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Project

Enhanced profitability of selected vegetable value chains in the southern Philippines and Australia

Component 3 - Integrated strategies for the management of bacterial wilt and other wilting diseases in Solanaceous crops in the Southern Philippines and Australia

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2 Executive summary

Bacterial wilt, caused by strains from the *Ralstonia solanacearum* species complex, is the most important disease of potatoes in Mindanao, Philippines. The project has developed a better understanding of the pathogen *Ralstonia solanacearum* enabling formulation of sustainable management strategies for bacterial wilt disease of solanaceous crops in Philippines and Australia and also bacterial canker of tomato in Australia.

Bacterial wilt: An extensive survey has confirmed the presence of bacterial wilt (BW) in all the potato and vegetable growing areas of Northern and Southern Philippines. Molecular and morphological studies have identified two strains of BW from the Philippines and Australia. The tropical strain, Phylotype I (race 1, bv 3) was found to be the major cause of bacterial wilt disease in Mindanao and Australia. The cooler strain, Phylotype II (race 3, bv 2) has been isolated from northern Philippines and highland areas of Mindanao, Philippines and potato growing areas of the Atherton Tablelands, Australia and indicates introduction through planting material. The optimization of a sensitive and reliable method for detection of BW from field samples has made it possible to determine the source of infection, and to make pre planting decisions to avoid the risk of introduction and spread of the disease to non infected areas. The management of bacterial wilt disease through chemicals is ineffective. Therefore use of other strategies such as soil amendment with biofumigant and non biofumigant crops has been evaluated for suppression of BW populations in the soil. Wild sunflower, a readily available common weed in Mindanao was found to be effective in bacterial wilt suppression and resulted in higher yields. These effects are attributed to increased organic matter content and build up of microbial communities in the soil. Likewise the use of other strategies including combinations of clean seed, crop rotation, tolerant varieties, planting time and clean cultivation have been evaluated as practical and cost effective options for bacterial wilt management for both countries. Availability of bacterial wilt free planting material is the major constraint for commercial potato cultivation in Mindanao. Aeroponic technologies are being evaluated as a method of producing clean potato seed in sufficient quantities and at a cost effective prices.

Bacterial Canker (BC) is a devastating disease of both field and greenhouse tomatoes, caused by the bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm). Cmm* isolates have entered Australia on many occasions, most likely with imported seed. Research has shown, for the first time, resistance to Cu fungicides in *Cmm*. The finding has significant implications for tomato growers, both in Australia and the Philippines. In particular, growers will be able to judge risks of *Cu* resistance. The chemical industry will also be able to respond and improve efficacy of copper-based products. Sodium hypochlorite and Calcium hypochlorite were the most effective disinfectants, their effectiveness depending on contact time with green house surfaces and tools.

Research and Extension: A participatory action research approach was implemented to disseminate technologies for management of bacterial wilt disease of solanaceous crops and bacterial canker disease in tomatoes. The research trials were conducted in collaboration with potato farmers, researchers, agriculture extension officers and Landcare groups. Results from the trials were used in the formulation of management strategies. Extension material was developed in the common dialect to promote best practices for BW management in potato Technology demonstrations and exhibits were presented during NOMIARC annual field day which were attended by approximately 3000 farmers from Mindanao plus some farmers from Luzon and the Visayas.In Australia fact sheets were developed for management of both diseases and delivered to farmers and industry representatives during field days and workshops.

This project improved facilities and equipment at both the University of the Philippines Los Baños (UPLB) and NOMIARC. This enabled the institutions to carry out further research activities and provided opportunity and support to three UPLB students to complete their bachelor'sand master's research studies in Plant Pathology. The support was also

extended to project team members to attend and present their research at national, international conferences and symposia. It is recommended that further research be conducted on aeroponic production of potatoes and integrated potato management systems.

3 Background

Bacterial wilt, caused by strains from the *Ralstonia solanacearum* species complex, is the most important disease of potatoes in Mindanao, Philippines. The impact of this disease has many repercussions, ranging from economic, social, and environmental to humanitarian. *R. solanacearum* persists in soil for many years and are transmitted via infected seed pieces, contaminated cultivation equipment and drainage water from affected land. An undersupply of clean seed and inadequacies in grower knowledge lead to the proliferation of bacterial wilt. Potatoes are among the most valuable crops grown in Mindanao in terms of economic return. While sweet potatoes may attract 5 peso per kilogram at the farm gate, white potatoes (chiefly Granola and Atlantic varieties) can attract between 25 and 80 peso per kilogram, depending on the time of the year (potatoes are much sought after during Christmas festivities). Being so potentially profitable, potatoes are an attractive crop for farming communities. However, in recent years many farmers have moved away from potato production in favour of bananas or other vegetable crops, mainly because of the impacts of the soil-borne disease bacterial wilt. When infection levels are moderate, farmers have to harvest after only six weeks of growth (compared to the recommended twelve weeks); when severe, farmers cannot grow potatoes at all.

In recent years considerable progress has been achieved through the use of biofumigation. Previous work has demonstrated that the biomass of certain plants, when incorporated into the soil, can release compounds that are lethal to *R. solanacearum* populations. There is developing awareness of this technology in Mindanao as selected leading farmers demonstrate the practice to others within the farming community. This is done through open days and other events that allow farmers to observe and participate in new management practices, referred to as Participatory Action Learning Groups (PALGs). However, while the technology has been developed to a certain extent, further progress requires improved biofumigation technology, greater dissemination to the farmers, and access to clean seed. Currently, the only source of certified clean seed in Mindanao is the tissue-culture facility at Bukidnon, run by the Northern Mindanao Integrated Agricultural Research Centre (NOMIARC). This centre produces approximately 240,000 clean seed pieces per year, equating to sufficient material to plant some four hectares. Each minituber costs PhP2.5 for the farmer, who has to pre-order to ensure supply. With insufficient clean seed to supply to the farmers, and a cost beyond the measure of many more, the undersupply of clean seed contributes to the perpetuation of bacterial wilt in the soil. Unfortunately there is no bulk remultiplication infrastructure in place, so while there is some effort to ensure that the clean seed goes only to farmers who employ biofumigation, there is currently no mechanism to ensure that the resultant seed pieces are also free of the disease. Greater supply of certified clean seed is an essential component of any bacterial wilt mitigation project.

There is a general absence of information concerning the strains of *R. solanacearum* causing the most damage in Mindanao. All of the major races and biovars are claimed to be present in Mindanao, but as each has distinct epidemiologies, it is important to establish the identity of the strains involved in order to make the best informed management decisions. Conflicting results of disease severity and soil populations of *R. solanacearum* have been reported by staff at the NOMIARC facility, who conduct limited pre-sowing soil testing for *R. solanacearum* using Enzyme-linked Immunosorbent Assays (ELISA). While generally there is a positive correlation between pathogen numbers in the soil and disease onset and incidence, cases have been reported where very high pathogen loads have caused little damage, while sometimes relatively low *R. solanacearum* populations have resulted in complete crop loss. Whether this represents varying virulence between strains of *R. solanacearum* or if different factors are involved is not known, but to address bacterial wilt in Mindanao a detailed profile of the causal strains and a suitable soil testing procedure needed to be established.

Although some farmers have adopted biofumigation technologies to control bacterial wilt, better results will not be achieved unless there is broader community participation. Much of this comes down to grower education about the epidemiology of bacterial wilt. For biofumigation to work properly farmers necessarily have to forgo a potentially profitable potato crop, so only certain plots are treated at any given time. Poor access to clean seed then results in the farmer planting infected seed-pieces in the biofumigated plots. Meanwhile, neighbouring farmers may not have adopted bacterial wilt control measures and their lands have become an inoculum source through runoff of contaminated water. Reducing the background inoculum level is also critical to addressing the bacterial wilt problem in Mindanao.

Improved farmer education is necessary to increase biofumigation uptake, as well as prevent the re-establishment of the disease. While growers generally know the most severely affected plots, one instance was observed where the grower planted certified clean seed in heavily infected soil; the rationale being that perhaps the clean seed would produce better results then presumed-infected seed pieces. The resultant crop was to be planted in a biofumigated plot, and the next crop was to be used for distribution among neighbouring farmers as clean seed. However, as bacterial wilt was observed in the first plot, not only has the clean seed been wasted, but bacterial wilt will be perpetuated in the biofumigated plot and among the members of the rest of the community.

In Australia, particularly north Queensland, bacterial wilt continues to impact on the production of many valuable crops. It has been recorded on potato and several other solanaceous plants, and has had deleterious impacts on a broad range of other crops, including native eucalypt plantations, palms and banana accessions. Its effects on potato are not limited to the actual yield losses associated with the disease, but also by preventing the export of potato into non-wilt areas such as Western Australia.

Previously, bacterial wilt has been treated by soil fumigants such as methyl bromide, which have since been prohibited. While these treatments killed much of the *R. solanacearum* population, they also destroyed much of the beneficial rhizospheral bacterial communities, resulting in long-term poor wilt reaction. Increasingly, Australian farmers are looking for cheap, low environmental impact methods of controlling bacterial wilt. Given hot, wet conditions, bacterial wilt can cause major problems in Australian agriculture.

A scoping study for management of bacterial wilt of potato in Mindanao was conducted in 2007 and identified the following key findings:

1. Further development and refinement of soil amendment technology:

- broaden the range of potential bioactive organic amendments and their incorporation methods;
- determine the role of soil organics in relation to soil micro biota, and the *R. solanacearum*-suppressing nature thereof.

2. Expansion of existing certified seed program:

- provision of *R. solanacearum*-free seed;
- establish links to Australian clean seed scheme;
- investigate on-farm best-bet seed multiplication;
- determine efficacy of broader range of seed pieces.

3. Assessment of different potato varieties:

- establish linkages with the regional arm of CIP, SAPRAD
- test the 26 available varieties for susceptibility/resistance to bacterial wilt, agronomic traits and marketability;
- undertake field trials to evaluate performance.

4. Better understanding of *R. solanacearum* epidemiology:

- collate epidemiological data;
- determine the impact of different strains.

5. Improved extension operations for the uptake of best management practice:

- improve extension materials and farmer participation
- evaluate alternate planting/rotation times.

Bacterial canker of tomato is one of the most serious diseases of tomato crops both in the Philippines and Australia, and it is an important cause of crop losses worldwide. It is caused by strains of the actinomycete bacterium, *Clavibacter michiganensis*, which is a diverse bacterial assemblage encompassing strains causing economically important disease losses on a range of plants, strains which cause disease on only one plant host, and strains which are incapable of causing disease (non-pathogenic). Strains that cause disease on tomatoes are classified as *C. michiganensis* subspecies *michiganensis* (*Cmm*). There are several reasons why *Cmm* causes serious crop losses: it can be spread internally and externally with seed; it forms chemical and heat resistant endospores which can survive in soil and infected plant material for over four years; it can have a long latent period after infection when no disease symptoms are expressed that would alert farmers to a problem; it is easily spread from plant to plant by handling, infected tools, and with soil and water via roots; and (similar to bacterial wilt) there are no effective chemical control options and infections result in plants dying. *Cmm* has also been shown to spread during grafting operations with tomatoes. Grafting susceptible but desirable cultivars onto resistant rootstocks has proven to be a useful means of limiting other tomato diseases such as bacterial wilt or *Fusarium* wilt in many countries. However, spread of *Cmm* during this operation and a long latent period has resulted in losses several weeks or even months later. This has been particularly costly to greenhouse producers in Australia where tomatoes are expected to crop for over twelve months to justify the expense of grafting and high infrastructure costs. Similar experiences have been recorded overseas. Breeding for bacterial canker resistance in tomatoes has commenced but there are currently no useful cultivars available in Australia. Furthermore, the relative tolerance of existing cultivars is unknown and the *Cmm* strains found in Australia have not been characterised. Some preliminary studies of these last two points have commenced but a broader range of management strategies are required for sustained and robust disease control both in the Philippines and Australia.

4 Objectives

The overall aim of this component was to improve the profitability of Southern Philippines' growers by developing a range of robust integrated management strategies for the management of bacterial wilt in potatoes (as a model for Solanaceous crops) and bacterial canker in tomatoes. The specific objectives were:

1. To better understand *Ralstonia solanacearum* epidemiology
    - Identify the distribution of *R. solanacearum* strains in potato and vegetable growing areas causing bacterial wilt disease in Philippines and Australia, to develop sustainable BW disease management strategies
2. To evaluate different potato varieties and vegetative propagation types for bacterial wilt susceptibility
    - Determine the feasibility of using different potato varieties with greater disease tolerance to BW and yield potential.
3. To further develop and refine biofumigation technology
    - Evaluate easily available plant sources as soil amendments to current biofumigation practice and test methods of incorporation to improve the suppression of BW disease and cost effectiveness of the technology for its immediate uptake.
4. To establish on-farm best-bet clean seed production and maintenance
    - Identify farmers (and agri-business companies) to participate in intensive training and co-operate with researchers for the establishment of model clean seed production practices. With the aim that these farmers will co-operate with other growers in their communities to facilitate the dissemination of best practices for the production and maintenance of clean seed.
5. To develop improved management strategies for bacterial canker of tomatoes.
    - Develop molecular detection method to test latent infection from tomato seed.
    - Evaluate, copper fungicides, disinfectants, fungal and bacterial biological agents and plant defence activator to explore more options for sustainable management of *Cmm*.
6. To implement appropriate adoption and dissemination strategies for the integrated management of bacterial wilt and other wilting diseases in Solanaceous crops.
    - Develop good communication channel and involve discussions with different stakeholder groups, researchers and Landcare groups to conduct activities with potato growers.
    - Develop extension material, for adoption of the project recommendations made through field experiments and research in collaboration with farmers.

5 Methodology

Objective 1: To better understand *Ralstonia solanacearum* epidemiology

1.1. Determine what strains are present and if there are significant differences between them

An extensive survey of vegetable growing areas of northern and southern Philippines was conducted from 2008-2011. Bacterial wilt isolates were collected from various hosts, soil and irrigation water to identify the bacterial wilt strain involved in disease development. A total of 383 *R.solanacearum* isolates (Appendix Table 1) were collected; 177 isolates were from the soil, 137 from plants and 69 isolates were from tubers. The most number of isolates were sourced from Dalwangan, Malaybalay, Bukidnon, followed by Buguias, Benguet and Talakag, Bukidnon. The remainder of the isolates were evenly distributed from many across locations where white potato and solanaceous vegetables are grown.

Sample collection

Samples were taken from wilted plants observed in the field, soils ranging from high to low to no disease incidence, and symptomatic and asymptomatic tubers. The most frequent external symptoms observed on infected plants were wilting, yellowing of the foliage and, sometimes, bending of leaves downward (Figure 1). When checked internally, the most frequent symptom was progressive discoloration of vascular tissues. On tubers, discoloration and decay of tuber especially in the vascular ring area (Figure 1) and bacterial slime oozing from the infected area were observed. All samples were placed separately in plastic bags, sealed tightly and brought to the laboratory for isolation and confirmation.

Figure 1. Symptoms of *R. solanacearum* infected potato plant (Left). Partial wilting of plants (Right) discoloration of vascular rings in tubers.

Bacterial isolation and Characterization

For plants: Wilted plant stems were immersed in distilled water and bacterial ooze was observed. A loopful of suspension was streaked onto TZCA medium (10 g/L Peptone, 5 g/L Glucose, 1 g/L Casein hydrolase, 18 g/L agar with tetrazolium chloride) and incubated for 48-72 hours at 32 °C.

For tubers: A modified method (Figure 2) was employed to isolate the bacterium from both symptomatic and asymptomatic tubers. Tuber tissue sampled by punching the tuber with a sterile 1 ml pipette tip, was mixed with sterile distilled water (approximately 1 ml) and suspension was streaked onto TZCA medium.

Figure 2. Laboratory isolation of the bacterium from infected tubers (Left) by punching a hole onto the tuber using a sterile pipette tip and dispensing distilled water (Right).

For soil: Ten (10) grams of soil sample was added into 90 ml of distilled water and vigorously mixed for 20 minutes. Standard dilution test followed and a 100 ml of suspension from each dilution was spread onto TZCA medium [amended with crystal violet (5 ppm), polymixin (100 ppm), thyrothricin (20 ppm), chloromycitin (5 ppm) and cycloheximide (5 ppm)]. Typical colonies of the bacterium were individually placed in 1 ml of sterile distilled water in sterile 1.5 micro centrifuge tube (3 sets) and stored in room temperature for further verification and characterization.

Characterization of BW isolates

Isolates were characterized based on appearance in selective media, TZCA (Casamino acid1.0g,Peptone10.0g,Glucose 5.0g,Agar17.0g,5ml of 1% stock solution of 2,3,5,triphenyl tetrazolium chloride /L).A loopful of suspension was streaked onto the media and plates were incubated at 32°C. Growth was observed after 32 hours of incubation at 32°C.

Race differentiation was made on host range studies; the biovar was determined by biochemical tests (Hayward et al 1990) and the phylotype through molecular techniques (Fegan and Prior2005)) Variability in the aggressiveness of isolates was determined by assessing disease severity on susceptible tomato seedling (Yellow Plum and Money Maker). RAPD (Random Amplifies Polymorphic DNA) studies were conducting to determine genetic diversity among bacterial wilt isolates and to determine relationships between strains among hosts and locations of Mindanao region. Similar studies were conducted with Australian bacterial wilt strains (Appendix 1, Part A & B).

1.2. Estimate the diagnostic efficiency for soil and plant testing

The sensitivity and specificity of various detection methods were evaluated for rapid detection of BW pathogen. The protocol for reliable detection was optimised for detection of BW pathogen from soil, water and planting material. Potato tuber, soil and water samples were artificially inoculated with different inoculum densities and enriched in SMSA broth for 48 and 72 hrs (Lin et al.,2009) and then serial dilution was made and the suspension was plated (50 µl) on semi selective media (SMSA- crystal violet 1% - 0.5ml, Polymyxin B sulphate 1% -10.0ml, Bactericin 1%-2.5ml, chloromycetin 1% - 0.5ml, Penicillin 0.1% -0.5ml, cycloheximide1% - 2.5m l/L of TZCA media). Bacterial counts were made to quantify the inoculum level and colony confirmation was done through Mplex PCR (Fegan and Prior 2005).

At NOMIARC, standard NCM Enzyme-linked Immunosorbent Assay (ELISA) was used for the detection of BW in planting material (Janse, 1988).

Sample collection, preparation and detection of BW from test samples

Soil: Ten soil samples (100 g each) were collected from 1000 m^2 area in a zigzag manner from infected tomato field at 10-30 cm depth from the weed rhizosphere. Ten grams of soil from each sample was mixed with 90 ml of sterile phosphate buffer and shaken for 30 minutes at 200 rpm at room temperature. The samples were rested to settle for 8 seconds before making a serial dilution. 1 ml of soil suspension was enriched with 9 ml of SMSA broth and incubated for 48 and 72 at 30 ºC and shaking at 160 rpm. 50 µl of each dilution was plated onto solid SMSA medium and 50 µl of each pre-enrichment sample was boiled and standard Mplex PCR protocol was followed to determine the pathogen population.

Potato Tuber: Tubers were washed thoroughly and surface sterilized with 0.5% sodium hypochlorite for 3 minutes then rinsed with sterilized water and allowed to dry. Samples were taken from the vascular ring from the stolen end of the tubers and put into a plastic bag. 3 ml of sterile citrate buffer per gram of tuber tissue were added and tissues were macerated using rubber mallet. The material was allowed to rest for 15-20 minutes. 50 µl of extract was plated onto SMSA for direct detection and 1 ml of extract of each sample was enriched with 9 ml of SMSA broth and incubated for 48 and 72 hrs and followed the serial dilution and Mplex PCR protocol as mentioned above.

Irrigation water: Water samples (100 ml) were collected from a creek; from known field infected from irrigation water; standing water in a field and from a field's drainage exit. Samples were processed directly. Initially they were centrifuged prior to detection (25ml of each sample was centrifuged at 11,100 rpm for 5-10 min at 28 °C (Tomy MX centrifuge). The supernatant was discarded and pellets resuspended into 1000 µl of SDW. One ml was added to 9ml enrichment SMSA broth and incubated for 48 and 72 hrs followed by direct plating. Mplex PCR was used for detection as in soil and planting material.

Objective 2: To evaluate different potato varieties and vegetative propagation types for bacterial wilt susceptibility

2.1 Evaluate the potential of different potato varieties for bacterial wilt resistance

Varietal Screening:

In glass house conditions:

Eighteen commercial varieties and four lines of potato in Philippines and 10 commercial potato varieties in Australia were evaluated for BW resistance. At Philippines varieties were revived and multiplied through tissue culture from existing potato germplasm at NOMIARC. The tissue culture plantlets were transplanted in sterilized media and mass propagated through stem cuttings (Pots; sterile soil + coir dust) in greenhouse. The sterilized potted soil was artificially inoculated with 10 ml pure culture of BW isolate (10^8 cfu/ml). 25 plants of each variety were planted in inoculated pots in randomized block design with 5 replications The varieties were assessed for BW tolerance under artificially inoculated and in non-inoculated (natural field) conditions at three different potato growing area. Disease incidence and disease severity was assessed at weekly intervals.

In Australia seed of ten commercial potato varieties were collected from local seed distributor and a pot experiment was conducted in glasshouse under controlled environmental conditions (RH70%&temp 28 ± 2 ºC).Potato seed of each variety was grown in plastic bags in three replications and one month old plants were artificially inoculated with BW culture(10 9 cfu/ml).Two methods of artificial inoculation were used for varietal screening.1) Bacterial suspension was injected into axils of third/fourth fully opened leaves and 2)by adding bacterial suspension to the soil. Data on disease severity was recorded at weekly interval using a scale 1 to 5 where 1 indicated absence of symptoms and five a whole wilted/dead plant. Wilt intensity was calculated using the formula: $I = [\sum (ni \times vi) / (V \times N)] 100$; where I = wilt intensity (%); ni=number of plants with respective disease rating; vi= disease rating(1,2,3,4,5);V=the highest disease rating(5);and N=the number of plants observed.

In field conditions: An experiment was conducted at NOMIARC bacterial wilt nursery for evaluation of potato varieties for bacterial wilt tolerance. Ten foundation seeds (G0) of

each variety/line were planted in 1m x 4 m plot at a planting distance of 40 cm between hills and 1 meter between rows in randomized block design with three replications. Signs for BW and other leaf diseases were monitored weekly. The number of BW-infected plants per plot was recorded weekly until 11 weeks after planting. At harvest all tubers per plot were sorted, counted and weighed.

The top ten varieties, tolerant to BW were further tested under natural field conditions in the farmers' field across three potato growing areas of Kibangay, Miarayon and Imbayao, Bukidnon. Percent wilting, weight and number of healthy and unhealthy tubers were taken in all trials. Agronomic characteristics and eating quality were also noted.

2.2 Evaluate different seed-piece types to bacterial wilt reaction

This activity was officially deleted from the study (January, 2009).

Objective 3: Further develop and refine biofumigation technology

3.1 Evaluate alternate amendments for bacterial wilt suppression

Field experiment (NOMIARC):

Broccoli, radish and wild sunflower (*Tithonia diversifolia)* were tested as biofumigant/soil amendment crops, for bacterial wilt suppression. Field trials were conducted on the research station at NOMIARC. The test field at NOMIARC was uniformly inoculated with pure culture of BW pathogen (10^8 cfu / ml). After seven days of inoculation plant materials were shredded and incorporated into the soil at rate of 5 and 10 kg / m^2 at the time of land preparation. The field was uniformly watered and allowed to sit for fifteen days for tissue decomposition. The clean planting material; G0 seeds of potato cv. Granola were planted in 7 m x 4 m plots at distance of 40 cm between hills and 1.5 m between rows in randomized block design with 5 replications.

Method of incorporation was also evaluated by applying radish and wild sunflower amendments in furrows and into whole plot at the rate of 5 and 10 kg/ m^2. The soil samples were collected from each plot, before biofumigation, after 15 and 30 days of incorporation and at the time of potato harvesting. Bacterial colony count was assessed by soil dilution test. Data on disease incidence and severity was noted at weekly interval. Data on weight and number of healthy and unhealthy tubers were taken at harvest.

Field experiment (Farmer's field):

In addition to NOMIARC, effect of biofumigation / soil amendment on BW suppression was also assessed at farmer's field (under natural field conditions) at three different sites, Kibangay, Miarayon and Imbayao, (Bukidnon province).The methodology used for experimentation was the same as described above for NOMIARC field trial.

Pot experiment

Biofumigation, using brassica and wild sunflower, as one of the management practices against BW was studied. Standardized bacterial suspension of 10^8 cfu/ml was drenched individually to potted soils. A week after, finely- chopped leaves and stem of Wild sunflower, radish and tomato plants were separately incorporated to the soil at specified rates. Bacterial colony counts were assessed weekly by soil dilution tests for a one month period. Thirty days after incorporation of biofumigants, tomato (cv. Yellow plum) plants were transplanted to potted soils with corresponding treatment (Table 1). The experiment was laid-out in a Completely Randomized Design (CRD) with 8 treatments replicated four times in the greenhouse. Good moisture condition was maintained throughout the experiment (Figure 3). Data were analysed using Analysis of Variance (ANOVA) and means were compared by LSD.

Table 1. Experimental treatments and rate of application.

Treatment No.	Treatments	Rate
1	No biofumigant (control)	0
2	Wild sunflower	5 kg/sq.m
3	Wild sunflower	10 kg/sq.m
4	Radish	5 kg/sq.m
5	Radish	10 kg/sq.m
6	Wild sunflower, surface	5 kg/sq.m
7	Radish, surface	5 kg/sq.m
8	Tomato	5 kg/sq.m

Figure 3. Greenhouse biofumigation experiment: incorporation of chopped wild sunflower plants (A), transplanting of tomato seedlings (B) and lay-out of experiment (C).

Evaluate biocontrol agents; plant extracts chemicals and organic manures on growth of BW pathogen

In vitro inhibition studies. For testing different test agents, TZCA plates were seeded with biovar 3 isolate (CTA 13754) by evenly spreading 0.1ml of suspension (10^9 cfu/ml). Four 6mm filter paper disc were dipped into each test agent preparation and placed at four different sites in a single plate. Average diameter of four inhibition zone was measured after 72 hrs of incubation at 30 ºC.

Pot Trial

Plastic pots 20 cm in diameter were used to study the effect of organic manures and biocontrol agent on suppression of bacterial wilt disease. Sterilized soil was drenched with a bacterial suspension (10^9cfu/ml) of BW isolate at the rate of 200 ml / pot to ensure complete infestation. After two weeks of inoculation, each biocontrol agent was incorporated at the rate of 5% v/w (bacterial antagonist-10 5 cfu/ml and fungal antagonist-

10 5 spores/ml) and organic manure was added at the rate of 5 and 10% w/w per pot and mixed thoroughly. Tomato plants (cultivar Money Maker) were planted in each pot and data on disease severity was recorded at weekly interval. The experiment was laid-out in a Completely Randomized Design (CRD) with 8 treatments replicated four times in the greenhouse.

3.2 Development of best crop rotation scheme for bacterial wilt suppression

The commonly practiced crop rotation scheme, potato-corn-legume-brassica was evaluated for bacterial wilt incidence. The area utilized for the experiment was the same as that used for biofumigation studies. It was known to have a more or less evenly distribution of BW. Brassicas' were incorporated before planting potatoes. BW colony counts were made before and one month after biofumigation and at the potato harvest to determine the amount of inoculum in the area. Corn, cowpea and radish were planted in rotation after potato. BW colony counts were made before planting and at harvest of each crop in rotation to determine the degree of bacterial wilt suppression and/or increase in bacterial wilt population. Yield and cost of production of each crop in rotation were taken.

Objective 4: Establish on-farm best-bet clean seed production and maintenance

4.1 - 4.2 Identify farmers (and agribusiness companies) willing to cooperate to set aside clean seed beds) and apply best-bet biofumigation /soil amendments and plant potato trials

Three sites with the lowest bacterial wilt incidence were identified for on-farm best-bet clean seed production. These sites were Miarayon, Imbayao and Kibangay, Bukidnon. The best bet strategies; use of soil amendment with wild sunflower, broccoli and cabbage; crop rotation with non-solanaceous crops and use of clean seed and equipment. G0 seed from NOMIARC was distributed to the farmers and soil amendment in furrows with wild sunflower, broccoli and cabbage was made 15 days before planting a seed potato crop. Data on disease incidence, yield and economic analysis was made to assess the potential of integrated strategies for the management of bacterial wilt disease and in maintenance of clean seed potato production system in Philippines.

4.3 Clean seed potato production through aeroponic technique

The commercial clean seed potato production and distribution system at NOMIARC was evaluated and realized need to develop soilless methods for seed potato production. To ensure a sustainable system it needs to be able to rapidly produce seed of high quality that is available to the Mindanao potato industry at a cost effective price.

A scoping study was conducted to identify a method of soilless seed potato production (Hughes and Trevorrow, 2011). A low cost aeroponic system was developed at Mareeba, Australia and successfully tested the potential of aeroponic systems for multiplication of clean planting material. A low cost, solar operated aeroponic unit has also been developed at Mareeba as a model that Philippines farmers could copy to produce clean planting material. A commercial aeroponic facility has also been set up at NOMIARC and is being optimised to assist meeting the demand for clean planting material (Appendix 2 Figure 4, 5 & 6.)

Objective 5: Develop improved management strategies for bacterial canker of tomatoes

This study was developed to improve management strategies for bacterial canker of tomatoes. Sound and robust management requires an understanding of the causal pathogen. In the case of Bacterial canker there are a number of known genetic factors that may influence the reliability of detection methods for *Cmm*, and the susceptibility of different strains to chemical and biological controls.

Genetic characterisation of Australian C*mm* isolates

A collection of Australian *Cmm* isolates were assembled from crop surveys and diagnostic samples. DNA was purified from colonies grown on KB media using a Qiagen DNeasy® blood and tissue extraction kit (Qiagen Ltd) as per the manufacturer's instructions. DNA was quantified using a NanoDrop™ spectrophotometer (NanoDrop Technologies, Wilmington, USA). DNA concentrations were adjusted with TE buffer to give a final concentration of 12.5 ng/µL. Arbitrarily-primed PCR (rep-PCR) was undertaken using BOX-A1R and ERIC primers that amplify repetitive element regions of the bacterial genome (Louws *et al*., 1998; Kleitman *et al*., 2008). A reference *Cmm* isolate (#1983) was included on every gel. PCR products (8µL) were loaded on 1.5%agarose/TBE electrophoresis gels and run at 120 V for 4.5-6 hours. DNA profiles were visualised after staining with either ethidium bromide (0.5µg/mL) or Biorad Gel Red (1:20,000 in TBE). A base pair (bp) ladder was also included to estimate of the size of electrophoresis bands. Individual bands were digitised and profiles analysed using Bionumerics v6.6 software. A similarity matrix was calculated using the Dice similarity co-efficient of the composite data set, and the dendrogram created using UPGMA algorithm for cluster analysis.

BOX amplification was performed in 20 µL reaction volumes containing 2 µL of 10x reaction buffer (Invitrogen), 0.2 mM each dNTP, 6 mM $MgCl_2$, 2U Taq polymerase (Invitrogen), 2.4 µM BOX1A primer and 3 µL of DNA 12.5 ng/µL. Amplification was performed on an Eppendorf Mastercycler thermocycler with an initial denaturation (94°C, 5 min), followed by 40 cycles of denaturation (94°C, 60 sec), annealing (53°C, 30 sec), and extension (72°C, 60 sec), then a final extension (72°C, 10 min).

ERIC amplification was performed in 20 µL reaction volumes containing 2 µL of 10x reaction Buffer (Invitrogen), 0.2 mM each dNTP, 3 mM $MgCl_2$, 2U Taq polymerase (Invitrogen),1 µM ERIC1R and 1 µM ERIC21 primers and 3 µL of DNA 12.5 ng/µL. Amplification was performed on an Eppendorf Mastercycler thermocycler with an initial denaturation (94°C, 5 min), followed by 40 cycles of denaturation (94°C, 60 sec), annealing (52°C, 30 sec), and extension (72°C, 60 sec), then a final extension (72°C, 10 min).

5.1 Evaluate disinfectants for efficacy to bacterial canker of tomato

The relative sensitivity of *Cmm* isolates to copper was determined, along with the sensitivity of the isolates to a range of commercial disinfectants commonly used in the greenhouse industry. *Cu* tolerance assays were performed using a method adapted from those described for *Pseudomonas syringae* (Scheck *et al*., 1996; Andersen *et al.,* 1991; Tesoriero *et al*., 1997). Casitone yeast extract-glycerol agar (CYEG) was prepared and the pH adjusted with NaOH to compensate for the drop in pH that occurred on addition of copper sulphate, giving a final pH of 6.5-7. A 50 mg/mL (approximately 200 mM) stock solution of $CuSO_4.5H_2O$, prepared in sterilized distilled water and filter-sterilised, was added to the cooled agar prior to pouring. Four final concentrations copper-amended media were used: 0, 0.1, 0.5, 0.75 and 1 mM and were assessed for 33 *Cmm* isolates. *Pseudomonas syringae* pv. *tomato* (isolate 2161= PT23, provided by D. Cooksey, USA) was included as a positive control.

