Biological Control of Weeds:

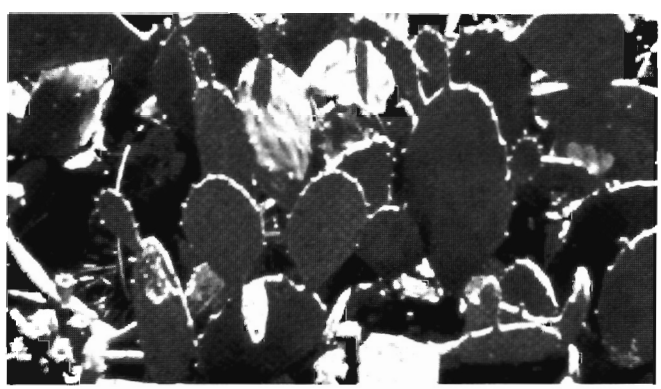
theory and practical application

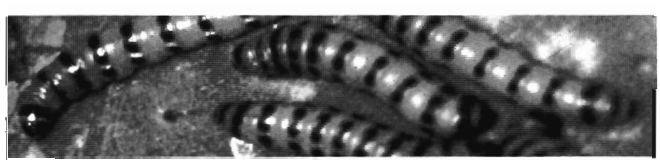
Mic Julien and
Graham White

Biological Control of Weeds:

theory and practical application

Mic Julien and
Graham White





The Australian Centre for International Agricultural Research (ACIAR) was established in June 1982 by an ACT of the Australian Parliament. Its mandate is to help identify agricultural problems in developing countries and to commission collaborative research between Australian and developing country researchers in fields where Australia has a special research competence.

Where trade names are used this constitutes neither endorsement of nor discrimination against and product by the Centre.

ACIAR Monograph Series

This peer reviewed series contains the results of original research supported by ACIAR, or material deemed relevant to ACIAR's research objectives. The series is distributed internationally, with an emphasis on developing countries.

Julien, Mic and White, Graham. 1997. Biological Control of Weeds: theory and practical application. ACIAR Monograph No. 49 192pp.

ISBN 1 86320 216 1

Typeset and layout: Design One Solutions, Canberra, Australia

Production management: Peter Lynch

Contents

Contents

Preface

Weed problems in the Asia-Pacific region are becoming increasingly difficult to manage. Most countries in the region are not only faced with a higher risk of exotic weed introduction, the result of a major increase in travel and trade, but are also attempting to reduce their reliance on herbicides for environmental, health and sustainability reasons. Against this background, biological control clearly has a major role to play in the region, either on its own or, increasingly, as part of an integrated weed management strategy.

In Australia, many of the major tropical weed problems are associated with extensive agricultural activities and biological control is often the only feasible means of control. As a result, considerable effort has been devoted to weed biological control, with considerable success. Indeed, it is generally recognised that Australia is a world leader in the development and implementation of practical weed biological control. During the past decade, CSIRO Entomology and Queensland Department of Natural Resources have been involved in a number of collaborative projects on weed biological control with neighbouring countries in the Asia-Pacific region, largely funded through the Australian Centre for International Agricultural Research (ACIAR).

However, having the will to collaborate and increase effort in biological control of weeds is one thing, to achieve practical implementation is another matter. It involves the cooperation of a number of key players and spans a range of disciplines, from taxonomy and ecology to political and socioeconomic science. To achieve increased cooperation in Australia in weed biological control and in other pest management areas, a joint venture — the Coooperative Research Centre for Tropical Pest Management (CTPM) — was established in 1991, involving CSIRO Entomology, Queensland Department of Natural Resources and Primary Industries and the University of Queensland.

It was decided very early in the life of CTPM that an international course in Biological Control of Tropical Weeds would enhance the international research effort in this field. The aim was to provide participants with the key knowledge, information and skills they require to implement a biological control project in their countries. To date, 52 participants from 21 countries have attended the four courses organised, with major funding coming from ACIAR, the Crawford Fund and GTZ.

Since the course was first established, we have received many requests for additional copies of the excellent lecture notes that were provided for course participants. This book is the result. We are extremely grateful to ACIAR for funding the production of this book and, in particular, to Paul Ferrar — Research Program Coordinator in ACIAR — for his initial and on-going encouragement and enthusiasm for the course and the book. I am sure this book will make an important contribution to an increase in the biological control of weeds in the Asia-Pacific region and worldwide.

Geoff Norton

Director, Centre for Tropical Pest Management, University of Queensland, Brisbane, Australia.

September 1997.

Introduction

The Cooperative Research Centre for Tropical Pest Management (CTPM) has presented four courses in Biological Control of Weeds since 1993. Each course included sessions at Centre for Tropical Pest Management headquarters on The University of Queensland campus, and at CSIRO Entomology at Long Pocket Laboratories and Queensland Department of Natural Resources at the Alan Fletcher Research Station which specialise in the biological control of weeds.

The course was designed for scientists and managers who are to be involved in the regulation, management or implementation of biological control of weeds but have limited experience in the area. The main aims of the course are to give participants a balanced understanding of the theory of weed biological control and a practical exposure to the procedures followed in a weed biological control program.

Participants to the courses received a set of notes. This book is a compilation of those notes, but with considerable expansion and modification and the addition of references, to make them suitable for publication.

The book covers in broad terms the theory underpinning some aspects of biological control of weeds, discusses the various stages of a project in some detail from its beginnings to evaluating the impact of agents after they have been released. It introduces techniques that may be usefully applied in a project. It also touches on some contemporary issues and methods such as host range testing, use of fungi as control agents and selection of the most appropriate agents. Some sections are purely methodology, e.g. propagating plants for rearing phytophagous insects and for host range testing, developing a list of plants for host testing, or climate matching using a computer model. Others are case studies illustrating the decision making processes required, e.g. interpreting results from host range tests. Relevant theory is introduced, but approaches that have proven not to be useful in practice, such as ecologically and biologically based ranking systems for potential agents, and speculative issues for which little data are available, such as negative environmental impacts of weed biological control agents, are not discussed in detail.

Some aspects of the course could not be captured in a book. For instance, the courses provided a forum to develop contacts to keep up to date with activities and to source control agents. It also provided an opportunity for additional formal and informal discussions with experienced scientists about specific weed problems and their management. For some chapters of the book, practical demonstrations during the courses assisted in clarification of techniques.

Altogether the papers in this book provide an overview of biological control of weeds with an emphasis on how to go about it. However, it is only a starting point and key material from the large literature on biological control of weeds is referenced throughout the book.

Graham White[1] *and*

Mic Julien[2]

[1] *CTPM, Gehrmann Laboratories, University of Queensland, 4072, Australia*

[2] *CSIRO Entomology, Private Bag 3, Indooroopilly, 4075, Australia*

Biological Control of Weeds: An Illustrated Seminar

Introduction

Presented here is a colour print copy of a set of slides that provide a precis of biological control of weeds. The set of slides was designed to assist participants in the course, upon which this book is based, to present an outline of biological control of weeds to their colleagues or to students. Included are: a definition, a list of the techniques used, explanation of the concept of biological control, descriptions of the steps involved in a project, some points about the strengths and limitations of biological control, and examples of weeds, the agents used to control them and the results achieved. Where applicable, next to each print copy, reference is made to the appropriate section(s) of this book for further information

The 38 colour prints are available as a set of slides for approximately $A55.00. Orders can be placed through the address below.

Mic Julien
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

1

Biological Control of Weeds

2

Slide assisted seminar

prepared by

The Centre for Tropical Pest Management

Brisbane **Australia**

3

Biological Control of Weeds

definition -

The utilisation of organisms for the regulation of host plant densities.

4

There are two techniques used

1. Non classical biological control

- *Inundative*
- *Augmentative*

2. Classical biological control

5

1. Non Classical Biological Control

(i) Inundative

- ***Release of large numbers of the agent to control the target weed, e.g. mycoherbicides.***
- ***Three formulations commercialised worldwide, several others under development.***

Refer to page 35, 129–134, 135–140

6

1. Non Classical Biological Control - continued

(ii) Augmentative

- ***Mass rearing and release of large numbers of a control agent that cannot be utilised as a mycoherbicide, i.e. cannot be grown* in vitro.**
- ***Five examples worldwide; none are in consistent use.***

Refer to page 35

2. Classical Biological Control

7

- *Most commonly used technique.*
- *Generally involves introduction of natural enemies from their native range into an exotic range where their host plant has become a weed.*
- *Example - the use of water hyacinth insects from South America to control that weed in other countries.*

Refer to page 35, 47-117, 119–127 (pathogens)

Concept of Biological Control

8

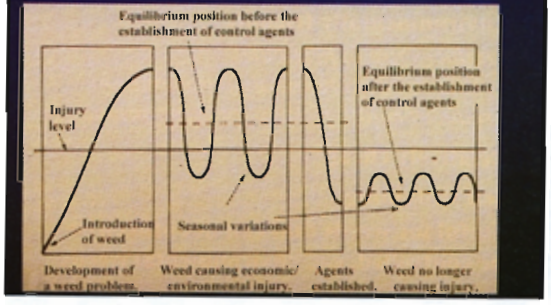
To introduce natural enemies of a target weed that will reduce the density of the weed to a level that is acceptable and that will maintain the weed density at that level.

[The next slide illustrates this concept graphically.]

Refer to page 34, 39–45

Changes in the population density of a weed before and after the establishment of biological control agents.

9



Refer to pages 39–45

Steps in a Biological Control of Weeds Project

10

1. Initiation
2. Approval to work
3. Foreign exploration
4. Surveys in introduced range
5. Ecological studies on weed and natural enemies

Refer to pages 47–49

Steps in a project - continued

11

6. Host specificity studies
7. Approval of agents
8. Importation
9. Rearing and release
10. Evaluation
11. Distribution

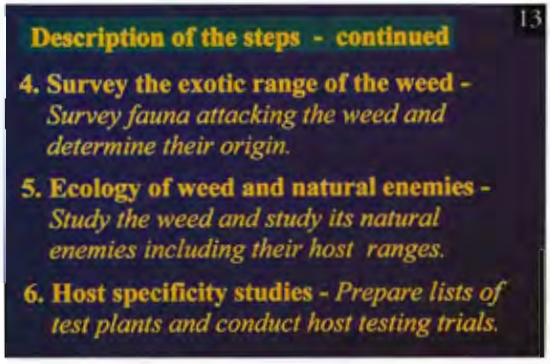
Refer to pages 47–49

Description of the steps in a biological control of weeds project

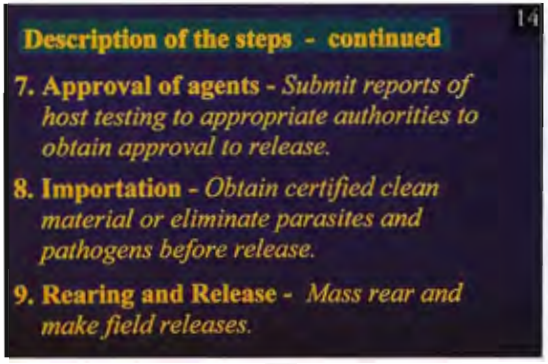
12

1. **Initiation** - *Review literature and compile existing knowledge about target weed and its natural enemies.*
2. **Approval to work** - *Seek approval and funds to work.*
3. **Foreign exploration** - *locate native range of the target weed and search for natural enemies of the target weed.*

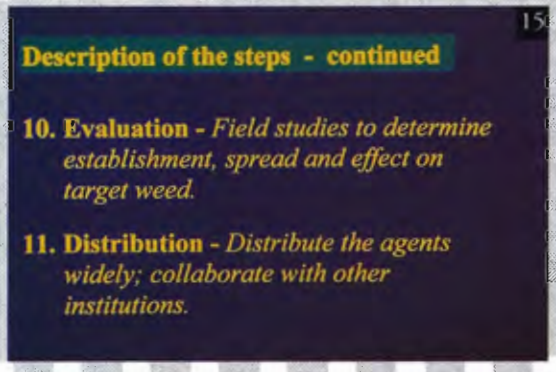
Refer to pages 47–48, 51–55



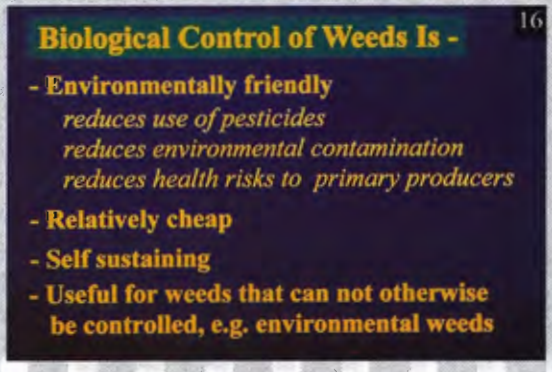
Refer to pages 48, 51–56, 57–62, 71–75, 77–82, 83–88

