=0.039) with immersion resulted in the most rapid death (Figure 24).

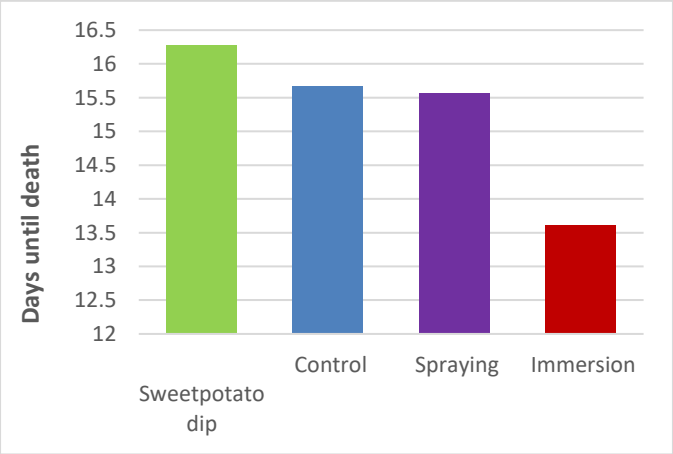

Figure 24. The effect of treatment on all *Euscepes postfasciatus*

1.1. Bioassay 1 Metarhizium sp. virulence on Euscepes postfasciatus

For female, male and sexes combined, only the effect of treatment was significant (all P<0.001). In all cases, immersed weevils were killed the fastest and this differed significantly to all other treatments (Figure 25, 26).

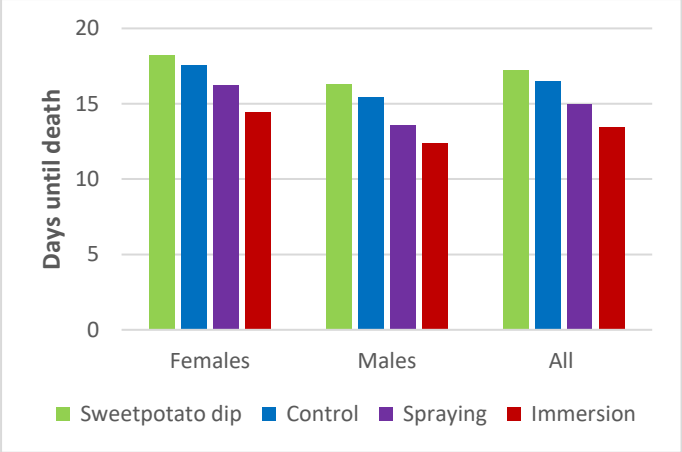

Figure 25. The effect of treatment on *Euscepes postfasciatus*

Figure 26. *Euscepes postfasciatus* with Metarhizium sp. outgrowth

1.2. *Bioassay 2: Metarhizium sp. virulence on Euscepes postfasciatus*

For female, male and sexes combined, only the effect of treatment was significant (all $P<0.001$). In all cases, immersed weevils were killed the fastest. However, for females and both sexes, the time taken to kill the weevils only differed to the control and sweetpotato dip (Figure 27).

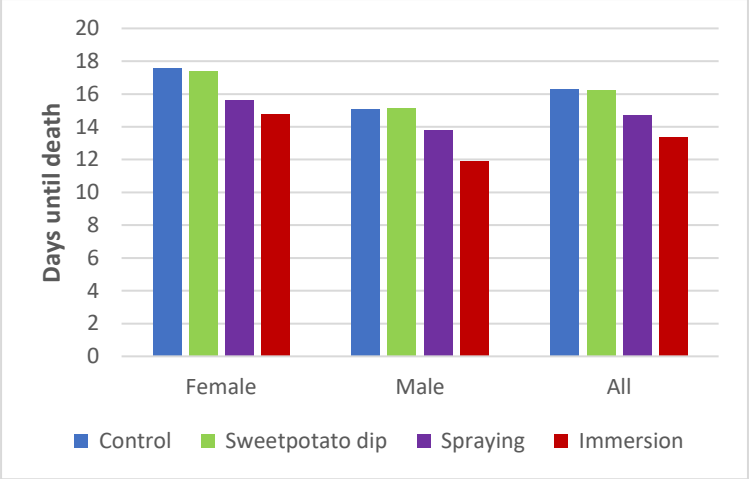

Figure 27. The effect of treatment on *Euscepes postfasciatus*

2. **Discussion/summary points**

To the best of our knowledge, this is the first detailed laboratory study examining the use of *Metarhizium* spp. and *Beauveria* spp. to manage *C. formicarius* and *E. postfasciatus* in Papua New Guinea. Moreover, this work has led to the creation of a large culture collection of entomopathogenic fungi that can be accessed for future research. Whilst *M. anisopliae* was the dominating species to be isolated from PNG soils (lowlands through to the highlands), other species such as *M. pinghaense*, *M. robertsii* and *M. lepidiotae* were also collected.

Across most bioassays, dipping (immersion) or spraying the weevils was the most effective application method; however, rarely were these methods significantly different to the control. It is not clear why dipping the sweetpotato in the conidia suspension resulted in increased weevil longevity. A very low dose of conidia (10^5 conidia per ml) was used in these experiments, which is much lower that what is commonly used in pathogenicity testing experiments (10^7 to conidia per ml). It is likely that the dose of conidia simply was not high enough to be effective, and this is a logical explanation as to why there was no difference in mortality between EPF treated and non-treated weevils. Death in the control was never attributed to EPF in these bioassays. Perhaps death in the control and all other treatments was age related (i.e. too old). Follow up work is recommended to screen the isolates at a higher dose (and dose-response bioassays) to better identify virulent isolates.

A series of bioassays were performed over time so it is difficult to ascertain the most effective isolate, instead, we are able to shortlist a few isolates of each genera that can be further pursued. Future work could examine the best-bet isolates (*Metarhizium* and *Beauveria* sp.) in the same bioassay to ascertain which is the most effective. Trends in these experiments suggest *Beauveria* sp. may cause more rapid death than *Metarhizium* sp. on *C. formicarius* and that *E. postfastiatus* are more 'resilient' to EPF than *C. formicarius* taking longer on average to die (differences in cuticle, behaviour and movement of insects in tubs?).

Appendix 6 Provisional best bet integrated pest and disease management (IPDM) strategies

This list (and brief justification for each method) is for use in the Asaro TEAM zone in 2017 and, subsequently, across additional TEAM zones in 2018-2021. The package of best bet IPDM methods will be compared with a control treatment in which standard farmer practice (as used in each zone) will be retained.

1. Pathogen-tested planting material – (part of the TADEP project 'package')

Sweetpotato virus is a major constraint in the industry, often reducing yield by 50% and even up to 90% (Gibson and Kreuze, 2015). The most common form of spread of viruses is through propagation material, in which a taxonomically diverse range of viruses can be present (Clark *et al*., 2012). Pathogen tested (PT) seeks to ensure that planting material is free from viruses. PT material will also provide more general benefits by carrying fewer pathogens and pests including weevils and gall mites.

2. Sanitation

Rogue sweetpotato plants and taxonomically related *Ipomoea* spp. weeds around fields can act as pest and disease reservoirs, particularly for viruses (Clark *et al*., 2012). Accordingly, crop damage can be reduced by removing and destroying any potential sources of inoculum (Clark *et al*., 2012; Johnson and Gurr, 2016) e.g. by destroying all crop residues (Coleman *et al*., 2009) or rotation to a non-host crop, even a fallow period (Clark *et al*., 2013).

ALL of the following needs to occur at every site:

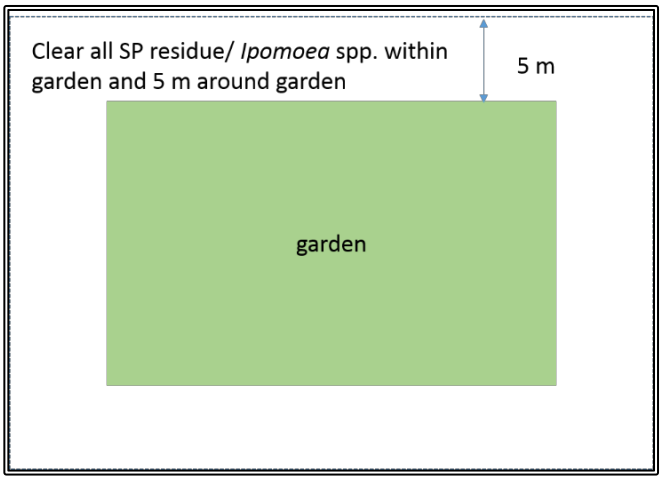

Fig. 1. Removal of sweetpotato residue and *Ipomoea* spp. from a garden and surrounds.

2.1. Careful removal of all sweetpotato crop residue*

2.2. Hand removal of *Ipomoea* spp. weeds from gardens and surrounding area to a distance of 5m around the plot (Fig. 1) before planting and encouraged during growing season.

2.3. Fallow/crop rotation: Plant new crops into land that has been fallow or planted to another crop type for at least 12 months (if a 'bush fallow is used, *Ipomoea* spp. weeds need to be hand removed).

***** To destroy pathogens or insects, sweetpotato crop residue or *Ipomoea* spp. weeds need to be fed to pigs or otherwise destroyed.

3. Crop isolation – plant new sweetpotato crops at least 5m away from other sweetpotato crops.

Contiguous planting of crops offers greater scope for pests and pathogen inoculum to travel into a newly planted crop from older, adjacent crops that are likely to already be infested/infected with pests and diseases. Weevil adults are a key example. Leaving a gap between sweetpotato new and existing crops minimises this transfer. The gap, which should be at least 5m wide around the whole garden should be occupied by non-crop vegetation and free of *Ipomoea* spp. weeds, or occupied by other types of crops (i.e., not sweetpotato). Later in the project selected barrier plants can be used; once these have been identified by studies in other sections of our project.

4. Pheromone mass trapping of sweetpotato weevil (*Cylas formicarius*) using commercial pheromone lures (0.1mg per lure) (provided by the project*) and locally made 'Coke bottle' traps (Fig. 2) filled with water with a couple of drops of liquid detergent (e.g., dish wash detergent). To avoid the base of traps becoming over-filled by rain, 2-3 holes need to be punctured part-way up the base. Density of traps is to be 1 trap per 200m^2 (10mx20m) which is consistent with the manufacturer's recommendations Based on work on the range over which *C. formicarius* responds to these lures (Reddy *et al*., 2014), our pheromone traps should be at least 100m away from the control plots/gardens (where standard farming practice is being used). The traps should be placed on the upwind side of each 10m x 20m area and the lures renewed every 6 weeks.

Pheromone mass trapping can result in significant reduction in sweetpotato storage root damage (Yasuda, 1995), increase in sweetpotato yield, decrease of application of insecticide.

* Pheromone lures are bought online Taobao (Chinese equivalent of eBay). Each lure contains 0.1mg pheromone. https://world.taobao.com/item/41779893131.htm?fromSite=main&spm=a312a.7700846.0.0.pFNP6B&_u=o41hvgc7c1f

Fig. 2 'coke bottle' pheromone trap

(Materials: pheromone lures *(provided by research team),* large paperclips (that measure 160mm when straightened) *(provided by research team)*, soft drink bottles (1 litre or larger capacity), knife or scissors for cutting bottle and cap, wire cutters and sticky, waterproof 'gaffer' tape. First cut the top quarter off of the bottle and invert it to serve as a funnel leading into the bottom part of the

bottle. Secure in place using tape. Punch a hole in the bottle cap. Straighten paperclip and put it through the hole in the cap. The end of the wire inside the cap needs to be bent into a small loop (to prevent the cap slipping down past it), and the tip of the wire bent into a simple 'U' shaped to hold the rubber pheromone lure. The other end of the wire should be bent so that it can be secured with tape to the outside edge of the bottle base. The length of the horizontal section of the wire needs to be such that the cap ends up in the centre of the trap. Puncture a few holes part-way up the base of the bottle (to allow drainage of rainfall) and half- fill with water plus a couple of drops of liquid detergent (e.g., dish wash detergent). Burry the bottom half of the bottle into the ground to prevent it blowing over. Wash your hands carefully then hang one pheromone lure on the wire beneath the cap of each trap.)

POSTSCRIPT

Area-wide management (eg synchronous fallow, planting & harvesting)

This will not be part of the IPDM best bet package because it is logistically impossible to include in a randomised, replicated experimental design that also includes conventional farmer practice (our control treatment). It is note here as a likely important strategy for future use in commercial production areas.

Area wide management (AWM) is when growers or advisers approach pest management as a group in a geographic area, rather than on individual farms. AWM is particularly relevant for pest species that are mobile, have a wide host range (crop and non-crop), and are locally generated in the farming system, and enables management strategies on a larger-scale that may be may be more effective than a paddock-by-paddock approach e.g. synchronous fallow, planting & harvesting in the large scale sweetpotato growing area.

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A proposal for field trial work in 2021: partitioning the separate effects of methods within the 'best bet' strategy.

The current best bet strategy has 4 methods for controlling pests & diseases:

a. **Pathogen-tested planting material**
b. **Sanitation**
c. **Crop isolation – plant new sweetpotato crops at least 5m away from other sweetpotato crops**
d. **Pheromone mass trapping of** sweetpotato weevil (*Cylas formicarius*)

To assess contribution of each of the component from best bet on sweetpotato pest & disease occurrence, an additional field trial will be conducted on farms.

Farm site selection

Farm sites should be selected where there is the highest possible chance of high **pest & disease pressure. These can be** within our TEAM zones or in other locations, best to be close to Aiyura station for the convenience of travel and data collection. If the site is in the TEAM zone, it is best to avoid using the previously best bet plot; aim to be far away from these plots (> 200m).

Treatment

The trial will have 5 treatments:

Treatment Abbreviation	Treatment detail
F	Farmer practice (negative control)
S	Sanitation only added to farmer practice
C	Crop isolation only added to farmer practice
P	Pheromone mass trapping only added to farmer practice
SCP	SCP combined (Sanitation, Crop isolation, Pheromone together as positive control)

Paired farm

We will use paired farms to accommodate all treatments for each replicate. In each pair, one farm will have treatments F, S and C, whilst the other farm in this pair will have treatments P and SCP. This also offers the advantage of the treatments with pheromones not interfering with the treatments that do not have pheromones. To avoid such interference, do ensure that all farms are at least 200m apart. 500m is even better.

Replication is required for the trial. So plan for at least 4 pairs of farms, but there's no need for more than 6 pairs. (In total 8 to 12 farms)

Each pair of farms should be in the same area and be as similar as possible in terms sweetpotato variety, farming practices, soil type etc. Also, each pair of farms will be the same in using EITHER pathogen tested planting material or non-PT. Do not mix these within a pair. It is ok for other pairs to use one or other type of planting material

Planting material.

Given NARI has been conducting trials to test PT effect, there is no need to repeat testing PT vs non-PT. So, either PT or non-PT can be used, but should be consistent within each pair of farms.

Trial design

Plot size should be as large as possible to be a realistic representation of farming conditions. Aim for at least 10m x 10m for each plot depending on availability of space offered by the host farmer. For each plot, one of the treatments (see below) will be used. A suggested layout for one site is shown in figure 1 below. The placement of treatment plots will differ on each site because of randomisation.

Randomise the plots within each of the two areas on farm (pheromone plots area and non-pheromone plots area).

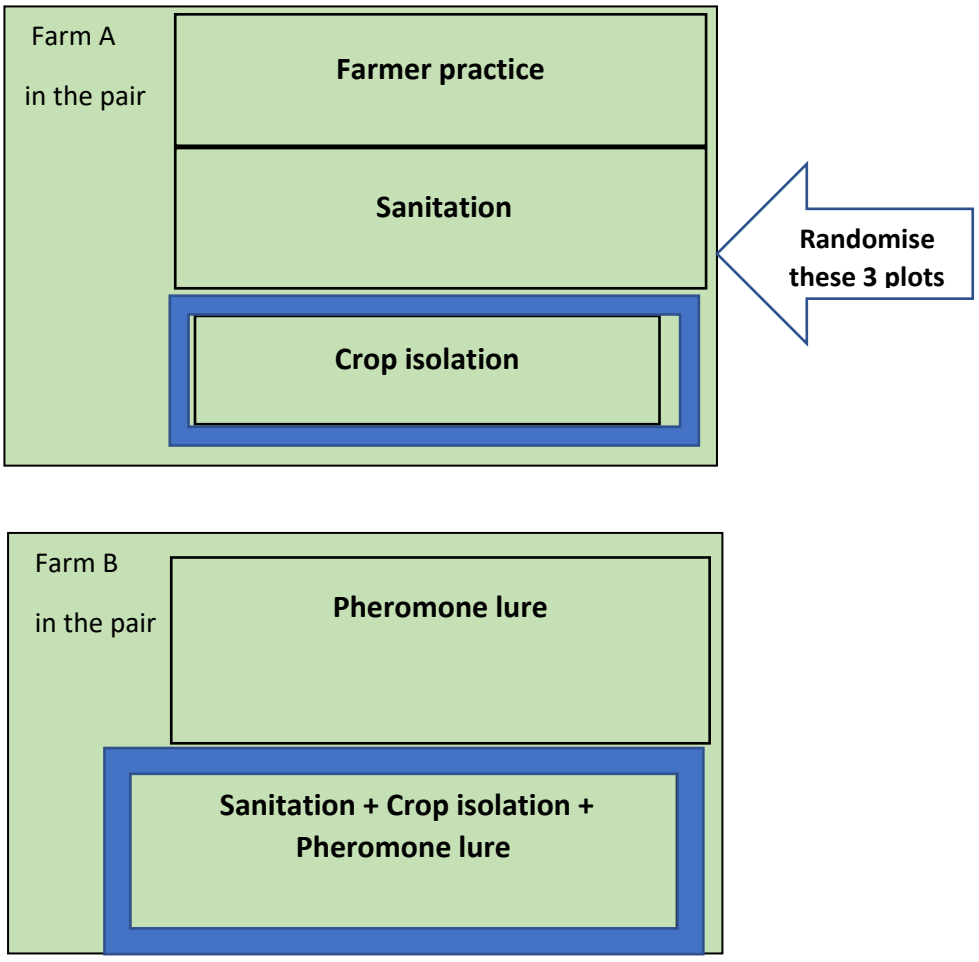

Figure 1. Illustration of trial design on farm site to assess single method. The thick blue border shows the isolation of that plot from other sweetpotato plants of at least 2 meters

Data collection

Given the large number of plots requiring assessment, data collection should be done in a more efficient and targeted manner than for earlier trials.

Mid-season survey

Weevils visualisation are difficult to be captured in the above ground visual survey so, at mid-season, the **main target of data are scab and gall mite symptom levels**. Methods for scoring will be as detailed in the earlier protocols used in TEAM zone survey.

Harvest time survey

Data collection at harvest time is critical to the trial. To save time and aiming accurate data collection, focus on the following data sets.

1. **Scab & gall mite symptom data**
2. **Destructive survey data**
3. **Incubation data**
4. **Yield data**

In addition, please liaise with the farmers to collect rainfall data and fortnightly pheromone trap weevil catches.

Appendix 4. Queensland sweetpotato grower *Cylas formicarius* weevil counts

At another organic farm in QLD, where weevils previously have not been a problem, a small trapping exercise to assess population (not efficiency of trap) was performed using bucket traps (sprayed red with paint) and modified yellow fly traps. Two paddocks were used: paddock 1 was 0.5 ha (sweetpotatoes ready for harvest) and had 4 traps and paddock 2 was 3 ha (sweetpotato being progressively harvested, volunteer sweetpotato and other weeds). Weevil counts were collected weekly for 4 weeks, with a total of approximately 2300 males trapped. After the initial large collection after the first lot of traps was installed (e.g. trap 8, Table 1 and Figure 2), overall pest pressure was relatively low. To ensure these numbers remain low the growers have continued to use these pheromone traps as a part of their pest management strategy for sweetpotato weevil and intend to install additional traps

Table 1. Various traps and their respective weevil counts

Trap	Description				
		18/09/2020	28/09/2020	8/10/2020	16/10/2020
1	Keith bucket trap with sweetpotato	11	2	4	1
2	Keith bucket trap empty	2	1	2	3
3	Keith bucket trap with tablet	12	2	3	5
4	Bree fly trap Bunnings	1	3	17	2
5	Bree fly trap Bunnings	47	3	12	1
6	Keith bucket trap with sweetpotato	38	2	6	15
7	Keith bucket trap empty	4	1	2	24
8	Keith bucket trap with tablet	1616	20	68	54
9	Bree fly trap Bunnings	3	6	20	9
10	Keith bucket trap with tablet	56	32	87	90
11	Keith bucket trap with sweetpotato	43	1	45	136
12	Keith bucket trap empty	2	8	148	83
	total each sampling date	**1835**	**81**	**414**	**423**

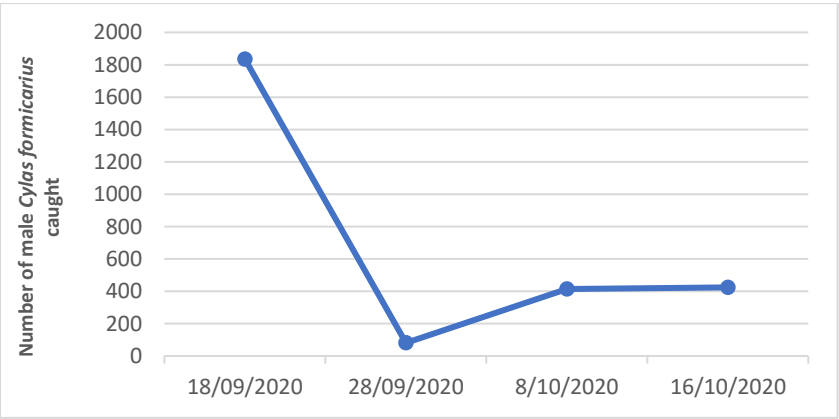

Figure 2. Total weevil counts over time

AARES Conference 2021

Economic impact of pathogen tested and conventional practice sweet potato production in the Highlands of PNG

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Abstract

Sweet potato (*Ipomoea batatas*) is a major staple food crop in Papua New Guinea (PNG). Around 90% of PNG's population consists of semi-subsistence smallholder farmers for whom sweet potato is a major food crop. It is grown extensively in the high-altitude highlands as a subsistence food crop by smallholder farmers and is developing into commercial production for cash income. The national demand for sweet potato is high and increasing and the income elasticity of sweet potato is positive. Sweet potato production is profitable and the profit from its production can be increased (doubled) when improved technologies are used by famers. Thus, PNG has a comparative advantage in sweet potato production. However, pests and diseases attack especially roots and foliar attack by a wide range of pests and diseases remains a major challenge. Majority of the PNG farmers do not practice an active pests and diseases management, which has a significant impact on the yield and farmers' income. Few farmers have opted to use cultural practices to manage the pests and diseases, though these methods are ineffective and expensive.

An alternative intervention technology through Pathogen Tested (PT) seeds as a part of Integrated Pest and Disease Management (IPDM) technology was trialled in three different zones in the Highlands of PNG and compared with the Conventional Practice (CP). The study was done using farmer participatory research approach (FPR). Pre-designed structured questionnaires were administered to twelve participating farmers to collect data for yield, costs, income and farmer's perception. The Cost Benefit Ratio (CBR) analysis was used to compare the costs, benefits and net incomes. T-test statistics was used to compare the mean yield. The farmer perception was summarised using descriptive statistics. Results revealed that marketable sweet potato yield was significantly ($P<0.01$) improved with implementation of the PT practice compared to the CP. The improvement in tuber yield had subsequent result in the net income doubled for the farmers. The yield and quality of tubers had significant influence on the farmers' perception towards adoption of the IPDM technology.

Keywords: Sweet potato, pests and diseases, IPDM, economic impact, PNG

1. Introduction

In PNG smallholder food crops farmers play a significant role in supplying to the local markets and sustaining the fresh fruits and vegetables needs of the people. Although food crops production data at this stage is fairly sketchy, there are some evidence to suggest that the food crops demand has increased over the years as the population increased rapidly in the main cities and towns (Chang and Spriggs, 2007). According to Allen et al. (2009), an average of 5,000-6,000 tonnes of food crops from the Highlands is shipped to coastal cities, especially Port Moresby, annually generating value of K10 million to K12 million with sweet potato being the highest earner followed by English potato. In 2003 it was estimated by Spriggs et al. (2003) that about 4,000 tonnes of fresh produce valued at almost K14 million shipped every year from the Highlands to Port Moresby alone. The overall volume of food crops shipments to major towns and cities has increased recently especially sweet potato.

Sweet potato (*Ipomoea batatas)* is the main traditional staple food crop in PNG in terms of area under cultivation and consumption. It has a long history of cultivation in the country and coupled with good local knowledge, it has evolved to become an ideal traditional food crop for promoting commercial scale production within the rural communities of PNG and perhaps creating industrial use benefits as alternative market to promote large scale production. Around 90% of PNG's population consists of semi-subsistence smallholder farmers for whom sweet potato is the primary food crop. It is cultivated extensively in the high-altitude Highlands as a subsistence food crop and developing into market- oriented commercial production for cash income. The national demand for sweet potato is high and increasing and the income elasticity of sweet potato is positive. The profitability of sweet potato is high and could double where improved technologies be used for improving yield. Thus, PNG has a comparative advantage in sweet potato production.

It is estimated that about 1 percent of total sweet potato production (i.e. 300,000 t) in the country is sold within the local market (Chang and Spriggs, 2007). Of these 2,000 tonnes shipped to Port Moresby from the Highlands and remaining is consumed locally in the Highlands provinces. In the recent years due to increase in local consumption, market-oriented sweet potato farming has increased among the rural subsistence farmers, especially in the Highlands. This has helped in providing income for the rural communities in meeting some of their cash needs, such as paying for children's education, medical care and other cash needs (Villano et al. 2016).

Although sweet potato has great potential in the country to transform the socio-economic status of the rural farmers, the national average yield remains relatively below the world average yield of 14.5 tonne ha^{-1} (FAO, 2016), for instance according to Bourke et al. (2000) the average yield is 14 tonnes per ha in the Highlands and 13 tonnes per ha in the lowland coastal regions. Furthermore, Australia's national average yield ranges from 30 - 35 tonnes per ha which is double the PNG's national average. There are several constrains identified as the cause of low yield output below the world average. Most notably, sweet potato pests (weevil)

and diseases (viruses) attack, especially roots and foliar damage by a wide range of pests and diseases remains a major challenge (Gurr et al. 2016; Regina et al. 2007). Majority of farmers do not practice an active pest and disease management, which has a significant impact on the yield and farmers' income. Few farmers have opted to use traditional cultural practices to manage the pests and diseases, though this method is ineffective to control the pests and diseases. There are also some farmers who use pesticides and insecticides, but they are not available close to the farmers and costly to purchase.

Therefore, an alternative intervention through combination of IPDM can be a promising approach to control pests and diseases. The IPDM intervention can be socially, economically and environmentally beneficial, while improving farmers' livelihood and contributing to food security objectives. A significant reduction in sweet potato weevil damage from an average of 45 percent to 6 percent was archived in Cuba (Lagnaoui et al. 2000). Half of total area planted to sweet potato in Cuba is under an integrated pest management program, using a combination of various locally available components resulted in yield increase from 6 tonnes per ha to 15 tonnes per ha nationally.

This paper discusses outcomes from an ongoing project on introducing IPDM strategies in PNG to improve yield, and transform the sweet potato production to a commercial food crop, given its socio-economic significance in the rural Highlands of PNG. Section 2 of the paper provides review of literature on farming systems practiced in PNG and the importance of growing sweet potato as a strategical crop. Section 3 details the research objectives and methodology. Results and discussion are given in section 4, followed by conclusion in section 5.

2. Literature review

Papua New Guinea's (PNG) economy is dominated by two broad sectors, the formal capital-intensive mineral resource (gold, copper, nickel, oil and gas) sector which accounts for 75 percent of the export earnings and 56 percent of the Gross Domestic Product (GDP) and, the

agriculture sector which contributes approximately 14 percent in foreign exchange earnings and accounts for 26 percent of the country's GDP (World Bank, 2018). The agriculture sector is broadly categorised into two main farming systems, as commercial crops farming systems and the food crops faming systems. The later farming systems comprises mainly of perishable food crops farming, which includes, roots crops, fruits, nuts and vegetables. This subsector is largely dominated by the smallholder semi-commercial farmers who supply fresh vegetables and fruits meeting the demand within the local economy (Figure 1). The sector sustains an approximate 80 percent of the population in the country and is the means of livelihood and provides food security for the bulk of the rural population (Bourke and Harwood, 2009).

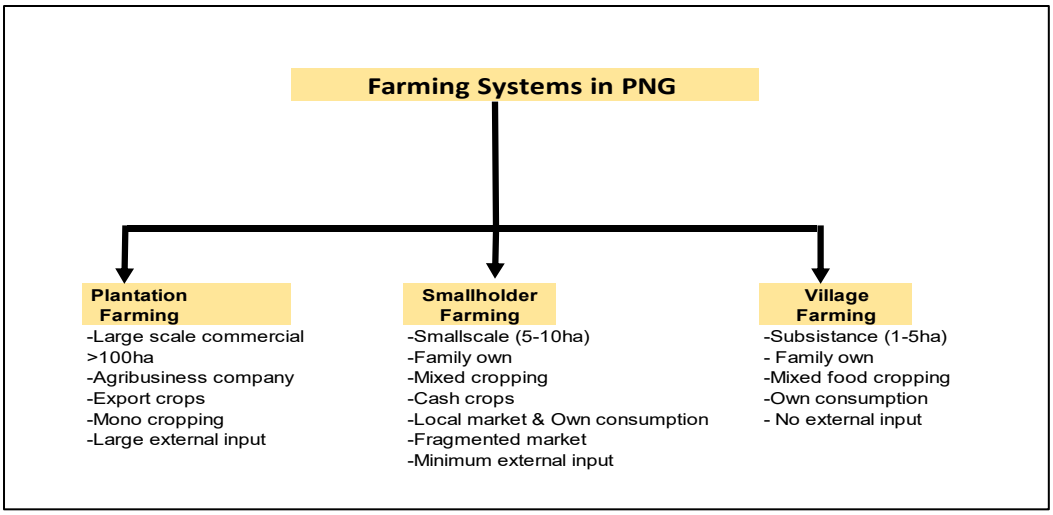

Figure 1: Farming systems in PNG

(Source: http://www.fao.org/farmingsystems/description_en.htm)

In the recent years, the government of PNG has put emphasis in empowering the rural economy through transforming traditional food crops to commercial food crops farming as a way towards addressing poverty, food security and improving socio-economic status of the rural

population (PNG Vision 2050, 2009). The government has realised the smallholder food crops commercialisation is the key to the development process for rural economic growth and poverty reduction (Pingali and Rosegrant, 1995). Furthermore, market-oriented smallholder is viewed as the most effective way to strengthen the linkages between technology, productivity and poverty reduction (De Janvry and Sadoulet, 2009).

The figures below show the trend in sweet potato production compared to other food crops (Figure 2) and cash crops (Figure 3). Though the trend shows that sweet potato production (in total) has increased over the time, its yield (productivity per ha of land) has been below the world average. Amongst the many factors contributing to yield reduction in sweet potato, several researchers have reported, pests and diseases lost are the major cause of yield reduction as high as up to 98 percent (Dotaona et al. 2015; Kapinga et al. 2007).

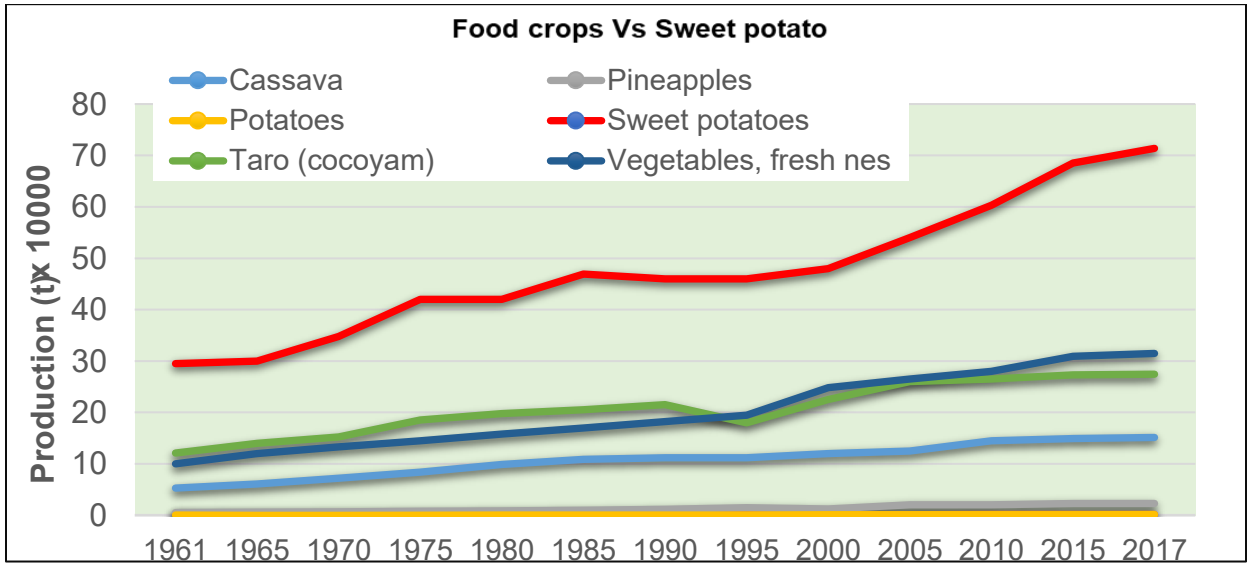

Figure 2: Trends in production of food crops vs sweet potato in PNG

(Source: FAO STATS 2016)

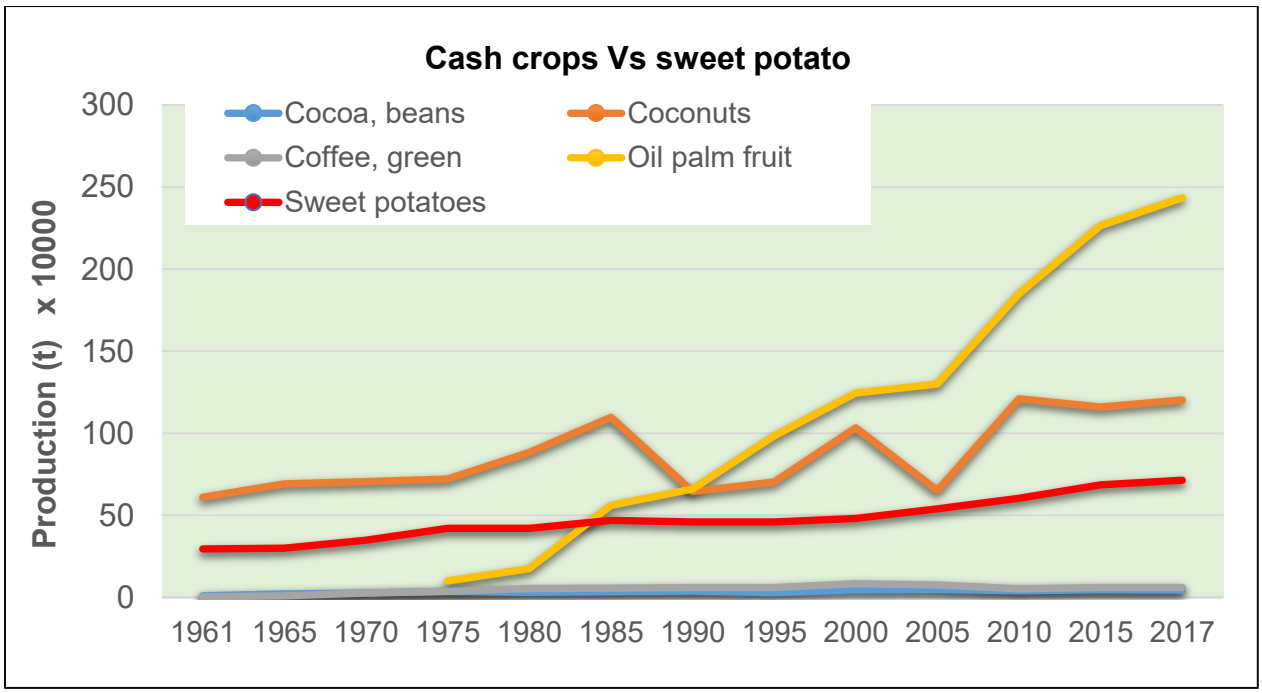

Figure 3: Trends in production of cash crops vs sweet potato in PNG

(Source: FAO STATS 2016)

In some African countries where sweet potato is grown, yield loses have been reported in the range of 30100% due to Sweet Potato Virus Disease (SPVD) (Mekonen et al. 2016), for instance in Ethiopia, according to Tadesse et al. (2013), the tuber yield was reduced to 37 percent due to synergistic infection of Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Chlorotic Stunt Virus (SPCSV). The yield loses due to pests and diseases damages have greatly reduced the income level for the rural farmers, which contributes to poor socio-economic status in many developing countries, where the traditional food crops sales such as sweet potato are the only means of generating income for their family's cash needs.

In PNG, although there are no current reports on sweet potato pests and diseases damage lost levels and their socio-economic impacts, evidence for high dependence on sweet potato for cash income (and as a buffer to food security risk) is clear. In a recent study by Gurr et al. (2016)

in the Highlands of PNG on the level of knowledge on pests and diseases, the farmers have expressed noticeable damage on tubers but not too sure of the level of damage. Their study also reported that West Indian Sweet Potato Weevil (WISPW) (*Euscepes postfasciatus*) was found to be much more widely distributed and common than previously suspected and probably a major problem than well-known Sweet Potato Weevil (SPW) (*Cylas formicarius*). In another study the farmers revealed that weevils (*Cylas formicarius)* are the biggest concern because it affects the quality of sweet potato and reduces the marketable tubers (Dotaona et al. 2015).

The scientists in the Pacific have also recognised the economic significance of the pests and diseases of sweet potato as they consider it as one of the important food crops in the region addressing food security (Beetham and Mason, 1992). Sweet potato pests and diseases management was one of the major focus of research as it affects the tubers quality for consumption and reduces marketable tuber qualities by approximately 75% in some instances. Quite recently the IPDM technology use is being researched in collaboration with PNG research institutions and universities in Australia, namely CSU and UQ under a project funded by Australian Centre for International Agricultural research (ACIAR) with the aim of improving sweet potato production in PNG. Hence this study is based on data collected from this a project in relation to introducing IPDM strategies to control pests and diseases damage in sweet potato production in PNG, and assessing the socio-economic significance of the sweet potato production as a staple food crop in the rural Highlands of PNG.

The IPDM is a concept that integrates various methods and techniques in the management of pests and diseases of agricultural crops in an integrated fashion in a farming system. Norton and Mullen (1994) define IPDM as an approach to making pest control decision with clear understanding and use of multiple techniques to manage pest population in an economically efficient and ecologically sound manner. It is also described as combination of a wide range of

cultural, mechanical, biological and chemical means to control targeted pests and diseases (Rao et al. 2008). The IPDM technology strategy has significant potential to reduce dependence on synthetic chemicals for control of pests and diseases and improve food quality and quantity (Pretty and Bharucha, 2015). Furthermore, this approach to farming keeps a balance between ecological and economic aspects of farm management and can ensure sustainability of the agriculture sector. Thus, they make good sense from public policy perspective.

The IPDM concept emphasizes the integration of pest suppression technologies such as biological control for example, use of beneficial organism against pest organism and cultural controls and crop rotational cultivation to break pest life cycle (Gapasin et al. 2016). The other methods include breeding and cultivation of pests and disease resistant crop varieties. The use of Pathogen Tested (PT) planting material for controlling virus diseases in vegetative propagated crops such as sweet potato is widely practiced in large scale commercial production (Loebenstein, 2015). The PT planting materials contributes to high quality tuber yield and lower the production cost and improve net income of farmers as a direct result of sustain virus diseases control. For example, in Australia, large commercial farmers using PT sweet potato reported archiving consistent yield of 30-35 tonne per ha.

3. Objectives and methodology

The control of sweet potato pests and diseases remains a current challenge for the subsistence rural sweet potato farmers in the Highland of PNG. Where farmers do not practice an active pests and diseases management, this can have a significant impact on the yield and farmers' income (Chang et at. 2016). Few farmers have opted to use traditional cultural practices to manage the pests and diseases, but this method is ineffective to control the pests and diseases of sweet

potato (Gurr et al. 2016). There are also some farmers who use pesticides and insecticides, but they are not readily available and costly to purchase for the farmers. Furthermore, lack of knowledge and adaptation of improved farming techniques and practices to control pests and diseases by rural farmers has also impaired the production level (Bang and Kanua, 2001).

Several studies have recommended the use of management strategies such as cultural practices, phytosanitary measures for controlling vectors to prevent or reduce the extent of damage in food crops, especially for rural farmers who are at times not able to meet expensive chemicals to control pests and diseases. Among these, use of IPDM strategies are ideal in terms of effectiveness in pests and diseases control as well as improvement in yield, quality and sustainability of managing pests and diseases (Maule et al. 2007; Valverde et al. 2007).

In this regard, this study aims to evaluate the socioeconomic impact of IPDM use in controlling pests and diseases of sweet potato and improvement in yield, quality and farmers' income by considering the following objectives:

- Assess and compare the cost of implementing IPDM technologies.
- Establish grower perception on the impact of IPDM performance.
- Evaluate the impact of IPDM on net income from sweet potato production.
- Measure and assess sweet potato yield, quantity and quality of output.

There are two hypotheses (in statistical context) which this study will disapprove:

- Adapting and use of IPDM in control of sweet potato pests and diseases does not lead to increase in marketable sweet potato yield.
- IPDM does not have incremental effect on net income in sweet potato farming.

Several crop protection options (practices) have been developed and are available to smallholder famers to adopt as novel pest management methods (IPDM). For example, prevalence

of sweet potato weevil is a critical issue and there is also a need for clean PT tested planting material to address the losses from viral diseases and nematodes. Postharvest losses to sweet potato roots (tubers) also constrain the development of commercial production and marketing into cities as a cash crop. Hence, development of a novel crop protection method would support commercial production as well as food security for smallholder farmers in PNG.

The activities of this study also include collecting household level data to analyse the socioeconomic impacts of the novel methods adopted by farmers (specifically the use of PT planting materials), as compared to those cultural (traditional) pests and diseases management methods practiced (CP) in the Highlands of PNG. Data was collected from three zones (in total of 12 farmers/field sites) where the farmers have adopted the novel methods, and analysed in relation to the research questions and hypotheses as mentioned above.

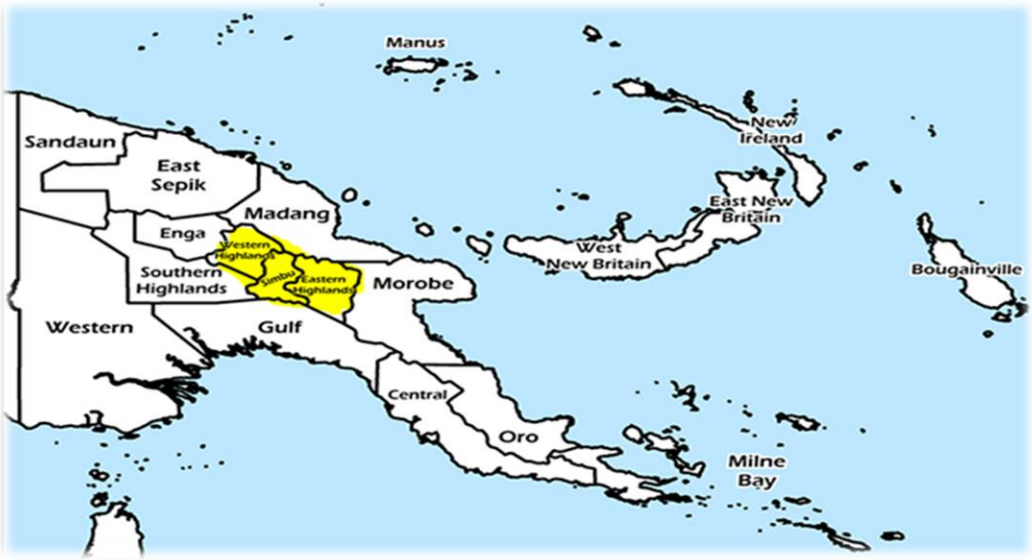

Figure 4: Highland of PNG (study area as highlighted)

(Source: olliedpng.wordpress.com/2013/07/25/the-islands-of-png/)

Farmer Participatory Research (FPR) approach field trails were conducted in the central high-altitude zones (provinces) of PNG for the ACIAR project (Figure 4). Twelve farmers (farm sites) selected from three different zones participated in the FPR for testing and comparing economic impact of PT method with CP method. Each farmer planted both PT and CP vines on a 12m x 16m plots (Figure 5). Socio-economic data collected in relation to crop yields, quality of tubers, income from tubers and their costs of production were used to calculate and compare the Benefit Cost Ratios (BCR) for both methods.

Data collected on farmers' perceptions towards adopting to PT sweet potato planting materials (as compared to the CP method) was also analysed. The farmers' responses were scored using perception rating scale from, 0-2, representing 0 being not effective, 1 for less effective and 2 being effective. Summary of these scores is given in percentages out of the four farmers from each zone.

Figure 5. Typical plot layout in the Highlands of PNG for sweet potato cultivation

(Source: PNG ACIAR project file)

4. Results and discussion

The economic impact of adopting novel method (PT) to control pests and diseases can increase yield and reduce the costs of production. This can therefore improve net farm income. The results demonstrated that the use of IPDM technology (PT planting material) has almost doubled the average marketable quality of sweet potato yield across all three zones compared to CP method used (Table 1). This result is in line with a recent study from Uganda where PT sweet potato planting material yielded 15.0 tonnes per ha compared to traditional sweet potato yield of 2.4 tonnes per ha (Namanda et al. 2019). The low yield from CP method also confirmed by another study reported in Iese et al. (2018).

Table 1. Mean comparison (T-test)

Particulars	Pathogen Tested	Conventional Practice	T-test	P value
Planting material cost (K/ha)	520.83	108.51	28.180	6.578
Labour cost (K/ha)	1323.78	1041.67	2.169	0.026**
Transport and marketing cost (K/ha)	338.25	228.52	2.599	0.012**
Marketable income (K/ha)	7061.75	1694.47	3.038	0.005***
Non-marketable income (K/ha)	890.99	471.58	1.400	0.093
Yield marketable (t/ha)	10.95	4.45	4.704	0.0006***
Yield non-marketable (t/ha)	1.16	1.25	-0.375	0.357

** Significant ($p<0.05$), *** highly significant ($p<0.01$). N= 12 farmers. Marketable and non-marketable yields are based on the quality of tubers. K = PNG currency (Kina).

The yield improvement was seen on both tuber number as well as fresh weight increase. The yield improvement in PT was attributed to low level of damage by pests and diseases on the tubers as compared to CP which had high non-marketable tubers because of pests and diseases damage on the tubers. The improved quality of sweet potato tubers and increased weight have subsequently enhanced income for the farmers by more than 70% on average as compared to CP method. The success results of PT sweet potato planting materials were clearly seen in the yield quality (marketable yield) and the income difference between the PT and CP method (Table 1).

The costs components analysed in this study include planting material cost, labour cost, and transport and marketing cost. Although the yield results were encouraging for farmers to adapt PT planting material, the average cost for PT planting material was four times higher (K520.83 per ha) than the CP planting material cost (K130.2 per ha) across all three zones. The survey results revealed that the transport and marketing cost on average for PT method were high in zone 1 (Jiwaka) and zone 2 (Goroka), ranging from K326.17 per ha to K331.71 per ha, as compared to CP method which showed the cost on average ranging from K191.80 per ha to K280.42 per ha. The transport and marketing cost averaged K353.50 per ha and K326.17 per ha for PT and CP methods respectively in zone 3 (Mt. Hagen) with no significant difference between the two planting material types. Following the compilations of costs and incomes and comparison between PT and CP method, the results showed that average costs of production of PT method across all three zones ranged from K1698.90 per ha to 2700.63 per ha compared to CP method which averaged from K1322.09 per ha to K2144.97 per ha (Table 2).

The average marketable yields compared showed highly significant ($p<0.01$) difference between PT and CP methods. The significance of difference was attributed to PT marketable yields doubled as compared to CP. The increase in yields resulted in significant ($p<0.05$) increase in incomes for PT method. However, the costs for labour, and transport and marketing costs were significantly ($p<0.05$) high for PT method than CP method. This was due to farmers preferring distance marketing which caused increased in costs for

transport and marketing. Despite that overall net return from PT method was higher than CP method (Table 2).

Table 2. Benefit Cost Ratio (BCR)

Zones	Production method	Average income (K/ha)	Average cost (K/ha)	Net income on average (K/ha)	Benefit Cost Ratio (on average)
Jiwaka (Zone 1)	PT	5312.69	2700.63	2612.06	2.09
	CP	2005.34	2144.97	-139.63	0.93
Goroka (Zone 2)	PT	13043.08	1698.90	11344.18	7.79
	CP	4542.92	1322.09	3220.83	3.51
Mt Hagen (Zone 3)	PT	5502.45	2149.08	3353.36	2.22
	CP	1976.14	1330.06	646.08	1.49

For each zone the average is from the four participating farmers. PT = Pathogen Tested; CP = Conventional Practice. K = PNG currency (Kina).

Non-marketable yields difference was statistically non-significant ($p<0.05$), however the mean yield for CP was slightly higher than the mean yield for PT method, reflecting high number of tubers damage caused by diseases in CP than PT method, confirming results reported by Richardson (2010). Similarly, the difference in incomes between CP and PT methods from non-marketable yields was non-significant ($p<0.05$). Furthermore, the planting material costs difference was statistically non-significant ($p<0.05$). Although the BCRs for both PT and CP methods were greater than one indicating a positive net income (except for CP method in zone 1), the BCRs for PT method as greater than CP method indicating that the net income has improved due to tuber yield and its quality (Table 2).

Table 3. Farmers' perception ratings and scores for PT method

Perception attributes	Zone 1	Zone 2	Zone 3	% Score of farmers' responses
Reduction in labour cost	0	0	0	0%
Reduction in pesticide use	n/a	n/a	n/a	0%
Reduction in pesticide cost	n/a	n/a	n/a	0%
Increase in yield	2	2	1	89%
High quality marketable yield	2	2	1	89%
Improvement in income	2	2	2	100%
Extension and support service	2	1	1	78%
Awareness of IPDM	2	1	1	78%
Reduction in insect pests and diseases damage	2	2	2	100%
Willingness of farmers to accept and use of PT method sweet potato production	Yes	Yes	Yes	100%

Ratings: n/a = not applicable; 0 = ineffective; 1 = less effective; 2 = effective. Question response: Yes/No.