Bacterial suspensions were prepared in 0.85% NaCl containing 0.02% Tween 20, from 48 hr cultures on King medium B, to an OD590 of 0.22-0.28. Aliquots (10 µL) were spotted onto the copper-amended media. Growth on the plates was scored following 2 and 6 days incubation at 25 C The upper *Cu* tolerance was determined for each isolate from triplicate aliquots on separate media plates and from three independent experiments. A suspension test with membrane filtration (Figure 4) was used to assess the efficacy of commonly available disinfectants against *Cmm.*

Figure 4. Filtration system and vacuum pump

The series of experiments were conducted to test disinfectants commonly available in Australia (Calcium hypochlorite 1%, Sodium hypochlorite 1%, Peratec 1%, Sporekill 1%, Virkon 1%, and Ethanol 70%) for their efficacy in sanitising water and a range of greenhouse surfaces and tools like; grafting clips, dripper line, string, secateurs, knife, weed mats and penda film. Test material was cut into 2 cm lengths with the dripper line also cut in half lengthways. String was cut into 4 cm lengths, knotted at one end to keep together. Panda Film (black and white plastic sheets used in hydroponic channels), Weedmat and Black plastic were cut into 1.5 cm squares. The tools tested were stainless steel blade knives and metal secateurs. Surfaces were inoculated by placing in bacterial inoculum for 1 min. They were allowed to dry in the incubator at 25 °C or at room temperature for 1 min and then placed into disinfectant treatment for length of contact time which usually included a Quick Dip (QD 1-3 sec), a length of time in minutes or hours, or sprayed and wiped with disinfectant (S&W). In the first series of tests Part A, the surface was placed in a wash after treatment, (sterile water for 2 min), but this approach was later discarded.

Control samples were inoculated and treated in sterile water for the longest contact time used in the test. Materials were placed directly onto a KB plate and / or swabbed with sterile cotton swabs and streaked onto KB plates. Plates were incubated at 25-26 °C for 3–5 days and checked for presence of *Cmm* colonies.

5.2 Evaluate plant defence activators for controlling bacterial canker of tomato

Three experiments were conducted to assess different biological and chemical products for the control of Bacterial canker Trials were conducted in temperature-controlled greenhouses and treatments were set out in randomised complete blocks. At regular intervals during the trial period and at the conclusion of each trial plants were scored for disease using the 0-5 rating scale (Appendix 1 Part C).

A bacterial suspension was made by adding freshly grown *Cmm* cultures into 10 mL of sterile water or a commercial tomato nutrient solution. A (10x) serial dilution was carried out and 10^{-5} and 10^{-6} dilutions were spread plated on KB medium to estimate bacterial counts. Counts were between 10^{7} – 10^{9} cfu/mL.

Disinfectants were made to the concentration to be tested. An 8 mL aliquot of water (control) or disinfectant was used with the addition or absence of Bovine Serum Albumin (BSA). BSA was used to simulate disinfectant quenching effects from organic matter present in greenhouse waste-water or dam water.

An 8 mL aliquot of sterile water/nutrient (control) or disinfectant was added to a 15 mL centrifuge tube. A 1 mL aliquot of 0.3% BSA (+BSA) or sterile water (-BSA) was added. A

1 mL aliquot of a *Cmm* suspension was added and a timer started to measure a prescribed contact time. Tubes were mixed by inverting 5 times and were then allowed to stand for the remaining time. The contents of each tube were poured into a sterilised filtration unit (Figure 4) containing a 0.2 µm membrane and filtered under vacuum. The membrane was removed aseptically and washed in 10 mL of sterile water in a Petri dish and shaken for 2 min. The membranes were placed on a KB plate. A 0.1 mL aliquot of the wash was spread on a KB plate. The filtrate was also plated in many samples to check the filtration efficacy. Plates were incubated at 25 C for 3-5 days and were checked for presence of *Cmm* colonies.

5.3 Evaluate microbial biocontrol for efficacy to bacterial canker of tomato

The microbial biocontrol product salicylic acid derivative product, Bion® (acibenzolar-s-methyl) and other biological and chemical products in combination were tested for the control of Bacterial canker. Trials were conducted in temperature-controlled greenhouses and treatments were set out in randomised complete blocks. At regular intervals during the trial period and at the conclusion of each trial plants were scored for disease using a rating scale (Appendix 1 Part D & E). Data on plant health and plant mortality was recorded and statistically analysed to assess the products efficacy for the control of bacterial canker of tomato.

Objective 6: Implement appropriate adoption and dissemination strategies for the integrated management of bacterial wilt and other wilting diseases in Solanaceous crops

6.1 Identify barriers to adoption and appropriate extension and dissemination strategies

Participatory rural appraisal using focus group discussion was conducted with potato farmers of Miarayon, Kibangay and Imbayao to determine the constraints in potato production. Results were analysed and used as basis for the dissemination of integrated disease management strategies.

6.2 Development of strategies to disseminate integrated management of bacterial wilt and other extension materials and activities in accordance with the findings in Activity 5.1.

Extension materials and activities were developed to disseminate strategies for integrated management of bacterial wilt in potato and other Solanaceous crops. Exhibits and extension materials such as leaflets and graphic narratives were developed and distributed to farmers during meetings and field days. Posters and papers were prepared for publication and presentation at international and national conferences and meetings. Research problems were also given to undergraduate and graduate students.

Field experiments on best practice for bacterial wilt management using clean and BW-tolerant potato seed, biofumigation, crop hygiene and proper sanitation were designed.

6.3 Disseminate materials and implement activities in accordance with Activity 6.1

Extension materials, graphic narratives, flyers and leaflets were distributed to participants attending the annual NOMIARC field days. Posters covering best practice for bacterial wilt management were also exhibited and discussed, as were healthy and infected plants and the different potato varieties. Detection of symptoms of BW in potato was demonstrated to attendees.

Presentations on the field experiments being conducted at NOMIARC were also made to farmers visiting during the year or those attending the annual field days. Workshops on best practice in bacterial wilt management were conducted with farmers at the three trail sites before the field trials were established.

6 Achievements against activities and outputs/milestones

Objective 1: To better understand Ralstonia solanacearum epidemiology

no.	activity	outputs/ milestones	completion date	comments
1.1	Determine what strains are present and if there are significant differences between them (PC)	1.1.1 Collect strains from potato and alternate hosts in potato-growing areas 1.1.2 Conduct genetic analyses at NOMIARC facility	December, 2010	Bacterial wilt isolates were also collected from Northern Philippines to compare and to generate information for mapping the geographical distribution of BW strains in potato and vegetable growing areas of the Philippines. Out of 383 isolates, molecular characterization has been performed on selected isolates, which represented each sample area. BW strains were confirmed through molecular techniques (Mplex PCR) and results revealed the presence of tropical strain of Ralstonia solanacearum (Rs); Phylotype I Race 1, biovar 3 (74%) and cooler climate strain Phylotype II race 3 bv 2 (26%). Pathogenicity test was performed and significant differences in aggressiveness were found among isolates, collected from different geographical areas of the Philippines. RAPD analysis was made at UPLB and great genetic variability among bacterial wilt strains has been observed among isolates collected from different places. These studies were also performed in Queensland, to determine the genetic variability /similarities among different BW isolates collected from different host and vegetable growing areas/ locations of Queensland, Australia

1.2	Estimate diagnostic efficiency for soil and plant testing (PC)	1.2.1 Conduct dilution series assays of seeded and naturally infected soil and plant samples at NOMIARC facility	April, 2012	Bio PCR has been found to be reliable method for the detection of Phylotype I bv3 BW strain but failed to detect Phylotype II bv 2 strain of Philippines and Australian BW isolates This method showed the detection sensitivity of 10^2 - 10^3 cfu/ml from naturally infected and seeded planting material Dilution plating method and Mplex PCR has been found more reliable and equally sensitive method for detection of all strains of BW pathogen. Dilution series assays and NCM ELISA are being used for detection of BW from soil and potato tubers at NOMIARC. Diagnostic efficiency can be increased by developing PCR facilities at the centre. Though serological methods has been found equally sensitive, but are more tedious and time consuming and sometime give false positive results due to non-specific antisera and needs confirmation bioassays. These detection protocols have been discussed and shared with researchers both at NOMIARC and UPLB. .

PC = partner country, A = Australia

Objective 2: To evaluate different potato varieties and vegetative propagation types for bacterial wilt susceptible for bacterial susceptibility

no.	activity	outputs/ milestones	completion date	comments
2.1	Evaluate the potential of different potato varieties at NOMIARC Tissue Culture Facility (PC)	2.1.1 Source potato varieties from NOMIARC facility in Mindanao 2.1.2 Plant replicate trials in areas of high bacterial wilt incidence 2.1.3 Evaluate trials for bacterial wilt incidence and severity, as well as general agronomic properties.	December, 2009 December, 2011 December, 2011	18 potato varieties and 4 NOMIARC lines were successfully multiplied at NOMIARC tissue culture laboratory. All varieties were tested in artificially inoculated bacterial wilt soil in glasshouse and at NOMIARC in BW nursery under field conditions for BW tolerance and yield potential. Top 10 potato varieties and one NOMIARC line (Igorata, Asterix, Raja, Columbus, Granola, Kennebec, Astra, Atlantic, Catani, Franz, and NOMPOB) were selected on the basis of tolerance to bacterial wilt and further evaluated at 3 sites (Miarayon, Kibangay, Imbayo) for bacterial wilt susceptibility, yield, and for processing and table quality traits. Nicola and Charlotte showed comparative tolerance to bacterial wilt among ten tested commercial Australian potato varieties
2.2	Evaluate different seed-piece types to bacterial wilt reaction (A)	2.2.1 Source tissue culture plantlets, microtubers, minitubers, tubers and cut tubers 2.2.2 Conduct laboratory trials for differences in bacterial wilt reaction	July, 2009 December, 2009	The activity was officially deleted from the study and funds available for this activity have been utilized to conduct preliminary studies of aeroponic technology of seed potato production.

PC = partner country, A = Australia

Objective 3: To further develop and refine biofumigation technology

no.	activity	outputs/ milestones	completion date	comments
3.1	Evaluate alternate amendments for bacterial wilt suppression (A,PC)	3.1.1 Identify potential plant biomass sources and organic and inorganic amendments 3.1.2 Identify sites for field trials and collaborating farmers 3.1.3 Establish field trial amendments 3.1.4 Establish field trial on potatoes 3.1.5 Establish field trials.	May 2009 May, 2012	Broccoli, radish and wild sunflower *(Tithonia diversifolia*) were evaluated as alternate soil amendment for the suppression of bacterial wilt. Being a soil pathogen and its random distribution in the field; soil amendment of whole field in comparison to furrows has been proved to provide better disease management. Wild sunflower is being adopted by the farmers due to its easy access, as it grows abundantly along the road and farm boundaries in Mindanao area. It is rich in N, P and K and contains a chemical substance sesquiterpene-lactone 1 which possibly effects the nematode population and suppressed bacterial wilt disease. The Australian strain of wild sunflower has been found to host BW and therefore collection of wild sunflower for soil amendment should be made from bacterial wilt free areas.
3.2	Evaluate the effect of different crop rotation duration and sowing times (A, PC)	3.2.1 Establish replicated trial of different alternate crop sowing and fallowing times (A,PC) 3.2.2 Evaluate trials	May 2012	Non-solanaceous crops such as corn, legumes and cucurbits are not known as alternate crops of bacterial wilt and thus growing these crops in rotation after solanaceous crops ideally reduces the bacterial wilt population in the soil. Brassica-Potato-Corn-Legume has been evaluated as economic and best crop rotation at NOMIARC, for reducing the bacterial wilt pathogen population from infected areas. Potato crop is mainly grown through out the year in Southern Mindanao i.e. August-November, October-December and Jan-April but October-December plantings are evaluated as best planting time (being the driest months of the year) to escape /reduce the BW incidence disease and allowed Mindanao farmers to obtain premium prices for their off season potato crop.

PC = partner country, A = Australia

Objective 4: To establish on-farm best-bet clean seed production and maintenance

no.	activity	outputs/ milestones	completion date	comments
4.1	Identify farmers (and agribusiness companies) willing to co-operate to set aside clean seed beds (PC)	4.1.1 Three farmers identified from Bukidnon	July,2010	Farmers from Kibangay, Miarayon and Imbayao and were trained for commercial seed production Clean seeds G0 from NOMIARC were provided to selected farmers to produce clean potato seed. The sites where no solanaceous crops were planted were identified and soil was amended in furrows with broccoli and wild sunflower. The bacterial wilt incidence was evaluated at each site.
4.2	Apply best –bet biofumigation /soil amendments and plant potato trials(PC)	4.2.1 Purchase clean seed from NOMIARC facility and sow 4.2.2 Evaluate crop for bacterial wilt incidence 4.2.3 Harvest crop and evaluate for bacterial wilt incidence 4.2.4 Reapply best-bet biofumigation/soil amendment 4.2.5 Sow new crop from best seed pieces 4.2.6 Evaluate crop for bacterial wilt incidence	July 2009	The Imbayao site was dropped due to high bacterial wilt incidence. Wild sunflower and broccoli were incorporated again into the areas before planting the G1 seeds. Bacterial wilt incidence was monitored in each site. Seeds G2 from the harvest were set aside for distribution to other farmers in the area. The seeds were tested for bacterial wilt and have not been found totally free from bacterial wilt. The aim to establish clean seed production under field conditions has been found to be risky due to the chance of spreading disease through latently infected planting material. The message of Integrated management strategies for the control of BW disease and sustainable production of solanaceous crops has been successfully spread among Mindanao farmers, researchers, students, agriculture workers, politicians through various extension activities. A number of field days have been conducted at different locations of regions and used the common dialect to deliver better understanding and knowledge among farmers to follow best bet technology for management of BW disease.

4.3	Clean potato seed production through aeroponic technique	3.1 Identify enthusiastic and resourceful farmer/s in cooler area of Mindanao 4.3.2 Establish aeroponic system 4.3.3 Purchase clean planting material (stem cuttings/minituber s from Australia 4.3.4 Standardize growing techniques for sustainable production 4.3.5 Monitor and guide to establish successful potato seed industry	December, 2012	Discussions on clean seed production through aeroponic technology have been made with Philippine university researchers, NOMIARC, government officials, land care workers, seed potato farmers and seed industry people. They showed great interest and support to develop this potential project. The scoping study for multiplication of clean planting through aeroponics has been completed and report submitted. Contact with Hanoi and Korean scientists has been made and practical viability of aeroponic systems has been assessed. Preliminary studies at Mareeba, Australia have tested the potential of aeroponic system for multiplication of clean planting material. The commercial facility for aeroponic seed potato production has been established at NOMIARC and studies are being initiated to standardise and refine the technology in both countries.

PC = partner country, A = Australia

Objective 5: To develop improved management strategies for bacterial canker of tomatoes

no.	activity	outputs/ milestones	completion date	comments
5.1	Evaluate disinfectants for efficacy to *Cmm*	5.1.1 Factsheet on disinfection guidelines	June 2012	A range of copper formulations are the only registered chemical control available for bacterial canker in Australia The relative sensitivity of *Cmm* isolates to copper was determined, along with the sensitivity of the isolates to a range of commercial disinfectants commonly used in the greenhouse industry. A wide range of Cu tolerance was found and several isolates from NSW, SA and WA were found strongly resistant and capable of growth on 0.75 mM Cu amended media. This has some implications to growers who are using IDM. Better control of Cu resistant bacteria can be achieved by tank mixing Cu with a dithiocarbamate fungicide but can affect the beneficial insects and mites. Better control may also be achieved by mixing Cu with iron chloride. Several other disinfectants; Calcium hypochlorite, Sodium hypochlorite, Peratec, Sporekill, Virkon, hydrogen peroxide, ethanol, and Menno Florades were evaluated to disinfect *Cmm* from water, dripper line, channel, grafting clips, string, greenhouse surfaces and tools; black plastic, penda film, weed mat, secateurs and knife. The efficacy of disinfectants was found to be strongly influenced by their concentration, time and time of exposure. Removal of dirt and organic matter from the dirty surface prior to disinfection improved the efficacy of disinfectants A disinfectant factsheet has been developed and guidelines were provided to greenhouse growers during various workshops and field days.
5.2	Evaluate plant defence activators for controlling bacterial canker of tomato	5.2.1 Completed chemical efficacy trials	June 2011	An experiment on testing efficacy of two plant defence activator chemicals for tomato canker disease has been completed. Unfortunately none of the treatments resulted in disease control at economic levels.
5.3	Evaluate microbial biocontrols for efficacy to bacterial canker of tomatoes	5.3.1 Completed biocontrol efficacy trials	December 2012	The biocontrol agents; Microplus, Mycormax and Phosscare were tested for the management of bacterial canker disease but none of them showed promising results.

PC = partner country, A = Australia

Objective 6: To implement appropriate adoption and dissemination strategies for the integrated management of bacterial wilt and other wilting diseases in Solanaceous crops

no.	activity	outputs/ milestones	completion date	comments
6.1	Identify barriers to adoption and appropriate extension and dissemination strategies	6.2.1 Finalize templates of graphic narratives, leaflets, and posters for bacterial wilt management	July 2010	Focused group discussion (FGD) with potato farmers of Imbayao, Kibangay and Miarayon were conducted by NOMIARC and constraints to commercial potato production were identified as follows: 1. Planting potato is dependent on the availability of seed rather than the growing season. 2. Productivity is dependent on seed quality; degenerated seed usually has low productivity due to increasing incidence of BW. 3. Traders dictate the price and varieties to grow. Varieties with long shelf life, yellow flesh and firm skin are preferred. 4. Trucking and hauling potatoes from farm to market contributed to high cost of production thus reducing farmer's profit. 5. Two production constraints identified by farmers were insufficient supply of clean seed potato and the high incidence of bacterial wilt.
6. 2	Development of strategies to disseminate integrated management of bacterial wilt and other wilting diseases in Solanaceous crops	6.2.2 Plan activities and prepare materials to disseminate integrated management for bacterial wilt	April 2011	Farmers suggested solutions to the problems such as: training on seed production and control of BW and the establishment of trading posts. Based on the findings of the FGD, potato production can be sustainable by: 1. sufficient supply of clean seed 2. presence of seed investors 3. availability of technology on BW management.:

6.3	Disseminate materials and implement activities in accordance with Activity 6.1.	6.3.1 Extension materials distributed, and activities implemented at NOMIARC and at farmers field 6.3.2 Feed back gathered	April 2012	Appropriate extension materials like graphic narratives, flyers, leaflets, posters (in English and local dialect) and PowerPoint presentations were developed to inform farmers on BW management. Various hands-on activities were done during field days and farmers' meeting in their respective areas. Simple disease diagnosis for bacterial wilt was demonstrated to farmers. Best practices such as use of clean and BW-tolerant seed, crop hygiene and field sanitation, and biofumigation were discussed and demonstrated. Extension material ;flyers, leaflets, handouts, graphic narratives and posters were developed and distributed to farmers during the NOMIARC field days which are attended by as many as 3000 farmers/year. Exhibits on how to diagnose bacteria wilt in potato and the different varieties were demonstrated during the field days. Techno demo plots were also conducted at NOMIARC and in farmer's fields to showcase the best practices for bacterial wilt management. Lectures and PowerPoint presentations were made during several workshops, farmer meetings at Bukidnon, South Cotabato and Davao del Sur and Atherton Tablelands (QLD) .Identification and management strategies for bacterial canker of tomato were discussed with greenhouse growers at Sydney (NSW), Adelaide (SA) and Wanneroo (WA) in Australia

PC = partner country, A = Australia

7 Key results and discussion

1.1 Better understanding of *Ralstonia solanacearum epidemiology*

Collection, distribution and analysis of BW isolates

Philippines:

383 isolates of *Ralstonia solanacearum* were sourced from Dalwangan, Malaybalay, Bukidnon, Buguias, Benguet and Talakag, Bukidnon (Figure 5) to determine the current distribution of BW strains and cause of bacterial wilt in potato and other solanaceous crops (Appendix 2.Table A1)

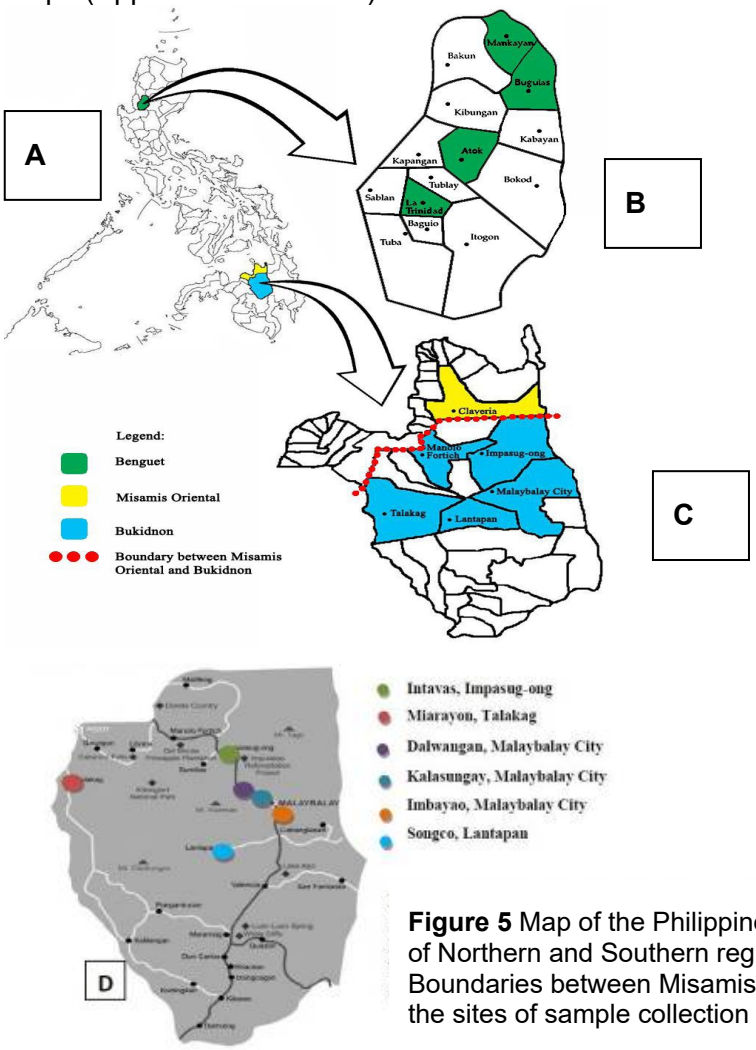


Figure 5 Map of the Philippines showing geographical locations of Northern and Southern regions (A), Benguet region (B), Boundaries between Misamis Oriental and Bukidnon (C), and the sites of sample collection in Bukidnon (D)

Figure: 5 Distribution of BW disease in Northern and southern Philippine

Characterization of BW strains

Phenotypic characterization

BW isolates were characterized based on its appearance in selective media (TZCA). Growth was visible as early as 36 hours of incubation and appeared as white to creamy-white colonies with pinkish to reddish centre that were fluidal or mucoidal and opaque (Figure 6).These characteristics usually indicate that the bacterium is virulent. Mucoid substances are produced by the accumulation of an exopolysaccharide (EPS) that causes colonies to exhibit irregularity of their surfaces (Smith, 1920). Characteristic whorls in the centre of the colonies were often observed from the different isolates.

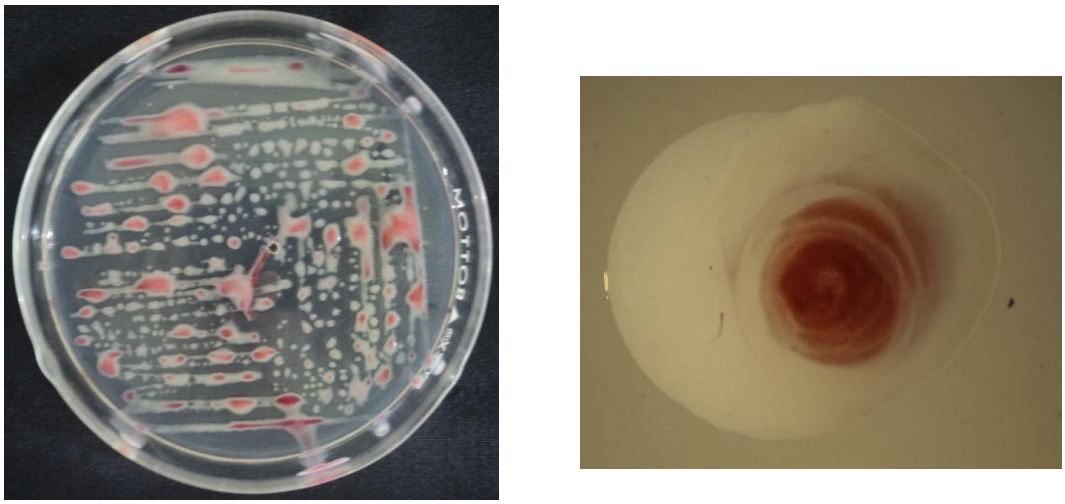


Figure 6. Pure Culture of *R. solanacearum o*n TZCA after 48 hours of incubation at 32 ºC (Right) Typical *R. solanacearum* colony with pink colour and whorls in the centre (Left).

The pathogenicity test with representative isolates collected from host and soil across the regions showed difference in aggressiveness among Bw isolates. Isolates collected from Bukidnon area showed wilting symptoms as early as 3 days after inoculation where as isolates collected from soil from Benquet area produced wilting symptoms at 4 DAI. The soil isolates were seen to be more aggressive on tomato plants than isolates collected from potato plant and tubers. Isolates from potato plants and tubers only induced yellowing of leaves on tomato plants rather than wilting symptoms. This difference in symptomatology has been attributed to difference in BW strain (bv 2) which is host specific and causes more losses in cool conditions so while it is able to infect potato crops it is able to induce yellowing in tomato plants (Appendix 2 Table A2).

Molecular Characterization of BW strains
Phylotyping

Isolates were further subjected to Multiplex PCR (Fegan and Prior, 2005) to verify the bacterial wilt strains. Isolates generated the expected 281bp fragment defined by 759/760 primer pair. A second fragment was used to detect phylotype, 144 bp for Phylotype I and 372 for Phylotype II (Table 1). Phylotype I include strains belonging to biovars 3, 4, and 5 and strains that are isolated primarily from Asia (Fegan and Prior, 2005). However, results of the phylotyping detected the presence of Phylotype II which is primarily isolated from America (Figure 7).

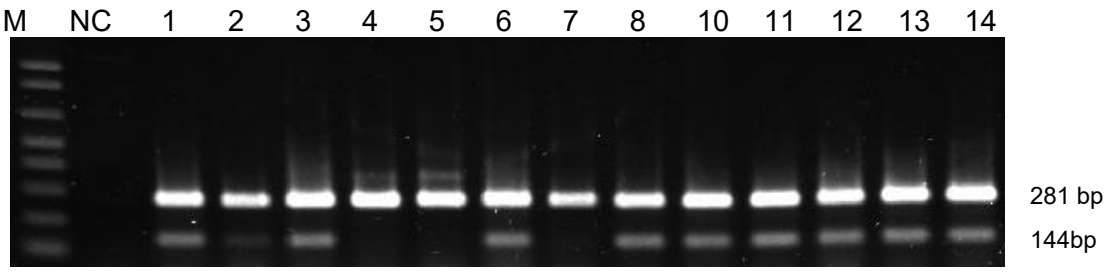


Figure 7. Agarose gel electrophoresis of PCR products from assay using primer pair 759/760 and Nmult primer mix. Lane M, Molecular Marker 1 kb plus ladder (Invitrogen, USA), Lane NC, negative check (water), lane 1-14, *R. solanacearum* isolates. The 372 bp fragment indicates Phylotype II and 144 bp fragment indicates Phylotype I. The 281 bp fragment is universal to *R. solanacearum*.

Biochemical analysis

Following the Phylotype-biovar detection, the isolates were classified into Biovar 2 (Phylotype II) and Biovar 3 (Phylotype I). To check these results, representative isolates were used for biovars classification using sugars and alcohols (Appendix 2 Table A 3). All biovar 2 isolates were found negative to trehlose, mannitol, sorbitol and dulcitol where as all biovar 3 isolates utilized sugars and alcohols showed the positive reaction. Change of basal medium colour to yellow indicates positive reaction. Few isolates took longer time to utilize sugar and alcohols. It was observed that most of the test isolates followed the rule that Phylotype I are Biovars 3 and Phylotype II are Biovars 2 isolates. Variations in some isolates observed can be attributed to contamination and other environmental factors. The results revealed that 190 isolates belong to Phylotype I (race 1, Bv 3) which is almost 50% of the total, and the remaining 193 isolates belonging to Phylotype II (Bv 2). Most of the Phylotype II isolates were observed in the northern region (Benguet) where the elevation is slightly higher than the southern region (Bukidnon). However, Phylotype II isolates were also collected from Bukidnon, where elevation is high and temperature is low as exemplified in Miarayaon, Talakag, and Bukidnon. Most of the Phylotype I isolates in the northern region were isolated from soil.

Genetic variability Analysis

RAPD-PCR analysis revealed 6 distinct groupings/clusters of *R. solanacearum* isolates based on binary scoring of reproducible bands with sizes that ranged from 340 to 1200 bp (Figure 8).

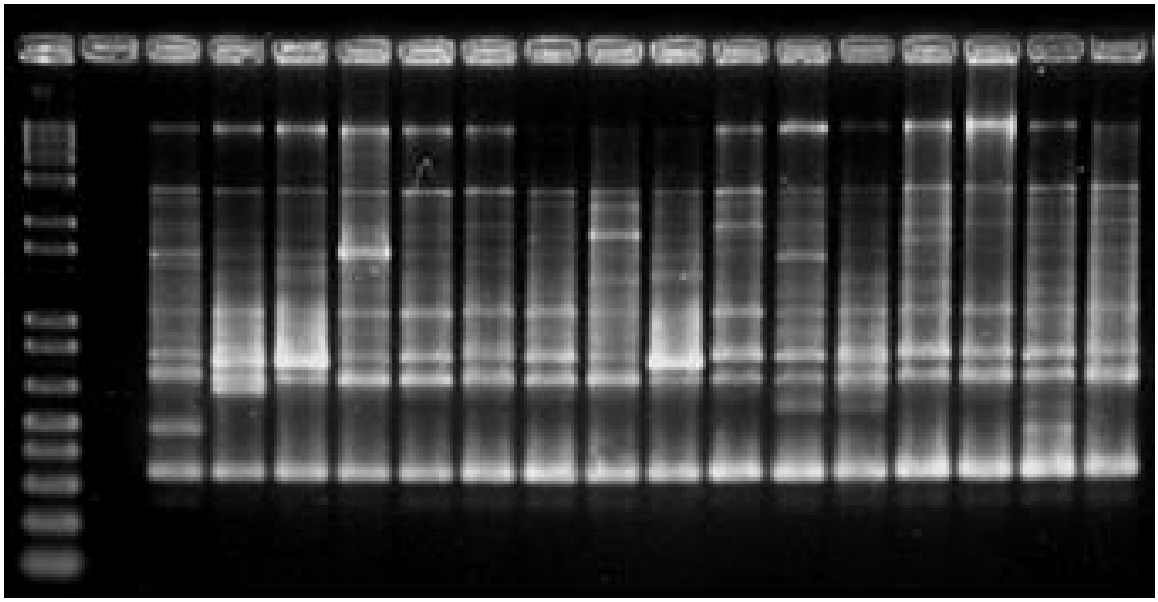


Figure 8. Banding pattern of *R. solanacearum* isolates generated using RAPD primer OPA-07 resolved in 1.5% Aragose gel.

The R2 and R6 were the largest clusters (Appendix 2; Figure 1&2) with 30 isolates each. R1 had 28 isolates that were more aggressive and were mostly isolated from elevated regions (Buguias and Talakag) and are Phylotype II with the exception of some isolates (S414-5, A49, WP233, 178T-1 and S90-2). Most isolates were also from plant tissues with few isolates from tubers and soil. S90-2 from Casisang, Malaybalay, Bukidnon, isolated from soil is grouped in this cluster; however it was further separated into another unique sub-cluster. The second cluster, R2, had isolates that were mostly Phylotype I except isolates 54T-2 and WP150. Most isolates also were collected from Bukidnon with exceptions of isolates from ampalaya and an isolate from Benguet (54T-2). Isolates belonging to this cluster were moderately aggressive (3-6 DAI). There were no trends

observed in cluster R3, R4 and R5 since isolates were distributed in these clusters regardless of source and aggressiveness. However, cluster R6 was very distinct since it included isolates from Bukidnon that were moderately aggressive and were all isolated from the soil. These isolates were from Dalwangan, Imbayao, Intavas, Talakag and one (1) isolate from Kalasungay. All isolates were found to be Phylotype I.

BOX genomic fingerprinting and clustering

A total of 25 BOX clusters (Figure 9) were defined from the *R. solanacearum* isolates by amplification using the BOX primer. Out of these 25, five (5) were large clusters. Fragments amplified from both Benguet and Mindanao isolates using BOX primers were observed to be mostly small sized fragments, with size ranging from 200 bp to 2000 bp. The 350 and 600-bp fragments were common to many isolates. The largest cluster is B1 consists of isolates all from Benguet, isolated from white potato plants and ascertained to Phylotype II. These isolates were also aggressive on tomato cv. Yellow plum and induced infection 2-3 DAI. The second large group is B5. In this cluster, isolates are Phylotype II, collected from Benguet and are isolated from soil. They are also aggressive (2-3 DAI) to moderately aggressive (4-9 DAI) isolates. B13 is also a large cluster comprising of Phylotype I isolates from Bukidnon and were isolated mainly from the soil planted with white potato and tomato.

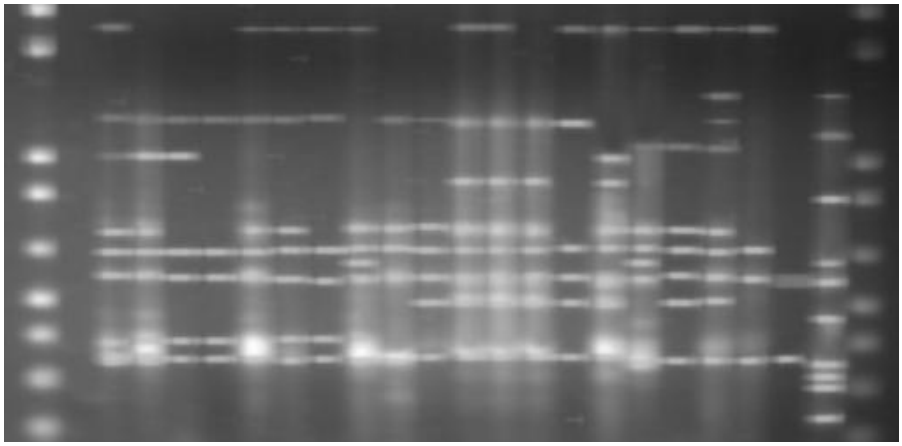


Figure 9 BOX haplotypes of isolates of *Ralstonia solanacearum* from Bukidnon obtained after DNA amplification using BOX primer.