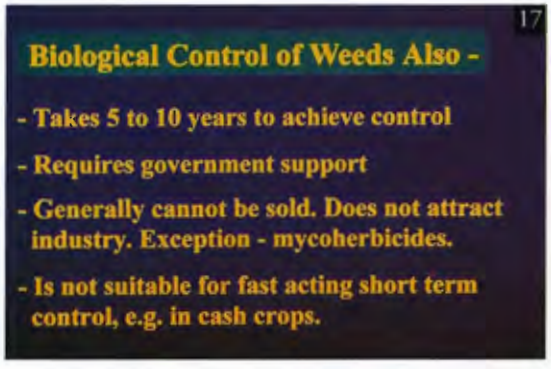


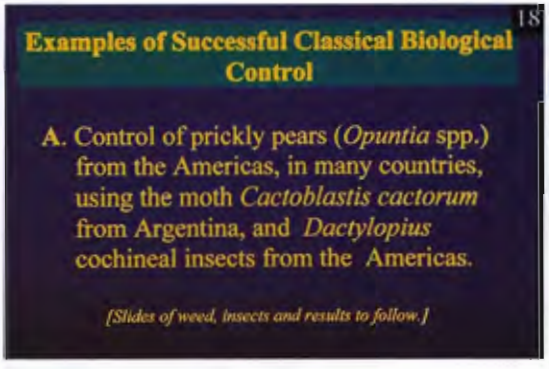
Refer to pages 48–49, 63–69, 89–96, 101–103



Refer to pages 49, 97–100, 105–118







Cactus (*Opuntia stricta*)
Queensland Department of Natural Resources

Larvae of the moth Cactoblastis cactorum.
G. White, Queensland Department of Natural Resources

Cochineal (*Dactylopius* spp.) on *Opuntia stricta.*
Queensland Department of Natural Resources

Before biological control–Cactus, Australia.
Queensland Department of Natural Resources

After biological control–Cactus, Australia.
Queensland Department of Natural Resources

Examples of Successful Classical Biological Control

B. Control of Alligator weed *Alternanthera philoxeroides* from South America, in Australia and USA using the alligator weed flea-beetle, *Agasicles hygrophila*, also from South America.

[Slides of weed, insect and results to follow]

Alligator weed, *Alternanthera philoxeroides.*
M. Julien, CSIRO Entomology

Alligator weed flea-beetle, *Agasicles hygrophila.*
J. Green, CSIRO Entomology

Before biological control–alligator weed, Australia.
M. Julien, CSIROEntomology

After biological control–alligator weed, Australia.
M. Julien, CSIRO Entomology

Examples of Successful Classical Biological Control

C. Control of the floating fern, *Salvinia molesta* from Brazil, in Australia and many other countries, using the salvinia weevil, *Cyrtobagous salviniae* from Brazil.

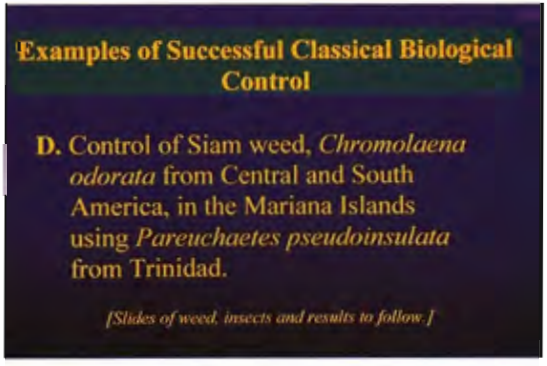
[Slides of weed, insect and results to follow]

Salvinia, *Salvinia molesta.*
M. Julien, CSIRO, Entomology

The salvinia weevil, *Cyrtobagous salviniae.*
P. Room, CSIRO Entomology

Before biological control–salvinia, PNG.
P. Room, CSIRO Entomology

After biological control–salvinia, PNG.
P. Room, CSIRO Entomology



Siam weed, *Chromolaena odorata.*
R. McFadyen, Queensland Department of Natural Resources

The moth *Pareuchaetes pseudoinsulata.*
R. Muniappan, University of Guam

Before biological control–Siam weed, Mariana Islands.
R. Muniappan, University of Guam

After biological control–Siam weed, Mariana Islands.
R. Muniappan, University of Guam

Success, and Failure, in Biological Control of Weeds

Introduction

The rate of success of projects aiming to achieve biological control is high considering the checks and constraints placed on biological control. The global success rates for classical biological control of weeds, estimated by Julien et al. (1984), using releases of agents from the first deliberate introduction to 1980 (Julien 1982), were as follows.

- Of 174 projects 39% were successful.
- 101 species of weeds were targeted of which 48% were controlled.
- 178 different species of organisms were released of which 71% established and 34% were effective.
- There were a total of 729 releases of control agents of which 64% established and 28% were involved in successful control of a weed.

Three factors were identified as positively affecting the rate:

- Organisations such as CSIRO and the Hawaiian Department of Agriculture, where detailed studies are conducted, had the highest rates of success. In comparison organisations or regions where mainly ad hoc releases have been made, had the highest failure rates.
- Utilisation of agents already proven successful in another country provided the best chance for successful control. Repeated use of proven agents is increasing as more countries increase their involvement in biological control. For example, the salvinia weevil, initially successful in Australia, was subsequently released and controlled salvinia in 13 other countries.
- Utilisation of native organisms to control weeds showed a far greater success rate, 62% of releases, compared to 28% for exotic organisms. These results should be treated cautiously because of biases in the comparison. The main limitation to using native organisms is that an ongoing commitment to rearing and making inundative releases is required.

One of the difficult aspects of assessing biological control is defining and describing success or failure. Outcomes of biotic interactions over the range in which the organisms (weed and agents) exist are usually variable hence describing the results can be complex. This is discussed in the next section. Then, the problem of how to ensure the success of a project and improve the overall (global) rates of success are considered. Discussion is particularly in relation to organisations entering the field and using agents that are already known.

Mic Julien
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Defining Success in Biological Control of Weeds

Generally a project is considered successful when the target weed has been significantly reduced. Success is usually reported using ecological data or descriptions of sociological or environmental benefits. Occasionally economics are used and cost:benefit analyses given.

The range in levels of control achieved

By definition, biological control will not eradicate a weed. Local extinctions may occur. In this respect, biological control is similar to other weed control techniques.

After biological control agents become established, a dynamic equilibrium is reached with the effects of this interaction ranging from no effect on the weed's density or biomass, to the weed being reduced to minor significance. When these extremes occur they are obvious. However, most cases fall between and require study to determine the level of control achieved and to make judgements about the requirement for further work.

In the past researchers have tended to use short descriptions such as: complete; substantial; partial or no control. These may be useful in some situations but over simplify reality that includes variation in time and space. In describing levels of control care should be taken not to mislead. Nothing is gained by understating the success of a project but future problems may arise if the level of success is overstated.

Biological control is driven by interactions with the environment and is dynamic and variable. Levels of control may vary between seasons or years. There may be periods when control is inadequate. Similarly, control may not be achieved throughout the range of the weed. Temperatures, for instance, may be too cold in some areas for the agents to develop damaging populations. In addition, the level of control may be adequate for one activity but not for others, for example, a reduction in water hyacinth may permit water transport but not net fishing. Control may be achieved in sun but not in shade (e.g. control of St John's wort in Australia), over water but not over land (e.g. control of alligator weed).

Knowledge of spatial and temporal variability is essential for realistic assessments of the level of control that has been achieved. Such assessments can only be made if the status of the weed has been quantified before and after control and throughout the range of the weed. This information is also useful if integrated management strategies are to be devised to provide control where biological agents are ineffective.

Describing control quantitatively using ecological information

Reporting of successful control varies from data describing the changes in abundance of the weed, obtained through simple field monitoring, to detailed studies of interactions between weed, agents and the environment. The later studies are particularly important because an accumulation of such information may provide the basis for improving the success rate in biological control.

Some researchers consider that ecological success occurs when the density or biomass of the target weed is reduced regardless of the economic impact. In a scientific sense this may be so and it provides a good argument to continue to search for additional agents to control the weed. Ecological success may not equate to successful control of the weed and this distinction is important. In most instances when an assessment of success of a project is being made it relates to the reduction in the status of the weed.

Describing control using sociological and environmental descriptors

The benefits of successful control sometimes occur where quantification is not possible, e.g. remote locations or conservation areas. Often resources to control a weed are not made available until severe infestations occur and the resulting problems are obvious. Then the resources are restricted to solving the problem and not quantifying the obvious damage caused by the weed. The result is that after control little or no data is available and the benefits of control can only be described subjectively. Such descriptions are enhanced significantly by using before and after photographs provided it is obvious that the photographs are taken at the same location.

Describing control quantitatively using economics

Reduction in the weed can be measured in terms of production increase and/or reduced costs of other control measures. It is sometimes possible to conduct simple assessments such as determining the value of reduced herbicide applications following successful biological control. For example, when alligator weed was controlled on a river in Australia the local council ceased herbicide applications which had cost $A 8000 per year for herbicide and labour (Julien 1981). Comprehensive cost: benefit studies require particular expertise. The economist Doleman (1989) studied the salvinia project and found that for Sri Lanka the costs to benefits ratio of successful control was 1 to 53 in monetary terms or 1 to 1673 in labour.

To convince governments, grants committees, and aid agencies that a project was successful, and that biological control projects are worthy of support, it is important to describe successes in economic terms. People deciding on the relative value of projects and providing the resources often do not understand ecological data. On the other hand they usually understand economics. The few studies of the economics of successful biological control have shown the benefits gained to be enormous and to far outweigh the costs of all failures.

Factors That Effect the Success of a Project

Achievements within a project

Success in biological control is dependant upon the accumulated achievements or outcomes during a project. Some stages are not critical and the failure to undertake a preferred aspect might not jeopardise the success of the project. For example, a successful agent may be found although the whole of the native range of the weed was not explored. Other stages are critical. For example, establishment of an agent is a necessary precursor to damaging the weed. While there are outcomes in a project over which the research has little influence, such as, the level of host selection of an agent, there are others over which the researcher has great influence, such as, methods of exploration, rearing techniques, selection of release sites, etc. It is most important that the researcher takes great care over the areas of the project that he/she can influence.

Projects, beginning with exploration for new agents, are undertaken by organisations in a few countries (South Africa, Australia, USA). Other countries utilise information and agents already known. In these latter instances, the first steps in the projects involve obtaining a colony of the desired agent, rearing it, sometimes undertaking tests to confirm host specificity, mass-rearing and release. Suggestions about how to gain the best overall result through careful management during these steps are discussed below.

Obtaining high quality material to begin a colony

It is important to ensure that the organisation supplying control agents provides individuals that are free of parasites and disease that might debilitate the colony and restrict the potential of released material. It is also important that the material received is from a colony with a broad genetic base and in general good health so that the ensuing generations will be genetically robust and able to reach their reproductive potential.

Rearing to maintain colony fitness, health and reproductive potential

Rearing is often considered a routine task and may not be given the attention required to ensure that the colony not only survives, but is well maintained, well fed and given the opportunity to produce generations of healthy, fecund individuals for release into the field. Poor hygiene, low quality food (host plant) and rearing under inappropriate environmental conditions all limit the general colony health. When colony health declines, numbers may fall so low that the genetic pool is restricted and progeny of the survivors may have lower genetic fitness. This could not prevent reproduction and survival but it may result in releases of individuals having less than optimum capacity to cope with the new environment, jeopardising the chances for establishment.