To understand farmers' perception towards using pathogen tested (PT) sweet potato planting material in the three zones, participating farmers were asked to report on the changes they had observed and their perception. They were asked to specify whether they had experienced: change in labour cost, reduction in pesticide use, reduction in pesticide cost, increase in yield, high quality marketable yield, income improvement, extension support service for adopting PT method, awareness of IPDM strategy, reduction in

pests and diseases damage, and the willingness to accept and use the PT sweet potato planting material. The results for their responses are reported in Table 3.

All the participating farmers in all three zones responded 100% for willingness to uptake the PT sweet potato production system after seeing the results. They had indicated that the PT planting material eliminated the pests and diseases damage on tubers, which has increased the yield and marketable quality of the tubers and subsequent increase in income. Hence the adoption of PT method can be promoted in PNG for improving rural farmers' income and livelihood through PT sweet potato production system. Sweet potato is also a strategical crop in PNG in relation to addressing food security in the country and that the adoption of PT method can be promoted in the country (Iese et al. 2018).

The farmers perception study revealed that farmers who saw reduction in pests and diseases damage, improved yield and quality and subsequent increase in their income were more (100%) likely to adopt the PT method. The perception study further indicated that adaptation of the PT method would be limited (70-90%) where farmers lack basic knowledge on IPDM and where no government extension services are limited. Need for knowledge and basic awareness of new technology adoption for farmers were found by studies conducted in relation to farmers' perception towards adopting to climate change (for example, Okonya et al. 2014; Urban and Culas, 2020). Another study (Gbetibouo, 2009) on evaluating farmers perception in adopting new farming technologies, found gender and education level had some influence in making decision to adopt the technology. Male farmers had the tendency to rush in to making decisions to adopt new ideas and technologies compared to the female farmers.

The questions on pesticides use and costs were not applicable to all the farmers, because all the farmers were rural farmers and were not able to afford them. About 89% of the farmers responded that there was increase in marketable yield and improvement in the tuber quality. The survey further indicated that 100% of the farmers responded noticeable decrease in pests and diseases damage on the tubers. The overall perception was that 100% of the farmers in all three zones responded their willingness to uptake the PT sweet

potato planting material after seeing the results. Similar perception study on adopting new farming technologies indicated that seeing the actual impacts of new technologies had more influence on farmers' perception and willingness to adopt (Astrid, 2003). Hence extension and agricultural policies should be promoted in PNG towards improving farmers' knowledge and building capacity for the adoption of IPDM.

5. Conclusion

PNG's agriculture is mostly rural based and largely remains as subsistence agricultural farming systems with very little modern farming practices and resource inputs. This has hindered, further improvements in PNG agriculture and improving majority of the rural people's livelihood. This study was done to improve production of sweet potato which is one of the major food crops grown mainly by smallholder farmers for own use as well as for marketing. The on-farm field trials were conducted to evaluate the economic value of adopting PT method compared to the CP method in sweet potato production in the Highlands of PNG. Although the costs per hectare for PT method was higher than the CP method, the net income was comparatively high for the PT method indicating that the use of pathogen tested planting materials is economically viable for the PNG rural farmers. Moreover, the benefit cost ratio for PT method was two times higher as compared to the CP method indicating positive net income for the famers. Hence the adoption of PT method can be promoted in the country for improving yield, quality of production and to enhance farm income and rural livelihood.

However, the ability to cultivate PT sweet potato planting material will only be harnessed if the clean planting material is readily and easily accessible for use by farmers. It is further suggested to use the local varieties for the cleaning and release to the farmers, because local consumers have shown preference to the taste and texture of the local varieties more. This is also good for pests and diseases control with the view that increased diversity of crop varieties corresponds to a decrease in average damage levels and reduces variance of diseases damage as reported with other food crops (Mulumba et al. 2012).

The study also discovered that the farmers' education level, knowledge of the PT method and access to extension services are likely to have more influence on the adoption of the PT technology. It is further concluded that farmer participatory research (FPR) approach, where farmers experience the yield benefits, reduction in pests and diseases damage and improved tuber quality, had more influence on the farmers' perception and willingness to accept the PT technology. Thus, any policy framework developed aimed at enhancing adaptive capacity of other farmers in the rural PNG should consider making use of the factors discussed above on famers' perception for further promotion and adaptation of the PT technology.

Acknowledgement

This research is an outcome of ACIAR funded projects implemented in PNG, under Horticulture Research Program, in relation to improving sweet potato production in the country. The authors acknowledge contribution of NARI, PNG for providing field trials yield data and FPDA, PNG for facilitating socioeconomic farm survey conducted in the study area during 2019-2020.

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Appendix 10. Can biopesticides be incorporated into integrated pest management programs for Australian sweetpotato?

1. Introduction

In Australia, the sweetpotato industry is worth about AU$ 100 million per annum. Sweetpotato production in Australia is centred around two main growing areas: Bundaberg in Queensland (about 90% of production) and Cudgen in northern New South Wales (ASPG 2021). In both regions, the crop can be attacked by many pest insects, but pest pressure can be sporadic and is seasonally dependent. There are many pesticides registered or available for use via a minor use permit for various pests and diseases of sweetpotato (Table 1). The Australian Pesticides and Veterinary Medicines Authority (APVMA) classifies sweetpotato as a minor crop in terms of access to pesticides and some of those used in sweetpotato pest management may be deregistered in the near future. Moreover, some growers have reported ineffectiveness of specific pesticides, for example those registered to control wireworm (bifenthrin, chlorpyrifos). There are no registered effective insect control options for organic sweetpotato growers.

For the simplicity of this discussion paper, for the most part, activity (laboratory, glasshouse, field) of entomopathogenic fungi (EPF) has been recorded against many pest insects (or related insects) that infest sweetpotato and these will not be discussed in further detail here. Whilst there is scope to incorporate softer options like biopesticides into integrated pest management program, there are however, a distinct lack of published field studies on the use of biopesticides in sweetpotato in Australia.

This paper discusses the pests of sweetpotato (*Ipomoea batatas* L.) and current control methods including pesticides and cultural practices and where biopesticides like entomopathogenic fungi might fit into commercial sweetpotato production systems, given sufficient demand for biologicals in integrated pest management. The paper discusses opportunities and constraints for the use of biopesticides in sweetpotato.

2. Pests of sweetpotato

2.1. Virus vectors and foliage pests

Australia has a sophisticated clean planting material program that supplies the majority of the industry with pathogen tested (PT) storage roots that are virus free. There are many sweetpotato viruses in Australia that infect both sweetpotato and related weeds (other Convolvulaceae) and the vectors that transmit these are a major concern (Wolfenden, Henderson et al. 2018) particularly in the generation and multiplication of PT plant material and on-farm nurseries (plant beds). Important vectors include aphids (for example cotton or melon aphid *Aphis gossypii* and green peach aphid *Myzus persicae*), silverleaf whitefly (*Bemisia tabaci*) and leafhoppers (also known as jassids for example *Austroasca* spp.) (Ekman and Lovatt 2015, Wolfenden, Henderson et al. 2018). At planting material multiplication sites, these vectors are controlled with intensive spray programs, although in the main field, these potential virus (and phytoplasma) vectors are less controlled (C. Henderson, pers. communication).

Because of its rapid growth rate, foliage pests are rarely a problem in sweetpotato. Insects such as cluster caterpillar/tropical armyworm (*Spodoptera litura*), Convolvulus hawk moth (*Agrius convolvuli*),

flea beetle (*Xenidia* spp.), green vegetable bug (*Nezara viridula*), mealybug (*Phenacoccus solenopsis*), Rutherglen bug (*Nysius vinitor*), sweetpotato tortoise beetle (*Aspidimorpha* spp.) and sweetpotato leafminer (*Bedellia somnulentella*) (Ekman and Lovatt 2015) occasionally occur; however, this is usually a result of adverse conditions affecting a standard insect control program. Mites (for example bean spider and two-spotted) (*Teranychus* spp.) can also occasionally be a problem (Ekman and Lovatt 2015). Fall armyworm (*Spodoptera frugiperda)* has been observed to consume sweetpotato shoots, leaves and storage roots in the laboratory (B. Wilson, pers. observation), but sweetpotato is unlikely to be preferred host in the field when grasses (e.g. crops like corn are abundant). Provided foliage pest numbers are managed, and particularly while the canopy is initially developing, they are not usually a problem for industry. Closer attention is usually paid to developing plant beds, as they can decimate planting material, and disrupt planting programs while the sprouts recover (C. Henderson, pers. communication).

2.2. Soilborne pests

Root knot nematodes (most commonly *Meloidognyne incognita* and *M. javanica*) are the most important pests for the industry (McCrystal 2010, McCrystal 2014, Ekman and Lovatt 2015). Other species, for example *Pratylenchus* spp. (root lesion nematode) and *Rotylechulus* spp. (reniform nematode) could be problematic under conditions where root knot nematode is less prevalent (C. Henderson, pers. communication).

The sweetpotato weevil (*Cylas formicarius*) can be a major pest in Australian sweetpotato (McCrystal 2010, McCrystal 2014, Uknown 2014, Ekman and Lovatt 2015). Although sweetpotato weevil adults can feed on the crowns and shoots weakening the plant, it is the feeding on storage roots and subsequent egg laying on storage roots that is most problematic. Developing larvae tunnel through the storage root causing cavities: this feeding injury causes necrosis and stimulates the plant to produce ethylene, leading to the release of terpenoids (Uritani, Saito et al. 1975), which make the sweetpotato unpalatable to humans or livestock.

There are other chewing pest insects that can cause significant damage to storage roots; however, feeding injury does not induce terpenoid production. The larval stage of these insects feed on the roots, causing holes in roots, making them unmarketable. Observed feeding damage can range from shallow to deep holes and from shallow to deep tracks along the storage root. Rarely is a single storage root completely damaged with feeding marks, more commonly, 1-3 feeding holes (sometimes quite elaborate) are observed on all the roots within a heavily infested area of the paddock. In conventional sweetpotato farming, these pests are usually well managed with pesticides; however, surface damage leads to product downgrading (sold for processing) or rejection at the market. Whilst there is more consumer 'tolerance' for imperfect produce in organic agriculture and market access to processing of surface damaged produce for organic baby food, damage can be so severe that one third of the harvested sweetpotato is discarded at the packing shed (B. Wilson, pers. observation).

The larvae of the whitefringed weevil (*Naupactus leucoloma* formerly *Graphognathus leucoloma*) causes shallow feeding holes and tracks along the storage root can be a problem, but usually reflects a breakdown in management practices, either via crop rotation ((whitefringed weevils are polyphagous although legumes are a preferred host (Barnes and De Barro 2009)), crop hygiene, or timely pesticide application (C. Henderson, pers. communication).

True (Elateridae) and false (Tenebrionidae) wireworms can be significant pests of Australian sweetpotatoes; however, the species infesting sweetpotato in Australia are not well defined (McCrystal 2010). Due to the proximity of sugarcane fields to sweetpotato production areas, the sugarcane wireworm (*Agrypnus variabilis)* and other true wireworms of sugarcane (species belonging to genera such as *Conoderus* or *Heteroderes*) are considered of agronomic importance (McCrystal, 2010). Based on their presence in Queensland field crops, false wireworms that may be of agronomic importance in sweetpotato include *Gonocephalum macleaya, Pterohelaeus alternatus* and *P. darlingensis* (Robertson 1993, McCrystal 2010)

Curl grubs or the larval stage of cockchafers or other scarabs (white grubs, cane grubs, peanut scarabs e.g. *Heteronyx* spp., *Heteronychus* spp.) (Ekman and Lovatt 2015) can be problematic, but again, usually reflects a breakdown in management practices (poor crop rotation or crop hygiene), or timely pesticide application. Curl grubs feed on lateral and storage roots in the field causing both shallow and deep holes and tracks. In the glasshouse, greyback cane grubs (*Dermolepida albohirtum*) fed on the belowground part of the stem that was developing roots, severing the stem from the roots (Wilson, pers. observation).

Black field crickets (*Teleogryllus commodus*) and mole crickets (*Gryllotalpa* spp.) (Ekman and Lovatt, 2015) can cause significant damage and affect the marketability of sweetpotato (for example almost every storage root at one particular farm in Queensland had at least one chewing mark from crickets, Wilson pers. observation) and but can also be very problematic in chewing holes in drip irrigation tape. Whilst management practices can mitigate those risks, organic growers have no chemical options for their control.

Table 1. Registered pesticides and selected minor use permits for Australian sweetpotato (blue is used commonly) (APVMA 2021, HIA 2021, Infopest 2021).

Target insect or disease	Active	Group (Insecticide)	Registered (R) or minor use permit
Aphids and silverleaf whitefly	Afidopyropen	Group 9D	R
Aphids, grasshoppers (wingless), leafhoppers (jassids), mites, thrips	Dimethoatem, Chlorpyrifos	Group 1B	R
Aphids, thrips, leafhoppers (jassids), organophosphate susceptible two-spotted mite and wireworm	Phorate	Group 1B	PER13902 Version 2
Aphids including green peach aphid & onion aphid	Pirimicarb	Group 1A	PER86443
Helicoverpa, cucumber moth, cluster caterpillar, loopers, webworm, Rutherglen bug, thrips (including Western flower thrip)	Methomyl	Group 1A	PER82428 Version 4
Fall armyworm	Spinosad	Group 5	PER89870
Fall armyworm	Sulfoxaflor	Group 4C	PER84743
Lepidoptera e.g. cluster caterpillar, fall armyworm	Emamectin as benzoate	Group 6	R, PER89263
Lepidoptera e.g cluster caterpillar	Flubendiamide	Group 28	R
Mole cricket, whitefringed weevil, wireworms (activity reported on sweetpotato weevil)	Fipronil	Group 2B	R
Nematodes	Fluensulfone	Nematicide	R
Silverleaf whitefly (likely to be active against other pests like weevils)	Imidacloprid	Group 4A	R
Silverleaf whitefly	Spirotetramat	Group 23	R
Silverleaf whitefly	Pyriproxyfen	Group 7C	R
Sweetpotato weevil and wireworms	Bifenthrin	Group 3A	R
Sweetpotato weevil	Carbaryl	Group 1A	R
Sweetpotato weevil and wireworms	Chlorpyrifos	Group 1B	PER14583 Version 4 under review
Tomato potato psyllid	Spinetoram	Group 5	PER84757 Version 2
Fungal diseases (scurf and root rot)	Thiabendazole	Group 1 Fungicide	PER12047 Version 3
Soil fumigant for weeds, nematodes, wireworms, symphylids, fungal and fungal-like diseases (pre-plant)	1,3-dichloropropene + chloropicrin, metham sodium		

3. Biopesticides in Australia

3.1. Registered products

Currently, there are few registered and available entomopathogenic fungi based biopesticides in Australia. One of these is the Australian indigenous *Metarhizium acridum* product Green Guard® SC Premium, targeting grasshoppers and locusts in the order Orthoptera. Previously, a product based on Australian indigenous *M. anisopliae* (BioCane™) was registered for canegrubs was quite effective; however, is no longer produced. Perhaps the production costs of BioCane™, inconsistency in the end-product (anonymous pers. communication) and the decreased efficacy compared to chemicals like imidacloprid (as a liquid or granule formulation e.g. suSCon maxi Intel), giving 1-4 years protection, made the biological option unfeasible. Often there are formulation and storage (temperature sensitivity) constraints that decrease a biological product's suitability to production and use in the field.

Velifer® Biological Insecticide based on an imported strain of *Beauveria bassiana* from South Africa is registered for suppression of thrips, whitefly, aphids and mites in protected cropping only (glasshouses, shade houses, plastic tunnels), not for open field use, presumably due to its non-indigenous status and unknown effects in the open-field environment (even though this species is ubiquitous in Australian soils as is likely entering the open field through movement of conidia with wind or water etc).

Based on the target pest or the registered uses, neither of these products can currently be incorporated into sweetpotato production (nor in many other non-protected horticultural or agricultural cropping systems). A market exists for the commercialisation of indigenous Australian derived biopesticides that show high efficacy across a range of pest insects associated with several agricultural and horticultural crops.

3.2. Grower interest in 'softer' options and scope for the commercialisation (and registration) of indigenous Australian isolates

In the interest of confidentiality, the identity of growers and potential commercial companies will remain anonymous and the information to follow has been gleaned from ongoing conversations. Organic and conventional growers (of many horticultural and agricultural crops e.g. sweetpotato, citrus, blueberry, macadamia, brassicas, maize, potato, sugarcane) are certainly interested in incorporating biopesticides into their respective pest management programs, should they become available in the future. Growers seem to be aware of the increased costs and associated with biopesticides compared to chemicals but an ongoing dialogue with growers about product efficacy and speed in which insects are managed (or suppressed) is required. Growers are aware of pesticide resistance and are aware that many currently available chemicals are likely to be deregistered in the near future. In the case of organic sweetpotato growers, there is no chemistry available to effectively manage important (and devastating) pests such as weevils, wireworm, curl grubs, crickets and root knot nematodes. These growers rely heavily on crop hygiene and other cultural practices. For example, sweetpotato weevil pheromone lures are used to monitor and potentially disrupt mating cycles by reducing males in the population. In one organic sweetpotato farm, approximately 80,000 male weevils were collected from 20 traps over the growing season and on another farm, other practices

like the installation of light trap 'stations' coupled with water sources around the property to lure an array of beetles or crickets away from the crop (e.g. African black beetle, adult cane grubs, cockchafers or other scarabs). However, it is not just organic growers that see a use for softer options. More sweetpotato growers are reporting increased damage to storage roots at the end of the season, when chemicals cannot be applied due to withholding periods and maximum residue limits. Biopesticides could be applied here to fill that gap, provided that effective control or more control than that recorded in untreated sweetpotato could be achieved. Supermarkets downgrade product (from 'premium' to 'composite' if insect feeding holes are present) based on consumer preference and demand for blemish/insect damage-free produce. Retailers (and consumers) get less fussy when sweetpotato is in short supply, although oversupply is the more common state of the market. In conventional sweetpotato production, roots with minor insect damage (or oversized roots) can be sent to facilities for processing (e.g. chips, or baby food) or to food supply businesses (e.g. centralised facilities that supply pre-cut vegetables to hospitals for cooking). Generally, there is more consumer tolerance for imperfect produce from organic farms, and perhaps this is the most logical starting point for biopesticides in Australia.

There is a gap in the organic certified and conventional markets for options like entomopathogenic fungi that are used individually or as a part of insect spray programs to decrease reliance on chemicals, for improved safety/health (humans and the environment), environmental sustainability and decreased pesticide resistance. The pre-commercialisation, commercialisation and registration processes are time-intensive (years of trials depending on target crop and pest insect) and come at significant costs (realistically >AU$2M). Whilst there has been significant government investment in biopesticide Startups in the USA, investment in small Australian companies is not comparable. Before an agricultural chemical can be supplied for use in Australia the active constituent must be approved, and the formulated product registered with an approved product label. Because of the cost involved in registration, prospective (smaller) Australian-based companies are probably more likely to develop or invest in a product that has proven activity against many insects and host crops.

Steps or information involved in the regulatory process to meet the requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) are extensive (https://apvma.gov.au/node/11196) and are detailed briefly below:

- Biology, manufacture
    - Specific data requirements for a biological active constituent (e.g. *Metarhizium or Beauveria* spp*.)* and product are often reduced and different to that of a chemical.
- Toxicology
    - Health hazards of active constituent and formulated product
- OH&S
    - The product to be registered is safe to workers using the product
- Environmental risk
    - Environmental fate and effects
    - Toxicity to mammals, birds, aquatic organisms, invertebrates and nematodes, bees, other microorganisms
- Efficacy and crop safety

- Label claims of efficacy and no phytotoxicity are supported by sound scientific evidence (laboratory assays and repeated extensive field trials on the target pest and target crop)
- For example, a minimum of two seasons of field data in the various major growing regions of each crop (and insect target).

4. Opportunities

4.1. Compatibility of sweetpotato production with entomopathogenic fungi: land preparation, fertilisation and pesticide application

In this section of text, the compatibility of biopesticides like entomopathogenic fungi (EPF) is discussed in terms of how a developed product could fit into the sweetpotato production system. *Metarhizium* spp. and *Beauveria* spp. are already ubiquitous in Australian soils, for example all of the isolates used in testing at the University of Southern Queensland were isolated from main sweetpotato growing areas, so there is already a degree of compatibility with current sweetpotato production systems if these fungi are able to persist. However, the concentration of indigenous infective propagules to manage pest populations just is not great enough to be effective and requires an inundative application (i.e. application of EPF throughout the crop growing season) approach for management or suppression.

4.2. Preparation of nursery beds sprout production

Typically, growers install nursery beds for sprout production away from the commercial production area, on land where the soil is well managed through crop rotation (at least 3 years between sweetpotato crops) and good cultural practices. Where there is a history of pests and diseases, some growers may opt to treat the soil where the plant beds will be formed using fumigants (e.g. metham sodium) or soil solarisation (rarely), which offer a temporary suppression of weeds, pests and diseases (as well as beneficial fungi /bacteria and invertebrates). Typically, the fumigant would be injected whilst the bed is mechanically formed, irrigated then left to activate. After the appropriate re-entry period, storage roots are positioned on a flat soil bed and covered with a shallow layer of soil. Fertiliser is applied on top of this soil layer (or during bed formation) and the bed would be sparingly irrigated until sprouting occurs. If soil borne pests are an issue (e.g. organic growers), there could be scope to apply a granular formulation of a biopesticide, especially if there was a risk that insect feeding activity could introduce pathogens that accelerated storage root breakdown (rot) and subsequent reduced sprout production in plant beds. Because the purpose of a nursery beds is to produce planting material (spouts) for the commercial field, the focus is usually on maintaining pest free sprouts chemicals like imidacloprid and spirotetramat to control sucking insects like whitefly or aphids that are virus vectors.

Liquid or emulsifiable concentrates (EC) or emulsifiable suspensions (ES) of EPF could be applied here in rotation with imidacloprid and spirotetramat after compatibility has been established, which is variable. Furlong and Groden (2001) showed synergism and compatibility of *Beauveria bassiana* with sub-lethal doses of imidacloprid for the control of Colorado Potato beetle, Russel *et al.,* (2010) demonstrated compatibility and synergism with imidacloprid and *Metarhizium brunneum* on Asian

longhorned beetles, and Abidin *et al.* (2017) demonstrated compatibility with imidacloprid and *M. anisopliae* and *B. bassiana*. James and Elzen (2001) showed that whilst imidacloprid did not negatively affect spore germination or colony formation of *B. bassiana in vitro,* combining the two resulted in reduced effectiveness of imidacloprid, possibly due to reduced feeding by whitefly (*Bemisia argentifolii*). Other studies found the interaction of imidacloprid or spirotetramat with *B. bassiana* and *Metarhizium* spp. be incompatible, resulting in decreased mycelium growth, spore germination and spore production (Sain, Monga et al. 2019, Yadav, Ranade et al. 2019).

4.3. Commercial production area

Soil preparation for the main sweetpotato growing area varies considerably between growers. Soil management practices are evolving in the industry, for example 'new' soil management approaches like permanent plant beds in the main growing area that sweetpotato and cover crops are planted into (Stirling, Stirling et al. 2020) these aim to reduce soil erosion, improve soil organic matter and soil 'health', minimise tillage and reduce the populations of root knot nematodes by encouraging beneficial microorganisms and free-living nematodes. Crops like forage sorghum (*Sorghum bicolor*) are regularly used as a cover crop; sorghum is mulched and can be incorporated into the soil with a rotary hoe. There is scope to add EPF to the soil when planting cover crops or prior to/as the crop is mulched, so there is time for the EPF to colonise the soil, grow saprophytically and infect insects prior to planting with sweetpotato. Whether this infection is great enough to offer suitable control remains to be seen.

Prior to planting the shoots, various pesticides are applied to these formed, main production hills (or beds or rows) including the pre-plant insecticide fipronil (to manage whitefringed weevil and wireworms) and the pre-plant nematicide fluensulfone. Fipronil is registered for application as a broadcast spray and incorporated before planting. A basal fertiliser may be applied after these chemicals and provided there was sufficient time between fipronil application (field compatibility is unknown or the time needed between spraying fipronil and incorporating biopesticides has not been established), a biopesticide might be incorporated as granules as targeted bands in the ridge of the hills (or along with fertiliser using a spreader) to allow the EPF to start 'acclimatising' to the soil to begin saprophytic growth or entomopathogenic growth should suitable hosts be present. The compatibility of fipronil with EPF is variable (owing to different formulation, variation in EPF and experimental conditions) and like other compatibility studies has mostly been examined *in vitro*. Although this may be seen as flawed, the EPF are exposed to the maximum dose of the pesticide, which is useful when assessing compatibility. Moino and Alves (1998) demonstrated that in the presence of 'an average' recommended rate and a sublethal rate (70% reduction of recommended rate), fipronil significantly reduced the growth of the colony and conidia production compared to the control for both *M. anisopliae* and *B. bassiana*. However, based on their chemical classification model for toxicity to EPF (Moino, Alves et al. 1998) fipronil was deemed to be compatible. In their work, Sain *et al.,* (2019) showed variability in fipronil toxicity depending on species of EPF and dose (surprisingly for some isolates, the toxicity was reduced when the full recommended rate was using, compared to using half rates).

After the shoots are planted and irrigation tape is laid (T-tape is usually laid at the same time shoots are planted), the shoots are irrigated immediately to decrease transplant stress. Granular EPF

formulations or EPF conidia-colonised grain (colonised rice, barley, maize that support the growth of EPF propagules in soil (Ekesi, Maniania et al. 2005, Ekesi, Maniania et al. 2011, Mayerhofer, Rauch et al. 2019) could be added as the shoots are being planted (there is some movement of soil which would cover over granules, protecting conidia from UV and desiccation) or a liquid formulation could be applied through the T-tape via direct injection (throughout the season if required). The irrigated soil provides an ideal environment (high humidity and optimal temperatures) for the proliferation of EPF. After some weeks, the sides off the rows are scuffled to remove weeds, simultaneously moving soil up the faces of the beds, providing additional coverage of soil around the shoots (and any exposed EPF).

During the growing season, several insecticides may be applied to the crop at various time points depending on registration/minor permit use. These include oxamyl (registered for nematode control) as well as chlorpyrifos (to reduce cricket damage to irrigation tape, weevils and wireworms) and bifenthrin (to control weevils and wireworms). Occasionally foliage feeders are treated especially in the first month or so after planting, when the shoots are establishing if large numbers of eggs are observed. One published study demonstrated no adverse impact of oxamyl on *B. bassiana* (Anderson and Roberts 1983); however, there is no literature on the effects of other EPF such as *M. anisopliae*. Amutha et al. (2010) demonstrated only a minimal toxic effect (colony radial growth and germination) of 20% EC chlorpyrifos on *B. bassiana* when used at the full recommended rate. In sugarcane, chlorpyrifos (Lorsban) and bifenthrin (Talstar) had little effect on colony radial growth in the laboratory or on spore germination from field retrieved spores (Samson et al., 2005). Although compatibility, synergy or antagonism may vary between EPF isolates and specific pesticides, there appears to be scope to include biopesticides in an integrated approach to managing pests in sweetpotato. Their success in providing effective pest management in the field; however, is unknown.

5. Considerations for successful biopesticides and constraints for their use

Effective production and formulation are major components that contribute to the success of a biopesticide; living microorganisms need protection against solar radiation and moisture and temperature fluctuations that cause desiccation, in order to maximise efficacy. The biopesticide also needs to be simple to produce, affordable and have an acceptable shelf-life (Jackson, Dunlap et al. 2010). The ability of the biopesticide (or its conidia in the case of EPF) to persist in the soil and remain virulent against insects is influenced by several factors. These include insect hosts, temperature (e.g. optimal temperature between 15-35 °C), availability of moisture in the soil and relative humidity to initiate germination, availability of nutrients for growth and the density of microbial antagonists and subsequent fungistasis (Jaronski 2010). In one trial in Bundaberg, soil temperature in sweetpotato plant beds was found to fluctuate between 20 °C and 35 °C in moist soil, and in drier soil, regularly spiked at 45 °C between late Spring and early Autumn (Wolfenden, Henderson et al. 2018). Although the soil temperature in the main growing area might be contrasting to that observed in this plant bed, the conditions here (good soil moisture supplied by irrigation tape and soil temperature) are likely to favour the persistence and potentially the resporulation of EPF like *M. anisopliae and B. bassiana*, especially if applied through the irrigation tape where conidia would be deposited in and around lateral and developing storage roots. Several isolates of *Metarhizium* spp. shown to be effective in laboratory and glasshouse studies were isolated from sweetpotato farms in Bundaberg and Rockhampton, demonstrating their ability to remain infective despite high soil temperatures (Wilson,

unpublished data). Many EPF have been shown to colonise the rhizosphere of plants (ie. are rhizosphere competent) and to colonise many species of plants endophytically (Vega 2018). Whilst *M. anisopliae and B. bassiana* appear to not be endophytes of sweetpotato (Wilson, unpublished data), it is unlikely that if they were in fact colonisers, would the level be great enough be effective in the field in high pest pressure situations. Whether the applied biopesticide successfully persists in the soil in great enough numbers to protect developing storage roots from insect damage is unclear. In a QLD based trial, the dose of applied *M. anisopliae and B. bassiana* in sweetpotato was not high enough to prevent storage root damage by a range of insects, likely owing to the fact that biopesticides are much slower to act than conventional pesticides, giving the insects ample opportunity to chew the storage roots before dying (if they actually died) (Wilson, unpublished data). However, despite the inability to prevent root damage (slow to act, not host specific, ineffective concentration despite being economically viable?), the conidia of both *M. anisopliae and B. bassiana* remained infective at the end of the season (4 months post-application), resulting in death of sweetpotato weevil and mealworm (used as a proxy for false wireworm) in a laboratory assay (Wilson, unpublished data), highlighting the possibility of effective persistence in the soil.

Wireworm activity is typically prevalent in the weeks leading up to harvest and the efficacy of soil insecticides generally does not persist in soil throughout the cropping period (McCrystal 2014). Research on soil incorporated pesticides in grains and sweetpotato (e.g. fipronil, bifenthrin) suggest they act more to repel wireworms rather than cause direct mortality (Robertson, 1983; McCrystal, 2010, Vernon & Van Herk 2013). Control is problematic when withholding periods exclude the use of pesticides when they are needed the most. Some *Metarhizium* spp. have been shown to repel insects, including wireworm (Kabaluk, Vernon et al. 2007) and sweetpotato weevil (Dotaona, Wilson et al. 2017). This repellence could be used to deter insects from entering commercial crops, either through inundative application of a biopesticide or through the use of the volatile organic compounds (or synthetically produced) for enhanced crop protection.

In a field experiment by McCrystal (2010), a local *M. anisopliae* isolate was tested in nursery plant beds to determine efficacy against the sweetpotato weevil, *Cylas formicarius*. Despite successful laboratory bioassays, at 'low' and 'high' rates, *M. anisopliae* was unable to reduce tunnelling in plant bed sprouts when sprayed over 4 occasions, compared to bifenthrin, which binds tightly to the soil, offering more protection to the plants when weevils come into contact with the soil. Whilst the author concluded that the *M. anisopliae* was ineffective, it is difficult to draw conclusions about the experiment given information about the viability of spores, and environmental and plant bed management data (soil moisture, temperature, irrigation etc) was not made available. It is possible that in the formulation and climate applied (e.g. insufficient humidity, high temperatures), the spores were unable to persist long enough to cause infection or insufficient contact was made between spores and the weevils to prevent sprout damage (storage root damage was not assessed). Likely, the rate of kill was just too slow to be effective and perhaps EPF are best combined with existing insecticides in an integrated pest management approach.

6. Concluding remarks

International deregistration of important pesticides, some of which are used in Australian sweetpotato production, will likely drive the increased uptake of biopesticides globally. The most

logical starting point to promote biopesticides is with organic sweetpotato growers and conventional growers that are reducing pesticide use (for example chlorpyrifos) and looking to complement their existing pest management strategies. However, it is unlikely that biopesticide manufacturers will invest in a product that targets pests of sweetpotato only, given that sweetpotato is considered a minor crop. Whilst there is scope to incorporate biopesticides into Australian sweetpotato production in terms of synthetic pesticide compatibility and application with existing equipment and technology, validation is required in the field. This validation requires trials with over many sites and seasons to capture adequate pest pressure, ideally with commercially formulated products that can withstand high temperatures and offer UV protection to the entomopathogenic fungi to ensure longevity, persistence and virulence.

7. Acknowledgments

Many thanks to all the sweetpotato growers and the sweetpotato researchers who engaged in conversations about the sweetpotato production system, pesticide use and the potential adoption of softer options like biopesticides for the control of important pests.

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West Indian Sweetpotato Weevil

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Description

Adults of the West Indian Sweetpotato Weevil (*Euscepes postfasiatus*) (Fig.1) are less than 4 mm in length, are red-brown to grey-black in colour, with white markings on the wing case.

Fig.1 Adult West Indian Sweetpotato Weevil

The larvae and pupae are creamy white and are found in the stems (Fig.2) or storage roots of sweetpotato (Fig.3).

Fig.2 Pupa in the crown of a sweetpotato

Fig.3 Larvae in a sweetpotato

Monitoring and detection

The size and colour of this weevil make it hard to see against the colour of the soil and colour of the sweetpotato roots (Fig.4). Look for holes and other damage at the base of the stem (crown) and break open the stem to inspect for weevil tunnelling and the presence of pupae/larvae.

Fig.4 Adult weevil on a sweetpotato

Management

- There is no pheromone to attract & trap the West Indian Sweetpotato Weevil.
- Use clean planting material and inspect vines for insect damage before planting.
- Avoid planting sweetpotato in gardens or on land that has had sweetpotato in the previous years.
- Keep the soil moist where possible.
- Regularly cover exposed roots or cracks in soil to stop weevils reaching the roots to feed and lay eggs.
- Remove volunteer sweetpotato plants in and around the planted crop.
- Remove vines and roots from the soil when the crop is harvested.
- Destroy infested roots by burning to kill developing weevils or give infested roots to animals for feed.

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NARI

Prepared by: HORT 2014/083 Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea

Appendix 12b. Preventative application of entomopathogenic fungi to manage soil dwelling insects in sweetpotato *Ipomoea batatas*

1. Introduction

Glasshouse experiments were established to test the efficacy of a crude inoculum of entomopathogenic fungi on mealworms, as a proxy for false wireworms in sweetpotato. In this experiment, the damage to sweetpotato storage roots, insect mortality and endophytic capacity of *Metarhizium anisopliae* and *Beauveria bassiana* was examined. In addition, the persistence of propagules and their subsequent virulence on an artificial 'second wave' of insects was assessed. An additional glasshouse experiment was set-up owing to the substantial cross-contamination between treatments (air movement in the glasshouse bay) that also sort to examine the damage to sweetpotato storage roots, insect mortality and endophytic capacity of *Metarhizium anisopliae* and *Beauveria bassiana.*

2. Materials and Methods

2.1. Production of fungal inoculum and preparation for experiments

Metarhizium anisopliae isolate ECS and *Beauveria bassiana* isolate KS1 were maintained on Sabouraud dextrose agar with yeast (SDAY) at 27 °C with a 12:12 day:night photoperiod. After 14 days, a 1 cm^2 square of sporulating culture was excised from each agar plate and used to inoculate 250 ml of SDY liquid broth. Flasks were incubated at 27 °C at 150 rpm for 7 days in a shaking incubator (New Brunswick 450, Eppendorf, Australia). Solid substrate was prepared by autoclaving 1 kg of Australian Certified Organic (basmati) long grain rice in a heat-sealed spawn bag (Microsac, Belgium part no.) at 121 °C for 20 min. A corner of each spawn bag was cut and 500 ml of tap water was added before autoclaving for a second time at 121 °C for 20 min. Cooled rice was inoculated with 100 ml of 7 day old liquid culture (approx. 10^9 blastospores per ml) and heat sealed under aseptic conditions. Spawn bags were massaged to evenly distribute the blastospores, then incubated at 27 °C for 14 days in a controlled temperature room. Conidiated rice was transferred to a paper bag (25 x 40 cm) and dried at low humidity (ca. 30% RH) at 20 °C for an additional 14 days to reduce moisture.

Forty-eight hours prior to use, conidia germination and conidia per g dried rice were calculated. Both isolates had >92% germination. Isolate ECS1 had 2.2 $x10^8$ conidia per g of rice and isolate KS1 had 2.8 $x10^9$ conidia per g of rice.

2.2. Preparation of sweetpotato planting material

Ipomoea batatas cv. 'Orleans', sourced as storage roots, were used in the glasshouse experiment. Storage roots were pre-sprouted in a controlled temperature room at 27 °C +/- 2 °C with a 12:12 day:night photoperiod at 50 % relative humidity for 5 weeks prior to experimentation. Roots were classed as 'mediums', had an average weight of 405 g (+/- 9 g) and average diameter of 6 cm +/- 0.87 cm.

2.3. Insects

Yellow mealworm larvae (*Tenebrio molitor*, Coleoptera: Tenebrionidae) were sourced from BioSupplies, Yagoona, NSW, Australia. The mealworms were reared at 27 °C in a diurnal light regime and were supplied with oatmeal and whole sweetpotato roots as a food source.

2.4. Experimental set-up and design

2.4.1. Glasshouse experiment 1

Plastic pots (10L) were half-filled with high quality potting mix (Searles, Bunnings, Toowoomba) and a sweetpotato storage root was placed horizontally. Additional potting mix was added to cover the roots, before the inoculum was added to the surface of the soil. The five treatments applied were i) control (sterilised mix of ECS1 and KS1, approx. 3 g per pot), ii) KS1 at 1 x10^6 conidia per g of soil, iii) ECS1 at 1 x10^6 conidia per g of soil, iv) KS1/ECS mix at 1 x10^6 conidia per g of soil and v) KS1/ECS mix at 1 x10^7 conidia per g of soil. Following application of inoculum, a 5 cm layer of potting mix was added to minimise cross contamination of conidia in between treatments. Prepared pots were placed onto a saucer inside an insect rearing cage (BugDorm-4S2260, Bugdorm, Taiwan) and received 250 ml of tap water. The pots were only watered again when at least one sprout was visible so as not to rot the storage root. There were 10 replicates per treatment and cages were arranged into blocks. The storage roots were allowed to sprout for 30 days prior to adding 10 larval mealworms (0.04 +/- 0.0025 g per individual, 13-15 mm) to the centre of each pot. Pots received 500 ml one a week, starting 24 h after the addition of the mealworms.

Storage roots were due for harvest, assessment of mealworm damage and assessment of mealworm mortality during COVID-19 lockdowns but it was not possible to complete this in a timely manner and

this was further restricted by personal circumstances. By the time the trial was due to be harvested, no live mealworms were found (either as larvae, pupae or adults), including in the control, where there was significant cross contamination (predominately *Metarhizium anisopliae*) and death (mycosis and evidence of bacterial infection). To check that there will still infective propagules in the soil, 50 g of soil was taken from each pot and placed into a sterile 70 ml plastic container. To this, 10 larval mealworms were added, with the addition of 2 ml of sterile water. A perforated lid was added to each container and the tubs were inverted weekly. All containers had evidence of infective propagules as measured by conidiation on mealworm cadavers. Even though there was evidence of cross contamination, which would inevitably cause death in the control pots, an additional 30 larval mealworms were added to the pots (11/2/2021).

2.4.2. Glasshouse experiment 2

Plastic pots (10L) were half-filled with high quality potting mix (Searles, Bunnings, Toowoomba) and a sweetpotato storage root was placed horizontally. Additional potting mix was added to cover the roots, before the inoculum was added to the surface of the soil. There were five treatments i) inside' control (sterilised mix of ECS1 and KS1, approx. 3 g per pot), ii) 'outside' control (as for inside control except placed into an isolated glasshouse bay, iii) KS1 at 1 x10^6 conidia per g of soil, iii) ECS1 at 1 x10^6 conidia per g of soil, iv) KS1/ECS1 mix at 1 x10^6 conidia per g of soil. Following application of inoculum, a 5 cm layer of potting mix was added to minimise cross contamination of conidia in between treatments. Prepared pots were placed onto a saucer inside an insect rearing cage (BugDorm-4S2260, Bugdorm, Taiwan) and received 250 ml of tap water. The pots were only watered again when at least one sprout was visible so as not to rot the storage root. There were 5 replicates per treatment and cages were arranged into blocks. The storage roots were allowed to sprout for 30 days prior to adding 30 larval mealworms (0.04 +/- 0.0025 g per individual, 13-15 mm) to the centre of each pot. Pots received 500 ml one a week, starting 24 h after the addition of the mealworms.

2.5. Assessment of mealworm damage to roots and growth on EPF on the soil substrate

Dead mealworms were removed weekly: cadavers were placed in a humid chamber (90 mm Petri dish containing moistened paper towel) and incubated at 27 °C to encourage conidiation. Cadavers were then scored as 'mycosed' or 'other' (bacteria). The presence of EPF actively growing on the soil surface was recorded.

2.6. EPF endophytic status

For experiment 1 and 2, the presence of *M. anisopliae* or *B. bassiana* colonising sweetpotato was examined across all treatments at the experiment's end. After all insects had been removed from the pots, the plant was divided into storage roots or shoots. For experiment 1, lateral roots and leaves were examined in addition to storage roots and shoots (stems). Storage roots were gently scrubbed before being photographed on the ventral sides, ensuring that all mealworm damage was captured. For experiment 1 only, the storage root from each pot was halved transversely, one ½ was used for endophytic fungi status and the other ½ was used in laboratory bioassays to challenge mealworms as 'infected' roots (see section 2.7 Infectivity of storage roots on mealworms).

In an endophyte-only experiment (glasshouse experiment 3), the presence of *M. anisopliae* (ECS1) or *B. bassiana* (KS1) colonising sweetpotato (cultivar white skin purple flesh or WSPF) was examined in sterile potting mix/sand. Shoots of WPSF were planted into 10 L citrus pots, burying two nodes. A 10^8 conidia per g potting mix layer was added, and additional potting mix/sand was added, burying a 3rd node. Sweetpotato plants were grown for 40 days before the shoots and lateral roots (no storage roots) were processed for endophyte assessment.

For each plant sample, the plant parts were halved and a sub-sample of storage roots (1 cm^3), lateral roots (1 cm) and shoots (1 cm) were washed in tap water then placed into separate sterile 70 ml containers with 70% ethanol in aseptic conditions. After 60 sec, the containers were drained and 3% NaOCl was added, covering the plant tissue. The various tissues were surface sterilised for 3 mins before being rinsed thrice in sterile tap water and subsamples from each tissue type of each plant were blotted dry on sterile filter paper (Whatman). For each plant, 5 samples of each tissue type were randomly selected and plated out onto MEA and SDAY Petri plates (90 mm) amended with antibiotics (chloramphenicol and cycloheximide) for a total of 10 individual samples per tissue type per plant. The effectiveness of the surface sterilisation process was tested by making an imprint of individual tissue types (leaves, stems, lateral roots and storage roots) on MEA with and without antibiotics. Plates were sealed with Parafilm and incubated at 25° C with a 12:12 light:dark photoperiod. Only outgrowth of fungi was of interest in this experiment. After 48 h, plates were inspected for growth and faster growing colonies were sub-cultured onto new MEA plates without antibiotics and incubated as described previously. Slower growing colonies were allowed to grow for up to another 7 days before being sub-cultured. The original plates were incubated at room temperature to allow other fungi to grow. Colonies that were not overgrown were sub-cultured as described above. All fungal cultures continued to be sub-cultured, until a pure culture was obtained. Representative cultures were described morphologically (colony colour) and photomicrographs were taken of fungal structures

using an Olympus X53 microscope. To prepare for DNA extraction, 3 x 0.5 cm^2 squares of pure culture of representative isolates (i.e. if morphologically inseparable cultures were present in every plant sample, a maximum of ten samples for that culture were prepared) were placed into 2 ml tubes in sterile water and frozen at -80 °C. The DNeasy® PowerSoil® kit (Qiagen, Australia) was used to extract genomic DNA from all samples. To identify the fungi inhabiting the various plant parts, the 5'region of elongation factor-1 alpha (EFT1) was amplified with primers EF1T/EF2T or ITS1 and ITS4 primers. Each PCR reaction was 25 µl and contained 12.5 µl GoTaq®2x GreenMaster Mix (Promega, Alexandria, NSW, Australia), 1 µl of each forward and reverse primers (10 mmol), 9.5 µl of nuclease-free water and 1 µl of fungal DNA (at 25–30 ng/µl). The PCR conditions were an initial denaturation at 94 °C for 3 min, then 34 cycles of 30 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C, with a final extension of 10 min at 72 °C. PCR products were sent to Macrogen Inc. (Seoul, South Korea) for PCR purification and DNA sequencing.

2.7. Infectivity of storage roots on mealworms

For glasshouse experiment 1 only, after the termination of the experiment, mealworms were re-exposed to storage roots that had been growing in control or entomopathogenic fungi treated soil (i.e. the same treatments and replicates as above as for experiment 1). The remaining part of the storage root from the endophyte assessment was scrubbed and surface sterilised with 70 % ethanol, sprayed with NaOCl, then rinsed with water. Storage roots (halves) were wiped down with paper towel and added to 500 ml capacity plastic containers with ¼ cup of oatmeal in the bottom. To each tub, 10 mealworms were added, and these were incubated at 27 °C in a 12:12 h photoperiod. After 14 days, mortality was recorded. Dead cadavers were no external conidiation were incubated using the same regime described above in a moist chamber to encourage external mycosis.

2.8. Statistical analysis

Mealworm mortality was analysed with ANOVA after checking for normal distribution using Genstat 21st Edition (VSN International Ltd). Where necessary, data were transformed prior to analysis.

3. Results

3.1. Glasshouse experiment 1: Insect mortality (1st release)

There were no surviving mealworms following the first release of insects, owing to the substantial delay in insect assessment. Mealworms (larvae, pupae or adults) that were not showing signs of mycosis had either disintegrated or were infected with bacteria. Those with mycosis often had had prolific mycelial growth and conidiation, which extended into the soil i.e. growth on the soil surface was evident and this often extended for several cm around the cadaver. Mycosis and fungal growth on the soil occurred across all treatments, including the control, owing to considerable cross contamination of fungal spores in the glasshouse.

3.2. Insect mortality (2nd release)

3.2.1. Glasshouse experiment 1

Mortality in the control was high over the course of the experiment, presumably due to significant cross-contamination in the glasshouse bay. Overall, death from mycosis was low (less than 55%) (Figure 1). The remaining insects either died from bacterial infection at the larval or pupal stages or became adults.

At 14 days after exposure (DAE), insects inoculated with KS1 or ECS had significantly greater mortality (P=0.008) compared to all other treatments. The lowest and significantly different morality was observed in the pots inoculated with the high concentration of ECS/KS1. At 21 DAE, insects inoculated with KS1 had significantly greater mortality compared to all other treatments (P=0.006); however, treatment was not significant at 28 DAE. At 35 DAE, there was significantly greater death in KS1 treated insects (P=0.044) although the other treatments did not differ significantly from each other (Figure 1).

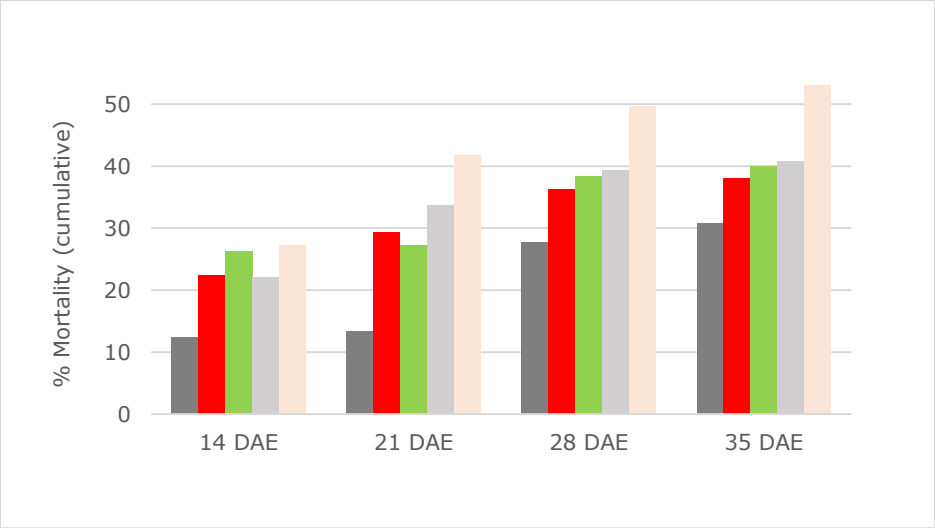

Figure 1. Glasshouse experiment 1 (2nd insect release). Effect of treatment on the mortality of mealworms over time. Values are the mean of 10 replicates. Dark grey (high concentration mix), red (control), green (*M. anisopliae*), cream (*B. bassiana*), light grey (low concentration mix).

3.2.2. Glasshouse experiment 2

There was no insect morality in the 'outside' control. By the experiment's end however, for the 'inside' control treatment, there was almost 15% morality caused by mycosis (predominately *M. anisopliae* or ECS1 was found sporulating on cadavers, but several individuals were sporulating with *B. bassiana* or KS1). At each harvest, the 'Inside' control always had significantly less mortality/mycosis that the EPF treated insects (Figure 2). At 11 DAE, the mortality of EPF treated insects (KS1, ECS1 or a mix of KS1/ECS1) did not differ significantly (P=0.013). At 16 DAE, insects treated with ECS or the KS1/ECS1 mix had significantly greater mortality than that recorded in KS1 treated insects (P<0.001). At 21 DAE, more than 50% mortality was recorded for all EPF treated insects; none of which differed significantly (also for 26 DAE and 32 DAE). By 37 DAE, the greatest mortality was achieved in ECS treated insects (73.3%), which different significantly to KS1 treated insects (60.6%) but not the KS1/ECS1 mix treated insects (70.6%) (P<0.001).