The B13 cluster was further divided into numerous sub clusters where one of the sub clusters came from soil from Songco, Lantapan planted with tomato. Another large cluster is B17 that include isolates from white potato that were collected in Bukidnon. These isolates were aggressive to moderately aggressive. Another unique cluster is B21, consists of Phylotype I isolates from soil planted with tomato and pepper. Of the two primers (RAPD and BOX) used for DNA fingerprinting of the isolates, the BOX primer had a higher discriminating capacity than the RAPD primer. More clusters/groups were defined by the BOX primer. Cruz et al (2002) explained that BOX had a higher discriminating capacity because it showed an increase in the number of polymorphic bands (Appendix 2; Figure 3).

Absence of cytochrome c1 signal peptide sequence in some *R. solanacearum* Philippine isolates

Preliminary tests performed using the *R. solanacearum* specific primer developed by Kang *et al.* (2007), RALSF/RASR, designed using the cytrochrome c1 signal peptide

sequences revealed that it could not amplify the expected product size of 932 bp in some *R. solanacearum* Philippine isolates. Counterchecking was done by adding primer pair 759/760 with a smaller product size of 281 bp in the master mix. Multiplex PCR analysis revealed that RALSF/RALSR is less sensitive in detecting some *R. solanacearum*. Moreover, the expected product size of 932 base pairs was only observed in test isolates belonging to Phylotype I. This result holds true both for Benguet and Bukidnon isolates (Figure10). Because of this inconsistency, primer pair 759/760 was used alongside Nmult primer mix for PCR detection and phylotyping of *R. solanacearum.*

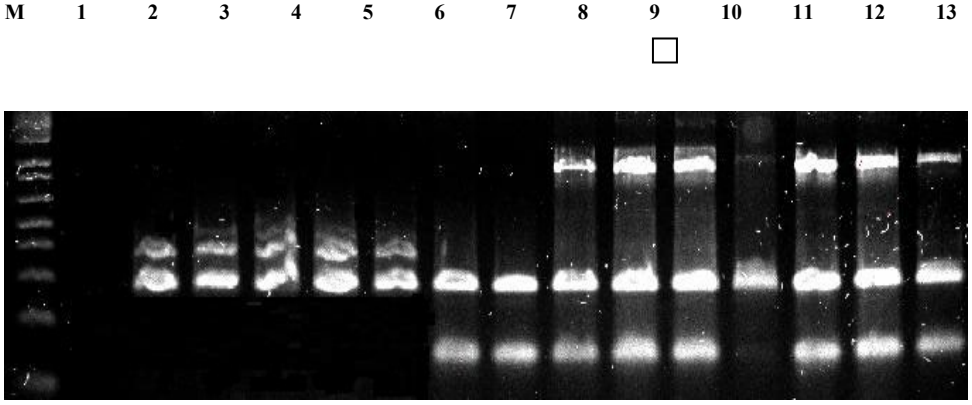


Figure: 10 Amplified PCR product using primers RALSF/RALSR resolved in 1.2% Agarose gel stained in GelRed. Lane 1, molecular weight marker (1 Kb plus ladder), lane 1, PCR negative check (water), lanes 1 to 6, *R. solanacearum* Phylotype II without RALSF/RALSR amplification. Lane 7-8, *R. solanacearum* from other crops, lanes 9-11 and 13-15, Phylotype I with RALSF/RALSR amplification. Box highlighted in red emphasize the absence of 932 bp of select *R. solanacearum* isolates.

PCR Detection of BW in symptomatic and asymptomatic potato tubers and their variations in phylotypes. Apparently healthy or symptomless potato tubers were collected and used in the isolation of R. solanacearum. A total of 68 *R. solanacearum* isolates were confirmed using colony morphology and PCR detection using primer pair 759/760. Phylotype analysis of the different isolates showed the existence of Phylotypes I and II. Variation in phylotypes was associated with geographical origin of the isolates. Phylotype I was mainly observed among the isolates collected Bukidnon while Phylotype II was observed among the isolates collected from highlands in Benguet and Bukidnon. It was interestingly noted that apparently healthy tubers harbour *R. solanacearum*.
The results indicating the presence of two phylotypes of R. solanacearum infecting white potato in the Philippines are very interesting. It was observed that most isolates from Mindanao belong to Phylotype I. According to Cellier and Prior (2010), this phylotype encompassed a majority of lowland or tropical strains with wide host range. Phylotype II, where all the isolates from Benguet were classified, encompassed highland and cold tolerant potato brown rot strains. This explains why the variation in phylotypes with regards to the geographic origin of the isolates is very distinct. Potato growing areas in Benguet were in higher elevation and the temperature in the area is relatively lower compared to some areas in Bukidnon. Potato farmers in Bukidnon,
tend to move to other area with higher elevation whenever they observe high bacterial wilt incidence in their current areas, leading them to Mt. Kitanglad

Australia:
Phylotyping of BW isolates

84 isolates of *Ralstonia solanacearum* collected over a period of time (1988-2011) from various hosts and locations of Queensland were subjected to molecular characterization (Multiplex PCR, Fegan and Prior, 2005) for phylotype genetic analysis. Results showed the presence of 91% of biovar 3 strain of bacterial wilt from most of the region of the state,

biovar 2 strain was found to be present in Atherton tablelands, potato growing areas and showed the possibility of introduction through latently infected potato seed tubers (Figure 11).

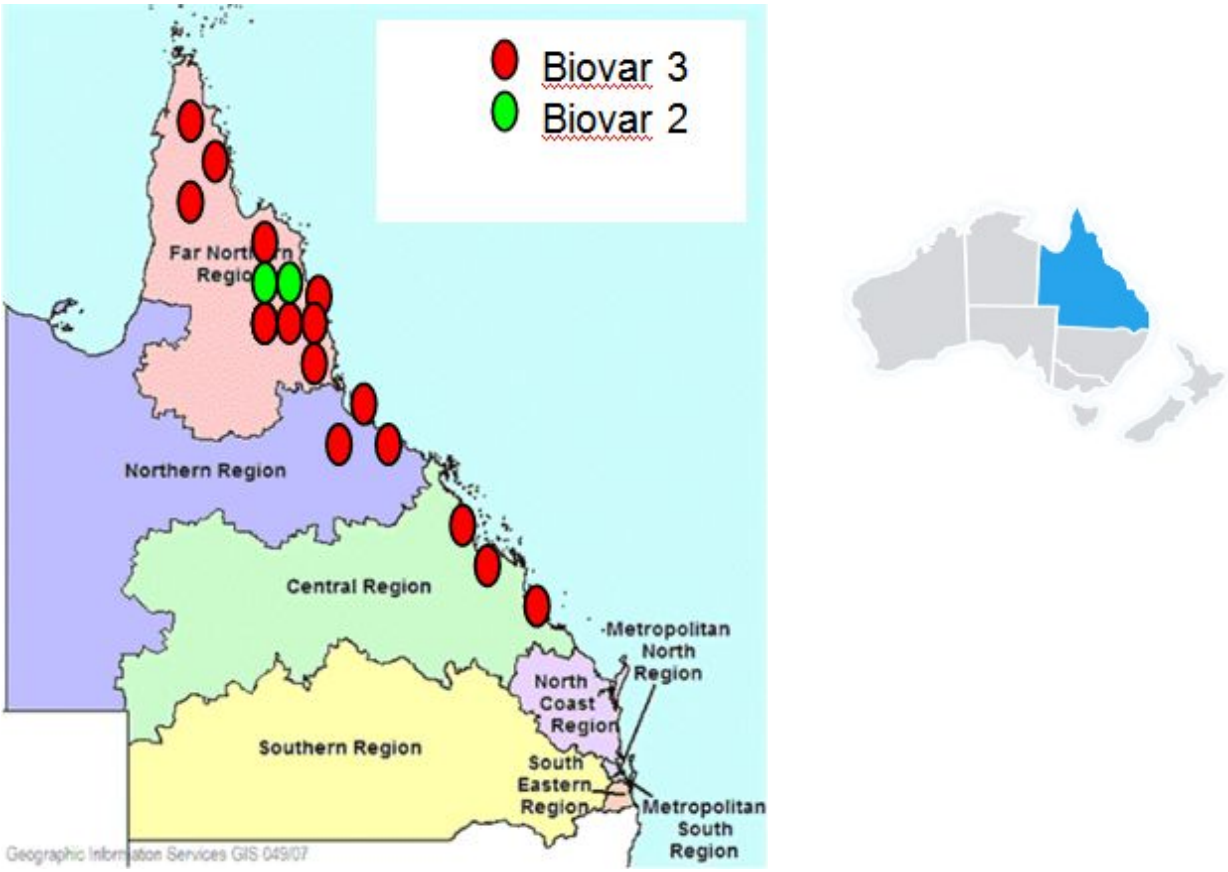


Figure11. Geographical distribution of Bacterial wilt strains in Queensland, Australia

Genetic Variability:

84 BW isolates were characterized by Random amplified polymorphic DNA analysis, to determine the genetic variation / similarity among *Ralstonia solanacearum* population with respect to host, aggressiveness and geographical areas. Three 10 mer primers OPA -7, OPF-8 and OPE-19 were used in study to generate the genetic profile of *R. solanacearum* isolates. Three primers gave a total of 31 band positions ranging from 300-2200 bp. with 11, 10 and 10 band position for OPF-8, OPA-7 and OPE-19 respectively. Among 84 isolates five distinct groups were formed on the basis of band based analysis. More genetic variability was found among biovar 3 isolates .The grouping of strains did not appear related to aggressiveness, but all the three primers individually as well as in combined analysis grouped biovar 3 strains and biovar 2 strains separately. Interestingly, isolates from different hosts from the same geographical origin clustered into same group except one group (group IV) in which isolates from different hosts from different geographical origin grouped together, which may be due to exchange of planting material from one place to another over a period of time (Appendix 2;Table A4). The study showed that environmental conditions (temperature, moisture and soil type) have great impact on the occurrence of strain diversity and development of bacterial wilt disease. Similar relationship between biovar and phylogenetic grouping have also been observed by other researchers (Horita and Tsuchiya, 2001; Poussier et al, 2000) and certain genetic clusters revealed close relationship between strain with high level of host specificity and strains were mostly clustered by their geographical origin. In this study number of isolates

were from tomato plant and from near by locations and needs large number of collection from other hosts to find genetic correlation between geographical areas and host population (Appendix 2; Figure A 3.)

The studies confirmed the presence of two BW strains; Phylotype I (biovar 3) and Phylotype II (biovar 2) from vegetable and potato growing areas of Philippines and Australia. Both BW strains have been found from high and low land areas and showed the introduction of disease through latently infected planting material.
Crop losses due to biovar 3 strain have also been observed in other solanaceous crops (Tomato, capsicum and egg plants) in Mindanao. The losses ranged between 30-60% and exceed to complete crop failure under warm and humid weather conditions. Similar crop losses were observed at Australia in solanaceous crops during 2009-2010 due to prolonged wet season.
The information derived through this study on distribution of BW strains in Mindanao and in Australia will be helpful in selection of suitable management strategies (region specific).The selection of management strategies, like avoiding the movement of planting material from infected area, isolating infested areas, removal of volunteer plants/weeds, tool decontamination, fallowing/crop rotation with non solanaceous crops ,soil amendments and regulation of quarantine measures will reduce the BW pathogen population in soil and will restrict further spread of disease to non infested areas.

1.2. Estimate the diagnostic efficiency for soil and plant testing

A simple and sensitive method is a prerequisite for conducting ecological studies and in devising suitable management strategies. The numbers of methods (Dilution plating, ELISA, PCR) developed by various workers were evaluated to test the sensitivity and specificity of the BW pathogen from seeded and naturally infested soil, planting material and from creek water (Pradhanang et al 2000; Fegan and Prior,2005; Lin et al 2009 Opina et al 1997 and Kang et al 2007).

Among tested protocols; Bio PCR has been found the effective method to detect Phylotype I bv 3 strain of BW pathogen from diverse field samples The advantages of this method includes elimination of false positive due to potential PCR inhibitors in sample extracts, elimination of false-positive resulting from dead cells or free DNA, and an increase of detection sensitivity due to increase in the number of target cells (Lin et al, 2009). The method has been found to detect as few as 10 cfu /ml from pure culture suspension and 10 2 cfu /ml from seeded soil as well as from naturally infected stem, root and plants. However Mplex PCR was found superior to Bio PCR as it detects all strains of BW pathogen at sub specific level.

The conventional serial dilution method using SMSA media was also compared to Bio PCR method and found equally effective in detection of BW pathogen from field samples. The sensitivity of plating method was further improved by ten folds using enrichment process and colony confirmation through Mplex PCR.

Some of the seeded soil samples having high organic matter content showed negative PCR test after 48 -72 hours of enrichment in SMSA broth and attributed to excessive growth of saprophytic bacteria or presence of inhibitory substances to PCR. Ten fold serial dilution of such samples after enrichment has improved the sensitivity of both plating and PCR test and detected as less as 10 2 cfu / ml from artificially seeded and naturally infected soils (Appendix 2; Table A5)

These studies confirmed that enrichment process and Mplex PCR is a useful tool for detection of BW pathogen and can be employed to check the presence of pathogen in soil, water and in planting material, which will help to make pre planting decision and will also strengthen the seed certification system.

2 Evaluate different potato varieties for bacterial wilt susceptibility

2.1. Evaluate the potential of different potato varieties at NOMIARC

18 commercial potato varieties and 4 NOMIARC lines available at Northern Mindanao Integrated Agriculture Research Centre were screened for bacterial wilt tolerance and yield performance. The test varieties were Alchip, Alpha, Asterix, ASN, Astra, Atlantic, Catani, CIP 30.5, Columbos, Franze, Granola, Igorota, IP, Kennebec, Nooksack, Raja, Raniag, Shepody. Igorota and Raniag .

NOMPO A, NOMPO B, NOMPO D and NOMPO E lines were developed at North Mindanao Integrated Agriculture Research Centre. Screening for tolerance to bacterial wilt was conducted in replicated trials in inoculated pots in greenhouse as well as in field at the NOMIARC BW Nursery.

Greenhouse evaluation

The test varieties were grown in inoculated pots (bv 3 strain of BW) and data on yield and BW incidence was recorded to compare relative tolerance among different varieties. Minimum incidence of BW was recorded in NOMPO D (17%), Igorota (22%), Asterix (23%) and in NOMPO B (25%), where as maximum BW incidence was observed in variety Columbos (75%) followed by CIP 30.5(72%), Astra (71%) and Franze (63%) and found to be most susceptible varieties to BW.

The most commonly grown variety Granola was found moderately tolerant to BW (45%) and produced maximum yield of 3 t/ha, while other varieties were found to be moderately susceptible with bacterial wilt incidence of more than 60% (Figure 12).

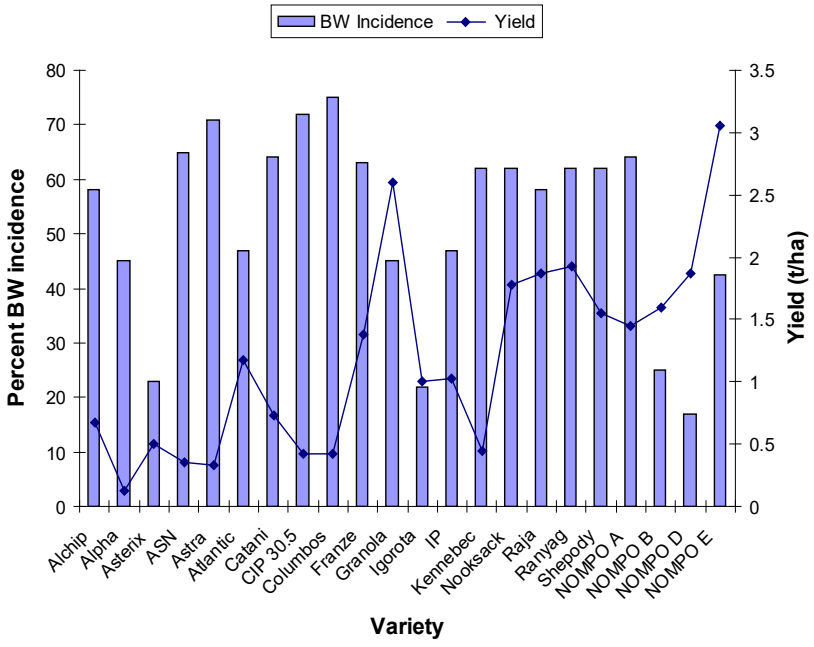


Figure 12. Comparative tolerance among varieties to BW disease in greenhouse conditions (NOMIARC)

Field evaluation

Varietal evaluation for tolerance to bacterial wilt disease in bacterial wilt nursery was conducted for two cropping seasons in August –November 2010 (1st crop) and January-April 2011(2 crop) at NOMIARC. Most of the varieties showed similar trend of tolerance to BW in both seasons, but varieties ASN, IP, Nooksack, Raniag, CIP 30.5, NOMPO A, and NOMPO D showed higher disease incidence in first cropping season ie. August – November.

Overall BW incidence was lowest in second cropping season, January-April has been evaluated as the best growing season for potato cultivation in Mindanao area (Figure13 &14).

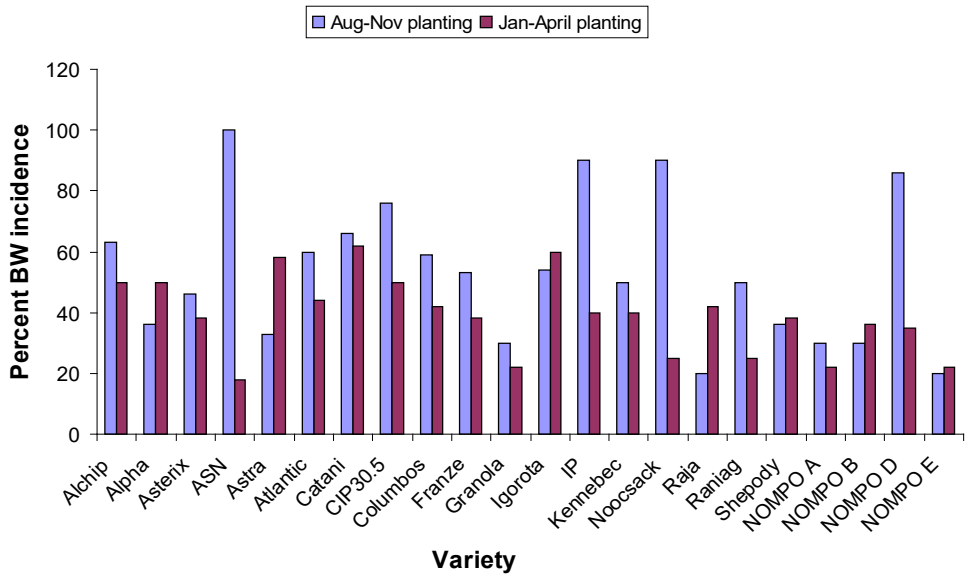


Figure 13. Comparative tolerance among varieties to BW disease under different cropping seasons

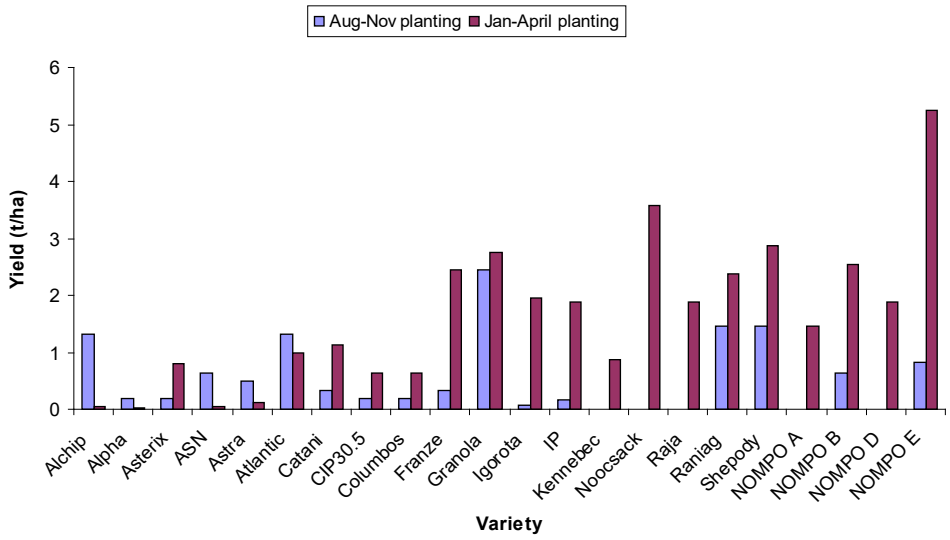


Figure 14. Yield potential of different varieties under different cropping seasons

Based on greenhouse and Bacterial Wilt Nursery evaluation, Granola, Asterix, Igorota, NOMPO B were evaluated as tolerant varieties while Alpha, Atlantic, Kennebec, Shepody, NOMPO D and NOMPO E were found moderately tolerant varieties and lines.

Multilocational evaluation

The different varieties and NOMIARC lines were further tested for bacterial wilt susceptibility; yield and for processing and table quality traits. All varieties and lines were assessed at the farmer' field in three different potato growing areas of Bukidnon, Imbayao, Miarayon and Kibangay.

In Imbayo (above (1200 masl), all varieties evaluated grew vigorously. However, the crop was infected with bacterial wilt at 45 days after planting. Bacterial wilt incidence ranged from 30% with Ranyag and NOMPO B to 80% with variety Igorota. The three varieties out

yielded Granola were Ranyag, NOM PO B and ASN with 13.49, 9.39 and 8.68 t/ha, respectively against Granola with only 8.12 t/ha (Figure15). Furthermore, varieties like Asterix, Columbos and Shepody produced significantly better yield than varieties Franze and Alpha.

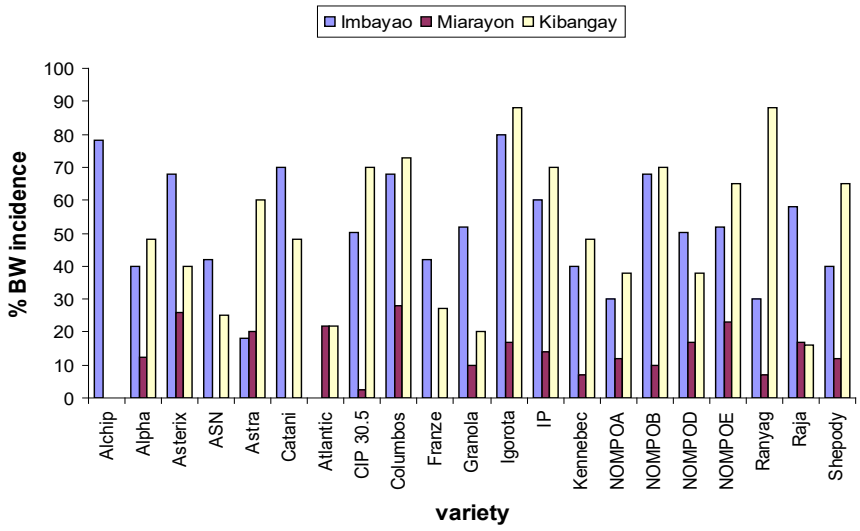


Figure 15. Comparative tolerance among varieties to BW disease in different potato growing areas (Imbayo,Miarayon and Kibangay).

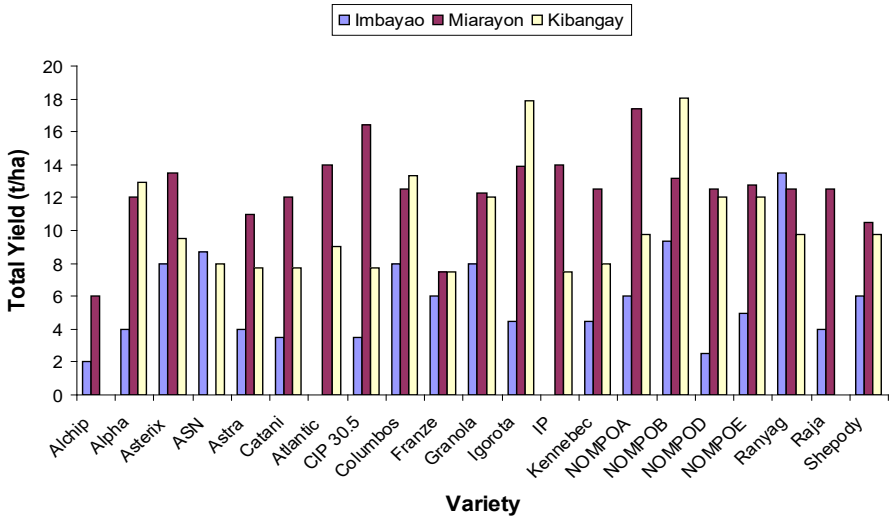


Figure16. Yield potential of different varieties in different potato growing areas (Imbayo, Miarayon and Kibangay)

In Miarayon, Talakag, Bukidnon (above 1400 masl), bacterial wilt incidence was low compared to Imbayao and Kibangay. Bacterial wilt symptoms were appeared late during the growing season i.e. 75 days after planting. In the test locations 3 varieties, Granola, Franze and Catani showed no symptom of bacterial wilt disease. The highest bacterial wilt infection was exhibited by Asterix with 26% infection. There were nine (9) varieties that out yielded Granola (12.28 t/ha) and Alpha (12.03 t/ha). These varieties were NOM PO A, CIP 30.5, IP, Atlantic, Igorota, Asterix, NOM PO B, NOM PO E and Kennebec with yield of 17.93, 16.39, 14.02, 13.96, 13.94, 13.48, 13.15, 12.78 and 12.78 t/ha, respectively Other varieties like NOM PO D, Ranyag, Columbos, Raja, Catani and Shepody were found significantly better than Franze (Figure 16).

In Kibangay, Lantapan, Bukidnon (above 1200 masl) , Ranyag variety significantly showed highest BW incidence (88.00%) followed by Columbos (73.00%) (Figure 15). The

lowest infection was expressed by Raja variety (16.00%). Four varieties significantly out yielded the variety Granola in this site, such as NOM PO B, Igorota, Columbos and Alpha with 18.04, 17.89, 13.33 and 12.89 t/ha.

All potato varieties exhibited different reaction to BW disease in test locations. Apart from genetic differences among varieties, it may be due to the difference in microclimatic conditions. The lower temperature and less humid conditions in Miarayon delayed the onset and severity of BW disease as compared to Kibangay and Imbayo. Potato varieties Igorota, Astrix, Raja, Columbos, Granola, Kennebec, Astra, Atlantic, Catani, Franz and NOM B performed better in test locations. These varieties showed comparative tolerance to BW and gave better returns, which showed possibility to introduce new varieties.

Potato farmers all over the country have been growing Granola ever since potato was introduced into the Philippines. It is one of the preferred varieties because of its tuber size, shape, skin colour and flesh colour. Participatory varietal selection was also conducted at each location to determine the preference of both consumers and growers. A local variety Igorota developed by Northern Root Crops Research Centre in Northern Philippine was been liked by farmers and consumers due to its similar characteristics to Granola and tolerance to bacterial wilt disease. The introduction of comparatively tolerant varieties Igorota, Raniag and NOM POB along with IDM technology may produce higher, returns to the farmers in future.

In Australia, ten commercial potato cultivars were assessed for bacterial wilt resistance in a glasshouse pot trial. Rapid wilting was observed after two weeks in stab inoculated plants and tolerance among cultivars could not be differentiated. However, cultivars Charlotte, FL 1867 and Nicola showed some tolerance to BW, while other cultivars were completely wilted after four weeks of inoculation.

The soil inoculation method was proved better for comparing varietal tolerance (Figure17). Disease intensity in FL1867 and Sebago was found significantly higher in beginning in comparison to other varieties but over eight weeks, complete wilting was observed in Atlantic, Kipfler, Lady Jane and Sebago.

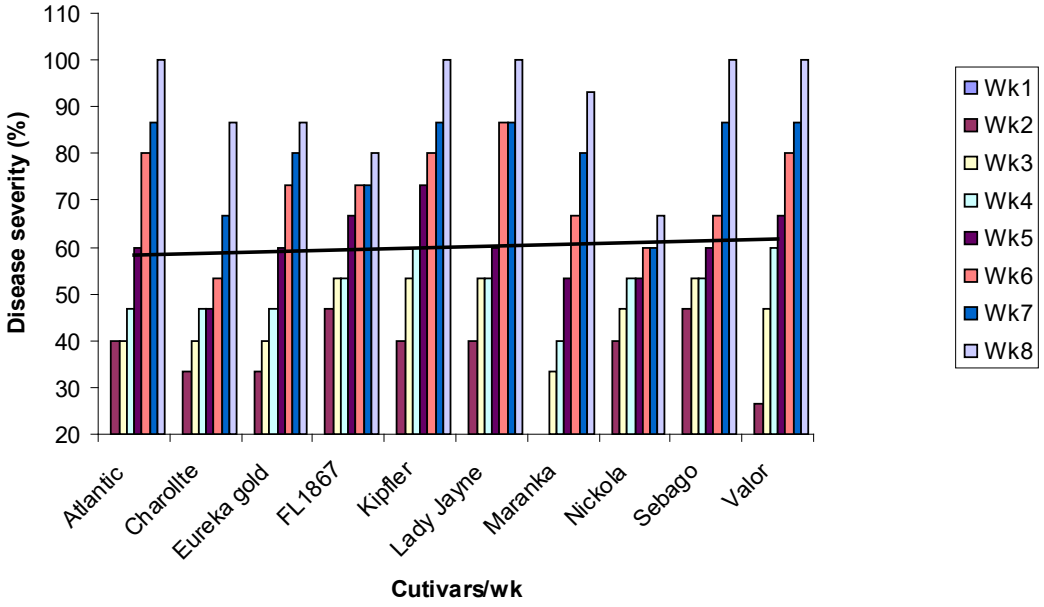


Figure 17. Comparative tolerance among Australian cultivars to BW disease (Phylotype I bv 3)

Overall Nicola, FL1867, Charlotte and Maranka showed the highest level of tolerance to BW followed by Eureka Gold. Cultivars Atlantic, Kipfler, Lady Jane, Sebago and Valour were found equally susceptible (Figure 17). The results showed low levels of resistance in

cultivars and none were found to have useful levels of resistance to bacterial wilt strain; Phylotype I bv 3.

These studies were restricted to glasshouse experiments because of quarantine disease and location of research centre within 20 km zone of commercial potato growing area. The varieties may show different reaction to BW strains (Phylotype I bv 3 and Phylotype II bv 2) under field conditions. At present, all varieties are being grown successfully in the region (Atherton Tableland).The regular practice of strict quarantine regulations, crop rotation with non solanaceous crops / fallowing, use of certified seed and cultivation during cool and dry months of the year (April-Sept.) are the key strategies for BW management and sustainable potato production in the region.

3. Further develop and refine biofumigation technology

3.1 Evaluate alternate amendments for bacterial wilt suppression

Glucosinolate hydrolysis products, isothiocyanates (ITCs) released by Brassica plants such as radish and broccoli suppress bacterial wilt, *R. solanacearum* in Solanaceous crops. Findings from the previous studies (ACIAR SCMN/2000/114) showed radish, broccoli, Chinese mustard and cauliflower have the highest amount of ITCs. Biofumigation is also found more effective for bacterial wilt suppression when plant tissues are chopped or shredded and well incorporated into the soil at land preparation. Watering after soil incorporation is also important so that tissues easily break and release ITCs. Maximum bacterial suppression can be attained at the rate of 5 kilograms/m^2. Biofumigation also is more effective in sandy soils than clay soils

Besides toxic ITCs, tissue decomposition provides high organic matter that promotes the growth of useful microorganisms that overcomes the pathogenic soil microbes. Wild sunflower, *Tithonia diversifolia,* a non-Brassica plant has been shown in previous studies to suppress bacterial wilt due to its high organic matter contribution to the soil upon decomposition. Field trials were conducted in farmer's fields to deliver the outcomes of biofumigation technology trials on the suppression of bacterial wilt disease. It was found that technology developed in an earlier ACIAR biofumigation project is popular among Filipino farmers but has only been partially adopted i.e. use of *Brassica* crop residues in furrows only due to limited land and economic resources. The biofumigation technology does not fit well into their crop rotations as a Brassica crop takes more than eight weeks to generate sufficient biomass for effective pathogen suppression and another two weeks for their residues to decompose efficiently to allow seeding of the next crop. It also requires management inputs. The farmers do not want to take their land out of cash production/cash flow by growing a biofumigation crop. Plants grown in abundance in the areas such as wild sunflower, *Tithonia diversifolia* was evaluated as soil amendment for BW suppression.

Field trial

An experiment was conducted at NOMIARC, using radish, broccoli, and wild sunflower as soil amendment crop to evaluate their effect on BW population and in disease suppression. Two weeks before fumigation, field was uniformly inoculated with BW strain (bv3) to a level of 10^7 cfu/g soil. Shredded plant tissues were incorporated at 5 kg/m^2 and irrigated. Clean potato seeds, G1 were planted after 3 weeks of plant tissue incorporation to ensure complete breakdown of plant material.