The importance of establishment of newly released agents

Once permission to release has been granted, success is dependant initially on the successful establishment of the control agent. However, in many instances too little attention is given to establishment of agents.

Successful establishment depends on:

a) interactions between the agent and the environment, over which the researcher has little control; and

b) ability of the researcher to organise releases to include: selecting the best release sites, making releases during the most favourable periods, rearing and releasing adequate numbers of individuals and releasing fit healthy agents (see section above).

If the researcher does not take responsibility to offer an agent the best conditions for establishment then the chances of establishment and of subsequently controlling the weed are reduced.

The weed, in its exotic range, may have a wider tolerance of the environment than the agents being released. Releases made into areas unsuitable for the agent are doomed to failure. Release strategies should be planned so that the agent is liberated into a wide range of habitats, ecoclimatic conditions and different seasons to assist establishment and increase the likelihood of successful control. For example, salvinia weevils released in temperate climates in mid and late summer had insufficient time to develop viable populations and survive winter. Releases made in early and mid spring resulted in establishment and eventual control of the weed.

Improving Success of Biological Control

Questions of how to improve the rate of success have vexed researchers for a long time. The difficulty is that each project has a unique set of circumstances and interactions and a large number of variables affect the outcome. In the absence of adequate numbers of well documented projects the development of useful generalisations and predictions has been limited. Some suggestions to improve efficiency or to improve success rates follow.

Choice of target weed

Burdon and Marshall (1981) looked at the correlation between reproductive biology for target weeds and level of control. They concluded that inbreeding

species were more likely to be controlled. The validity of the conclusion has been questioned on the basis that the weeds assessed were not representative and that their assessment was based only on correlation (Chaboudez and Sheppard 1996). The choice of target will continue to be driven by the costs and practicality of other methods of control, the relationship of the weed to important plant species and the likelihood of finding host specific agents.

Choice of area for collection of agents

Hokkanen and Pimental (1984) suggested that natural enemies and their host plants that had coexisted for long periods evolved together to their mutual benefit, developing a homeostasis. For the natural enemies, this evolution tended away from traits that are detrimental to the plant and hence they are less likely to be the best agents. They concluded that the epicentre of evolution of the weed may not be the best place to search for potential control agents.

On the other hand, searching in areas relatively new to the plant may provide natural enemies that are more damaging and potentially better control agents, the hypothesis being that there had been less time for natural enemies to evolve homeostatic traits with their host plant. They suggest that exploration should take place on the edge of the weed's native range, or natural enemies would be sought on closely related plant species that have different geographical ranges to the target but that will also attack the target weed.

This concept may have a theoretical basis and there are practical examples, such as the most famous moth, *Cactoblastis cactorum*, from Argentina that controlled prickly pears that originated in Central America. However, exploration during modern projects included collections from as wide a geographical source as possible and most successful control agents have been found near the epicentre of the weed's native range.

Choice of the best natural enemies to study

Harris (1973) and Goeden (1983) proposed sets of criteria against which potential control agents could be scored and compared for selection for further study. These scoring systems generated considerable debate, have been widely tested, but have not been found universally useful. Because of the uniqueness of biological interactions, such systems tend to understate the importance of some characters in relation to a particular weed or agent. On the other hand, characters that contribute to the overall score for another weed or agent may not be relevant.

Cullen (1996) discussed the above suggestions and concluded that "If we rely on any or all of these to guide our predictions, we run an enormous risk of excluding potentially effective species and of simply getting it wrong." He proposed that during research a series of questions be asked, hypotheses be formulated and tested, and the answers should help determine the proceeding hypotheses to test. The accumulation of questions and answers should aim to understand the three major factors that influence success. They are:

- the damage an individual or population unit of an agent can produce on a plant;
- the ecology of the agent in determining its density and therefore the total damage produced;
- the ecology of the weed in determining whether that damage is significant in reducing its population.

Such a systematic approach to understanding ecological systems is not new. It requires researchers with the training and resources to develop the appropriate questions and pursue the answers. As the number of case studies conducted increases, researchers will at least be able to better formulate the questions that when answered will provide useful information. Eventually accumulated knowledge may

allow answers to be predicted and generalisation to be developed concerning the best strategies to achieve successful biological control.

Improving Host Specificity Assessments

The demand to be sure that only safe organisms are released and the emphasis placed on cage studies in host specificity tests has, in the past, forced very conservative assessments of potential control agents. As a result, some insects have not been released because they showed interest in non host plants when confined in cages with that test plant. With improved understanding of plant/insect interactions and better methods to assess likely hosts, the number of rejected agents will decline. In addition, a greater tolerance is being given to the use of oligophagus species when the possible negative impact of releasing an agent is minor compared to the considerable impact caused by allowing the weed to grow without control (e.g. McFadyen and Marohasy 1990). These changes should help increase the chances of gaining control of the target weed and increase the global rates of success.

The Importance of Adequate Resources

Personnel with appropriate training in biological control are essential for the effective management of a project. Projects that commence with little information on the target weed and no information on associated natural enemies will require about 11 to 24 scientist years (Andres 1977; Harris 1979) and cost approximately $1–2 million each (Menz et al. 1984). However, where a weed has been controlled successfully in one country, the cost of introducing the successful agent into another country is comparatively little, the time to achieve control reduced (5 to 10 years) and chances of success are increased (Julien et al. 1984). The degree of success in controlling a weed is proportional to the amount and thoroughness of the research carried out (Waterhouse and Norris 1987).

References

- Andres, L.A. 1977. The economics of biological control of weeds. Aquatic Botany 3, 111–123.
- Burdon, J.J. and Marshall, D.R. 1981. Biological control and the reproductive mode of weeds. Journal of Applied Ecology 18, 649–658.
- Chaboudez, P. and Sheppard, A.W. 1996. Are particular weeds more amenable to biological control?—A reanalysis of mode of reproduction and life history. In: Delfosse, E.S. and Scott, R.R. ed. Proceedings of the VIII International Symposium on Biological Control of Weeds, Canterbury, 1992. CSIRO Publishing, Melbourne. 95–102.
- Cullen, J.M. 1996. Predicting effectiveness: fact or fantasy. In: Delfosse, E.S. and Scott, R.R. ed. Proceedings of the VIII International Symposium on Biological Control of Weeds, Canterbury, 1992. CSIRO Publishing, Melbourne. 103–109.
- Doleman, J.A. 1989. Biological control of *Salvinia molesta* in Sri Lanka. An assessment of costs and benefits. ACIAR Technical Report 12. 14pp.
- Goeden, R.D. 1983. Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. Protection Ecology 5, 287–301.
- Harris, P. 1973. The selection of effective agents for the biological control of weeds. Canadian Entomologist 105, 1495–1503.
- —1979. Cost of biological control of weeds in Canada. Weed Science 27, 242–250.
- Hokkanen, H. and Pimental, D. 1984. New approaches for selecting biological control agents. Canadian Entomologist 116, 1109–1121.

- Julien, M.H. 1981. Control of aquatic *Alternanthera philoxeroides* in Australia; another success for *Agasicles hygrophila*. In: Del Fosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds, Brisbane, 1980. CSIRO, Melbourne. 507–514.

- — 1982. Biological Control of Weeds. A World Catalogue of Agents and Their Target Weeds. Commonwealth Agricultural Bureaux, England. 108pp.

- Julien, M.H., Kerr, J.D. and Chan, R.R. 1984. Biological control of weeds: an evaluation. Protection Ecology 7, 3–25.

- McFadyen, R.E. and Marohasy, J.J. 1990. A leaf feeding moth, *Euclasta whalleyi* [Lep.: Pyralidae], for the biological control of *Cryptostegia grandiflora* [Asclepiadaceae] in Queensland, Australia. Entomophaga 35, 431–435.

- Menz, K.M., Auld, B.A. and Tisdell, C.A. 1984. The role for biological weed control in Australia. Search 15, 208–210.

- Waterhouse, D.F.W. and Norris, K.R. 1987. Biological Control Pacific Prospects. Inkata Press. Melbourne. 454pp.

A History of Biological Control Of Weeds

Early Examples of Classical Biological Control of Weeds

The earliest intentional biological control of a weed was the use of the cochineal mealybug *Dactylopius ceylonicus* against the cactus *Opuntia vulgaris* in southern India in 1863 and in Sri Lanka in 1865 (Tryon 1910). Cochineals were used in several countries to produce a red dye, but this was the first time they were used to control their host cactus, which had become a weed.

The first significant program of classical biological control, involving the import of agents following a search in the country of origin of the weed, was the program against *Lantana camara* in Hawaii. In 1902, the entomologist Koebele, who had previously been responsible for the successful control of cottony cushion scale *Icerya purchasi* on citrus in California, was employed to search for insects attacking lantana in its native range in Mexico. Twenty three different insect species from Mexico were shipped to Hawaii, of which 14 were released and eight of these established to give adequate control of lantana in most areas (Waterhouse and Norris 1987).

Rachel McFadyen and Brian Willson
Alan Fletcher Research Station
Queensland Department of Natural Resources
PO Box 36, Sherwood, QLD 4075
Australia

The next major program was the successful control of the prickly pears *Opuntia* species in Australia. Huge areas of valuable land were being progressively overrun by prickly pears, introduced to grow cochineal and as hedges or drought fodder. In 1912, two scientists were sent overseas to search for control agents and five agents were introduced between 1913 and 1914. The cochineal *Dactylopius ceylonicus* successfully controlled the drooping tree pear, *Opuntia vulgaris*, but the other major pear, *Opuntia stricta*, continued to spread.

In 1920, the Commonwealth Prickly Pear Board was set up, with the sole aim of attempting to control the prickly pears biologically. The Board Headquarters were in Brisbane, in a converted house at the site of the Alan Fletcher Research Station. Entomologists were based for several years in Mexico and the southern USA, where the cactus originated, and in Argentina where other related prickly pears occurred. A total of 48 different insect species were imported for testing in Brisbane, 12 of which were released and established. The most important was the moth *Cactoblastis cactorum*, introduced from Argentina in 1925. At the time, 60 million acres (24 million hectares) of valuable land had been infested by the cactus. By 1933, only a few years after the first release of the cactoblastis moth, the last big cactus areas had been destroyed. Ever since, the cactus continues to be controlled by the moth, with only occasional small outbreaks due to unusually dry weather which favours the cactus over the moth. These are controlled as soon as wetter summers return.

The enormous success of the prickly pear program led to requests for the moth from other countries, and cactoblastis was introduced to South Africa and the West Indies, as well as other smaller countries. In nearly all areas, the moth established easily and rapidly controlled the pest cacti, though it is more successful against some species than others. In the West Indies, the moth was first introduced in the 1960s, and spread slowly north and west until in 1989 it was found in Florida in the mainland USA (Bennett and Habeck 1996; Pemberton 1995). Here it threatens native cacti and is regarded as a serious pest. It can be expected to continue spreading into Mexico and the western USA.

Countries Involved in Classical Biological Control of Weeds

Since these first programs, the use of classical biological control against weeds has steadily increased, and Julien, in his 'World Catalogue of Agents and their Target Weeds' (1992), lists 117 target plants against which 729 exotic invertebrates and fungi have been deliberately released. The five most active countries, in numbers of weed species targeted and agents released, are the USA, Australia, South Africa, Canada and New Zealand, in that order, with the USA and Australia nearly twice as active as the others (Table 1). All these countries have a long history of successful weed biological control. For example, Hawaii has a success rate close to 50%, with seven out of 21 weed species targeted under 'complete' control, and significant partial control of three more (Gardner et al. 1995). Originally agricultural weeds were targeted, but there is an increased emphasis now on using biological control for weeds of natural ecosystems (here called environmental weeds), which are having a major impact on native ecosystems in Hawaii (Markin et al. 1992). Hawaii undertakes its own foreign exploration programs, and increasingly introduces pathogens as well as insects.

Continental USA is actively involved in several programs. Overseas surveys and testing are undertaken through various USDA-ARS laboratories or through the International Institute of Biological Control (IIBC). Canada also has an active weed biological control program, and usually employs IIBC for overseas surveys. Canada and the USA work closely together in both overseas exploration and introductions.