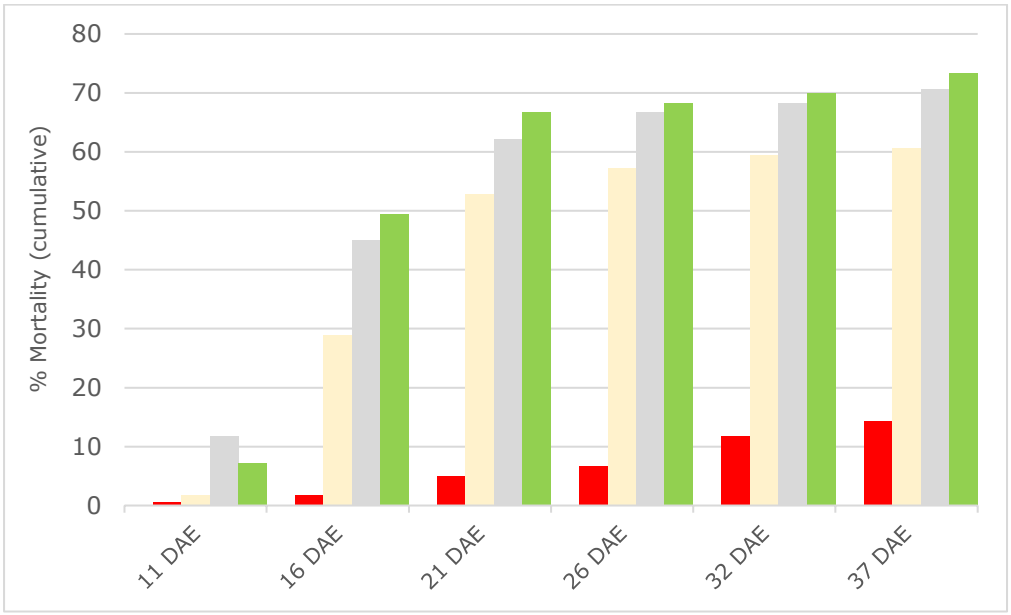

Figure 2. Glasshouse experiment 2. Effect of treatment on the mortality of mealworms over time. Values are the mean of 5 replicates. Red (control), green (*M. anisopliae*), cream (*B. bassiana*), light grey (mix of *M. anisopliae* and *B. bassiana*). At each harvest: 21/5/21 (LSD = 7.29, P=0.013), 26/5/21 (LSD = 15.83, P<0.001), 31/5/21 (LSD = 13.94, P<0.001), 5/6/21 (LSD = 12.34, P<0.001), 11/6/21 (LSD = 12.76, P<0.001) and 16/6/21 (LSD = 12.13, P<0.001).

The relative proportions of ECS1 or KS1 contributing to insect mortality in the KS1/ECS1 mix treatment were are illustrated in Figure 3. At 11 DAE, there were almost twice as many insects with external *B. bassiana* sporulation than there were with *M. anisopliae* sporulation. Overtime, this evened out and by the time the experiment was terminated, ECS1 (*M. anisopliae*) induced-mortality represented over 40% of all insects, whilst KS1 (*B. bassiana*) induced-mortality represented about 30% of all insects.

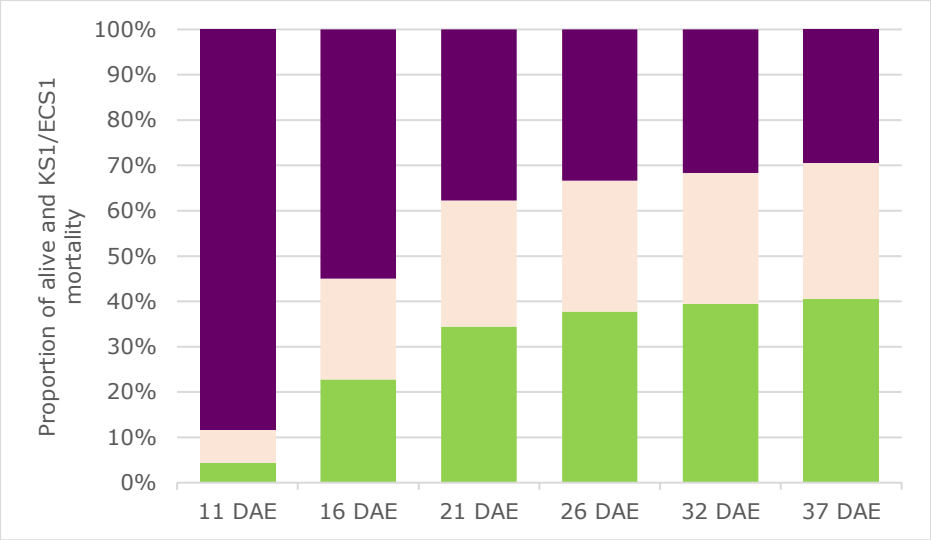

Figure 3. Glasshouse experiment 2. Cumulative mortality in the KS1/ECS1 mix treatment represented by % mortality of ECS1 (green, *M. anisopliae*) and KS1 (cream, *B. bassiana*) (alive insects in purple).

3.3. Assessment of mealworm damage to roots

3.3.1. Glasshouse experiment 1

Overall, there was little damage to the sweetpotato storage roots from mealworm feeding, despite their voracity in the laboratory (Figure 4). Generally speaking, the mealworms tended to stay on the surface of the soil, hiding underneath senesced leaves. The effect of treatment on root damage was not significant (P=0.89). On average, roots had only between 1-2 lesions associated with mealworm damage (Figure 5).

Figure 4. Typical damage seen on sweetpotato in the laboratory (noting high population)

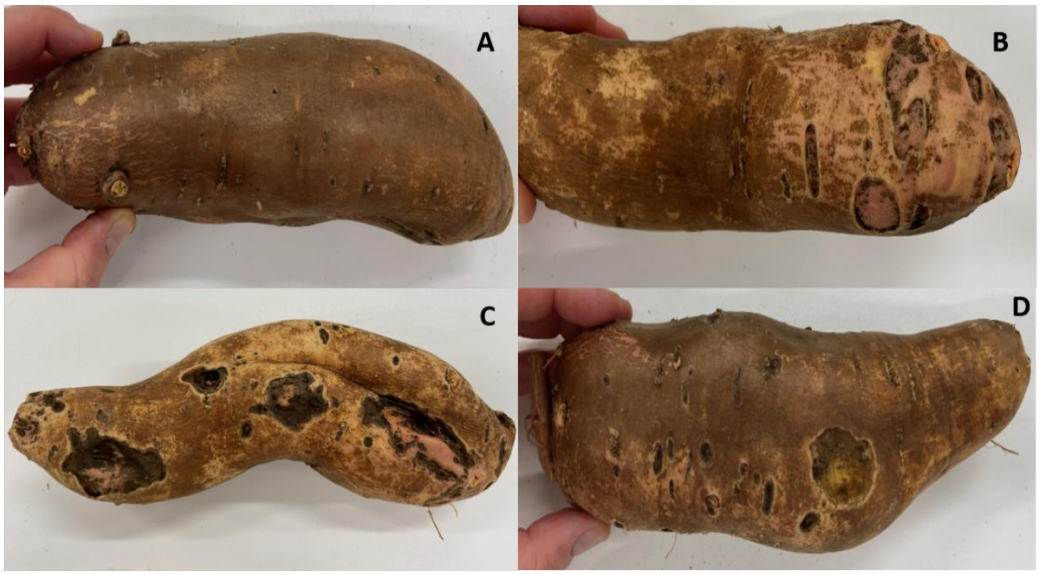

Figure 5. A storage root with no damage (A), new shallow feeding holes (B), deeper feeding holes (C) and older shallow feeding holes (D).

3.3.2. Glasshouse experiment 2

Similar to what was found in glasshouse experiment 1, there was no significant differences between treatments in terms of feeding damage ($P>0.05$).

3.4. EPF endophytic status

There was no fungal or bacterial growth on the surface sterilisation process check plates. There was no evidence of endophytic *Metarhizium anisopliae* or *Beauveria bassiana* in any of the >400 samples isolated and sequenced. The sequencing of the isolates showed that *Trichoderma asperellum*, *T. atroviride*, *T.neokoningii, T. longibrachiatum,* which are saprophytic fungi and common endophytes

dominated leaf, shoot, lateral root and storage root samples (>70% of the sections had *Trichoderma* spp. isolated). Other fungi that were present in many plant part sections included potential plant pathogens or other saprophytes and endophytes including many *Fusarium* spp. (despite no apparent evidence of wilting or rot normally associated with Fusarium) e.g. *Fusarium oxysporum, F. proliferatum, F. solani, F. falciforme, F. denticulatum, F. incarnatum, F. coeruleum, F. equiseti, Macrophomina phaseolina*, *Albifimbria verrucaria, Colletotrichum truncatum, C. gloeosporioides, Penicillium chrysogenum, P. raperi, Chaetomium globosum, Aspergillus magaliesburgensis, Aspergillus alliaceus, A. terreus, A. niger, Talaromyces sp., Setophoma sp. Talaromyces pinophilus, Ceratobasidium sp./Rhizoctonia sp., Acrocalymma vagum, Alternaria longissimi, A. alternata, A. tenuissima, Scedosporium apiospermum, S. aurantiacum, Acrocalymma vagum, Myceliophthora thermophila* and *Mucor circinelloides.*

3.5. Infectivity of storage roots on mealworms post-harvest

Although only occurring on in a few containers (and therefore not analysed statistically), death of mealworms when exposed to the clean storage root was observed, resulting in conidia of both *Metarhizium anisopliae* or *Beauveria bassiana* on many individuals. This was unexpected due to the inability to recover the EPF from the sweetpotato plants, but demonstrates the possibility that storage roots exposed to EPF may harbour a low infection of *M. anisopliae* or *B. bassiana* that are able to infect and kill more susceptible hosts (e.g. false wireworm). We also observed *Trichoderma* spp. to be growing on the cut surface of the sweetpotato.

4. Summary and discussion

- In glasshouse experiment 1, both *M. anisopliae* (ECS1) and the low concentration mix of *B. bassiana* (KS1) with *M. anisopliae* (ECS1), caused about 40% mortality of mealworms at low concentration 10^6 conidia g of soil (in a targeted application band), whereas KS1 was more effective than ECS1 throughout most time points in the experiment, with a maximum of 53% mortality. There was no additive effect of combining the two EPF in terms of increased mortality in mealworm. The higher dose of the ECS1/KS1 mix resulted in the least death by mycosis. This was unexpected and could be an anomaly in this experiment.
- In glasshouse experiment 2, high mortality of mealworms was achieved when pots were inoculated with the equivalent of 10^6 conidia g of soil (in a targeted application band). After 37 days, more than 70% mortality was achieved in the ECS1 and ECS1/KS1 treatments. Although more death was achieved with the ECS1/KS1 mix compared to the KS1 alone, the

differences were not statistically significant. However, in the field, it may be beneficial to have a mix of EPF species that target different hosts (for example different weevil species, wireworms, African black beetle) and which occupy varying environmental niches.

- There was no evidence of colonisation of sweetpotato tissues (shoot, leaves, storage root, lateral roots) by either *M. anisopliae* or *B. bassiana* in this experiment.
    - Only a few individuals of mealworms exposed to ex-experimental roots acquired an infection by *M. anisopliae* or *B. bassiana.*
    - It is unlikely that the plant parts could be sufficiently colonised with high enough levels of *M. anisopliae* or *B. bassiana* to cause insect mortality; however, low levels of colonisation or the association of *M. anisopliae* or *B. bassiana* with the rhizosphere of plants may increase plant growth and improve plant defence in response to plant disease (Barelli et al., 2020) or herbivory.
- Only the presence of fungi (rather than bacteria) was recorded from plant tissue in this experiment. Fungi re-isolated from plant tissues was dominated by several species of Trichoderma- a known endophyte, saprophyte and biocontrol agent of other soil microbes. However, another study has recorded *T. asperellum* (the dominant species observed in the work presented here) to be a postharvest pathogen, causing 'green mold disease' through wound infection (Yang et al., 2021). Growth of *T. asperellum* on cut surfaces was also recorded in the work presented here, highlighting its potential to be a postharvest disease in Australia if roots are wounded during the washing and packing process.
- In Petri-plate assays (see Figure 6 below), co-inoculation of *M. anisopliae* or *B. bassiana* with *Trichoderma* sp. resulted in the EPF being out-competed by Trichoderma, eventually leading to complete overgrowth on the plate.
- It is possible that the dominance of *Trichoderma* sp. in these plants (variety Orleans) may have prevented any co-colonisation by EPF.
- It is difficult to determine if EPF colonisation would ever be possible, considering the re-isolation of non-entomopathogenic fungi from every piece of plant tissue examined (> 30 species isolated and identified with DNA sequencing).

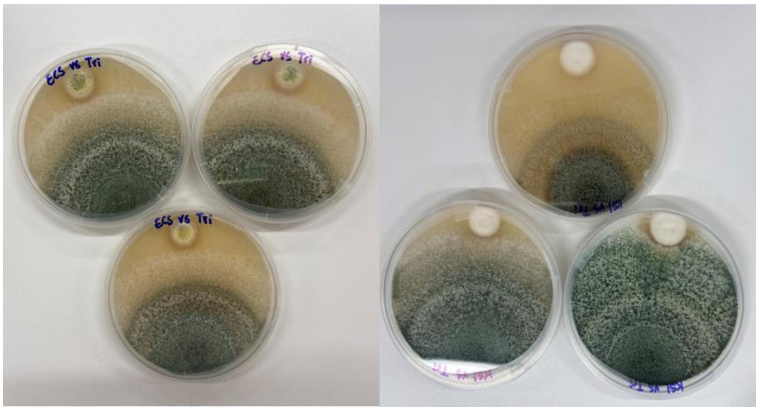

Figure 6. *Trichoderma asperellum* outcompeting ECS1 (left) and KS1 (right)

- Eight species of Fusarium were isolated from sweetpotato tissues although further molecular testing with additional primers is required to confirm these species (owing to the complexity of Fusarium). The results of this endophyte research contributes to the knowledge on *Fusarium* spp. occupying sweetpotato tissues in Australia. It is established that Fusarium root rot is caused by *F. solani*; however, the specific Fusarium species causing wilt is unclear (Ekman & Lovatt, 2015).
- It is possible that these Fusarium-based diseases remain dormant in the sweetpotato until environmental conditions (or internal conditions within the sweetpotato) are favourable for disease development.
- Other studies have demonstrated non-pathogenic strains of Fusarium (e.g. *F. oxysporum*, which was identified from sweetpotato here) to be biological control agents of pathogenic strains causing wilt (Kaur et al., 2010, Sutherland et al., 2018).
- Considering species of Fusarium (and many other of the endophytes found in this study) was reisolated from all plant parts, it is clear that the fungi (with the ability to be pathogenic or beneficial) could be easily moved around from bedding roots to shoots in the field.

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Appendix 13a. Reduced *Cylas formicarius* emergence from *Ipomoea batatas* storage roots in the presence of *Metarhizium pinghaense*

1. Introduction or rationale

A report detailing the set-up of use of Metarhizium and Pathogen-tested material at PNG Unitech and Poahom Village in Situm can be found in the Appendix detailing the "*Metarhizium* and Pathogen-Tested Planting Materials Trial Establishment in PNG Unitech Agriculture Farm (On-station) & Poahom Village (On-Farm) in Situm, Morobe Province"

2. Materials and Methods

2.1. Site description

There were two sites selected to establish this trial, one on-station trial in PNG Unitech's Agriculture Farm and another site was in Poahom village in Situm area. The Unitech Agriculture Farm is located in the Taraka Campus of the Papua New Guinea University of Technology, near Lae, while Situm is situated about 20 km north-east of Lae.

2.2. Planting material

The sweetpotato variety Beauregard was used in both trials. The vines used for planting were obtained from pathogen-tested (PT) Beauregard sweetpotato from NARI – Aiyura (Highlands Regional Centre). Two vines were planted in each mound.

2.3. Entomopathogenic Fungi

Two isolates of *Metarhizium* were examined: 'Highlands' *Metarhizium pinghaense* and the 'Lowlands' *M. pinghaense* (Table 1). These isolates performed similarly to others screened in the laboratory bioassays on sweetpotato weevils; however, they were selected for use based on their growth on media (potato dextrose agar) and on rice for production of conidia.

Table 1. Details of isolates used in the field trials in PNG

Isolate name	Species based on sequencing the EFT gene region	Location	Cropping and isolation method
Highlands	*M. pinghaense*	Mt. Hagen- Hagen Central District	Sweetpotato- baited from soil using Sago grub larvae
Lowlands	*M. pinghaense*	Unitech Agriculture Farm	Corn- baited from soil using Sago grub larvae

After pure cultures were obtained, the two isolates detailed above were mass-produced on rice. For the semi-solid fermentation, liquid cultures each of the isolates were inoculated into spawn bags contained 1 kg of twice sterilised rice and incubated at room temperature for 3 weeks. After sporulation was complete, spawn bags were refrigerated until use at 5 °C. By the time all the inoculum has been produced, some of the inoculum was 4 months old due to availability of laboratory equipment for rice sterilisation. Prior to their use, germination of the resulting conidia was calculated. A serial dilution was made from 1 g of conidiated rice and 9 ml of distilled water containing Tween 20 (0.05%) solution and adjusted to 2.3×10^6 conidia/ml for each bag. For each suspension, 100 µl was plated on prepared SDAY media and spread using a spreader. For each bag of conidiated rice, three plates were plated with the spore suspension, sealed with parafilm and incubated at room temperature. On average, the germination for the bags of 'Highlands' isolate had 82% germination and the 'Lowlands' isolate had 79% germination. To calculate the number of total conidia per g of rice, a subsample of 5 g of homogenous conidiated rice samples was taken from each bag and transferred to 9 ml of sterilized distilled water containing Tween 20 (0.05%) solution. The suspension was adjusted to 10^{-3} conidia per ml. The counting of conidia was made after the third serial dilution of the suspension using the Neubauer heamocytometer for determining the number of conidia. The 'Highlands' isolate had approx. 1.05×10^9 conidia per g of rice and the 'Lowlands' had 1.14×10^{10} conidia per g of rice.

2.4. Assessment of weevil numbers prior to installing the trials

To estimate the number of *C. formicarius* weevils (males) at both trial sites, just before planting, pheromone lures were set at three random locations around the trial plot area. The number of weevils lured by the pheromone within the first 15-20 minutes was counted. After counting the weevils, the pheromone lures were removed in order to get an estimate of numbers. During the harvest, natural occurring weevils were counted, without using the pheromone lures.

Table 2. Number of weevils trapped in pheromone lures prior to planting and the number of weevils counted at harvest (not trapped)

Trial site	Number of weevils counted		Time of day	
	Pheromone trapped weevils (males) before planting (sum of 3 traps)	'Natural' roaming weevils in plots (both sexes) at harvest	Before planting	At harvest
Poahom Village, Situm	567	256	11-12 noon	10:45-2:00 pm
PNG Unitech Ag Farm	62	23	9:30-10:00 am	10:00-12 noon

2.5. Trial establishment measurements: yield, weevil emergence

Randomized Complete Block Design (RCBD) was used for the establishment of the Best-Bet Plus trial. On a prepared land area, 120 m^2 (12m x 10m) was marked and the trial was setup. Each shoot planted was spaced 1 m apart and 1 m between the rows of the mounds. Two shoots were planted per mound. Treatments were then applied respectively and replicated six (6) times. About 250 g of conidiated rice inoculum (*M. pinghaense*) was applied to a depression created around the mounds. The trial was established/harvested on the 7/10/20-16/2/21 and the 8/10/20-17/2/21 at Poahom Village and Unitech Ag Farm respectively (approximately 4 months). Over the trial period, the two sites differed substantially in rainfall (Figure 1).

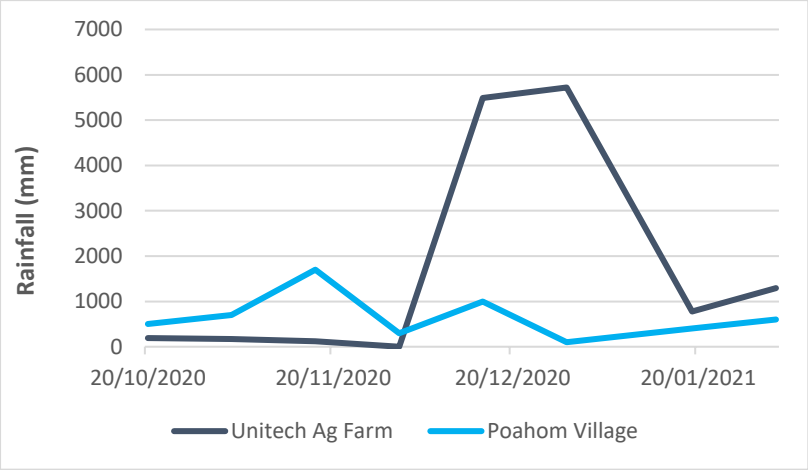

Figure 1. Fortnightly rainfall for the two trial sites

2.6. Statistical analysis

Yield and weevil emergence was analysed with an ANOVA after checking for normal distribution using Genstat 21st Edition (VSN International Ltd).

3. Results

3.1. Weevil emergence from storage roots and stems and yield from the Unitech Ag Farm

Overall, the pest pressure and subsequent weevil damage to storage roots and vines was low at the Unitech Ag farm. The data shows that although a greater number of *C. formicarius* emerged from storage roots in the control plots, these were not significantly different (P = 0.262) to the number of weevils that emerged from the two *Metarhizium* treatments: Lowlands and Highlands (Figure 1). Only 3 individuals of *C. formicarius* emerged from stems at the Unitech site from one Lowlands replicate root set and only 3 roots from the Lowlands treatment had emerged *E. postfasciatus* recorded from the roots; however, the numbers were so low the data did not meet the requirements of normality and was not analysed statistically.

At the Unitech farm, there was no significant difference between marketable yield (kg) (Figure 3). However, for the non-marketable yield, the plants treated with the Highlands isolate had the lowest non-marketable yield (P = 0.037). For the number of marketable or non-marketable roots, there were no significant differences between treatments (data not shown).

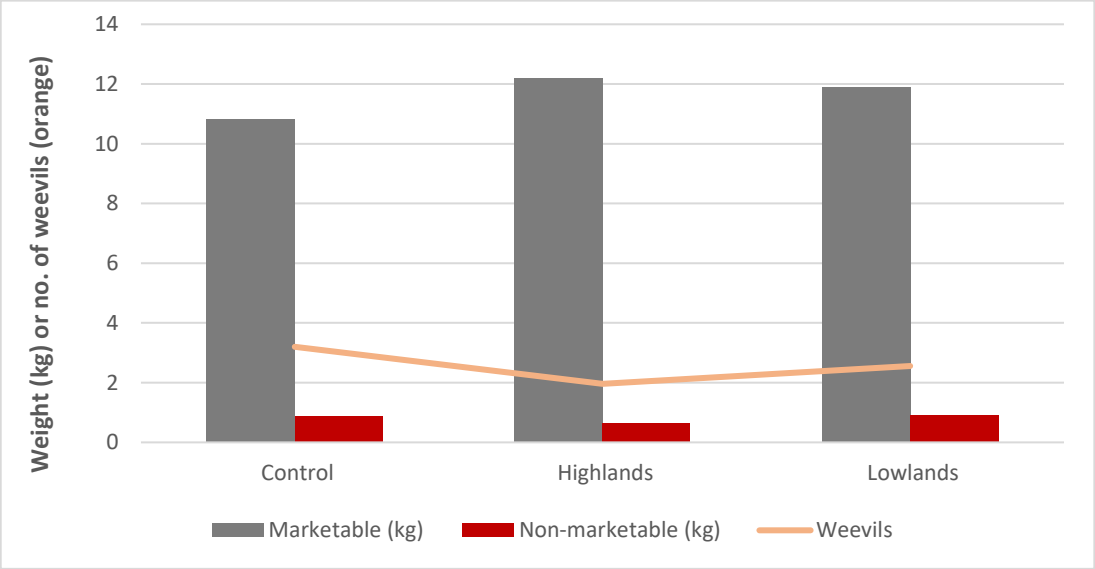

Figure 1. Yield and emerged *Cylas formicarius* from individual storage roots at the Unitech farm. Values are the mean of 6 replicates, each with 5 roots assessed, across three plots (LSD =0.211 for non-marketable yield at P<0.05).

3.2. Weevil emergence from storage roots and stems and yield from Poahom

Overall, the pest pressure and subsequent weevil damage to storage roots and vines was high at the Poahom Village. The storage roots and base of the vines were exposed across many mounds and the destructive harvest on farm revealed extensive weevil tunnelling, feeding marks and the presence of larvae, pupae and adults in the developing storage roots.

At the Poahom site, there was a significantly greater number of weevils that emerged from storage roots in the control plots (P = 0.012) compared to the number of weevils from the two *Metarhizium* treatments. Significantly more *Cylas formicarius* emerged from stems (P = 0.042) compared to *Metarhizium* treated plots (data not shown). No *E. postfasciatus* were recorded to have emerged from storage roots or stems. At Poahom, there was no significant differences between treatments for the yield of marketable or non-marketable roots (Figure 2). There were however, more roots eaten by rodents or infected by bacteria than retrieved for yield measurements from the Control (7), Highlands (8) and Lowlands (15)

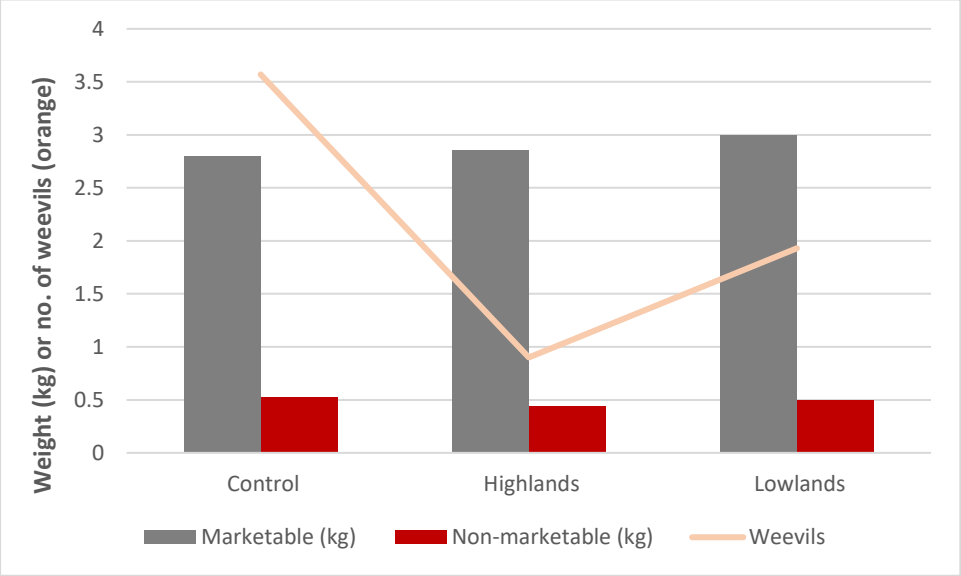

Figure 2. Yield and emerged *Cylas formicarius* from individual storage roots at the Poahom farm. Values are the mean of 6 replicates, each with 5 roots assessed, across three plots (LSD= 1.63 for weevils P<0.05).

3.3. *Infectivity of field inoculum*

Although *Metarhizium* was observed to be growing on the soil and sugarcane at the trial sites, infected cadavers were not seen in or around the mounds, possibly due to complete disintegration. Because of this, weevils were incubated in field collected soil to examine the infectivity and persistence of applied inoculum (Highlands or Lowlands).

There was no significant difference between treatments (Control, Highlands, Lowlands) in death of *Cylas* or *Euscepes* weevils at either field site when weevils were 'incubated' in field collected soil to test EPF infectivity in the laboratory. Overall, death was rapid and caused extensive sporulation on the cadavers, which is encouraging in terms of persistence of EPF in the field. Death from the control, uninoculated plots, could be explained by the presence of indigenous *Metarhizium* spp. or through from cross-contamination of applied isolates through insect, air or water transfer.

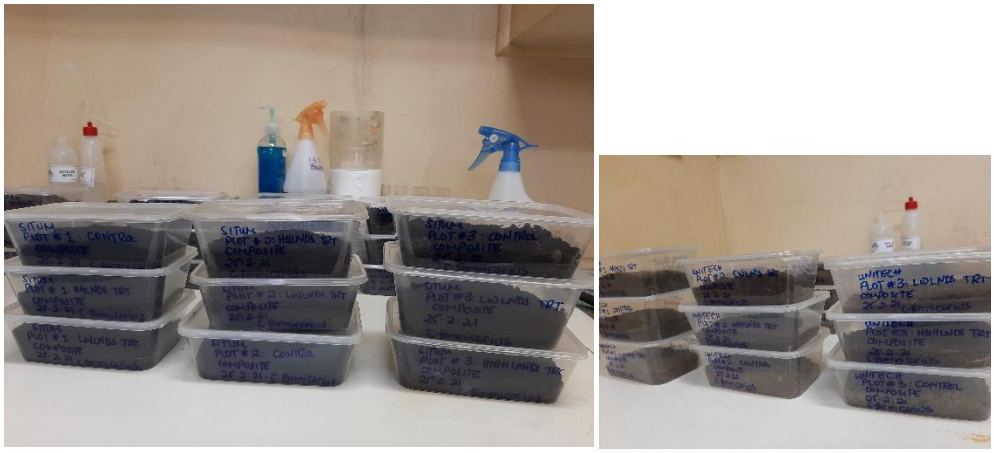

Figure 3. Insect baiting to examine the infectivity and persistence of Metarhizium sp. in the soil from the field trials at the Unitech farm and Poahom.

4. Discussion

- The trials at both sites were the first to use indigenous strains of *M. pinghaense* isolated from the Lowlands and Highlands of PNG to examine efficacy on important weevils of sweetpotato: *Cylas formicarius* and *Euscepes postfasciatus.*
- Observations from both sites of the inoculum showed that the *M. pinghaense* was actively growing (hyphae and conidia) on both the rice substrate and the soil that the EPF was in contact with. Whilst *M. pinghaense* -infected weevils were not captured directly from the field, this important observation is important for ongoing infectivity and persistence of the EPF in the field, for ongoing pest control.
- Weather conditions (soil moisture) and temperature (soil and ambient) are essential for the success of EPF to infect pest insects and also play a role in the rhizosphere competence of the applied inoculum.
    - There was substantially less rainfall in Poahom Village compared to that recorded at the Unitech Ag Farm, which may explain higher weevil numbers and decreased yield. Despite the extensive rainfall at Unitech in December, infective propagules remained in the soil, but may have easily been spread between inoculated and uninoculated mounds.
- Where the weevil numbers were high (Poahom Village) and the number of mounds with exposed roots and crowns were prevalent, significant damage from rodents and weevils was observed. However, at the same site where soil was adequately hilled up, there was no damage, highlighting the importance of regularly placing soil over the roots during the growing season.
- We only applied the *M. pinghaense* conidiated rice once, with each mound receiving between 2.5 x 10^{11} (highlands) and 2.5 x 10^{12} (lowlands) conidia depending on the isolate.
    - Although this initial concentration of conidia was high, perhaps it was not sufficient to provide ongoing weevil control, especially if the inoculum was washed away.

    - Or the positioning of the inoculum around the sweetpotato mound (creating a ring-like 'barrier') was not suitable for high levels of weevil control.
- Further work could examine:
    - More frequent inspection of the trial to collect sporulating weevil cadavers, if present
    - Better vertebrate pest control to ensure the trial was not decimated by rats
    - The effectiveness of applying the *M. pinghaense* multiple times throughout the season, including spraying the vines when weevils were detected.
    - The effectiveness of incorporating different isolates into trials, for example different species of *Metarhizium* and *Beauveria* to provide more weevil control throughout the growing season (e.g. a different EPF may work better at lower temperatures or work better on a different weevil species).

Appendix 12a. Isolation of entomopathogenic fungi from Australian sweetpotato farms and laboratory screening against the yellow mealworm (*Tenebrio molitor*) and the sweetpotato weevil (*Cylas formicarius*)

Background

In a series of laboratory experiments, the pathogenicity of various entomopathogenic fungi (EPF) isolated from the soil of sweetpotato farms in NSW and QLD was evaluated. *Tenebrio molitor* (yellow mealworm) belongs to the family Tenebrionidae, the same family to which some false wireworms also belong. Considering false wireworms are an important pest of sweetpotato, the yellow mealworm was used a test insect. Mealworms (larvae and adults) are easily killed by EPF in controlled conditions; they are an effective means to bait EPF from soil and to test pathogenicity. If an isolated EPF could not cause mortality to mealworms, then it was discarded. If an isolated EPF caused mortality to mealworms but resulted in poor sporulation on the cadaver, then that isolate was also discarded. After the list of isolated EPF was narrowed down, bioassays challenging adult sweetpotato weevil were performed. The data that is shown below is representative of the results found in many bioassays.

Materials and methods

Soil collection and baiting/isolation of entomopathogenic fungi

In the Bundaberg region, soil was collected from one organic sweetpotato grower (red ferrosol/light clay) and four conventional growers with contrasting soil types (red ferrosol/light clay to heavier dark clays, sandy loam). In addition to the soil samples retrieved from Bundaberg for the isolation of EPF, further collections were made from sweetpotato farms in the Atherton Tablelands, Rockhampton, Gatton Esk and Cudgen (NSW). Accepted methods to isolate EPF from soil were used. Briefly, collected soil was placed into individual 70 ml containers, moistened with sterile water and baited with 10 larval yellow mealworms (*Tenebrio molitor).* Entomopathogenic fungi were then isolated from dead sporulating larvae (identified morphologically as *Metarhizium* spp. or *Beauveria* spp.) and processed until a pure culture was obtained (Figures 1 and 2).

Figure 1. Baiting for EPF: mealworm larvae were placed into soil then processed to obtain clean cultures (far right).

Figure 2. Baiting for EPF: mealworm beetles and larvae showing profuse conidiation with *Metarhizium anisopliae*

Single-spore cultures were made for each isolate; however, isolates that showed poor growth (slow or poor sporulation after three weeks) or sectoring were discarded (Figure 3). Purified cultures were grown on Sabouraud Dextrose agar with yeast (*Metarhizium* spp.) or malt extract agar (*Beauveria* spp.) Cultures were stored securely in multiple locations as agar cubes (0.5 cm^2) in 2 ml tubes containing sterile water at -80 °C. Every 3 months, the cultures were checked for purity and viability. Cultures that grew poorly after several subs were passaged back through an insect host to enhance sporulation and potential virulence. A selection of cultures was deposited in the Queensland Plant Pathology Herbarium (BRIP).

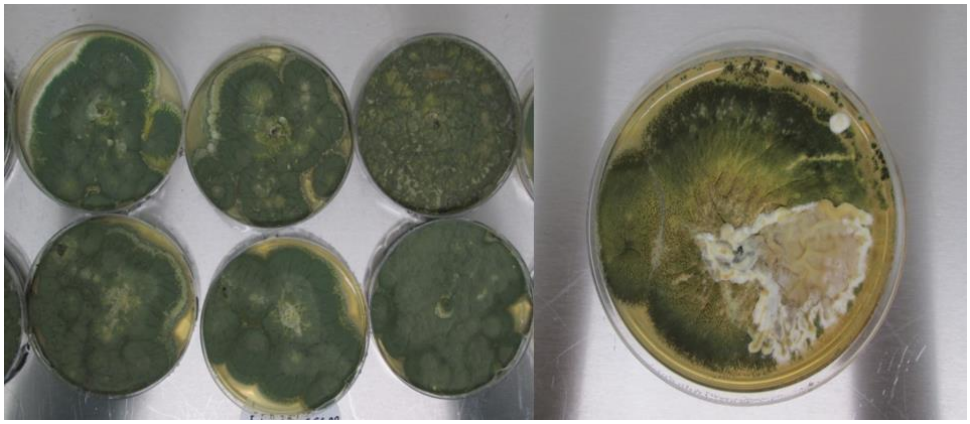

Figure 3. Cultures with good growth (left) and one culture with undesirable growth (right)

Entomopathogenic fungal cultures, preparation of suspensions and conidiated rice for bioassays

For the production of conidia for experiments, *M. anisopliae* was maintained on Sabouraud dextrose agar amended with 1% yeast extract (SDAY) and for *B. bassiana*, malt extract agar (MEA) was used. Cultures were incubated at 27 °C in a controlled temperature room with a 12:12 h light and dark photoperiod for 15-20 days (Figure 4). The conidia were harvested by gently scaping the surface of the cultures using a sterile spatula in a biohazard cabinet (Esco class II BSC).

For conidiated rice production, the various *M. anisopliae* isolates and the one *B bassiana* isolate were cultured as described above. After 14 days, a 1 cm^2 square of sporulating culture was excised from each agar plate and used to inoculate 250 ml of SDY liquid broth. Flasks were incubated at 27 °C at 150 rpm for 7 days in a shaking incubator (New Brunswick 450, Eppendorf, Australia). Solid substrate was prepared by autoclaving 1 kg of grain rice in a heat-sealed spawn bag (Microsac, Belgium) at 121 °C for 20 min. A corner of each spawn bag was cut and 500 ml of tap water was added before autoclaving for a second time at 121 °C for 20 min. Cooled rice was inoculated with 100 ml of a 7 day old liquid culture (ca. 10^9 blastospores per ml) and heat sealed under aseptic conditions. Spawn bags were massaged to evenly distribute the blastospores, then incubated at 27 °C for 14 days in a controlled temperature room. Conidiated rice was transferred to aluminium trays and dried at low humidity (ca. 30% RH) at 20 °C for an additional 14 days to reduce moisture (Figure 4).

Figure 4. *Metarhizium* spp. and *Beauveria bassiana* cultures (left) and conidiated rice drying (right)

Forty-eight hours prior to use, conidia germination and conidia per g of dried rice were calculated. Various concentrations of conidia were used throughout the experiments. All liquid suspensions were made in sterile 0.05% Tween® 80. Each suspension of conidia was homogenised using a vortex (Vortex-Genie® 2, Mo Bio Laboratories, INC) at maximum speed for 4 min before being diluted, vortexed again and counted. The number of conidia in the suspensions were quantified using a hemocytometer (Neubauer improved double net ruling) at 400× using a compound microscope (Model BX53) equipped with a digital camera (Model DP74, Olympus Australia Pty Ltd, Victoria, Australia). The viability of conidia was checked prior to all bioassays. A 1 x 10^4 conidia/mL suspension was prepared in sterile Tween® 80. For each isolate, 100 µl of the suspension was spread evenly over the surface of SDAY or MEA plates (depending on the species) using a L-shaped sterile spreader. Plates were sealed with Parafilm® and incubated at 27 °C with 12:12 h light and dark photoperiod. After 18 h of incubation, the percentage conidial germination was determined. From each Petri plate, two pieces of agar (20 mm^2) were cut out and placed onto microscope slides with 22 mm^2 coverslips and a minimum of 200 conidia were counted per square per slide using an Olympus BX53 compound microscope (400×). Only isolates with >85% germination were used in bioassays.

Molecular identification of entomopathogenic fungi

The DNeasy® PowerSoil® kit (Qiagen, Australia) was used to extract genomic DNA from all samples following the manufacturer's protocol with the exception that 3 x 0.5 cm^2 squares of pure fungal culture on agar was used rather than soil. For *Metarhizium* spp., the 5' region of the elongation factor-1 alpha (EFT1) gene was amplified with primers EF1T/EF2T. The B locus nuclear intergenic region (Bloc)

was used to identify species of *Beauveria* with primers B22U/B822L. Each PCR reaction was 25 µl and contained 12.5 µl GoTaq®2x GreenMaster Mix (Promega, Alexandria, NSW, Australia), 1 µl of each forward and reverse primers (10 mmol), 9.5 µl of nuclease-free water and 1 µl of fungal DNA (at 25–30 ng/µl). The PCR conditions were an initial denaturation at 94 °C for 3 min, then 34 cycles of 30 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C, with a final extension of 10 min at 72 °C. PCR products were sent to Macrogen Inc. (Seoul, South Korea) for PCR purification and DNA sequencing.

Insect colonies

Larval and adult yellow mealworms (*Tenebrio molitor*) were used as a model insect to evaluate the pathogenicity and screen the various entomopathogenic fungi isolated from sweetpotato soil (Figure 5). Mealworms were acquired from BioSupplies, Yagoona, NSW and reared in controlled temperature rooms at 25 ° C with 12:12 h light and dark photoperiod. A plastic tub (30 cm x 20 cm x 15 cm) was used to house the larvae, which were supplied with wheat germ and Gold sweetpotato roots purchased from the supermarket as a food source. Adult mealworms that had been in the beetle life stage for 14 days were used in experiments.

Figure 5. *Tenebrio molitor* (yellow mealworm) larvae colony

A colony of sweetpotato weevils (*Cylas formicarius*) from Wagga Wagga (ex-Bundaberg weevils) was supplemented with new weevils from Bundaberg and Cudgen for pathogenicity testing. This involved both pheromone trapping and collection of male weevils as well as collection of infested storage roots from the field to obtain female (and male) weevils. Rarely were there infested storage roots around as most growers adhere to strict hygiene practices to limit infestations (i.e. roots are disced into the ground or deeply buried). A cohort of mixed weevils was obtained from infested storage roots from

one farm in the Bundaberg region. Pheromone traps were constructed from modified yellow fly traps (Envirosafe, Bunnings) or Bucket traps (PHEROCON® Unitraps, Trécé Inc.). For the fly traps, the fly bait was removed and holes were drilled into the plastic under the lid to secure the lid (and trap) to a post using a cable tie. This allowed for easy access and removal of live weevils. The female *Cylas formicarius* sex-pheromone *Z*3-dodecenyl-*E*2-butenoate was acquired in bulk (Sapphire Bioscience, NSW, Australia). Lures were made by pipetting 20 µL (equivalent to 1 mg per lure) of pheromone into the inside of 2 cm lengths of rubber tubing (orange natural rubber tubing, 5 mm internal diameter). One lure per trap was suspended inside the trap with metal wire (1 mm diameter). For the bucket traps, the lure was positioned within the lid in the designated compartment for lures. Pheromone traps were installed on wooden stakes or metal star pickets 50 cm from the ground, on the outer edge of a crop. Typically, pheromone traps were installed for 24 hr before collecting live male weevils and transporting them back to Toowoomba to be reared in the laboratory (Figure 6). Weevils were fed store bought sweetpotato roots and shoots. Five generations of weevils were completed before using the offspring for experiments. Ten day old weevils (mixed sex) were used for all experiments (Figure 7).

Figure 6. Collection of pheromone trapped male weevils from traps (left), infested field collected storage roots with emerged weevils (middle) and a laboratory colony of mixed weevils.

Figure 7. Weevil colonies in the laboratory

Isolate screening and pathogenicity testing

Replicate experiments in the screening and pathogenicity testing differed in their set-up depending on isolate tested, concentration of conidia and application of conidia. For mealworm larvae and beetle experiments, there were typically 10-20 beetles per replicate and 3-6 replicates per treatment. Application of conidia was always in sterile 0.05% Tween®80 and was either applied directly by pipetting 30 µl of suspension onto individual beetles (on the thorax and abdomen area) in 90 mm Petri plates or by pipetting a larger volume (400 µl) into the base of a 90 mm Petri plates and allowing beetles to walk around for 5 mins before being transferred to the experimental tubs. Plastic tubs (⌀ 9 cm, height 10.5 cm) with lids with ventilation (⌀ 7 cm voile fabric inserted in lid) were the experimental tubs. To each tub, 30 g of wheat germ and a 50 g disc (typically ⌀ 7 cm) of sweetpotato (Gold) was added, followed by incubation at 25 ± 1 °C, 35 ± 3% RH and 16L:8D photoperiod in a Conviron® A1000 growth chamber (Conviron Asia Pacific Pty Ltd., Melbourne, Australia).

Weevils were collected from the main colony, placed into tubs described above and chilled at 6 °C for 5 min to reduce weevil mobility. Weevils were then allocated to replicate experimental tubs, as a mixed sex cohort of 10-20 weevils depending on the experiment. Replicate tubs with weevils were rechilled (maximum 5 min), before opening the tub and spraying conidial suspensions (1 ml) or sterile 0.05% Tween®80 using an X-Press It® micro-atomiser (Xpress Graph-X Pty. Ltd., Victoria, Australia) directly into the tub, then fastening the lid. Tubs were immediately inverted to allow excess suspension to drain through the voile fabric insert in the lid. The lid was removed again and a 50 g disc (typically ⌀ 7 cm) of sweetpotato (Gold) was added to each tub, followed by incubation at 26 ± 1 °C,

35 ± 3% RH and 16L:8D photoperiod in a Conviron® A1000 growth chamber (Conviron Asia Pacific Pty Ltd., Melbourne, Australia).

For bioassays with conidiated rice, sterilised sand/potting mix (50:50) was used. To achieve the required rates of conidia, conidia per g were counted for each isolate and the amount required weighed for each replicate/treatment. To each tub, 200 g of sand/potting rice was added, conidiated rice was then applied to the surface and sterile water was applied to achieve 80% field capacity. Food was added to each tub as described above. Every 3rd day, tubs were sprayed with 5 ml of sterile water (Figure 8, Figure 9).

Figure 8. Sand/potting mix bioassay set-up for weevils showing various concentrations of conidia per gram of sand/potting mix: 10^6 conidia per g (left), 10^7 conidia per g (middle) and 10^8 conidia per g (right).

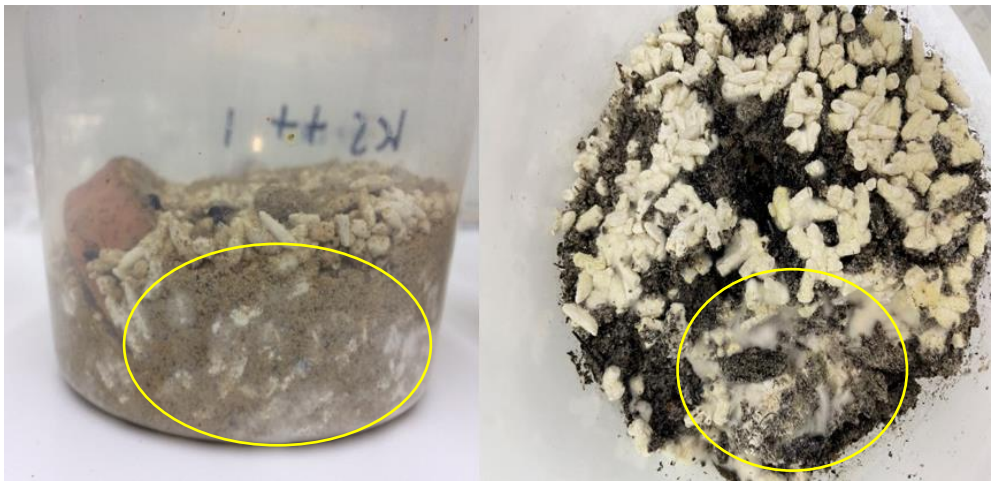

Figure 9. Conidiated rice on sand/potting mix substrates were the preferred method for bioassays as mycelium and sporulation (yellow circles) was observed in both *B. bassiana* (KS1) and *M. anisopliae* isolates (not shown).

For both mealworm and weevil experiments, tubs were arranged in randomized blocks. Each tub was provided with a new disc of sweetpotato every 4th day. Tubs were examined daily for dead insects; if

found they were removed with clean forceps, placed in 90 mm Petri plates containing filter paper dampened with sterile distilled water and sealed with Parafilm®. These plates were incubated as above to stimulate mycosis to verify fungal infection.

Statistical analysis

Morality data was transformed where necessary and was analysed using ANOVA. Regression analysis (Probit analysis) was performed for the dose-repsonse bioassay. All analyses were performed using Genstat (various editions including 21st) VSN International Inc.

Results

Entomopathogenic fungi

Of the 35 soil samples processed (some of which were from the same site), six *Beauveria* spp. and 29 *Metarhizium* spp. were isolated. From these, the collection was narrowed down to focus on one *Beauveria bassiana* isolate from Cudgen, NSW and 5 isolates of *Metarhizium* spp. from QLD soils (Table 1). One isolate from previous sweetpotato weevil research (e.g. Dotaona et al., 2015) was used in some bioassays as a positive control.

Table 1. List of entomopathogenic fungi isolated from Australian sweetpotato farms used in screening experiments

Species	Isolate/accession	GenBank Accession	Location	Year	Collector
M. anisopliae	QS155/DAR 82480	-	Mapuru, NT	2015	Unknown
M. anisopliae	B4A1/BRIP 70268	MN966532	Bundaberg, QLD	2017	B. Wilson
M. anisopliae	DA1/BRIP 70271	MN96653	Bundaberg, QLD	2017	B. Wilson
M. anisopliae	ECF1/BRIP 70270	MN966529	Rockhampton, QLD	2017	B. Wilson
M. anisopliae	ECS1/BRIP 70272	MN966530	Rockhampton, QLD	2017	B. Wilson
M. pinghaense	RM1	-	Bundaberg, QLD	2017	B. Wilson
B. bassiana	KS1	-	Cudgen, NSW	2017	B. Wilson

Isolate screening

Only selected examples of bioassays for selecting isolates-screening against mealworms and weevils are shown below to avoid repetition. For larval mealworms, the top 4 isolates (3 x *M. anisopliae* and 1 x *B. bassiana*) caused more than 90% mortality in ten days, whereas application of the positive

control *Metarhizium* spp. only resulted in about 65% mortality (Figure 10). In an adult mealworm bioassay, the three tested isolates did not differ significantly, resulting in 89-97% mortality after 8 days (Figure 11).

Figure 10. Mortality of larval mealworms at 10 days after inoculation (n = 20), 3 replicates per treatment. Control is 0.05% Tween 80 solution. Larvae were exposed to 400 µl of a 10^7 conidia per ml solution in a 90 mm Petri plate. Treatments with a different letter are significantly different (P<0.05).

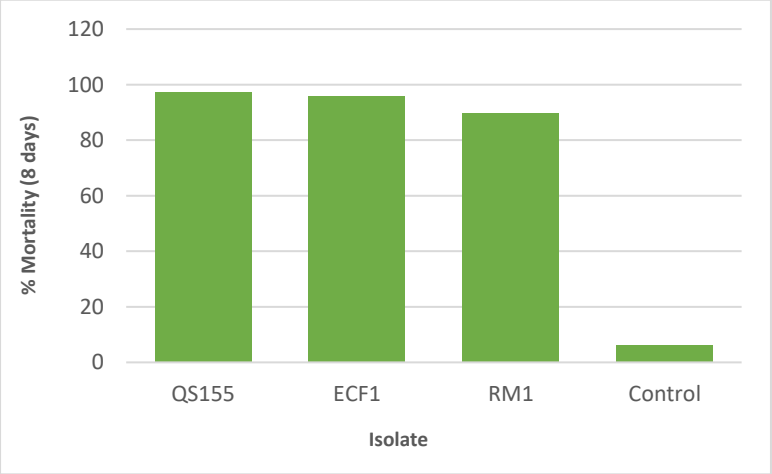

Figure 11. Mortality of adult mealworm beetles at 8 days after inoculation (n = 20, 3 replicates per treatment). Control is 0.05% Tween®80 solution. Beetles were exposed to 30 µl of a 10^7 conidia per ml solution. Isolates did not differ significantly from each other, only to the control (P<0.001).

When screening isolates against weevils, mortality was much lower (<45%) compared to that observed for mealworms (Figure 12). After 7 days, the greatest mortality was observed when weevils were sprayed with ECS1 (42.8%), which was significantly different to all other treatments (P<0.05) (Figures

12-14). In another experiment, weevils sprayed with KS1 showed the highest mortality (68%) after 13 days, which was significantly different (P<0.001) to all other treatment (Figure 15). The *Metarhizium* spp. treatments generally performed poorly, resulting in 11-46% mortality.