At harvest, percent reduction in pathogen population was observed in broccoli, radish and wild sunflower treatment. The results showed 24.5, 39.3 and 57.4 percent reduction in broccoli, radish and wild sunflower respectively in comparison to 57.5% increase in pathogen population in control treatment. After second crop incorporation, highest reduction in bacterial wilt population was observed in wild sunflower (95.2%) followed by broccoli (91.2%) and radish (90.6%), however only 5.3% reduction was observed in

control treatment. Suppression in BW population caused corresponding decrease in bacterial wilt disease

Bacterial wilt incidence was significantly less in wild sunflower amended soil than radish and broccoli incorporation. Bacterial wilt infection caused the reduction in yield of potato (Table 2) during the first season, August-November 2009. Increased yield in wild sunflower amended soil could be due to increased potassium and phosphorus from wild sunflower. Soil samples at harvest were collected for analysis of nutrients to determine the amount of the nutrients contributed to the soil upon decomposition of plant biomass. Analysis showed soil amendments increased the soil pH, phosphorus and potassium (Table 3). These two elements are important nutrients needed for the development of tubers. In year 2010 trial was failed due to early outbreak of late blight of potato.

Table 2. Yield (tons/ha) of potato, cv. Granola grown in soil amended with broccoli, radish and wild sunflower (NOMIARC, Bukidnon, August-November 2009)

	August-November 2009		
Treatment	**Marketable**	**Non Marketable**	**# Infected tubers**
Broccoli	3.95 ^{c}	0.95 ^{b}	136
Radish	4.47 ^{b}	0.85 ^{c}	131
Wild sunflower	6.62 ^{a}	0.82 ^{cd}	110
Control	2.98 ^{d}	1.8 ^{a}	188

*Means followed by the same letter are not significantly different (P< 0.05).

Table 3. Soil analysis of samples taken from plots amended with broccoli, radish and wild sunflower (NOMIARC, Bukidnon, August-November 2009)

Treatments	**Nutrient elements and soil pH**				
	pH	**OM (%)**	**N%**	**P (Bray) ppm**	**K c mol(+)/kg soil**
Broccoli	4.8	4.52	.24	12.5	.76
Radish	4.9	4.62	.24	19.5	.57
Wild sunflower	4.9	4.57	.22	24.0	.92
Control	4.7	5.04	.24	15.8	.97

Soil amendment trials

From 3 potato growing areas (Kibangay, Miarayon and Imbayao) farmer collaborators were identified for conducting field trials. Their fields were selected on the basis of less bacterial wilt infection as this study also aimed to produce clean seeds for farmer distribution. Biofumigation technology was introduced to the farmers during a workshop conducted before planning the study. Paul Cabaledas, Erol Nabe and Gabriel Banadao, were trained for commercial seed production. P. Cabaledas incorporated broccoli from harvest in his farm while E. Nabe and G. Banadao harvested cabbage and used the crop residues for biofumigation. These crops are commonly produced in their barangays or villages. Wild sunflower is growing abundantly along the road, field borders, and peripheries and utilized for this study. Potato cv. Granola foundation seeds were planted in plots and amended with broccoli/cabbage and wild sunflower.

The results showed (Table 4) very low bacterial wilt incidence in potato crop from all three areas. In Kibangay and Miarayon, higher numbers of tubers were harvested per plant in the wild sunflower amended plots. All in all, higher yield was obtained from the wild sunflower amended soil than in cabbage or broccoli amended plots and untreated plots .This difference in yield is likely to be due to its green manure effect and aligned with research in western Kenya. Jama et al (2000) reported the potential of wild sunflower green biomass in improving soil fertility. They also reported that green leaf biomass of wild sunflower is high in nutrients averaging about 3.5% N, 0.3% P and 4.1% K on dry matter basis.

Table 4. Bacterial wilt incidence, yield and yield parameters of potato grown in soil with broccoli/ cabbage and wild sunflower amendments at Miarayon, Imbayao and Kibangay, Bukidnon, Philippines (July-November 2011)

Location	Parameters	Soil amendment		
		Wild sunflower	**Cabbage/broccoli**	**Control**
Miarayon	BW incidence (%)	0.0 [b]	0.0 [b]	2.0 [a]
	# Tuber/plant	6.8 [a]	6.2 [a]	5.6 [b]
	Average tuber weight (gm)	27.0 [a]	23.0 [b]	30.0 [a]
	Yield (tons/ha)	11.7 [a]	8.7 [c]	10.6 [b]
Imbayao	BW incidence (%)	0.0 [b]	0.0 [b]	8.0 [a]
	# Tuber/plant	4.9 [b]	5.6	4.7 [b]
	Average tuber weight (gm)	4 [a] 2	33 [b]	37.0 [a]
	Yield (tons/ha)	12.6 [a]	10.9 [a]	10.5 [a]
Kibangay	BW incidence (%)	0.0 [b]	5 [b]	7.0 [b]
	# Tuber/plant	7.2 [b]	6.3 [b]	6.0 [b]
	Average tuber weight (gm)	50.0 [a]	31.0 [a]	31.0 [a]
	Yield (tons/ha)	20.4 [a]	10.9 [a]	11.2 [a]

*Means followed by the same letter are not significantly different (P< 0.05).

Influence of soil amendment on colony count, crop stand, and disease Incidence.

Pot trial

A pot trial was conducted to determine the effect of different application rate of biomass incorporation and mode of application in soil for suppression of BW pathogen. Results showed the significant differences between biofumigated and non-biofumigated soil in terms of bacterial colonies, crop stand (Table 5), disease incidence and severity (Table 6).

Table 5 Colony count and crop stand of tomato cv. Yellow plum after biofumigation

Treatment No.	Treatment 4WAI	Colony count initial		Crop Stand	
		Initial	4WAI	Initial	4WAI
1	No wild sunflower, no radish	12	2.75[a]	9.50	2.50[b]
2	Wild sunflower, 5 kg/sq.m	12	0.00[c]	10.0	6.00[ab]
3	Wild sunflower, 10 kg/sq.m	12	0.00 [c]	10.0	9.00 [a]
4	Radish, 5 kg/sq.m	12	0.25 [c]	10.0	5.75[ab]
5	Radish, 10 kg/sq.m	12	0.00 [c]	10.0	6.00[ab]
6	Wild sunflower surface, 5 kg/sq.m	12	0.25[bc]	10.0	6.00[ab]
7	Radish surface,5 kg/sq.m	12	0.25[bc]	10.0	5.00 [b]
8	Tomato,5 kg/ha	12	1.75[ab]	10.0	3.25 [b]
		61.23%	29.47%	3.55	46.66
CV		ns	*	ns	*

*Means followed by the same letter are not significantly different at 0.5 level of significant
WAI, weeks after inoculation

Table 6 Incidence and severity of bacterial wilt of tomato cv.Yellow Plum after biofumigation

Treatment No.	Treatment	Percent Incidence		Percent severity	
		Initial	4WAI	Initial	4WAI
1	No wild sunflower, no radish	5.00	75.00 [a]	3.00	74.00 [a]
2	Wild sunflower, 5 kg/sq.m	0.00	40.00 [ab]	0.00	32.00
3	Wild sunflower, 10 kg/sq.m	0.00	10.00 [b]	0.00	10.00 [c]
4	Radish, 5 kg/sq.m	0.00	42.50 [ab]	0.00	40.50
5	Radish, 10 kg/sq.m	0.00	40.00 [ab]	0.00	34.50
6	Wild sunflower surface, 5 kg/sq.m	0.00	40.00 [ab]	0.00	38.00[a] [c]
7	Radish surface, 5 kg/sq.m	0.00	50.00 [a]	0.00	44.50[a] [bc]
8	Tomato,5 kg/ha	0.00	67.50 [a]	0.00	67.50 [ab]
		87.50	55.61%	87.50%	57.34
CV		ns	*	ns	*

* Means followed by the same letter are not significantly different at 0.5 level of significant
WAI, weeks after inoculation

Numbers of colonies were significantly higher in non-biofumigated (treatment 1) and tomato- amended soil compared to wild sunflower and radish-fumigated treatments. No colony was observed 4 WAI in radish-treated and wild sunflower-treated soil while an average of 0.25 means colonies were recorded in treatments 4, 6 and 7. No significant difference was observed among different concentrations of biofumigants applied.
Test plants were vigorous among tomato plants grown in soil-fumigated with wild sunflower at the rate of 10 kg/sq.m (treatment 3) with a rating of 9.0 (10 as the highest rating). Poor crop stand was observed in both non-biofumigated (2.5/10) and tomato-amended (3.25/10) soils. No significant difference was observed from other treatments (2, 4, 5 and 6) except for treatment 7 (5.0/10) with radish applied in the soil surface.

Consequently, incidence and severity of wilting were high in non-biofumigated (treatment 1) and tomato-treated soils. Lowest incidence (10%) and severity was recorded in wild sunflower-treated soil (treatment 3). No significant difference was again observed among other treatments (2, 4, 5 and 6 with 40, 42.5, 40, and 40 % respectively) except for treatment 7 (radish, surface) with 50% incidence. Incidence and severity is very high in treatment 1 (75% severity and 74% severity) followed by treatment 8 (67.5% incidence and severity). It was noted; however, that incidence of bacterial wilt was still observed even though the colony count was zero. This can be attributed to the existence of viable but non-culturable state of the bacteria. The bacterium may not be culturable in selective media but remain viable. In the presence of its host, the bacterium became infective and causes the wilting symptoms. In general, this experiment showed the potential of wild sunflower in suppression of bacterial wilt disease and this was mainly attributed to the green manure effect or possibly due to chemical sesquiterpene lactone 1 that was shown to have phytotherapeutic agent against some bacterial infections (Obafemi, 2006).This need further testing of chemical against BW pathogen which may help to determine application rate of biomass incorporation for BW infested fields.

The results showed that increased rate of biomass incorporation of biofumigation and non biofumigation crops provided better BW management. No significant difference in disease incidence has been observed in treatments between surface and sub surface mulching of wild sunflower. Therefore mulching of wild sunflower on soil surface could be opted to reduce labour cost.

Effect of organic manures and biocontrol agents on suppression of BW disease

In Australia a pot trial was conducted to investigate the effect of different biocontrol agents and organic amendments on suppression of bacterial wilt disease. The result showed the significant differences between treatments at all time points except the final three sampling events, 49, 56 and after 63 days of soil amendment. The control treatment had significantly more disease severity than all other treatments at the first time point. Disease severity was found significantly lower in chicken manure 10%, cow manure 10% and in *Trichoderma* amended soil (Figure 18). Significant differences were observed between rates of manure application on suppression of bacterial wilt disease. It has shown possibility to reduce disease by further increase in manure application for soil amendment.

In this experiment biocontrol agent were tested individually but further testing and combined use of biocontrol agents with manures/composts may prove beneficial to improve efficacy and reliability of BW disease suppression

There have been several reports to relate the disease suppressions of compost to particular biological, chemical and/or physical characteristics. Lazarovits et al (2005) described that volatiles containing compounds, organic acids and ammonia are mainly attributed for disease suppression. The most important factor affecting disease control is

soil pH; as the toxic products responsible for killing pathogen exist only when the soil pH is below 6 or above 8. From an understanding of the mode of action (organic soil amendments), amendments can be formulated to improve efficacy and reduce variability in management of soil borne pathogen. This includes basing application rates upon soil properties, manipulating amendment and/or soil pH, altering the rate of nitrification and organic matter contents, screening of amendments for appropriate chemistries, improving storage condition, altering their microbiology and improving the time and mode of application.

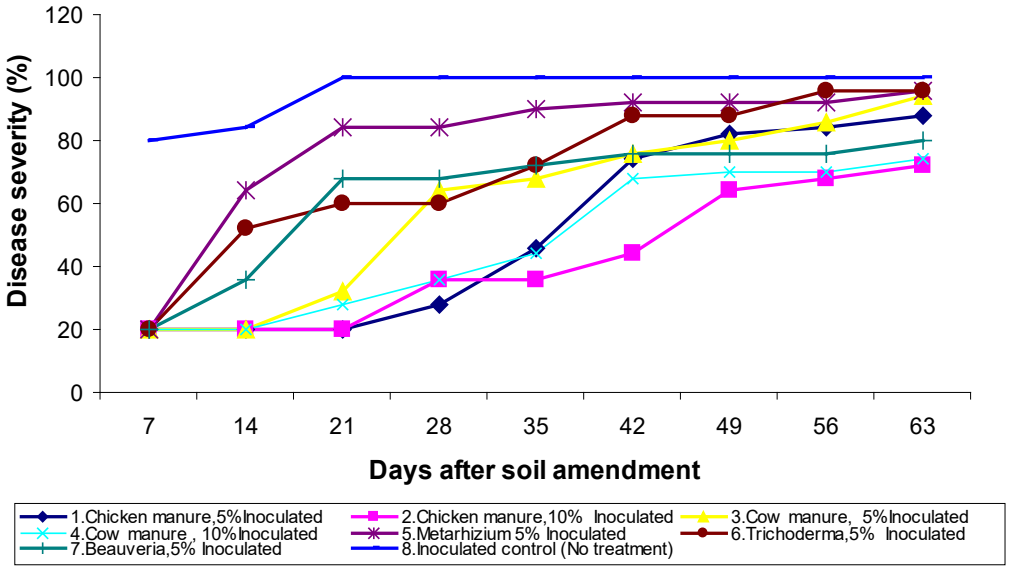


Figure 8. Effect of organic manures and biocontrol agents on suppression of BW disease

Effect of biocontrol agent, plant extract, chemicals, and organic manures on growth of BW pathogen

In Australia, number of biocontrol agent, plant extract, chemicals, and organic manures on growth of BW pathogen were screened *in vitro* conditions to see their possibility to use in integrated management strategies. Significant differences were found between the treatments .The results suggested that all treatments other than canola oil Japanese Wild sunflower and different manure tea have a significantly larger mean inhibition zone than the untreated control (Appendix 2; Table A 6). Organic manures did not show direct effect on suppression of BW pathogen *(in vitro)* and supported the other findings of the project that disease suppression is mainly due to improvement in biological, chemical and/or physical characteristics of the soil.

A number of biocontrol agents; *Enterobacter sp, Bacilllus sp, Trichoderma sp* , plant extracts in 10 % alcohol; Tea tree oil (commercial formulation), bamboo shoots, paw paw seeds and ghost chilli and chemical / fertilizer lime sulphur and copper sulphate inhibited the growth of BW pathogen. The study explored more options for management of bacterial wilt diseases and possibility to develop new bactericides/chemicals or to use plant /plant part as soil amendment for suppression of soil borne pathogens.

3.2 Development of best crop rotation scheme for bacterial wilt suppression

The crop rotation, crucifers-potato-corn-legume, commonly practiced by farmers in Bukidnon was evaluated for reduction in bacterial wilt infection in the field. Before planting and at harvest of each crop in rotation, bacterial wilt colony counts were made. Initial

population counts of BW pathogen was 3 x10 ^{3} (cfu/g of soil) in all plots. After biofumigation, potato was planted and BW population counts were reduced at harvest by 91.2%, 90.4%, 95.2% in broccoli, radish and wild sunflower incorporated plots, respectively compared to only 5.3% in untreated plots. Lower percent reduction in BW counts, 24.5%, 39.3%, 57.4% were observed in the second potato crop after biofumigation with broccoli, radish and wild sunflower, respectively. There was a 4.61% increase in BW pathogen *counts* in the untreated plots. When planted with corn after potato, the BW pathogen counts were reduced in all treatments by 24.3%, 19.0%, 1.1% and 0.9% in broccoli, radish, and Wild sunflower incorporated plots and untreated plots respectively. At harvest of cowpea planted after corn, BW pathogen counts in the biofumigated plots all decreased but increased in untreated plots. However, planting radish after cowpea again reduced the BW pathogen counts. Non-host plants such as corn and cowpea planted after solanaceous crops were not found favorable to increase the BW pathogen population. These studies showed that impact of disease could be reduced by changing the cropping sequence. Longer crop rotation with non solanaceous crops, eradication of weed and volunteer plants and education are key factors in management of BW infected areas/field.

4. Establish on-farm best-bet clean seed production and maintenance

4.1- 4.2. Focused group discussion (FGD) with potato farmers of Imbayao, Kibangay and Miarayon were conducted by NOMIARC collaborators. These three areas were identified as the key potato production areas of Bukidnon. Findings were summarized as follows:

- Planting of potato was dependent on the availability of seeds rather than suitable planting time.
- Productivity is dependent on seed quality; degenerated seeds usually have low productivity due to increasing incidence of BW.
- Traders dictate the price as well as varieties to grow; varieties with long shelf life, yellow flesh and firm skin are preferred.
- Trucking and hauling from farm to market contributed to high production cost thus reducing farmer's net profit.
- Two important potato production constraints identified were insufficient supply of disease-free potato seeds and incidence of bacterial wilt.
- The suggested solutions to these problems were training on seed production and control of bacterial wilt and the establishment of trading post in the locality.

Based on the findings from the FGD conducted, potato production can be sustainable if the following will be addressed:

- Insufficient supply of clean potato seeds
- Presence of seed investors to buy the seeds
- Availability of technology on bacterial wilt management

To address the constraints in potato production, clean potato seed production was established in identified collaborators farmers' fields of identified collaborators. Granola seeds (G0), the preferred variety were planted at Roberto Sulatan and Erol Nabe's farm in Miarayon, at Paul Cabelada's farm in Kibangay and at Gabriel Banadao's farm in Imbayao.

Areas selected were not previously grown to solanaceous crops to ensure none or very low incidence of bacterial wilt. The farmers were trained on biofumigation and best practice for bacterial wilt management. The areas selected were biofumigated with broccoli and Wild sunflower. Highest yield, 18.1 tons/ha was obtained in plots with Wild sunflower amendment at Kibangay, 11.3 tons/ha in Miarayon and 10.6 tons/ha in Imbayao compared to broccoli-incorporated plots. Seeds harvested were set aside for another planting. The next batch of seeds produced, G2 were agreed to be distributed to other interested farmers who will grow potatoes. This seed distribution scheme is being practiced because NOMIARC tissue culture facility is inadequate to provide enough seed

supply for potato growers in Mindanao. Thus, farmers have to source out their seeds from other farmers or suppliers; however, there is no guarantee for its cleanliness.

There is a prospect for the establishment of an aeroponics potato seed production so that the demand for seeds can be satisfied. Aeroponic seed production is already being practiced in other countries like China and Vietnam. There was a discussion with Landcare, ACIAR and NOMIARC for the possibility of developing the system. UPLB is already using the solution for aeroponics and this can be utilized for aeroponic potato seed production. After the scoping mission to visit Hanoi's aeroponics facility, a design was developed for a simple aeroponics system that would utilize readily available materials in the region. The solution available at UPLB will also be tested and if possible formulate a less expensive solution but equally effective solution for potato seed production.

4.3. Clean potato seed production through aeroponic technique

Aeroponic technology (soil less) was experimented at Centre for Tropical Agriculture Mareeba (Oct.2011) to assess its potential for clean potato seed production. Initial studies included development of a low cost aeroponic system; evaluation of varietal response and nutritional requirements, and assessment of insect pests related issues.

A small aeroponic unit 2.0 x 1.5 m and solar powered aeroponic system was constructed (Appendix 2; Figure 5 & 6) and tested for minituber production. Details for construction and material used for both aeroponic units are described in Appendix 2; Figure 5 & 6.

Initial planting material (Tissue culture; minitubers 2-2.5g) of two popular Australian varieties Sebago and Nicola were procured from the reputed source; Victorian Certified Seed Potato Australia (VicSPA). The minitubers were grown in sterilized sand in plastic tubes (67-120 mm) for 15 days and then roots were washed with clean water and treated with fungicide Mancozeb (0.25% for 15 minutes) before transplanted to the aeroponic unit (Figure 19).

The timer was fixed to run the nutrient solution for 60 seconds after every 10 minutes. The plants were securely staked and preventive fungicide/insecticide sprays were applied to avoid the incidence of insect pests. The pH (6.0-6.5) and EC (2-2.5) of the nutrient solution were checked weekly and adjusted accordingly.

Two commercially available nutrient solutions, Flora series (Flora Gro - total N 2%, P_2O_5 1%, K_2O 6%, Mg 0.5%; Flora Micro N 5%, K_2O 1%, Ca 5%, B 100ppm, Co 5.5ppm, Cu 100ppm, Fe 1000ppm, Mn 500ppm, Mo 8ppm, Zn 150ppm and Flora Bloom - P_2O_5 5%, K_2O 4%.Mg 1.5%,S 1%) and Plantastic hydroponic solution A and B (total N 2.55%, P 0.38%, KNO_3 3.68%, Ca 2.6%, S 0.84%, Mg 0.65%, Fe 400ppm, Mn 100ppm, Bo 42ppm, Zn 30ppm, Cu 18ppm Mo 6ppm) were evaluated. The Flora grow combined with Flora Micro and Flora Bloom nutrition have been found excellent for supporting plant growth and tuberization in the aeroponic system.

The Plantastic nutrient solution, supported good vegetative and root growth, but failed to produce potato tubers. This may be due to the application of the same nutrient concentration through out the growing period, whereas in the Flora series the precise formula was used to mix Flora Micro, Flora Grow and Flora Bloom as per manufacturer recommendations to make tailor-made nutrient mixes for each growth stage of the crop.

Differential response to plant growth and minituber production was observed among varieties in aeroponic system which may be attributed to their genotypic and phenotypic characteristics and/or nutritional requirements.

Figure 19. Tuber growth after 40 days of transplanting into aeroponic system; variety Nicola (L), Sebago (R)

Tuber initiation in Nicola was started 10 days earlier than Sebago. The first harvest was made 50 days after transplanting (Figures 18).Nicola variety produced more number of tubers (42 minitubers/plant) than Sebago (35 minituber/plant). Tubers of both varieties were harvested at an interval of 10-15 days in the weight range of 8 - 30g. Field studies will be further conducted to evaluate the yield difference with respect to seed size under field conditions, and standardize the tuber seed size for harvesting for direct commercial plantings.

The raw estimation has been made on the basis of these initial studies that 80 square meter area of aeroponic facility in the Philippines may cost PhP 150,000 and require another PhP 50,000 as operational cost per crop to grow 1000 plants, to produce more than 180,000 mintubers per year (3 crops a year), which in return will provide clean planting material for 4.5 ha land. This would give a profit of PhP 130,000 in first year and then in second year and onward around PhP 300,000.

Introduction of this technology at farmer's level will allow them to produce their own clean seed. from 600-800 plants from an area of less than 80m^2 ^{lt} will provide enough seed tubers (40,000 approx) in 3-4 months for their one hectare of plantings. It will avoid the risk of bacterial wilt contamination and further spread of the disease through seed to non-infected areas.

It was concluded that scaling up of seed multiplication rate in Mindanao will require continuous functional research and development support programs to run the system efficiently. There is also a requirement for research into field multiplication at the farm level, and development of best agronomic practices (e.g. improvement in plant nutrition and disease management). For its commercialization there is need to involve the private sector and educating the farmer population to understand the value of high health seed and get best out of clean seed.

5. To develop improved management strategies for bacterial canker of tomatoes

Genetic characterisation of Australian *cmm* isolates

DNA profiles of 130 isolates from rep-PCR demonstrated a high degree of genetic diversity within *Cmm* isolates. Isolates were placed into four broad clades (Figure 20 and (Appendix 2 Table A 7). There was some correlation between genotypes and geographic

origin. In particular, members of Clade 4 were almost exclusively from the Sydney Basin while there were only three Australian isolates in Clade 1

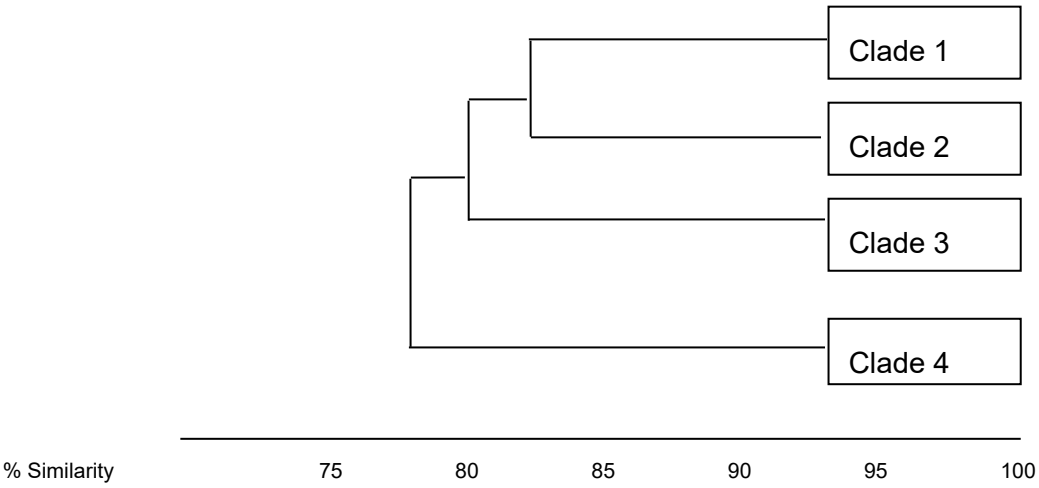


Figure 20. Consensus tree of *Cmm* isolates from UPGMA cluster analysis of DNA profiles generated by rep-PCR

Four broad genetic groups of *Cmm* isolates were resolved in Australia. A majority of isolates in Clade 4 were obtained from greenhouse tomatoes growing in the Sydney basin over the past decade. In contrast, *Cmm* isolates in Clades 1 and 2 isolates had wider geographical and temporal origins. This supports the hypothesis that *Cmm* isolates have entered Australia on many occasions, most likely with imported seed.

The findings of an homogenous group (Clade 4) from the Sydney basin is consistent with a similar study of *Cmm* isolates (Kleitman *et al*., 2008) who found four distinct genetic groups of which the two major ones were confined to greenhouse production in the Besor region located in the south-west of Israel, and the Gaza Strip. Kleitman *et al*. (2008) found that both major *Cmm* groups were equally pathogenic on tomatoes. On analysis of all their combined data, these authors concluded that these *Cmm* strains probably persisted in residual plants and soil between seasons despite extensive control measures taken by growers, including soil fumigation with formalin. The two remaining Israeli genetic strains were isolated from the northern and north-eastern regions. It was inferred from DNA fingerprinting that all four Israeli strains were highly homogenous (>92% similarity) within each group, suggesting that they may each have been clonal lineages. The objective 5 team noted the surprising finding that other exotic strains were not isolated over the 10 year survey period despite the likelihood that infested seed was an important source of disease outbreaks. They suggested that incoming exotic strains could not establish in these production areas. Alternative hypotheses for both the Israeli and Australian findings are that disease outbreaks are due only to local strains, or that exotic strains donate virulence genes to endemic strains, giving rise to pathogenic strains that are better adapted to local environmental conditions. Further studies are required to understand the epidemiology of different *Cmm* strains, particularly when different genetic strains become occupants of the same ecological niches and hosts.

Several reports in the literature state that *Cmm* does not survive well in soil, except where it is associated with plant debris. This poor survival is thought to be due to its growth requirement for reduced sulphur compounds and the lack of an ability to reduce nitrates. However, it should also be noted that nutrient solutions used for greenhouse fertigation may contain sufficient reduced forms of sulphur and nitrogen to allow *Cmm* to survive. In particular, degrading roots in substrate media would provide a ready niche for *Cmm* survival. Use of microbial biocontrol bacteria such as certain strains of *Pseudomonas* and *Bacillus* could be useful to out-compete *Cmm* in these substrate niches.

5.1 Copper sensitivity of Australian *Cmm* isolates

A wide range in *Cu* tolerance was found among several isolates from NSW, SA and WA (Figure 21).The isolates were found strongly resistant and capable of growth on 0.75 mM *Cu*-amended media. The positive control test organism, *P. syringae* pv. *tomato* (#2162) which has plasmid-mediated *Cu* resistance, also tolerated 0.75 mM *Cu* but not 1mM *Cu*-amended media. *Cu* tolerance was found in several of the overseas reference isolates. Isolates from Kenya, UK, Belgium, USA, Canada and Bulgaria tolerated 0.75 mM *Cu*. The earliest isolated *Cmm* with this level of *Cu* tolerance was a Kenyan isolate (#4067) from 1945.

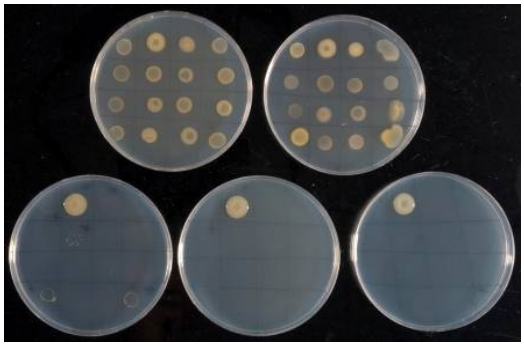
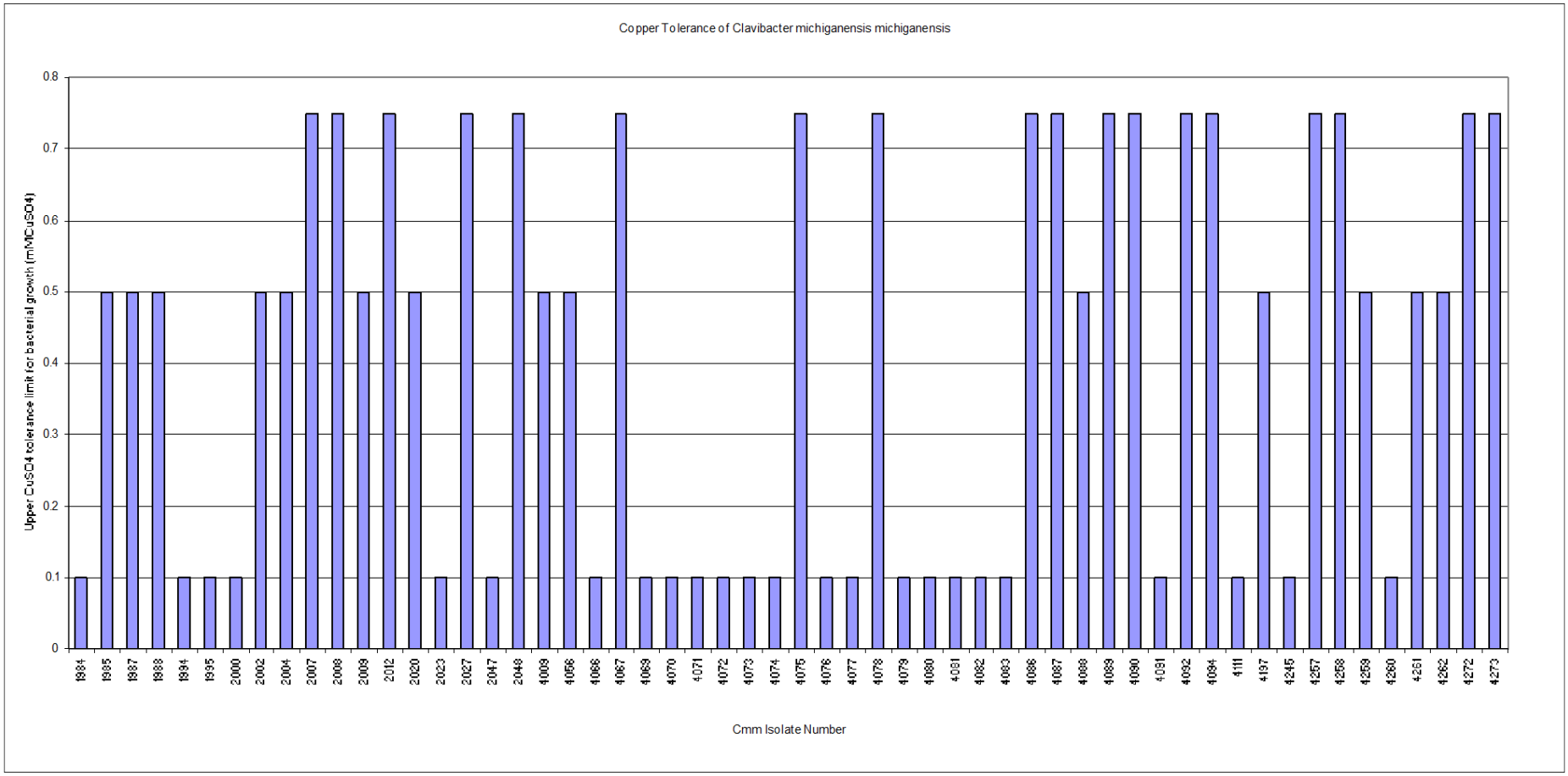


Figure 21. Copper tolerance screening assay with bacterial isolates on CYEG media amended with increasing copper concentrations. Top left: 0 *Cu*; top right 0.1 mM *Cu*; bottom left 0.5 mM *Cu*; bottom centre 0.75 mM *Cu*; bottom right 1 mM *Cu*

Fifty-seven percent (32/56) of the *Cmm* isolates grew on ≥0.5 mM *Cu*-amended media. Of these more than half (23) tolerated 0.75 mM *Cu*-amended media. *Cu* resistance has not previously reported in *Cmm* isolates (Figure 22). This has some implications for tomato growers, particularly greenhouse growers who are using IPM. Better control of *Cu*-resistant bacteria can be achieved by tank-mixing *Cu* with a dithiocarbamate fungicide (Appendix 2; Table A 8). However, chemicals from that class disrupt certain beneficial insects and mites. One alternative might be to tank-mix *Cu* with iron chloride (Lee et al., 1993, Scheck & Pscheidt, 1998). Zevenhuizen et al. (1979) determined that the only accurate predictor for the efficacy of copper products was the amount of free cupric ions (Cu^{2+}) in solution. Moreover, they found that metallic copper content did not correlate with product efficacy. It appears that the availability of soluble copper for bacterial toxicity is influenced by chemical interactions once it has been applied and other chemicals in the product formulation. Arman & Wain (1958) showed that *Cu* forms complexes with organic compounds that leach from plants. Some of this bound *Cu* is unavailable for further reactions and therefore does not affect bacteria. Equilibrium establishes between the complexed and free Cu^{2+} giving rise to an effective concentration of Cu^{2+}.