Australia is the second most active country. Foreign exploration is usually undertaken by Australian scientists based overseas, or by employing IIBC,

Table 1. Number of agent species released and weed species targeted by 1990 in the five most active countries (adapted from Hoffmann 1995)

Country	Agent species released	Weed species targeted
USA including Hawaii	130	54
Australia	123	45
South Africa	61	28
Canada	53	18
New Zealand	24	15

particularly to test pathogens which are increasingly used. New Zealand has several programs underway, cooperating with Australia, undertaking their own overseas research, or contracting IIBC. New Zealand also uses exotic pathogens.

South Africa has a very active program, with an excellent success rate of 83% overall, with six weeds out of 23 targeted under complete control and a further 13 under substantial control (Hoffmann 1995). South Africa carries out its own overseas exploration and cooperates closely with Australia, both for shared weed problems and because many plants from each country have become weeds in the other.

Other countries involved in classical biological control are Malaysia, Thailand, India, Indonesia, Vietnam, Papua New Guinea and China. In Africa; Uganda, Zambia, Tanzania, Kenya, Ghana, Côte d'Ivoire and Benin have active biological control projects. FAO now promotes biological control of weeds as a preferred option, and is currently supporting programs for the biological control of water hyacinth in Latin America and Africa, itchgrass (*Rottboelia* spp.) in Central America and the Caribbean, *C. odorata* in West Africa, and the parasitic weeds *Orobanche* and *Cuscuta* species in North Africa (Labrada 1996).

International cooperation has been a feature of biological control from the start. For example, the lantana seed fly *Ophiomyia lantanae*, collected by Koebele and established in Hawaii, was sent to New Caledonia in 1908, in 1911 to Fiji, and in 1914 to Australia. This cooperation has continued through joint projects, supply of nucleus colonies of proven agents, and sharing of test data and information on rearing methods etc.

Changes in Host Testing

Attitudes towards the risk of damage by biological control agents to plants other than the target weed, and the use of host-specificity testing to minimise that risk, have changed through the history of biological control of weeds.

In the early programs by Hawaii, no host-testing was undertaken (Waterhouse and Norris 1987). Observations were made in the field in Mexico, and if the insects were seen feeding only on lantana, it was assumed that they were sufficiently host-restricted for safe introduction. Hawaii continued to rely on field observations in the country of origin to determine host-specificity, until at least the 1950s. The unique and restricted flora of Hawaii, with few relationships to plants in other continents, probably reduced any problem of attack on plants other than the target weed. However, problems did arise when insects were introduced into other countries on the basis of their use in Hawaii and without further tests. For example, the lantana tingid *Teleonemia scrupulosa* was introduced into East Africa in the 1960s without further testing, where it caused problems by moving onto the crop sesame when the lantana was defoliated. There was also attack on teak trees. Economic damage was not great, but the attack caused problems and it is likely that the insect would not have been introduced if host-testing had been carried out first.

Host-specificity testing was first used in Australia in the 1920s, in the major campaign against the prickly pears (Dodd 1940). Field observations, where insects were seen to be feeding only on Cactaceae in the wild, together with the known association of the insect type with Cactaceae, were still seen as the chief proof of host specificity. Host-testing was undertaken mainly to satisfy the general public that crop plants would not be damaged. Initially, both choice tests and no-choice tests were used, but the choice tests were quickly abandoned in favour of no-choice tests. In choice tests, candidate agents are confined for varying periods

of time with test plant species together with the target weed. In no-choice tests, candidate agents are confined usually with only one test plant species and without the target weed. The tests used were starvation tests, that is, the insects were confined on the test plant until they died or developed through to the next stage. As the purpose of the tests was to prove that crop plants were safe, only plants of economic importance were tested; there was no concern for native Australian plants other than their economic value.

The conservative principle was adopted from the start (Dodd 1940): that is, an insect was rejected if it could complete development on a test plant, even if oviposition on the plant would not normally occur, and even where the insect was known not to attack the test plant in the field in its native range. Similarly, if one species in a genus was considered unsafe, the whole genus was rejected. Several stages were tested; always newly-hatched larvae or nymphs, and usually half-grown larvae and adult oviposition as well.

By the 1950s, the next major period of activity, host-testing was seen as an essential part of a weed biological control program. No-choice or sequential host-specificity tests of varying duration became the accepted methodology for the determination of host specificity until the late 1960s (Harris and Zwolfer 1968). Sequential tests involve the sequential presentation of a series of test plants in a no-choice situation, with each plant species usually exposed to the candidate agent for a relatively short time.

In the late 1960s Harley (1969) advocated choice as opposed to no-choice tests. It was argued that choice tests are a more 'natural test' of host range, as the target weed will be usually be present in mixed stands with other plants in the field situation (Cullen 1990). Choice tests may lead to fewer incidences of feeding on test plant species and less rejection of 'safe' insects. Discussions on the 'best' methods for host-specificity testing continue, and a mixture of choice and no-choice tests is usually used.

Changes in Test Plant Lists

Initially, only plants of economic importance were tested. Test lists included many plants quite unrelated to the host weed, and which were most unlikely to be attacked. In 1968, Harris and Zwolfer (1968) proposed that testing should concentrate on plants related to the known host, and should aim to determine the range of plants acceptable to the insect rather than demonstrate that certain valued plants were immune to attack. This approach was seen to be sensible, and test lists progressively changed to focussed lists of plants botanically or chemically related to the host weed.

The next major change was the growing concern over environmental issues, and possible damage to native plants, and from the 1980s these began to be included in test lists. In many cases, this has led to conflict over whether feeding on native plants of no economic significance is a sufficient reason to block release of a potentially valuable agent of a major agricultural or environmental weed. Discussion on these issues continues (McEvoy 1996; McFadyen 1998).

Pathogens

In the early days, pathogens were not seen as suitable biological control agents. Early workers were primarily entomologists and searched for insects only (Wilson 1964). Pathogens attacking prickly pears were studied but were not deliberately introduced, though two appeared in Australia, probably introduced on or with the insects. In the 1960s, the USDA was studying a rust *Uromyces rumicis* for the control of *Rumex crispus,* but it was not introduced. The first deliberate introduction of a pathogen was of the rust fungus *Puccinia chondrillinae* into Australia from Italy in 1971, to control skeleton weed *Chondrilla juncea.* This fungus was extremely successful in controlling the most common narrow-

leaf form of the weed but the remaining two forms were unaffected. New strains which will attack these forms are being sought in south-eastern Europe, the centre of origin of *Chondrilla*.

Despite this success, doubts continued regarding the safety of importing pathogens, and the next introduction was not until 1991 when the rust *Puccinia abrupta* var. *partheniicola* was introduced into Australia for the control of parthenium weed *Parthenium hysterophorus*.

It is now accepted that pathogens are as narrowly host-specific as insects, and equally safe to use as biological control agents. Several have now been imported into the USA as well as Australia, New Zealand and South Africa.

Other Organisms

Phytophagous fish have been introduced as biological control agents. The grass carp *Ctenopharyngodon idella* has been spread world wide from China. Although it had potential for aquatic weed control and fish production (Van Zon 1981), it has been ecologically disruptive through reducing aquatic vegetation generally and displacing native fish species. Fish are no longer considered suitable for biological control.

Legal Controls on Importation

In most countries, a permit from quarantine authorities was required for importation of plant-feeding insects. Rules for issuing these permits and enforcement of them varied greatly. The Hawaiian scientists worked for the Department of Agriculture, and Australian scientists for the Prickly Pear Commission or the Queensland Government. Both groups therefore had official sanction. However, *Dactylopius opuntiae* on *O. stricta* was introduced into Australia by a private individual in 1921 (Dodd 1940 p.68). In the USA, releases were made by individuals in universities and elsewhere, and it is not clear to what extent their views represented general scientific opinion (e.g. *D. opuntiae* was introduced from Hawaii into California by H.S. Smith in 1951 (Goeden et al. 1967)). IIBC programs were generally requested by the Department of Agriculture of the country concerned. In all these cases, the rules governing release were usually unwritten, and depended on a letter of approval from the Chief Entomologist or Chief Agricultural Scientist of the country or state. Little or no consultation with other groups may have taken place; however, no disasters occurred either.

Today biological control introductions in most countries are carried out under the supervision of quarantine authorities (McFadyen 1998).

References

- Bennett, F.D. and Habeck, D.H. 1996. *Cactoblastis cactorum*: a successful weed control agent in the Caribbean, now a pest in Florida? In: Delfosse, E.S. and Scott, R.R. ed. Proceedings VIII International Symposium on Biological Control of Weeds. Canterbury, 1992. CSIRO Publishing, Melbourne. 21–26.
- Cullen, J.M. 1990. Current problems in host-specificity screening In: Delfosse, E.S. ed. Proceedings VII International Symposium on Biological Control of Weeds. Rome, 1988. Instituto Sperimentale per la Patologia Vegetale, Rome. 27–36.
- Dodd, A.P. 1940. The Biological Campaign against Prickly-Pear. Commonwealth Prickly Pear Board, Brisbane, 177pp.

- Gardner, D.E., Smith, C.W. and Markin, G.P. 1995. Biological control of alien plants in natural areas of Hawaii. In: Delfosse, E.S. and Scott, R.R. ed. Proceedings VIII International Symposium on Biological Control of Weeds, Canterbury, 1992. CSIRO Publishing, Melbourne. 35–40.
- Goeden, R.D., Fleschner, C.A. and Ricker, D.W. 1967. Biological control of prickly pear cacti on Santa Cruz Island, California. Hilgardia 38, 579–606.
- Harley, K.L. 1969. The suitability of *Octotoma scabripennis* Guer. and *Uroplata girardi* Pic (Col.: Chrysomelidae) for the control of Lantana (Verbenaceae) in Australia. Bulletin of Entomological Research 58, 835–44.
- Harris, P. and Zwolfer, H. 1968. Screening of phytophagous insects for biological control of weeds. Canadian Entomologist 100, 295–303.
- Hoffmann, J.H. 1995. Biological control of weeds: the way forward, a South African perspective. Proceedings of the British Crop Protection Council Symposium. Weeds in a Changing World. Brighton 1995. British Crop Protection Council, Farnham. 77–89.
- Julien, M.H. 1992. Biological Control of Weeds. A World Catalogue of Agents and Their Target Weeds. Third Edition. CAB International, Wallingford. 186pp.
- Labrada, R. 1996. The importance of biological control for the reduction of the incidence of major weeds in developing countries. In: Moran, V.C. and Hoffmann, J.H. ed. Proceedings of the IX International Symposium on Biological Control of Weeds. Stellenbosch, 1996. University of Cape Town, South Africa. 287–290.
- Markin, G.P., Lai, P-Y. and Funusaki, G.P. 1992. Status of biological control of weeds in Hawai'i and implications for managing native ecosystems. In: Stone, C.P., Smith, C.W. and Tunison, J.T. ed. Alien Plant Invasions in Native Ecosystems of Hawai'i: Management and Research. Honolulu: University Hawaii Press. 466–482.
- McEvoy, P.B. 1996. Host specificity and biological pest control. Bioscience 46, 401–405.
- McFadyen, R. Cruttwell. 1998. Biological control of weeds. Annual Review of Entomology. 43, 369-393.
- Pemberton, R.W. 1995. *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States: an immigrant biological control agent or an introduction of the nursery industry? American Entomologist 41, 230–232.
- Tryon, H. 1910. The wild cochineal insect, with reference to its injurious action on prickly pear (*Opuntia* spp.) in India, etc., and to its availability for the subjugation of this plant in Queensland and elsewhere. Queensland Agricultural Journal 25, 188–97.
- Van Zon, J.C.J. 1981. Status of the use of grass carp *(Ctenopharyngodon idella* Val.). In: Del Fosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds. Brisbane, 1980. CSIRO, Melbourne. 249–260.
- Waterhouse, D.F. and Norris, K.R. 1987. Biological Control: Pacific Prospects. Inkata Press, Melbourne. 454pp.
- Wilson, F. 1964. The biological control of weeds. Annual Review of Entomology 9, 225–244.