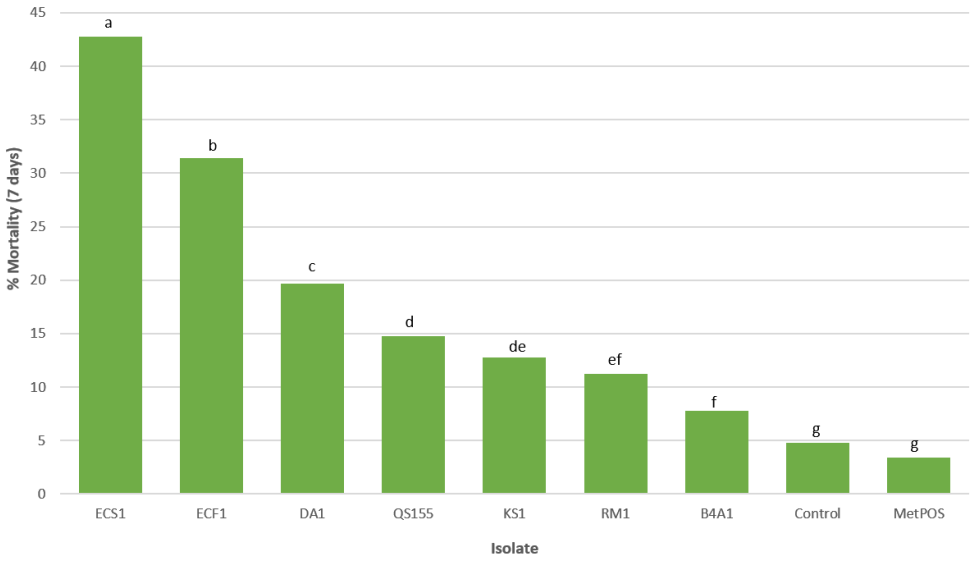

Figure 12. Mortality of weevils at 6 days after inoculation. Weevils were exposed to a 10^7 conidia per ml solution via spraying (n = 40, 3 replicates per treatment). Control is 0.05% Tween®80 solution. Treatments with a different letter are significantly different (P<0.001).

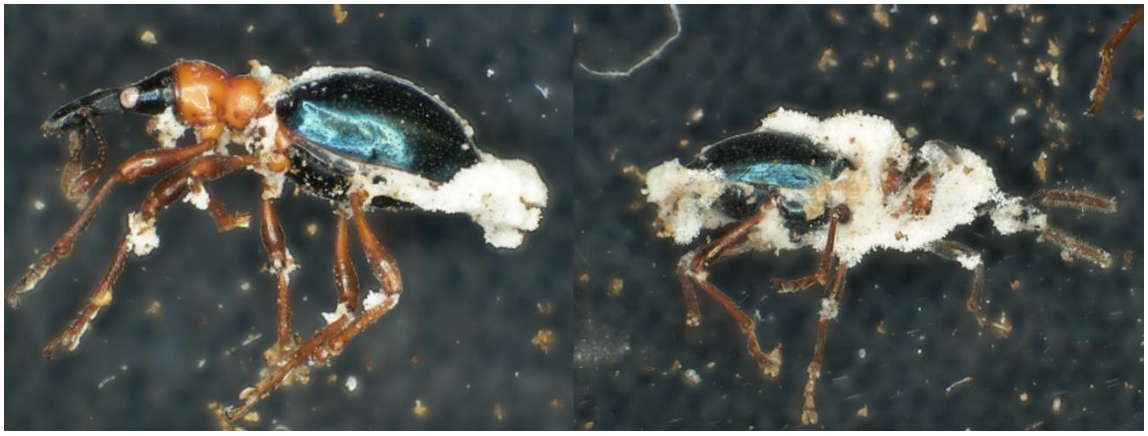

Figure 13. Female (left) and male (right) weevils with KS1 (*B. bassiana*) sporulation

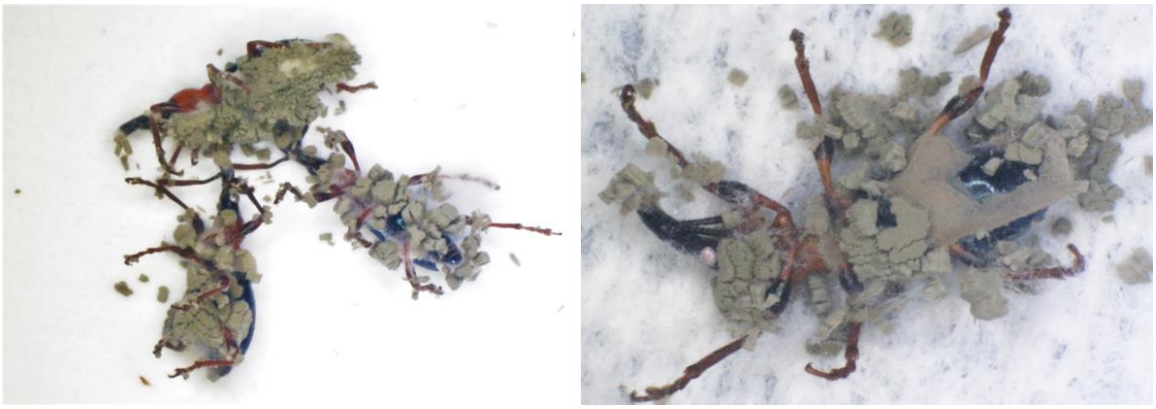

Figure 14. Mixed sex weevils with ECS1 (*Metarhizium anisopliae*) sporulation

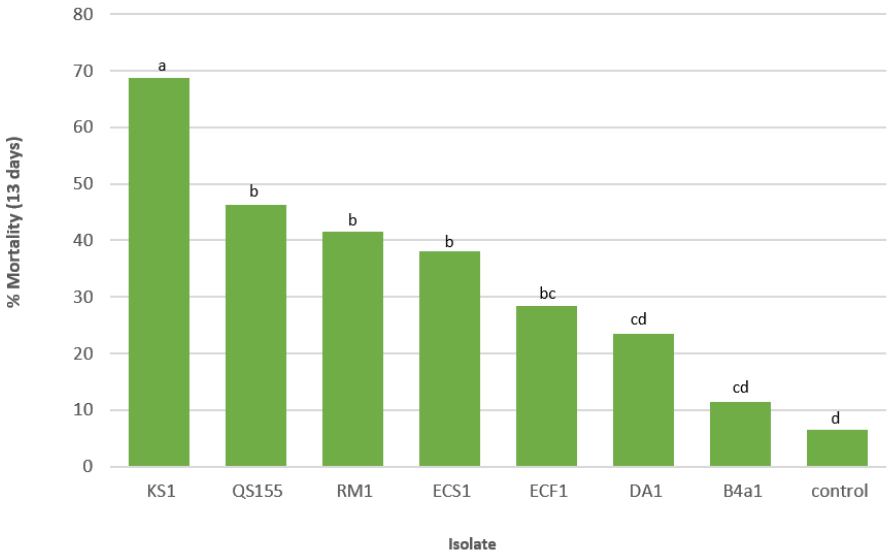

Figure 15. Mortality of weevils 13 days after inoculation. Weevils were exposed to a 10^7 conidia per ml solution via spraying (n = 15, 4 replicates per treatment). Control is 0.05% Tween®80 solution. Treatments with a different letter are significantly different (P<0.001).

In another small experiment where B*. bassiana* isolate KS1 was combined with various *M. anisopliae* isolates (i.e. 50% KS1 and 50% other M. anisopliae to achieve 10^7 conidia per ml) or trialled alone, greater death was achieved in the KS1/QS155 treatment then when KS1 was applied alone. However, combinations with other *M. anisopliae* was not significantly additive (P<0.001) (Figure 16). Although it was not analysed statistically, sporulating cadavers in the KS1/mixed treatments were generally dominated by *Metarhizium* spp., with fewer *B. bassiana* sporulating cadavers (Figure 17). Overall, mortality in this experiment was low, with a maximum of 45% death achieved. In a final experiment before changing to sand/potting mix-based bioassays, a 10^8 conidia per ml solution of various isolates was sprayed on weevils (Figure 18). After 7 days, weevils inoculated with isolate ECS1 resulted in the

greatest mortality (65%), which was significantly different to all other treatments (P<0.001). The other isolates did not differ significantly from each other and mortality ranged from about 50 to 57% after 7 days (Figure 18).

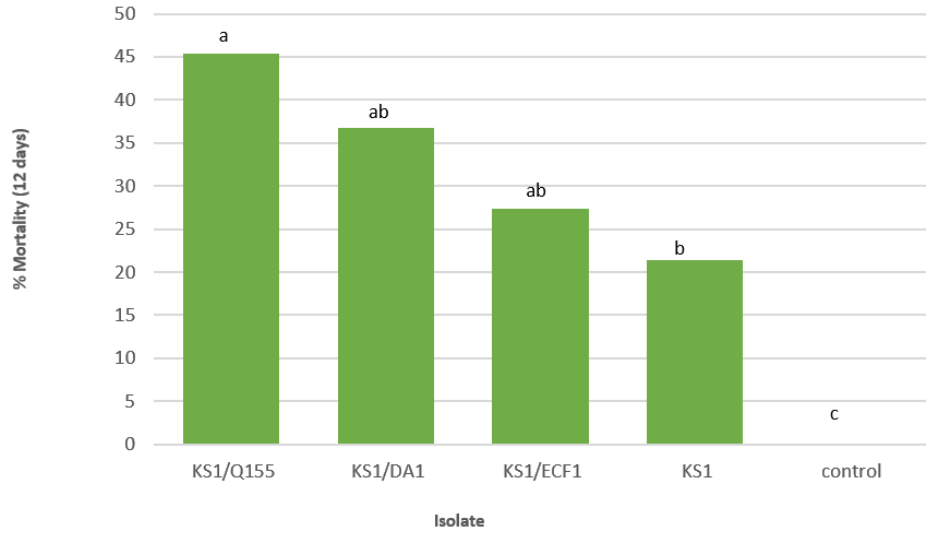

Figure 16. Mortality of weevils 12 days after inoculation. Weevils were exposed to a 10^7 conidia per ml solution via spraying (n = 15, 4 replicates per treatment). Control is 0.05% Tween®80 solution. Treatments with a different letter are significantly different (P<0.001). Data was $\log_{10}$ transformed to meet the requirements of an ANOVA. Data presented are back-transformed means.

Figure 17. Weevils dual inoculated with KS1/QS155 (left) and weevils inoculated with KS1 alone (right).

Figure 18. Mortality of weevils 7 days after inoculation. Weevils were exposed to a 10^8 conidia per ml solution via spraying (n = 15, 4 replicates per treatment). Control is 0.05% Tween®80 solution. Treatments with a different letter are significantly different (P<0.001). Data was $\log_{10}$ transformed to meet the requirements of an ANOVA. Data presented are back-transformed means.

A preliminary experiment as set-up to test conidiated rice as the inoculum source was effective (Figure 19). There was no significant difference between the isolates/rates tested (P<0.001) but isolates were significantly different to the controls.

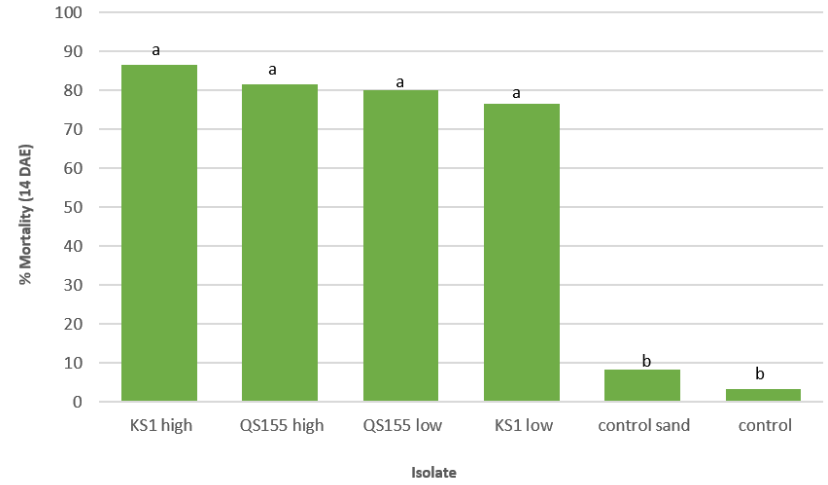

Figure 19. Mortality of weevils 14 days after inoculation. Weevils were exposed to various concentrations of conidiated rice. KS1 high was 6 x 10^8 conidia per g, QS155 high was 3 x 10^8 conidia per g, KS1 low was 3.5 x 10^8 conidia per g and QS155 low was 1.2 x 10^8 conidia per g (n = 15, 4 replicates per treatment). Control either sand alone or no sand. Treatments with a different letter are significantly different (P<0.001).

The results of a multi-dose response bioassay showed that by the end of the experiment, there was no significant difference between the LD_{50} of the two isolates ECS1 and KS1 (Table 2). Moderately high (c. 75%) to high (c. 90%) mortality was achieved for both isolates by 30 days after inoculation (DAI), although for KS1 10^8 conidia per g, this was achieved at 14 DAI (Figure 20).

Table 2. LD_{50} estimates of isolates (conidia per gram soil)

Isolate	LD_{50}	lower 95%	upper 95%
ECS1	2.72×10^5	9.09×10^4	6.46×10^5
KS1	2.54×10^5	8.41×10^4	6.08×10^5

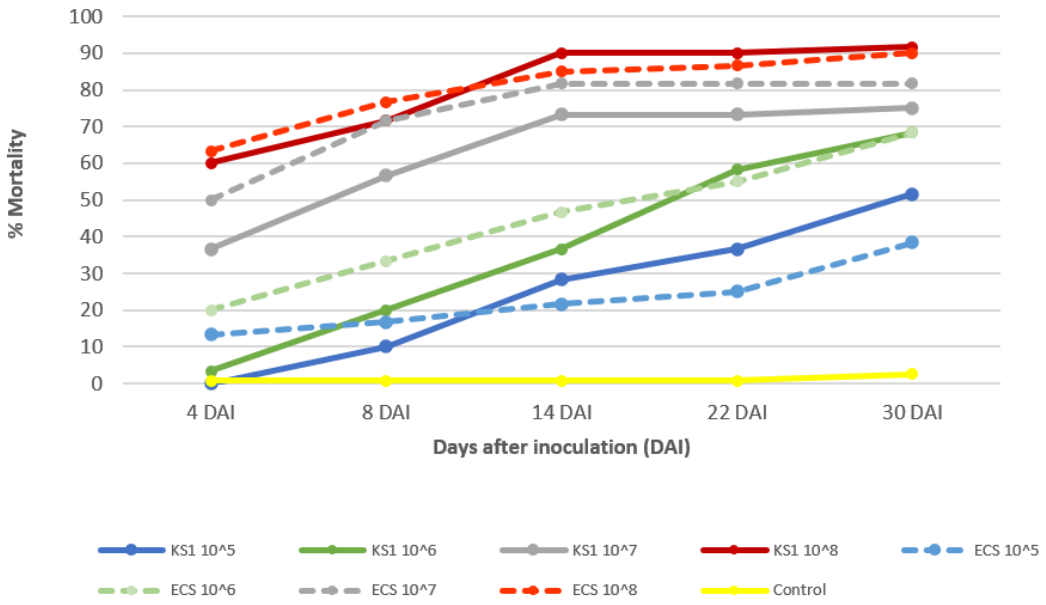

Figure 20. Mortality of weevils over time when inoculated with ECS1 or KS1 (concentrations ranging from 10^5 to 10^8 conidia per gram) as conidiated rice in a dose-response bioassay (n = 20, 6 replicates per treatment). Control is sterilised sand/potting mix alone.

Discussion points

- Although a large number of entomopathogenic fungi were isolated from sweetpotato field soil, this number was quickly narrowed down to 6 isolates, based on their ability to sporulate effectively, growth after storage in the freezer and their ability to sporulate heavily on the cadaver after killing the host, which is important for secondary infection.
- These 6 isolates, one *B. bassiana*, 4 *M. anisopliae* and 1 *M. pinghaense* were used to challenge larval and adult mealworms (as a proxy for false wireworms, as an 'easy' test host)
- Generally speaking, the isolates performed similarly across most experiments, with some variation over time. Because of this, other properties for example, their ability to grow and produce high amounts of conidia in solid-state fermentation (i.e. on rice) was assessed. Inconsistent and problematic conidia production on rice removed RM1 and B4A1 from further testing, although neither isolate was discarded.
- Although conidia production was problematic for KS1, the isolate was maintained in the collection, and the fermentation system was modified to enhance harvest of conidia from the grain (i.e. to improve the harvest of conidia from the grain).
- In their most basic formulation in 0.05% Tween®80, isolates were only 'moderate to poor' in their effectiveness against the sweetpotato weevil when sprayed at 10^7 conidia per ml, with less than 50% mortality achieved after 13 days. When isolates were sprayed at a rate of 10^8 conidia per ml, greater mortality was recorded although only 1 isolate achieved > 65% mortality.
    - The results here contrast drastically with other studies on *Cylas formicarius* although application methods and formulations differed. In this study, perhaps the volume of spray was insufficient to deposit enough conidia to be transferred effectively to cause high death or more importantly, the low relative humidity (35% RH) used strategically in this experiment to test the activity of the EPF in dry environments, was too low to be effective (although high mortality was achieved with *Tenebrio molitor* at the same relative humidity).
    - For example in their research, Dotaona et al., (2015) achieved 100% mortality with many isolates (including isolate QS155 used in the work presented here) after 12 days when weevils were individually dipped in suspensions containing 10^7 conidia per ml. In the research by Reddy et al., (2014), commercially available isolates (from which EPF were derived, although the formulation of the carrier was not disclosed) caused 100% mortality after 5 days when incubated at 70-80% RH.
- A 'pilot' bioassay demonstrated the potential of combining two isolates in terms of compatibility (and synergism), which has been demonstrated elsewhere for *Cylas formicarius* using EPF from

commercially available products (Reddy et al., 2014). A commercial biopesticide with multiple EPF species may be useful in targeting different pest insects that occupy the same environment. The experiment was problematic because it did not examine the pathogenicity of the *Metarhizium* spp. isolates alone, making it impossible to determine the relative contribution of each species. However, a greater mortality was achieved when combining isolates KS1 and QS155 compared to KS1 alone.

- Despite the relatively 'poor performance' of these isolates when sprayed as formulations of 0.05% Tween®80, it is well established that formulation plays a critical role in enhancing the activity of entomopathogenic fungi in commercial products for field or protected cropping application (e.g. in their paper, Khun et al., (2020) showed a reduction in mortality on macadamia seed weevil when a commercial product (80% mortality) compared to the EPF isolated (70% morality) from the commercial product was used.
- Bioassays using conidiated rice as the inoculum source appeared to be more successful than spraying conidia, although the two application methods cannot be compared directly as they were not set-up in the same experiment.
    - We noted that both isolates tested were able to grow on sand/potting mix and this has important implications for the persistence and infectivity of EPF in insect control.
- Overhead or irrigation tape administered liquid formulations and granular formulations (in its most basic form as conidiated rice) could play a role in weevil management.
- In another 'pilot' bioassay, conidiated rice was tested using KS1 and QS155. A high rate (various concentrations in the magnitude 10^8 conidia per gram soil) achieved between 78% and 88% mortality after 14 days. Moreover, both isolates were actively growing on the sand producing mycelium and conidia required for potential reinfection of insects (secondary infection) and increasing the supply of inoculum in the soil. Considering that weevils occupy both the foliage and soil surface and soil-sub surface when adults emerge from infested storage roots in the ground, it is logical to consider multiple formulations for effective weevil control.
- In the final experiment presented here, a dose-response bioassay examined the virulence of KS1 and ES1 over time. Overall, the dose to kill 50% of the population was low (10^5) conidia per g soil, suggesting that using these isolates in a commercial context could be viable (at a higher concentration to kill significantly more than 50% weevils).
    - In their experiment on macadamia seed weevils, Khuy et al., (2020) demonstrated that the same isolate ECS1 was highly virulent, resulting in 97.5% mortality at 10^7 conidia per ml in laboratory conditions, although similar to what was described here, there was

considerable cross-over between isolates, i.e. non-significant differences, meaning many isolates performed similarly.

Conclusions and future opportunities

Beauveria bassiana isolate KS1 and *M. anisopliae* isolate ECS1 cause high mortality on *C. formicarius* in laboratory conditions when applied as conidiated rice. Whilst better performance was achieved at a higher rate of 10^8 conidia per gram soil could appear economically prohibitive, targeted banded application of granular formulated EPF (i.e. formulated conidiated rice) in commercial sweetpotato farms (e.g. along the irrigation tape when planting shoots) may be an economically viable option, especially for organic growers.

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ACIAR SWEET POTATO PEST & DISEASE TECHNICAL FIELD REPORT

Metarhizium and Pathogen-Tested Planting Materials Trial Establishment in PNG Unitech Agriculture Farm (On-station) & Pahoam Village (On-Farm) in Situm, Morobe Province.

HORT/ 2014/083
Developing improved crop protection options in Support of intensification of sweet potato production in Papua New Guinea

By Melanie Pitiki

INTRODUCTION

The need to establish an on-station trial in Unitech was to focus only on three components of the Best Bet and Best Bet Plus Practices and they are; 1)the use of entomopathogenic fungi (EPF) 2) pathogen-tested planting materials and 3) sanitation. The **Best Bet + Practices** used out in the team zone sites include: the use of mulch as barrier materials (Mexican sunflower, Leuceanna and Sugar-cane), living barrier plants (Marigold, Desmodium, and Smooth Senna). Furthermore, the **Best Bet Practices** include: the use of pathogen-tested planting materials, pheromone traps and general sanitation. Apart from setting this trial in PNG Unitech Agriculture Farm as an on-station trial, another site in Pahoam village in Situm was selected as the on-farm trial. The project partner involved in this trial establishment was Mr. Wilfred Wau from National Agricultural Research Institute (NARI) Highlands Regional Centre, Aiyura.

AIM/OBJECTIVE/PURPOSE

1. Establishment of EPF (*Metarhizium*) and Pathogen-Tested planting materials trial in PNG Unitech Agriculture Farm and Pahoam village in Situm area.
2. To test the effects of entomopathogenic fungi (*Metarhizium*) on causing weevil mortality in field conditions.
3. To test the effects of entomopathogenic fungi (*Metarhizium*) on causing weevil avoidance to sweet potato tubers.

ITINERARY/WORK PLAN

The itinerary and workplan for the Unitech and Situm trial establishment.

Day	Date	Time	Task	Description
Sunday	4/10/2020	10:00 am	Unitech Driver travels to Aiyura.	Unitech Driver leaves Lae and travel to Aiyura to pick Mr. Wilfred Wau and the planting materials.
Monday	5/10/2020	- 8: 30 am - 1: 30 pm	1. NARI staff's (Mr. Wilfred Wau) travel to Lae. 2. Mr. Wilfred Wau and Unitech Team (Dr. Dotaona, Dr. Ban and Melanie Pitiki) travel to Situm site to for sight- seeing and discussion with the farmer.	- Leaving Aiyura and will be travelling to Lae with planting materials. - After Mr. Wilfred Wau checks-in at the Unitech Guesthouse, he and and Unitech team travels to Situm site to see the planting area and inform the farmer of the date of establishment so the farmer can prepare the planting area.
Tuesday	6/10/2020	8: 30 am – 4:00 pm	Establishment of Trial at the Unitech Agriculture Farm	- Preparing and measuring the land area. - Making mounds. - Planting the Sweetpotato vines. - Applying the entomopathogenic fungus.
Wednesday	7/10/2020	9:00 am – 4:00 pm	Establishment of Trial at Situm site.	- Preparing and measuring the land area. - Making mounds. - Planting the Sweetpotato vines. - Applying the entomopathogenic fungus.
Thursday	8/10/2020	- 8:30 am – 11:30 am - 11:00 am	1. Melanie Pitiki to give Mr. Wilfred Wau a brief run-down of activities being undertaken by the Unitech Team at the Unitech Biotech Centre. 2. Mr. Wilfred Wau's travel back to Aiyura.	- Melanie Pitiki to give Mr. Wilfred Wau a brief run-down of activities being undertaken by the Unitech Team at the Unitech Biotech Centre. - Mr. Wilfred Wau's departure to Aiyura.
Friday	9/10/2020		- Departure of Unitech Driver to Lae.	- Unitech Driver returns to Lae.

MATERIAL AND METHODS

Study Site

There were two sites selected to establish this trial, one on-station trial in PNG Unitech's Agriculture Farm and another site was in Pahoam village in Situm area as an on-farm trial. The Unitech Agriculture Farm is located in the Taraka Campus of the Papua New Guinea University of Technology, and is about 10 -15 minutes' drive from the Lae city. While, Situm is situated about 20 km north-east of Lae, across the Busu River.

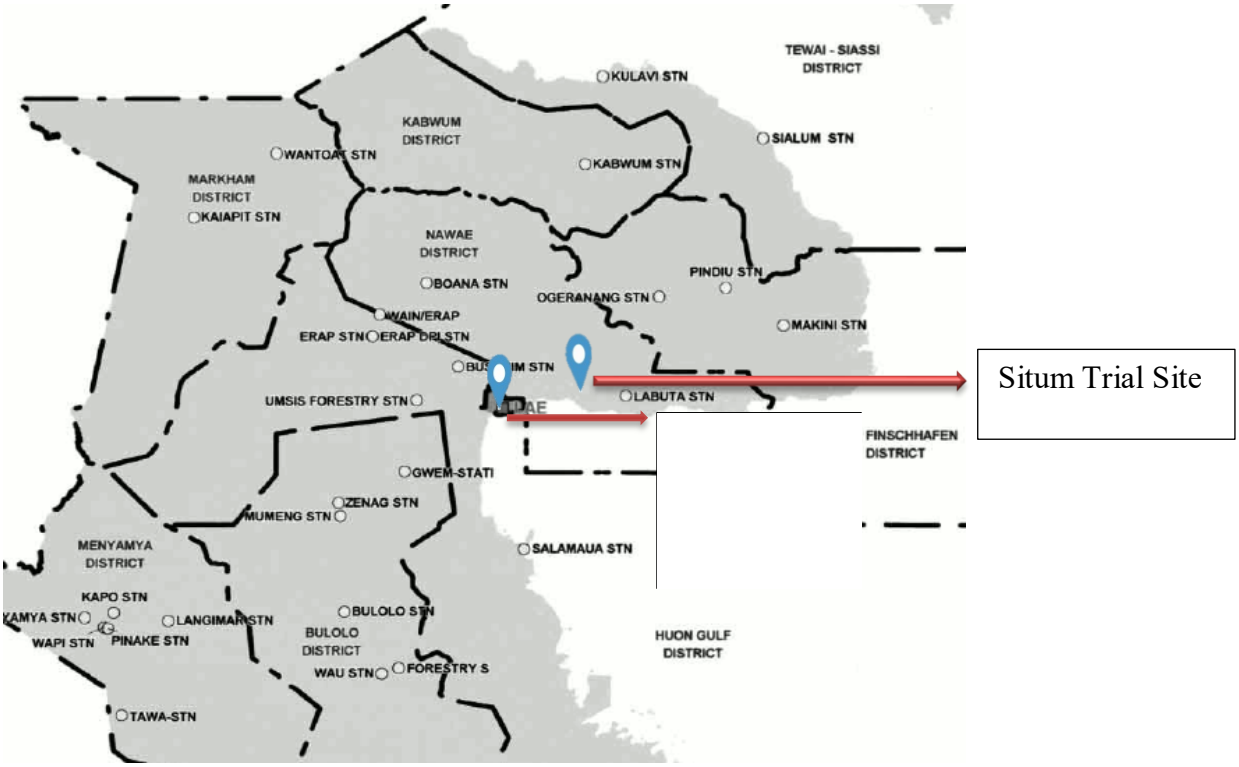


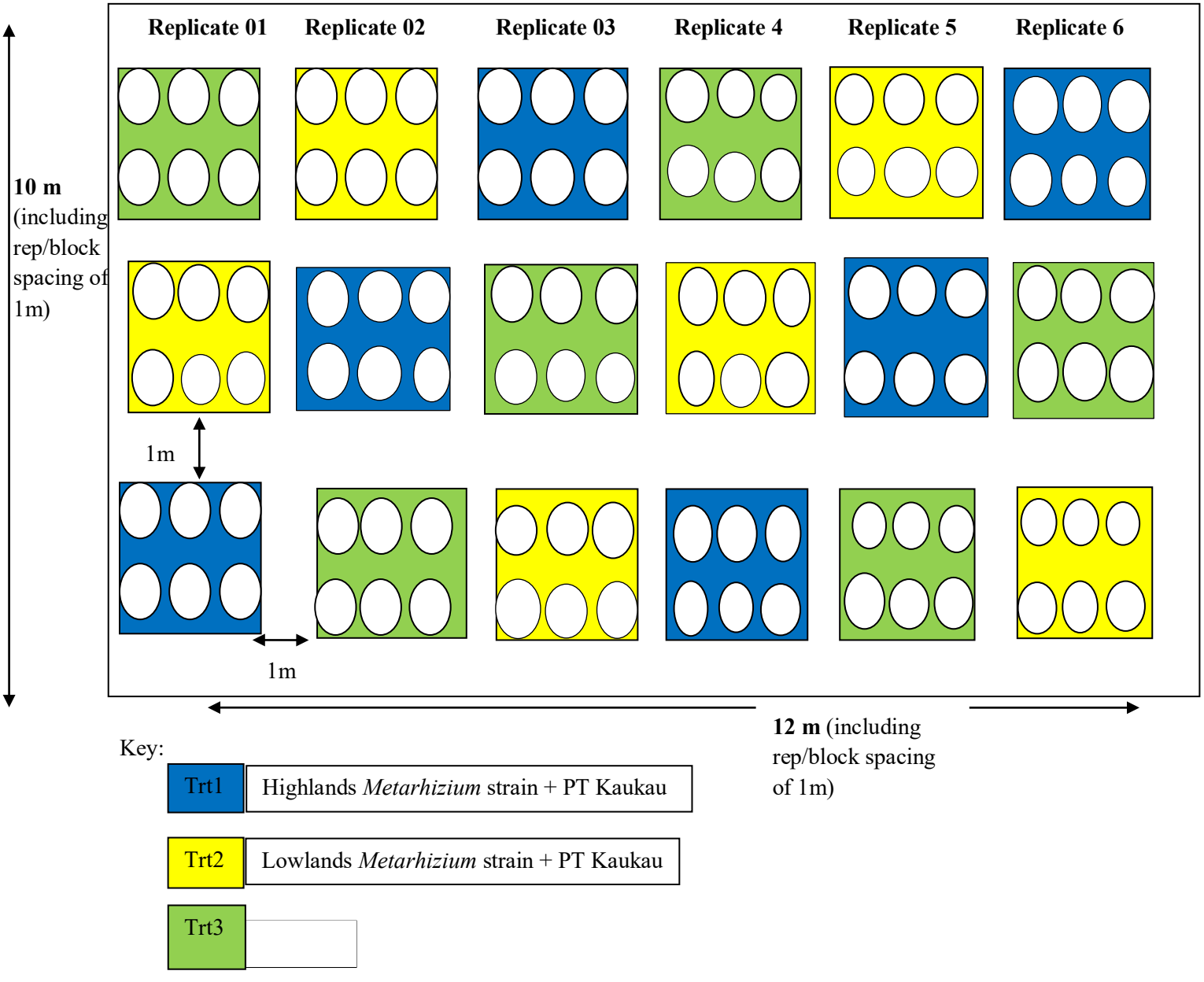
Figure 1: Map of the districts of Morobe Province in PNG showing the approximate location of the two trial sites. Note: The points of the location are not accurate but used just to show the approximate locations of the two trial sites. **Image Source**: Internet.

Sweet Potato Variety

The sweet potato variety used in this trial is Beauregard. The vines used for planting were obtained from pathogen-tested (PT) Beauregard sweet potato from NARI – Aiyura (Highlands Regional Centre). Two vines were planted in each mound.

Experimental Design

Randomized Complete Block Design (RCBD) was used for the establishment of the Best-Bet Plus trial. On a prepared land area, 120m^2 (12m x 10m) was marked and the trial was setup. Each plant planted was spaced 1 m apart and 1m between the rows of the mounds. Treatments were then applied respectively and replicated six (6) times.



Estimates of Weevil Population

To obtain the estimates of the sweet potato weevils' population in both trial sites, pheromone lures were set at three random locations around the trial plot area before planting the sweet potato vines. The number of weevils lured by the pheromone within the first 15-20 minutes was counted. After counting the weevils, the pheromone lures were removed. This was done basically to get an estimate of the sweet potato weevils' population in both trial sites.

Trial Site	Number of Sweet potato Weevil Counted	Time of Day
Pahaom Village, Situm		
PNG Unitech Agriculture Farm	62	Between 9: 30 am – 10:00 am

Entomopathogenic Fungi

There were two strains of entomopathogenic fungi used. They were both morphologically characterized as *Metarhizium* strains. The two *Metarhizium* strains were isolated from two different locations, where one strain was isolated from soil samples collected at the Unitech Agriculture Farm and the other strain was isolated from soil samples collected in the project's TEAM Zone 3 farm sites in Mt. Hagen in Western Highlands province. The *Met.* strains were multiplied on rice grain as the solid media substrate at the PNG Unitech Biotechnology Centre. About 250 g of *Metarhizium* was applied to a depression created around the mounds (Fig. 1a & 1b).

Entomopathogenic Fungi	Site Collected	Cropping Site
Metarhizium strain 1		Corn
Metarhizium strain 2	Mt. Hagen- Hagen Central District (Highlands/ Higher Altitude strain)	Sweet potato

Fig. 1a

Fig. 1b

Figure 1: **a)** Picture of the measuring cup used to measure 250g of conidiated rice to be applied. **b)** Melanie Pitiki applying the condiated rice into the depression created around the top of the mound.

i) Determination of Conidia per Gram of Rice Substrate

A total of six (6) conidiated rice bags per strain (Higher altitude and lower altitude strain) were used, i.e., three of each in each trial site.

Conidia number per gram of rice was calculated following Dr. Bree Wilson's formula. For example, 5g of homogenous conidiated rice samples were taken from each bag of about 1kg conidiate rice bags and transferred to 9 ml of sterilized distilled water containing Tween 20 (0.05%) solution. The suspension was adjusted to 10^{-3}. The counting of spores was made after the third serial dilution of the spore suspension using the Neubauer heamocytometer for determining the number of conidia in 5g of the sample.

Bag #	Highlands *Metarhizium* strain – Mt. Hagen	Lowlands *Metarhizium* strain – Unitech Agriculture Farm
1	2.0×10^9 conidia/g	6.7×10^9 conidia/g
2	1.8×10^9 conidia/g	9.4×10^9 conidia/g
3	2.1×10^8 conidia/g	1.2×10^{10} conidia/g
4	2.0×10^9 conidia/g	1.3×10^{10} conidia/g
5	1.3×10^8 conidia/g	1.2×10^{10} conidia/g
6	1.6×10^8 conidia/g	1.4×10^{10} conidia/g

Note: The bags of conidiated rice are two (2) to four (4) months old and have been stored in the fridge.

ii) Germination

A serial dilution was made from 1g of conidiated rice and 9ml of distilled water containing Tween 20 (0.05%) solution and adjusted to 2.3×10^6 conidia/ml for each bag. 100µl of the spore suspension was plated on prepared SDAY media and spread using a spreader. For each bag of conidiated rice, three plates were plated with the spore suspension, sealed with parafilm and incubated at room temperature.

Conidiated Rice Bag #	Plate Replicate	Highlands strain % Germination (n=200)	Lowlands strain % Germination (n=200)
1	1	81.5 %	75.0 %
	2	79.8 %	79.5 %
	3	84.0 %	78. 3 %
2	1	88.1 %	80.2 %
	2	82.7 %	74.9 %
	3	80.1 %	85.3 %
3	1	77. 5 %	78. 4 %
	2	82.0 %	69.5 %
	3	85.3 %	79.9 %
4	1	91.2 %	75.7 %
	2	89.7 %	80.7 %
	3	90.5 %	75. 3%
5	1	83.9 %	79.2 %
	2	72.8 %	79.5 %
	3	75.5 %	82.6 %
6	1	80.4 %	78. 4 %
	2	83.0 %	75. 9 %
	3	85.2 %	73.5 %

Note: The spore suspension was prepared from two (2) to four (4) months old conidiated rice.

ACTIVITIES UNDERTAKEN

Trial Site 1: Pahoam village, Situm.

Figure 2: a) The farmer Mr. Ano Gielo's family clearing the land area for the trial set-up. **b)** Melanie Pitiki and the farmer's family making mounds for planting.

Figure 3: a) Wilfred Wau and the farmer's son digging drains. **b)** Melanie Pitiki laying out two vines per mound before demonstrating to the farmer's family on how to plant the vines into the mounds and why NARI recommended it.

Figure 4: a) Women planting out the vines after the demonstration and explanation of planting technique. **b)** Melanie Pitiki making depression around the top of the mound for applying the entomopathogenic fungi. **c)** About 250 g of entomopathogenic fungi was applied into the depression around the mound and the depression was covered with soil afterwards.

Trial Site 2: PNG Unitech Agriculture Farm.

Figure 5: a) A Unitech Agriculture Farm worker and training students from Umi Technical Vocational School and Finchafen Vocational Technical School assisting in making the mounds after the land area has been cleared and marked out. **b)** Wilfred Wau demonstrating 'how' and the 'direction' to plant the sweet potato vines based on sweet potato agronomy.

Figure 6: a) Wilfred Wau, farmer worker James Kwanua and the training students from Umi Technical Vocational School and Finchafen Vocational Technical School planting the sweet potato vines. **b)** Melanie Pitiki about to apply the entomopathogenic fungi into the depression created around the top of the mound.

Figure 7: a) Melanie Pitiki applying the entomopathogenic fungi into the depression around the mound top. **b)** Farm worker James Kwanua covering the depression with soil.

RAINFALL MEASUREMENT

Other parameters could not be measured or determined. However, the rainfall measurement will be recorded on a daily basis determine the average amount of rainfall throughout the trial.

A 1000ml measuring cylinder, a funnel and a cylindrical storage container was used to construct a rain gauge (**Figure 8** on the right) and set at the middle of the plot to be used to record rainfall measurements daily.

Note: This figure is the rain gauge set-up in Unitech Agriculture Farm. At Situm farm, a 1L container was used. However, it will be replaced with a similar one in the picture during the next visit to Situm trial site.

Fig. 8

GENERAL REMARKS

- The trial was successfully established at Pahoam village in Situm area on Wednesday 7th of October, 2020 and the trial at Unitech Agriculture Farm was also successfully established on Thursday 8th of October, 2020.
- The establishment of the trial at Unitech was supposed to be on Tuesday, however, it rained heavily the whole day and therefore we were not able to do any work on that day.
- However, on Tuesday 7th of October, 2020, Melanie gave a run-down of all the processes involved in the entomopathogenic fungal isolation to characterization, to multiplication on rice and briefly on molecular characterization of the EPF strains.
- The farmer at Pahoam village Mr. Ano Gielo was very excited to see this trial through. His family was very supportive and helped throughout the establishment of the trial.
- A locally constructed rain gauge using 1L container was set-up to collect daily rainfall data on both sites.
- I will be doing fortnightly visits to check the trial and give a new rainfall measurement data collection sheet to Mr. Ano Gielo and his family for rainfall measurement recording.
- The application of the entomopathogenic fungi will be done monthly.

ACKNOWLEDGEMENT

The establishment of this trial at the two trial sites was done successfully and I would like to sincerely thank the people who have helped in clearing the planting area, marking, making mounds, planting and digging the drains. Therefore, on behalf of the Unitech Team (Dr. Ronnie Dotaona, Dr. Gwendolyn Ban and myself;

I would like to firstly acknowledge and thank Mr. Wilfred Wau for his help in planning the trial, providing technical assistance and details in coming up with the design of the field plan, and actually establishing the trial too. Also thank you for negotiating with the farmer Mr. Ano Gielo and making the arrangements for us to work with him in setting up our trial at his garden. Thank you.

Secondly, I would like to acknowledge and thank Mr. Ano Gielo for agreeing to work with us and giving us his land area to establish an on-farm trial. I also like to thank his family for assisting in clearing the planting area and making mounds, as well as planting the sweet potato vines.

Thirdly, I would like to acknowledge and thank the Unitech Agriculture Farm staff, especially Mr. James Kwanua and the four training students (boys) from Umi Technical Vocational School and Finchafen Technical Vocational School who helped in clearing the land area for planting, making mounds, digging the drains and planting the sweet potato vines.

Finally, I would like to thank our driver, Mr. Steven Atuak, for driving us around safely.

Appendix 14. Are entomopathogenic fungi effective in controlling sweetpotato pests in the field?

1. Background

A small field trial was established on a sweetpotato farm in Queensland to examine the pathogenicity of two entomopathogenic fungi isolates on various pests of sweetpotato as a proof-of-concept exercise. The grower had history of minor *Cylas formicarius* damage and wireworm, but significant curl grub, cricket, and white fringed weevil damage to cultivars Bellevue (gold) and Northern Star (red). It was not unusual for this grower to lose 30% of the yield to pest damage.

2. Materials and methods

2.1. Preparation of the field for the trial

The field was planted with shoots of cv. Bellevue as per standard growing practices in Australia in November 2020. The transplanted shoots were watered with an overhead irrigation for the 1st week before the installed T-tape irrigation system was used. The shoots had been established in the sandy-loam field soil for 2 weeks prior to being inoculated with conidiated rice. Three rows (25 m long) were allocated randomly to each treatment: control, KS1 (*Beauveria bassiana*) and ECS1 (*Metarhizium anisopliae*) for a total of 9 rows.

2.2. Production of fungal inoculum and application of conidiated rice to the field

Metarhizium anisopliae isolate ECS1 and *Beauveria bassiana* isolate KS1 were maintained on Sabouraud dextrose agar with yeast (SDAY) at 27 °C with a 12:12 day:night photoperiod. After 14 days, a 1 cm^2 square of sporulating culture was excised from each agar plate and used to inoculate 250 ml of SDY liquid broth. Flasks were incubated at 27 °C at 150 rpm for 7 days in a shaking incubator (New Brunswick 450, Eppendorf, Australia). Solid substrate was prepared by autoclaving 1 kg of Australian Certified Organic (basmati) long grain rice in a heat-sealed spawn bag (Microsac, Belgium part no.) at 121 °C for 20 min. A corner of each spawn bag was cut and 500 ml of tap water was added before autoclaving for a second time at 121 °C for 20 min. Cooled rice was inoculated with 100 ml of 7 day old liquid culture (approx. 10^9 blastospores per ml) and heat sealed under aseptic conditions. Spawn bags were massaged to evenly distribute the blastospores, then incubated at 27 °C for 14 days in a controlled temperature room. Conidiated rice was transferred to a paper bag (25 x 40 cm) and dried at low humidity (ca. 30% RH) at 20 °C for an additional 14 days to reduce moisture.

Forty-eight hours prior to use, conidia germination and conidia per g dried rice were calculated. Both isolates had >92% germination. Isolate ECS1 had 2 x10^9 conidia per g of rice and isolate KS1 had 2.8 x10^9 conidia per g of rice. The concentration of conidia applied to the rows along the planted shoots and t-tape, (informed by laboratory and glasshouse studies) was approximately 1 x10^6 conidia per cm^3. This was calculated based on the 10 cm wide strip in which the conidiated rice was applied using a plastic cup, which was then covered with a layer of field soil to a depth of 10 cm to protect against UV radiation.

Figure 1. *Metarhizium anisopliae* (ECS1) conidiated rice (left) and *Beauveria bassiana* (KS1) conidiated rice (right) used for the field trial.

Figure 2. *Metarhizium anisopliae* (ECS1) conidiated rice inoculated along the T-tape irrigation/transplanted shoots (left) and *Beauveria bassiana* (KS1) conidiated rice (right) inoculated along the T-tape irrigation/transplanted shoots

2.3. Harvest and assessment of insect damage

Harvested sweetpotatoes from each treatment/replicate row were harvested in April 2021 and were placed into separate mega bins filled with water to aid washing. For each treatment/replicate row, 80 storage roots were randomly selected from the mega bin, positioned on a crate, and photographed for damage (and rotated to capture damage on the underside etc). Insect damage was classified into that damage by *Cylas formicarius* (sweetpotato weevil), damage caused by crickets (mole or black cricket), damaged caused by wireworm (true and false) and damaged caused by curl grubs (e.g. African black beetle *Heteronychus arator* or whitefringed weevil *Naupactus leucoloma*. Symptoms of disease was also recorded (see Figures 4-8). In some of the images, symptoms on cv. Northern star (red) were provided for comparison and reference. Various online resources (PestNet, CABI, LSU AgCenter), books (Ekman & Lovatt, 2015) and sweetpotato growers were consulted to establish the origin of damage symptoms. In some cases, insects were collected from the field (whitefringed weevil and curl grubs) and reared for a short period on sweetpotato roots to assess damage symptoms.

Figure 3. Harvest of Bellevue

2.4. Soil collection for assessment of infective entomopathogenic fungi (EPF) propagules

At harvest, three samples of soil were collected randomly in 70 ml capacity tubs from each row and treatment. The soil samples were brought back to the laboratory, where each tub received six larval mealworms (*Tenebrio molitor*), 2 ml of sterile water and a lid with ventilation holes. The tubs were gently inverted twice and incubated at 25 °C ±2 in a controlled temperature room. Tubs were incubated for up to two weeks and checked every other day for dead or infected mealworms, which were removed from the tub and placed into a humid chamber to encourage sporulation. Entomopathogenic fungi if present, was recorded; however, this was not analysed statistically.

2.5. Statistical analysis

Data was checked for normality using the W-test for normality and analysed with ANOVA after using GenStat 21st Edition (VSN International Ltd). Where necessary, data were transformed prior to analysis. Data that were not distributed normally were not analysed owing to the little power in non-parametric tests.

Figure 4. Healthy roots (a), root with sweetpotato weevil damage (b), root with old wireworm damage (c and d), roots with cricket damage (e and f). It is not clear if mole cricket (*Gryllotalpa* spp.) or black field cricket (*Teleogryllus commodus*) or other insects cause the damage presented here.

Figure 5. Root with curl grub or whitefringed weevil damage (a), root with curl grub and cricket damage (b), root with curl grub damage (c), roots with Southern blight lesions (d, e, and f). Here, curl grub damage may represent white grub, cane grub or peanut scarab.

Figure 6. Roots (cv. Northern star) with curl grub or whitefringed weevil damage (a, b, and c), unidentified curl grubs (possibly African black beetle) collected from field (d), root with curl grub and cricket damage (e, f).

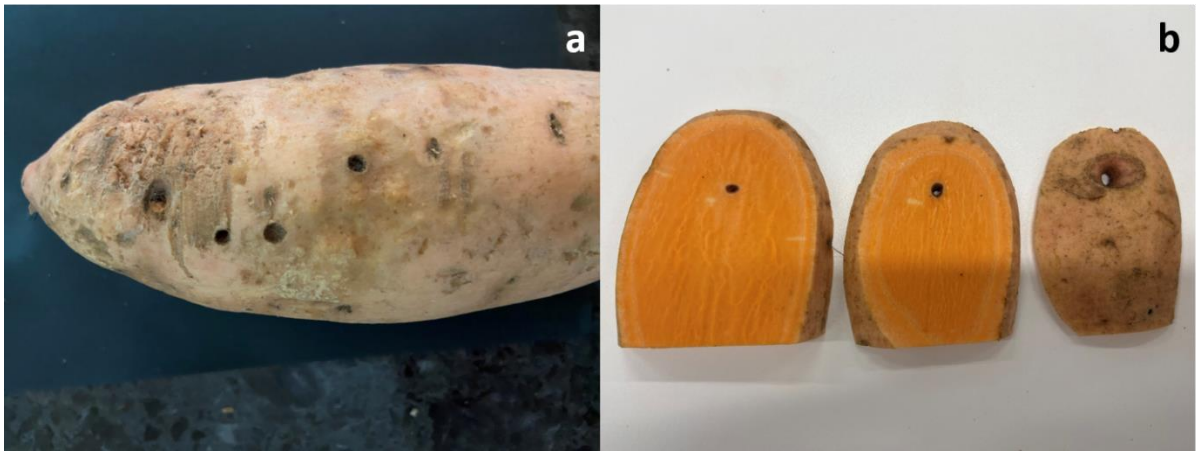


Figure 7. Roots (cv. Bellevue) with wireworm damage (a) and sectioned root with wireworm damage (b)

Figure 8. Roots (cv. Northern star) with cricket damage

3. Results

3.1. Disease

There was no evidence of root knot nematode or other nematode damage on the roots examined. Storage roots only had symptoms of Southern blight, caused by the fungus *Sclerotium rolfsii.* There was a higher incidence (9%) of Southern blight in the control roots; however, incidence did not differ between treatments, owing to the large variation within treatments (Figure 6).

3.2. Insect damage

For cricket damage, the effect of treatment was significant (P=0.015). There was significantly more cricket damage in control roots (ca. 13%), although the EPF treated rows did not differ significantly

from each other. The effect of treatment on wireworm was not significant (P=0.1) despite almost 14% of control roots having 1 or more wireworm lesions. The effect of weevil was significant (P=0.003), where there was significantly greater weevil damage in sweetpotatoes treated with Metarhizium. Finally, for curl grub/white fringed weevil, there was no significant differences between treatments (P=0.624) (Figure 9).

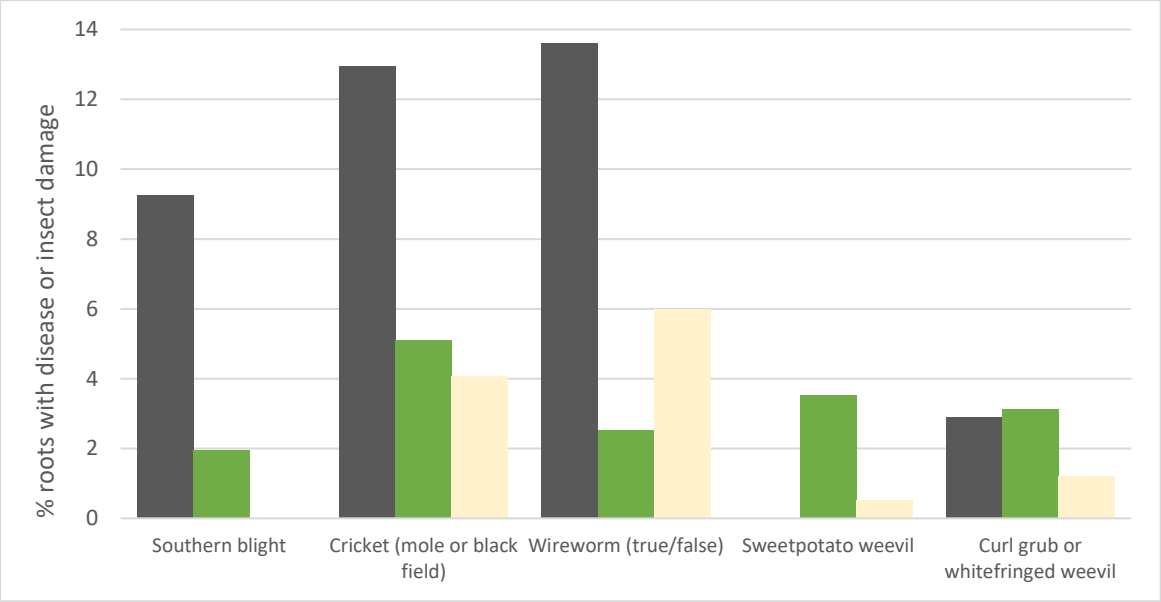


Figure 9. Percent roots with Southern Blight or various types of insect damage

The effect of treatment on all damaged roots (damage from insects or pathogens) damage was significant (P=0.012) (Figure 10). More damage was recorded on control roots compared to the EPF treated roots, which did not differ significantly from each other. For insect-only damage, the trend was as for damaged (P=0.037). The effect of treatment on the roots without damage (non-damage) was also significant (P=0.012). Significantly more roots treated with EPF were damage free compared to control roots.