The results from this study will assist the Australian vegetable industry to better manage bacterial diseases. In particular, growers will be able to judge risks of *Cu* resistance. The chemical industry will also be able to respond and improve efficacy of copper-based products. Field assessment of tank-mixing *Cu* with iron chloride could be the subject to future studies. It is initially required to confirm its efficacy to Australian crops affected by *Cu* -tolerant bacteria. Efficacy data may also be required to support registration of a formulated product.

Figure 22. Tolerance levels to Cu^{2+} of 65 Australian and overseas reference isolates of the bacterium, *Cmm* from tomato



Disinfection of water, green house surfaces and tools

Part A

The first series of tests looked at dripper line and hose, string, drippers, clips, plastic channel, panda film and tools. Disinfectants were given a 1 or 2 min contact time. Four *Cmm* isolates were tested for most of these surfaces. The disinfectants used are shown in Appendix 2 Table 8.

Part B.

Further tests concentrated on panda film, weedmat, black plastic and tools. Disinfectants tested are listed in Appendix 2 Table 9.

(i) Disinfection of water

Calcium hypochlorite at 500ppm and sodium hypochlorite at 4% were able to eliminate *Cmm* from clean water + BSA with a 5min contact time. These results are shown in Table 7 and Figure 23.

Table 7. Water disinfectant testing using calcium and sodium hypochlorite

Disinfectants 5min contact time	**Conc.**	**Test**	**Results for each culture - = no *Cmm* detected + = *Cmm* detected**					
***Cmm* isolate**			**2097**		**2000**		**2008**	
			nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite	100ppm	T1	-				-	-
		T2	-	+			-	+
	250ppm	T1	-	-			+	+
	500ppm (0.05%)	T1	-		-	-	-	-
		T2	-	-				
Sodium hypochlorite	1%	T1					-	+ 1 colony
	4%	T1			-	-	-	-

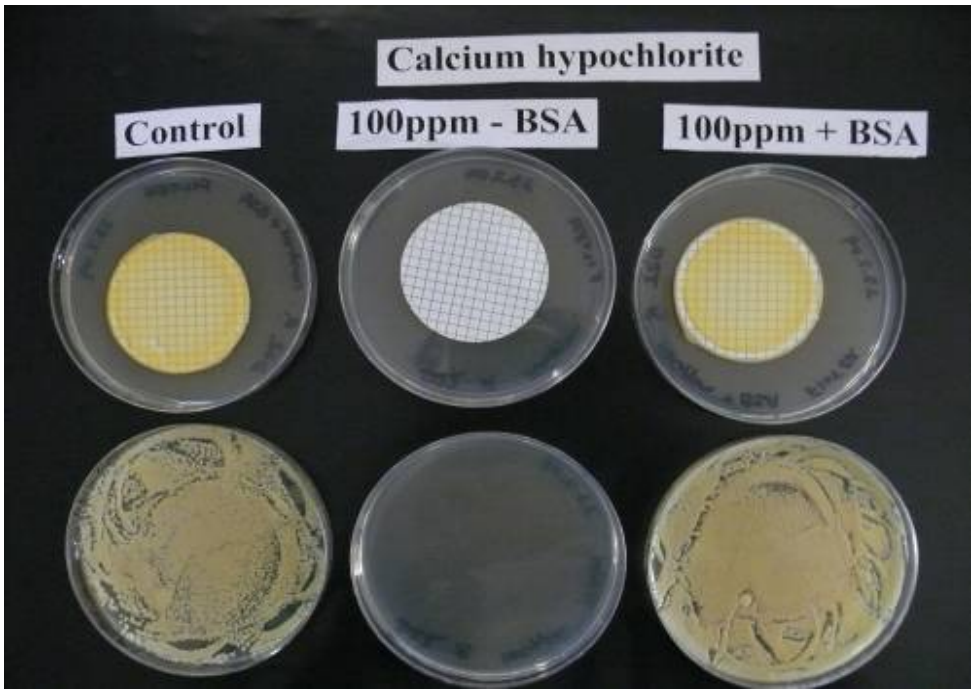


Figure 23. Plates showing positive *Cmm* growth for treatment of water + BSA with Calcium hypochlorite. Note efficacy without BSA.

The results for 1% Peratec®, Sporekill® and Virkon® were found more promising, showing that with a contact time of 2 min they were able to eliminate *Cmm* in a clean water sample with or without BSA. Hydrogen peroxide at 6% was not effective in removing *Cmm* from water using a 5 min exposure time. *Spray 'n Wipe* was effective at 2 min and 5 min (Table 8).

Table 8. Results of water disinfectant testing using hydrogen peroxide and *Spray n Wipe*

Disinfectant	**Test**	**Results** **- = no *Cmm* detected** **+ = *Cmm* detected**	
		***Cmm* isolate 2000**	
		nil	**BSA**
3% Hydrogen peroxide	T1 5 min	+	+
6% Hydrogen peroxide	T1 5 min	+	+
Spray n Wipe *filtered for 5 instead of 2mins	T1 5 min	-	-
	T2 5 min*	-	-
	T2 2 min	-	-

(ii) Persistence of commercial Virkon® dip solution

All reused samples tested negative for *Cmm* on both day 1 of testing and when retested 7 days later (Data not shown).

(III)Surface disinfection trials – Part A

Efficacy data from plastic surfaces was variable (Appendix 2 Tables A 9-15). In some cases they were completely efficacious in one of the duplicate trials and in others they were only effective against certain *Cmm* isolates. All the products used in Part A completely killed *Cmm* on plastic clips and plastic channel (Table 12), based on a single experiment. Only 1% Peratec successfully killed all *Cmm* isolates in dripper lines, plastic irrigation hose and string with a 2 min exposure time over duplicated trials, while other products were more variable in their efficacy.

A 10^3ppm solution of sodium hypochlorite was completely efficacious with a 2 min exposure time on Panda Film whereas none of the other products successfully killed three *Cmm* isolates (Table 9). There was sometimes difficulty (shown by negative or low amount of *Cmm* on control plates), getting the *Cmm* to 'stick' to the Panda Film which has a very shiny, slippery surface.

Table 9 Disinfectant efficacy for *Cmm* on Panda film

Disinfectants 2min contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		-		+			+ 500ppm	
1%Sodium hypochlorite	T1		-		-			-	
1% Peratec®	T1		-		-	+		-	-
1% Sporekill®	T1		+		+	-		-	-
1% Virkon®	T1		-		-	+		-	-

Surface disinfection trials – Part B

Greenhouse surfaces

In Part B 70% ethanol, denatured ethanol and 1% Virkon treatments were able to eliminate *Cmm* with a 1 min contact time on Panda film and Black plastic. The Weedmat proved harder to clean which is most likely due to its plastic woven texture.

Menno Florades (1%) was able to eliminate *Cmm* from all surfaces tested when treated for 16 hr. This is the recommended rate for Menno Florades in treating bacteria on hard surfaces. It was also successful when used for 1 hour on Panda Film and Black plastic but again Weedmat proved harder to disinfect.

A 3% solution of Menno Florades for 1 hr was successful in treating all surfaces and 1 min eliminated *Cmm* from Panda film and Black Plastic with a small amount of *Cmm* isolated from the Weedmat (Tables 10 -14).

Table 10. Surface disinfectant efficacy for *Cmm* using 70% ethanol

70% Ethanol							
Treatment	***Cmm* isolate**	**Panda film**		**Weedmat**		**Black Plastic**	
		-BSA	**+BSA**	**-BSA**	**+BSA**	**-BSA**	**+BSA**
Quick Dip	**2008**	-	-	-	+	+	-
	2004	-	-	-	+	-	+
1 min	**2008**	-	-	+	-	-	-
	2004	-	-	-	-	-	-
S&W	**2008**	-	-	+	+	-	+
	2004	-	+	+	+	-	+

Table 11. Surface disinfectant efficacy for *Cmm* using 70% denatured ethanol

70% Denatured Ethanol							
Treatment	***Cmm* isolate**	**Panda film**		**Weedmat**		**Black Plastic**	
		-BSA	**+BSA**	**-BSA**	**+BSA**	**-BSA**	**+BSA**
Quick Dip	**2008**	-	-	-	-	-	-
	2004	-	-	+	+	-	+
1 min	**2008**	-	-	-	-	-	-
	2004	-	-	+	+	-	-
S&W	**2008**	-	-	+	+	+ 1	-
	2004	+	-	+	+	+	+

Table 12. Surface disinfectant efficacy for *Cmm* using 1% Virkon®

1% Virkon®							
Treatment	***Cmm* isolate**	**Panda film**		**Weedmat**		**Black Plastic**	
		-BSA	**+BSA**	**-BSA**	**+BSA**	**-BSA**	**+BSA**
Quick Dip	**2008**	-	-	-	-	-	-
	2004	-	-	-	+	-	+
1 min	**2008**	-	-	-	-	-	-
	2004	-	-	-	+ 1 colony	-	-
S&W	**2008**	-	-	+	+	+	+
	2004	+	+	+	+	-	-

Table 13. Surface disinfectant efficacy for *Cmm* using 1% Menno Florades®

Treatment	***Cmm* isolate**	**Panda film**		**Weedmat**		**Black Plastic**	
		-BSA	**+BSA**	**-BSA**	**+BSA**	**-BSA**	**+BSA**
1 min	**2008**	-	-	+	+	-	+
	2008	+	+	-	-	-	-
	2004	+	-	-	-	-	-
	2004	-	-	-	+	-	-
1 hour	**2008**	-	-	-	-	-	-
	2008	-	-	-	-	-	-
	2004	-	-	+	-	-	-
	2004	-	-	-	-	-	-
16 hours	**2008**	-	-	-	-	-	-
	2008	-	-	-	-	-	-
	2004	-	-	-	-	-	-
	2004	-	-	-	-	-	-

Table 14. Surface disinfectant efficacy for *Cmm* using 3% Menno Florades®

Treatment	*Cmm* isolate	Panda film		Weedmat		Black Plastic	
		-BSA	+BSA	-BSA	+BSA	-BSA	+BSA
1 min	**2008**	-	-	-	+ 1 colony	-	-
	2008	-	-	-	-	-	-
	2004	-	-	-	-	-	-
	2004	-	-	-	+ 1 colony	-	-
1 hour	**2008**	-	-	-	-	-	-
	2008	-	-	-	-	-	-
	2004	-	-	-	-	-	-
	2004	-	-	-	-	-	-
16 hours	**2008**	-	-	-	-	-	-
	2008	-	-	-	-	-	-
	2004	-	-	-	-	-	-
	2004	-	-	-	-	-	-

Greenhouse Tools

When compared with the cheaper metal secateurs the stainless steel knife appeared to be easier to sterilise (Tables 15 & 16). Results from two preliminary trials on cheaper metal secateurs (Table 16) suggested that none of the products was completely effective for all bacterial isolates tested with a 1-minute exposure time; although a 10^3ppm solution of sodium hypochlorite was completely efficacious with a 2-min exposure time on a stainless steel knife.

Table15. Disinfectant efficacy for *Cmm* on a stainless steel knife

Disinfectants 1 min contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		+		-		+	+ 500ppm 5min	
1%Sodium hypochlorite	T1		-		-		-	- 2min	
1% Peratec®	T1		-		+		+	-	-
1%Sporekill®	T1		-		-		+	-	-
	T2						-		
1% Virkon®	T1		-		-	+ 1colony		-	-
	T2						+ 1colony		

Table 16. Disinfectant efficacy for *Cmm* on metal secateurs

Disinfectants 1 min contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		+		+		+	+ 500ppm 2min	
	T2								+ 5min
1%Sodium hypochlorite	T1		-		+		-		- 2min
	T2							+ 2min	
1% Peratec®	T1		+		+		+	+	+
1% Sporekill®	T1		+		-		+		+
	T2							-	-
1% Virkon®	T1		+		+		+		+ 5min
	T2							+	+

Neither the 70% ethanol nor 70% denatured ethanol were able to consistently eliminate *Cmm* in 1min. It did appear that with a 1 min from metal blades (Table 17 &18) contact

time the amount of *Cmm* was reduced particularly for the stainless steel knives where a low level was detected at 1 minute. Results were also better without the addition of BSA.

A Quick Dip and 1 min treatment of 1% Virkon on the stainless steel knives was successful in eliminating *Cmm*, although in Part A, (Table 19, Figure 24), a low level was detected.

A 1% Menno Florades® treatment was able to eliminate *Cmm* in 3 minutes for the stainless steel knife. A low level was however detected on the secateurs at 3 minutes particularly with the addition of BSA (Table 19, Figure 25). The recommended rate for treating tools with Menno Florades® is 1% for 3 min.

There was no *Cmm* detected using a 3% solution of Menno Florades® for 1-3 sec, 1 and 3 min on the stainless steel knife or 1 and 3 min on the secateurs.(Table 20).

These results confirmed those in part A that the smooth surface of the stainless steel knife is easier to clean than the metal secateurs.

Table 17. Disinfection of cutting tools with 70% ethanol (wash technique shaded)

70% Ethanol					
Treatment	**Test / Isolate**	**Secateurs**		**SS Knives**	
		-BSA	**+BSA**	**-BSA**	**+BSA**
QD	**T3 2000**	+		+	
	T4 2097	+	+	+ 2 colonies	+
	T5 2097	+	-	-	+
	T6 2008	-	+	-	-
	T7 2004	-	-	-	-
1 min	**T3 2000**	+		+	
	T4 2097	+	+	-	+ 2 colonies
	T5 2097	-	-	-	-
	T6 2008	-	-	-	-
	T7 2004	-	-	-	-
S&W	**T3 2000**	+		+ 4 colonies	
	T4 2097	+	+	+ 1 colony	+
	T5 2097	+	+	-	+
	T6 2008	-	+	-	-
	T7 2004	+	+	+	+

Table 18. Disinfection of cutting tools with 70% denatured ethanol

Treatment	Test / Isolate	Secateurs		SS Knives	
		-BSA	+BSA	-BSA	+BSA
QD	T8 2097	-	-	-	-
	T9 2008	-	-	-	-
	T10 2004	-	+	-	-
1 min	T8 2097	-	-	-	-
	T9 2008	+	-	-	+ 1 colony
	T10 2004	-	+	-	-
S&W	T8 2097	-	-	-	+
	T9 2008	+	+	-	+
	T10 2004	+	+	+	+

Table 19. Disinfection of cutting tools with 1% Virkon

Treatment	Test	Secateurs		SS Knives	
		-BSA	+BSA	-BSA	+BSA
QD	T11 2008	+ 1 colony	+	-	-
	T12 2008	-	+	-	-
	T13 2004	+	+	-	-
1min	T11 2008	-	-	-	-
	T12 2008	-	-	-	-
	T13 2004	-	-	-	-
S&W	T11 2008	+	+	-	+
	T12 2008	+	+	-	+
	T13 2004	+	+	+	+

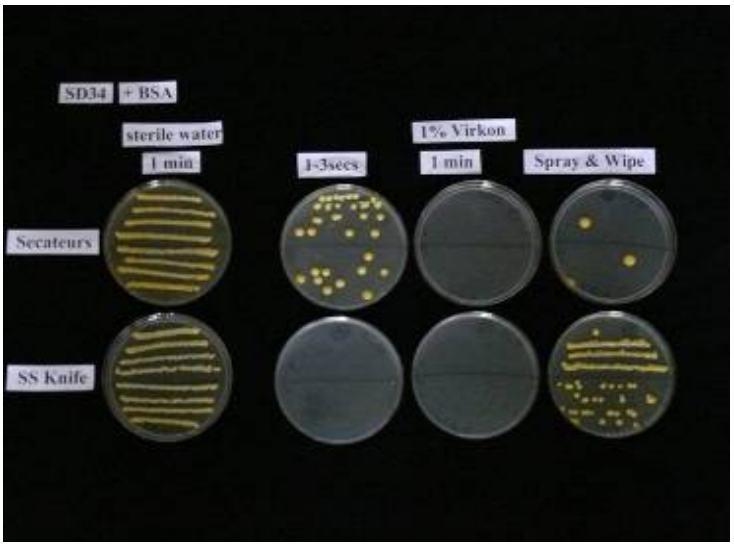


Figure 24. Plates from T11 showing no *Cmm* recovery from cutting tools when treated with 1% Virkon® for 1 minute

Table 20. Disinfection of cutting tools with 1% Menno Florades®

Treatment	Test / Isolate	Secateurs		SS Knives	
		-BSA	+BSA	-BSA	+BSA
QD	**T14 2008**	+	+	-	+
	T15 2008	+	+	-	+
	T16 2004	+	+	-	+
	T17 2004	+	+	-	+ 2 colonies
1 min	**T14 2008**	-	+	-	-
	T15 2008	-	-	-	-
	T16 2004	+ 3 colonies	+ 1 colony	-	-
	T17 2004	-	-	-	+ 4 colonies
3min	**T14 2008**	-	+ 1 colony	-	-
	T15 2008	-	-	-	-
	T16 2004	-	+ 2 colonies	-	-
	T17 2004	-	-	-	-

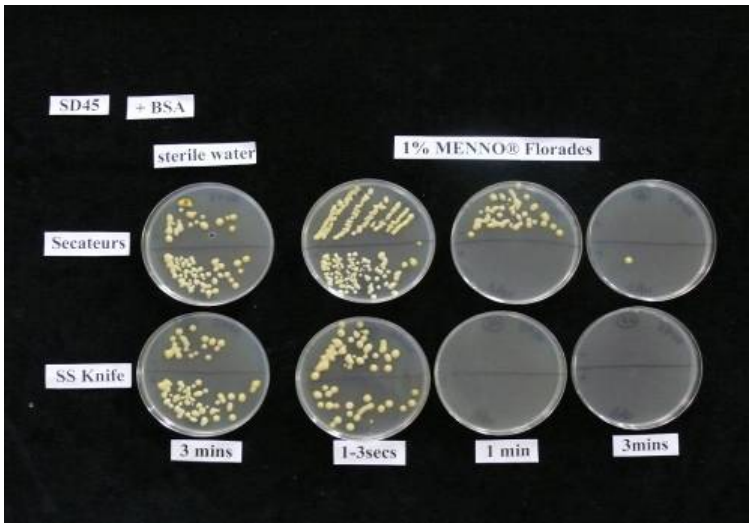


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Figure 25. Agar plates from Test 14 showing no *Cmm* recovery from a stainless steel knife and positive results for secateurs using 1% Menno Florades® for 1 & 3

Table 21. Disinfection of cutting tools with 3% Menno Florades®

Treatment	Test / Isolate	Secateurs		SS Knives	
		-BSA	+BSA	-BSA	+BSA
QD	T14 2008	+	+	-	-
	T15 2008	-	-	-	-
	T16 2004	-	+	-	-
	T17 2004	-	-	-	-
1 min	T14 2008	-	-	-	-
	T15 2008	-	-	-	-
	T16 2004	-	-	-	-
	T17 2004	-	-	-	-
3min	T14 2008	-	-	-	-
	T15 2008	-	-	-	-
	T16 2004	-	-	-	-
	T17 2004	-	-	-	-

The efficacy of disinfectants was demonstrated to be strongly influenced by their concentration, and time of exposure. No single product was consistently able to eliminate *Cmm* from all plastic surfaces, volumes of water or metal blades. There are, however, clear trends for developing best practice guidelines. The use of BSA generally

demonstrated that dirty surfaces or at least the presence of organic matter where attempting disinfection reduces chemical efficacy. It is therefore important to remove organic matter and dirt prior to disinfecting. The cheapest option to disinfect water is to treat it with a 1% hypochlorite and allow sufficient time for the chlorine level to dissipate before using on plants. Five minutes was sufficient to eradicate *Cmm* from water but substantially longer periods (over-night) are generally recommended for the chlorine level to drop.

When compared with the cheaper metal secateurs the stainless steel knife appeared to be easier to sterilise. This is likely to be due to its smoother surface as previously suggested by Kleinhampel *et al*. (1987). They showed that disinfectant solutions will not remove bacterial slime from the surface of cutting tools as the blades are covered with microscopic pits in which bacteria reside. A 3% solution of Menno Florades® was the best disinfectant for metal blades, but it required a 1 minute period for it to kill all *Cmm*. When working in a crop it is recommended that the worker has a few pairs of secateurs soaking in a container and they are rotated between plants or at least every minute to minimise the risk of spreading *Cmm*.

5.2-5.3 Efficacy of microbial biological controls and plant defence response chemicals

Experiment 1: Single treatment with the plant defence activator, Bion®

Analysis 1: Average disease scores on day 75 for plants treated with *Cmm* were significantly higher (3.354) than disease scores for uninfected control plants (0.354). No other significant treatment effects were detected.

Analysis 2: The association between *Cmm* and score category was significant (deviance=297.62; df=5; p<0.001). No other significant treatment effects were detected.

Plant height after 4 weeks for plants treated with Bion® as a seed dressing was on average significantly shorter (8.07 mm) than the heights of those plants which were not (10.49 mm). No other significant treatment effects were detected.

Plant height after 7 weeks for plants treated with Bion® as a seed dressing was on average significantly shorter (28.91 mm) than the heights of control plants (41.49 mm). No other significant treatment effects were detected.

Plant height increase between 4 and 7 weeks for plants treated with Bion® as a seed dressing was on average significantly less (20.83 mm) than the heights of those plants which were not (31.00 mm).

The interaction between *Cmm* and Drench was significant. The average height increase for the *Cmm* treated plants was less (24.52 mm) for drenched plants than for plants not drenched (27.06 mm). For *Cmm* treated plants, drenching did not affect height increase.

The treatments with no deaths were omitted from the data and GLMM analysis was conducted. The treatment effect on plant mortality was significant with a significant LRT observed (chi square=13.8693,df=4, p=0.007724). Differences in the proportions of dead plants for the four *Cmm* treatments were not significant.

Experiment 2: Microbial biocontrols and phosphorous acid

There was no significant treatment effect on disease rating score for the *Cmm* treated plants when data was analysed using either method.

Dry weights for plants inoculated with *Cmm* were on average significantly lighter (64.4 g) than the dry weights of those plants which were not (103.2g). The treatment effect and interaction between *Cmm* and treatment was not significant at the 5% level.

For fruit weight the interaction between *Cmm* and treatment was significant (df=3, 49;p=0.017), suggesting that the response of fruit weight to the 4 treatments in the presence of *Cmm* was different to the response without the bacteria. When *Cmm* was

present fruit weight in the control treatments was significantly heavier (0.518kg) than Phoscare treated fruit (0.343kg). When no *Cmm* was present fruit weight from the control treated plants was significantly less (0.482kg) than fruit from the Phoscare treated fruit (0.657kg), l.s.d.=0.161g. A similar trend was found for wet weight data.

There were no significant treatment effects on stem diameter while stem lengths for plants treated with *Cmm* were on average significantly shorter (162.0cm) than the stem lengths of those plants which were not (182.0cm). The treatment effect and interaction between *Cmm* and treatment was not significant at the 5% level.

The treatment effect of plant mortality was not significant (LRT p= 0.5072)

Experiment 3: Bion®, X-Press®, Potassium silicate and Fulzyme® Plus

There was no significant treatment effect on disease rating score and plant mortality for the *Cmm* treated plants when data was analysed using either method.

The main effect of treatment on plant height was significant ($F=13.05$; $df=4,63$; $p<0.001$). The average heights of plants in the Xpress-Sett and Potassium silicate treatments were significantly shorter than plants in the other 3 treatments. This response was consistent regardless of whether the *Cmm* was present. The effect of *Cmm* on plant height was not significant.

The main effect of *Cmm* on fruit weight was significant ($F=691.42$; $df=1,57$; $p<0.001$). The weight of fruit from plants treated with *Cmm* (0.406) was significantly less than the weight of fruit from the non- *Cmm* treated plants (2.213). The effect of treatment and the interaction between *Cmm* and treatment was not significant.

6.1- 6.3 Development and implementation of appropriate extension and dissemination strategies for the integrated management of bacterial wilt and other wilting diseases in Solanaceous crops

Extension materials such as graphic narrative (Comic), 4 leaflets and poster in English and Visaya were drafted, finalized, printed and distributed to farmers, technicians, students and extension workers during the annual NOMIARC field days and in farmer's forum. Tubers and plants of the different potato varieties were also exhibited. healthy and infected solanaceous plants were also exhibited. Simple diagnosis for bacterial wilt such as the ooze test was demonstrated during field days.

Field experiments at NOMIARC were always visited during field days. Project staff provided visitors a briefing of the experiment. Demonstration of soil incorporation for biofumigation was shown to visitors. A total of 3000 farmers/annually visit NOMIARC field day and the project trials have been showcased for 3 years. Oftentimes, biofumigation was discussed in farmer's meetings and workshops conducted by various organizations and projects.

Collaboration with farmers was established in the conduct of field experiments. This step facilitated the extension of biofumigation technology, varietal screening for tolerance to bacterial wilt and the best practice for potato production. The farmer's of Kapatagan, Imbayao, Kibangay, and Miarayon were invited during field days held at harvest of the crop. Results of the experiment were presented and discussed during the farmer's field days held during harvest of the crop.

Papers on biofumigation were also presented at the International Biofumigation Workshop at Canberra, Australia in 2008; at the FAO's Workshop on Potato Production during the International Year of the Potato held in Dalat, Vietnam in 2008 and a poster at the Asian/Australasian Conference in Plant Pathology at Darwin, Australia in 2011. Six papers and 4 posters were presented in local conferences and meetings. A poster on genetic diversity won the best research paper award in the ACIAR–Bohol meeting in 2011.

The number of BW management strategies being utilised ineffectively through a lack of understanding of the technique and language barriers has been identified as major constraint in transfer of technology. Discussions with researchers and Landcare workers during this project indicated that many Filipino farmers have a clear preference for seeing on-farm results to other forms of extension .A number of farmers were interviewed about their farming practices and problems relating to Bcterial wilt.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The causative strain of BW in different regions of both Australia and Philippines was identified and used to formulate sustainable BW and *Cmm* management strategies. The genetic differences and similarities among BW strains helped to explain the characteristic symptoms and incidence in varying host crops, cropping systems and geographic regions. This study is novel both in the Philippines and in Australia and will contribute in mapping (GIS) the phylotypes of *Ralstonia solanacearum* in the vegetable growing regions and will further strengthen to develop more stable and region specific management strategies.

The optimization of molecular method for detection of bacterial wilt pathogen and real time PCR for bacterial canker of tomato will allow making planting decisions based on the inoculum level of soil and planting material and will be useful to regulate and improve the seed certification and quarantine regulations.

The research conducted in this project has shown, for the first time resistance to copper fungicides in *Cmm.* The results from this study will assist the Australian and Filipino vegetable industry to better manage bacterial diseases. In particular, growers will be able to judge risks of *Cu* resistance and chemical industry to respond and improve efficacy of copper based products.

Similarly the publications of other findings, optimization of molecular detection method, evaluation of tolerant varieties, use of common weed as soil amendment for BW suppression and evaluation of locally available plant /plant parts, organic manures for BW suppression, and aeroponic technology for multiplication of clean planting material will be the guidelines for the future refinement of management strategies of bacterial wilt disease as well for other soil borne diseases. Extract of Bamboo shoots (cyanide), tea tree oil and papaya seeds (Benzyl isothiocyanate) are possible alternatives for making commercial products as all are available locally and proved to inhibit the growth of bacterial wilt pathogen *in vitro* conditions. These findings would lead to further investigations or research on other aspects or application.

8.2 Capacity impacts – now and in 5 years

Three researchers in the Philippines and Australia have enhanced their skills in molecular characterization of the pathogen and have increased their diagnostic capabilities. Eighteen staff members were supported through this project to attend national and International symposia. Three project team members from Philippines and Australia and one collaborator from Landcare Foundation of Philippines travelled to Hanoi Agriculture University and the Field Crops Research Institute in Vietnam to gain an understanding of Vietnamese use of aeroponic technologies in seed potato multiplication and its applicability to the Philippines situation.

Four research assistants and students have enhanced skills in molecular techniques and research capacity such as conducting field experiments, gathering and interpretation of the data. They also were able to attend and present papers in meetings and conferences and conduct extension activities for farmers.

The project supported three university students with their research work (UPLB; Los Banos, Philippines). They successfully completed their research work, submitted thesis and published research papers in various workshops and symposia.

- Ms Danah Jean Concepcion, MSc student conducted research on, “Molecular characterization and analysis of pathogenic variation of *Ralstonia solanacearum* in the Philippines”

- Ms Aira Waje, BSc student completed research work on," Genetic variability of *Ralstonia solanacearum* isolated from soil planted with different solanaceous crops in the Philippines".
- Ms Pearl Jean Binahon, BSc student conducted studies on,"DNA fingerprinting of *Ralstonia solanacearum* isolates from soil collected from potato *(solanum tuberosum)* growing areas in Bukidnon, Philippines".
- Mark Balendres, Research associate got 2nd best paper award in research paper category for research paper entitled,' Molecular Detection, phylotyping and genetic diversity analysis of *Ralstonia solanacearum* isolated from white potato in the Philippines during 4th ACIAR-PCARRD Meeting, Bohol, Philippines, July 20-22, 2011.

More than five hundred farmers, industry representatives and agriculture officials have benefitted through various field days, workshops, conferences and field demonstrations held in both the countries. The technologies for BW management and other wilting diseases in solanaceous crops in the Southern Philippines and Australia were showcased at the 18th NOMIARC field day on 20-22 September 2011. This included biofumigation against BW and rotational cropping systems for BW management. An exhibition booth was erected and lectures and on-site demonstrations given to students, farmers, and local government officials. Leaflets and information materials were produced and distributed to the participants. Vegetable farmers were also interviewed about their farming practices and problems relating to BW. 3,000 farmers and 200 students visited the trial sites and observed the effectiveness of integrated management strategies on BW suppression resulting in production of a high quality potato crops. They realized that a single strategy is not enough to combat BW and that integration of a number of strategies is required.

The project has also been able to improved the tissue culture facilities, of the commercial aeroponic unit at NOMIARC, Philippines, and enhance the molecular diagnostic facility for BW at UPLB. Aeroponic research facilities were also developed at Mareeba, Australia with the aim to standardize the soilless seed potato production technologies enabling tropical countries to reduce the seed potato degeneration problems (due to increased incidence of viruses and soil-borne diseases) and to increase the supply of clean planting material of potato at affordable prices.

In the future this increased awareness, knowledge and skill base among young researchers, students and farmers will help improve disease diagnosis, and education of farmers to adopt appropriate management strategies that will restrict the further spread of diseases within and across their international boundaries.

8.3 Community impacts – now and in 5 years

The research trials conducted on farmer's fields have increased the awareness of Integrated Disease Management (IDM) technology for sustainable potato production. The adoption of these ecofriendly strategies for the management of bacterial wilt and other wilting diseases of solanaceous crops has increased in both the countries and has increased both farm yield and profitability. The major focus of the project was to identify the actual strains of the pathogen; to find out the sources of infection and to find practical solutions for the management of bacterial wilt and other wilting diseases of solanaceous crops.

Project outcomes have been successfully delivered and well communicated among growers through extension activities and field demonstrations at various locations in Mindanao, Philippines and Australia. Farmers have realized the prime importance of clean seed and clean cultivation for successful crop production. The multi-locational testing of potato varieties for BW tolerance and better yield has created interest among farmers to further potato production.

The project has increased awareness and knowledge of soil remediation through biofumigation, soil amendment, crop rotation with non solanaceous crops, field sanitation, and proper disposal of BW infected crop residues and quarantine measures. In future, the adoption of these strategies in integration will definitely reduce the bacterial wilt and other soil borne pathogens, increase yield and stop deforestation in the mountains for opening up of new areas for potato cultivation.

The efforts to increase clean seed supply through aeroponic technology along with use of Integrated Crop Management (ICM) technologies will strengthen development of a sustainable potato industry in the Philippines.

8.3.1 Economic impacts

Having clean seed available will increase the number of potato growers. Growing higher yielding varieties that are tolerant to bacterial wilt and applying potato production best practices will result in improved yield and higher profit. Market price and supply of potato would become more stable. An opportunity to grow other varieties such as processing varieties would create new opportunities for livelihood programs and reduce importation costs of potato products.

Similar benefits will follow from increased yields in tomatoes in both Australia and the Philippines. Greater production efficiency will make Australian exports of truss tomatoes more competitive and negate need for imports.

Initially, the project will generate considerable value to the farmers who can now return to successful potato cropping. This will allow both an increase in the production of potatoes and flexibility for the farmer in terms of replacing lower-return crops with higher-return potatoes.

Reduced tomato crop losses from bacterial canker will increase the viability of greenhouse and field production. As an example, in Australia, losses in a high-technology production greenhouse facility where the crop was expected to yield for over a one year were estimated at 100 plant deaths per week. Cumulative losses reached over one-third of the entire crop within six months and the crop was terminated early. Clearly, these losses are unsustainable with high-cost infrastructure and any significant reduction will increase the viability of this industry sector. Similar losses have been experienced with field tomato production where soil populations can give rise to persistent and endemic disease occurrence. Alternative disease control options now available will increase the economic sustainability of these productions systems.

8.3.2 Social impacts

On-farm demonstration trials for BW management showed increases in crop yield. The Sunstar newspaper documented this positive impact on the farmer's economy in the article "Growing healthy potatoes via bio-fumigation technology", January 18, 2010. In this article Rogelio Gualberto, an experienced vegetable farmer and project co-operator, mentioned that, he adopted biofumigation technology and attained 50% increase in marketable yield from his potato crop and earned over P 60000 net income from just 2,000 sq meter of crop.

Meetings and field days conducted during the project enabled farmers to become active and responsible members of the community by participating in activities.

8.3.3 Environmental impacts

Application of eco-friendly approaches to bacterial wilt management will improve the structural, nutritional and microbial status of the soil, help to reduce the BW population in soil and will decrease the spread of the disease resulting in less virgin land being cleared for potato production. There will be less clearing of native forests for the production on virgin soils free of disease. This in turn will deliver greater prosperity to the community by

preserving sources of high value tourism attractions. Indeed, the indigenous peoples of Mindanao, who are custodians of the forests, will have their way of life preserved. Naturally, food security will be improved for the people of Mindanao, leading to increased social stability.