Introduction to Weed Science

What Is a Weed?

Although the word 'weed' means something to almost everyone it is not easy to give a wholly satisfactory definition of this class of plant. Common definitions of weeds (Blatchely 1912; Harper 1944; Oxford English Dictionary 1988) include the very important and central idea that they are exclusively associated with people and their activities, and that they are undesirable and have negative value.

A plant may be useful in some situations but a weed in others. For example, Bermuda grass (*Cynodon dactylon*) is quoted as one of the world's worst weeds but in some places it is better known as a valuable lawn grass or as a major fodder grass. Similarly, a crop plant which carries over into the next crop in a rotation is a weed.

The best simple definition of a weed is:

'Plants existing at places and/or times in which they are considered undesirable'

Importance of Weeds

Crop losses

Losses caused by uncontrolled weed growth are measured in billions of dollars per annum.

Annual losses of crops to weeds, pests and diseases differ depending on the crop (Table 1) and geographic region (Table 2). Some of the greatest losses are recorded in the tropics where good growing conditions and poor weed control measures result in good weed establishment. British agriculture has spent in excess of $250 million per year from 1980 to 1990 on herbicides to alleviate damage that would otherwise be caused by weeds. This outstrips that of either insecticides or fungicides.

Steve Adkins
Department of Agriculture
University of Queensland, QLD 4072
Australia

Table 1. Annual losses in potential production of three crops due to various agents

Crops	Potential production (Mt)	Losses (Mt) due to: Pests	Diseases	Weeds
Cereals	1468	204 (40%)	135 (27%)	167 (33%)
Sugar crops	1330	228 (36%)	232 (37%)	175 (26%)
Vegetables	280	23 (29%)	31 (40%)	24 (31%)

Table 2. Annual losses in production due to various agents in different parts of the world

Area	Percentage losses due to: Pests	Diseases	Weeds
Worldwide	14	12	10
Europe	5	13	7
North America	9	11	8
Africa	13	13	16
Asia	21	11	11

Table 3. Effect of wild oats (Avena fatua) on wheat yield

Wild oat density (plants / m²)	Yield loss (%)	Example (t / ha)
0	0	0
40	25	3
100	50	6
200	67	8
600	75	9

Problems Weeds Cause

Yield losses through weed interference (direct)

Competition

Weeds compete with desirable plants for space, water, nutrients and light, and so reduce the yield of desirable plants. Characteristics of the weed influence the outcome of competition. Relative rooting depths will influence competition for water and nutrients. Some weeds are particularly effective in the uptake of certain nutrients, e.g. black grass, (*Alopercurus myosuroides*) and this may be due to selection pressure acting on the plant which came from a type that lived on disturbed land (low in nutrients). Mode of growth affects competition for light. For example, broad-leaved weeds spread out relatively close to the ground inhibiting light from reaching others at the soil surface. Table 3 illustrates the relationship between yield loss and weed density under competition.

It is during the very early stages of crop growth that weeds are particularly competitive. For example, in a field bean crop the first four weeks are the most critical; keeping fields free of weeds for five weeks can improve yield by as much as 300%.

Interference with crops by means of toxic exudates

Many plants produce chemicals which may, when released from their roots or leaves, adversely affect the growth of other plants. For example, a weed of flax, gold-of-pleasure (*Camelina alyssum)* has no effect on flax yield when both are grown together in pots watered from below. However, if water is allowed to fall onto its leaves a toxic material is leached (a phenolic compound) which can affect the growth of the flax.

A second example would be the extracts (breakdown products) of old root and rhizomes of couch grass (*Agropyron repens)* which are able to inhibit the germination and growth of oil seed rape seedlings.

Parasitic upon crop plants

This property is not of great significance in Australia, but extremely important in other parts of the world. Witchweeds (*Striga* spp.) can be a parasite on sorghum and maize (Africa, India and USA). Some forms of mistletoe (*Amyema* spp.) are locally important in tropical countries.

Summary 'Competition'

The total effect of the weed on the crop/pasture is often referred to as 'competition'. The balance of competition can favour the weed or the crop. It depends on the combined effect of all factors (Fig. 1) including several important environmental parameters.

Increased production costs (indirect)

Weed infestations will often lead to increased tillage operations, greater herbicide usage and may damage farm machinery (plants with long wiry stems which spread close to the ground). As some weeds may cover others the efficacy of herbicides may be reduced and more herbicide will have to be used to achieve good control. Finally some weed products (e.g. seeds) may infest crop products and increase transport costs of the crop.

Quality loss in marketable products (indirect)

This is likely to be true with crops grown for seed, when contamination by weeds greatly increases the costs of the cleaning process. In some cases contaminated seed will not be acceptable in crop seed at all, e.g. the poisonous black nightshade (*Solanum nigrum*) in crops of peas grown for canning or freezing industries. Other losses could result from the weed producing off-flavours in the harvested product or the weed may reduce the quality of the soil (e.g. linseed) or protein (e.g. barley) in the harvested product.

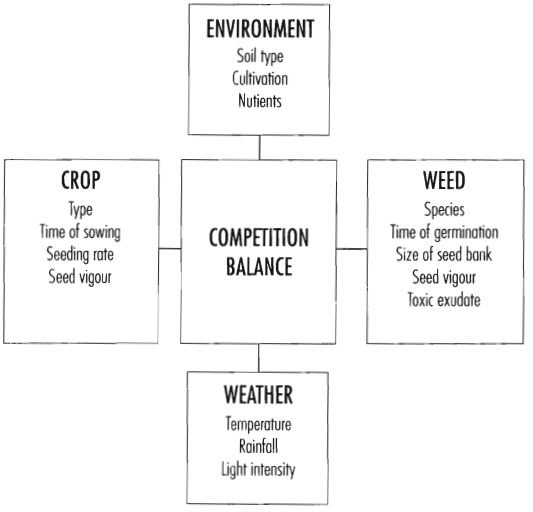


Figure 1. Influence of the crop, weed and the environment on the balance of competition

Table 4. Weeds which act as hosts to various crop diseases

Weed	Crop	Disease/pest
Black grass (*Alopercurus myosuroides*)	Rye	Ergot fungus
Chickweed (*Stellaria media*)	Many crops	Cucumber mosaic virus
Fat hen (*Chenopodium album*)	Field beans	Black bean aphids
Numerous weeds	Numerous crops	Nematodes
Wild oats (*Avena fatua*)	Cereals	Smuts and mildews

Harbour of pests (insects) and diseases (indirect)

Weeds may act as host for diseases and pests which affect crop plants (Table 4).

Reduced value of land and other property (indirect)

Some weeds have an unattractive appearance (e.g. *Panicum* spp.) and may put off a prospective buyer. Weeds may also block drains, ditches and irrigation channels (e.g. water hyacinth, *Eichhornia crassipes*) as well as cover road verges and railway lines (e.g. many grasses). Such properties of weeds will lower the value of the land.

Physical characteristics may be problematical (indirect)

Parts of plants such as the hooked fruit of cleavers (*Galium aparine*) which become tangled in sheeps' wool can be a serious nuisance and thorns or spines may cause injury to animals.

Poisonous nature (direct/indirect)

The poisoning of humans is rare. Grazing animals often avoid poisonous plants in pastures but may be unable to discriminate against them in hay or silage. It has been reported that the annual losses of livestock in USA due to plant poisoning is approximately 15 million US dollars.

Toxic to animals

In the USA alone, some 700 plant species have been declared poisonous to livestock. Poor stock management is the primary factor determining the probability of stock poisoning, e.g. overgrazing pastures, producing nutritional stress in sparse feed areas. It has been found that half of the broadleaf plants on rangeland can be poisonous.

There are various groups of chemicals which are produced by weeds which can affect stock:

- Alkaloids (*Crotalaria, Datura*) – Sudden death.
- Glucosides (*Cryptostegia*) – Salivation, difficulty in breathing and death.
- Oxalates (*Amaranthus, Cenchrus*) – Muscle trembling and death.
- Cyano-glycosides (*Sorghums*) – Muscular spasms and death.
- Terpenoids (*Pimelea, Lantana*) – Loss of appetite, difficulty in breathing.
- Phenols (*Acacias*) – Frothing at the mouth, sudden death.
- Saponins (*Sarcostemma*) – Champing of the jaws, vomiting.
- Nitrates/nitrites (*Amaranthus, Portulaca*) – All stock rapid respiration/death.

Unpalatable to animals

Weeds may be unpalatable, nutritionally poor or may cause tainting of animal products. For example, the wild onion (*Allium* spp.) causes unacceptable flavours in meat and milk.

Toxic to humans

Some weeds may have two effects on human health, poisonous or allergenic. Human deaths from poisonous plants are 40 times greater than from pesticide poisoning. The most common poisoning is due to inedible mushrooms. Allergenic plants can cause problems via contact when contact allergens are produced or via inhalation allergens such as pollen.

Weeds in public lands and forests

Unlike weeds in cropping situations, environmental weeds on public lands are difficult to access and control. The best methods of control are biological, chemical or mechanical. It is generally accepted that the major threat weeds have on such land is to alter biodiversity and to reduce appearance of public places.

Beneficial characters of weeds

Weeds are said to have various beneficial characters. These characters include:

- Weeds grow quickly over unsightly scars on the landscape caused by people or nature.
- Some weeds provide excellent forage for livestock, especially in the spring.
- Weeds provide shelter and food for birds and wildlife.
- Weeds serve as hosts for beneficial insects and provide nectar for bees.
- Weeds play an important part in nutrient cycling as they tap nutrients from the lower soil depths and return them to the soil surface as litter.
- Weeds add organic matter to the soil.
- Some weeds are used as food (e.g. herbs)
- Weeds prevent or reduce wind and water erosion of the land.
- Weeds may be the source of drugs used in tropical public health to cure headaches, skin diseases and syphilis.
- Weeds have been the source of some natural pesticides (e.g. pyrethrum from chrysanthemum).
- Weeds act as a source of genetic materials for crop improvement (e.g. breeding for insect pest and plant disease resistance).
- Weeds may beautify the landscape. Some house and garden plants have come straight from the wild state (e.g. *Bryophyllum* spp.).
- Weed eradication projects may provide employment for otherwise unemployed people.
- Weeds which cause allergies, dermatitis and poisoning may be considered to provide physicians and veterinarians with afflicted patients.

Major Weeds of the World

Taxonomy

Out of the whole world flora only a very small fraction (fewer than 250 species) is composed of major weeds. About 80 weed species are thought to be responsible for 90% of crop losses attributable to weeds.

Some families of plants contain a disproportionate number of weeds. The major families are the Poaceae, Asteraceae, Cyperaceae and the Brassicaceae (Table 5).

Most serious weeds of the world

The ten most serious weeds of the world, found in 17 or more countries are ranked in order of total number of countries, in which each species is considered to be a 'serious weed' (Table 6).

Table 5. The major weed families. (Adapted from Hill 1977 and Akobundu 1987)

Family	Number of major weed species in the world	Number of major and minor weed species in North America
Poaceae	44	65
Asteraceae	32	112
Cyperaceae	12	*
Brassicaceae	*	61
Lamiaceae	*	60
Fabaceae	6	54
Amaranthaceae	7	37
Scrophulariaceae	*	30
Polygonaceae	8	*
Convolvulaceae	5	*
Euphorbiaceae	5	*
Malvaceae	4	*
Solanaceae	4	*
Total	126	435

* *Data not available.*

Table 6. The top 10 most serious weeds of the world based on total number of countries infested (Holm et al. 1977)

Species	No. countries
Nutgrass (*Cyperus rotundus*)	52
Barnyard grass (*Echinochloa crus-galli*)	32
Bermuda grass (*Cynodon dactylon*)	28
Summer grass (*Digitaria sanguinalis*)	23
Barnyard grass (*Echinochloa colona*)	22
Johnson grass (*Sorghum halapense*)	22
Crowsfoot grass (*Eleusine indica*)	20
Dirty dora (*Cyperus difformis*)	19
Blady grass (*Imperata cylindrica*)	18
Pigweed (*Portulaca oleracea*)	17

Some important grass weeds

Bermuda grass (*Cynodon dactylon*), a perennial of cosmopolitan distribution often used as a forage, is the most widespread grass weed affecting 90 countries. The two other perennial grasses, Johnson grass (*Sorghum halapense*) and blady grass *(Imperata cylindrica*) are widespread in tropical areas.