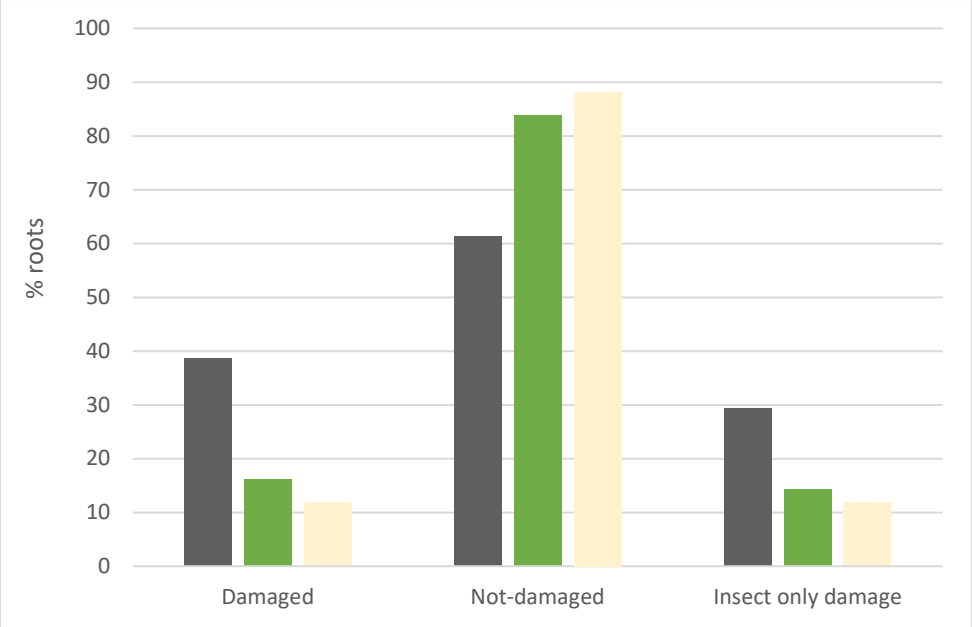


Figure 10. Percent roots with overall damage (damaged), not-damaged or insect-only damage.

When soil was sampled from treated (*Metarhizium anisopliae* (ECS1) or *Beauveria bassiana* (KS1)) or control rows to check for infective EPF propagules, there was 100% mortality of the 'bait' mealworms in all EPF treated tubs (9 tubs of soil collected per treatment). However, there was 70% mortality across the control treatments. Both EPF applied were recovered from their respective treatments (Figure 11); however, a darker green indigenous *Metarhizium* sp. was also recovered from the field site and was present in some of the ECS1 and KS1 soil (as sporulating cadavers) and frequently present in the control tubs.

Figure 11. Insect baiting using mealworms on treated field soil post-harvest. *Metarhizium anisopliae* (ECS1) treated soil and sporulating mealworm cadavers (left). The darker green sporulating mealworm is an indigenous *Metarhizium* sp. from the field site. *Beauveria bassiana* (KS1) treated soil and sporulating mealworm cadavers (right), showing colonisation of the soil (smaller cream colonies).

4. Discussion/summary points

Whilst many online resources were accessed to correctly classify insect damage, it was not always clear which insect was responsible for specific lesions. For example, there was a distinct lack of resources on what sweetpotato damage by crickets looked like, so it is very possible that this damage has been misidentified. Both mole crickets (and their burrows) and black field crickets had been sighted at the property.

Whilst treatment did have a significant effect on many different types of insect damage, it was not significant for sweetpotato weevil or curl grubs/white fringed weevil. According to the grower, pest pressure appeared low this season. Pest pressure was likely uneven throughout the experimental area and this would have contributed to the high level of variation between replicate rows. A large insect attracting light/water trap installed by the grower mid-experiment was closer to the EPF treated rows than the control rows (despite being allocated randomly) and as a consequence, insects occupying those rows may have moved out of the crop. Whilst there were no insect cadavers with sporulation recovered from the field there was evidence post-harvest that would suggest that infective propagules remained in the soil, which may have been responsible for mortality of crickets for example. Many species of entomopathogenic fungi have been isolated from mole crickets and have been shown to

cause varying mortality and behavioural changes in tunnelling that may indicate avoidance (Thompson & Brandenburg, 2005, Xia et al., 2000, Sonmez et al., 2016).

Future trials, with more replication are required to better establish efficacy of EPF in the field. A greater concentration of conidia should be used or multiple applications of conidia used to increase the chance of insects intercepting EPF, causing mortality or avoidance of the crop.

5. References

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Sweetpotato pest & disease management for Papua New Guinea: a toolbox approach

A brief manual produced by HORT/2014/083: Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea

Australian Centre for International Agricultural Research | Australian Aid

Charles Sturt University | University of Southern Queensland | NARI | PDFA | The Papua New Guinea University of Technology

A manual produced by HORT/2014/083: Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea. Version 1.0. November 2021.

Introduction

Pests and diseases can cause great loss to sweetpotato production in both home food gardens and commercial farms. There are many pests (such as weevils and mites) and diseases (caused by viruses, fungi and bacteria) that affect sweetpotato in Papua New Guinea (PNG). In this manual, we pay attention to the management of a selected few pests and diseases that have been identified as priorities in recent studies, with a focus on those easily spread in new gardens by infected planting material. Bedding root diseases and post-harvest diseases are not covered here.

Weevils are considered the most important pest of sweetpotato and includes both the sweetpotato weevil and the West Indian sweetpotato weevil shown in Figure 1, Figure 2 and Figure 3, both of which are widespread in many provinces. Feeding and tunnelling of larval stage weevils in storage roots makes them inedible.

Figure 1. The sweetpotato weevil (a) and (c) (see red circle) is easy to spot on storage roots. The West Indian sweetpotato weevil (b) (c) (see yellow circles) is easy to spot when there is good colour contrast, but is difficult to see against soil (e), both weevils cause similar damage to storage roots (f, g).

Figure 2. The sweetpotato weevil and feeding damage to stems (see orange circles) is easy to spot on close inspection. Do not use planting material with this damage, as the stems may harbour developing weevils.

Figure 3. Tunnelling in old sweetpotato stems and crowns caused by sweetpotato and West Indian sweetpotato weevils. In the middle photograph, a white pupa can be seen easily. Damage to the crown and stems weakens the plant and affects yield. Weevils are easily spread through infected planting material.

Gall mite is another important and widespread pest of sweetpotato, despite it being so small in size. The gall mite lays eggs in the plant tissue and the adults that eventually emerge feed at these sites and go on to create new galls. Galls reduce plant vigour and yield of storage roots (Figures 4 and 5).

Figure 4. Gall mite symptoms on sweetpotato leaves and stems. Gall mite is easily spread to new areas through infected planting material

Figure 5. Gall mite damage on shoots and petioles (within blue circles)

There is limited information on true or false wireworm infesting sweetpotato in PNG. True wireworms cause shallow feeding holes in storage roots, reducing the quality and marketability of the produce (Figure 6) and false wireworms tunnel deeper into storage roots causing 'shot' holes (Figure 7).

Figure 6. An example of wireworm larvae and an adult (a) and a corn wireworm on sweetpotato causing deep holes (b). *The collection of images in (a) have been used with permission for education purposes from Cesar Australia and image credit is given to Andrew Weeks, Cesar Australia. Image 5605710 (Image b) is permitted for use under a Creative Commons Licence, with permission and is credited to Gerald Holmes, Strawberry Center, Cal Poly San Luis Obispo, Bugwood.org.*

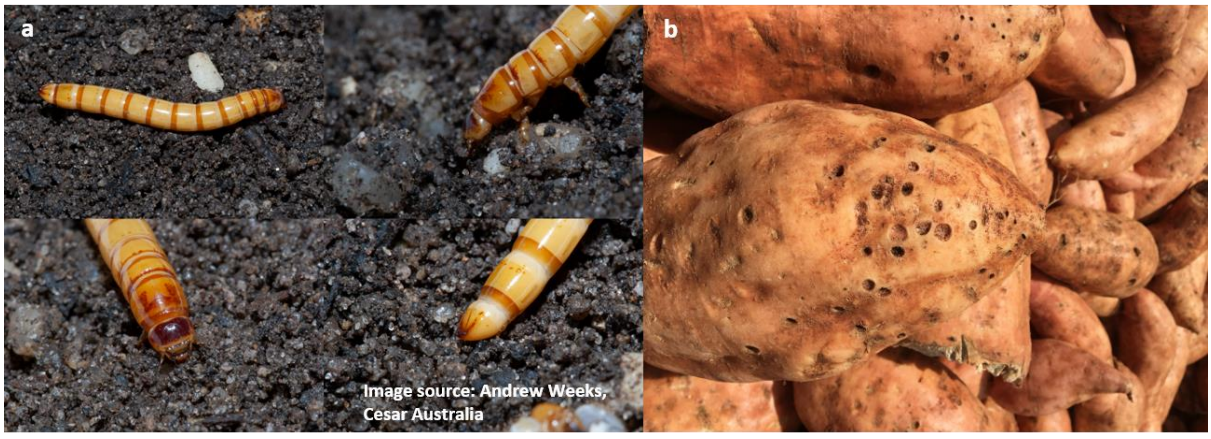

Figure 7. The eastern false wireworm larvae (a) and false wireworm damage on sweetpotato (b). *The collection of images in (a) have been used with permission for education purposes from Cesar Australia and image credit is given to Andrew Weeks, Cesar Australia.*

Root-knot nematodes are a major pest for sweetpotato globally but the extent of their problem in PNG is not clear. Root-knot nematodes are more common in sandy soil. Shoots become yellow and wilt and galls appear on lateral and storage roots. Storage roots become blistered due to root-knot nematode feeding and development, roots can become cracked and have a rough surface (Figure 8).

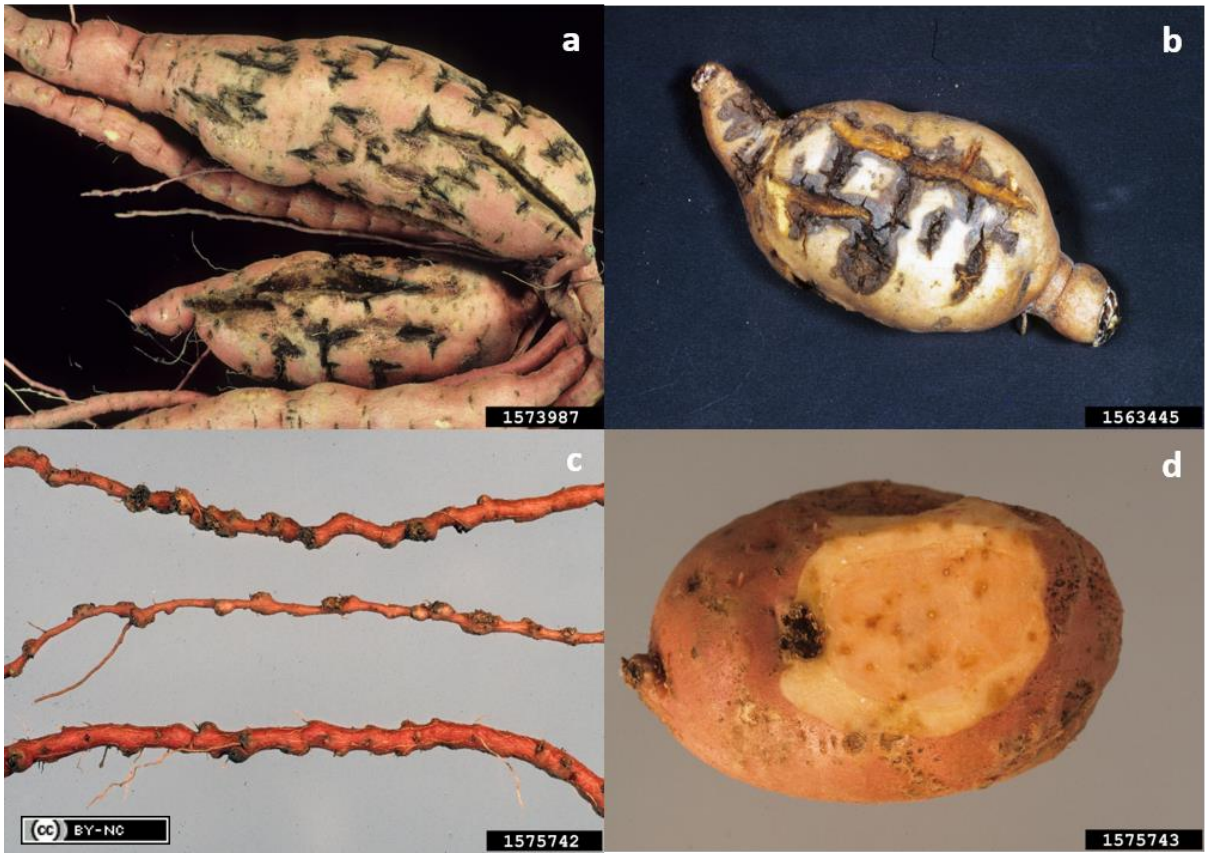

Figure 8. Root-knot nematode symptoms on sweetpotato storage roots (a and b), on developing storage and lateral roots (c) and internal damage to a storage root when the flesh is exposed (d). *All images permitted for use under a Creative Commons Licence, with permission. Images 1573987, 1575742 and 1575743 credited to Gerald Holmes, Strawberry Center, Cal Poly San Luis Obispo, Bugwood.org and image 1563445 credited to Charles Averre, North Carolina State University, Bugwood.org*.

Insects that are vectors for sweetpotato viruses are major concern for sweetpotato productivity. Whiteflies and aphids spread viruses between plants and are easily moved to new areas on planting material (Figures 9-11).

Figure 9. Whiteflies can be seen in both small and large numbers on the underside of sweetpotato leaves. Whiteflies transmit viruses between plants during feeding.

Figure 10. Aphids can be seen in both small and large numbers on the underside of sweetpotato leaves and stems. Aphids transmit viruses between plants during feeding.

Figure 11. Aphids can feed on other plants in the garden, such as cabbages (blue circle). These aphids may also transmit viruses important for sweetpotato. In the photo on the right, aphids (green) and dead parasitised aphids (brown) can be seen. The brown dead aphids are a good sign that 'natural' biological control is occurring by a beneficial wasp.

Viruses can severely reduce plant vigour and yield and there are many sweetpotato viruses of concern. One example is sweetpotato feathery mottle virus (SPFMV), which is spread by insects and through infected planting material (Figure 12).

Figure 12. Sweetpotato feathery mottle virus (purple mottling, halos) can appear differently depending on the variety, age of plant and growth conditions

Scab is a widespread fungal disease of sweetpotato that affects plant vigour, shoot formation and yield of sweetpotato (Figure 13). The tips of growing stems grow distinctively upright and have crinkled leaves. These are brittle and break off easily.

Figure 13. Scab is caused by a fungal pathogen that can be seen on the leaves and shoots of sweetpotato. Brown scabbed lesions occur on leaf veins, petioles and stems causing the leaves to become cupped and deformed

Management options for the sweetpotato grower's toolbox

Tool number 1. Sanitation: "cleaning-up" the garden

Sanitation is a great prevention tool for many pest and disease issues. Like us washing our hands to avoid getting sick, a crop can be protected by sanitation. In practical terms, this involves the following:

- Carefully remove all sweetpotato crop residue from the field (vines, damaged storage roots). Not removing old vines and damaged storage roots will encourage pests such as weevils to breed in the crop residue and some pathogens that cause plant diseases to proliferate.

- Ideally this is done immediately after harvesting, otherwise pests and pathogens can move to nearby gardens.

- Ideally, the land that you intend to plant with sweetpotato should not have had sweetpotato planted in it for at least 12 months or longer if possible.

- It is also a good idea to re-check the area of land just prior to planting a new crop.

- It is especially important to remove volunteer sweetpotato plants that might be growing from a discarded stem or piece of storage root.

- Crop residues, volunteer sweetpotato and weeds (see below) removed from a garden can be used to feed pigs or can be burnt.

Figure 14. Old vines (inside yellow circles), unwanted storage roots and volunteer sweetpotato plants need to be removed promptly after harvest to avoid these areas becoming a breeding site and reservoir for unwanted pests and diseases.

The second aspect of sanitation is to remove all weeds that are members of the same family as sweetpotato.

- Scientists call these 'Convolvulaceae' but in plain English it means plants that have large, trumpet-shaped flowers like sweetpotato plants.

- If you are unsure about identity of the weeds (for example if they are too young to have flowered), then to be on the safe side and remove these anyway!

- If using a crop rotation (growing another type of crop for a while, or just 'resting' the ground), it is important to keep the area free of sweetpotato volunteers and related weeds.

Figure 15. Weeding is an important component of integrated pest management

Tool number 2. Crop isolation

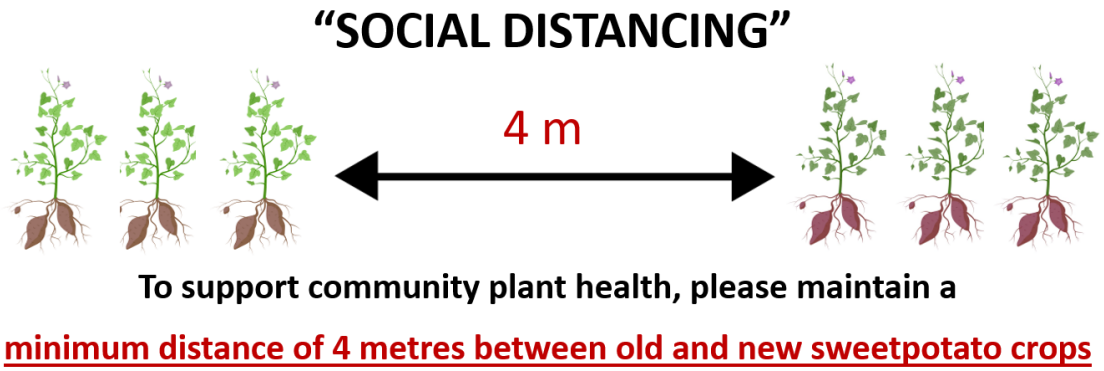

Figure 16. "Social distancing" for crops. Created with BioRender.com

"Social distancing" for a new sweetpotato crop is a useful way to reduce the risk that pests such as weevils might otherwise walk-in from an older crop field, which might already have built-up high levels of these pest insects.

- It is best to not have a new sweetpotato crop positioned next to an old sweetpotato crop. The wider the spacing the better, but our trials suggested a 4 metre-wide gap between old and new crops is large enough to be useful.
- This need not be bare ground; the area can be used to grow an alternative crop such as carrot or corn because these crops are not host to the most serious pests and diseases of sweetpotato.
- If root-knot nematodes (RKN) are a problem, carrot is not a good crop as it is a host to RKN.

Tool number 3. Klin kaukau (pathogen tested planting material)

Diseases like scab and viruses and pests such as gall mite and weevils, can found on (or within) sweetpotato cuttings (sprouts). If planting material is collected directly from the field, there is a good chance it is already infected (with a pathogen) or infested (with insects, which may be inside the shoot).

- Your new crop will have a better start if you are able to obtain clean planting material (klin kaukau)

- Increasing numbers of sweetpotato varieties are becoming available from the pathogen tested (PT) planting material program. If you live in an area where this is accessible, the extra cost is worthwhile.

Figure 17. Field cut vine on (a) may harbour pests and diseases. If possible, use 'clean' storage roots grown in an insect proof cage nursery for generating disease and insect free sprouts for field planting

- A crop established using PT material is likely to give significantly cleaner looking roots and higher yields (Figure 18-19). The benefits of investing in PT planting materials can also extend for another season or two because cuttings taken from a PT crop are likely to be 'cleaner' than those taken from a regular crop (which might harbour pests and diseases).

Figure 18. Some growers use plant beds to generate planting material for field planting. It is important that only healthy bedding roots (clean or PT) to generate these sprouts to maximise the number of successive collections that can be made and to minimise spread of pests and diseases. Crop sanitation and crop isolation recommendations apply to plant beds too.

Figure 9. Klin kaukau (PT) gives better yield and higher quality sweetpotato

Tool number 4. Weevil trapping with pheromone lures

This approach is specifically for the sweetpotato weevil (Figure 1a: colourful adults). It does not extend to other pests, not even the West Indian sweetpotato weevil (Figure 1b: drab colouration). This approach uses an empty plastic bottle into which is placed a small rubber 'lure' that contains a compound that mimics the scent produced by female weevils to attracts male sweetpotato weevils for mating (Figure 20).

Figure 20. The plastic bottle 'weevil trap' containing the pheromone lure deployed in the field (a), checking the trap in the field (b), a close-up of a male sweetpotato weevil on the pheromone lure that is placed inside the plastic bottle (c), live male weevils in the lid of a commercial weevil trap without water (d), drowned weevils in a weevil trap (e).

The trap has two uses:

Monitoring

- The trap can serve as an alert that this species of weevil is present in your crop and can give an idea of the relative density of the weevils and the risk to the crop.

- If large numbers of weevils are caught:

    - This signals an elevated risk of damage to the storage roots.
    - The crowns and shoots of the sweetpotato plant may be infested; do not use weevil-infested shoots as a source of planting material for new crops.

- If high numbers of sweetpotato weevils are being caught in traps, ensure there is good cover of developing storage roots using either soil or mulch to cover over exposed roots and any gaps in the soil that will allow weevils access to storage roots.

- The weevils are powerfully attracted to the weevil trap from a distance of 60 m away (down-wind and even upwind). Unless the field size is very large, monitoring for weevils requires only one trap per field.

- If you have multiple fields/gardens that are outside this 60 m distance, then use a weevil trap for each of these fields/gardens.

Mass trapping

- If multiple traps are used in the garden, they can catch so many adult males that the local density of these is potentially reduced to the extent that females then have trouble finding mates, so fewer eggs are likely to be laid.

- The trials in PNG used one weevil trap per 200 square meters. This is equivalent to about 10 traps for a typical commercial crop of 50 paces long and 40 paces wide. For typical smallholder garden (20 paces long and 10 paces wide) a single trap can be installed.

- Because weevils traps are relatively cheap, and there is no downside to using 'too many'. At the very minimum, use one weevil trap per 200 square metres of sweetpotato.

- Overseas work that used two and a half times as many weevil traps (2-3 traps per 200 square metres) showed an almost complete reduction of sweetpotato weevil feeding holes in storage roots.

- Always place the weevil traps at the edge of the garden or at the end of the row to avoid attracting weevils into the middle of the crop

Tool number 5. Mulching

Mulching is simply placing dead organic matter on the soil surface. This can be an effective barrier to protect storage roots from pests such as weevils and is especially important if the soil is dry and cracked because pests will use these openings to reach the storage roots.

- A trial of mulches in PNG also showed that these materials can help increase the local numbers of beneficial predators that prey on pests. Our trials made use of various locally available waste materials such as chopped sugarcane foliage and plant material cut from nearby uncultivated areas (Figure 21).

- Potential mulches that are widely available for free collection include Mexican sunflower and the shrub leucaena. As mulches breakdown they will provide the additional advantages of increasing soil organic matter, water holding capacity and releasing nutrients for crop growth.

- If mulch materials are not readily available, the normal practice of heaping up field soil to provide coverage of storage roots and fill cracks in the soil can be performed.

Figure 21. Mulches like sugarcane (a, b, c, e) can be placed on sweetpotato mounds to provide a barrier to pests that may access the storage roots through cracks in the soil and also break down to increase soil organic matter, nutrients and soil moisture (b, c). Various mulches were examined in field trials (d, e).

Tool number 6. Barrier plants

Strips of plants can be grown around a crop to provide a barrier to the movement of pests into a sweetpotato crop. This can be especially important if a new crop is planted adjacent to an older sweetpotato crop that is likely to contain pests and diseases. It is even more important if the new crop is not isolated (see Tool number 2. Crop isolation).

- Ideally the barrier crop is repellent to one of more important pests. Our trials used 50 cm wide barriers of Silverleaf desmodium, Smooth senna and Marigold (Figure 22.)

- Potentially, these plants can provide a secondary source of income (e.g., by picking the flowering stems of marigold flowers).

- Another potential advantage of the flowering plants is that the nectar and pollen they produce can help nourish beneficial predators of pests and even boost local densities of pollinators such as bees.

Figure 22. Living plant barriers such as Smooth Senna (left), Silverleaf Desmodium (middle) and French Marigold (right) were used in our field trials.

Remember what's in your toolbox

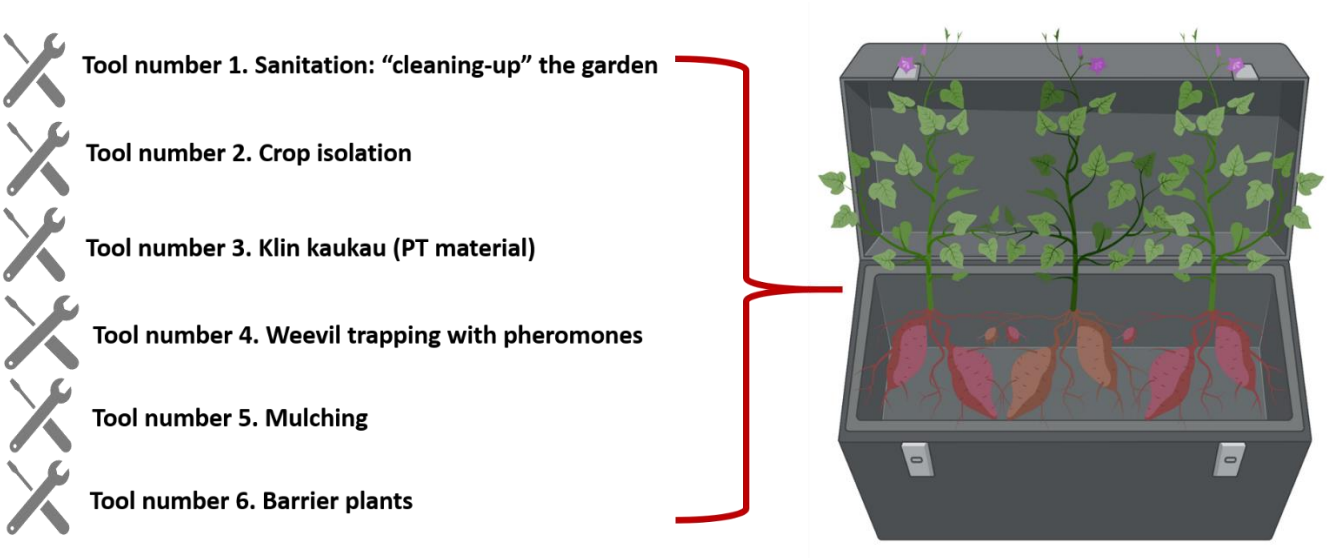

Figure 23. Toolbox for improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea. Made with BioRender.com

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A manual produced by HORT/2014/083: Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea. Version 1.0. November 2021.

SCIENTIFIC REPORTS
nature research

OPEN

Organic mulches reduce crop attack by sweetpotato weevil (*Cylas formicarius*)

Received: 15 April 2019
Accepted: 3 September 2019
Published online: 16 October 2019

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Mulching with organic materials is a management practice with long history for weed suppression, soil water conservation and erosion control. Its potential impact on crop pests is less well explored. Here we report its utility for reducing crop damage by the serious pest, sweetpotato weevil (*Cylas formicarius*). Laboratory bioassays measured the response of adult female weevils to sweetpotato storage roots beneath mulches of fresh or dried plant materials. Weevils were significant repelled by fresh basil, catnip, basil lime and dry eucalyptus, cypress, lucerne and sugarcane. A subsequent field study found that mulches of dry cypress, eucalyptus and lucerne reduced movement of weevils from a release point to reach sweetpotato plants and lowered level of damage to storage roots. Results demonstrate that mulching with organic materials merits further testing as part of the integrated management of sweetpotato weevil, particularly to protect developing storage roots during dry periods when soil cracking can facilitate access by pests.

Mulches are defined as organic or synthetic materials that are applied as a cover to the soil surface and are widely used in various agricultural systems[1]. Mulches can suppress weeds, conserve soil moisture and reduce erosion but those consisting of organic materials offer an additional range of potential benefits. These include enhancing soil organic matter and associated soil biological activity, soil nutrient status, and moderated soil temperature[1,2]. Much less well explored is the potential of mulches to contribute to pest suppression via phenomena such as constituting a physical barrier to pest access to vulnerable crop parts, or making host plant detection more difficult by virtue of the chemical composition and volatile production by mulch material[3].

Sweetpotato weevil, *Cylas formicarius* (Coleoptera: Apionidae), is globally the most destructive pest of sweetpotato, *Ipomoea batatas*[4,5]. Adult *C. formicarius* feed on sweetpotato foliage and the larvae damage stems but the most serious impact results from larval attack to the storage roots that are the harvested commodity. Direct feeding damage to the storage roots is compounded by the plant producing defensive sesquiterpenes that make the roots strongly distasteful, as well as by promoting damage by fungi and bacteria[6]. Losses to weevils are especially severe under dry seasons when soil cracking makes access to the roots easier for gravid females[7]. Storage roots may also be exposed to pest attack when their swelling, possibly combined with soil erosion from rainfall, leads to the cover of soil being breached. Sweetpotato weevil adults have limited capacity to dig through the soil[8] so, in the absence of these conditions, they can reach storage roots only with difficulty. Though *C. formicarius* adults are known to fly, dispersal is chiefly via the use of infected planting material or by adult weevils walking from infested crops to newly planted nearby areas[9]. Accordingly, preventing the initial movement of *C. formicarius* into a crop and minimising subsequent access to storage roots are key to managing this pest[4,10].

Insecticides, the use of pathogen tested planting material, and trapping with sex pheromones are used with success in sweetpotato productions systems in developed countries but are less available or affordable in developing countries where it is necessary to develop low-cost approaches[4,11], potentially including pest-deterring mulches of locally-available materials. Even developed countries may benefit from the availability of new methods

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to lessen reliance on costly inputs and slow the development of insecticide resistance. Mulches have previously been used in sweetpotato cropping to suppress weeds, provide nitrogen, minimise soil erosion, encourage beneficial insects[11,12] and to reduce pest attack[7].

The aim of the present study was to explore a range of organic mulch treatments to determine their potential utility for sweetpotato weevil management, specifically by preventing their movement through layers of mulch and reducing levels of infestation achieved in storage roots. Initial laboratory screening of mulch treatments were designed to address two scenarios of field use by farmers. First, the application of mulch over partially exposed storage roots with the control treatment consisting of storage root covered by potting mix. This control reflected the farmer manually cultivating the field soil to repair cracks whilst the mulch treatments represented the less laborious application of mulch to cracked areas. The second study compared mulches with a control in which the storage root remained partially exposed. This reflected the farmer not cultivating to repair cracked soil and compared the mulch treatments with this 'no-action' scenario. A smaller number of mulch treatments was subsequently evaluated in small field plots, again with covered and uncovered storage root fragments to address both management scenarios.

Methods

Insect and plant materials. *Cylas formicarius* were collected from farms in Cudgen, New South Wales and Bundaberg Queensland as immatures within infested storage roots and supplemented by adult males caught using sex-pheromone traps. A laboratory colony was maintained at the Orange campus of Charles Sturt University (33.2465°S, 149.1173°E) using growth chambers ran at 26 °C ± 2 °C, 60 ± 5%RH, 12:12L:D photoperiod. Insects were supplied with supermarket-purchased sweetpotato storage roots that were replaced on a weekly basis. Infested sweetpotatoes were kept in separate containers to give specific cohorts of emerging adults. Females were discriminated from males based on antennal morphology and only these were used in experiments. Preliminary studies identified a need to move adult females from the high density rearing/emergence vessels for a period of 24 hours in lower density vessels in order for them to exhibit biologically faithful responses to various plant stimuli. Without this measure, females exhibited very strong dispersal irrespective of external conditions. Accordingly we use a protocol that took cohorts of females 10–15 days after eclosion and held these under low density conditions without food for 24 h at 26 °C ± 2 °C prior to use in all studies.

The mulch materials used were selected based on a literature review that indicated their likely production of compounds with activity against herbivore Coleoptera[13–18]. Each was either (i) a potential secondary crops that could be cultivated in conjunction with sweetpotato so that crop residues or excess plants (e.g., thinnings, prunings) could be used as mulch or (ii) organic materials that are likely to be cheaply available or readily produced locally.

Potential secondary crops that were tested as freshly chopped fragments were: spring onion (*Allium fistulosum* L.), basil (*Ocimum basilicum* L.), catnip (*Nepeta cataria* L.), chilli (*Capsicum annuum* L), lime basil (*Ocimum americanum*, L.), tobacco (*Nicotiana tabacum* L.), oregano (*Origanum vulgare* L.), French lavender (*Lavandula stoechas* L.), white onion (*Allium cepa* L.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), marigold (*Tagetes patula* L.), Mexican sunflower (*Tithonia rotundifolia* (Mill.) S.F. Blake) and spearmint (*Mentha spicata* L.). Dried mulch materials were: sugarcane (*Saccharum officinarum* L.), lucerne (*Medicago sativa* L.), wheat straw (*Triticum aestivum* L.), eucalyptus (*Eucalyptus albens* Benth.) and cypress (*Cupressus* × *leylandii* A. B. Jacks. & Dallim).

Fresh materials were purchased as live plants from a plant nursery (Thompson's Garden Centre, Orange, New South Wales). The mulch was prepared by chopping all aboveground plant parts into 2–3 cm long pieces with clean scissors. Dry mulch materials, lucerne (Oreco Group, Organic Lucerne Mulch), sugarcane (Oreco Group, Sweet Garden Organic Sugar Cane Mulch), eucalyptus (ANL, Eucy Mulch) and cypress (Ki-Carma, Cypress Mulch) were purchased from Bunnings Warehouse (Orange, New South Wales) and used directly from the pack in their original, proprietary form. Wheat straw was purchased as a bale from Mullion Produce (Orange, New South Wales). An additional treatment of whole fresh cabbage leaves was included in the field experiment and this used freshly-collected plants from the University farm.

Laboratory screening of mulches. The mulch materials were divided into groups for testing: Group A (spring onion, sugarcane and lucerne); Group B (basil, catnip and chilli); Group C (lime basil, tobacco and oregano); Group D (French lavender, white onion and lemongrass); Group E (marigold, Mexican sunflower and spearmint); Group F (wheat straw, eucalyptus and cypress).

The initial screening of the mulch materials was carried out in multiple-choice test mesocosms made with plastic plant pots, 31 cm in diameter, and 26 cm in depth. The pots were half -filled with proprietary potting mix (Osmocote Professional Premium Plus Potting Mix, from Bunnings Warehouse, Orange, New South Wales), then a 90 mm diameter Petri dish base placed centrally on the potting mix surface of each. The area surrounding the Petri dish was then divided into four, equal sized quadrants. In the centre of each quadrant, a 50 g (±10 g) piece of sweetpotato storage root was positioned on the potting mix surface. This was then covered by one of the mulch materials, with the fourth quadrant as control. The first type of control left the root fragment uncovered to simulate exposure of storage roots in cracked field soil. The second type of control covered the root fragment with 2 cm of potting mix to approximate field conditions in which soil cover had been retained over developing storage roots.

Each group was replicated 3 or 4 times with the uncovered control and 3 or 4 times with the covered control. Mulches were applied to a 2 cm depth. Forty naive adult female *C. formicarius* were placed in each central Petri dish at around 3 pm and allowed to disperse into each of the equidistant mulch treatments. A Fluon (INSECT-A-STOP, Queensland) barrier was applied to the top edge of pots to prevented escape from mesocosms. After 24 hours, each piece of sweetpotato storage root was visually inspected and the number of weevils and feeding holes were recorded.

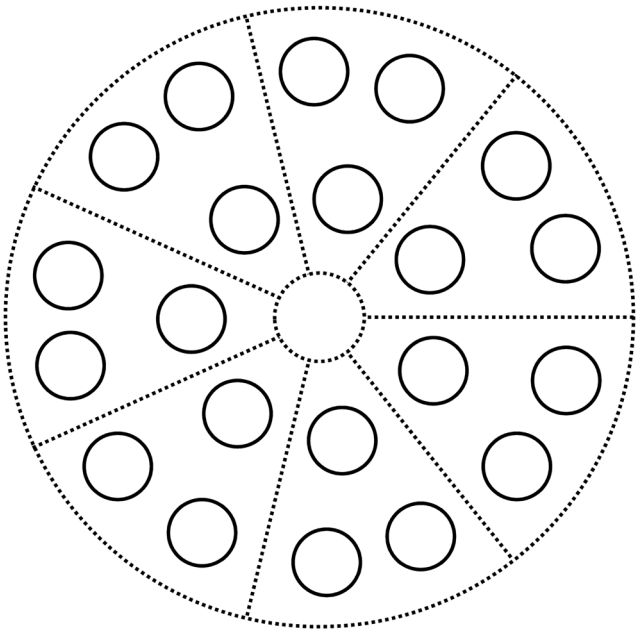

Figure 1. Illustration of multiple-choice test field design. Each block was 2 m in diameter with a central release point for weevils, surrounded by one plot of each mulch treatment. Plots each contained three sweetpotato plants illustrated in solid circles, one in the inner ring and two in the outer ring.

Field testing of mulches. Mulch materials of lucerne, sugarcane, eucalyptus, cypress, wheat straw and cabbage leaves were selected for field testing. An area of grassland on the university campus farm, which does not have sweetpotato weevil, was cultivated to prepare four round blocks, each 2 metres in diameter and 2 metres apart. Each block was surrounded by a 60-cm-tall black plastic barrier (Whites Recycled Garden Edging), the base of which was sunk into the soil to prevent passage of weevils. Each of the four blocks was divided into 7 equal-sized wedges-shaped plots, with the central area as the weevils release point (Fig. 1). Within each plot, three soil mounds, each 15 cm-tall and 25 cm in dimeter, were formed using hand tools. Accordingly, each block had 21 mounds with seven forming a ring in the inner part of the block and equidistant form the weevil release site. The remaining 14 mounds made up an outer ring (Fig. 1). Sweetpotato plants were transplanted singly into each mound at the end of January 2018. The control plot in each block had bare soil. Thereafter, plots were watered and hand weeded twice a week. Six weeks later, shop-purchased sweetpotato storage roots were selected with comparable size (each 200 ± 50 gram) and placed in plots. At this time, mulch materials were applied, one treatment to each plot, to a depth of 3 to 5 cm. A piece was placed either side of the inner sweetpotato plant, one fully covered by the existing mulch and a second piece sunk into the mulch but with the upper surface exposed. In the outer part of each plot, the two pieces of storage root were positioned equidistant between the two sweetpotato plants, one fully covered and one partly exposed. This method was adopted to assess the effects of mulching on two types of behaviour by the weevil: (i) lateral movement over the mulch surface to reach uncovered storage root, and (ii) vertical movement through the mulch layer to reach storage root covered by mulch after travelling laterally. In the control treatment where no mulch material was applied, field soil was used to partially or fully cover the storage roots. The mulch materials were given 24 h to settle before 140 adult female *C. formicarius* were released into the centre of each block.

Initial Assessment was done two days after weevil release. Sweetpotato foliage was visually inspected and the number of weevils present on the leaves and stems of each plant were recorded. All storage roots were visually inspected for weevils, then removed to the laboratory for inspection of feeding holes.

Immediately after the initial assessment, two new pieces of storage root were placed in each plot, one beside the sweetpotato plant in the inner ring and one between the two sweetpotato plants on the outer ring. These were fully covered with mulch except in the control treatment (no mulch) where storage roots were left uncovered.

Second Assessment was conducted 10 days after the weevil release. Sweetpotato foliage and storage roots were inspected for the presence of weevils a second time. The storage root pieces were removed to be inspected for feeding holes in the laboratory.

Statistical analyses. Analysis of variance (ANOVA) and LSD test were conducted to compare the effect of mulch treatments on number of weevils reaching the sweetpotato and number of feeding holes using software IBM-SPSS. In the laboratory studies, the mesocosms of each control type were analysed separately. In the field study, a GLM univariate analysis of variance was conducted on the influence of three independent variables, mulch treatment, and distance from the central release area of weevils and whether or not the storage root fragment was covered. Figures were generated using Microsoft 2013 excel and package ggplot2 in R version 3.4.4[19].

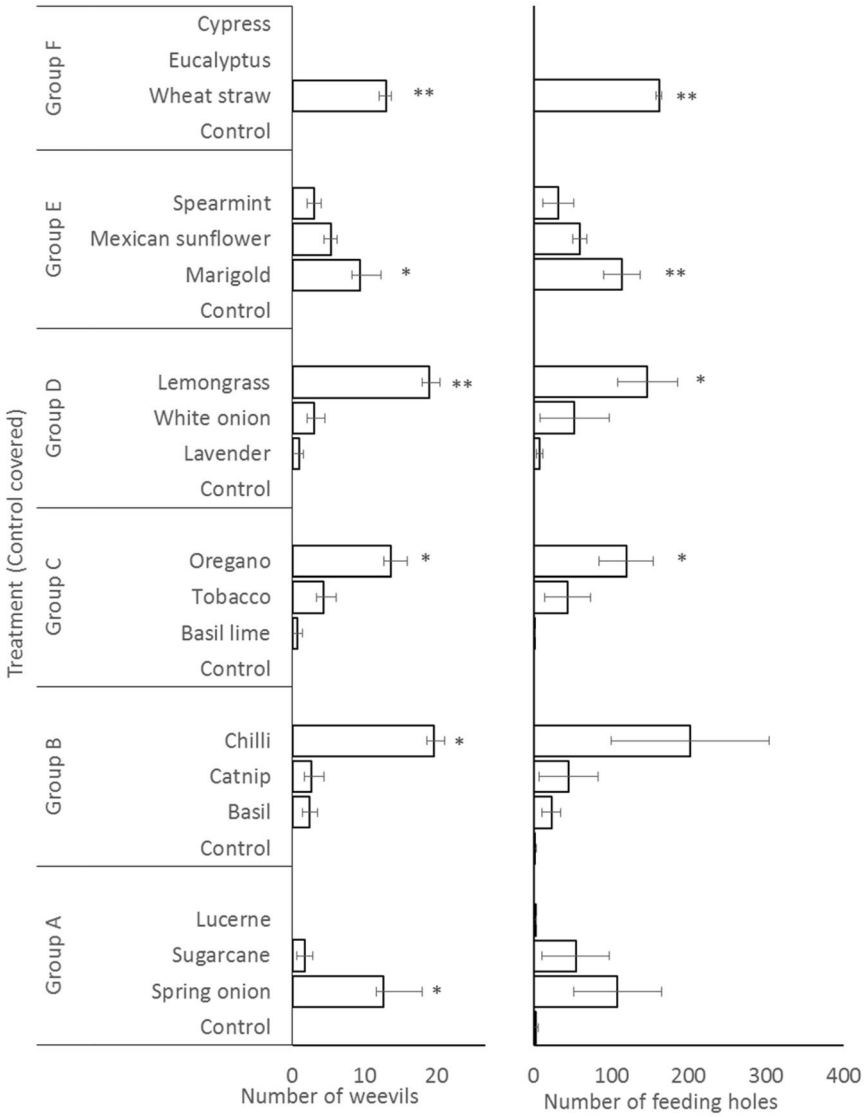

Figure 2. Effect of mulch materials in protecting sweetpotato storage root (Control covered by potting mix) (laboratory screening of mulches). (*means $p < 0.05$ when compared to control within the group; **means $p < 0.01$ when compared to control within the group).

Results

Laboratory screening of mulches. Experimental treatments had large and statistically significant effects on numbers of *C. formicarius* and incidence of their feeding holes on sweetpotato storage root pieces. Weevil numbers were low (0 been observed) in the control consisting of root pieces covered by potting mix, and significantly elevated, to >9 weevils per root fragment, by spring onion, chilli, oregano, lemon grass, marigold and wheat straw (Fig. 2). In the study in which the control had uncovered storage root pieces, relatively large numbers of weevils (between 4 and 16) were present on the storage root pieces and significant reductions (to <3) were evident in treatments of spring onion, sugarcane, lucerne, basil, catnip, basil lime, spearmint, eucalyptus and cypress (Fig. 3).

Numbers of weevil feeding holes on sweetpotato storage root fragments followed a broadly similar trend to the foregoing results for weevil numbers. In the covered root control (Fig. 2), <5 holes were recorded per root fragment but this rose significantly, to >100, in treatments of spring onion, chilli, oregano, lemongrass, marigold and wheat straw. This demonstrated that no mulch treatments gave weevil control that was more effective than covering storage roots with soil and that some mulches exacerbated damage.

For the study comparing mulches with an exposed fragment of storage root, numbers of feeding holes were high in the control, falling between 121 and 369. In contrast, significant reductions, less than 48 feeding holes, were evident for mulch treatments spring onion, sugarcane, lucerne, basil, catnip, basil lime, tobacco, lavender, marigold, Mexican sunflower, spearmint, eucalyptus and cypress. This demonstrates that a majority of the mulches tested gave significant levels of protection to exposed storage roots compared with a scenario of the farmer not having the time or labour to manually cover the exposed roots with soil.

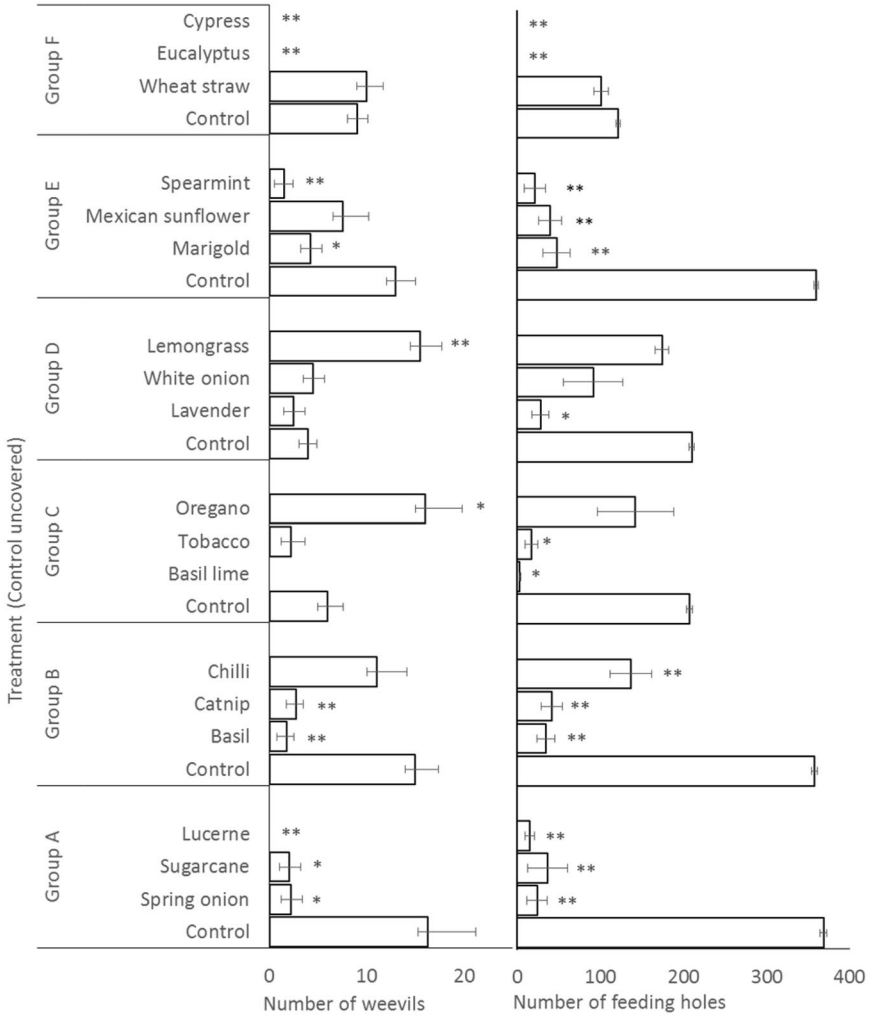

Figure 3. Effect of mulch materials in protecting sweetpotato storage root (Control uncovered by potting mix) (laboratory screening of mulches). (*means $p < 0.05$ when compared to control within the group; ** means $p < 0.01$ when compared to control within the group).

In the first group of mulches when the piece of sweetpotato was left uncovered a significantly higher number of weevils and feeding holes were found on the control (no mulch) treatment. However, when the sweetpotato piece was buried in the potting mix the piece of sweetpotato under the spring onion was the least successful in masking the sweetpotato and attracted the highest number of weevils. There was no differences in the number of feeding holes for any of the treatments spring onion, sugarcane, lucerne or control when the control was covered (Fig. 2).

Field testing of mulches. Reflecting the earlier laboratory results, mulches had strong effects on weevils under field conditions. Numbers of weevils on the foliage of sweetpotato plants was reduced by all mulch treatments, irrespective of the distance of plants from the central release zone ($F_{mulch}(6,42) = 3.4$, $p < 0.01$; $F_{distance}(1,42) = 0.2$, $p < 0.7$) (Fig. 4). This response variable did not, however, consistently agree with the more economically important variables of numbers of weevils reaching storage roots and levels of damage. Weevil numbers on storage root fragments were significantly affected by mulch treatment and distance ($F_{mulch}(6,84) = 4.9$, $p < 0.001$; $F_{distance}(1,84) = 13.8$, $p < 0.001$) with lower numbers in the outer distance and for the mulches lucerne, sugarcane, eucalyptus and cypress mulches (Fig. 5). For numbers of feeding holes, the same variables as well as whether the storage root was covered or not, all had significant effects (($F_{mulch}(6,84) = 9.5$, $p < 0.001$; $F_{distance}(1,84) = 29.8$, $p < 0.001$; $F_{cover}(1,84) = 7.2$, $p < 0.01$). Numbers of holes in the inner distance were very much higher than in outer distance and here damage was greater in the control, wheat straw and cabbage treatments with damage also tending to be higher for unexposed storage root fragments (Fig. 6).