Integrated management options for bacterial canker of tomatoes based on plant defence activators and microbial biocontrol will also be more sustainable than use of antibiotics or soil fumigants as have been used in some countries to manage this disease.

8.4 Communication and dissemination activities

Six research papers and four posters were submitted for publication and presented at international and local scientific conferences, meetings and workshops, which will help other groups, better understand these widespread and economically important diseases (Section 10.2).

Field demonstrations/training activities/workshops were conducted where results of this project were reported to farmers, industry representatives and a group of greenhouse tomato growers both in the Philippines and in Australia. In particular, discussion was focussed on soil amendments, biofumigation, crop rotation, use of disease free planting material, field sanitation and disposal of infected crop residues.

- Field demonstration in NOMIARC, Malaybalay City, Philippines - September,2009
- Field day at Ravenshoe, Qld, Australia - January, 21,2010
- Field day in NOMIARC, Malaybalay City, Philippines - April, 2010
- Field day at Claveria, Philippines – April,26,2010
- Field day in Sydney, Australia -July,20,2010
- Field day in Adelaide,South Australia – September 2010
- Demonstration Trial Imbayao, Malaybalay City, Philippines– October, 12, 2010
- Demonstration Trial Miarayon , Bukidnon ,Philippines – October, 23, 2010
- Demonstration Trial Kibangay, Malaybalay City, Philippines – October, 26, 2010
- Field day in NOMIARC, Malaybalay City - September,20-23, 2011
- Workshop in Wanneroo, Western Australia, October 2011
- Field day in Atherton, Qld. ,Australia –November,15,2012

Produced and distributed 500 copies of graphic narratives on BW management in English and Visaya at the NOMIARC field days. Fact sheets, flyers and poster were printed and distributed during field days.

Fact sheets

Bacterial Wilt Management of Solanaceous Vegetable Crops (Biovar 3)

Bacterial canker of tomato and its management

Flyers

1) What is bacterial wilt?

2) Symptoms and spread of bacterial wilt disease

3) Economic importance of bacterial wilt

4) Bacterial wilt, a major problem of potato in region 10

5) Biofumigation technology for potato production,

6) Steps to successful biofumigation

7) Integrated strategies for bacterial wilt management in solanaceous crops

Poster - 'Bacterial wilt – What we should know about it'

Social media like web, magazine and local newspaper were used for disseminating information on BW disease management and for delivering project outcomes.

1. Growing healthy potatoes via bio-fumigation technology. Sunstar January 18, 2010.

2. Farmers struggle with crop disease. Mareeba Express Aug, 4, 2010.

3. Unseasonal warm weather increases bacterial wilt risk - News release, August, 5, 2010

http://www.dpi.qld.gov.au

4. Identification and control of Bacterial wilt in solanaceous crops in Australia and inthe Philippines. ausveg.worldsecuresystems.com/publications/PA/PA_FebMar2013_LR.pdf

9 Conclusions and recommendations

9.1 Conclusions

Epidemiological studies of *Ralstonia solanacearum*

The molecular identification of *Ralstonia solanacearum* isolates, has confirmed the presence of tropical strains phylotype I biovar 3 and cool climate strain phylotype II biovar 2 from both the countries.

It is highly unlikely that farmers will be able to identify the type of BW present on their land, therefore IDM strategies were formulated on the basis of distribution of the pathogen (BW strain) tested and rated to use in a BW wilt management plan in both countries. Due to the nature of this soil borne disease, an individual management strategy alone is unlikely to provide effective sustainable management of BW; however when these strategies are used in combination, effective disease management is possible.

Evaluation of Potato varieties to bacterial wilt susceptibility

The use of tolerant varieties alone will not be the complete solution to the problem but along with ICM strategies will definitely contribute to a decrease the inoculum potential. However it should be kept in mind that potato tuber of tolerant varieties should not be saved for seed as they may carry latent infection and introduce disease to unaffected land.

Develop and refine biofumigation technology

Soil amendment with biofumigation and non-biofumigation crops and compost made from readily available farm waste can be a practical and cost effective solution for BW management and could be used as part of an IDM strategy.

Some of the biocontrol agents, plant products and plant extracts from bamboo shoots, pawpaw seeds, and tea tree oil used in the study inhibited the growth of bacterial wilt pathogen (*in vitro*). There now needs to be further studies to evaluate locally available plant/plant extracts/crops/organic products for their anti bactericidal activity on BW pathogen and effect on disease severity.

Evaluate the effect of different crop rotation duration and sowing times

In Mindanao temperatures are generally constant throughout the year. October to December has been evaluated as the best sowing time for potato crop to escape the BW disease as these are the driest months of the year.

The brassica-potato-corn-legumes rotation in Bukidnon has been found effective in reducing the bacterial wilt population in the soil and longer crop rotations (3-4 years) which include non-solanaceous crops for bacterial wilt infected areas is one of the best and cheapest bacterial wilt management strategies for tropical and subtropical areas.

Establish on farm best –bet clean seed production and maintenance

The positive tests for BW pathogen (seed potato) from a number of sites highlighted the risks of seed production under field conditions and potential to spread disease through latently infected planting material.

It was concluded that a critical component of developing a seed system in BW prone areas is isolation of high quality seed potatoes from any source of contamination. These contamination sources may be the soil the seed and/or seedlings are planted into and water used for irrigation. The preliminary studies into soilless production methods; using aeroponic technology has shown to be appropriate for rapid seed multiplication and may provide sustainable supply of clean seed in BW free areas.

Develop improved management strategies for bacterial canker of tomato

The real-time PCR molecular assay has been validated and adopted after collaboration with Dutch and German researchers and could be used to screen imported tomato seed to ensure that it is free of *Cmm*.

A wide range of copper tolerance was observed among *Cmm* isolates and showed need to develop chemical and biological control options; use of bacteriophages (bacterial virus) to minimize the risk of spreading *Cmm* during grafting.

Transfer and adoption of Integrated Disease Management strategies for BW

The language barrier has been identified as major constraint in transfer of technology. Based on farmer feed back, flyers, leaflets, handouts, graphic narrative and posters were developed in local dialect (Visaya) and distributed to farmers during the field days. Technical demonstration plots were conducted at NOMIARC and in farmer's fields to showcase the best practices for BW management.

9.2 Recommendations

Further research

1. That analysis of BW isolates at sequevar level should be further investigated for better understanding of pathogen and for the development of region specific management strategies.

Improved farm management practices

2. That the use of organic and inorganic soil amendments should be promoted as a method to reduce BW incidence. These amendments improve soil structure and build up microbial communities in soil and reduce crop losses.

3. That late blight resistant varieties and IDM practices be used to reduce late blight disease in potato crops.

4. That potassium peroxomonosulphate, benzoic acid and 70% denatured ethanol should be used to disinfect glasshouse equipment and tools to maintain crop hygiene and to reduce the spread of bacterial and viral diseases during glasshouse operations

5. That aeroponic technology for potato seed production be developed and commercialized to strengthen the potato industry in the Philippines.

6. That strict seed certification and quarantine measures be followed to manage BW spread through latently infected planting material.

Improving adoption of results

7. That specialized training courses for researchers and extension workers to be provided to develop and improve diagnostic skills in biotic and abiotic diseases. Further training of Landcare and Agriculture department staff in extension processes would assist in the transfer of new knowledge to potato producers ensuring a more sustainable industry.

8. That a smart Power Point or video should be developed to shows a number of IDM strategies. That could include how to adopt the 14 point plan to improve BW management for potato production.

9. That the concept of Model farms to be introduced to promote sustainable vegetable farming systems in Mindanao. This approach enables fast-tracking of best practice in supply chains, soil health, pest and disease management, and agronomic practices on the model farms while providing detailed data on performance and change. It also enables other growers to see on-farm the benefits of these changes.

10 References

10.1 References cited in report

Andersen GL, Menkissoglou O. and Lindow SE 1991. Occurrence and properties of copper tolerant strains of *Pseudomonas syringae* isolated from fruit trees in California. *Phytopathology*, 81:648-656.

Arman P and Wain RL 1958. The role of leaf exudates in the solution of copper from Bordeaux mixture. *Annals of Applied Biology*, 46:366-374.

Cellier G and Prior P 2010 . Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. *Phytopathology* 100 (11): 1250-61.

Cruz R, Tenreino R, Costa M, Diogo E, Eloy M and Oliveira H. 2002. Genetic diversity of Portuguese isolates of Ralstonia solanacearum by minisatellite primer PCR and BOX-PCR. Abstract of paper presented at the Third International Bacterial Wilt Symposium held from February 4-8, 2002 at South Africa. Posted at http://ibws.nexenservices.com

Fegan M and Prior P 2005. How complex is the "*Ralstonia solanacearum* species complex?" In: Allen C, Prior P and Hayward AC (eds). Bacterial wilt disease and the *Ralstonia solanacearum* species complex. (APS Press: St Paul) pp.449-461.

Hayward AC, El-Nashaar HM, Nydegger U and De Lindo L 1990. Variation in nitrate metabolism in biovars of Pseudomonas solanacearum. *J. Appl. Bacteriol*. 69:269-280.

Hughes M and Trevorrow P 2011. A scoping study on clean seed production and integrated crop management options for potato production in bacterial wilt prone areas of Southern Philippines. Submitted to ACIAR pp13.

Jama B, Palm CA, Buresh RJ, Niang A, Gachengo C, Nziguheba G and Amadalo B. 2000.Tithonia diversifolia as a green manure for soil fertility improvement in Western Kenya; A review Agroforestry systems 49:201-221.

Janse JD, 1988. A detection method for *Pseudomonas solanacearum* in symptomless potato tubers and some data on its sensitivity and specificity. *EPPO/OEPP Bulletin*, 18: 343 – 351.

Kang MJ, Lee MH, Shim JK and Seo ST 2007. PCR-based specific detection of Ralstonia solanacearum by amplification of cytochrome c1 signal peptide sequences. *J. Microbiol.Biotechnology.* 17(11), 1765-1771.

Kelman A 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology.* 44: 693-695.

Kleinhempel H, Nachtigall M, Ficke W and Ehrig F 1987. Disinfection of pruning shears for the prevention of the fire blight transmission. *Acta Horticulturae*, 217 211-218.

Kleitman F, Barash I, Burger A, Iraki N, Falah Y, Sessa G, Weinthal D, Chalupowicz L, Gartemann KH, Eichenlaub R and Manulis-Sasson S. 2008. Characterisation of a *Clavibacter michigasnensis* subsp. *michiganensis* population in Israel. *European Journal of Plant Pathology*, 121:463-475.

Lazaroviits, GC, KL, Abbasi, PA and Tenuta M. 2005. Understanding the mode of action of organic soil amendments provides the way for improved management of soil borne plant pathogens.*Acta Horticulture.*698:215-223.

Lee YA, Schroth MN, Hendson M, Lindow SE, Wang XL, Olson B, Buchner RP and Teviotdale B. 1993. Increased toxicity of iron-amended copper-containing bactericides to the walnut blight pathogen *Xanthomonas campestris* pv. *juglandis*. *Phytopathology* 83:1460-1465.

Lin CH, Hsu ST, Tzeng KC, Wang JF, 2009. Detection of race 1strain of *Ralstonia solnacearum* in field samples in Taiwan using a BIO – PCR method .*Eur. J. Plant Pathology* 124:75-85.

Louws FJ, Medina-Mora CM, Smart CD, Opgenorth D, Ishimuru CA, Hausbeck MK, de Bruijn FJ and Fulbright DW. 1998. Rep-PCR- mediated genomic fingerprinting: a rapid and effective method to identify *Clavibacter michiganensis*. *Phytopathology*, 88:862-868.

Obafemi CA, Sulaimon TO, Akinpelu, DA and Olugbade 2006. Antimicrobial activity of extracts and a germacranolide type esequiterpene lactone from Tithonia diversifolia leaf extract.African *Journal of Biotechnology* 5(12):1254-1258.

Opina N, Tavner F, Hollway G,Wang JF, Li T H, Maghirang R, Fegan M, Hayward AC, Krishnapillai V, Hong WF, Holloway B W and Timmins J 1997. A novel method for development of species and strain-specific DNA probes and PCR primers for identifying *Burkholderia solanacearum* (formerly Pseudomonas solanacearum). Asia *Pac. J. Mol. Biol. Biotechnoogyl*. 5:19-30.

Pradhanang PM, Elphinstone JG and Fox RTV 2000. Sensitive detection of *Ralstonia solanacearum* in soil: a comparison of different detection techniques. *Plant pathology*.49, 414-422.

Scheck HJ and Pscheidt JW 1998. Effect of copper bactericides on copper-resistant and sensitive strains of *Pseudomonas syringae* pv. *syringae. Plant Disease*, 82:397-406.

Scheck HJ, Pscheidt JW and Moore LW 1996. Copper and streptomycin resistance in strains of *Pseudomonas syringae* from pacific northwest nurseries. *Plant Disease* 80: 1034-1039.

Tesoriero L and Milligan M 1991. Identifying and managing vegetable diseases. In *Australian Vegetable Growing Handbook*, Publisher IREC ISBN 0 909677 02 6, pp 73-125.

Zevenhuizen LPTM, Dolfing J, Eshuis EJ and Scholten-Koerselman IJ 1979. Inhibitory effects of copper on bacteria related to the free ion concentration. *Microbial Ecology*, 5:139-146.

10.2 List of publications produced by project

Balendres MAO, Justo VP, Pathania N, Trevorrow P and Dela Cueva, FM 2012. Characterization of *Ralstonia solanacearum* Phylotype I and Reduction of its population in Biofumigated soil. Paper presented at 43rd Pest Management Council of the Philippines Scientific Conference at Mallberry Suites, Cagayan de Oro, Philippines. May 8-11, 2012.

Binahon PJQ, Ardales EY, Dela Cueva FM and Justo VP 2012. DNA Fingerprinting of *R. solanacearum* (E.F.Smith) Yabuuchi *et al.* Isolates from Soil collected from Potato (*Solanum tuberosum*) growing areas in Bukidnon, Philippines. Paper presented at 43rd Pest Management Council of the Philippines Scientific Conference at Mallberry Suites, Cagayan de Oro, Philippines. May 8-11, 2012.

Balendres MAO, Dela Cueva FM, Vergara MJ, Concepcion DAJ, Tiongco RL, Justo VP, Pathania N and Trevorrow P 2011. Molecular Detection, Phylotyping and Genetic Diversity Analysis of *Ralstonia solanacearum* Isolated from White Potato in the Philippines. 4th ACIAR-PCARRD Meeting, Bohol, Philippines, July 20-22, 2011.

Balendres M A O, Dela Cueva FM and Valeriana P Justo (2011). Bacterial Wilt: What You Should Know About It Poster presented during the 18th NOMIARC Field Days and Technology Forum. NOMIARC, Dalwangan, Malaybalay City, Bukidnon, September 20-23, 2011.

Concepcion DJ, Ardales EY, Justo VP, Pathania N, Dela Cueva FM 2012. Phylotype and Pathogenic Analysis of *Ralstonia solanacearum* isolated from white potato in the Philippines. Paper presented at 43rd Pest Management Council of the Philippines Scientific Conference at Mallberry Suites, Cagayan de Oro, Philippines. May 8-11, 2012.

Concepcion DJA, Dela Cueva FM, Ardales EY, Vergara MJ, Balendres MAO, Rizalina L Tiongco RL, Justo VP and Pathania N 2011. Variation in Phylotypes of *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* Isolated from White Potato Tubers. 4th ACIAR-PCARRD Meeting, Bohol, Philippines, July 20-22, 2011.

Concepcion DJA, Dela Cueva FM, Vergara MJ, Tiongco RL, Justo VP and Pathania N 2011. Comparative analysis of *Ralstonia solanacearum* from Mindanao and Northern Luzon. 2011. Paper presented at the 42nd Annual Conference of the Pest Management Council of the Philippines. Bacolod City, May 4-7, 2011.

Dela Cueva FM, Justo VP, Vergara MJ and Concepcion DJ (2011). Genetic Diversity and Pathogenic Variability among isolates of *Ralstonia solanacearum* from white potato-growing areas in Northern Mindanao. Asian/Australasian Conference in Plant Pathology, April 27-29, 2011. Darwin, N. Territory, Australia.

Forsyth LM, Crowe T, Deutscher A and Tesoriero L. (2009) Bacterial canker of tomato: Australian diversity of *Clavibacter michiganensis subsp. michiganensis.* 16th Biennial Australasian Plant Pathology Conference,Newcastle, Australia.

Forsyth LM and Tesoriero L (2009). Bacterial canker of tomato in Australia. Australian Hydroponic Greenhouse Association Conference, Sydney showground, Australia, July19-22, 2009.

Hughes M and Trevorrow P 2011. A scoping study on clean seed production and integrated crop management options for potato production in bacterial wilt prone areas of Southern Philippines. Submitted to ACIAR pp13.

Justo VP, Kirkigaard J, Akiew S, Bayot R, Zorilla R, Lando LA, Abragan F and Dangan JJG 2008. Farmer's Experiences on Biofumigation for Bacterial Wilt Management in Solanaceous Crops in Southern Philippines Paper presented at the 3rd International Biofumigation Symposium at CSIRO Discovery Centre, Canberra, Australia, July 21-25, 2008.

Justo VP 2008. Biofumigation for soil-borne disease management in potatoes. Paper presented at the Workshop on Opportunities and Challenges for Promotion and Sustainable Production of Potato Crop in Vietnam and Elsewhere in Asia, Dalat, Vietnam. November 25-28, 2008.

Pathania N, Trevorrow P, Young A, Wright C and Marton T (2010). Evaluation of biofumigant crops on suppression of *Ralstonia solanacearum* population Abstract .In Annual Scientific Conference at Davao Philippines, 9-12 March 2010.

Pathania N and Trevorrow P (2010). Report on comparative tolerance of commercial potato varieties of North Queensland to *Ralstonia solanacearum* prepared for local potato seed distributor, Sept, 2009.

Pathania N, Trevorrow P, Hughes M, Marton T, Justo VP and Salvani J 2012 Preliminary research to develop low technology aeroponic system for clean potato seed production in The Philippines. ACIAR- PCAARRD End of Programme Horticulture conference, July, 3, 2012, Parklane International Hotel Cebu.

Tesoriero L, Terras MAT and Forsyth LM (2009). New and emerging disease threats to the Australian greenhouse and ornamental industry. Australian Hydroponic Greenhouse Association Conference, Sydney, Australia.

Tesoriero L and Forsyth LM (2011). Characterisation of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the cause of tomato bacterial canker in Australia (Submitted).

Vergara, MJ, Rizalina L T, Dela Cueva FM & Justo VP (2010). PCR Detection, Biochemical Characterization, and Aggressiveness of *Ralstonia solanacearum* Collected from Northern Mindanao. Poster and paper presented during the PMCP Annual Convention and Scientific Conference, Mar 6-9, 2010. Waterfront Hotel, 2010, Davao City.

Vergara MJ, Tiongco Rizalina L, Cueva Dela FM and Justo VP (2010). PCR detection, biochemical characterization and aggressiveness of *R.solanacarum* collected from north Mindanao .Poster presented during the Philippine Phytopathological Society, Inc. Scientific Session .41st Anniversary and Scientific Conference of the Pest Management Council of the Philippines, Inc. at Waterfront Insular Hotel, Davao City, Philippines on March 12, 2010.

Vergara MJ, Concepcion DJ, Tiongco RT, Dela Cueva FM, Justo, VP and Pathania N (2011). Comparative Nucleic Acid- based Analysis of Isolates of *Ralstonia solanacearum* collected from Mindanao and Northern Luzon. Paper presented in 42nd PMCP Annual Convention and Scientific Conference. May 3-6, 2011. Bacolod City.

Waje AF, Ardales EY, Dela Cueva FM and Justo VP 2012. Genetic Variability of *R. solanacearum* (E.F.Smith) Yabuuchi *et al.* Isolated from Soil Planted to Different Solanaceous Crops in the Philippines. Paper presented at 43rd Pest Management Council of the Philippines Scientific Conference at Mallberry Suites, Cagayan de Oro, Philippines. May 8-11, 2012.

11. Appendices

11.1 Appendix 1: Methodology

A. Genetic diversity analysis
DNA extraction and quantification
Extraction of the bacterial genomic DNA was done using the method of Chen and Kuo (1993). Culture of *R. solanacearum* was transferred in a microcentrifuge tube containing one ml of sterile distilled water. The bacterial suspension was centrifuged for 3 minutes at 12,000 rpm. After centrifugation, the supernatant was discarded to obtain the cell pellet. The cell pellet was resuspended in 200 µl of lysis buffer (40mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1 % w/v SDS) and was lyzed by vigorous pippetting. After lysis, 66µl of 5M NaCl solution was added. The contents of the tube were mixed thoroughly by inverting the tubes several times to remove most proteins and cell debris. The resulting viscous mixture was centrifuged at 12,000 rpm for 10 minutes at 4oC. The supernatant was transferred to another clean microcentrifuge tube and was placed on ice. An equal volume of chloroform was added to the supernatant. The tubes were mixed gently at least 50 times until a milky solution was formed. The mixture was centrifuged at 12,000 rpm for 3 minutes to separate the mixture into three different phases: aqueous phase (top), interphase (middle), and organic phase (bottom). The aqueous phase containing the DNA was transferred to another clean microcentrifuge tube. The bacterial DNA was precipitated out of solution by the addition of 2 volumes of 100% ethanol and gentle mixing. A stringy white DNA precipitate was formed that condensed into a tight mass. Centrifugation at 10,000 rpm for 15 minutes was done to obtain the pellet and the alcohol was discarded. The pellet was washed twice with 500 µl of 70% ethanol. The alcohol was removed and the pellet was air dried and dissolved in 50-100 µl TE buffer (pH 8). The quantity and quality of DNA extracted were determined using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Delaware, USA) at the Virology Laboratory, International Rice Research Institute (IRRI), College, Laguna.

B. DNA fingerprinting and Cluster Analysis.
RAPD fingerprints and clustering RAPD primer OPA-07, (GAAACG GGTG) was used for DNA amplification of *R. solanacearum*. A total of 25 ul reaction mixture consisting of 1X buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.67 uM of primer OPA-07, 1 unit of *Taq* polymerase (Invitrogen), and 1 ul volume of bacterial DNA solution as DNA template was assayed in PC-960 thermal cycler (Corbett Research). The assay was performed following the thermal cycling conditions; initial denaturation of 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for1 minute, annealing of 37 °C for 1 minute, and extension of 72 °C for 2 minutes, and a final extension of 72 C for 5 minutes. RAPD products were separated in a 1.5% Agarose gel in 0.5X TAE buffer for 80V for 70min. Only reproducible bands were scored using a binomial system of scoring (1-present, 0-absent). NTSYS software was used to calculate Jaccard's (1908) similarity coefficients. These coefficients were used to construct a dendrogram by the unweighted pair-group method of arithmetic average (UPGMA).

C. Disease severity Ratings (Cmm)

0	No symptoms
1	Slight marginal wilting 1-10% of leaves with wilt
2	11-25% of leaves with wilt
3	Sectored wilting 26-49% of leaves showing wilting associated with chlorosis

4	Pronounced leaf collapse, 50-74% of leaves showing wilting
5	Whole plant wilted

D. Microbial biocontrols and phosphorous acid

The biocontrols used were MicroPlus (*Streptomyces lydicus* WYEC108, Organic Farming Systems), Mycormax® **(**Vesicular-arbuscular mycorrhizal fungi, JH Biotech Inc) and Phoscare® (Phosphorous acid, JH Biotech Inc). There were 8 treatments as follows:

Negative *Cmm* – Treatments 1-4 inoculated with sterile water

1. Control – nil treatment
2. MicroPlus – seed soak followed by weekly drench.
3. Mycormax® – treated at seeding and at transplanting
4. Phoscare® – drenched weekly after germination

Positive *Cmm* – Treatments 5-8 inoculated with *Cmm*

5. Control + Cmm - nil treatment
6. MicroPlus + Cmm – seed soak followed by weekly drench
7. Mycormax® + Cmm – treated at seeding and at transplanting
8. Phoscare® + Cmm – drenched weekly after germination

Statistical analyses methods were as described for Experiment 1 taking into account the 2 x 4 factorial structure of the 8 treatments. In addition to the measurements listed for Experiment 1, plant weight (fresh and dry weight), basal stem diameter and fresh fruit weight were measured and analysed.

E. Bion®, X-Press®, Potassium silicate and Fulzyme® Plus

Design: 10 treatments x 8 replicates x 2 plants. An experimental unit comprised 2 tomato plants. Tomato seed (cv. Petula) were planted into Cocopeat Easyfil Planter Bags set out in 4 channels (rows) as in Experiment 1. Temperature in the greenhouse was recorded on a data logger and averaged 25.6ºC. Plants were grown for 69 days before being harvested and assessed.

The treatments used were Bion® 500 SC (500 g/L acibenzolar-s-methyl- Syngenta Crop Protection), Fulzyme® Plus (*Bacillus subtilis* -JH Biotech Inc), Potassium silicate (Liquid silica K/Si) and X-Press (Copper hydroxide, Manganese and Zinc oxides - Stoller Australia Ltd) + Sett Enhanced (Calcium and Boron – Stoller Australia Ltd). There were 10 treatments as follows:

Negative *Cmm* – Treatments 1-5 inoculated with sterile water

1. Control –nil treatment – weekly spray with water
2. Bion – weekly spray
3. Fulzyme® Plus – weekly spray
4. Potassium silicate – weekly spray
5. X-Press + Sett Enhanced- weekly spray

Positive *Cmm* – Treatments 6-10 inoculated with *Cmm*

6. Control + *Cmm* – nil treatment, weekly spray with water
7. Bion + *Cmm* – weekly spray
8. Fulzyme® Plus + *Cmm* – weekly spray

9. Potassium silicate + *Cmm* – weekly spray

10. X-Press + Sett Enhanced + *Cmm* - weekly spray

Statistical analyses methods were as described for Experiment 1 taking into account the 2 x 5 factorial structures of the 10 treatments.

11.2 Appendix 2: Results from research trials

Table A1 Summary of BW isolates from Northern and Southern Philippines

Area of	Plants	Soil	Tuber
Abatan, Buguias,	-	19	-
Abiang, Atok, Benguet	3	2	-
Bacolongan	3	-	5
Balili, La	9	-	-
Balili, Mankayan,	9	8	-
Bangaio, Buguias,	10	7	-
Bauco, Sinto, Mt.	1	-	-
Buyacoan, Buguia Benguet	29	23	8
Casisang, Malaybalay	1	2	-
Claveria, Misamis	-	2	-
Dalwangan, Malaybalay, Bukidnon	6	53	2
Guinaoang, Mankayan,	-	2	-
Imbayao, Malaybalay,	5	10	-
Impalutao, Bukidnon	-	1	-
Intavas, Impasug-ong,	-	16	-
Kada, Balili, Mankayan, Benguet	-	2	-
Kalasungay, Malaybalay,	1	3	-
Loo, Buguias, Benguet	4	9	13
Los Banos Laguna	6	-	-
Malaybalay City,	-	1	-
Managa, Bansalan,	1	-	-
Manolo Fortich, Bukidnon	-	1	-
Miarayon, Talakag,	34	5	10
Modayan, Buguias,	9	-	1
Mt. Kitanglad, Impasug-ong, Bukidnon	3	2	-
Neptune, Managa,	-	3	7
Salingaw, Buguias,	-	2	17
Sitio Maharlika,	3	-	-
Songco, Lantapan,	-	4	-
Cotcot, Bangao, Bugias	-	-	6
TOTAL (383 Isolates)	**137**	**177**	**69**

Table A 2 List of aggressive isolates

Isolate	Source	Host	Collection site	Initial infection (Days)
WP8,WP15.WP19,WP25 WP28,WP29WP25,WP35 ,WP38,	Plant	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	2
WP44,WP46		*Solanum tuberosum*	Loo, Buguias, Benguet	2
WP49,WP50,WP51,WP5 2,WP54,WP57	Plant	*Solanum tuberosum*	Modayan, Buguias, Benguet	2
WP136	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	2
WP12	Plant	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	3
WP72	Plant	*Solanum tuberosum*	Bangaio, Buguias, Benguet	3
WP137	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	3
WP154,WP162,WP175, WP178	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	3
WP196	Plant	*Solanum tuberosum*	Imbayao, Malaybalay, Bukidnon	3
WP201,WP,202,WP,203, WP207,	Plant	*Solanum tuberosum*	Dalwangan, Malaybalay, Bukidnon	3
WP230,WP231,WP233, WPM2G,WPM2H	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	3
T24,S414-1 P	Soil	*Solanum tuberosum*	Intavas, Impasug-ong	3
T24 -2	Plant	*Lycopersicum esculentum*	Mt. Kitanglad, Impasug-ong, Bukidnon	3
S414-1 P	Soil	*Solanum tuberosum*	Intavas, Impasug-ong	3
WP180T-1,WP180T-3	Tuber	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	3
WP7,WP16,WP17,WP33	Plant	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	4
WP100	Plant	*Solanum tuberosum*	Balili, Mankayan,	4

			Benguet	
WP130	Plant	*Solanum tuberosum*	Abiang, Atok, Benguet	4
WP139,WP140,WP142	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	4
WP165,WP176,WP177	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	4
WP189,WP191,WP192, WP195	Plant	*Solanum tuberosum*	Imbayao, Malaybalay, Bukidnon	4
WP206,WP213,WP216, WP217,WP222,WP232, WPM2F	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	4
S257 - L	Soil	*Solanum tuberosum*	Sitio Bekes, Buyacaoan, Buguias, Benguet	4
S416-2 P,S416-3 P	Soil	*Solanum tuberosum*	Intavas, Impasug-ong	4
S466-2 P	Soil	*Solanum tuberosum*	Imbayao, Malaybalay City	4
S474-1 P,S474-2, S474-6P,S481-10 P,S481-3 P	Soil	*Solanum tuberosum*	NOMIARC, Malaybalay City	4
WP162T-2	Tuber	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	4
WP31T-1,	Tuber	*Solanum tuberosum*	Bekes, Buyacaoan, Bugias	4
WP135	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	5
WP145,WP163,WP181	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	5
WP190	Plant	*Solanum tuberosum*	Imbayao, Malaybalay, Bukidnon	5
WP200	Plant	*Solanum tuberosum*	Dalwangan, Malaybalay, Bukidnon	5
S207 - 3	Soil	*Solanum tuberosum*	Sitio Loo, Brgy. Abatan, Buguias, Benguet	5
S257-1	Soil	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	5

S262 - 1,S265-4,S267-7-2,S267-L,S267-L	Soil	*Solanum tuberosum*	Sitio Bekes, Buyacaoan, Buguias, Benguet	5
S343 - 1	Soil	*Solanum tuberosum*	Kada, Balili, Mankayan, Benguet	5
S414-2 P,S414-4 P,S414-5 P,	Soil	*Solanum tuberosum*	Intavas, Impasug-ong	5
S464-4 P	Soil	*Solanum tuberosum*	Imbayao, Malaybalay City	5
S474-4 P,S474-5P.S474-7P,S479-5P,S481-12 P,S481-13 P,P,S481-14 P,P,S481-5 P,P,S481-6 P,P,S481-7 P,P,S481-8 P	Soil	*Solanum tuberosum*	NOMIARC, Malaybalay City	5
WP33T-14,WP33T-2,WP34T-5	Tuber	*Solanum tuberosum*	Salingaw, Buyacaoan, Bugias	5
WP64T-4,	Tuber	*Solanum tuberosum*	Naduguan, Bacolongan Norte, Buigias, Benguet	5
S495T-4	Soil	*Solanum tuberosum*	Neptune, Managa, Bansalan	5
WP21,WP23	Plant	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	6
WP69	Plant	*Solanum tuberosum*	Bangaio, Buguias, Benguet	6
WP132,WP133	Plant	*Solanum tuberosum*	Abiang, Atok, Benguet	6
WP134	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	6
WP160,WP211	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	6
A48,A49,A50	Plant	*Momordica charantia*	Los Banos Laguna	6
P5-2,	Plant	*Capsicum annum*	Mt. Kitanglad, Impasug-ong, Bukidnon	6
P5-3	Plant	*Capsicum annum*	Casisang, Malaybalay, Bukidnon	6
T35	Plant	*Lycopersicum esculentum*	Kalasungay, Malaybalay, Bukidnon	6

S207 - 7 - 1,S208-1,S258-7-1	Soil	Solanum tuberosum	Sitio Loo, Brgy. Abatan, Buguias, Benguet	6
S406-4 P	Soil	Lycopersicum esculentum	Songco, Lantapan,	6
S408-3 P	Soil	Lycopersicum esculentum	Kalasungay, Malaybalay City	6
S413T-3	Tuber	*Solanum tuberosum*	Intavas, Impasug-ong, Bukidnon	6
S458-3 P	Soil	*Solanum tuberosum*	Brgy. Miarayon, Talakag	6
S479-1 P,S418-11P	Soil	*Solanum tuberosum*	NOMIARC, Malaybalay City	6
S55-1		*Capsicum annum*	Manolo Fortich, Bukidnon	6
S6-1-1,S6-1-2	Soil	Lycopersicum esculentum	Malaybalay City, Bukidnon	6
S75-1,S75-2	Soil	Capsicum annum	Claveria, Misamis Oriental	6
S90-1	Soil	*Lycopersicum esculentum*	Sitio Gabunan, Casisang, Malaybalay City, Bukidnon	6
WP210T-1,WPT210T8,WP210T-9	Tuber	*Solanum tuberosum*	Neptune, Managa, Bansalan	6
WP31T-2	Tuber	*Solanum tuberosum*	Bekes, Buyacaoan, Bugias	6
WP33T-3	Tuber	*Solanum tuberosum*	Salingaw, Buyacaoan, Bugias	6
WP46T-6	Tuber	*Solanum tuberosum*	Loo, Bugias, Benguet	6
WP84T-2,WP84 T-3	Tuber	*Solanum tuberosum*	Cotcot, Bangao, Bugias	6
WP138	Plant	*Solanum tuberosum*	Balili, La Trinidad, Benguet	7
WP159	Plant	*Solanum tuberosum*	Miarayon, Talakag, Bukidnon	7
A1	Plant	Momordica charantia	Los Banos Laguna	7
S207 - 4,S207-5,S258-1	Soil	*Solanum tuberosum*	Sitio Loo, Brgy. Abatan, Buguias, Benguet	7