Among the annual grasses, winter grass (*Poa annua*), of European origin, is the most cosmopolitan as a weed of crops, lawns and waste land. Crowsfoot grass (*Eleusine indica*) is mainly found in tropical regions. The two Barnyard grasses spp. (*Echinochloa*) are annuals and are major weeds of irrigated tropical crops.

Wild oats (*Avena fatu*), which is of Mediterranean origin, is found particularly wherever the annual temperate cereals; wheat, barley, oats and rye are grown. Summer grass (*Digitaria sanguinalis*) is found in both tropical and temperate regions in both crop and non-crop situations.

The trait of seed dormancy is strongly associated with the persistence of six of the top nine grass weeds (those that are annuals). These plants rely on a period of dormancy in the seed bank to effect population renewal over several seasons. Other traits such as competitive ability at the seedling and vegetative stage and seed number will have a strong influence on the success as a weed.

Some important broad-leafed weeds

Common ragweed (*Ambrosia artemisiifolia*) is an annual weed throughout the northern hemisphere in cropping and rangeland and causes hayfever in many individuals.

Redroot pigweed (*Amaranthus retroflexus*) is a coarse, erect annual widely distributed around the globe in cultivated lands, gardens and waste areas. Germination can occur at any time during the growing season when soil moisture is sufficient.

Canada thistle (*Cirsium arvense*) is a colony-forming perennial native of south-eastern Eurasia. It has been introduced to many new areas as a contaminant of crop seed. This aggressive weed is very difficult to control as roots are often broken up by ploughing and this seems to increase the numbers of new plants.

Biological Characteristics of Weeds

Many characteristics which are likely to make a plant successful as a weed are self-evident.

Seed output

A species which can produce a large seed population, will be at an advantage in a competitive situation and many weeds are notable for producing large numbers of seeds (Table 7). Perennial plants are not under the same pressure to produce large numbers of seeds and are less prolific than annuals. Abilities to set seed on

poorly developed or young plants are an advantage when unfavourable environmental conditions limit growth or prevent further development.

Table 7. Seed production of various weeds

Temperate species	No seeds/ plant
Groundsel (*Senecio vulgaris*)	1 000
Chickweed (*Stellaria media*)	2 500
Common poppy (*Papaver rhoeas*)	16 000
St Johns wort (*Hypericum spp.*)	30 000
Shepherds purse (*Capsella bursa-pastoris*)	38 000
Hard rush (*Juncus inflexus*)	220 000
Wormwood (*Artemisia biennis*)	1 000 000
Tropical species	
Summer grasses (*Digitaria spp.*)	12 000
Barnyard grasses (*Echinochloa spp.*)	42 000
Witchweeds (*Striga spp.*)	90 000

It is an advantage to continue flower production for a long time to take advantage of favourable conditions. This may be achieved by producing seed over a very large portion of the growing period, or by a spread of flowering within the population.

Seed dormancy

The ability of weeds to spread their germination over a long period of time is vital to avoid unfavourable conditions. This is the phenomenon of seed dormancy.

Weeds may be contrasted with most crops which have been selected both consciously and unconsciously by scientists for rapid, uniform and immediate germination without any dormant period. Such behaviour in an annual weed would mean that it could be eliminated entirely by one or two years of careful cultivation.

Dormancy in seeds is often considered to fall into three categories.

Innate dormancy (dormant when shed from plant)

This form of dormancy can be caused by one, some or all of the following:

- Impermeable or mechanically resistant seed coat.
- Endogenous germination inhibitor(s).
- Rudimentary embryo or physiologically immature embryo.

Induced dormancy

In this form of dormancy seeds that would normally germinate are prevented by an unfavourable environment parameter. However, when the seeds are returned to a favourable environment they are unable to germinate.

Enforced dormancy

In this form of dormancy seeds are prevented from germinating by the lack of a key environmental factor such as moisture, oxygen, or low temperature. However, the seeds are able to germinate when the missing factor is supplied. Many weed species show a marked periodicity of germination because of enforced dormancy during part of the year.

Seed longevity

Many weeds produce seed with extended longevity (Table 8).

Table 8. Germination percentage (viability) of seed buried for different periods of time (Duvel 1902)

Species	Germination (%)	Burial time (year)
Shepherds purse (*Capsella bursa-pastoris*)	47	16
Greater plantain (*Plantago major*)	84	21
Black nightshade (*Solanum nigrum*)	83	39
Rye (*Secale cereale*) (crop)	0	1

The population of viable weed seeds in the soil seed bank is controlled by the rate of input from mature plants and the rate of mortality.

Seeds in soil die due to external factors such as disease and decay organisms and predation by animals, and to internal factors such as:

- Respiration of food supplies stored in the seed.
- Enzyme action leading to breakdown of stored food.
- Coagulation of proteins.
- Accumulation of toxic products (ethylene, ethanol).
- Degeneration of DNA.

The number of weed seeds in the soil is influenced by farming practices (Table 9).

Table 9. Number of weed seeds found in soil under different cropping systems

Cropping regime	Seed m^2
Continuous wheat	34 000
Mixed crops	28 000
Derelict arable land	46 000
Old arable land	250
Commercial vegetable crops	>86 000

No special germination requirement

Plants with no special germination requirements, or variability in germination requirements, may exploit a wider range of environments. However, as some weeds compete with crop plants it may be advantageous if germination is controlled by some factor which is linked to the cultivation operation (i.e. light stimulated or flowering controlled by photoperiod).

Rapid seedling growth

Rapid and effective establishment of seedlings is important both for crops and weeds. However, it is not always necessary for a weed to have this character as second and third chances for germination may occur later in the season.

Tolerance of variation in the physical environment

Plants that tolerate environmental extremes are likely to be successful weeds.

Adaptations for dispersal

The ability to produce offspring which may establish some distance from the parent plant is a characteristic of fundamental importance to all plants; especially those colonising new areas. Mechanisms include wind dispersal of seeds with parachute-like pappus, dispersal of floating fruit in water, movement of hooked fruit by attachment to animals and equipment (Table 10).

Table 10. Dispersal mechanisms of some common weeds

Species	Dispersal structure	Medium
Dandelion (*Taraxacum officinale*)	Parachute-like pappus	Wind
Hard rush (*Juncus inflexus*)	Floating fruit	Water
Morning glory (*Ipomoea indica*)	Floating fruit	Water
Cleavers (*Galium aparine*)	Hooked fruit	Animal
Sensitive plant (*Mimosa pigra*)	Hooked fruit	Animal
Noogoora burr (*Xanthium pungens*)	Hooked fruit	Animal
Wild oat (*Avena fatua*)	Awned fruit	Equipment

Vegetative growth (reproductive propagules)

Plants that rely on vegetative methods of reproduction may produce one or several of the following.

- Rhizomes (*Sorghum, Cyperus, Pteridium*)
- Tubers (*Cyperus*)
- Stolons (*Salvinia, Tradescantia*)
- Stem joints (*Opuntia*)
- Bulbs (*Oxalis*)
- Roots busa (*Lantana*)
- Leaf buds (*Kalanchoe*)
- Apomictic seeds (*Lantana*)

Physiological and morphological attributes

Physiological attributes, including rapid ion uptake and rapid root growth, together with morphological and behavioural features such as the ability to climb up other plants (e.g. bindweeds) or to scramble over competitors (chickweeds) confer a competitive advantage on weeds.

Life cycle

Most annual weeds have a very adaptable life cycle (Fig. 2) able to respond to any farmer-induced or environmental pressure that may cause a problem to their continued existence.

The ideal weed

Fortunately the ideal weed does not exist. It would be a plant with all the above-listed characters. Both Johnson grass and nut grass are very close to being ideal weeds.

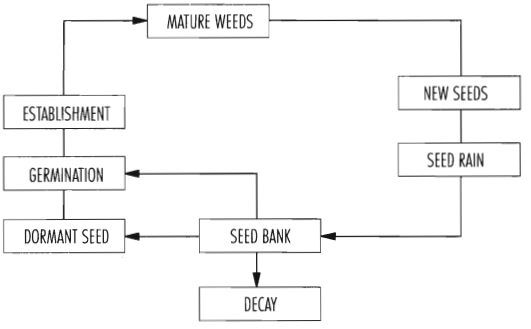


Figure 2. A schematic population cycle of an annual weed

Methods of Weed Control

Preventative (good management) methods

Weed-free seed

Planting crop seed contaminated with weed seed is one of the most common ways of introducing weeds into crop land. Clean, tested and tagged crop seed has many fewer weed seeds. The kind and percent of weed seeds present in commercial crop seed is listed on the tag on the seed bag, based on a representative sample.

Crop rotations

Rotations are practiced as a means of weed control to prevent or reduce the build up of high populations of weeds common to a particular crop. The kind of crop plants to include in a crop rotation sequence should have growth and cultural characteristics in sharp contrast to those of the previous crop and the problem weed. Fallow periods may also be used.

Clean tillage and harvest implements

All tillage and harvest implements should be cleaned before moving to the next field to prevent transfer of weeds.

Sanitation measures

This involves weed control in waste areas, roadsides and fences to ensure reinfestation does not come from these sources.

Prevention of seed production

This is a common method of preventive weed control and may include mowing before weeds shed pollen or the use of fire to destroy some types of seeds.

Smother crops (live mulch)

These are crops that are especially highly competitive with the weed species infesting an area and compete with the weed for light, nutrients and moisture. Crops most used include barley, millet, rye, sorghum, alfalfa, clovers, cowpeas, buckwheat, sesbania, peanuts and sudan grass.

Controlled movement of vehicles

Most vehicles can carry weed seeds in many places, or seeds blow from loaded trucks without tarps.

Controlled movement of stock

Seed can be transported within the digestive tract of many animals. Domestic animals can be ranked from most to least destructive of weed seeds as follows: chickens, sheep, horses, swine, cattle.

Controlled movement of plants locally

Weeds should not be hauled in with sod for lawns, potted plants, irrigation water from ditches. Quarantine laws may prevent this sort of plant movement.

Controlled movement of plants internationally

Exotic plants should not be cultivated if there is a chance of their escape into the natural environment. Quarantine laws are often in place to prevent this.

Mechanical harvesting

Mechanical harvesting methods often scatter weed seeds over a field as well as into new fields.

Animal feed

Animal feed may carry numerous weed seeds, however seed viability can be destroyed by processing the feed. For example large seeds can be killed by grinding while small seeds can be cooked. Ensilage will also destroy most weed seeds. Composting can destroy some weed seeds but this depends on the size of pile, type of manure, moisture content, temperature, location and condition of weed seeds, and length of storage.

Mechanical (physical) methods

Manual cultivation

Hard pulling is the oldest and one of the most effective as human energy is directly utilised. It is of minor value to the control of established perennials as underground parts are usually not disturbed. Hoeing is a widely used technique in the tropics. It is a highly effective method of weed control, however it is labour intensive.

Machine tillage (= cultivation)

This can involve any implement powered by animals or machines and the method is often less effective than manual methods. It is generally effective against annuals and shallow rooted biennials but may not kill deep rooted biennials. It may be applied before (cultivation) or after (tillage) crop emerges and the mode-of-action is to bury small weeds, pull up others by loosening the soil around the roots and killing the plant by desiccation. When tillage is used to prepare the land for furrow irrigation but tends to create favourable conditions for weed seed germination. Deep rooted perennials are controlled only by depletion of food reserves through continuous destruction of top growth.

Mowing and cutting

Sickles, scythe, axes, mowers have only a limited value as a means of weed control. Their primary aim is to restrict unsightly weed growth. They are commonly used along road sides and in waste places. These methods may help control weeds when applied at or before the bud stage because they prevent seed production. They also deplete food reserves especially in perennials. Generally, mowing is ineffective against prostrate or short weeds growing close to the ground.