For the second assessment, the weevils distribution was significantly affected by mulch treatment, with few present on storage roots covered by lucerne, sugarcane, eucalyptus or cypress mulches ($F_{mulch}(6,42) = 5.0$,$p < 0.01$) (Fig. 7). Compared with the earlier assessment date, relatively large numbers of weevil feeding holes were evident on storage roots in the outer zone of plots. Notwithstanding this, feeding damage was significantly greater to storage roots in the centre part of each plot ($F_{distance}(1,42) = 12.5$, $p < 0.01$) (Fig. 8). For this variable also, mulch treatment also had an effect ($F_{mulch}(6,42) = 4.4$, $p < 0.01$) such that lucerne, eucalyptus and cypress mulches

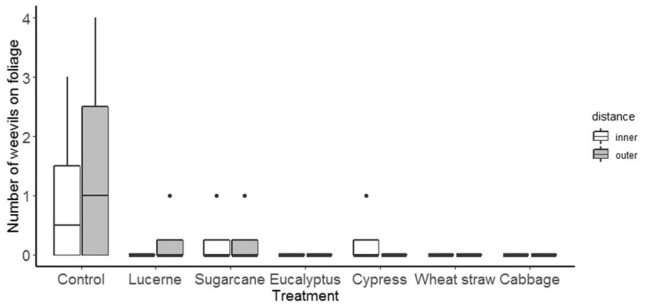

Figure 4. Effect of mulch on weevils' movement towards sweetpotato foliage at initial assessment in the field test.

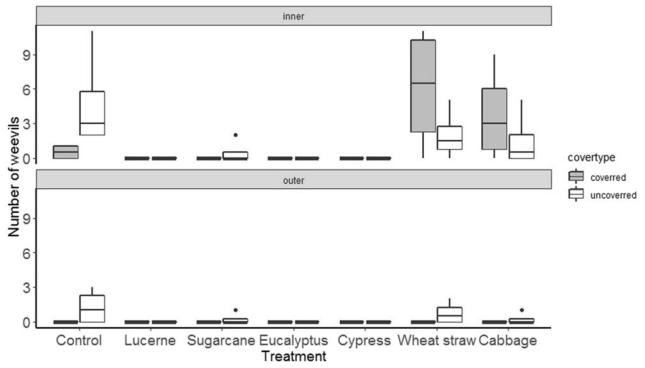

Figure 5. Effect of mulch on weevils' movement towards sweetpotato storage root at initial assessment in the field test.

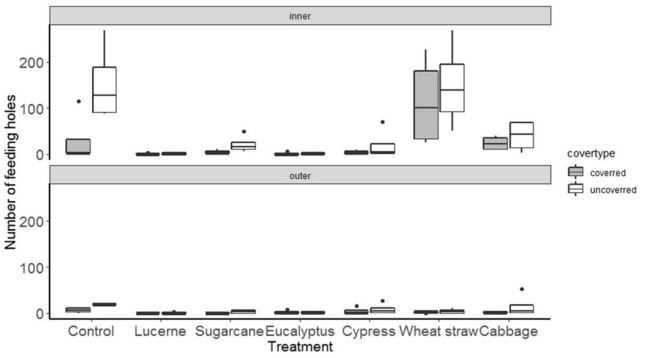

Figure 6. Effect of mulch on weevils' feeding damage on sweetpotato storage root at initial assessment in the field test.

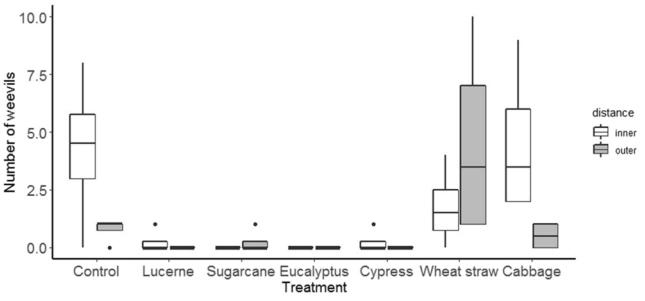

Figure 7. Effect of mulch on weevils' movement towards sweetpotato storage root at 2nd assessment in the field test.

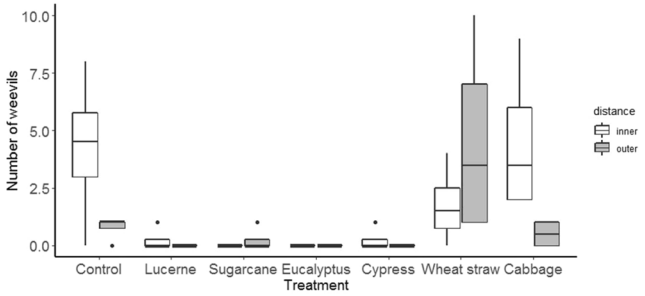

Figure 8. Effect of mulch on weevils' feeding damage on sweetpotato storage root at 2nd assessment in the field test.

significantly reduced the number of weevils reached sweetpotato storage root compared to control treatment. Cabbage leaf and, especially, wheat straw and mulches afforded a poor level of protection to sweetpotato roots.

Discussion

Mulches of 9 of the 18 types of plant material tested in the initial laboratory screen exhibited protective effects for sweetpotato against *C. formicarius*. Among the six treatments subsequently tested under field conditions, four provided significant levels of control of weevil colonisation of the crop and reduced feeding damage. This high incidence of biologically active plant species in both studies is of practical significance because it suggests that future testing of the plant materials that are readily and cheaply available to growers in a given region are likely to reveal a number of suitable candidate mulches with protective properties.

Mulches of synthetic material or of living or dead plant material are widely used in agriculture for a number of purposes, most commonly weed suppression and water conservation. This level of prior acceptance is likely to predispose farmers to be prepared to adopt mulches to serve a wider range of functions including pest management. In sweetpotato production, straw or plastic mulches have been used to reduce weed growth and extend the season in cooler climates[20] and there has been one study suggesting utility against a congeneric of the species we studied, *C. puncticollis*[7]. In that African field study, freshly harvested, dried, chopped aerial parts of elephant grass (*Panicum maximum*) were applied to plots at 0, 1, 3 and 5t/ha. Mulching intensity was significantly and inversely associated with pest infestation rate of *C. puncticollis* and led to higher storage root yields. Though the present study did not include elephant grass, the apparent repellence of several of the mulches tested and reductions in feeding hole numbers are consistent with the effects reported by Mansaray *et al.*[7]. The Poaceae species tested in the present study, sugarcane, lemongrass and wheat straw, had inconsistent effects on *C. formicarius*, suggesting that taxonomic identity of the plant from which mulch material is sourced is likely to be less important as a predictor of efficacy than specific plant traits. Among the traits most likely to be key to efficacy in pest management is the chemical composition, particularly the production of volatiles. Indeed most of the plant materials showing effects in the present study were – to human perception – strongly smelling. Significantly, however, the diversity of plants that yielded efficacious mulches suggests that there is no single class of volatile compounds responsible for the observed effects on *C. formicarius*.

There is little information available on the chemistry of *Cupressus* × *leylandii*, the source of the cypress mulch that was effective in laboratory and field studies but it is anecdotally repellent to termites (http://www.cypress-mulch.com.au/). Members of the genus reportedly have pinene- and -myrcene-rich terpeneoid volatiles[21,22] that have widely-reported repellent effects on arthropods[23] that are likely to be key to the effects observed on *C. formicarius*. Mulch of *Eucalyptus albens* was potent in laboratory and field tests and the members of this genus are known to produce volatile terpenes with activity against arthropod pests[24,25]. The sugarcane mulch tested in the present study was produced from leaves and tops rather than being the 'bagasse' by product of sugar processing. Though the plant nutrient composition of this material has been studied[26] and insecticidal properties reported from lignin emulsion extracted from sugarcane waste[27], there is no available information on the volatiles that are likely to be responsible for effects on *C. formicarius* in the present study. For lucerne hay, the fourth mulch material exhibiting potency in the present laboratory and field studies, a total of 147 peaks was reported in GC-MS studies of livestock feeding preferences[28]. That study did not seek to identify the compounds responsible for the GC-MS peaks and there is a lack of other studies of this topic. Among the species that were active in the laboratory studies, basil has previously been reported as useful suppressing other pests and is considered a useful companion plant due to the volatile is produces[29–31]. There is, however, no literature on its effects on pests when used as a mulch.

Sweetpotato weevil responses to volatiles are known to vary according to chemical composition[32,33]. The foregoing brief overview of the volatiles of the mulches with efficacy against *C. formicarius* illustrates that much further work needs to be conducted to establish a knowledge of the chemistry of mulches and to link this with results from behavioural studies of this species and other target pests of interest. Key to this is establishing the concentration and identity of volatile compounds in various types of mulches for this will affect the duration of effect after a mulch is applied. A short period of efficacy reflecting, for example, highly volatile active compounds, may not preclude utility in use against *C. formicarius* if application coincides with a period of high levels of crop protection such as during a drought when soil cracking becomes serious.

A wider range factors is likely to affect the net effect of mulches on pest management. Mulch application may lead to microclimatic changes within the crop canopy and to the soil as well as within the mulch itself and these could encourage natural enemies. In apples, for example mulch treatments including composted poultry manure led to lower soil temperature and higher soil moisture levels as well as an increase in edaphic detritivores and predators[28]. Cover crops that remain on soil surface after dying off, effectively resulting in a cover of mulch, increase the structural complexity of the soil surface and – combined with other factors – can lead to greater abundance of natural enemy species and lower abundance of pests[34]. The organic matter in mulches can enhance natural enemies independent of effects on physical structure and microclimate by promoting the densities of detritivore prey available to generalist predators, an effect that can translate into enhanced control of the focal pest is systems such as rice[35,36]. Cypress mulch has also been shown to promote predatory insects which in turn reduces insect pest incidence[37]. The addition of organic matter can also have a more general promoting effect on soil invertebrate biodiversity, for example sugarcane promoting ants and earthworms[38,39]. More widely, the presence of organic matter can have an effect on entomopathogens through protection from desiccation or ultra-violet light, an effect evident for the persistence of the entomopathogenic nematode, *Steinernema carpocapsae*, in plots of soybean[36]. Entomopathogenic nematodes of sweetpotato weevil are known[40] therefore mulches could potentially be used to promote these. A further, potential beneficial effect of mulches is that the organic matter can promote entomopathogenic fungi by serving as substrate[41]. It is important to note that the foregoing, natural enemy-mediated effects were not operable in the present laboratory studies and were unlikely to have been significant in the subsequent field study. They do, however, need to be considered in future field evaluations.

A final set of factors that favour the potential value of organic mulches in sweetpotato production systems relate to broader agronomic issues. Stone *et al.*[42] reported sweetpotato yields were promoted by a treatment involving a killed cover crop of vetch, with this likely to have resulted from the resulting mulch layer reducing soil temperature and promoting the development and bulking of storage roots[42–44]. Further, the decomposition of the organic matter in mulches adds nutrient to the soil[4,45,46]. Sweetpotato is not a good competitor of early season weeds[45] and smallholder farmers often hand weed at this stage[47], therefore mulches can help suppress weeds with the extra labour required to collect and apply the mulch offset by reduced need for labour to weed the crop.

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Acknowledgements

This work was funded by the Australian Centre for International Agricultural Research (grant Hort2014/083).

Author Contributions

J.L. and G.M.G. conceived the study, M.R. and J.L. conducted the work with assistance from T.E.D., J.L. analysed the data and prepared figures. All authors prepared the manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

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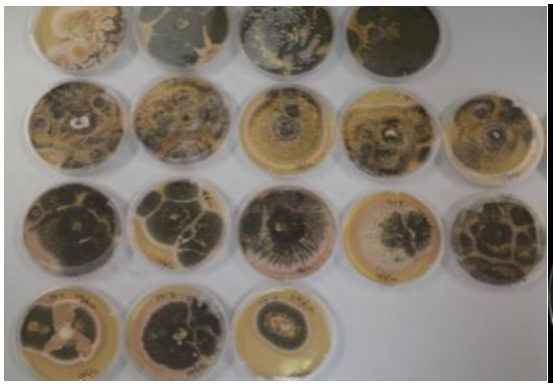
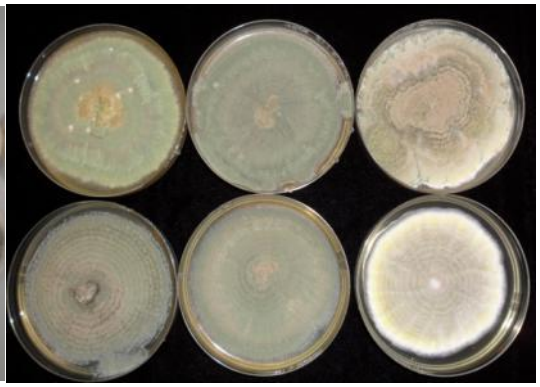
HORT 2014 083 Fungal entomopathogen research methods

Dr Bree Wilson, University of Southern Queensland

1. Isolation of *Metarhizium* spp. from the soil- baiting and soil dilution series

Field soil sampling

Materials

Plastic bags

Trowel or small spade or corer.

Pencil and paper for labelling and notes on site, conditions.

Paper towel and 70% ethanol for cleaning trowel between samples

GPS if possible for location of field

Esky if possible to keep soil cool after sampling

1. Entomopathogenic fungi can be isolated from many types of soil; areas that are commercially cropped, community gardens and family gardens (PNG). It can be soil that is currently cropped or is currently fallow.
2. Select fields for sampling that have contrasting cropping history and that are geographically disparate (for example soil in Lae up to Goroka, Toowoomba to Bundaberg).
3. There are no set rules for sampling soil, it depends on why you are sampling. Is it to try to isolate something or to compare entomopathogen populations between sites?
4. For a quick survey of the soil, take 6 separate samples (e.g. walk along a diagonal transect through a plot and sample every 10 m. For a more extensive survey, take at least 30 samples for comparison. The soil sampling is relatively quick. It's the downstream processing that is time consuming.
5. Remove the equivalent of about 4 handfuls of soil or use a measure (e.g. 500 ml capacity container).

6. Place into a bag, label using paper and a pencil, seal the bag and place into the esky.
7. Clean the trowel/container between samples.
8. Store soil at 4 °C until processed. The soil should be processed within a few weeks of sampling to avoid overgrowth of undesirable fungi.

Insect baiting method

1. Use live mealworm larvae of *Tenebrio molitor* (buy from the pet store). Do not use giant mealworms, they are a different species and are impossible to kill with soil baiting.
2. Transfer moist soil to 70 ml or tall sterile urine containers filling ½ to ¾ full OR rectangle takeaway containers, does not matter. Use multiple containers per soil if required.
3. Punch breathing holes in the containers
4. Place 10 larvae per container
5. Incubate in the dark at 25 °C
6. Every day, gently invert the containers to encourage movement of larvae.
7. Inspect the samples after 7 days and remove any dead larvae.
8. If you have small number of soil samples and larvae, then surface sterilise dead larvae (otherwise don't bother, time consuming and not everyone recommends it because if it has been killed by *Metarhizium* or other entomopathogens then it will grow out of them anyway)
    a. Use fine forceps to handle larvae
    b. 30 s dip in 70 % ethanol
    c. 30 s dip in 1 % NaOCl (sodium hypochlorite)
    d. Rinse in sterile water
    e. Transfer surface-sterilised larvae to petri plates containing moistened filter paper and incubate at 25 °C.
    f. Check for sporulation on cadavers. Green normally is *Metarhizium* and white/purple could be *Beauveria* and *Isaria* spp.
    g. Once sporulation has occurred, aseptically transfer the whole larvae to SDA plates or transfer conidia with a loop or needle to a plate.

h. Grow out at 25 °C and identify based on colony, spores and eventually molecular work.

i. Sub-culture onto new plates as necessary to get rid of any contaminants

j. If *Metarhizium anisopliae* is identified then put into long term storage as for the other collection.

- Continue incubating for another 2-3 weeks, inverting the soil every three days and inspecting for dead larvae at the same time. Process dead larvae as described above.

Soil dilution method

1. Make up SDAY plates with the exception that plates should be supplemented with antibiotics or bactericides to decrease growth of undesirable bacteria. In Veen's medium (selective) Cycloheximide (0.25 g per L) and chloramphenicol (0.5 g per L) can be used (recipe below)
2. 1 g of soil vortexed in 9 ml of water in a McCartney bottle (10^0)
3. 1 ml taken out and vortexed in 9 ml of water in a McCartney bottle (10^{-1})
4. Continue to do dilutions until 10^{-8} is obtained
5. Plate out a selection of the dilutions
    a. E.g. plate out in duplicate strong, medium and weak dilutions.
6. Incubate at 25-28 °C for a week or so. Monitor plates every other day to check for colonies that show early Metarhizium morphology (clear/white mycelium that look like mini cyclones).

7. Sub-culture desired colonies on fresh SDAY plates

8. Keep those showing *Metarhizium* sp. characteristics only.

Veen's medium	**g**
Peptone	10
Dextrose	10
Agar	15
Cycloheximide	0.25
chloramphenicol	0.5

Maintenance of fungal cultures

Media

All sub-culturing is carried out on Sabouraud Dextrose Agar (SDAY)

Peptone 10 g (not animal based)

Yeast extract 10 g

Dextrose 40 g (d-glucose)

Agar 15 g

Water to 1000 ml

Boil water. Weight out reagents and add to laboratory bottle (1L) or beaker. Add boiling water and dissolve reagents (magnetic stirrer). Divide contents into 2 x 1L laboratory bottles for autoclaving (15 mins, 121 °C).

After sterilising, pour approximately 20-30 ml into sterile Petri plates. Allow to dry thoroughly before packing into sterile petri plate sleave (bag) and storing at 4 °C.

2. Storage of cultures

Background on necessity of culture collection

A viable culture collection and good record keeping of that collection is imperative for research. A culture collection must be regularly maintained (checked for viability, desirable morphology) and it is vital that specimens are replicated across different storage methods to insure against culture loss.

Culturing (short-term storage)

Using aseptic technique, transfer a small square of sporulating culture onto a fresh SDAY plate. Ensure the culture has desirable morphology before sub-culturing. Label, with date, your initials and culture details. Seal with Parafilm M and incubate at 25-28 °C.

Agar cubes in water (medium term storage)

Materials required

Sterile 1.5-2 ml tubes

Sterile water

Pipette

Scalpel

Using aseptic technique, cut multiple squares into a sporulating culture using a scalpel. Transfer the agar squares to sterile containing approx. 700 µl of sterile water. The water needs to be covering the agar squares. Seal, label appropriately and store at 4 °C.

The agar squares in water are stable for approx. 12 months.

When required for sub-culturing, aseptically remove one square of agar per Petri plate and culture as described above.

Slopes (long-term storage)

For slopes, use a commercial Sabouraud Dextrose Agar (Bacto) for better results. Add the required amount of agar and boiling water to a beaker and stir until dissolved. Decant approximately 10 ml of liquid per McCartney bottle. Screw lids on loosely, transfer to suitable container and autoclave as above. Before the agar sets, store container with bottles on a suitable angle to create slopes. If this is done in a laminar flow, then there is no need to tighten the lids while the bottle is still hot. Seal bottles and store at room temperature away from direct sunlight.

Once the desired culture morphology is achieved on a petri plate, using aseptic technique, cut a small section of agar with spores, add to the agar slope and lightly screw the lid on. Incubate at 25-28 °C (light, dark or light:dark cycle is fine).

Once the slope is covered with a sporulating culture, tighten lid, seal with Parafilm M and label suitably on a freezer resistant label. Store at -80 °C if possible.

3. Spore germination methods

Hydrating spores before use (for dried spores, not spores direct from culture)

Materials required

1L box with lid

70 ml specimen jars (sterile)

Boiling water

Heat block, kettle

Thermometer

1. Boil water
2. Weigh or measure the desired amount of spores in a specimen jar
3. Place the jar in the corner(s) of the 1L box
4. Place a specimen jar with boiling water in the middle of the box
5. Seal the box and leave for 30 mins
6. Before use, spores should be mixed with water at 33 °C to avoid imbibitional damage

Slide method

Materials required

Sterilised slides (sterilise a whole box at once, wrap in foil to maintain sterility)

SDAY

Pipette and tips

Spreader

Petri dishes

Sterilised cotton buds

Sterile water

Microwave

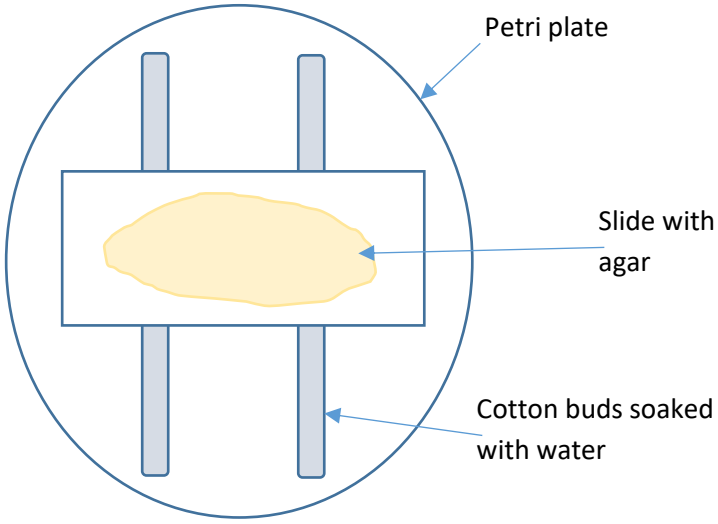
1. Dispense 500 µl molten agar onto a slide. Sliding the tip on the glass prevents too many from forming. The agar should cover about 2/3s of the slide surface.

2. Place two sterile cotton buds in a sterile Petri dish and add sterile water to soak the buds. For each isolate, prepare 3 slides for examination in separate Petri plates.

3. Inoculate the agar on the slide with 15 µl of spore suspension (approx. 10^6 conidia per ml or less). Spread using a sterile spreader.

4. Place the slide on top of the two cotton buds, which raises the slide above the surface of the dish and provides a humid environment for germination.

5. After 18-24 h, check the slides for germination using an inverted microscope (if possible) at 200-400 X magnification. Calculate germination percentage as described below in the 'plate method'.



<u>Plate method</u>

1. Start task in the late afternoon (e.g. 3 or 4 pm) as the *Metarhizium* isolates germinate quite quickly. Germination can then be assessed at about 18 h then at 24 h.

2. Have SDAY plates made up and ready to inoculate (take out of fridge into laminar flow to ensure dry).

3. Make up a 10^5 conidia/ml suspension of spores in 0.05 % Tween 20/80.

4. For example, start with 1 mg of spores per 1 ml 0.05 % Tween 20/80. Vortex for several minutes.

5. Make a 1/10 dilution. Count conidia per ml using a haemocytometer
6. Adjust solution to get desired concentration (might need to do a 1/100 dilution, really needs to be quite dilute so that you can see whether an individual conidia has germinated [germ tube visible] rather than hyphae growing over each other).
7. Pipette about 50 µL of the conidia suspension onto 6 replicate plates per isolate (use three for the 18 h check and three for the 24 h check). Use a sterile spreader to distribute the suspension evenly over the entire plate.
8. Seal and incubate plates at 25-28 °C.
9. At 18 h (and 24 h) check for germination.
    a. Cut out squares of agar that fit under a coverslip
    b. Use a compound microscope at x 200- x400 magnification.
    c. Have a look to see if you can view the conidia. If hard to see, place a drop of stain (lactophenol or other) on the agar and place a cover slip over the droplet (gives you an area to concentrate on).
    d. Count at least 100 conidia (more is better) keeping a separate tally for germinated (visible germ tube) and non-germinated conidia (draw a table like this) for each of the three replicate plates.

Germinated (a)	Non-germinated (b)

    e. Calculate the % germination using the formula below
        i. % germination = [a/(a + b)*100
        ii. a = germinated and b = non-germinated
    f. Calculate the average % germination of the three plates
    g. Germination should be > 85 % otherwise do not continue with bioassay

4. Single spore cultures (for culture collection and molecular work to ensure only one isolate is being used not a mix)

1. Prepare 2-3 % SDAY strong agar plates (the firmer the better. i.e. add more agar).
2. Prepare 'regular' SDAY plates
3. Under aseptic conditions, make a dilute suspension (e.g. 10^5, 10^6 conidia per ml) in 0.05% Tween 80 of the desired fungi.
4. Spread about 50 µl of the suspension onto about 10 plates per isolate (strong agar).
5. Incubate at 25 °C for 18 hrs (so set-up in the afternoon before)
6. This time does differ depending on the isolate, so you may have to incubate for shorter or longer times. Check periodically. Keep some of the plates in the fridge after incubation so prevent further growth.
7. The morning of the single spore collecting, put the stereo microscope etc in the laminar flow and sterilise.
8. Under the stereo microscope, pick out germinated conidia (so aim for the agar surrounding the conidia) that are well separated from other conidia/hyphae with sterilised ultra-fine forceps (or whatever you feel comfortable with, the smaller the better).
9. Gently dip the tips of the forceps into regular agar plates. You can put about 4 conidia per plate to save on plates. Do at least 20 plates to increase probability of success.
10. Incubate at 25-28 °C for several days.
11. Check plates for germination. Aseptically transfer one colony to new plates (one per plate). Do not use any that are running into another. Aim for at least 30 single spore cultures (so do more, say 40).
12. Incubate at 25-28 °C for several weeks.
13. Check plates for correct growth morphology and morphology of conidia.
14. Choose desired isolates for further work.
15. For molecular work, scrape to get a loopful or two of spores and inoculate SDAY broth (100-250 ml). Grow for 3-4 days at 25-28 °C at 150 rpm. Harvest as per the harvesting protocol.

5. Extraction of DNA from spores for PCR (PCR using ITS, EFT, B-tubulin, Bloc etc not described)

Commercial DNA extraction kits can be used or other methods as described below

1. Collect the spores from the surface of colonies using a sterile toothpick and suspend them in 0.1 ml of breaking buffer (2% Triton X-100, 1% SDS, 100 mm NaCl, 1 mm EDTA, and 10 mm Tris-HCl, pH 8) in screwcap 1.5 ml microcentrifuge tubes. Alternatively spores can be collected with a wire loop wetted with the buffer.

2. Add 150 mg of 0.45-0.5 mm glass beads and vortex for 30 sec.

3. Incubate for 30 min at 65°C (or 70 ºC) vortexing 30 sec every 10 min.

4. Add 0.1 ml of phenol/chloroform/isoamyl alcohol, (25:24:1) and mix by vortexing for 5 min. Centrifuge at maximum speed in a microcentrifuge for 5 min.

7. Collect 80 µl from the upper phase and transfer it to a new tube. Dilute an aliquot in 1:10 in TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and use 2 µl as a template for a 50 µl PCR reaction.

6. Harvesting protocol of mycelium for DNA extraction (for larger amounts of total DNA)

1. Place sterile Whatman filter paper into Buchner funnel (fold to fit) on a Buchner flask.
2. Attach to vacuum via the rubber hose (the sucking one not the blowing one)
3. Turn on vacuum pump and gradually pour in culture. I have been pouring in about ¼ at a time, spreading it around the filter. About ½ way through use a sterilised spatula to scrape growth on sides into broth.
4. Once all poured in, allow broth to filter off and then wash mat with MQH_2O at least twice.
5. Allow free liquid to filter off under vacuum (i.e. no more drips coming from funnel)
6. Switch off pump and break vacuum to remove filter paper/culture mat.
7. Place culture mat onto 2 clean filter papers (also have some paper towel beneath these) and place a further two filters over the top.
8. Gently press to remove excess water. This may need to be repeated depending on how much water comes off. Remove culture mat to new filter and place on paper towel in the Laminar flow. Leave for approximately 90 min.
9. Cut culture mats in half and place each half onto a strip of foil. Wrap and weigh.
10. Freeze in liquid nitrogen for 5 mins, then place at -80°C

7. Rice production protocol and notes (*Metarhizium anisopliae*)

1. Culture desired isolate on SDAY plates.

2. Prepare liquid cultures of the isolate (SDAY broth without the agar)

    a. Sterilise SDAY broth in flasks (do about 200 ml per flask) with a cotton bung/foil, silicon stopper or appropriate lid.

    b. Scrape off one-two loopfuls of conidia and inoculate flasks. Grow for at least 4 days at 25 °C at 150 rpm.

    c. After this time, spread a small amount (e.g. 100 µl) of broth culture onto SDAY plates and incubate at 25-28 °C for at least 2 days to check for contamination. In the meantime, the rice can be processed so that it is ready (see below).

3. Long grain rice produces the best results with *M. anisopliae*.

4. Use the breathable bags that have several regions for gas exchange. The bags we use are Saco2 Microsac bags PP75-BEU6-X32-57 (http://www.saco2.com/en/index_home.html)

5. For the rice, I use a 2:1 ratio of rice to water and generally use a plastic container with a 1 kg capacity. I use a volume ratio, saves weighing it out but is still accurate (so 2 volumes of rice to 1 volume of water).

6. First, put I tub or 1 kg or rice into the bags and seal with bag heat sealer. Autoclave for 20 mins at 121 °C. Four to five bags can be autoclaved in the one basket at a time, depending on your autoclave. Stand them up and avoid crushing the tops of them (makes it easier to reseal them after).

7. Cut off a corner of the bag (it can still be hot, there is no steam), insert a funnel (to direct water away from where the bag is sealed) and add ½ the volume of water (can use boiling water to speed things up). Seal the corner with the bag sealer. It's really important that a good seal is achieved or you'll get contamination. Seal several times if necessary.

8. Autoclave for 20 mins at 121 °C. It is best if the rice is taken out as soon as the run is over and the bags are allowed to cool down a bit. While the rice is still warm, massage the rice to break up most the lumps. It'll also mean that the bits of rice at the top (and less wet) are incorporated into the wetter rice = more consistent substrate.

9. Bags can be placed into the fridge at this point or continue with point 10.

10. Allow to cool entirely before inoculating with a liquid culture that is at least 5 days old. Massage the bag to incorporate the condensation, it will be absorbed.

11. Put the bag sealer, scissors, 10 ml pipette and sterile tips in the laminar flow and turn the UV light on.

12. Swab scissors with ethanol and cut of a small corner of the bag. Carefully add 20 ml of liquid culture, being careful to not let the liquid culture enter the barrel of the pipette. Avoid aiming the tip at the bag, aim for the rice.

13. Seal the bag ensuring that openings are present. Massage the rice, label and incubate at 25-28°C for about 3 weeks (again, the temperature and time is dependent on your system).
14. After a few days, there should be mycelial growth and the rice grains will clump. Massage every few days to distribute mycelium to promote sporulation.

8. Drying rice and spores in preparation of spore harvesting

1. After approximately 3 weeks in the incubator the rice/spores are ready to be dried.
2. All work is to be performed in the laminar flow
3. Collect aluminium trays, muslin cloth to fit (doubled over), clips to secure muslin onto trays and marker pens. Store these outside the laminar flow.
4. If all the rice bags belong to the same batch (and isolate) transfer bags to the laminar flow.
5. Mark on the lip of the tray the isolate (e.g. WW3, SSC1), the date of inoculation (I = 1/10/2016) and the date of drying (D = 30/10/2016). Right this on two sides of the tray.
6. Cut a large corner of the bag
7. Carefully pour the contents of the bag into the tray, avoiding creating too much spore dust.
8. If there is any liquid, then mop it up with a tissue.
9. Discard the tissue and the bag in an autoclave waste bag.
10. Place a double layer of clean muslin over the rice/spores and fasten with clips.
11. Transfer to a cool (<20 °C), dry environment (low humidity) and dry for at least 3 weeks.
12. Every week, take trays back into fume hood and use a spoon or spatula to agitate the rice/spores so that the top goes to the bottom. This will enable more even drying.

9. Harvesting spores from rice cultures (*Metarhizium anisopliae*)

Mechanical sieve shaker

1. After three weeks the spores should be ready for harvest.
2. If the rice does not look dry then do not use in the sieve shaker. Dry for another couple of weeks if necessary.
3. All work is to be performed in the fume hood*
4. Collect the sieves for the sieve shaker (top shelf to the left of the laminar flow)
    a. Use a nest of sieves including 1mm, 250 µm (can be smaller) and 32 µm.
    b. As well as the lid and the collecting tray.
5. Collect Schott bottles, funnel and paint brush (about 2 cm wide).
6. Label the bottle with all the information on the aluminium tray including the harvest date (H = 31/10/2016) and the empty bottle/lid weight.
7. Form the nest of sieves in descending order of aperture. Put a teaspoon full (approx.) of clean silica gel into the 250 µm sieve.
8. Carefully pour the contents of the tray into the 1 mm sieve. Use a brush to get the spores into the sieve. Do this carefully to avoid creating too much spore dust.
9. Transfer the nest of sieves to the sieve shaker.
10. Fasten all bolts.
11. Turn on and make sure that all fasteners are secure. The machine should be pretty quiet.
12. Turn on for 60 minutes (dependent on equipment)
13. If necessary, check the rice in the top sieve and agitate it by stirring with a spoon. Also check to see if the 250 µm sieve is clogged.
14. Continue sieve shaking if necessary.
15. When complete, turn of the shaker, undo the bolts and lift off the nest of sieves.
16. Remove the lid and top two sieves
17. Place the funnel in the Schott bottle and use a spoon and the brush to carefully transfer the spores.
18. Weight the bottle/lid
19. Cover the mouth of the bottle with a small square of muslin (double layer) and fasten with a rubber band.

20. Put into a lettuce plastic container that has a layer of silica gel. Label outside container with ‘Biological: *Metarhizium anisopliae’*, ‘dry spores’, ‘do not breathe dust’, your name and date.

21. Place the lid on the lettuce container and put into the fridge.

22. After a few weeks, place the lid on the bottle and store as before.

❖ If a fume hood is not available, a facemask must be worn during sieving. Preferably a non-disposable mask with twin filters and respirator is used because sieving spores can be time consuming.

Mechanical sieve shaker, photo for illustrative purposes only
http://www.marctech.com.au/laboratory-products-solutions/particle-sizing-systems/haver-boecker-sieve-shakers-and-sieves-2/

https://www.bunnings.com.au/protector-medium-large-half-face-twin-respirator_p5820154

Manual harvesting of spores

1. As above with the exception that a mechanical sieve shaker is not used and the nest of sieves is shaken manually.
2. Correct PPE to be worn
3. A spoon might be used to encourage spores to fall off the grain in the coarse sieve but paint brushes to be used in the fine sieves of the mesh will be damaged.

10. Rearing sweetpotato weevils

1. SPW are obtained from an infested field site.
2. SPW are reared in large (e.g. 30 x 20 x 20 cm) plastic containers with aeration holes (e.g. cage below, www.amazon.co.uk). A fine mesh needs to be fixed to the cage if the aeration holes are large as SPW crawl up the sides easily.
3. SPW are kept at approximately 27 °C with a 12:12 h photoperiod. Insects should be reared at low humidity if possible <60 %.
4. Feed SPW on the roots of any sweetpotato cultivar, but keep the food regime constant and buy from the same supplier. For example, Beauregard Gold is readily available in Australia from most greengrocers or supermarkets.
5. A fresh root is supplied every week. Old roots are removed after larvae have emerged. If the timing is uncertain, place old roots into a separate container, monitor for emergence, then dispose of the old root. Roots can be broken open to examine for larvae but this can injure larvae if present.
6. To obtain age consistent cohorts, after oviposition roots can removed from the main storage container and placed into a new container. Adults weevils can then be sexed using antennal morphology (at the distal end of the antennae, males have straight antennae and females have egg shaped-antennae).

11. Rearing mealworms

1. Larvae are obtained readily from pet stores (e.g. Biosupplies) and are reared in large (e.g. 30 x 20 x 20 cm) plastic containers with aeration holes (e.g. cage below, www.amazon.co.uk)

2. Larvae/pupa/beetles are kept at approximately 27 °C with a 12:12 h photoperiod. Insects should be reared at low humidity if possible <60 %

3. Insects are supplied with fresh carrot weekly and the bedding of wheat germ is changed fortnightly. To obtain age-uniform cohorts for bioassays, freshly emerged adults are removed from the colony and placed into a new container as described above.

4. After 48 hours as emerged adults, beetles are used in bioassays (allowing for cuticular melanisation).

12. Insect bioassays- examples only- consult a statistician too

Materials

Fungal culture or dried spores (depends on the size of experiment)

Pipettes and tips

70 ml sterile containers

Haemocytometer (e.g. neubauer new improved brightline) and correlating coverslip

Spatula

Soft baby spoon

Funnel

Muslin cloth

Vortex

Tween 80 0.05%

Compound microscope

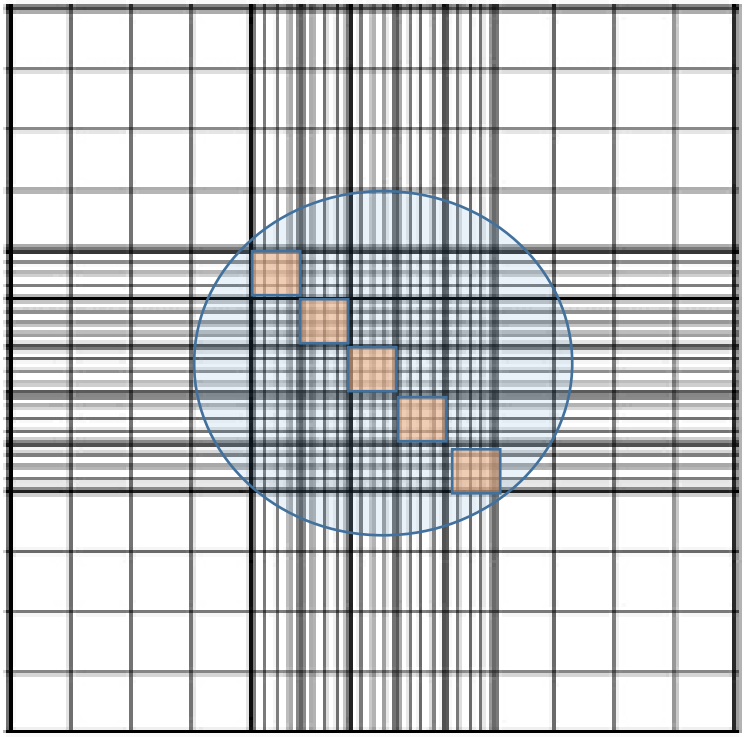
Methods

Dried spores should be rehydrated using method detailed previously before use. After rehydration, transfer approximately 0.1 g of spores to a 70 ml sterile container with Tween 80 0.05%. Vortex and dilute if necessary before quantifying spores. A 1/10 dilution is often necessary, the solution should only be a light green for ease of counting.

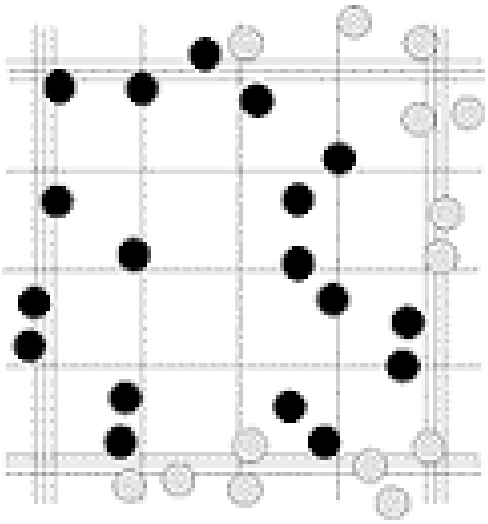
Spores from plates are carefully harvested by flooding the plate with about 10-20 ml of sterile Tween 80 0.05%. A soft baby spoon can be run over the surface of the plate. If the suspension is very lumpy, pass through a muslin cloth, and rinse with Tween 80 0.05%.

A capillary tube or pipette is used to transfer the solution to a haemocytometer. Vortex the solution and immediately draw up some solution and deliver to the haemocytometer. Vorex again and draw up more for the other chamber. Count and repeat.

Using a haemocytometer



Count conidia in 5 of the squares in the blue circle. Each square has 16 smaller squares (below). Do this for the top and bottom



Conidia cells touching the upper and left limits but not those touching lower and right limits.

Record the number of conidia in each of the 5 squares in both top and bottom for a total of 10 squares

Top Bottom

1	45	**6**	48
2	66	**7**	51
3	41	**8**	34
4	32	**9**	39
5	64	**10**	61

A = Average is 48.1 spores per square (per 0.004 mm^3)

Convert to number of spores in 1 mm^3

A X 250 =B, where B = number spores per 1 mm^3

Convert to spores per ml

B X 10^3 = C = spores/ml.

Or A X 250000 = spores per ml

48.1*2500000 = 1.2 X 10^7 spores per ml in solution

If this was a dilution, need to multiply. E.g. 1:10 dilution, X 10 = 1.2 X 10^8 spores per ml in original

Spraying insects using pressurised Nalgene bottles

1. The Nalgene bottles have the capacity for approximately 100 ml of solution. Anymore and the bottle can't be pressurised properly.
2. **********Nalgene bottle now discontinued**********
3. Become familiar with the bottles before use- e.g. use water to calibrate them for yourself
4. Every time you spray a replicate plate (we spray them separately for statistical reasons), pump the bottle the same number of times. For example, I would pump 15 or 20 times (but keep consistent). It definitely gets tougher to get a full pump at the end.
5. I normally deliver 2-3 ml per replicate plate (about 5 seconds)
    a. This is the amount that is sprayed into the Perspex cylinder or bucket and the plate doesn't receive every drop (if there is solution pooling on the filter paper that the insects are on then they are receiving too much and will drown).
    b. Calibrate it for yourself and keep consistent between treatments.
6. Set-up the spraying rig Perspex cylinder or bucket and retort stand) in a fume hood or well ventilated area. Angle the bottle on the retort stand and practice with water so that the spray is being delivered into the Perspex cylinder/bucket.
7. If this set-up doesn't work, then do away with the retort stand and keep your hand/bottle position consistent between replicate plates of sweet potato weevils (or whatever) that you are spraying (e.g. 10 cm above lip of Perspex, resting on Perspex). You do want some distance between the spray bottle and the insect).

Inoculating insects using a pipette

1. Bioassays have been developed using the non-model target *Tenebrio molitor* (Coleoptera: Tenebrionidae) to rapidly screen a large number of isolates.
2. Insects belonging to the Tenebrionidae are major pests of stored grain and have been frequently used in bioassays with biopesticides (Rangel et al., 2008, Oppert, 2010, Vinokurov et al., 2006, Bharadwaj & Stafford, 2011, Milner et al., 2002, Barnes & Siva-Jothy, 2000, Haine et al., 2008, Michalaki et al., 2006, Michalaki et al., 2007).

Example of experimental design
6 replicates (ca. 30 mealworms per container) or 40 replicates for dose-response, individually housed.

Treatments: control 1 (no spray), control 2 (carrier e.g. Tween 80 0.05% or formulation only), and 3-7 concentrations of a particular isolate (e.g. 10^9, 10^8 10^7, 10^6, 10^5, 10^4 conidia per ml etc) or testing of multiple isolates.

Materials and equipment

- 500 ml capacity round clear plastic containers (takeaway) (Figure 1) or 20 ml sample cups with holes punched (Figure 5)
- Lids with a 70 mm ⌀ circle removed, and replaced with a 90 mm ⌀ circle of very fine voile (glued) (Figure 1)
- Fresh carrot (whole for cultures, 3 cm^3 for 500 ml containers, 1 cm^3 for 20 ml cups)
- Mealworms
- Wheat germ
- Petri plates and parafilm
- Pipette and tips
- Forceps
- Soft baby spoon
- Spatula
- Trays
- Plastic bags

Methods

1. Spore germination tests as described previously
2. Conidial suspensions are described previously
3. Label all the required containers or cups and place onto trays
4. Using the soft baby spoon, transfer the required number of beetles to the container (or for the cups, one beetle/container).
5. Beetles are inoculated with 30 µl conidial suspension between the head and thorax
6. After 30 min, (allowing for the suspension to dry), a piece of carrot is added to each cup or 3 pieces of 3 cm^3 for 500 ml containers.
7. After 60 min, one spatula scoop of wheat germ is added per cup or ¼ cup to the 500 ml container.

8. Containers containing mealworms are arranged in a completely randomised design in the incubator and then incubated at 25 °C at 100 % RH for 24 h (by placing a wet paper towel inside the tray and sealing the tray in a plastic bag) then at 35 % RH for duration of experiment (removing the tray from the bag).

9. Carrot is replaced every 3rd day.

10. Assessment

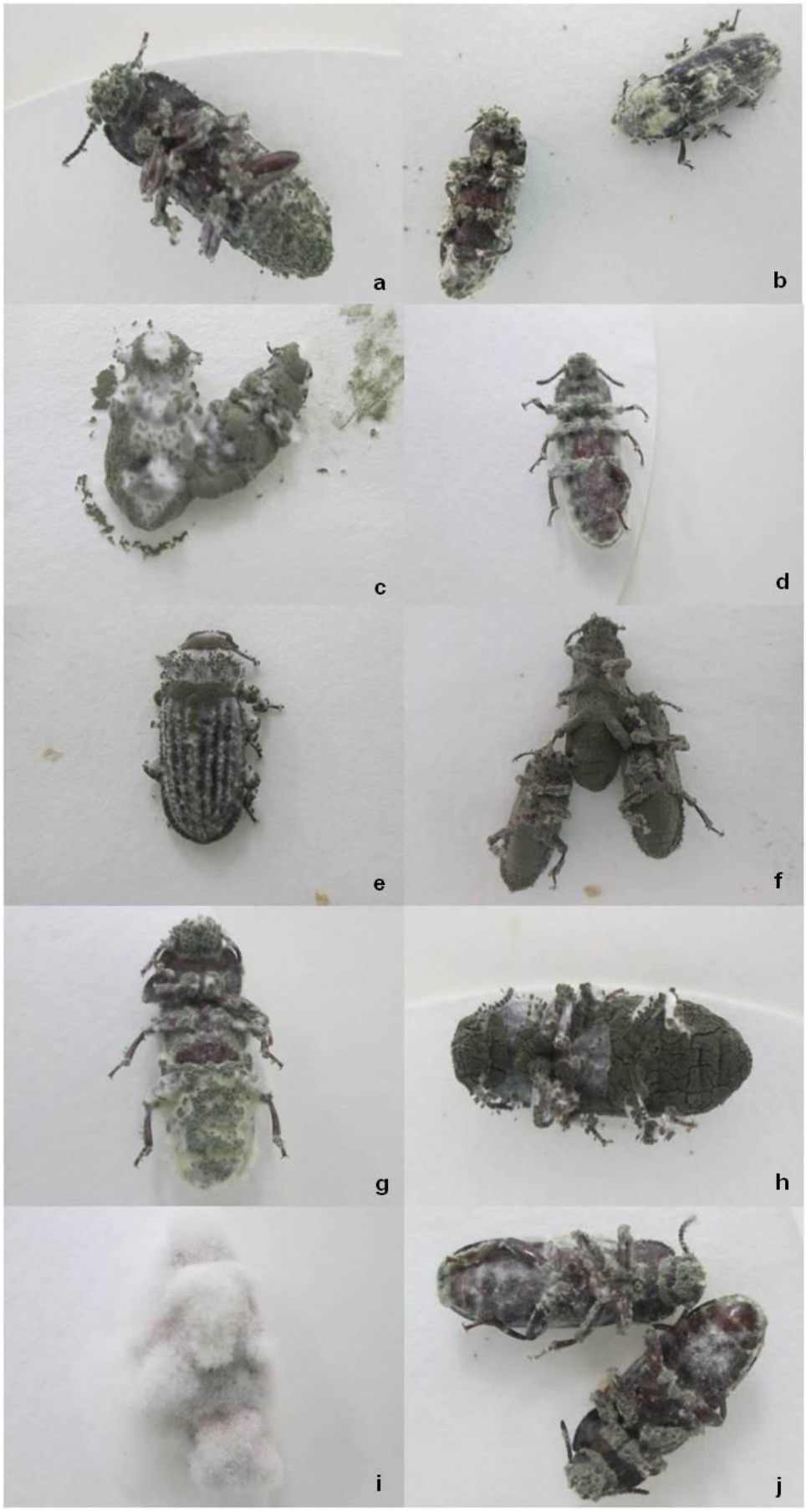
    a. After 3 days, assess for mortality every 24 hours for up to 14 days. Keep a separate moist chamber (Petri dish) for each replicate of each treatment. Add mealworms to this every 24 h, just record on a spreadsheet how many died at each time point.

    b. After a week, check for white mycelial growth and typical sporulation of the entomopathogen.

    c. Keep incubating if necessary. Record total dead per rep and the number of cadavers with entomopathogen.

    d. Statistics: Analysis of variance (ANOVA) or Probit analysis with estimation by generalized linear models where appropriate (GenStat).

Figure 5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Sweet potato weevil (Cylas formicarius)

1. SPW can be sprayed as above, inoculated using a pipette, dipped individually (using soft forceps) or dipped in a group of replicates using a tea-strainer (below).
2. If dipped, the solution containing conidia should be constantly agitated (e.g. stir bar) to ensure solution is homogeneous.
3. The time that insects are immersed must remain consistent.
4. If necessary, insects may need to be transferred to filter paper to mop-up excess solution before placing them in containers for bioassays.
5. Food-each experimental unit should receive the same amount of sweetpotato- for example 50 g of sweetpotato cube or a whole root that is of consistent size. Use the same supplier/cultivar of sweetpotato for all experiments if possible or grow your own.
6. Small cubes of roots should be replaced every three days at minimum.

https://www.thegoodguys.com.au/davis-and-waddell-mesh-tea-ball-on-chain-d2031?CAWELAID=620013790000409365&CAGPSPN=pla&CAAGID=22187282246&CATCI=pla-140215815686&gclid=CP_lg5n8otICFQsFKgodp0QPFw&gclsrc=aw.ds&dclid=CIXl4Jn8otICFc0vlgodhOwBOA

Appendix 27. Notes from Bree from PNG trip May 21-26th Asaro Valley visits: Eastern Highlands Province

Bree Wilson, Jian Liu, Mike Hughes, Robert Kei Geno, Wilfred Wau, Alexa Agiwa, Enopa Lindsay, Johnny Wemin-farm visits

Site 1: Nipuka

Grower: Wasi

Soil and field

- Collected soil samples for UniTech (Lae) to bait entomopathogenic fungi (using Taro beetle larvae or soil dilutions) from the best-bet and conventional trial area. Best-bet uses PT material, pheromone traps for SPW and weeding. Three sandwich bag size snap lock bags of soil from each (total of 6). Pheromone traps removed from field-I would like to see them reinstated in the field so the farmer can see what it's doing, giving visual feedback. If possible, NARI and/or FPFA could count weevils to see the insect pressure over time.
- Jian and I met with Robert L FPDA to catch-up and we chatted about the possibility of someone in PNG importing the lures she's organised from China so they can be sold to the farmers-everyone seems keen on the SPW lures

Screen house

- Land around screen house clear from plants.
- No aphids (inside or out)
- Major infestation and leaf damage by caterpillars (likely to be the tropical armyworm or cluster caterpillar *Spodoptera litura*)
- Plants overgrown inside (shoot length of 60-80 cm not uncommon-waiting for FPDA to come back to harvest, this was the general trend in all the screen houses: overgrown and waiting).
- Shoots have been cut twice already.
- I asked Mike about replacement of soil in screen houses-he suggested every 12 months but this prompted discussion about the need for that frequency (can we push out to 18 -24 months as much effort required to replace soil) and ways we could test to see if the soil was performing (not nutrition as chicken manure applied and nutrition relatively easy to correct?) but how certain beneficial microbe communities may build up to support plant growth (i.e. soil C and structure) but also potential for deleterious communities to build up.