S258-3	Soil	*Solanum tuberosum*	Buyacoan, Buguias, Benguet	7
S265-7-1,S267-4	Soil	*Solanum tuberosum*	Sitio Bekes, Buyacaoan, Buguias, Benguet	7
S412T-1	Tuber	*Solanum tuberosum*	Intavas, Impasug-ong, Bukidnon	7
S458-2 P	Soil	*Solanum tuberosum*	Brgy. Miarayon, Talakag	7
S466-1 P	Soil	Solanum tuberosum	Imbayao, Malaybalay City	7
S479-14 P,S481-1P	Soil	Solanum tuberosum	NOMIARC, Malaybalay City	7
S495T-2,S495T-3	Soil	Solanum tuberosum	Neptune, Managa, Bansalan	7
WP178T-1	Tuber	Solanum tuberosum	Miarayon, Talakag, Bukidnon	7
WP20T-6	Tuber	Solanum tuberosum	Neptune, Managa, Bansalan	7
WP210T-3	Tuber	Solanum tuberosum	Neptune, Managa, Bansalan	7
WP21T-1	Tuber	Solanum tuberosum	Bekes, Buyacaoan, Bugias	7
WP33T10,WP33T15,WP 33T4,WP34T-6	Tuber	Solanum tuberosum	Salingaw, Buyacaoan, Bugias	7
WP45T-3	Tuber	Solanum tuberosum	Loo, Bugias, Benguet	
WP64T-3	Tuber	Solanum tuberosum	Naduguan, Bacolongan Norte, Buigias, Benguet	
WP30	Plant	Solanum tuberosum	Buyacoan, Buguias, Benguet	8
WP81	Plant	Solanum tuberosum	Bangaio, Buguias, Benguet	8
S257 - 3,S258-6-2,S258-7-2,S265-5,S270-1	Soil	Solanum tuberosum	Sitio Bekes, Buyacaoan, Buguias, Benguet	8
S406-2 P	Soil	Lycopersicum esculentum	Songco, Lantapan,	
S479-2 P,S479-3P,S479-4P,S481-2P,S481-4P	Soil	Solanum tuberosum	NOMIARC, Malaybalay City	8

S88-1	Soil	Lycopersicum esculentum	Mt. Kitanglad, Impasug-ong, Bukidnon	8
WP210T-2,WP210T-4,	Tuber	Solanum tuberosum	Neptune, Managa, Bansalan	8
WP33T-11,	Tuber	Solanum tuberosum	Salingaw, Buyacaoan, Bugias	8
WP46T-5	Tuber	Solanum tuberosum	Loo, Bugias, Benguet	8
WP33T-1,WP34T-2	Tuber	Solanum tuberosum	Salingaw, Buyacaoan, Bugias	8
WP34T-2	Tuber	Solanum tuberosum	Salingaw, Buyacaoan, Bugias	9
WP45T-1	Tuber	Solanum tuberosum	Loo, Bugias, Benguet	9
WP64T-1	Tuber	Solanum tuberosum	Naduguan, Bacolongan Norte, Buigias, Benguet	9
WP36	Plant	Solanum tuberosum	Buyacoan, Buguias, Benguet	9
WP119	Plant	Solanum tuberosum	Balili, Mankayan, Benguet	9
A47	Plant	Momordica charantia	Los Banos Laguna	9
S267 - 5	Plant	Solanum tuberosum	Sitio Bekes, Buyacaoan, Buguias, Benguet	9
S458-1 P	Soil	Solanum tuberosum	Brgy. Miarayon, Talakag	9
S464-1 P,S464-3P	Soil	Solanum tuberosum	Imbayao, Malaybalay City	9
WP20T-1	Tuber	Solanum tuberosum	Bekes, Buyacaoan, Bugias	9

* Isolates, which showed initial symptoms of BW after 9 days of inoculation are not included in this table

Table A 3 Biochemical analysis for biovar determination

Isolate	Phylotype	Biovar Based on Phylotyping	Biovar based on Biochemical Test
WP140	1	3	3
WP180	1	3	3
WP195	1	3	3
WP202	1	3	3
WP207	1	3	3
WP211	1	3	3
WP216	2	2	2
P5-3	1	3	3
S258-3	2	2	2
S481-2	1	3	3
S90-2	1	3	3
S88	1	3	3

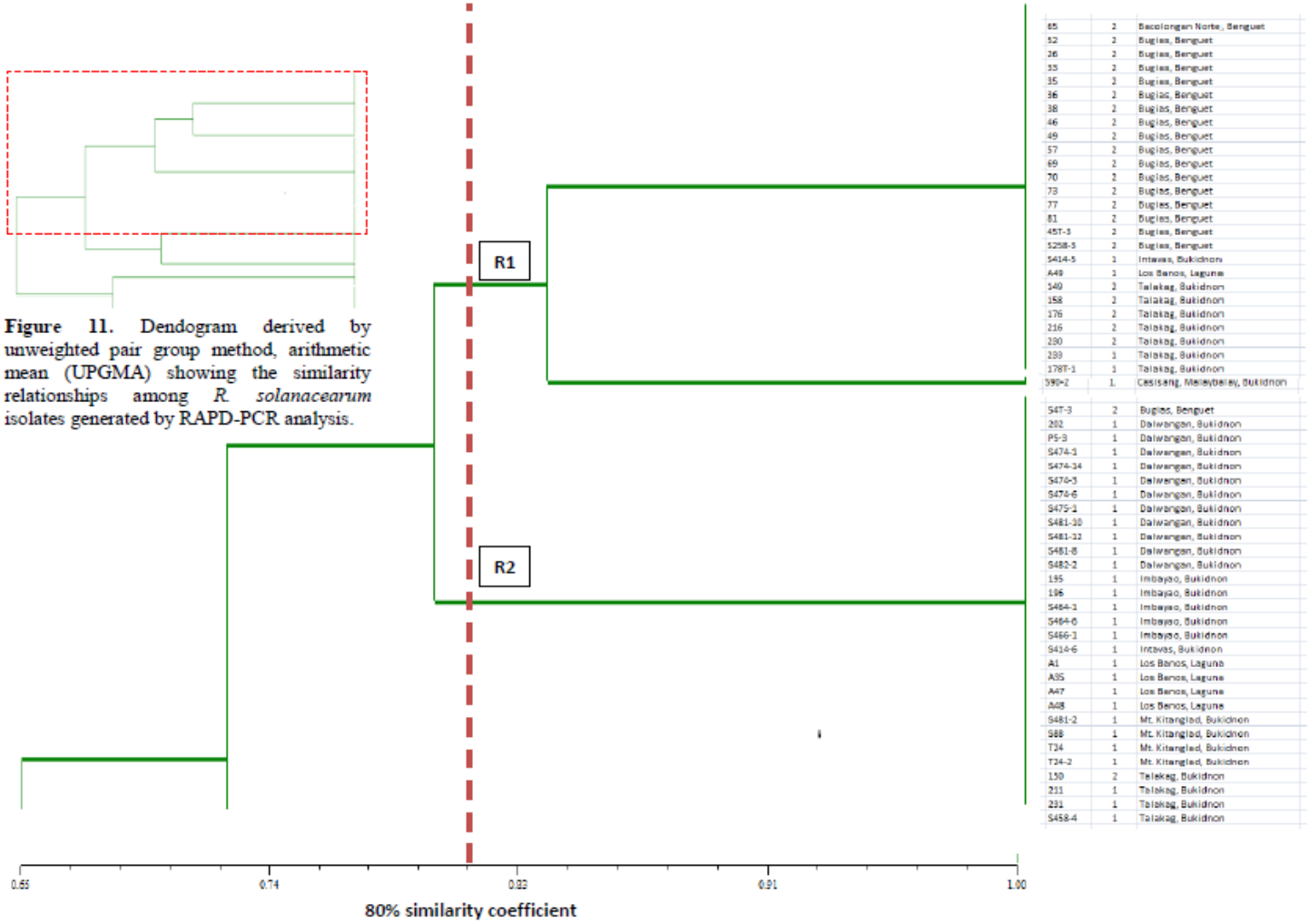


Figure 11. Dendogram derived by unweighted pair group method, arithmetic mean (UPGMA) showing the similarity relationships among *R. solanacearum* isolates generated by RAPD-PCR analysis.

65	2	Bacolongan Norte, Benguet
52	2	Buglas, Benguet
26	2	Buglas, Benguet
33	2	Buglas, Benguet
35	2	Buglas, Benguet
36	2	Buglas, Benguet
38	2	Buglas, Benguet
46	2	Buglas, Benguet
49	2	Buglas, Benguet
57	2	Buglas, Benguet
69	2	Buglas, Benguet
70	2	Buglas, Benguet
73	2	Buglas, Benguet
77	2	Buglas, Benguet
81	2	Buglas, Benguet
457-3	2	Buglas, Benguet
5258-3	2	Buglas, Benguet
S414-5	1	Intavas, Bukidnon
A49	1	Los Banos, Laguna
149	2	Talakag, Bukidnon
158	2	Talakag, Bukidnon
176	2	Talakag, Bukidnon
216	2	Talakag, Bukidnon
230	2	Talakag, Bukidnon
233	1	Talakag, Bukidnon
178T-1	1	Talakag, Bukidnon
590-2	1	Casisang, Malaybalay, Bukidnon
S4T-3	2	Buglas, Benguet
202	1	Dalwangan, Bukidnon
P5-3	1	Dalwangan, Bukidnon
S474-1	1	Dalwangan, Bukidnon
S474-14	1	Dalwangan, Bukidnon
S476-3	1	Dalwangan, Bukidnon
S476-6	1	Dalwangan, Bukidnon
S475-1	1	Dalwangan, Bukidnon
S481-10	1	Dalwangan, Bukidnon
S481-12	1	Dalwangan, Bukidnon
S481-8	1	Dalwangan, Bukidnon
S482-2	1	Dalwangan, Bukidnon
195	1	Imbayao, Bukidnon
196	1	Imbayao, Bukidnon
S464-1	1	Imbayao, Bukidnon
S464-6	1	Imbayao, Bukidnon
S466-1	1	Imbayao, Bukidnon
S414-6	1	Intavas, Bukidnon
A1	1	Los Banos, Laguna
A35	1	Los Banos, Laguna
A47	1	Los Banos, Laguna
A48	1	Los Banos, Laguna
S481-2	1	Mt. Kitanglad, Bukidnon
S88	1	Mt. Kitanglad, Bukidnon
T34	1	Mt. Kitanglad, Bukidnon
T34-2	1	Mt. Kitanglad, Bukidnon
150	2	Talakag, Bukidnon
211	1	Talakag, Bukidnon
231	1	Talakag, Bukidnon
S458-4	1	Talakag, Bukidnon

Figure A1 Dendrogram derived by unweighted pair group method arithmatic mean showing the similarity between BW isolates(Contd. -p82)

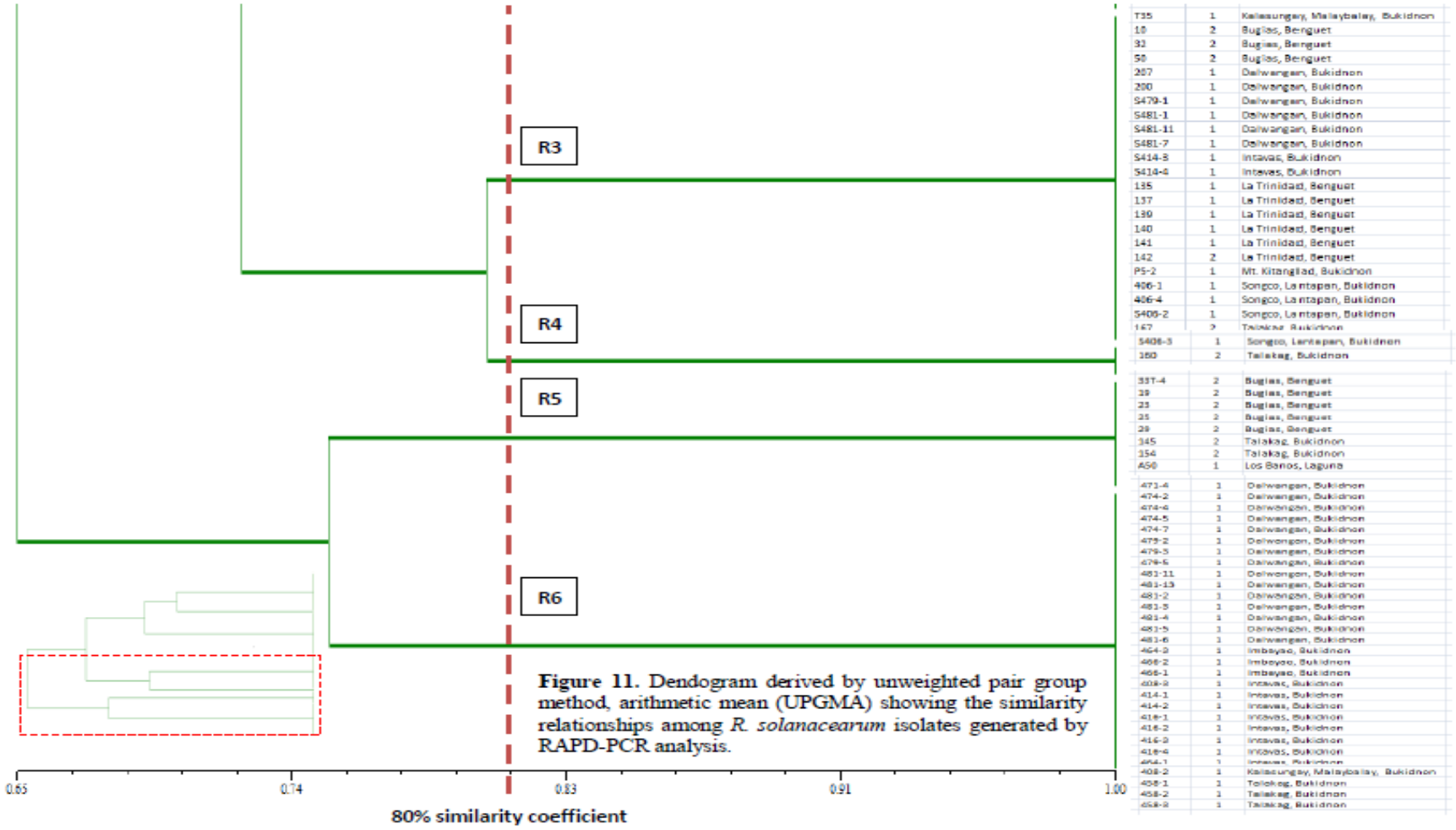


Figure 11. Dendogram derived by unweighted pair group method, arithmetic mean (UPGMA) showing the similarity relationships among *R. solanacearum* isolates generated by RAPD-PCR analysis.

Figure. A1 Dendrogram derived by unweighted pair group method arithmatic mean showing the similarity between BW isolates

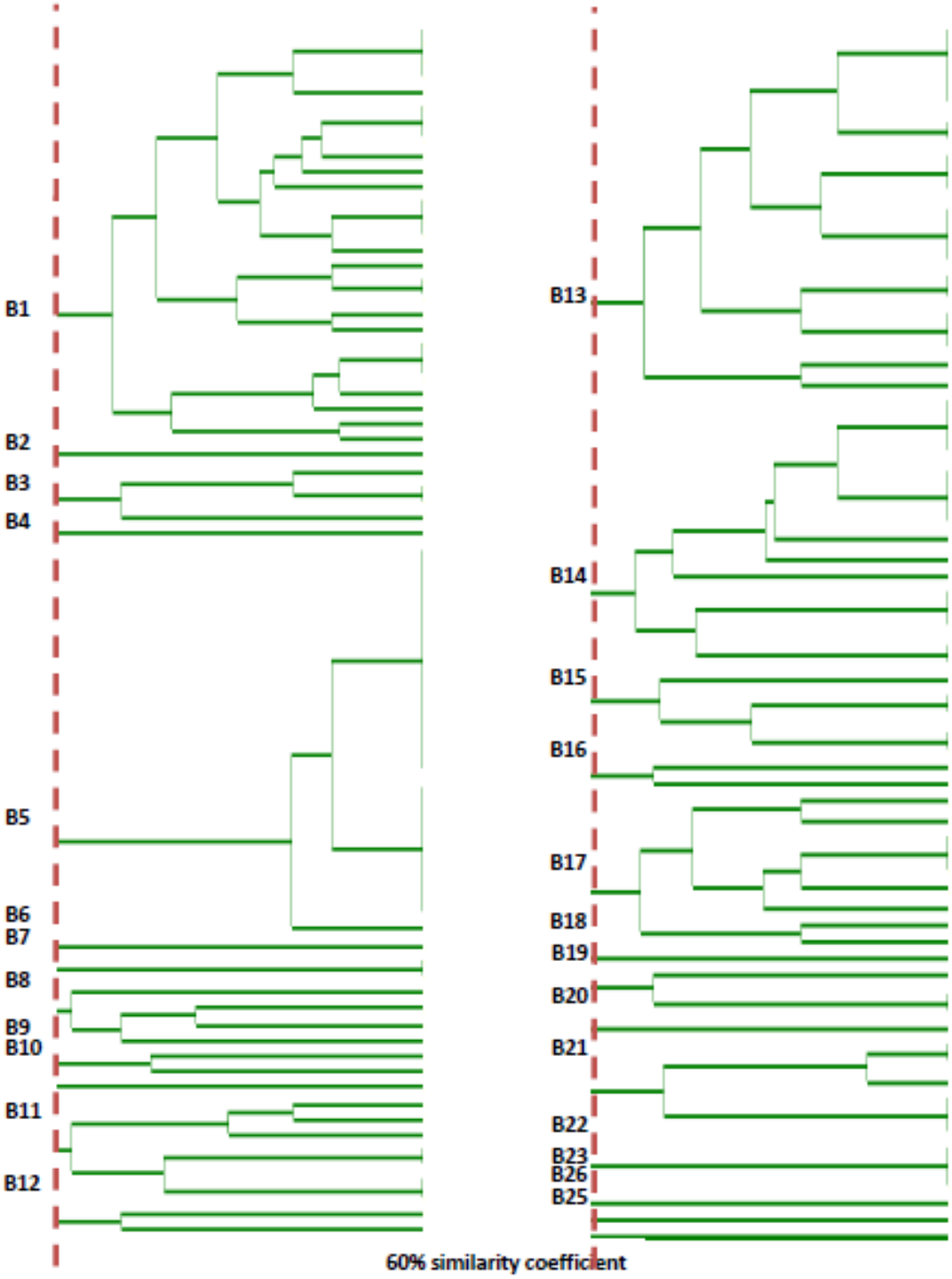


Figure A 2. Unique Box cluster identified from BW isolatesobtained from Bukhidnon and Benbuet. Cluster designation consists of a first letter denoting the primer used followed by a number indicating the cluster

Table A 4. List of BW isolates from CTA collection for Phylotyping and genetic variation studies

	ID	Biovar	Host	Location				Group
1	6935	2	Potato	N/A				Biovar 2
2	6942	2	Potato	Tolga	Atherton Table			Biovar 2
3	2029	2	Eggplant	Home Hill	Coastal Tropics			Biovar 2
4	5525	2	Potato	Atherton	Atherton Table			Biovar 2
5	5334	2	Potato	Evelyn	Atherton Table			Biovar 2
6	14056-VG	2	Potato		Victoria			Biovar 2
7	14056-AG	2	Potato		Victoria			Biovar 2
22	5289	3	Tomato	South edge	Atherton Table			Mixed origin
25	5336	3	Orn. Ginger	Gordonvale	Coastal Tropics			Mixed origin
35	5463	3	Tomato	Mareeba	Atherton Table			Mixed origin
42	5760	3	Snakeweed	Lochart	Cape York			Mixed origin
43	5779	3	Billygoat weed	Cape York	Cape York			Mixed origin
48	5852	3	Tomato	Bundaberg	Southern Coastal Qld			Mixed origin
50	5867	3	Tomato	N Bundaberg	Southern Coastal Qld			Mixed origin
55	6143	3	Antherium	Aloomba	Coastal Tropics			Mixed origin
56	6158	3	Antherium	Aloomba	Coastal Tropics			Mixed origin
58	6192	3	Tomato	Walkamin	Atherton Table			Mixed origin
76	9832	3	Lisianthus	Mareeba	Atherton Table			Mixed origin
78	13754	3	Potato	Tolga	Atherton Table			Mixed origin
79	13842	3	Eggplant	Hopevale	Coastal Tropics			Mixed origin
80	13843	3	Tomato	Hopevale	Coastal Tropics			Mixed origin
82	Cont B 2	2						Mixed origin
40	5691	3	Tomato	Julatten	Atherton Table			Mixed origin
8	4938	3	Eggplant	Ingham	Coastal Tropics			Atherton Tablelands-1
24	5303	3	Tomato	Dimbulah	Atherton			Atherton

					Table			Tablelands-1
30	5380	3	Eggplant	Lochart River	Cape York			Atherton Tablelands-1
37	5671	3	Tomato	Tolga	Atherton Table			Atherton Tablelands-1
45	5818	3	Tomato	Bundaberg	Southern Coastal Qld			Atherton Tablelands-1
46	5825	3	Tomato	Bundaberg	Southern Coastal Qld			Atherton Tablelands-1
47	5851	3	Tomato	Bundaberg	Southern Coastal Qld			Atherton Tablelands-1
53	6094	3	Tobacco	South edge	Atherton Table			Atherton Tablelands-1
54	6125	3	Tomato	Emerald Creek	Atherton Table			Atherton Tablelands-1
57	6165	3	Tomato	Ravenshoe	Atherton Table			Atherton Tablelands-1
60	6251	3	Tomato	Dimbulah	Atherton Table			Atherton Tablelands-1
62	6441-1	3	Eucalyptus	Innisfail	Coastal Tropics			Atherton Tablelands-1
64	6456	3	Tomato	Mareeba	Atherton			Atherton Tablelands-1
67	6664	3	Tomato	Herberton	Atherton			Atherton Tablelands-1
70	6708	3	Chillies	Mareeba	Atherton			Atherton Tablelands-1
71	7070	3	Strelitzia	Malanda	Atherton			Atherton Tablelands-1
72	7484	3	Capsicum	Walkamin	Atherton			Atherton Tablelands-1
73	7494	3	Tomato	Malanda	Atherton			Atherton Tablelands-1
77	13394	3	Eggplant	Mackay	Coastal Tropics			Atherton Tablelands-1
83	Cont B 3	3						Atherton Tablelands-1
9	4981	3	Tomato	Paddy's Green	Atherton			Atherton Tablelands-1
31	5389	3	Heliconia	Redlynch	Coastal Tropics			Atherton Tablelands-1
34	5457	3	Heliconia	Redlynch	Coastal Tropics			Atherton Tablelands-1
38	5673	3	Tomato	Paddy's Green	Atherton			Atherton Tablelands-1
65	6563	3	Potato	Evelyn	Atherton			Atherton Tablelands-1
12	5175	3	Alexandra Palm	Clifton Beach	Coastal Tropics			Coastal Tropics
14	5240	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics

15	5244	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics
16	5245	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics
18	5271	3	Heliconia	Crystal Cascades	Coastal Tropics			Coastal Tropics
19	5272	3	Heliconia	Crystal Cascades	Coastal Tropics			Coastal Tropics
20	5273	3	Heliconia	Crystal Cascades	Coastal Tropics			Coastal Tropics
26	5358	3	Heliconia	Mossman	Coastal Tropics			Coastal Tropics
32	5402	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics
33	5446	3	Eggplant	Paddy's Green	Atherton			Coastal Tropics
36	5512	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics
39	5663	3	Heliconia	Redlynch	Coastal Tropics			Coastal Tropics
41	5752	3	Tomato	Herberton	Atherton			Coastal Tropics
44	5774	3	Diploid banana	Sth Johnstone	Coastal Tropics			Coastal Tropics
51	6039	3	Alexandra Palm	Mission Beach	Coastal Tropics			Coastal Tropics
52	6039 (2)	3	Alexandra Palm	Mission Beach	Coastal Tropics			Coastal Tropics
61	6397	3	Eucalyptus	Innisfail	Coastal Tropics			Coastal Tropics
63	6441	3	Eucalyptus	Innisfail	Coastal Tropics			Coastal Tropics
74	9431	3	Eggplant	Mossman	Coastal Tropics			Coastal Tropics
75	9826	3	Marigold	Cairns	Coastal Tropics			Coastal Tropics
17	5263	3	Heliconia	Crystal Cascades	Coastal Tropics			Coastal Tropics
21	5274	3	Heliconia	Crystal Cascades	Coastal Tropics			Coastal Tropics
81	13970	3	Tomato	Mareeba	Atherton			Coastal Tropics
10	4991	3	Tobacco	Springs Rd	Atherton			Atherton Tablelands-2
11	4992	3	Tobacco	South edge	Atherton			Atherton Tablelands-2
13	5230	3	Tobacco	Springs Rd	Atherton			Atherton Tablelands-2
23	5290	3	Tobacco	Springs Rd	Atherton			Atherton Tablelands-2
27	5361	3	Tobacco	South edge	Atherton			Atherton Tablelands-2
28	5370	3	Tobacco	South edge	Atherton			Atherton Tablelands-2
29	5372	3	Cobblers	South edge	Atherton			Atherton

			Peg					Tablelands-2
49	5360	3	*S. nigrum*	South edge	Atherton			Atherton Tablelands-2
66	6603	3	Tobacco	Springs Rd	Atherton			Atherton Tablelands-2
68	6689	3	Peanut	South edge	Atherton			Atherton Tablelands-2
69	6690	3	Peanut	South edge	Atherton			Atherton Tablelands-2
59	6194	3	Tomato	Moreton P.O.	Cape York			Atherton Tablelands-2

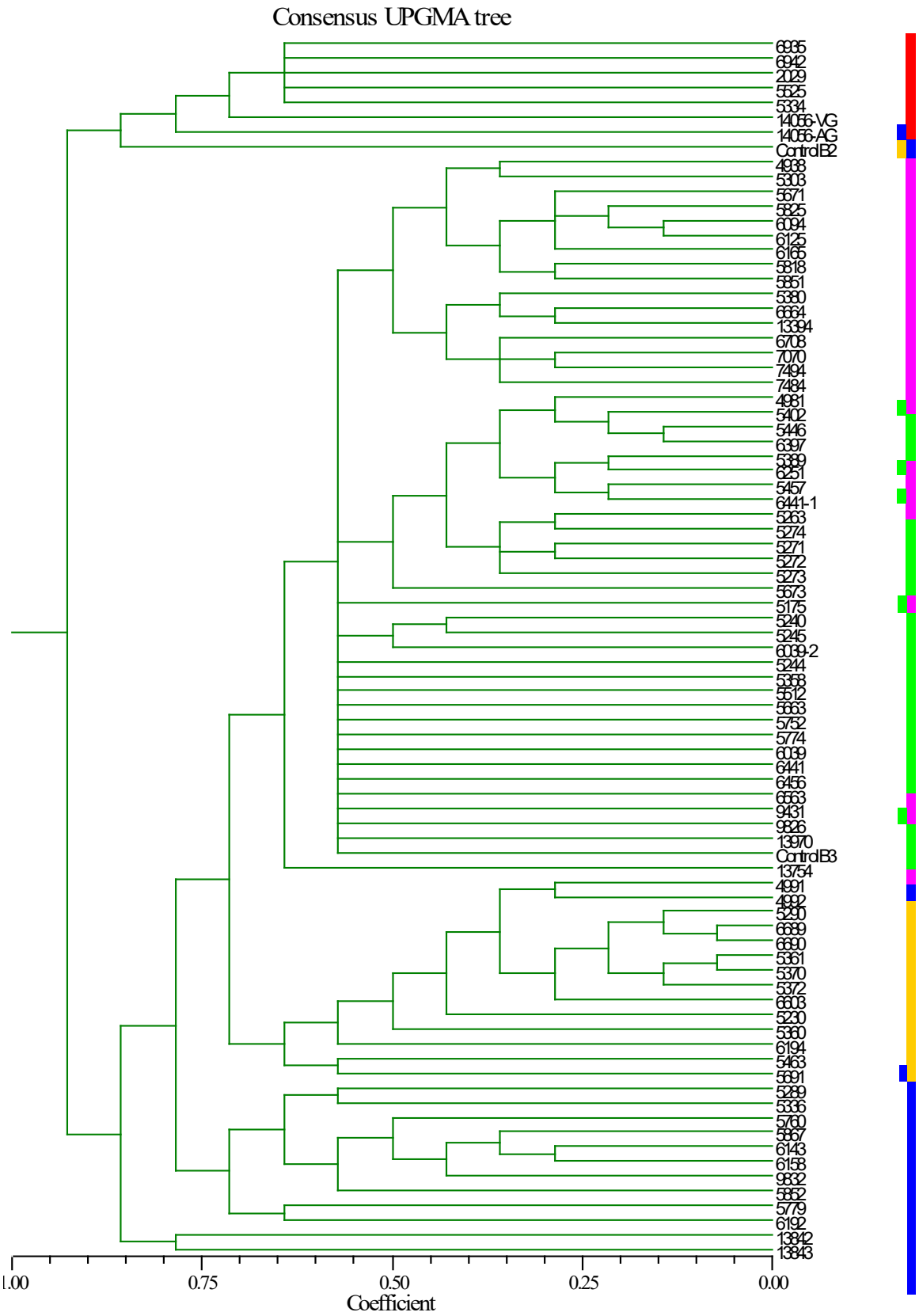


Figure A 3 .Dendrogram derived by unweighted pair group method arithmetic mean (UPGMA) showing the similarity relationship among BW isolates derived by RAPD-PCR analysis

Table A 5. Detection of BW pathogen from naturally and artificially inoculated water, potato tuber and infected plant parts using direct plating, enrichment and Mplex PCR

Test sample	**Detection Level (cfu/ml)**		
	Plating Method (pre enrichment)	**Plating Method (post enrichment)**	**Multiplex PCR**
Sterilized Distilled Water (artificial inoculation)	10^1	10^1	10^1
Creek water (Artificial inoculation)	10^2	10^4	10^2
Sterile Soil (Artificial inoculation)			
Clay soil	10^4	10^2	10^3
Sandy Loam soil	10^3	10^2	10^3
Sandy soil	10^3	10^2	10^2
Non sterile soil (Artificial inoculation)			
Clay soil,	10^4	10^2	10^3
Loam soil	10^4	10^2	10^2
sandy soil	10^3	10^2	10^4
Soil (Naturally infested Tomato field)	10^4	10^2	10^3
Tomato			
Leaf,	10^3	10^1	10^1
Root	10^1	10^1	10^1
Stem	10^2	10^1	10^1
Potato tuber	10^3	10^3	10^4

Table A 6 Screening of biocontrol agent, chemicals, plant extract and organic manures on growth of BW pathogen

Treatment	Concentration used	Growth inhibition (mm)
Biocontrol agents		
Enterobacter spp	10^5 cfu / ml	19.0 ^{g}
Bacillus spp	10^5 cfu / ml	16.0 ^{f}
Trichoderma spp	10^5 spores/ ml	12.0 ^{de}
Baeuveria bassianna	10^5 spores/ ml	9.3 ^{cd}
Metarhizium spp	10^5 spores/ ml	6.0 ^{b}
Plant extract		
Nemfix	10% alcohol extract	14.3 ^{ef}
Radish	10% alcohol extract	15.3 ^{f}
BQ mulch	10% alcohol extract	11.7 ^{de}
Japanese Wild sunflower	10% alcohol extract	6.0 ^{b}
Paw paw seed	10% alcohol extract	26.7 ^{ij}
Bamboo shoot	10% alcohol extract	25.7 ^{i}
Ghost chilli	10% alcohol extract	29.3 ^{jk}
Tea tree oil	Commercial formulation	30.0 ^{k}
Canola oil	Commercial formulation	7.0 ^{bc}
Neem oil	Commercial formulation	6.0 ^{b}
Chemical/Fertilizer		
Urea	1000 ppm	0.0 ^{a}
Lime sulphur	1000 ppm	24.0 ^{hi}
Copper sulphate	1000 ppm	27.0 ^{ij}
Streptomycin sulphate	500ppm	21.3 ^{gh}
Organic Manure		
Compost	Tea (brewed for 7 days)	0.0 ^{a}
Chicken manure	Tea (brewed for 7 days)	6.0 ^{b}
Sheep manure	Tea (brewed for 7 days)	0.0 ^{a}
Cow manure	Tea (brewed for 7 days)	0.0 ^{a}
p-value	<0.001	
SED	1.36	
95% LSD	2.78	

Figure A 4. Construction of aeroponic units for potato seed production

	
Old iron structure utilized for outer frame of aeroponic box; wood or bamboo may be used to make it cost effective	Lined with polystyrene (2 inch thick) to maintain optimum temperature and moisture condition for optimum development of root and tuber formation
	
Installation of nutrient tank and pump for recirculation of nutrient solution	Lined with black plastic to avoid light penetration for root growth and to make it leak proof for collection of nutrient solution
	
Aeroponic unit ready to fix curtains (inner curtain to prevent the nebulized nutrient solution from escaping the box and outer black curtains to prevent light entering the box).	Installation of nutrient feeding system, nebulizer fitted to the main pipe; Back up irrigation system (run on gravity) to cover the risk of pump failure and electricity

Figure A 5. Construction of solar powered aeroponic system

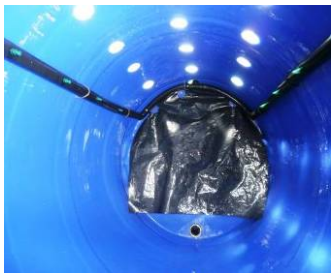
		
Blue chemical /fertilizer drum used for making aeroponic system	Coated with black paint to block light and silver to reflect light to keep drum cool	Solar panel used to run aeroponic system
		
Micro jet sprinklers for nutrition feeding	Fixed two black polythene curtains outer to block light penetration and inner to prevent nutrition spilling	Battery for storing solar power Trickle charger to use under continuously rainy period and timer
		
Nutrient tank	Bilge pump for recirculation of nutrient solution	Chiller for cooling of nutrient solution

Figure A 6. Commercial Aeroponic set up at Northern Mindanao Integrated Agriculture Research Centre (NOMIARC) Malayblay, Philippines

Table A 7. List of bacterial canker isolates for genetic characterization studies.