Flooding, dredging, draining, and chaining

Flooding deprives weeds of air and the ability to carry out photosynthesis. It is only effective when the roots and shoots are covered and when the situation prevails for a sufficiently long period of time. Its success is confined to terrestrial plants. Dredging, draining, chaining are techniques that can be used on aquatic weeds.

Heat

Fire is often used to burn accumulated debris that is a health hazard or is unsightly especially on railroads, canal banks, drainage ditches and road sides. Flaming kills young plants if their cambium reaches 50°C. To use this technique the crop plant must be larger than the weeds, have woody stems, be resistant to intense heat and the flame should be directed towards the ground. The technique is used in cotton, sugarcane and soybean.

Smothering with non-living materials

Hay, manure, grass clippings, straw, sawdust, wood chips, rice hulls, paper, and plastic film can be used to completely exclude light from the growing weed. This prevents photosynthesis and further growth. Cost of materials and residues make this an expensive option.

Biological control methods

General principles of biological weed control

Biological control is defined as, 'the action of predators, pathogens, and/or parasites in maintaining another organism's population density at a lower average level than would occur in their absence'. Biological weed control involves the utilisation of natural enemies for the control of weeds. It may be achieved via direct or indirect action of the biotic agent which can either;

a) bore into the weed and weaken its structure,

b) consume or destroy vital plant parts,

c) reduce weed vigour and reproduction, or

d) enhance conditions that favour plant pathogen attack.

The objective in biological control is never eradication; it is reduction of weed density to non-economic levels.

Methods used in biological control

Classical
Natural enemies are introduced from their native range into another country to control an exotic weed. Most common method. For example, the control of salvinia (*Salvinia molesta)* in Australia using the weevil *Cyrtobagous salviniae.* Both the weed and the weevil came from Brazil.

Inundative
When an agent, usually native to the country of application, is mass reared and released in an ongoing program. There are few examples and none which are current.

Bioherbicides or mycoherbicides
Preparations containing pathogens which are applied as sprays.

Agents (natural enemies) used for biological control

Insects
The reason insects are often good biological control agents is that they have great diversity, high degrees of host specialisation and intimate adaptations to hosts. Examples of successful programs include *Cactoblastis cactorum* (moth on several cacti), *Chrysolina quadrigemina* (beetle) on St John's wort and *Cyrtobagous salviniae* (weevil) on salvinia.

Fungi, mites, nematodes
Rusts such as *Puccinia chondrillina* have been used to control several weed species such as skeletonweed (*Chondrilla juncea).* Other agents include *Collectotrichum gloesporioides* f.sp. *aeschynomene*, an agent to control northern jointvetch (*Aeschynomene virginica*). Mites and a nematode have also been released to control particular weeds.

Fish
Some species of fish have been released specifically to control aquatic weeds. Others are used as multipurpose weed control and food source.

Other organisms used for weed control

The following are sometimes used for weed control although it is not their primary use. They are not normally included in a discussion about biological control of weeds.

Vertebrates
Sheep and goats can be used to control ragwort (*Senecio jacobaea*) and blackberry (*Rubus* spp.).

Geese
Water birds such as geese can be used to control weeds in cotton.

Other higher plants
Higher plants may have an allelopathic effect on weeds and be considered live mulches.

Grazers
Grazers such as fish and ducks can be used for aquatic weeds. Snails and manatees have been assessed but found unsuitable.

Chemical control methods

Introduction
The use of chemicals for weed control offers the greatest possibilities for relieving the physical effort which, in the past, has been necessary for this tedious chore. Chemicals function on the basis that certain

chemicals are capable of killing plants and that they kill some kinds of plants without significant injury to other kinds. As a group, these phytotoxic chemicals are called Herbicides. The use of chemicals for weed control has been practiced largely since 1944 following the discovery of the phytotoxic phenoxy group of chemicals. Today there are more than 180 different chemicals available for use as herbicides. They offer an almost bewildering array of trademark products of which there are thousands each varying from the other as to the active ingredients, concentration and/or formulation constituents. Chemical control of weeds has paved the way to mechanisation and modern farm systems with large increases in productivity. As a result, today a farm worker 'feeds' 120 people worldwide compared with less than 20 in 1940.

Advantages of chemical weed control

Herbicides can be applied prior to the emergence of the crop, they can permit closer crop row spacing, lower labour involvement, they minimise crop root damage, they can be applied over a wide range of weather conditions and at a very fast rate.

Disadvantages of chemical weed control

Herbicides are costly to produce and to apply (e.g. incorporation), they may provide inadequate levels of weed control or cause crop damage. Herbicides need sophisticated equipment for application, they tend to change weeds towards resistant types and may pollute soil, water and air with their residues.

Types of herbicides

Inorganic salts

This group has a long history but they have largely been replaced by modern organic herbicides. Sodium chlorate still remains a substantially used chemical for total weed control in non-cropping areas.

Organic herbicides

For an orderly study organic herbicides may be grouped on the basis of one or more common characteristics such as chemistry, biological effect, application or use. Most text books group herbicides based on their chemical similarity. A number of chemicals will comprise a group or herbicide family. These families are listed below.

Phenoxy-carboxylic acids (e.g. MCPA, 2,4-D)

Substituted benzoic acids (e.g. Dicamba)

Picolinic acids (e.g. Picloram)

Benzonitriles (e.g. Bromoxynil)

Sulphonylureas (e.g. Chlorsulfuron)

Dinitroanilines (e.g. Trifluralin)

Carbamates (e.g. Propham, Asulam)

Bipyrazolum compounds (e.g. Difenzoquat)

Diphenyl ethers (e.g. Diclofop)

Substituted cyclohexanes (e.g. Sethoxydim)

Acid amides (e.g. Propanil)

Triazinones (e.g. Metribuzin)

Dintrophenols (e.g. Dinoseb)

Bipyridilium compounds (e.g. Paraquat)

Organophosphates (e.g. Glyphosate)

Imidazolinones (e.g. Imazaquin, Imazapyr)

Glufosinates (e.g. Glufosinate)

Integrated weed management

Introduction

This is a weed management system that, in the context of the associated environment and the population dynamics of the weed species, utilises all available techniques and methods in as compatible a manner as possible (Fig. 3), and maintains the weed population at levels below those causing economic injury. Therefore, in order to implement integrated

weed management (IWM) programs successfully it is necessary for the weed scientist to be trained in all aspects of IWM.

Weed management systems

Western world

At the moment there is too much dependence on chemical control. Herbicides provide effective weed control under a variety of conditions and often allow farmers increased flexibility in the selection of crops, cropping practices, and overall farming operations under many conditions. However, there is now widespread concern about the use of such large amounts of chemicals and the fact remains that they are not absolutely necessary.

Methods for reduced 'herbicide' input

When plants are grown close to each other, they may interact in several ways. In three of the 10 ways they may interact, an adverse effect is observed on the growth of one or both of the interacting species. These interactions are: Amensalis, Competition and Parasitism. Amensalism is, 'an interaction in which growth of one is depressed while that of the other is unaffected'. A type of amensalism is where a plant species releases a chemical that adversely affects the growth of another. Allelopathy is therefore, 'a toxic interaction of plants (including living and decaying tissues) resulting in the reduced growth of one member of the interaction'. The chemicals can be produced by a number of plant parts including the leaves (washed to the soil), the roots (secreted directly to the soil) or from, decaying tissues such as leaves or roots.

The most commonly produced toxins are phenols and these usually reduce germination or slow growth. An example is the juglone produced by the leaf litter of walnut which prevent seed germination and seedling growth.

Naturally occurring compounds

A naturally occurring compound is one that is biosynthetic or is a breakdown product of a natural compound that could be expected to be found in a natural environment. There is interest in this area because such chemicals prove less time consuming and expensive to register and they are easily and rapidly degraded or detoxified in the environment. Additionally, many phytotoxic natural compounds have chemistries unlike those synthetic herbicides. Thus, study of these compounds may lead to the discovery of new herbicide classes that affect sites of action hitherto untouched by currently used herbicides.

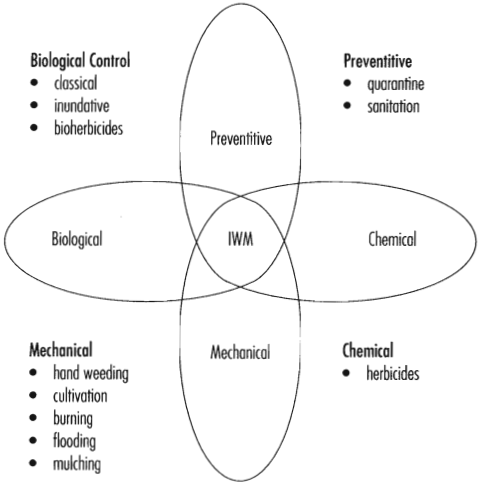


Figure 3. Components of integrated weed management systems.

References

- Akobundu, I.O. 1987. Weed Science in the Tropics. Principles and Practices. John Wiley and Sons Ltd, New York. 522pp.
- Blatchley, W.S. 1912. The Indiana Weed Book. Nature Publishing Company, Indianapolis. 191pp.
- Duvel, J.W.T. 1902. Seeds buried in soil. Science, New York 17, 872–873.
- Harper, R.M. 1944. Preliminary report on the weeds of Alabama. Bulletin of Geological Survey. Alabama University, Alabama, No. 53, 275pp.
- Holm, L.G., Plucknett, D.L., Panchap, J.V. and Herberger, J.P. 1977. The World's Worst Weeds: Distribution and Biology. University Press of Hawaii, Honolulu. 609pp.
- Hill, T.A. 1977. The Biology of Weeds. Edward Arnold, Ltd, London. 68pp.

Population Ecology and Biological Control of Weeds

Introduction

A single plant is unlikely to have 'weed' status, but a population of the plants at a density that interferes with our use of the land or water will change that perception. An individual biological control agent may damage part of a weed, but this only becomes significant if the population of the agent reaches a density at which the total damage by all the individuals reduces the weed population. An understanding of how weed and biological control agent populations change to reach levels that are significant will help in management of weeds, and in biological control of weeds.

This paper provides a brief checklist of factors and processes to be considered when interpreting changes in populations of weeds and biological control agents. More detailed explanations may be found in publications on ecology (e.g. May 1981; Ricklefs 1990), population ecology (e.g. Begon and Mortimer 1981; Harper 1977), insect–plant interactions (e.g. Crawley 1983; Strong et al. 1984), and in some texts on biological control (e.g. van Driesche and Bellows 1996). Aspects of the population ecology of weeds are discussed by Adkins (this volume), and methods of investigation of populations of weeds are discussed by Farrell and Lonsdale (this volume).

Graham White
Cooperative Research Centre for Tropical Pest Management
Gehrmann Laboratories, University of Queensland
St Lucia, QLD 4072
Australia

Population Change

The process of change in populations, including weeds and biological control agents, can be summarised by the equation:

population change =
(births + immigrants) – (deaths + emigrants).

All species have genetically determined limits to the rates of these processes (birth, death, migration), and environmental factors limit the processes or modify the rates within the genetically determined limits.

Some environmental factors cannot be controlled, but their effects on population change may be significant. Other factors can be controlled, so manipulation of these factors may be included in management of the weed (Adkins this volume).

The objective of pest management is to reduce population growth rate of the pest, to levels at which pest impact is insignificant, by:

- reducing birth rate,
- reducing immigration rate,
- increasing death rate, or
- increasing emigration rate.

Biological control of weeds is usually aimed at reducing birth rate or increasing death rate. Migration rates could be affected by biological control agents that interfere with the biology or mechanics of seed or fruit dispersal, but the aims of weed biological control are usually more direct.

Populations of insects and plants differ in that all adult insects in a population are usually the same shape and roughly the same size, but plants of the same age within a population are highly variable in shape and size. It is useful to think about plant populations in two ways. Taking the broader view, plant populations are made up of individual plants. At a more detailed level, individual plants are made up of populations of modules (e.g. shoots on trees, leaf and bud units on annuals, tillers on grasses). The 'birth' and death of these modules lead to the growth, and decline, of individual plants. Biological control agents affect specific parts of plants and hence influence the births and deaths of these modules.