- Another obvious area of research (after proof of concept at USQ-Bree, in process) is looking at the endophytic status of sweetpotato and beneficial fungi like entomopathogens and mycorrhizal fungi-scope to optimise the shoot health before it's out-planted in the field.

Figures 1 -4: Left to right- Bree Wilson (USQ) and Robert Kei Geno (NARI) sampling soil at Wasi's farm for isolation of fungal entomopathogenic fungi at UniTech, Lae by Ronnie Dotaona, the best-bet trial at Wasi's farm Robert Kei Geno and Jian Liu (CSU) at Wasi's farm.

Figures 5-13: Left to right- Farmers sweetpotato field with screen house in distance (5), Mike (QDAFF) and Wasi (farmer) outside the screen house (6), screen house alongside conventional treatment (pest and disease project) (7), Iron and possibly zinc deficient shoots, although not representative of shoots in whole screen house (8), Mike Hughes, Wasi, Johnny (FPDA) and Jian Liu inside screen house (9), plant beds (10), Mike demonstrating to Wasi the desirable length of shoots (these were ca. 70 cm) (11), relatively insect-free trap (replaced that day) (12), Johnny inspecting shoot length (13).

Figures 14-21: Left to right- Sing sing-and Mudmen attacking us…very very cool (14-18), Mike chatting to the village about the klin seed and business and market and (pidgin, so only understood half of it!) (19-20), sweetpotato and other crops in the village (21).

Site 2: Kuka

Grower: Benjamin

Soil

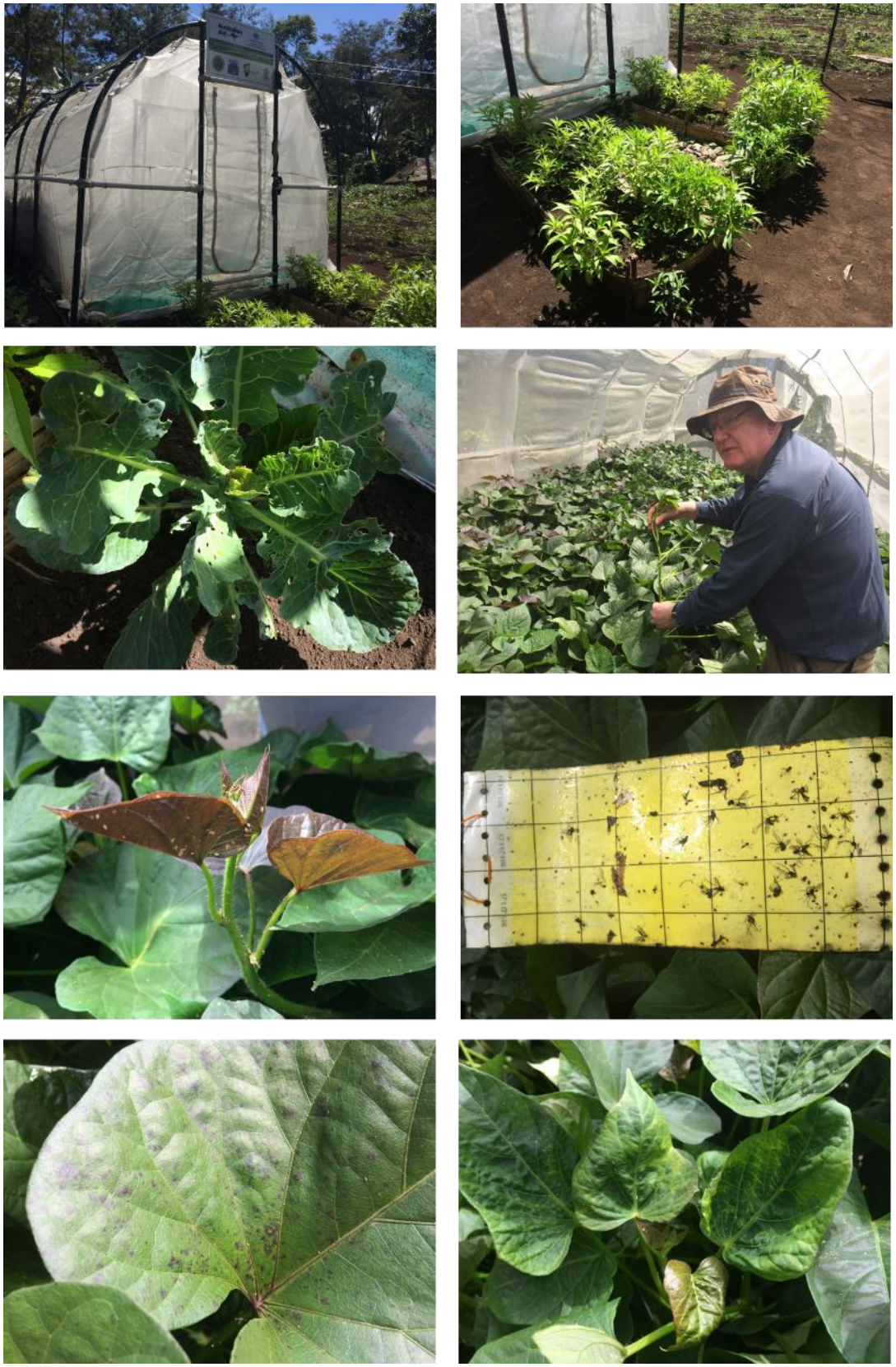
Soil collected as above from NARI implemented trial for UniTech to extract entomopathogenic fungi

Screen house

- Loaded with aphids (Green peach *Myzus persicae*) inside. No winged forms yet, which was surprising given how dense the population was on individual leaves (normally prompting alate aphids to be produced).
- Outside, say 4 m away, fewer aphids on sweetpotato field plants but still present in abundance.
- Ornamentals and brassicas planted at entrance and alongside the screen house. The care and passion into these plantings is lovely but obviously a potential problem for attracting aphids or silver leaf whitefly. Strongly advise to remove the brassicas.
- We later (Jian, Mike, Birte and I) later talked about the potential of planting repellent barrier plants instead-or the push pull idea-pushing pests away from the screen house and pulling to another plant to then target spraying.




Figures 22-25: Left to right- Jian accessing fence to Ben's screen house (22), screen house klin vines in the field (23), Green peach aphids on field klin vines (24), established sweetpotato crop (25).



Figures 26-33: Left to right- Ben's screen house (26), ornamentals at entrance to screen house (with moths) (27), *Brassica* sp. (cabbage) alongside screen house (currently no aphids) (28), Mike demonstrating ideal shoot length (29), new shoot with Green peach aphids (representative of all shoots) (30), sticky insect trap with aphids and other unidentified insects (31), early purpling and potential mottling, observed on a few shoots (32), leaf distortion, observed on a few shoots (33).

Site 3: Gimisave, Daulo district

Farmer: Kuman Giregire

Soil

Soil collected as above from NARI implemented trial for UniTech to extract entomopathogenic fungi (no NARI trial here, just collected some the rhizosphere of sweetpotato plants)

Screen house

- Aphids (GPA)
- 1st cut done
- Over grown shoots
- Some possible chemical damage to some leaves at front of screen house (minor)
- Using cuttings in single and double plantings
- Trying to introduce flat/ridge planting

Figures 34-41: Left to right- screen house (34), Mike, Kuman and Jian inspecting shoots (35), plant beds (too dense to walk through!) (36), green peach aphids on new leaves (37, 38), potential chemical damage on leaves at entrance to screen house (39), Kuman and Mike discussing sweetpotato (40), Kuman and Robert in the field adjacent to screen house (41).

Site 3: Meteyufa

Farmer: Aku

Soil

- Soil collected as above from NARI implemented trial for Unitech to extract entomopathogenic fungi (from best bet and conventional trial soil).
- To better explain to the farmer why the soil was taken, I showed a photo of a *Metarhizium* sp. infested insect-with the help of Johnny Wenham or Robert Kei Geno, explained to the farmer the principle of the fungi killing the insect as it passes through the soil.
- Is there scope to put this sort of image onto the CommCare system etc, so that the farmers can be shown images of pest, diseases, beneficial microorganisms?

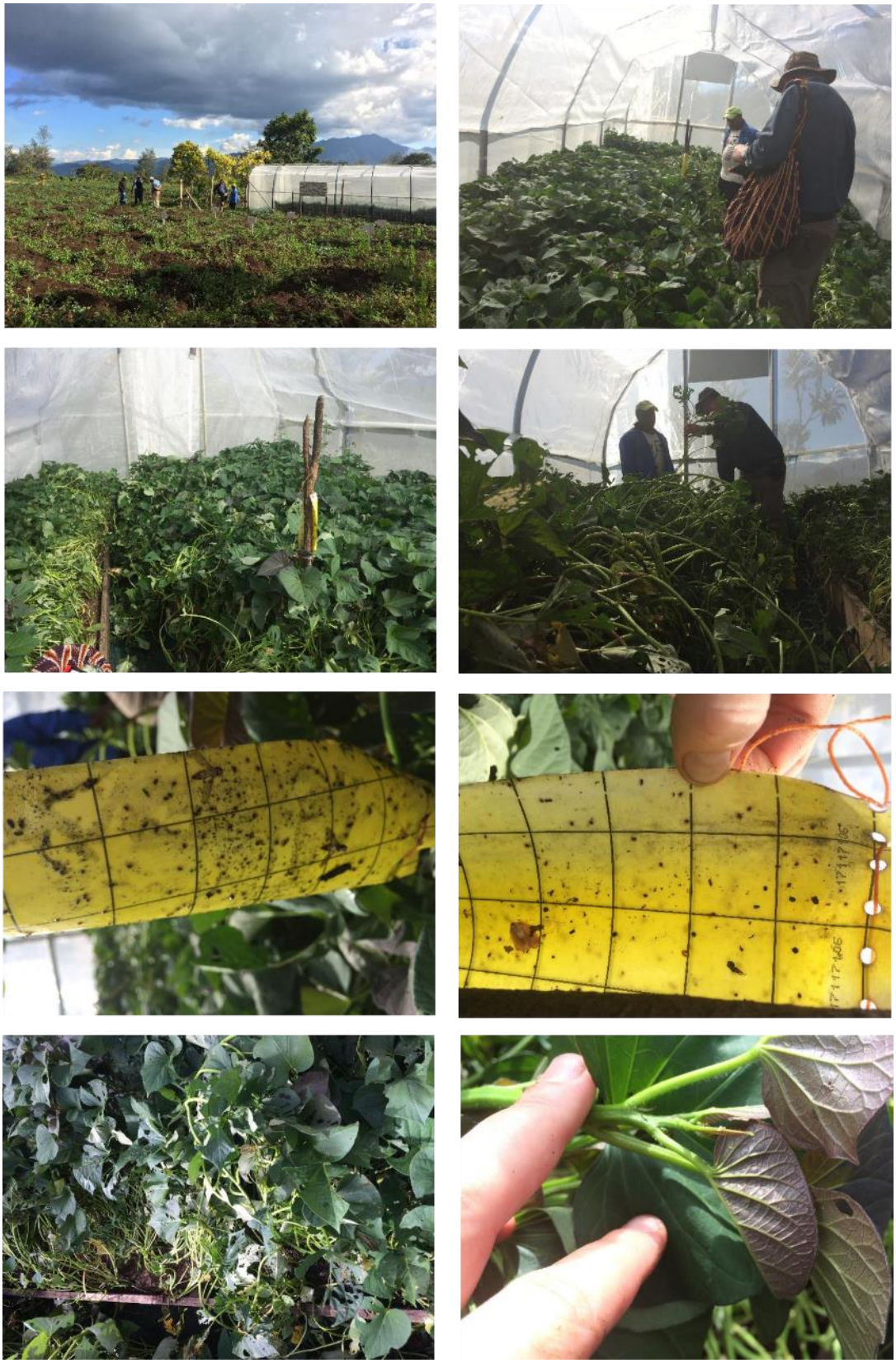
Screen house

- Aphids- Green peach *Myzus persicae*
- Healthy looking shoots but major infestation and leaf damage by caterpillars (likely to be the tropical armyworm or cluster caterpillar *Spodoptera litura*)
- Research why or how this pest is infiltrating the screen house
- Do a fact sheet or CommCare information on controlling for this caterpillar/moth?
- Scope for fungal biocontrol if growers want to avoid chemical-foliar spray. Slow acting but I don't think it needs to be super-fast unlike aphid control.
- Over grown shoots-Mike demonstrated ideal length

Other notes

- Nematode damage observed…NARI extracted nematodes from the soil for identification
- Wireworm adults (true) in field
- Aku is extremely frustrated with NARI and FPDA, more so NARI.
- Feels like the research is 'bullshit' and everyone wants to be on his land but he gets nothing for it (he actually does get paid)
- Threatened NARI staff (guns and punching threats) -we agreed that NARI doesn't need to go back to Aku's land, too risky

- He wants bigger trials done (if we go back of course) as he can't see the value in the small trails as he can't see the bags of sweetpotatoes that come out of it. He seems only concerned with the market, doesn't see the value in PT material (let alone other research being done).
- He shouted at the group for about 10 mins. Didn't need to be fluent in pidgin to understand the gist of it. He was very pissed off.
- Johnny went back for 2 hours that night to help smooth things over-Johnny thinks he made some progress.
- Aku's wife gave us bags of fruit-seemed like a semi-peace offering (but only given to Jian and I).



Figures 42-49: Left to right- Screen house and sister projects trials in foreground at Aku's property (42), Mike and Aku in the screen house (43), plant beds and discussing shoot length (44-45), sticky insect traps with aphids and other unidentified insects (46-47), shoots showing considerable caterpillar damage (49), new shoots with green peach aphids (49).

Figures 50-57: Left to right- wireworm? (50), Wilfred and Robert standing in the best-bet pest and disease trial with soils team trial in foreground (52), NARI trials best bet (53 and conventional (54), Enopa, Aku, Johnny, Mike, Jian and Alex in field (55), Mike and Johnny discussing plantings (56), Aku, Mike, Jian in field, team in field with mountains in background (57).

***Facilities tour, Brief presentations at Airuya by Robert Kei Geno and William* (Bree, Jian, Mike, Myla, Winnie, Robert, Wilfred, William, Enopa and Alex)**

- We visited the old screen houses, new screen houses, the indexing plants, greenhouse with dirty field plants and insect rearing facilities (not control;ed temperature).
- Mike talked about the need to potentially replace the mesh on the old screen house in the near future.
- Mike also talked with Myla and Winnie (and Birte) about getting an additional section of mesh to act as the vestibule for the two new screen houses.
- Probably a good idea considering aphids were sighted on nearby grasses and virus field trials in progress in close proximity to the screen houses (15 m).
- Mike questioned the lack of partitions in the new screen house-he thought they were ordered with the option to partition a screen house into 2-3 sections.
- The pest and disease team are still acquiring their collection of both weevil species for laboratory experiments-subsequent harvests of trials should provide an additional supply.
- There was talk of separating the species on larval life stages-if NARI was interested, I could develop LAMP primers to help with this. Rapid diagnostic.
- After seeing the potential of weevil (*Cylas formicarius*) at UniTech (see Unitech notes), it should be relatively easy for Ronnie and colleagues to supply NARI Airuya with weevils (or infested roots) for the laboratory.
- This is important too as in the near future, the entomopathogenic fungi and barrier plant work will be combined and the two groups will need to work together.

- PT material/tissue culture laboratory
    - Chats with Winnie, Mike and Myla (and further chats with Birte at Bubia) highlighted the need to get LAMP diagnostics underway sooner rather than later based on the length of time for indexing, or finding somewhere suitable to grow more *I. setosa* plants or bulking up phase of tissue culture.
    - Farmers in some regions are ‘waiting’ for PT material (e.g. Kerot Hgu) and clearly molecular ID would help hasten the process.
    - Winnie and I chatted about Gou Rauka in Bubia and her experience with the LAMP with phytoplasma and how we could try to do a training session ASAP.
    - I’m very happy to help out where possible here.
    - Robert mentioned that the coffee research people were going to bring in entomopathogenic fungi work for borer into the tissue culture lab. Unless there was

substantial segregation, I would not recommend such dirty work mixing with such clean work as tissue culture. Something to chase.

- Robert gave a short presentation on progress of the field trials-best-bet and forecasted barrier plant work-most work on track for the field in Jiwaka, Western and Eastern Highlands Provinces.
- William gave a brief update of the soils team experiments-focus on soil fertility management and PT material and increased yields compared to non PT material, non-amended soil.
- We asked NARI what they saw as their major constraints-resounding answer was the poor (or aggressive) attitude of some of the farmers.
- We chatted about the pheromone traps (*Cylas formicarius*) that had be placed in some fields-the pest and disease team collect and count weevils (or Enopa-collecting in Hagen). Most traps removed from fields when the trial finished (best-bet), but I'd like to see the odd trap left in the field as a visual of what's going on. Soapy water could be used in the bottom of the bottles to kill off the males and counts could be done by FPDA or NARI as the opportunity arose.
- I think it's best if FPDA arrange for these extra traps to go out into fields. I'm happy to do an information sheet on these in collaboration with NARI, FPDA and Mike etc to add to the folder that NARI is preparing for the screen house extension.
- We talked about the weevils present at the different districts-Asaro has *Cylas formicarius* only, Jiwaka both types, Western highlands province-not sure yet but more likely just *Cylas formicarius.*
- We also talked briefly about the incidence of gall mite and scab at both Jiwaka and WHP.
- NARI has prepared posters/boards to go out into the field (?) summarising the best-bet strategies (nothing rocket science here, just highlighting the use of the pheromone traps as the main attraction, PT material and removal of weeds etc).
- One possibly major problem with the field trials by pest and disease and soils team is the lack of standardisation of vines used-node number, shoot length. This is something that needs to be addressed and will probably (or hopefully) reduce the error that's being observed in the data (i.e. looks different between PT and no PT material but not statistically significant).
- Mike and I chatted to the group about the findings in the VG13004 project and work done by Arthur Villordan. Perhaps we can get a copy of his presentation to send to the NARI/FPDA teams? Craig Henderson's sharepoint?

- We had a big discussion about the aphids in the screen houses.
    - Myla and Winnie have written a management protocol for aphids
    - Mike has it and I'm happy to help him further with it-can Mike circulate it?
    - My thoughts are a more concise version or initial summary page would be good as the current one is very detailed but perhaps overwhelming.
    - Winnie has suggested that a training session on aphid control will occur in the near future. Perhaps she/we can prepare a poster too?
    - Or can we load the information for control options onto CommCare? (chemicals, keeping screen houses closed: timing of chemical spraying e.g. early morning so it doesn't get too hot in the screen house increasing the chances of the door being left open for cooler air, insecticide resistance and importance of rotating chemicals, close-up images of the aphid species that could be present?

Meeting with Birte at NARI Bubia (Mike, Bree, Jian, Birte) 24/5/18

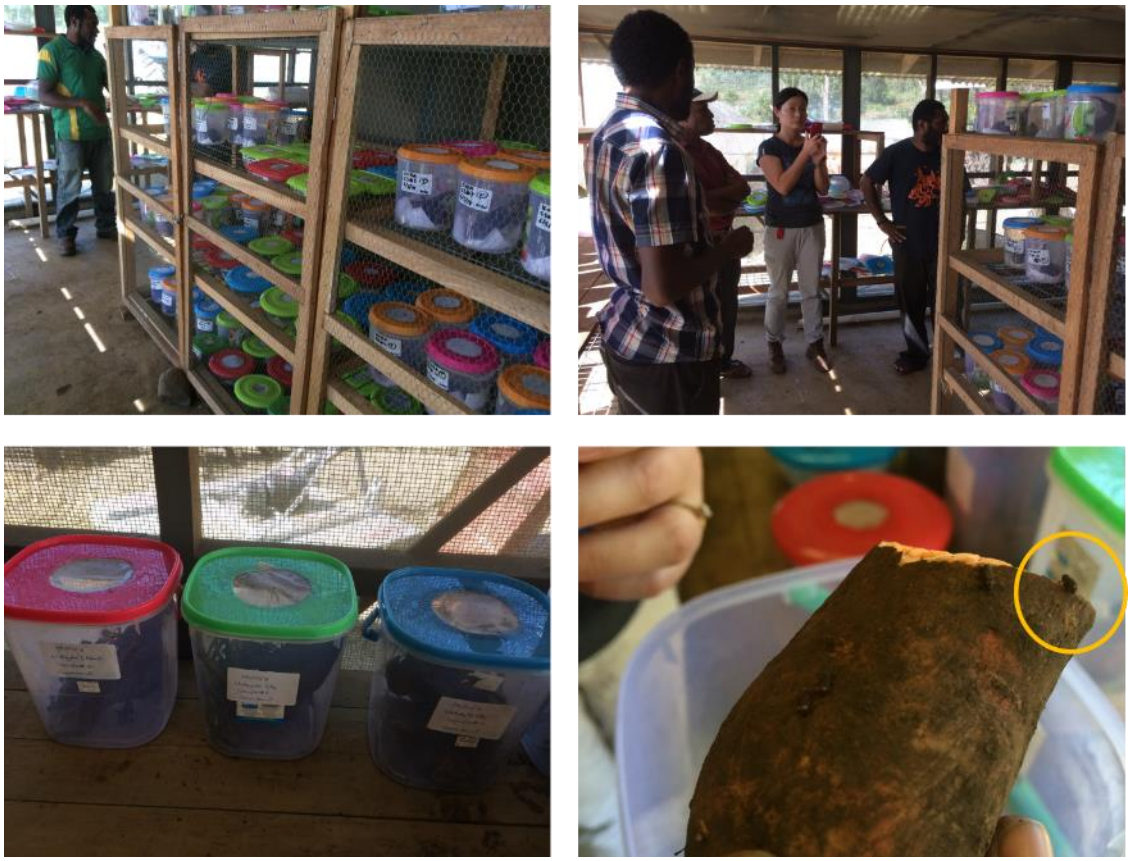
- Mike talked about needing to put out some fires in some districts regarding farmer misconceptions that NARI/FPDA/Australia are bringing in KauKau in screen houses in an effort to make farmers by insecticides and fertilisers-rumours because farmers are already convinced that NARI brought in Late Blight.
- Mike commented that Johnny did a really good job talking to farmers in an attempt to quash the rumours.
- Mike highlighted to Birte that several bits of equipment needed repairing- if it needs repairing, the request has to come from Airuya, not Mike.
- Birte questioned the baseline value of PT material when it goes out into the field and gets a virus anyway.
- Birte suggested that cultivars such as Kerot (Hgu) even though it's not quite clean, to get it out anyway-just as a starting point, not to replace the final klin product. She said we need to stop farmers waiting as they are already questioning the science.
- Birte also suggested to just put the Kerot onto *I. setosa* to avoid the long wait with thermotherapy.
- Another reminder that the lab really does need the LAMP.
- Mike talked about the importance of not letting not fully tested material out.
- Building on Mike's powerpoint presentation comment on the need to control *Ralstonia solancearum* in soil in Tambul-and the need to control.

    - Unsure of who's role this may be to help rectify: NARI or FPDA
    - This is something the pest and disease team could look at (PNG and Australia), not affecting sweetpotato but could have indirect effects on rotating crops and build-up of other diseases that could affect sweetpotato?
    - Mike talked about crop rotation with monocots (non-hosts), using broad leaf herbicides to knock back vegetation and bacterial populations.
- Mike also talked about barrier plants to repel aphids around screen houses-obvious collaboration between Jian of CSU.

Figures 58-65: old screen house

Figures 66-73: new screen houses, noting aphids in monocots exterior to screen house

Figures 74-81: Left to right- *Ipomoea setosa* indicator plants including Mike trying to hide in them (74-77), dirty field plants in the greenhouse (78-79), sweetpotato lines (with gall mite 80) and rest of collection (81).

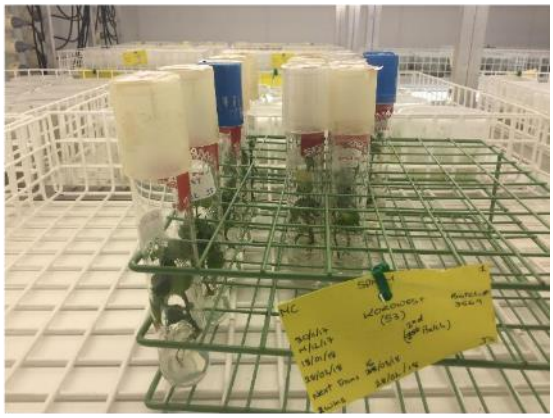


Figures 82-85: NARI insect house assessing emergence of weevils both *Cylas formicarius* and *Eucepes postfaciatus* (yellow ring) from field trials, for subsequent rearing.




Figures 86-89: Left to right- Discussing potential barrier plants in the shed (86-87), Jian explaining experimental set-up for barrier plant work in pots and olfactometer work for live barrier plants (88-89).



Figures 90-95: Tissue culture lab

Meeting with Ronnie at Crossroads in Bubia (24/5) and at UniTech Lae (25/5, just Bree)

- Jian, Bree and Ronnie chatted about project finances and generation of acquittals and invoices…some things have progressed since that meeting.
- We are still waiting for the junior scientist to be appointed at UniTech. Ronnie has someone in mind and hopefully we're just waiting on the formality of the appointment (and replacement of Robert, who moved to NARI).
- Ronnie has sourced quotes for incubators and fridges and has purchased consumables (awaiting delivery). Strongly encouraged Ronnie to start making purchases-big and small and we spent a bit of time collating a list.
- Gerega Maiga is a Masters student who will be working on the isolation and identification of entomopathogenic fungi from soil around the district.
- There is scope for Ronnie to take on additional 4th year students to run mini projects within this project. I'll work with Ronnie for get project ideas to benefit both the student and the project.
- Whilst I was at UniTech, we started preparing the soil collected from the Asaro Valley for baiting of fungal entomopathogens. Gerega and Ronnie were awaiting the receival of Taro grubs to serve as the bait, which should have arrived on the 27/5.
- Lab facilities are ok at UniTech, but the front room where insects are reared has a rat problem (rats eating roots with larvae/pupae/adults waiting to emerge).
    - Aim to rectify by getting metal mesh and larger vessels (and more of them).
    - There is scope to move into an additional lab in the near future. If that's not possible then I suggested moving an old dysfunctional incubator to the rearing area to protect the roots from rat predation.
    - In the absence of an incubator, I've suggested that grow on of fungi isolated from insect baiting methods or soil dilutions is carried out in the hot blue-benched lab (28 °C at least).
- We installed a Jian/NARI pheromone trap in the field at UniTech. It'll stay in the field to fast track their collection, which will obviously be supplemented with infested roots for acquisition of females. No shortage of males.
- For field trials in the area, given the load of weevils, we'll need to think carefully about treatments like lure and kill (pheromone and entomopathogenic fungi) in the design and proximity to the germplasm etc.

- I have some small concerns about the potential quality of shoots that can be produced at UniTech for field work (based on the 'state' of plants in the germplasm, and loading of weevils).
- Hopefully Unitech can get access to glasshouse space to grow shoots for planting but I'd also like Ronnie to visit Airuya to see how NARI produce SP shoots *en masse*.
- Can NARI supply Ronnie with documentation about fertiliser, soil etc?
- Can Ronnie get PT shoots from Airuya-probably Beauregard etc?

Figures 96-101: Left to right- Installation of pheromone trap (courtesy of Jian and NARI Airuya) in the sweetpotato germplasm collection at UniTech (96), trap after about 10 min (97), Bree, Ronnie Dotaone (grey stripes) and Gerega Maiga (purple) in the germplasm discussing the need for healthy PT material for trials (98-99), collecting weevils for the laboratory colony (literally hundreds after 30 mins) 100-101.

Figures 102-105: Left to right- collection of storage roots for weevil emergence (102), preparing vessels of soil for Taro grub baiting of fungal entomopathogens for collection and bioassays (Bree, Gerega, Ronnie) (103-105).

Summary of potential synergies with the mother and sister project (pest and disease)

- Insects inside screen house:
    - Probably difficult to assess the insect species present on yellow sticky traps unless photos taken when quite fresh etc, and macro setting images.
    - Wouldn't be hard to get a total insect count though-photos taken very time the traps are changed over?
    - I like the idea of putting traps outside the screen house, in the vestibule and inside the screen house for that visual feedback.
- I think it's best if FPDA arrange for these extra traps to go out into fields. I'm happy to do an information sheet on these in collaboration with NARI, FPDA and Mike etc to add to the folder that NARI is preparing for the screen house extension.
- Is there scope to put this sort of image onto the CommCare system etc, so that the farmers can be shown images of pest, diseases, beneficial microorganisms?
- Research why or how moths are infiltrating the screen house-soil, bad timing with open doors (in multiple sites though).
- Do a fact sheet or CommCare information on controlling for this caterpillar/moth?
- Scope for fungal biocontrol if growers want to avoid chemical-foliar spray. Slow acting but I don't think it needs to be super-fast unlike aphid control.
- Ideal shoot length-fact sheet or poster to help dictate shoot harvest?
- Can UniTech get access to PT material for trials in Lae?
- Building on Mike's powerpoint presentation comment on the need to control *Ralstonia solancearum* in soil in Tambul-and the need to control.
- Barrier plants around screen houses-Jian
- Bree helping with LAMP diagnostics or training in (being opportunistic with visits?).
- Ways we could test to see if the soil in screen house was performing, but how certain beneficial microbe communities may build up to support plant growth (i.e. soil C and structure) but also potential for deleterious communities to build up.
- Another obvious area of research (after proof of concept at USQ-Bree, in process) is looking at the endophytic status of sweetpotato and beneficial fungi like entomopathogens and mycorrhizal fungi-scope to optimise the shoot health before it's out-planted in the field.

Appendix 18. Examining project derived entomopathogenic fungi on other insect hosts

Background

In 2019, a relationship with a potential biopesticide industry partner (Biological Ag) was formed to examine the efficacy of project derived entomopathogenic fungi on insects other than those attacking sweetpotato. The rationale behind this was the sweetpotato industry is so small, it is unlikely that a biopesticide company (big or small) would invest in a product that only benefitted one industry. Therefore, opportunistic and strategic testing was conducted on insects of interest other than those found in sweetpotato. Regular contact and experimentation over the last 2 years has developed that relationship further.

Insects tested- preliminary observations only

1. Poinciana longicorn beetle larvae (*Agrianome spinicollis*)
    a. Larvae were collected from a citrus farm in the Bundaberg region. The larvae had been decimating various varieties of citrus following floods in the region. These huge larvae (up to 10 cm in length) were extremely hard to extract from the trees due to the tunnels in which they hid. As a consequence, sample numbers were low and testing was done in batches. No statistical analysis was performed.
    b. Various isolates of EPF including KS1 (*Beauveria bassiana*) and ECS1 (*Metarhizium anisopliae*) were trialled in the laboratory as a proof-of-concept. Larvae required two applications of a 10^7 conidia to cause death (Figure 1). An indigenous *Metarhizium brunneum* was isolated from an unsprayed larvae designated as an untreated control (Figure 1).

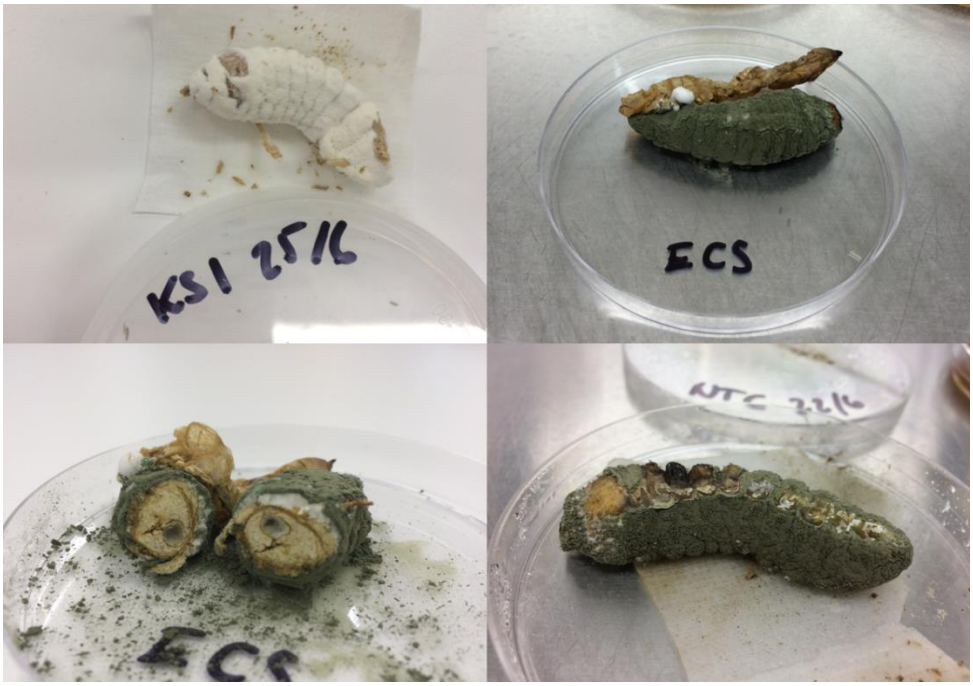


Figure 1. Longicorn larvae sprayed from top left (*B. bassiana*) to right (*M. anisopliae*), bottom left ((*M. anisopliae* sectioned) to right (unsprayed control harbouring *M. brunneum*)

2. Fruit fly

    a. Low mortality in fruit fly, but isolate KS1 showed pathogenicity (as did other, non-project derived *M. anisopliae*) (Figure 2)

Figure 2. Fruit fly with KS1 *B. bassiana* sporulation, sprayed at 10^7 conidia per ml

3. Fall armyworm

    a. Isolates including KS1 and ECS1 were either sprayed onto 3rd instars or inoculated as conidiated rice into soil for pupae (Figure 3).

    b. Fall armyworm were extremely difficult to kill and required 2-3 applications of conidia. Later work with a University of Queensland student showed variable results when maize leaves were sprayed with EPF before exposing 3rd instar larvae.

    c. Results variable, project isolated EPF are pathogenic but more work required on application and formulation

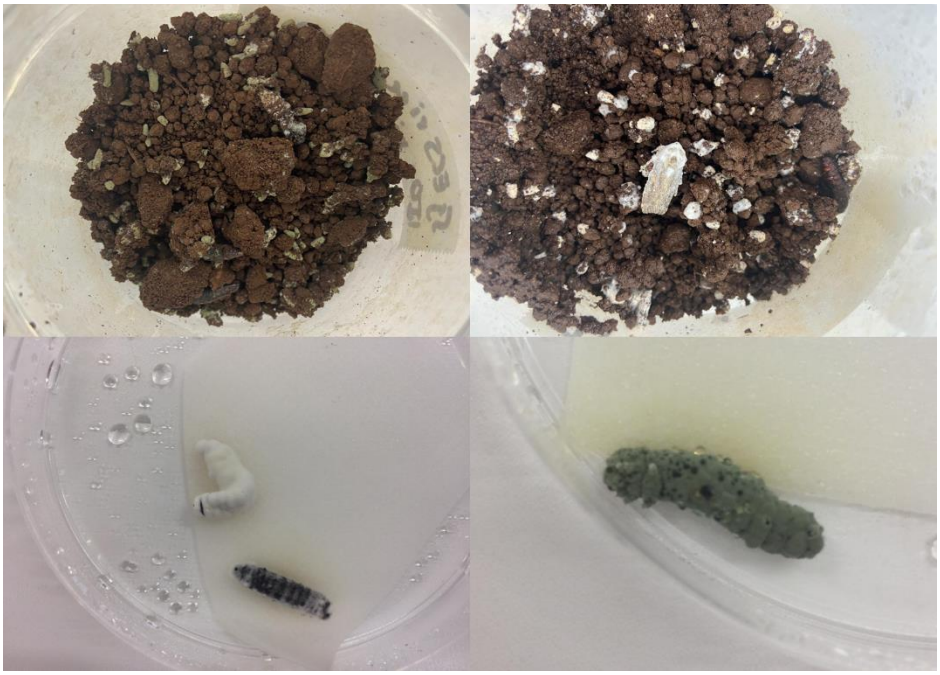


Figure 3. Fall armyworm from top left (*M. anisopliae* on conidiated rice), top right (*B. bassiana* on adult) bottom left (non-project *B. bassiana*) to bottom right (non-project *M. anisopliae*)

4. Elephant weevil (ex blueberry, QLD)

    a. Excellent mortality (50-100%) from project and non-project EPF in laboratory bioassays

Figure 3. Elephant weevil from top left (*M. anisopliae* non-project isolate), top right (*M. anisopliae* ECS1), bottom left (*B. bassiana* KS1) to bottom right (*M. anisopliae* DA1). Photos: Keith Danckwerts, Biological Ag, with permission.

5. Citrus gall wasp and Fullers rose weevil- awaiting results from collaborator

Appendix 19. Publications arising from association with the entomopathogen side of the project

Supervised by and co-authored by Dr Wilson and Prof Ash, Dr Kim Khuy Khun (USQ) published four scientific papers examining the use of entomopathogenic fungi on the macadamia weevil using strains isolated in the sweetpotato project and one review paper on the integration of entomopathogenic fungi into IPM (involving sweetpotato weevils). These papers are relevant to the current project as they provide valuable data that is required as a part of the requirements for biopesticide registration.

Khun, Kim Khuy and **Ash, Gavin J.** and Stevens, Mark M. and Huwer, Ruth K. and **Wilson, Bree** A.L. (2021) *Compatibility of Metarhizium anisopliae and Beauveria bassiana with insecticides and fungicides used in macadamia production in Australia.* Pest Management Science, 77 (2). pp. 709-718. ISSN 1526-498X https://doi.org/10.1002/ps.6065

Khun, Kim Khuy and Wilson, Bree A. L and Stevens, Mark M. and Huwer, Ruth K. and Ash, Gavin J. (2020) *Integration of entomopathogenic fungi into IPM programs: studies involving weevils (Coleoptera: Curculionoidea) affecting horticultural crops.* Insects, 11 (10):659. https://doi.org/10.3390/insects11100659

Khun, Kim Khuy and **Ash, Gavin J.** and Stevens, Mark M. and Huwer, Ruth K. and **Wilson, Bree A.** L. (2021) *Interactions of fungal entomopathogens with synthetic insecticides for the control of Kuschelorhynchus macadamiae (Coleoptera: Curculionidae).* Journal of Applied Entomology, 145 (6). pp. 553-566. ISSN 0931-2048 https://doi.org/10.1111/jen.12879

Khun, Kim Khuy and **Ash, Gavin J.** and Stevens, Mark M. and Huwer, Ruth K. and **Wilson, Bree** A.L. (2020) *Response of the macadamia seed weevil Kuschelorhynchus macadamiae (Coleoptera: Curculionidae) to Metarhizium anisopliae and Beauveria bassiana in laboratory bioassays.* Journal of Invertebrate Pathology, 174:107437. pp. 1-7. ISSN 0022-2011 https://doi.org/10.1016/j.jip.2020.107437.

Khun, Kim Khuy and **Ash, Gavin J.** and Stevens, Mark M. and Huwer, Ruth K. and **Wilson, Bree A.** L. (2021) *Transmission of Metarhizium anisopliae and Beauveria bassiana to adults of Kuschelorhynchus macadamiae (Coleoptera: Curculionidae) from infected adults and conidiated cadavers.* Scientific Reports, 11:2188. pp. 1-12. https://doi.org/10.1038/s41598-021-81647-0

Australian Government
Australian Centre for International Agricultural Research

Assessing virus and its associated vectors, host plants and beneficial insects using appropriate diagnostic tools at sweetpotato commercial farmer's fields in Highlands of Papua New Guinea

Jiwaka Sweetpotato Farmers' Field Day_ 28th Feb – 5th Mar 2021

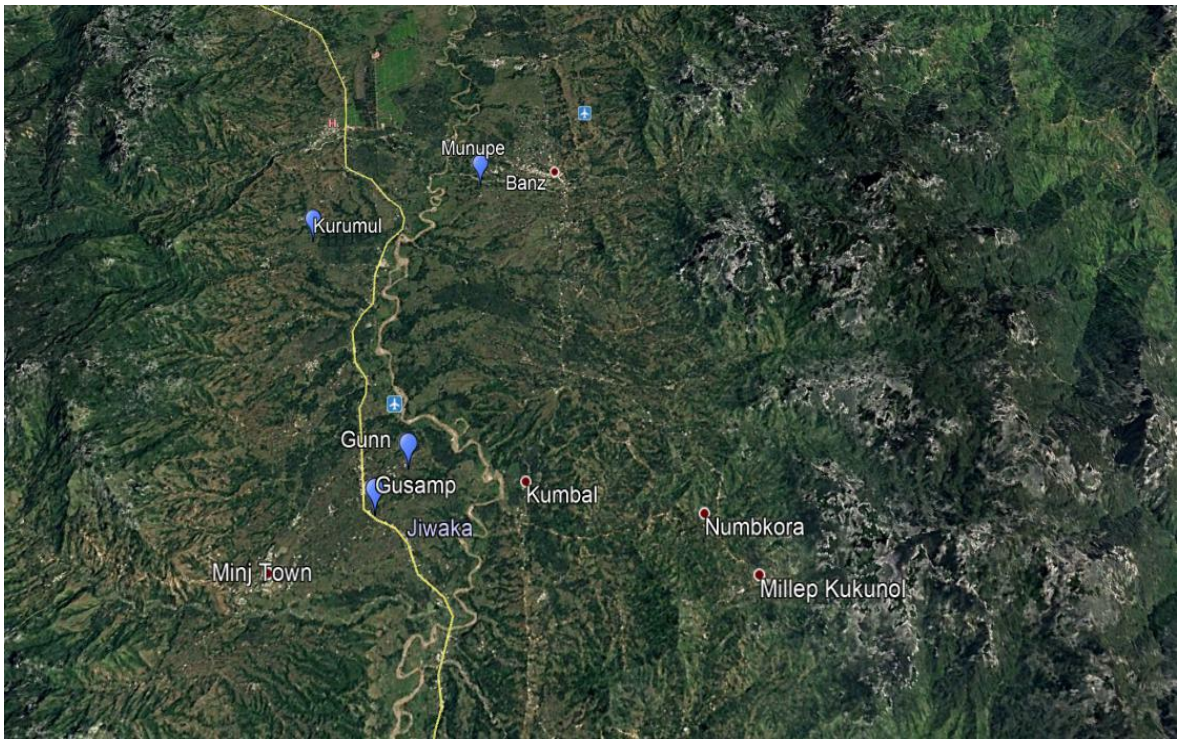
To: Mr Alex Agiwa
Cc: Mr John Kewa and Mr Chris Mathew
From: Wilfred Wau
Project: ACIAR Pest & Disease Project

Name	Wilfred Wau – Project Research Officer
Main Objective	• To lead-out Farmer's Field Day at four trial sites in Jiwaka TEAM zone#2, i.e. in Banz, Gunn, Gusamp and Kurumul • Conduct socioeconomic survey at those sites
People met	• Mr John Kewa (ACIAR Project Manager - FPDA) • Mr Alex Agiwa (ACIAR Pest & Disease Project Coordinator – FPDA) • SP commercial farmers: Agnes - Gusamp, Rachael - Gunn, Susan – Banz and Mrs. Isaac – Kurumul
Major events	❖ **Sunday (28/02/21)** o FPDA vehicle traveled to Aiyura for pickup and travelled to Jiwaka o Overnight at GK Lodge as it was late to continue travelling to Jiwaka because the vehicle arrived late for pickup (around 1pm) and also vehicle encounter some technical problem which delayed traveling time ❖ **Monday (1/03/21)** o Before departing to Jiwaka; ▪ A brief meeting regarding project's plans was held at FPDA Extension office with Mr Kewa and Mr Agiwa ▪ Socioeconomic survey forms were printed and made copies, i.e. twenty (20) copies of five (5) page: 5 forms per trial site ▪ Departed at 11 am and arrived after 5 hours o Sorted accommodation at Molka Lodge, Minj for the week ❖ **Tuesday (2/03/21) - Thursday (4/03/21)** o As per the itinerary, sweetpotato commercial farmers have had organized their contact farmers and general farming communities for the field days at strategic location (central/public spots) in their respective sites. o At Munupe (Banz) and Gunn (Minj), an open air

Facts/Results	❖ Nine (9) packets of pheromone lures containing twenty (20) pheromones each (540 pieces in total) were distributed to the farming communities. Each site was given three (3) packets (180 pieces) each. ❖ There is increasing demand for Pheromone lures and PT

	planting materials ❖ Banz farmers in North Whagi Electorate have raised their strong concern on accessibility of PT planting materials as current three (3) igloo farmers are situated in South Whagi Electorate. There was high cost involved to travel across to access those materials and material cost and somewhat limit our interest for continue production. Hench, they requested for an Igloo must be set at Commercial farmer (Susan) site so for easy accessibility. ❖ Shortage of pheromone lures stock; hence, farmers were given 1 each or even shared especially those farmers on same area. A new order is required. ❖ It is to my surprise that Potato Seed Scheme was never known by those farmers. They've shown amazing faces and expressed frustration that why they were derived after they lost interest when PLB strikes in 2003. The farmers requested for Potato team in FPDA to assist them as soon as possible as they claim the crop to be an alternative cash crop to sweetpotato and an insurance crop.
Challenge/Constraint	• Unpredictable wet weather condition has delayed set time for field date so we have to start whenever weather is fine. Thus, affected the attendance. • There was death in Gunn so many of contact farmers expected didn't turn up. • Socioeconomic survey forms for Gunn were left with the igloo farmer as expected contact farmers to fill didn't turn up due to a death in the community. She (igloo farmer) will organize later and will be picked-up during next duty travel
Way forward	• An awareness and mass distribution of PT vines and Pheromone lures is required to be done in main markets in Minj, Kudjip and Banz • A request to FPDA Sweetpotato Program to construct an Igloo for Banz farmers in North Whagi Electorate has there is great need • A video footage need to be done to capture the impact of Pheromone lures in farmer's field
Resolution/Suggestion	• Immediate need to order more pheromone lures • Video documentation is required to file concrete support to persuade the commercial company such as Brian Bell for possible imports of the pheromone lures • Socioeconomic survey forms are shelve and once the remaining sites covered then all forms will be scanned and sent it to Richard Calus • Next Travel to final site (WHP) will be on 28/03 – 4/4/21
Photo Gallery	✓ Pictures below were taken during the Field Days at respective commercial farmers sites
Jiwaka SP Farmers Field Day	• Farmer's Field Day was conducted from 28th February to 5th March 2021 • Report submission date: 18/03/21

Trial site map	• The blue colored flag/point showed the trial sites where the Farmer's Field Day was conducted



Farmers Field Day – One (1)

Munupe, Banz – North Whagi Electorate

There were total of 108 farmers attended the field day: 28 were male (above 18 years of age), 56 were female (above 18 years of age) and 24 were girls and boys (below 18 years of age). This site recorded the highest attendance compared to other two sites. Collated pictures of the event shown below.

Contact farmers were gathered under a shade and got busy filling the socioeconomic survey form. Chris Mathew (FPDA field offer) assisted the male farmer interpreting the questionnaire while I assisted the females.

Farmers Field Day – Two (2)

Gunn, Minj – South Whagi Electorate

There were total of 97 farmers attended the field day: 21 were male and 53 were female, all above 18 years of age and 23 were girls and boys (below 18 years of age who joined us after school). Good number of expected contact farmers didn't turned up due to death in community.

Collated pictures of the event shown below.

Igloo farmer, Ms Rachael (green colored cap) thankfully accepted the packets of Pheromone lures given while other farmers looked on. She then addressed the crowds and distributed the pheromone 1 each to a farmer and those that have gardens at same location, shared.

Farmers Field Day – Three (3)

Gusamp, Minj – South Whagi Electorate

There were total of 32 farmers attended the field day: 13 were male (above 18 years of age), 17 were female (above 18 years of age) and 2 children (a boy and a girl). Although there was less number attended but the discussion was in-depth and very interactive.

Collated pictures of the event shown below.

Mr. Wau (NARI Research Officer) demonstrated the process involved in construction of the pheromone trap to farmers.

ACIAR Pest and Disease Project HORT/2014/083
"*Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea*"

Sweetpotato Farmer's Field Day – TEAM zone #3:
Western Highlands Province

Integrated Pest Management (IPM) Package
Mini-Show

Trip Report#:03

To: Dr Komolong (PD1) and Mr Johannes Pakatul (RDC)
Copy: Robert Geno (ACIAR Pest & Disease Project Coordinator)

Report Compiled By: Wilfred Wau (Project Scientist)
Date: 4th June 2021

Farmers crowded over limited supply of pheromone lures at Nipuka trial site in Asaro TEAM zone #1

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Figures

Introduction

After successful completion of consecutive trials of best-bet and best-bet plus (+) trials conducted across the TEAM zone sites (Asaro, Jiwaka & WHP), recommended best-bet practices have to be made known to the wider farming communities. Hence, Fresh Produce Development Agency (FPDA) has taken the initiative in organizing Farmers' Field Days as an avenue for promotion, dissemination and fair discussions on recommended best-bet practices and other pressing issues for the sustainability of the ACIAR Pest and Disease Project HORT/2014/083 "*Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea*".)

Farmer's Field Day is very much a mini-show displaying IPM package derived from best-bet and best-bet (+) trials being experimented under the project. The use of pheromone lures was one of the best-bet practices trialled and apparently effective in reducing populations of sweetpotato weevil (Cylas formicarius). Commercial farmers and their contact farmers and even wider farming community have expressed their need of accessing the pheromones as they enthusiastically witnessed the impact. The pheromone traps are unavailable in country so farmers currently need to be supplied with the pheromone lures and instructed in how to construct the traps using locally available materials.