Clade 1	4031	Qld	2008
	4081	NZ	1967
	4083	Tonga	1968
	4094	Rossmore, NSW	2009
	4088	Canada	1982
	4089	Bulgaria	1983
	4072	Zimbabwe	1961
	4074	Zambia	1963
	4067	Kenya	1945
	4073	Italy	1961
	4092	Pheasants Nest, NSW	2009
	4066	Hungary	1963
	4091	Hungary	1957
	4077	Romania	1970
	4071	U.S.A.	1960

	4090	Bulgaria	1983
	4087	Canada	1982
	4079	NZ	1961
	4082	NZ	1968
	4086	U.S.A.	1983
Clade 2	4012	Yendon, Vic	2004
	4069	U.K.	1956
	4080	NZ	1967
	2089	Somersby, NSW	1981
	2091	Logie Brae, NSW	1992
	2097	Bowen, Qld	1990
	2107	Lake Munmorah, NSW	2004
	4009	Two Wells, SA	2008
	4020	Bundaberg, Qld	2004
	4021	Lake Munmorah, NSW	2004
	2098	Murrumbateman, NSW	2004
	1984	Qld	1999
	1987	Qld	2008
	1994	Lake Munmorah, NSW	2004
	2000	Port Macquarie, NSW	2004
	2008	Guyra, NSW	2007
	2012	Joondalup, WA	2007
	2016	Leeton, NSW	2007
	2055	Warragul, Vic	2008
	2048	Murrumbateman, NSW	2004
	2050	Guyra, NSW	2007
	2035	Virginia, SA	2005
	2007	Guyra, NSW	2007
	2053	Bringelly, NSW	2004
	4075	Channel Islands, UK	1962
	4078	E.U.	1967
	2090	Glenorie, NSW	1992
	2096	Kenthurst, NSW	1994
	1986	Qld	2005
	1983	Qld	1999
	4013	Qld	1999
	4260	Virginia, SA	2007
Clade 3	4245	Lakesland, NSW	2010
	4278	Dareton, NSW	2011
	2030	Kemps Creek, NSW	2007
	4111	WA	2009
	4197	Mansfield, Vic	2009

	4262	Drysdale, Vic	2010
	4272	Virginia, SA	2011
	4258	Guyra, NSW	2010
	4257	Guyra, NSW	2010
	4273	Rossmore, NSW	2011
	4259	Guyra, NSW	2010
Clade 4	2027	Rossmore, NSW	2007
	4261	Rossmore, NSW	2007
	2029	Rossmore, NSW	2007
	1995	Rossmore, NSW	2007
	2004	Dural, NSW	2006
	2009	Rossmore, NSW	2007
	2001	Guildford, NSW	2004
	2019	Rossmore, NSW	2007
	2002	Rossmore, NSW	2006
	2003	Rossmore, NSW	2006
	2020	Rossmore, NSW	2007
	2023	Rossmore, NSW	2007
	2024	Rossmore, NSW	2007
	2108	Rossmore, NSW	2004
	2015	Bringelly, NSW	2007
	2032	Virginia, SA	2007

Table A 8. *Clavibacter michiganesis (Cmm)* isolates screened for tolerance to Cu^{2+}

Isolate Number	Specimen Number	Host	Location	Date of Isolation
1984	J1578	Tomato	Qld	2005
1985	J1677	Tomato	Qld	2005
1987	4011	Tomato	Qld	2005
1988	4018	Tomato	Qld	2005
1994	04/019	Tomato	NSW	2004
1995	04/068	Tomato	NSW	2004
2000	04/529	Tomato	NSW	2004
2002	06/875	Tomato	NSW	2006
2004	06/890	Tomato	NSW	2006
2007	07/163	Tomato	NSW	2007
2008	07/192	Tomato	NSW	2007
2009	07/193	Tomato	NSW	2007
2012	07/231	Tomato	WA	2007
2020	07/911	Tomato	NSW	2007
2023	07/917	Tomato	NSW	2007
2027	07/922	Tomato	NSW	2007
2047	04/438B	Tomato	Vic	2004
2048	04/388	Tomato	NSW	2004
4009	08/684	Tomato	SA	2008
4056	08/924	Tomato	Vic	2008
4066	LMG2891	Tomato	Hungary	1963
4067	LMG3679	Tomato	Kenya	1945

4069	LMG3681	Tomato	U.K.	1956
4070	LMG3683	Tomato	Sicily	1956
4071	LMG3685	Tomato	U.S.	1939
4072	LMG3686	Tomato	Zimbabwe	1960
4073	LMG3687	Tomato	Italy	1961
4074	LMG3689	Tomato	Zambia	1962
4075	LMG3690	Tomato	U.K.	1962
4076	LMG3694	Tomato	South Africa	1967
4077	LMG3695	Tomato	Romania	1970
4078	LMG3696	Tomato	Belgium	1967
4079	LMG5597	Tomato	New Zealand	1961
4080	LMG5602	Tomato	New Zealand	1967
4081	LMG5603	Tomato	New Zealand	1967
4082	LMG5604	Tomato	New Zealand	1968
4083	LMG5605	Tomato	Tonga	1968
4086	LMG5616	Tomato	U.S.	1983
4087	LMG5643	Tomato	Canada	1982
4088	LMG5644	Tomato	Canada	1982
4089	LMG5726	Tomato	Bulgaria	1983
4090	LMG5727	Tomato	Bulgaria	1983
4091	LMG7333T	Tomato	Hungary	1957
4092	09/281	Tomato	NSW	2009
4094	09/212	Tomato	NSW	2009
4111	09/703	Tomato	WA	2009
4197	09/841	Tomato	Vic	2009
4245	10/334	Tomato	NSW	2010
4257	10/494	Tomato	NSW	2010
4258	10/494B	Tomato	NSW	2010
4259	10/494C	Tomato	NSW	2010
4260	07/581	Tomato	SA	2007
4261	07/911	Tomato	NSW	2007
4262	10/627	Tomato	Vic	2010
4272	11/111	Tomato	SA	2011
4273	11/122G	Tomato	NSW	2011

Table A 9. Disinfectants used in Part A surface testing

Product	Active ingredient	Concentration
Calcium hypochlorite	Hypochlorous acid	250ppm
		500ppm
Sodium hypochlorite	Hypochlorous acid	10^3ppm
Sporekill®	Didecyldimethylammonium chloride	1%
Peratec®	Hydrogen peroxide plus peroxyacetic acid	1%
Virkon®	Potassium peroxomonosulphate plus other salts	1%

Table A 10. Disinfectants used in Part B surface testing

Product	Active ingredient	Concentration
Ethanol	Ethyl alcohol	70%
Denatured ethanol (methylated spirits)	Ethyl alcohol	70%
Virkon®	Potassium peroxomonosulphate plus other salts	1%
Menno Florades®	Propan-1-ol, propan-2-ol and benzoic acid	1%
		3%

Figure A 7. The surfaces and tools that were examined for *Cmm* disinfection

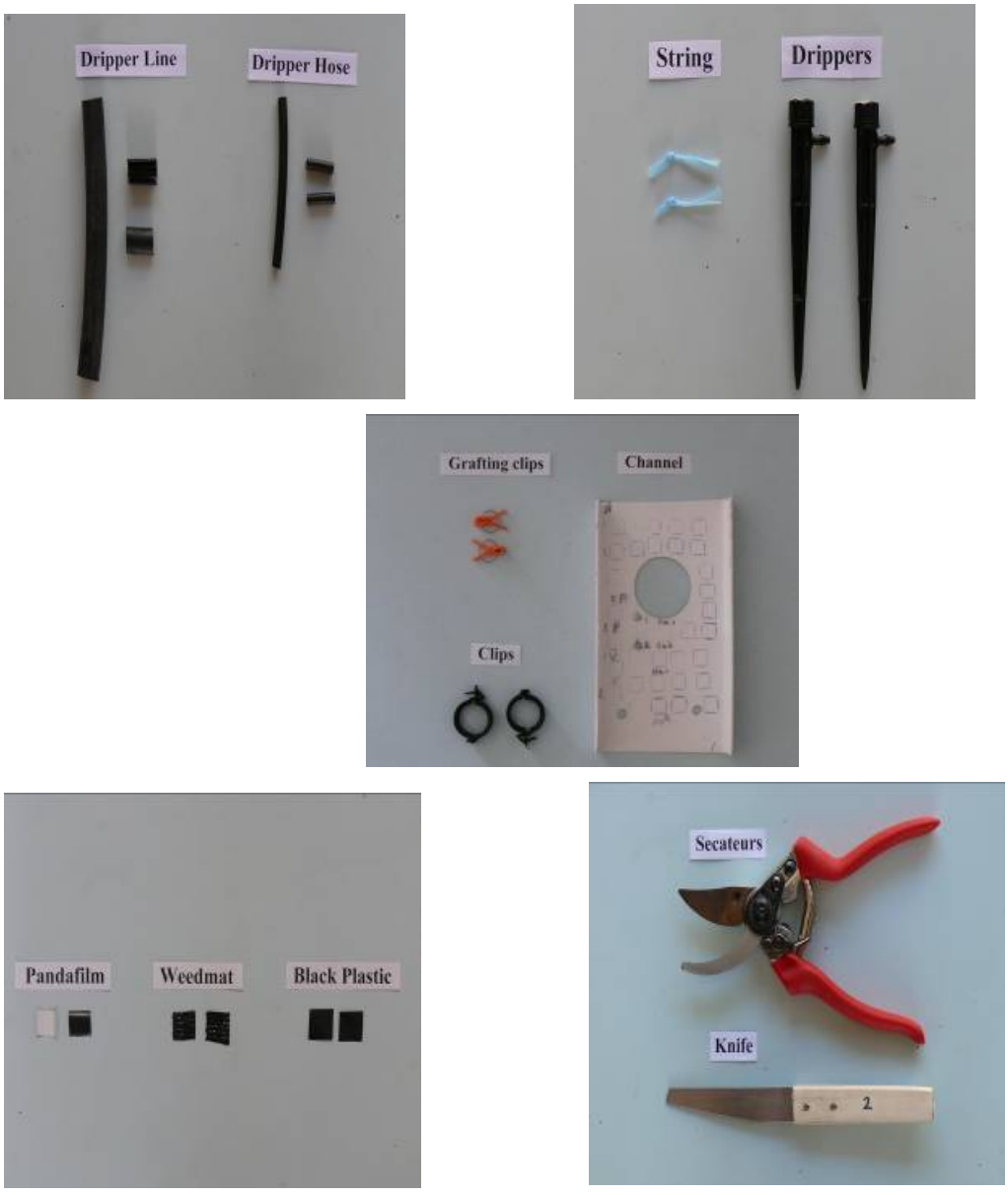


Table A 11. Disinfectant efficacy for *Cmm* on dripper hose

Disinfectants 2min contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		_		+	+			+
	T2		+		+		-		+
1% Sodium hypochlorite	T1		-		-	-			-
	T2		-		-		-		-
1% Peratec®	T1		-		-	-			-
	T2		-		-		-		
1% Sporekill®	T1		-		-	-			-
	T2		+		+		-		
1% Virkon®	T1		-		-	-			+
	T2		-		-		-		-
	T3				+				

Table A 12. Disinfectant efficacy for *Cmm* on dripper line

Disinfectants 2mins contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		-		-	-			-
	T2		-		+		+		-
1% Sodium hypochlorite	T1		-		-	-			-
	T2		-		-		-		-
1% Peratec®	T1		-		-	-			-
	T2		-		-		-		
1% Sporekill®	T1		-		-	-			-

Disinfectants **2mins contact time**	**Test**	**Results for each *Cmm* isolate** **- = no *Cmm* detected** **+ = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
	T2		+		+		+		
1% Virkon®	T1		-		-	-			+
	T2		-		-		-		-

Table A 13. Disinfectant efficacy for *Cmm* on drippers

Disinfectants **2min contact time**	**Test**	**Results for each *Cmm* isolate** **- = no *Cmm* detected** **+ = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		-		-	-			-
	T2		-		-		-		-
1%Sodium hypochlorite	T1		-		-	-			-
	T2		-		-		-		-
1% Peratec®	T1		-		-	-			-
	T2		-		-		-		
1% Sporekill®	T1		+		-	-			-
	T2		-		+		-		
1% Virkon®	T1		-		-	-			-
	T2		-		-		-		-

Table A 14. Disinfectant efficacy for *Cmm* on string

Disinfectants 2min contact time	**Test**	**Results for each *Cmm* isolate - = no *Cmm* detected + = *Cmm* detected**							
***Cmm* isolate**		**2092**		**2097**		**2000**		**2008**	
		nil	**BSA**	**nil**	**BSA**	**nil**	**BSA**	**nil**	**BSA**
Calcium hypochlorite 250ppm	T1		-		-	-			-
	T2		-		+		+		-
1%Sodium hypochlorite	T1		-		-	-			-
	T2		-		-		-		-
1% Peratec®	T1		-		-	-			-
	T2		-		-		-		
1% Sporekill®	T1		-		-	-			-
	T2		-		+		-		
1% Virkon®	T1		-		-	-			-
	T2		-		-		-		-

Table A 15. Disinfectant efficacy for *Cmm* on plastic channel, plastic clips and grafting clips

Disinfectants 2min contact time + BSA	**Test**	**Results for *Cmm* isolate 2092 - = no *Cmm* detected + = *Cmm* detected**		
		Channel	**Plastic clips**	**Grafting clips**
Calcium hypochlorite 250ppm	T1	-	-	-
1%Sodium hypochlorite	T1	-	-	-
1% Peratec®	T1	-	-	-
1% Sporekill®	T1	-	-	-
1% Virkon®	T1	-	-	-

11.3 Appendix 3 : Fact Sheets

Integrated Management of Bacterial Wilt disease of Solanaceous Vegetable Crops (Biovar 3)

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Background

Bacterial wilt is caused by *Ralstonia solanacearum* and results in severe economic and environmental losses in tropical, subtropical, and warm temperate regions of the world. The disease can also occur in cooler climates such as relatively higher elevations in the tropics. World wide spread of the disease in potato has been associated with the distribution of latently infected planting material.

Over 400 plant species have been recorded as hosts of the bacterium. It is a complex pathogen having various strains. These strains differ from one another, depending upon host and geographical origins. Race 3 (biovar 2A) strains of *R. solanacearum* are most common in higher elevations of the tropics (up to 3400 masl) and affect mainly the potato crop, but occasionally tomato and some weeds. At lower elevations, race 1 strains are most prevalent and affect a wide range of crops and weeds. Crops highly susceptible to race 1 (biovar 3) of *R. solanacearum* are potato, tobacco, tomato, eggplant, chilli, bell pepper, and peanut.

Disease distribution

In tropical Queensland, bacterial wilt disease causes severe losses to tomato, potato and other solanaceous vegetable crops under favourable environment conditions (28-35 ºC and relative humidity over 70%). The several incursions of disease have been observed from time to time and assessed to cause 20-60% of crop losses in tomato and potato crop. The majority of isolates were collected in Queensland from 1988 to 2011, from various hosts and from different agro-climatic zones of the state. The majority of isolates were from Atherton Tablelands (49%), Costal tropics (40%) followed by southern costal Queensland (6%), and Cape York (5%).The recent analysis of these collected isolates showed the presence of Race I, biovar 3 strain (94%) and Race 3, biovar 2 (6%) of the pathogen.

The biovar 3 strain is endemic to tropical areas and has a wide host range. It is associated with a number of plant species in the Queensland, (**Vegetable and other crops**: potato, tomato, tobacco, capsicum, chilli, eggplant, peanut, and eucalyptus; **Ornamental plants**: Strelitzia, Anthurium, Heliconia, marigold, Lisianthus, Alexandra palm and ornamental ginger and **Weed plants**: black night shade, common chick weed, Cobblers peg, snakeweed and billygoat weed). However, the presence of biovar 2 from potato growing area of Atherton Tablelands shows the possibility of introduction through latently infected potato seed tubers. Latently infected tubers are those that do not show any external and internal visible symptoms. Contaminated soil, weeds, rain, irrigation water and machinery keep on, never ending cycle of disease development and spread in infected and non infected areas (Figure 4).

Symptoms

Plant Symptoms

Characteristic symptoms of the disease include curling and partial wilting of leaf and stem. Wilting of plants can be easily identified during the warmest part of the day. Under favourable environmental conditions disease development is fast (30-35 ºC); plants wilt

rapidly and collapse within 3-4 days after infection, without showing any yellowing of leaves (Figure 1).

Figure 1.Typical symptoms that include partial wilting of plant canopy in bacterial wilt infected tomato (Top) and potato (Bottom).

Under slow disease development conditions(when day temperature do not exceed 30 ºC), infected tomato plants produce yellowing of lower leaves and develop large number of adventitious roots on the main stem. On cutting of root and lower portion of the stem at ground level shows a brown discoloration of the water conducting tissues. Milk exudates/bacterial strands can be easily observed from a cut stem end, when placed in water (Figure 2).

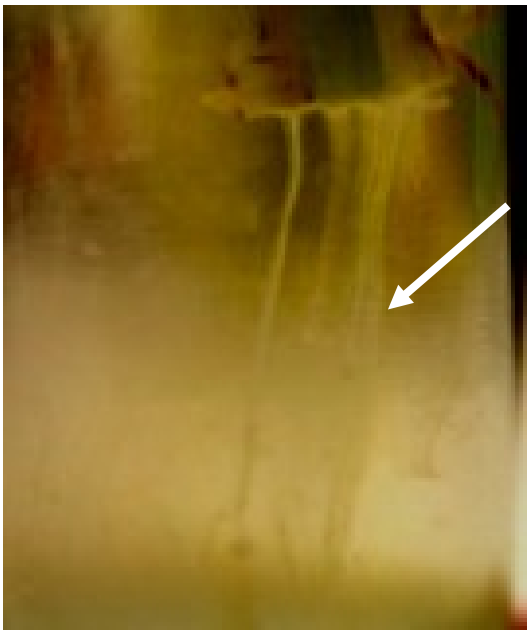
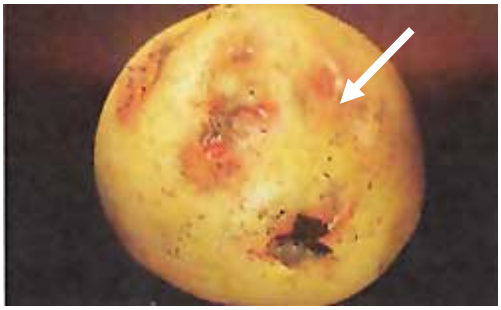


Figure2. White, slimy strands of bacteria from vascular bundles can be noticed when cut stem of infected plant are submerged in water.

Tuber symptoms

The symptoms of severely infected potato tuber show oozing of bacterial slime from eyes and stolen end. These symptoms can easily be distinguished at harvesting time by soil sticking to the eyes and stolen end. Disease is best identified when tuber is cut across the vascular ring. Infected tubers show light brown discoloration and the breakdown of water conducting tissues. Milky fluid oozes out from discoloured area if the tuber is squeezed (Figure 3).



(Photo: Courtesy of International Potato Centre)

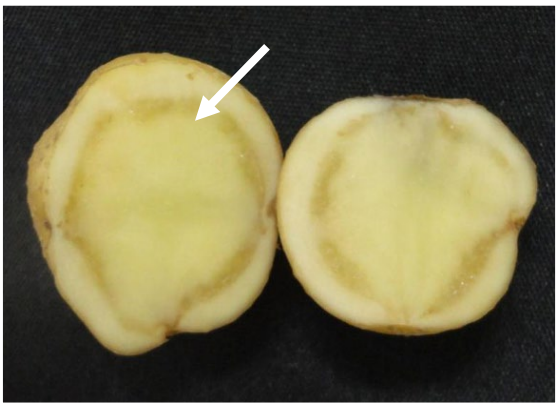


Figure 3. Typical symptoms (bacterial slime oozing from eyes of potato) of bacterial wilt infected tuber (Left). Brown vascular discoloration (Right)

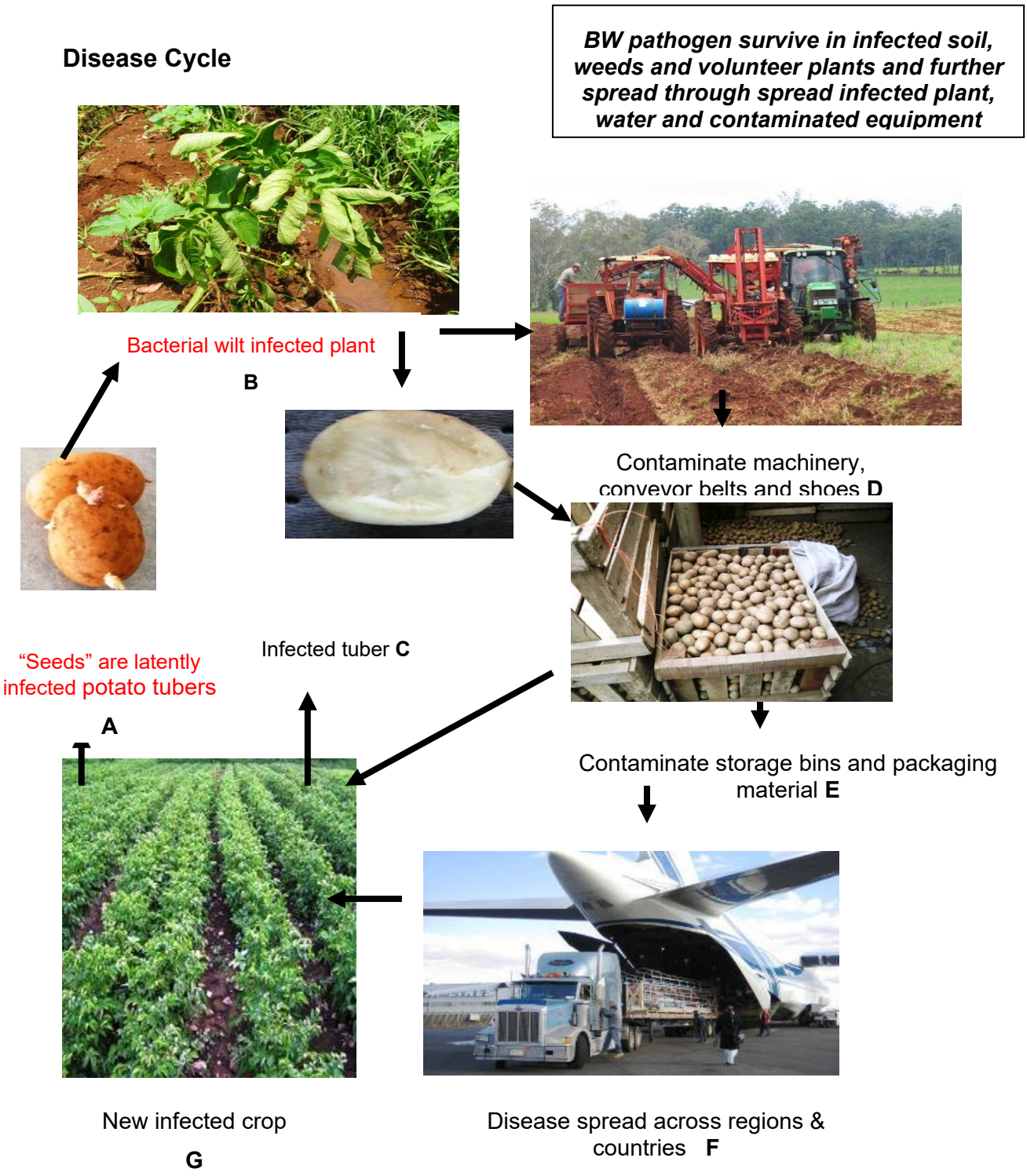


Figure 4. **A** Infected seed tubers (latent infection) are source of primary infection, **B and C** Diseased plants /tubers contaminate soil, irrigation water, weed hosts, water ways, tools and machinery etc. and spread disease in adjoining fields/area, **D** Machinery used for harvesting and transporting crop, introduce disease to storage facilities and spreads disease on use in new areas, **E and F** Transport of latent infected tubers result in long distance dissemination of disease (across regions and countries) and spread disease in new areas. Several factors help maintain the disease cycle leading to the persistence of the disease in infected areas and spread to new, not infected areas. These factors include soil, irrigation water and machinery contaminated with the pathogen, alternative

plant hosts (e.g. weeds) infected with the pathogen, and warm and humid environmental conditions.

Disease Management

Bacterial wilt control of biovar 3 strain is difficult due to its broad host range. In absence of chemical control, no single strategy has ability to control the disease; but can be managed by adoption of integrated management strategies

Use of certified planting material and following strict quarantine regulations help to stop further spread of the disease to non infected areas.

There are various potential management strategies (Table 1), which can be followed for developing a bacterial wilt management programme. These management strategies have been weighted from 1 to 7 (Priou et.al, 2004 and Janse, 2005) on the basis of disease management of the biovar 3 strain of bacterial wilt. Higher numbers relate to a greater potential to manage the disease. Strategies selected should add up to at least 14 points, for better disease management and sustainable potato production.

Table1. Key strategies suggested for managing bacterial wilt (Race 1 Biovar 3) disease listed according to their effectiveness of managing the disease successfully. Levels 7 relate to a more effective management and level 1 to a less effective management.

Key Management Strategies	Race 1, Biovar 3
Certified Bacterial wilt free seed	**7**
Bacterial wilt free soil	**7**
Biofumigation (Brassica spp.-radish, Bioquire mulch, Nemfix etc.)	**4**
Rotation with non-hosts (3-4 Years)	**3**
Flooding of paddy rice	**3**
Rouging of wilted potato, tomato, capsicum, eggplant and other weed plants	**3**
Removal of infected haulms and/or harvest leftovers	**3**
Soil amendments	**3**
Intercropping	**2**
Nematode control	**2**
Minimal post-emergence cultivation	**2**
Resistant or tolerant variety	**2**
Weed control and Rouging of volunteering plants	**2**
Suppressive soil	**2**
Minimum till	**2**
Solarisation	**1**
Cold climate (Elevation>2500 m in tropical area)	**1**

Date of planting	**1**
Control of spread in water	**1**
Tool decontamination, washing hooves and shoes	**1**

This factsheet is primarily designed for farmers and extension workers to provide information on potential management strategies of bacterial wilt disease. This information will help users to identify less and more effective disease management strategies and create their specific management plan according to their environments and cropping systems. An improved bacterial wilt management programme includes the use of clean seed, tolerant varieties, crop rotation with non host crops, an effective management of weeds and plant parasitic nematodes control, as well as good farm management practices listed in Table 1.

Future Research

The soil-borne and virus diseases are identified as major threats for potato cultivation in tropical areas. These diseases are often introduced through latently infected material. The seed is the primary requirement for a successful crop production .In absence of quality seed it is impossible to have quality production. The soilless technology of potato seed production could be analysed for rapid production and availability of seed at affordable prices. Preliminary studies on the development and cultural techniques for an aeroponic system indicate that it can produce seed tubers of an acceptable size, making it possible for a direct distribution of aeroponic seeds to farmers or certified seed growers. Adoption of this technology by small-scale seed producers in the cooler production areas could be tested across the Mindanao region of Philippines and could be applied in Australia. The development of a cost effective aeroponic system may provide an opportunity for the private sector to multiply quality seeds at faster rates and supply seeds to farmers at an affordable cost. The development and adoption of aeroponic technology along with IDM strategies are envisaged to build a sustainable potato industry in tropical countries.

Acknowledgement

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References

Priou S, Aley P,Chujoy B, Lemaga,B and French ER (2004).Integrated control for bacterial wilt of potato, CIP publication. www,fao.org/sd/erp/toolkit/BOOKS/integr-control-of-bacterial-wilt-in-potato.pdf

Janse JD 2005.Phytobacteriology Principles and Practices CABI publication pp361

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NSW DPI Factsheet

Bacterial canker of tomato and its management strategies

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Background

Bacterial Canker is a devastating disease of both field and greenhouse tomatoes and is caused by the bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). First found in Michigan, USA in 1909, *Cmm* is found in all the major tomato growing areas of the world including seed production regions. In recent years Bacterial Canker has been the most important disease affecting tomato production in the Australian greenhouse industry.

Leaf Scorch symptoms

Typical symptoms to look for

Disease symptoms vary widely depending on when infection occurs, the tomato variety's genetics, and environmental and cultural factors such as temperature and nutrient management.

Seedling or seed-borne infection may lead to no obvious symptoms developing for up to 90 days or plants may quickly wilt and die. Early symptoms in greenhouse crops generally occur when plants have set the first few trusses of fruit. Typical symptoms are scorching marks on leaves which are similar to those caused by nutritional disorders such as potassium deficiency or phosphorous toxicity. Advanced disease symptoms are typically wilting plants with yellow to dark brown discolouration of the vascular tissue. This is most notable in the main stem near ground level or at the junction between stems and leaf bases. Marbling of green fruit

and stem lesions are common on affected greenhouse crops while bird-eye cankers on fruit and stem and leaf cankers are more common on field crops.

Cmm infected tomato seedling wilting on one side

Brown vascular tissue inside tomato stem

How is *Cmm* spread?

Cmm can be introduced onto a farm with infected seed and seedlings, or as a contaminant of media, machinery, tools or people. *Cmm* survives on tomato plant surfaces or inside infected crop residues. Bacteria are spread primarily with cutting tools and when plants are handled. Potential spread also occurs along rows in waste water with subsequent infection through roots. Poor greenhouse climate control can result in higher numbers of *Cmm* on leaf surfaces in moisture films or in guttation droplets.

The long latent period between infection and symptom development means that *Cmm* may have been introduced and spread unknowingly throughout a greenhouse or farm long before disease symptoms are noticed. *Cmm* can also infect solanaceous weeds and crops such as capsicum. This needs to be noted when attempting eradiation.

Wilting plants with advanced *Cmm* infection

Control options

Preventative strategies are the most important aspect to an integrated management approach. In Europe there are regulations that help to restrict seed borne infections. Quality management systems in seed production have improved in recent years following canker disease outbreaks in different parts of the world. However, there are still critical control points in Australia where *Cmm* can be spread in seedling production. In particular, sanitation and disinfection during grafting plants need to be effective and carefully maintained. Good crop hygiene is also essential to minimise the spread of *Cmm* on farm. Quarantining infected areas of the greenhouse and removing affected plants may help to reduce *Cmm* spreading but remember that by the time symptoms are observed bacteria may have already spread considerable distances from primary infections. Continuous removal of diseased plants may yield diminishing returns as more plants begin to express disease symptoms. Disinfectants need to be in contact with surfaces for 1-2 minutes to kill all *Cmm* bacteria.

Copper is the only registered chemical control option for tomato bacterial canker. However, our research has demonstrated that many Australian *Cmm* isolates tolerate high copper concentrations. Other bacteria that carry known genetic resistance can grow at equivalent copper concentrations.

Future Research

There are a number of research gaps that need to be addressed to improve the control of tomato Bacterial Canker in Australia. Firstly independent auditing for the *Cmm* health status of seed and seedlings could reduce the risks of spreading infected plants. In addition to effective hygiene, disinfection and sanitation strategies there are possible chemical and biological control options that could be evaluated to minimise the risks of spreading *Cmm* during grafting. For instance, a recently commercialised bacterial virus (bacteriophage) is highly specific to *Cmm* and related strains. It has shown promise in controlling tomato canker in North America. The Australian greenhouse tomato industry is encouraged to contribute to developing these strategies in a research project proposal developed by the authors and Cherie Gambley, Senior Plant Pathologist, DAFF Queensland.

Further Reading

D. Persley, T. Cooke & S. House, 2010. Diseases of vegetable crops in Australia. CSIRO Publishing.

J. Badgery-Parker, 2009. Keep it Clean – Reducing costs and losses in the management of pests & diseases in the greenhouse. NSW DPI Publication.

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11.4 Appendix 4 : Abbreviation and Acronyms

ACIAR	Australian Centre for International Agricultural Research
BW	Bacterial Wilt (*Ralstonia solanacearum*)
Bv	Biovar
Cmm	*Clavibacter michiganensis* subspecies *michiganensis*
CPG	Casamino Peptone Glucose media
Cu	Copper
CYEG	Casitone Yeast Extract Glycerol Agar
ELISA	Enzyme-linked Immunosorbent Assays
EPS	Exo Poly Saccharide
FGD	Focused group discussion
GIS	Geographic information system
ICM	Integrated Crop Management
IDM	Integrated Disease Management
Mplex	Multiplex
masl	Meter above sea level
NOMIARC	Northern Mindanao Integrated Agricultural Research Centre
OD	Optical Density
PALGs	Participatory Action Learning Groups
PCCAARD	Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development
PCR	Polymerase chain reaction
SMSA	Semi selective Media South Africa
TZCA	Tetrazolium Chloride Agar media
UPLB	University of the Philippines Los Banos