Several Farmers' Field Day was conducted already in TEAM zone #1 (Asaro) and #2 (Jiwaka). WHP sweetpotato farmers in TEAM zone #3 were privileged to have their field days as reported below. The presentation was done in the following manner:

- Life display of weevils (Cylas & Euscepes) and poster display
- Pheromones distribution and method in construction of the traps
- PT scheme: TC plantlets display and the cycle of process involved (poster display)
- Demonstration of sprouting technique and disinfection of tubers
- Farmer's success stories presentation to general public
- General discussion: farmer's questions and answers

Objective:

- Showcase IPM package
    - Specifically to conduct mini-show displaying Best-bet practices conducted under ACIAR Pest and Disease project
- Conduct socioeconomic survey
- Distribution and sales of pheromone lures throughout the TEAM zone sites

Travel itinerary

Staffs involve				
1. Wilfred Wau (Project Scientist) – NARI officer				
2. Chris Mathew (Project Cadet Officer) and Samuel (Driver) – FPDA officers				
Itinerary				
Day	**Date**	**Time**	**Event**	**Accommodation**
Sunday	23/05/21	Wilfred's travelling day to Goroka	- Pickup by FPDA vehicle - Travel to Minj, Jiwaka - Sort-out accommodation - Make prior arrangement with sweetpotato igloo farmers via phone communication	Molka Lodge
Monday	24/05/21	Travelling to each site for publicity	- Farmers' Field Day awareness at the TEAM zone sites - Stick flyers on stores/buildings situated at public places and roadsides with assistance from local igloo farmers - Physically inform the farmers of the event and the expectation	Lutheran Guest House
Tuesday to Friday	25/05/21 to 28/05/21		• From 8 am to 5 pm/late: o Conduct farmer's field day at Kurumul in Jiwaka and at Bomrui, Mul, Koge, Kelua and Tonga in Western Highlands Province. o Subject showcase includes the IPM package: ▪ Life display of weevils (*Cylas* & *Euscepes*), pheromones, construction of traps, posters ▪ PT scheme: TC plantlets display and the cycle of process involved (poster display) ▪ Demonstration of sprouting technique and disinfection of tubers ▪ Sales and distribution of pheromone lures ▪ Demonstrate method in construction of a pheromone trap ▪ Farmer's success stories presentation to general public ▪ General discussion: farmer's questions and answers o Conduct socioeconomic survey on Best-bet plus	
Saturday	29/05/21	Wilfred's travelling day to Aiyura	• Drop-off by FPDA vehicle	

Publicity

Reaching out to wider farming communities in disseminating improved knowledge and skills is paramount for the sustainability of the project. Hence, publicity plays an important role in any convenience. Following are some medium being used to drive the message across the igloo farmers plus their contact farmers and the interested farmers in the respective TEAM zones sites.

Group text message

- An open group text message on promotion and invitation was sent prior to the Farmers' Field Day to the five (5) sweetpotato igloo farmers to keep them inform for participation and to extend the invitation to the general farming communities.

Flyer

- Two different flyers were created using Adobe Photoshop CS6: one was for the Farmers' Field Day and the other for public notice on sales of pheromone lures (see figures below). About 15 colored copies on Farmers' Field Day was printed, i.e. 3 per site and about 39 colored copies printed for Pheromone Sale notice, i.e. also 3 per sites. The flyer was simple outlining the key points for clear understanding and capture readers' attention with color full pictures of PT Beauregard (both peeled and unpeel), pheromone lures and weevils (*Cylas and Euscepes*).
- With the help of the commercial farmers, we've covered the designated neighboring farming communities via farther roads making public awareness and promotion. The flyers were pinned on store walls at market places, schools and roadside after every public encounter. It took us a day to completely cover the expected farming communities.
- Below is an example of both flyers. Note: on pheromone sales notice, local dialect in describing the damage done by sweetpotato weevils was stated on the notice, for example; "segesege" for Asaro farmers.

Figure 1. Farmer's Field Day flyer sample

Figure 2. Pheromone sales flyer sample

FPDA notice board

- A copy of colored printed flyer on the *Pheromone Sales Notice* was pinned at the FPDA office notice board for officers' information and could possible buy if needed for their back home farming or for their sweetpotato farmers.

Face Book (FB)

- Since most of our farmers are using Face Book, both flyers were posted on personal FB account and tagged those farmers and project staffs who were my friends. The post was also shared in 5 public forums such as EHP Development Forum for further awareness. It was overwhelming to notice Robert and Melanie shared the post on their wall.

Farmers Field Day

Farmers attendance record

The column graph below shows the farmers attendance at the respective sites. The legend on top indicated Male (dark blue), Female (orange) and Children (yellow). It was unexpected that there were 3 sites (Koge, Mul & Bomrui) that Farmers' Field Day didn't eventuate as planned due to death in the community.

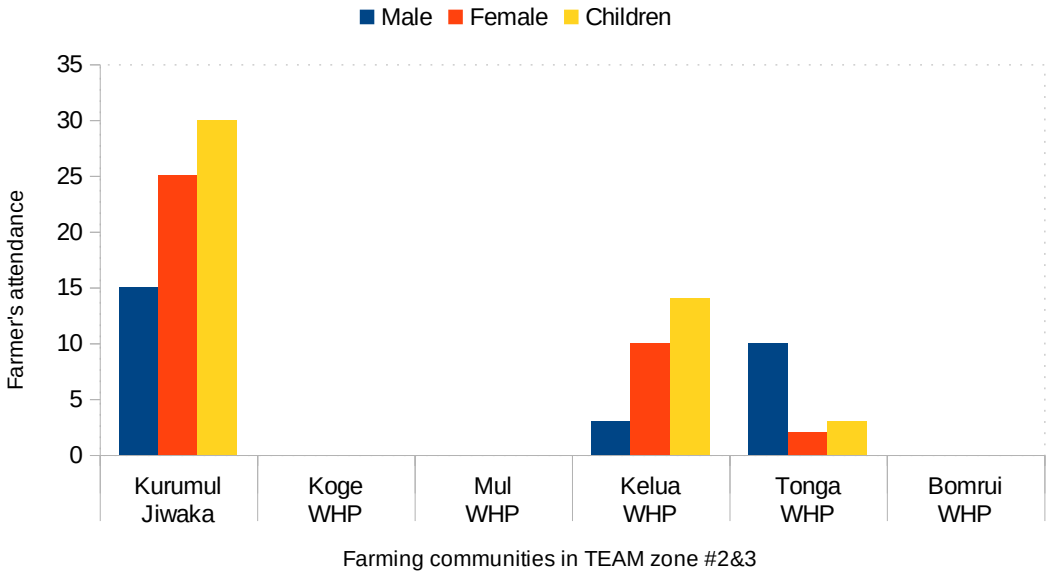


Figure 3. Cumulative graph of farmers attendance

Pheromone lures distribution and sales

- Depending on the Farmers' Field Day attendance at each site, pheromone lures were distributed. A total of 9 packets of pheromone lures distributed to 3 sites that the event took place (4 packets contained 20 pieces were given to farmers at Kurumul (Jiwaka) and 3 packets for Kelua and 2 for Tonga in WHP) and 1 packet each for unattended sites to be distributed. A sum of 240 (12 *20) pheromones lures has been distributed freely to the farmers in those farming communities.
- Pheromone lures for sales were given to each of the sweetpotato igloo farmers with specific instruction as expected in this exercise. Detail information is tabulated as follows.

TEAM zones sites	Trial sites	District or Electorate	SP Commercial Farmer	Pheromone Packets Sales	Revenue
Zone #1 – Asaro, EHP	1.Meteyufa	Goroka	Aku Kulo	3 packets	K60.00
	2.Nipuka	Daulo	Wasi Waukave	3 packets	K60.00

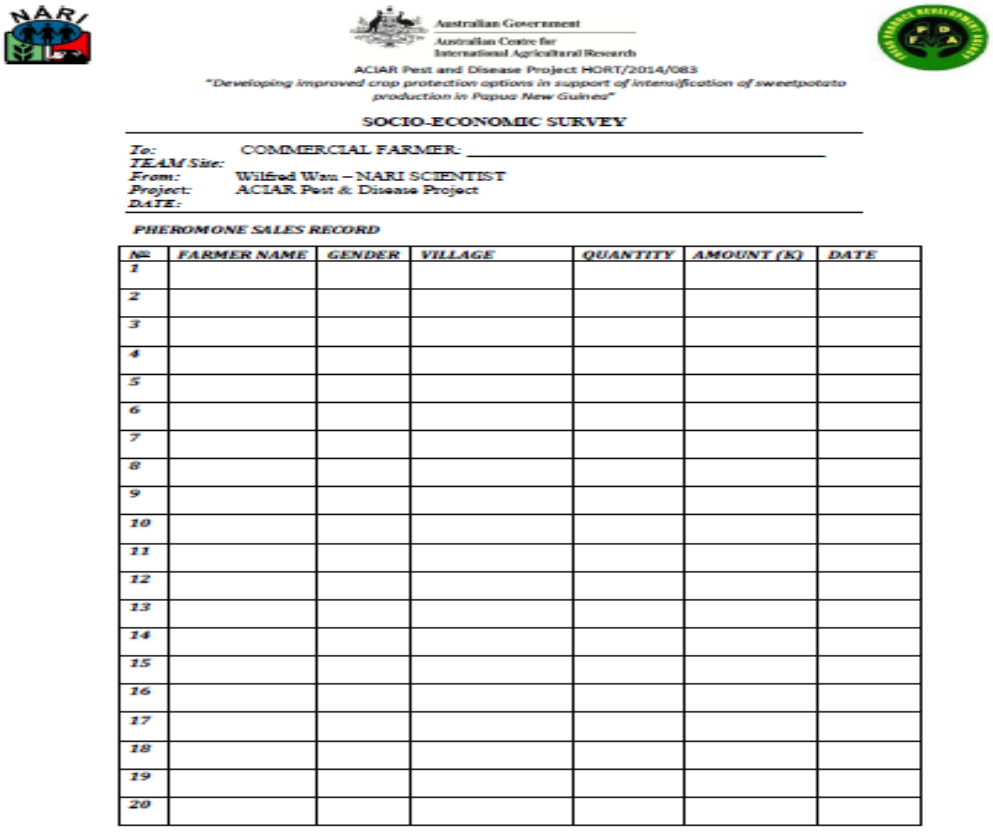
	3.Gimisave	Daulo	Kuman Giregire	3 packets	K60.00
	4.Kuka	Daulo	Ben Iseho	3 packets	K60.00
Zone #2 - Jiwaka Province	1.Gusamp	South Whagi	Agnes Merep	3 packets	K60.00
	2.Gunn	South Whagi	Rachael Kaman	3 packets	K60.00
	3.Kurumul	South Whagi	Isac Monam	3 packets	K60.00
	4.Banz (Kongabil)	North Whagi	Susan Ben	3 packets	K60.00
Zone #3 – Western Highlands	1.Bomrui	Hagen Central	John Worinu	2 packets	K40.00
	2.Koge	Hagen Central	Jacob Timbil	2 packets	K40.00
	3.Mul	Mul Baiyer	Win Moni	2 packets	K40.00
	4.Kelua	Hagen Central	Paul Kupariu	2 packets	K40.00
	5.Tonga	Tambul Nebilyer	Paul Berem	2 packets	K40.00
				Total	**K680.00**

Pheromone lures sales record form

A sample of the data sheet is found below. It's pretty basic, outlined farmers name, gender, village, quantity, amount (K) and date.

- The information generated should contribute some answers to whether the pheromone lures is economically feasible in the marginal farming communities.
- The amount of money generated should be collected and deposited to ACIAR Pest and Disease Project Account.

This exercise is actually the plan B if our attempts to convince business houses for possible imports are unsuccessfully or should take longer period than expected then FPDA partner should import the pheromones under the their Seed Program as agreed by Mr Agiwa as the program manager. FPDA now will facilitate and then engage the respective igloo farmers to be the sole distributor and do the sales till such a time when the business houses such as Brian Bell, Tininga, Farmset, and Chemica can take onboard as initially expected.



Australian Government
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ACIAR Pest and Disease Project HORT/2014/083
"Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea"

SOCIO-ECONOMIC SURVEY

To: COMMERCIAL FARMER: _____
TEAM Site:
From: Wilfred Wan – NARI SCIENTIST
Project: ACIAR Pest & Disease Project
DATE:

PHEROMONE SALES RECORD

№	FARMER NAME	GENDER	VILLAGE	QUANTITY	AMOUNT (K)	DATE
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Figure 4. Pheromone sales record sheet

Stock-take of the pheromone lures

The parcel of shipment was received on 10th April 2021 at NARI HQ, Bubia 10 Mile. I received the parcel two weeks later (23/04/21) during my duty travel to HQ.

The parcel consisted of 3 batches of pheromone packets, containing 25 packets each. It was a total of 75 packets altogether in the parcel. Off these, 12 pheromone packets were distributed for free during Farmers' Field Days and 34 were given to sweetpotato igloo farmers for sales, totalled to 46 pheromone packets despatched and currently we available stock of 29 pheromone packets (1 batch contained 25 packets and 4 loose packets).

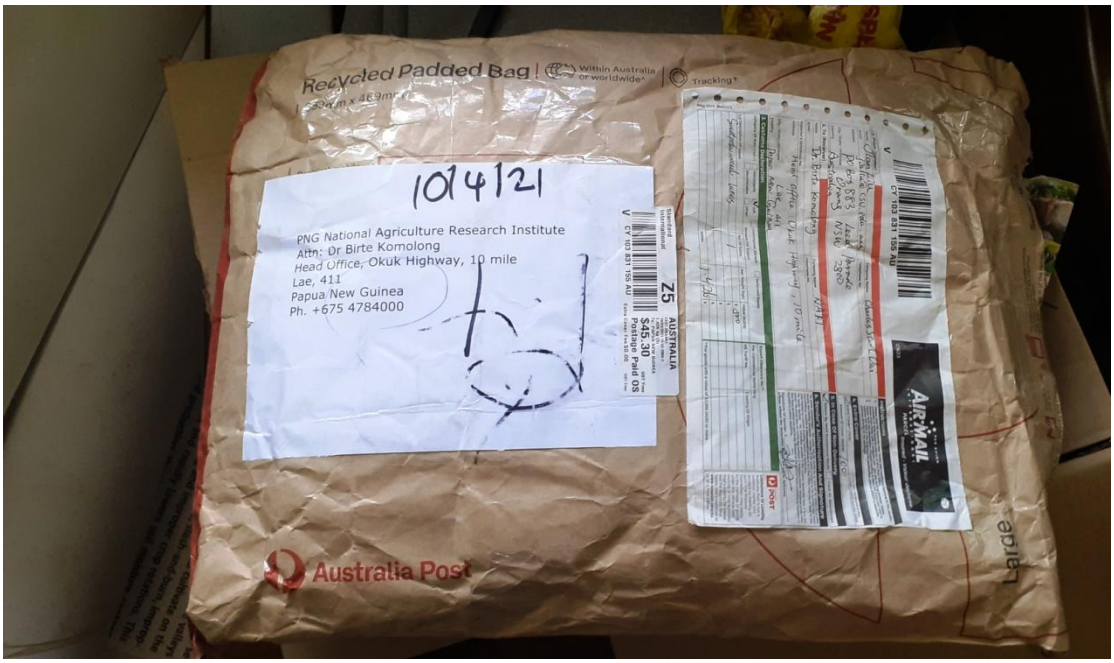

Figure 5. Pheromone parcel shipment

Socioeconomic survey

It was an initiative taken to assist Mr Agiwa where we can especially when the project is towards completion for the socioeconomic survey. We managed to conduct the socioeconomic survey alongside with the Farmers' Field Day. About 5 farmers per site were interviewed after every field day. A total of 15 farmers were interviewed except 3 unattended sites. Previous survey forms left with farmers in Jiwaka were collected and all sorted. On my way to Aiyura, the survey forms were delivered to Mr Alex Agiwa to be submitted to Richard Calus.

Challenges

- Farmer's do have priorities, traditional obligation and cultural restriction in their communities which was highly respected and its very challenging to adjust for their convenience to conduct the farmer's field day.
- There were three igloo farmers (John Worinu, Jacob Timbil and Win Moni) who have death in their community which prevent us from organizing the Farmer's Field Day.
- Farmer's having the perception that PT sweetpotato and pheromone lures are like foreign pathogens introduced and it's a treat to their farming communities. "It definitely will like Potato Late Blight (PLB) outbreak that we had experienced before, farmers ended up saying that". They had also other issues that such field days created a platform for discussion so be harmonized for the sustainability of the project.

Recommendation/suggestion

- Chris Matthew and Mr Agiwa was advised to conduct the Farmer's Field Day for unattended sites (Bomrui, Mul & Koge) which was disrupted due to death in the community.
- Mr Enopa Linsay was also informed of challenges faced on the ground for information sake as he is the current manager for FDPA Hagen office. Maybe can involved in supporting Chris and Alex in addressing the farming community.
- Presentation to Brian Bell and other commercial companies should be done within the month of July.

Next plan of action

- Short video shooting for selected igloo farmers at respective TEAM zone sites whom use of pheromone lures have impacted in his/her farming and the community at large. About two farmers should be selected per site: an igloo farmer and a contact farmer.
- Conduct public awareness in sweetpotato market places.
- Collect pheromone lures sales data
- Proposed date for next duty travel should be ***14th to 18th June 2021*** to achieve the above mentioned activities.

Acknowledgement

- Firstly, my sincere gratitude to the igloo farmers and their farming community who were welcoming and participated exceptionally during the Farmers' Field Days. Without your presence such event shouldn't eventuated successfully.
- My deepest thanks to Mr John Kewa (ACIAR Projects Manager) for facilitating approval for this exercise to be executed as planned in the absence of Mr Agiwa (ACIAR pest & Disease Project Coordinator), who was out of the province for other project activities.
- I should also thank Chris Mathew (Project Cadet Officer) for his undivided support during the Farmers' Field Days and the photos (some) provided for this report
- Finally, my thanks to NARI for giving me the approval to continue work with collaborating partner in delivering this worthy task.

Farmers Field Days Pictorial Highlights

Kurumul#2, South Wagi Jiwaka Province

Figure 6. Wilfred demonstrated the Mulch and Living Barrier plants and its benefits to the farmers at Kurumul market place

Tonga, Tambul Nebilyer WHP

Figure 7. Igloo farmer, Paul Berem explained to the farmer in their mother tongue while Wilfred pointed to the poster.

Kelua, Hagen Central WHP

Figure 8. Paul Kupariu explained to the farmer in their mother tongue on how to construct a pheromone trap during the presentation

KAUKAU FARMER'S FIELD DAYS – ASARO TEAM ZONE

INTERGRATED PEST MANAGEMENT PACKAGE MINI-SHOW

Trip Report#:01

Implemented by: Mr Alex Agiwa (Project Coordinator – FPDA), Chris Mathew (Cadet Officer – FPDA and Wilfred Wau (Project Scientist)

To: Dr Komolong (PD1) and Mr Johannes Pakatul (RDC)
Copy: Robert Geno (ACIR Pest & Disease Project Coordinator)

Report Compiled By: Wilfred Wau
Date: 24th September 2020

Farmers crowded over limited supply of pheromone lures at Nipuka trial site in Asaro TEAM zone

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Introduction

Fresh Produce Development Agency (FPDA) took the lead in organizing this Farmers' Field Day as part of promotion and adaptation of recommended Best-bet practices after successful completion of trials conducted by project partner [National Agricultural Research Institute (NARI)] under the ACIAR Pest and Disease Project HORT/2014/083 "*Developing improved crop protection options in support of intensification of sweetpotato production in Papua New Guinea*".

Use of pheromone trapping was one of the Best-bet practices trialled and apparently effective in reducing populations of sweetpotato weevil (*Cylas formicarius*). Commercial farmers and their contact farmers have expressed their need of accessing the pheromones as they enthusiastically witnessed the impact. The pheromone traps are unavailable in country so farmers currently need to be supplied with the pheromone lures and instructed in how to construct the traps using locally available materials.

After successful completion of Phase 1 & 2 Pheromone Trapping Training at TEAM zone sites (EHP, Jiwaka and WHP) involving sweetpotato commercial farmers and their contact farmers from immediate farming communities, the training program now reached out wider farming communities through Field Day.

Farmer's Field Day is very much a mini-show displaying IPM package derived from Best-bet and Best-be (+) trials being experimented under the project. The most significant best-bet practice promoted included Pheromone lures and Pathogen Tested (PT) technology. The presentation done involves the following:

- Life display of weevils (Cylas & Euscepes) and poster display
- Pheromones distribution and construction of traps
- PT scheme: TC plantlets display and the cycle of process involved (poster display)
- Demonstration of sprouting technique and disinfection of tubers
- Farmer's success stories presentation to general public
- General discussion: farmer's questions and answers

Objective:

- Showcase IPM package
    - Specifically to conduct mini-show displaying what ACIAR Pest and Disease project has done and doing to this date and the recommended best practices:
        - 1) **Pheromone lures** and
        - 2) **PT technology**

Travel itinerary

Staff involved				
1. NARI_ Wilfred Wau (Project Scientist)				
2. FPDA_ Alex Agiwa (Project Coordinator) 2. Chris Mathew (Cadet Officer) 3. Tuls (Driver)				
Itinerary				
Day	**Date**	**Time**	**Event**	**Accommodation**
Sunday	16/08/20	Wilfred's travelling day to Goroka	-Pickup by FPDA vehicle -Travel to Goroka - Accommodation sorted -Purchase consumables and confirm itinerary with farmers	GK Lodge
Monday	17/08/20	Travel to each site per day	-Conduct awareness and promotion of the Farmers Field Day -Post flyer at public places and roadsides -Physically inform respective commercial farmers	
Tuesday to Friday	18/08/20 to 21/08/20		• From 10 am to 3 pm/late: o Conduct farmer's field day at Nipuka, Kuka, Gimisave and Meteyufa trial sites. o Community awareness and promotion on two Best-bet practices mostly preferred by farmers: ▪ Pheromone lures ▪ PT technology o Subject showcased includes: ▪ Life display of weevils (*Cylas* & *Euscepes*), pheromones, construction of traps, posters ▪ PT scheme: TC plantlets display and the cycle of process involved (poster display) ▪ Demonstration of sprouting technique and disinfection of tubers ▪ Farmer's success stories presentation to general public ▪ General discussion: farmer's questions and answers	
Saturday	22/08/20	Wilfred's travelling day to Aiyura	• Drop-off by FPDA vehicle	

Publicity

It was very important that the wider farming communities are reached as much as possible for good coverage of the farmers creating demand. Following are the medium being used to drive the message across to the farmers, colleagues and general public.

Group text

- A group text on promotion and invitation for Farmers Field Day was sent to the four (4) commercial farmers to be informed and extend the invitation to their farming communities.
- From the phone directory of contact farmers registered during previous Pheromone Trapping trainings, similar text message was forwarded to them. More than 50 farmers have received the message and couple of them responded with acknowledgement.

Group email

- FPDA as being partnering institute in current ACIAR projects, an open invitational email was sent to every project staffs: Mr John Kewa (ACIAR Projects manager), Mr Alex Agiwa (Pest & Disease Coordinator), Dr David Minemba (Sweetpotato Program Manager), Mr Chris Bugajim (TADEP Project Officer), Mr Bennie Atingu (ANU Project Officer). The email was attached with a flyer detailing the specification of the Field Day.
- A color printed flyer was pinned at the FPDA office notice board for general information

Flyer

- A flyer was created using Adobe Photoshop CS6 and 28 colored flyers printed (i.e. 7 per site). The flyer was simple outlining the key points for clear understanding and capture readers' attention with color full pictures of PT Beauregard and weevils (*Cylas and Euscepes*).
- With the help of the commercial farmers, we've covered the designated neighboring farming communities via farther roads making public awareness and promotion. The flyers were pinned on store walls at market places, schools and roadside after every public encounter. It took us a day to completely cover the expected farming communities.
- Below is an example of flyer used for Nipuka trial sites

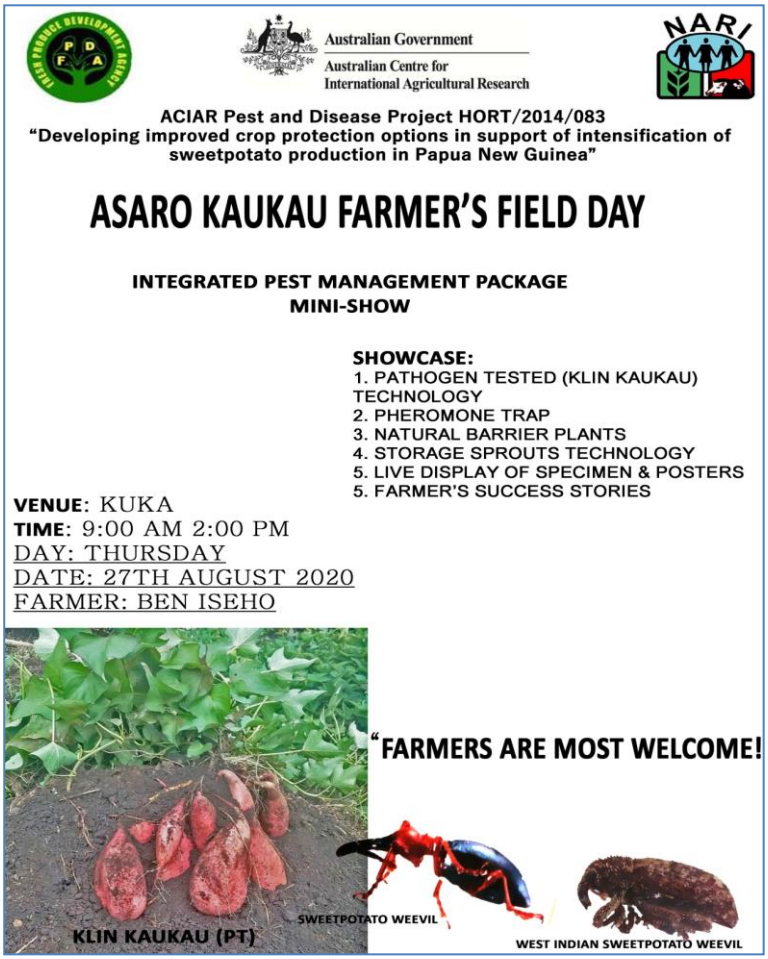

Figure 1. Sample of the Flyer

Community awareness and promotion

Figure 2. Commercial farmer, Mr Wasi Waukave from Nipuka made awareness and promotion in their dialect at Kasena community while flyer was sticked by locals onto a store wall at the roadside

Figure 3. Flyers sticked onto a store at the roadside while the public looked on at Kuka community

Figure 4. Crowds gathered at Asaro station in front of the trade store after flyers being posted. The commercial farmer, Mr Wasi was in action trying to get the message across to the farmers in their local dialect.

Field Days attendance per sites

Figure below (5), indicated the sum of farmers attended in each of the sites at Asaro TEAM zone: blue depicted MALE, red depicted FEMALE, green depicted Children and purple depicted Community Leaders.

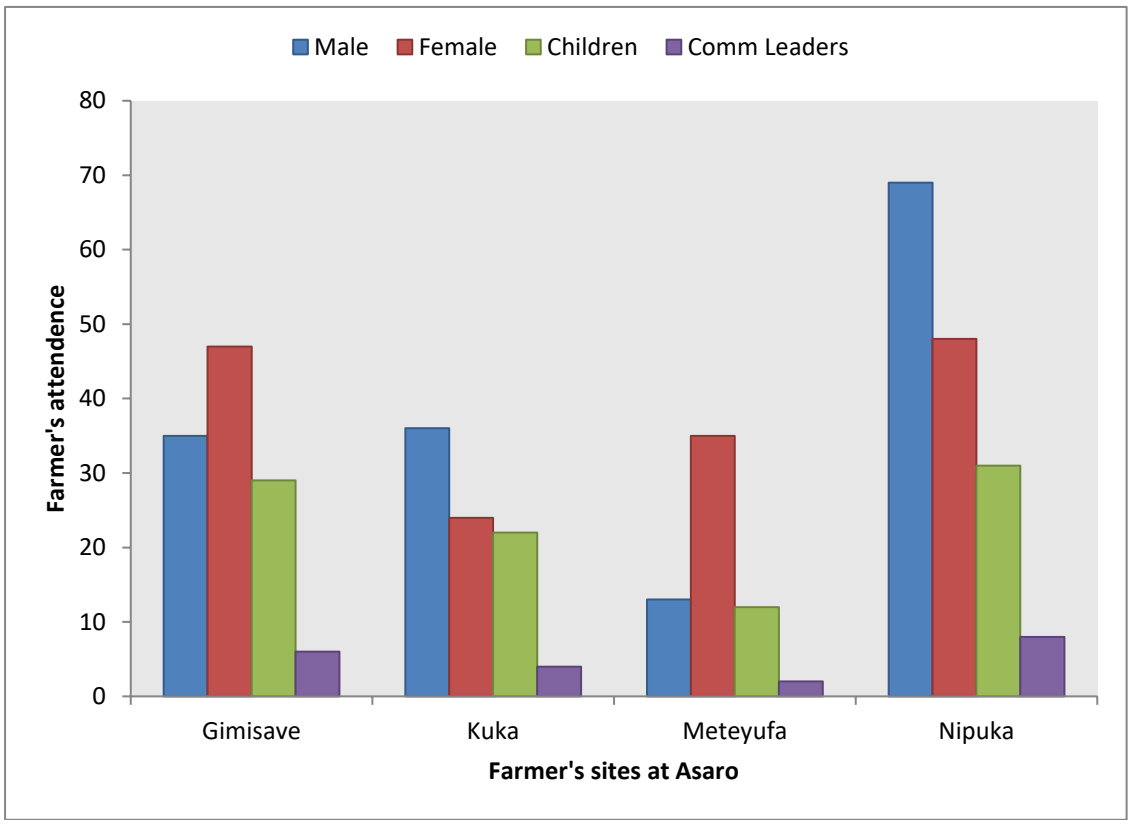

Figure 5. Cumulative graph of farmer attendance

Descriptive analysis of the farmer's attendance:

- Total number of farmers attended in each sites were:
    1. Nipuka = 156
    2. Gimisave = 117,
    3. Kuka = 86
    4. Meteyufa = 62
- Grand total of farmers attended for Asaro TEAM zone was 421
- There were gender equality distributed across the sites (F=153:M=154)
- A quarter of grand total of the farmers was children. It's a good sign for future generation being integral part of the Field Day.
- Field day at Meteyufa site clashed with a training conducted by FPDA lead by Bennie Atingu but managed to conduct after them. It was unfortunate, the commercial farmer, Mr Aku stated that 50% of the trainees left for Goroka District by-election campaign.
- There was couple of deaths at Nipuka and Kuka which directly affected the farmer's attendance. Otherwise, the attendance should have doubled
- At Gimisave trial sites, most of the farmers gather on Wednesday as was informed not knowing that it was the National Repentance Public Holiday. Later released and postponed to Friday which affected the attendance as many didn't make it as before.

Pheromone lures distribution

- Total of 8 packets of pheromone lures distributed: 2 packets contained 20 pieces being given to farmers attended at each site. A grand total of 160 pieces of pheromones have been distributed at Asaro TEAM zone sites.
- Stock was limited to give more so a single piece of pheromone was given to each farmer but still not enough for all farmers. Demand for more has increased.

Figure 6. Distributed limited pheromones lures farmers at Nipuka site

Asaro Kaukau Farmers Field Days

Nipuka farming community

Figure 7. Nipuka farming communities sat on the bare ground and attentively to the presentation

Gimisave farming community

Figure 8. Gimisave farmers enjoyed watching the displays

Kuka farming community

Figure 9. Kuka farmers paid attention during the Field Day as Mr Wau in action

Meteyufa Field Day

Figure 10. Meteyufa farmers waited for the Field Day to be conducted soon

Recommendation and Suggestion

- There were lots of impact stories shared by farmers which should be captured by video. Needed a proper camera with high definition for the course.
- It would be appropriate to organize media crew for publicity (FPDA can organize) for next 2 TEAM zones (Jiwaka and WHP)

Acknowledgement

- My sincere gratitude to Mr Agiwa for making this Asaro Sweetpotato Farmers Field Days a success
- Should also thank Chris Mathew for his assistance during display and photos (some) provided for this report
- My deepest thanks to Mr John Kewa for facilitating approval for this exercise to be executed as planned
- Finally, my thanks to NARI for giving me the approval to work with collaborating partner in delivering this worthy task.

Sweetpotato industry clues up for the future

https://www.goodfruitandvegetables.com.au/story/5110142/sweetpotato-industry-clues-up-for-the-future/

15 Dec 2017, 5 a.m.

News

ENGAGED: Sweetpotato growers and researchers tune into the paddock discussion over growing techniques and plant health.

Aa

SWEETPOTATO growers are aiming to “own their own destinies” after recent workshops held in Bundaberg, Queensland and Cudgen, NSW.

Growers representing about 85 per cent of Australia’s sweetpotato production attended the workshops and field demonstrations over the two days, attending from as far away as Atherton, Rockhampton and the Locker Valley to get involved.

The agronomic material that made up the bulk of the days was presented by scientists from the Queensland Department of Agriculture and Fisheries (DAF), ASPG and the University of Southern Queensland (USQ).

Australian Sweetpotato Growers (ASPG) principal horticulturist, Craig Henderson, there was a considerably positive atmosphere within both meetings.

“You just couldn't get a more enthusiastic group of people, committed to sweetpotatoes as a sustainable, healthy contributor to the Australian economy and community,” Mr Henderson said.

TUNED IN: The sweetpotato workshops provided the opportunity for growers to engage with Horticulture Innovation Australia staff over funding and projects.

The days began with growers discussing broader industry development activities with Horticulture Innovation Australia leaders.

Hort Innovation's Craig Perring and Christian Patterson outlined diverse marketing activities promoting the health benefits of sweetpotatoes to Australians, especially young families with active, growing children.

A highlight was the story of growers Jane Prichard and Chanel Kennedy talking to eager mothers at the Sydney Baby Show.

The growers emphasised the enthusiasm of parents to find out all things sweetpotato.

Growers were keen to see how their marketing levies were being spent, and contributed positively to discussions on future activities.

Investment plan

CONSULTANT Brian Ramsay summarised progress on the Sweetpotato Investment Plan, which will influence industry research and development activities for the next five to eight years.

While growers have already contributed through initial consultation, they organised additional conversations with Mr Ramsay to make sure as levy payers they continue to heavily influence research activities.

Mr Henderson rounded out the morning session outlining ASPG's commitment to effective industry development for its members, as well as the broader sweetpotato community.

In particular, he discussed how important it was for research efforts across organisations to be coordinated, to maximise the industry benefits of current and future work.

ALL IN: Growers attended from the major sweetpotato growing regions in Australia, including Cudgen, Bundaberg, Atherton and the Lockyer Valley.

ASPG is committing funds and resources to make this happen, as well as strongly facilitating grower engagement and partnering in research activities.

Mr Henderson also ran through biosecurity issues impacting the sweetpotato industry, stressing preparations for exotic pest incursions, and effective engagement with regulatory organisations.

In an innovate twist, DAF scientist, Rachael Langenbaker, provided Lego figurines to spice up the points being made.

The afternoon sessions were more practically focussed, showcasing research and outcomes from Hort Innovation Project VG13004 “Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry”, as well as allied sweetpotato R&D activities.

Weather hindrance

DUE to the heavy rain in Bundaberg in mid-October, the scheduled field walk was replaced by indoor presentations and discussions.

However, Cudgen growers got the chance to visit the Prichard and Kennedy farms, to look at plant beds, discuss best agronomic practices, and figure out how to maximise the benefits of their clean plant material scheme.

The industry attendees saw how their research project has changed the way growers construct and manage their plant beds.

RELATED READING

- Sweetpotato industry tackles viruses through science
- Sweetpotato research looks to healthy roots
- TIA researchers help potato farmers manage powdery scab

Compared to three years ago, growers are making their beds higher, and only covering their bedding roots with a 3-5 cm layer of soil, to maximise aeration and reduce losses from rots.

Mr Henderson and Ms Langenbaker discussed how new sweetpotato cultivars were probably more difficult to manage in plant beds, and that irrigation precision, preventing over-heating under plastic, and preferencing smaller bedding roots, were probably key components of a sustainable system.

They also discussed how nitrogen nutrition could be adjusted for the different cultivars, and that complete fertilisers (organic or inorganic based) were probably a good insurance for optimal productivity.

There were some grower observations and spirited discussions on several of these points.

Industry threats

IN both Bundaberg and Cudgen, the industry heard from DAF Virologist Sandra Dennien about the current sweetpotato virus threats in Australia, their distribution and seasonal occurrence.

Growers appeared enthusiastic to hear their clean plant material scheme had actually removed two viruses from commercial presence in the main growing areas.

Ms Dennien told growers about recent improvements in diagnostic capability, with the hope that molecular techniques could provide more rapid, routine capacity to screen for a range of current and potential virus threats.

OUTSIDE: Workshop attendees take the opportunity to shift the classroom outside for more hands-on demonstrations.
The industry attendees watched demonstrations of LAMP molecular diagnostic units, by both Ms Dennien, and USQ scientist Bree Wilson.

Whilst Ms Dennien was testing sweetpotato leaf samples for viruses, Ms Wilson was demonstrating the potential for the machine to test soil samples for nematodes, critical pests, or beneficial organisms (depending on species), in sweetpotato production.

Both scientists reinforced it was still early days but expressed excitement that the technology could bring laboratory precision to field sites, and really improve the turnaround time for diagnostics.

More timely analytics could prove very useful for key decision making on farms, as well as regional management of new pest incursions.

Sensing soil

MR Henderson and Ms Langenbaker also described how they were evaluating Chameleon soil moisture and temperature sensors (developed by CSIRO) in sweetpotato plant beds.

Given how important irrigation and temperature management are in plant bed performance and root breakdown, both researchers and growers are excited about a cost-effective tool that may assist industry learning.

The sensing technology is still very much a work in progress, with some issues of connectivity and capturing data.

However, already seven growers are participating in evaluations. The research team is seeing big differences in irrigation strategies, with many growers opting to err on keeping soil dry, to reduce breakdown of roots in plant beds.

Both the LAMP and Chameleon research activities are undertaken by projects substantially funded through the Australian Centre for International Agricultural Research (ACIAR), supporting collaborative sweetpotato research in Papua New Guinea and Australia .

It demonstrated the benefits of coordinating sweetpotato R&D activities between organisations such as DAF, CQU, USQ, ASPG and funding bodies like Hort Innovation and ACIAR.

Feedback with attendees following the sweetpotato industry days indicated they were happy to have come along, and took ideas and practices away that they could immediately try out.

Several researchers and growers talked about setting up an annual “sweetpotato week”, so people could program in getting to workshops and field days.

Sex pheromone trap Construction guide

INTRODUCTION

Pheromones are chemicals produced naturally by insects of the same species to communicate with each other or attract an individual of the opposite sex for mating. Synthetic pheromones have been used to manage wide range of insects, including the sweetpotato weevil which severely damage sweetpotatoes. It was reported that sweetpotato weevil adults and larva can cause 50% to 90% damage to storage roots. The effects of weevil damages are drastic and produce odor 'terpene phytoalexins' that are unpleasant to humans and animals.

Female weevils produce a chemical 'perfume' which attracts male weevils. This chemical is called a "Sex Pheromone". Sweetpotato weevil sex pheromone can be used to trap male weevils present in the sweetpotato field. Trapping male weevils disrupts the weevil production cycle. Less weevil's population will mean less damage to the crop.

Many commercial sweetpotato farmers and contact farmers in Eastern Highlands (Asaro Valley), Jiwaka (South & North Whagi) and Western Highland Provinces (Nebilyer, Hagen Central and Mul Baiyer) have been using Sex Pheromone Traps in sweetpotato production between 2018 – 2021 under the project and have found that weevil damage has been reduced significantly.

8.The trap should be check weekly or fortnightly depending on weevil's population density and discard the solution containing the dead weevils. Reset the trap with new soapy solution as done in step 5. For knowledge sake, record the number of dead weevils by empting the solution onto a filter cloth and count.

The pheromone trap is effective up to six weeks as per manufacture warranty before it is replaced with a new trap.

PRODUCED BY: WILFRED WAU (PATHOLOGIST)
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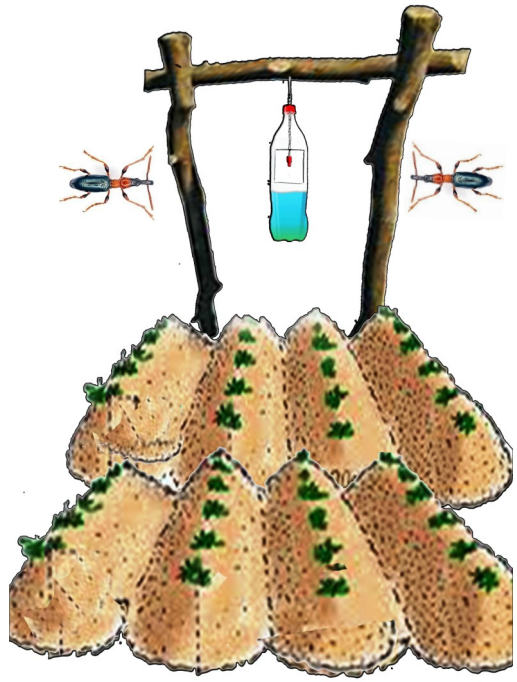
Integrated Pest Management (IPM)

SWEETPOTATO WEEVIL SEX PHEROMONE TRAP

CONSTRUCTION GUIDE



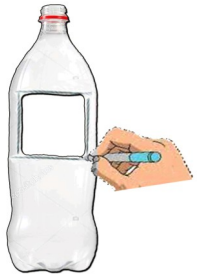
Step-by-Step Procedure in Construction of Sweetpotato Weevil Sex Pheromone Trap

Materials required

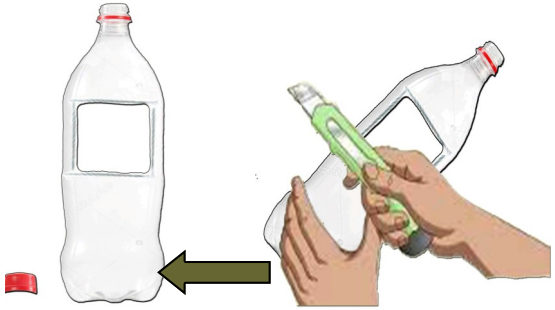
- Sweetpotato Weevil Sex Pheromone
- Empty plastic container (1L coke container)
- Ruler (30cm)
- Cutting Blade/Knife
- Marker (any color)
- Nail (3 piece of 2inch nail)
- Hammer
- Stick/Post (3x 1.5m length)
- Wire/Rope (25cm)
- Bush knife
- Soap/Detergent (any form)
- Water (500ml)
- Bucket (9L)

Steps

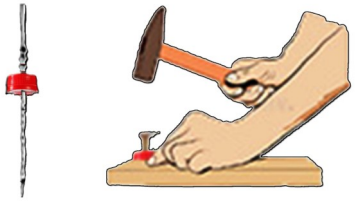
1. At each end of the cylindrical surface of an empty plastic container, measure 10cm x 10cm and mark along with a marker. Then do the same at the opposite side, forming a curved square on both sides, 3cm apart.



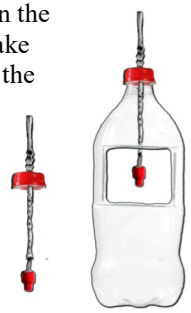
2. Remove the lid of the container and cut along the square lines while pressing firmly at each side of the container.



3. Punch a hole at the centre of the lid. Insert a 25cm wire from inside-out and tie a knot at 13cm as stopper beneath the lid to support hanging.

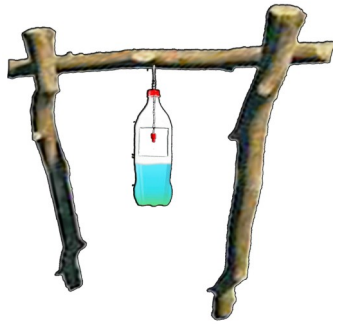


4. With the sharp end of the wire below the lid, pierce a sex pheromone. Screw back the lid to close lure hanging on the wire and fasten firmly. Make sure the lure is hanging in the center of the square cut.

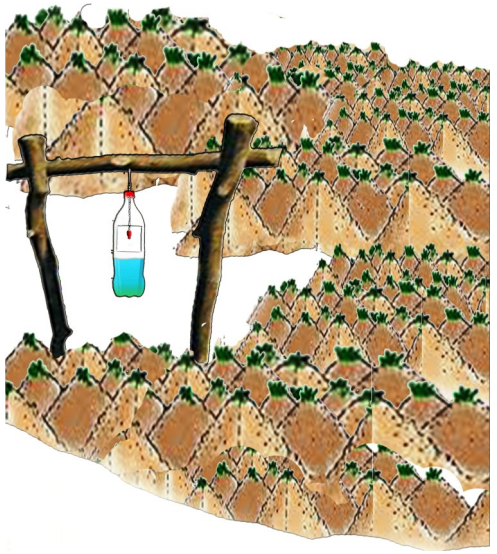


5. Make a soapy solution by mixing a detergent or soap in a bucket of tap water, and then pour the solution into the empty container to ¾ capacity, just below the cut.

6. Nail a post across the other two, forming letter 'H' at 1m spacing. At the cross post, punch a nail half way through and hang the Pheromone Trap by tying the wire to it.



7. Set the trap in the middle or edge of the sweetpotato field where it's free from any obstruction in weevil's movement.



Major Sweetpotato Disease Causing Economical Yield Decline in Sweetpotato Production in Papua New Guinea

National Agriculture Research Institute (NARI)

Australian Government
Australian Centre for International Agricultural Research

Sweetpotato Scab

Casual organism: *Elsinoe batatas*

Description

Scab or *Elsinoe batatas* is probably the most serious fungal disease of sweetpotato throughout the tropical world. It is essentially a wet weather disease, with severity increasing with rainfall. In Indonesia, it is considered a limiting factor to increasing sweetpotato production. The fungus has a sexual stage, *Elsinoe*, and an asexual stage, *Sphaceloma*.

Symptoms

Young leaves are infected along the veins, and infections also occur on the leaf stalks and stems. As the leaves grow, the damage to the veins prevents expansion, and they twist, curl and tear. On older leaves, infections produce pinpoint spots between the veins, and the leaves are twisted to expose the under surfaces. In severe, but rare cases, shoot-tips are killed.

Young shoot stunted

Brown lesion on leaf stalks and stems

Leaves twisted and curled

Shoot tips are killed

Impact

Scab is the most severe fungal disease of sweetpotato in South East Asia and the Pacific. In Papua New Guinea highlands, comparisons between healthy and diseased plants, showed a 60% difference in the yield of storage roots.

Management

Pathogen-free (PT) planting material of the most resistant varieties and good sanitation practices should be used.

Cultural Control

- Use healthy planting materials (PT), produced through a healthy "seed" scheme.
- Crop sanitation- manual removal of symptomatic plants at first sign and do regular weed control

Resistant varieties

- Use resistant varieties if available.
- If growers want to grow susceptible varieties, because of their taste and/or high market value, for instance, then they should do the following:

Generate tuber sprouts

Planted tuber spouts at nursery pots

Healthy and vigorously grown vines

During growth: Vigorous early growth will reduce the impact of the disease when the plants become infected later.

After harvest: remove old or harvested vines and burn or bury them.

Before planting: Produce disease-free planting material from tuber sprout:

Chemical Control

Scientists have recommended the use of fungicide 'Mancozeb' for commercial farmers who grow susceptible varieties for the market. The recommendations is to use Mancozeb on planting material before planting: Dip the cuttings for 15 minutes in Mancozeb before planting. Spay with Mancozeb at the first sign of symptoms. Repeat spraying at 14-day intervals, depending on the weather, until 1-2 months before harvest.

Mancozeb Fungicide

Disinfect the planting materials before planting

Spraying after planting

Major Sweetpotato Disease Causing Economical Yield Decline in Sweetpotato Production in Papua New Guinea

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Sweetpotato Viral Disease

Description

Viruses are amongst the smallest organisms known and can be seen only using a very powerful electron microscope. They are very simple organisms and only have the capacity to live and multiply inside their hosts/ victims. Most also need to be carried from plant to plant, usually by an insect which feeds on plant sap, such as aphids or whiteflies.

The impact from virus infection is not known well in Pacific island countries. Single infection of virus does little compared to multiple infection. Recent studies in PNG have shown more than 50% yield reduction in some of the local varieties. In most cases, susceptible varieties are severely affected.

Distribution and Detection

Worldwide, probably wherever sweetpotato is grown: Asia, Africa, North, South and Central America, The Caribbean, Europe (restricted), Oceania. In PNG, the following viruses have been recorded:

I. *Sweetpotato Feathery Mottle* (SPFMV), transmitted by Aphids
II. *Sweetpotato Virus G* (SPVG), transmission unknown
III. *Sweetpotato Mild Mottle* (SPMMV), transmitted by Whitefly
IV. Sweetpotato Chlorotic Fleck (SPCFV), transmission unknown
V. *Sweetpotato Caulimo-like* (SPCV), transmission unknown
VI. *Sweetpotato Ring Spot* (SPRSV), transmission unknown
VII. *Sweetpotato Begomovirus*, transmitted by Whitefly


Aphids

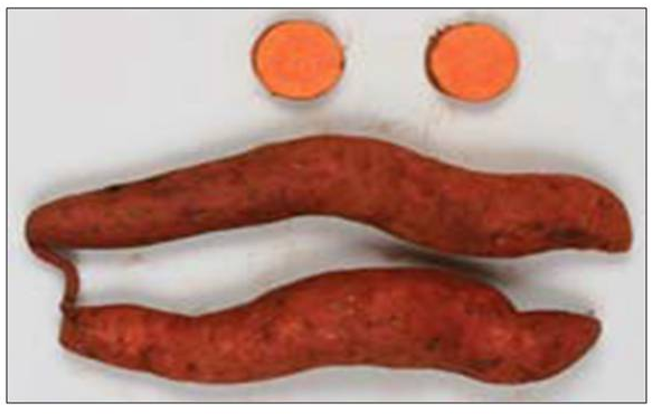

Whiteflies

Symptoms


Purpling spots


Veinal chlorosis


Small brown to black corky spots


Irregular shapes

Hosts

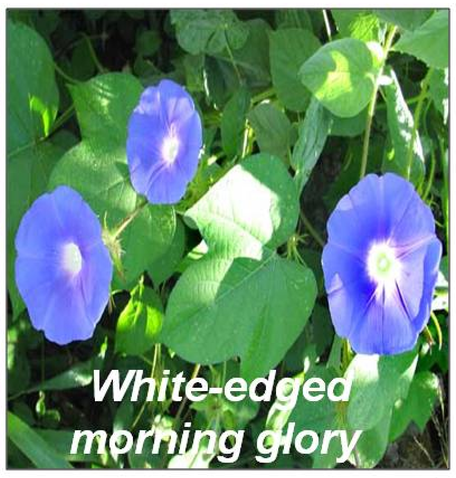
Sweetpotato and other (wild) *Ipomoea species.*


Coastal morning glory


Kang Kong


Hummingbird vine


Star Ipomoea Trompillo


White-edged morning glory

Management

Sweetpotato viral disease is difficult to control. Symptoms are often unclear, so that removal of infected plants cannot be done with confidence. Also, it is not practical to use an insecticide to kill vectors (aphids and whitefly) that spread the virus. Transfer the virus from vectors to plants occurs quickly; this means that by the time insecticide have kill the vectors, infection has already taken place.

Cultural Control

- Use healthy planting materials, '*Pathogen Tested* '(PT)
- Remove wild *Ipomoea* species
- Remove/burn old, harvested, vines
- Plant new crops away from older ones that might be the source of vectors and viruses.


PT planting material harvested