Abiotic Environmental Factors Affecting Population Change

Temperature

Growth rate

Within high and low temperature limits, growth rate increases with temperature to an optimum, then decreases sharply near the upper limit. Development of insects and growth of plants may be switched to a state of diapause or dormancy at unsuitably high or low temperatures to minimise damage to the organisms.

Birth rate

The birth rate of some insects and plants is affected by temperature in a similar pattern to effects on growth rate.

Mortality rate

Mortality rates are high at temperatures near the upper limit for growth and, for some organisms, in frosts or freezing temperatures.

Behaviour

Key behaviours of insects, such as flight, mating and feeding are temperature limited. The lower temperature thresholds for these behaviours may be much higher than the limits for growth and development.

Moisture

Growth rate

Water is essential for development of weeds, insects and pathogens. Moisture content of the air affects plants and externally feeding insects, but insects in living plant tissues are not normally limited by moisture. Moisture content of the soil affects growth of terrestrial plants. Surface moisture from dew or rain is usually necessary for infection by fungal pathogens.

Rain and increasing soil moisture, as indicators of favourable plant growing conditions in the future, switch on the development of many insects and plants after diapause or dormancy.

Birth rate

Moisture availability has a major effect on 'birth' rates of plants. Some trees are stimulated to flower by water stress. Flowering and seed set by other plants are inhibited by water stress. Initiation of new modules by individual plants is usually restricted by water stress. Rainfall may prevent oviposition by insects.

Mortality rate

Mortality of insects and plants increases in dry environments due to desiccation and wilting. High moisture may indirectly increase mortality rates of insects and weeds by favouring pathogens. Rainfall may increase mortality of adult and immature insects.

Dispersal/migration rates

Floods are an important means for dispersal of many plants.

Light

Growth rate

As the energy source for photosynthesising plants, light is crucial for growth. Some plants require high light intensity, while others are adapted to shade.

Photoperiod, or period of daylight, is used by insects and plants as a trigger for many processes including initiation or termination of diapause or dormancy.

Birth rate

Photoperiod may also trigger flowering in plants and reproduction by insects.

Behaviour

Mating, flight and other key behaviours of insects can be controlled by light intensity or photoperiod. Some are attracted by light but others are repelled.

Soil and nutrients

Growth and mortality rates

The quantity and availability of nutrients greatly influence weed growth (see Fichera this volume; Winterton this volume). Mechanical structure of soil, particularly heavy clays, can limit seedling emergence, root growth, movement of insect larvae, formation of pupal cocoons and emergence of adult insects from pupal cocoons. Soil structure also affects moisture and gas interchange, which in turn affects insects and plants.

Wind

Dispersal/migration rates

Wind may be a major factor in dispersal or migration of insects and plant seeds or spores.

Biotic Interactions Affecting Population Change

Intra-specific competition

Competition with other members of the same species within a population may be severe because all members of the population are generally competing for the same resources. Increasing population density results in increasing mortality, slower rate of growth, and reduced fecundity, all of which result in a lower rate of population increase. These effects are termed 'density-dependent' because their effects increase with density.

Inter-specific competition

Other species competing for the same resources as the population under consideration may have effects varying from minimal, for poor competitors, to local extinction of the population if competitors are successful in taking all of one of the necessary resources.

Plants become weeds through successful competition with desirable plants for water, light and mineral nutrients. They may also be detrimental to the desirable plants through release of allelopathic chemicals that inhibit growth of other plants.

Parasites, predators and pathogens

Insect predators preying on other insects kill and consume their prey. Parasites and pathogens live in close association with their host, feeding on their host and reducing host fitness but not necessarily killing the host. Parasitoids may be free-living as adults but immatures develop on their host and usually kill it.

Parasites, predators and pathogens may reduce populations of potential biological control agents in the country of origin and in the country to which the agents are introduced. In the country of origin the effect may be to reduce the agent to insignificant

densities. When introduced to the new country free of those parasites, predators and pathogens, agent populations may increase to much higher densities than in the country of origin.

Herbivores

Insect herbivores feeding on plants may be similar to parasites, especially the insects selected for biological control which live in close association with their host. The reduction in host plant fitness due to feeding by the herbivore varies with different insect/ plant combinations. Insect attack on meristems, or the growing points of the plant, would be expected to have a greater effect on plant growth than feeding on leaves, but compensatory growth may overcome the effect of meristem damage. Many sap sucking and gall forming insects have little effect on the host plant, but some greatly reduce fitness by acting as an energy sink, diverting nutrients away from growth points in the plant. Flower and fruit feeders appear to greatly reduce 'birth' rate of plants, but this loss of potential progeny may merely substitute for the normal shedding of excess flowers or fruit.

The effect of insect herbivores on the population of their host plant depends not only on the type of damage inflicted by individuals, but also on how many individuals are present (i.e. density relative to the host plant). Factors such as unfavourable abiotic conditions or predators and parasites of the herbivore may prevent the herbivore population increasing to levels that significantly affect the plant host.

Quality of the plant host as food for herbivorous insects

Food quality of host plants can have a major influence on population change in insects. Food quality can be so poor that few larvae survive, and most of the dispersing phase may leave the poor quality food. Herbivores are indirectly vulnerable to effects of water and nutrient availability on their host plant, but effects on the insects are variable. Phloem feeders may be provided with a richer nutrient supply if the host plant is under stress, particularly water stress, whereas leaf feeders may develop more slowly with increased mortality if the host plant is water stressed. Herbivores are particularly sensitive to the nitrogen content of their host.

Herbivores are also influenced by secondary plant substances—metabolic products that act as deterrents or, occasionally, as attractants. Plants that would otherwise be suitable hosts may be rejected as food because of these substances.

Population Regulation

When a species is introduced into an environment in which food and space are abundant, the population increases exponentially at the intrinsic rate of increase, *r*, characteristic of the species under the particular environmental conditions of temperature, moisture, food quality, etc. (Fig. 1). If conditions remain favourable for long enough, resources become limiting at high densities. Population growth slows through density-dependent intraspecific competition as the carrying capacity of the environment, K, under the

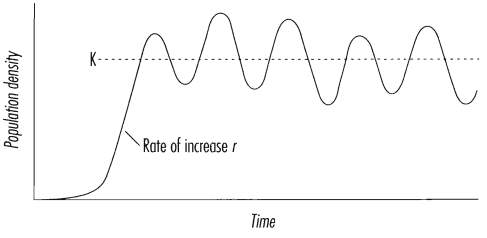


Figure 1. Change in population density of an organism after introduction into an environment with a mean carrying capacity of K.

particular environmental conditions is approached or exceeded (Fig. 1). The population may stabilise at, or fluctuate about, the carrying capacity (Fig. 1).

The discussion of *r* and K assumed stable environmental conditions, but there are major seasonal changes in conditions and shorter term weather influences may also be significant. As abiotic components of the environment become less favourable they limit development of all individuals in the population, and thus reduce *r*, whether the density is high or low (i.e. density-independent limitation). The population may decrease until favourable conditions return and the population again increases. Seasonal changes and weather influences may also affect biotic components of the environment, such as food, parasites and predators, so altering the carrying capacity of the environment K and inducing density-dependent limitation. Populations increase and decrease through time because of environmental changes. Both density-dependent and density-independent regulation affect most populations, but they may act at different times and in different places.

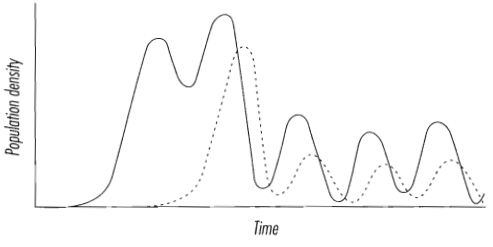


Figure 2. Change in population density of a weed (solid line) after introduction to a new environment, and a successful biological control agent (dashed line) after its release.

If biological control of weeds is to be successful, the biological control agent must build up to densities that significantly affect the host, and the herbivore and the host then become locked into a mutual density-dependent relationship (Fig. 2). If the host density increases, the herbivore population will rapidly increase in the presence of excess food to eventually reduce the density of its host. When the host density is low, the herbivore experiences local food shortage resulting in a decrease in population growth rate, and most dispersing individuals will die without finding another host plant. Considering the relationship from the other point of view, if the herbivore density is low, the host plant population will increase. If herbivore density is high, the host plant density will decrease through attack by the herbivore. This alternate cycling of herbivore and host densities usually occurs at different times in different places across the distribution of the plant resulting in an overall density of the host plant much less than would exist if the herbivore was not present.

Cactoblastis cactorum and *Opuntia stricta*—an analysis of population ecology

Control of prickly pear, *O. stricta*, by the moth *C. cactorum* was one of the most successful examples of biological control of weeds (Dodd 1940). Numbers of the biological control agent and the weed followed the pattern shown in Figure 2.

Prickly pear infestation increased rapidly in Australia from five million hectares in 1905 to 25 million hectares in 1925, when it had occupied most of the area in which conditions for its growth were favourable. It was present at high densities over about half its range. The prickly pear was probably approaching the carrying capacity of the Australian environment.

Following release in 1926, numbers of *C. cactorum* increased rapidly. Feeding by the larvae, accompanied by microbial rots, destroyed most, but not all, plants by 1934. Huge numbers of larvae starved to death. The insect population exceeded the carrying capacity of the environment and experienced massive density-related mortality. Surviving parts of plants were able to regrow while insect numbers were low, but the moth population again increased and attacked the prickly pear. By 1939 the infestation of prickly pear had been reduced by over 99% in Queensland.

Viewed on an Australia-wide scale, the system has now settled to an equilibrium situation with low densities of prickly pear and *C. cactorum*. However, the 'equilibrium' densities of plant and insect vary with weather, climate and region.

In southern areas where *C. cactorum* populations are limited by temperature, prickly pear survives at higher densities than in Queensland. During droughts the prickly pear population at the arid western limit to its range may die back. In more favourable areas the prickly pear continues to grow during a drought, but plants growing under water stress are much less suitable for survival of larvae (White 1981). The effect of plant quality in reducing *C. cactorum* populations may be magnified by high temperature-induced mortality of larvae. Thus prickly pear density may increase and *C. cactorum* populations may decrease until wetter seasons prevail.

On soil types with low nutrition, prickly pear is resistant to attack by *C. cactorum*—the physiology of the plant is modified to produce highly mucilaginous sap in which larvae drown. Application of nitrogen fertilizer results in normal plant growth, making these plants susceptible to *C. cactorum*.

At a local level, populations of plant and insect may fluctuate widely. Prickly pears may be carried by flood as vegetative pieces or by birds or other animals as seed a long way from the original prickly pear source and *C. cactorum* infestation. A plant and its progeny may develop for a long time into a locally dense infestation before a moth finds the infestation, lays eggs and begins destruction of the infestation. This cycle of local increases in plant density followed by rapid increase in insect density and destruction of the plants occurs in many places and at many times.

Conclusions

Insect and plant populations are influenced by many factors in complex ways. To date it has only been possible to understand all of these effects and all of this complexity for any insect/plant combination once the relationship has been established and observed over many years in the field. Introduction and release of a weed biological control agent is an experiment with an unpredictable outcome. The most significant questions to be asked are:

- Will the insect feed on the plant?
- Will the insect increase in numbers under conditions in the field, and will density-independent factors limit its increase?
- Will parasites and/or predators limit the population of the insect?
- At densities achieved in the field, will damage inflicted on the plants significantly reduce the plant population?

An understanding of general population ecology and particular knowledge of the population ecology of the weed and the biology of the potential biocontrol agent will help in answering these questions. Answers to the first two questions can be found to some extent before field release through studies of taxonomy, host specificity, climate matching, and temperature effects. Answers to the third question, and therefore the fourth, cannot be found until the major experiment in biological control, i.e. field release, has been carried out.

References

- Begon, M. and Mortimer , M. 1981. Population ecology: a unified study of animals and plants. Blackwell Scientific Publications, Oxford. 200pp.
- Crawley, M.J. 1983. Herbivory: the dynamics of animal–plant interactions. Blackwell Scientific Publications, Oxford. 437pp
- Dodd, A.P. 1940. The biological campaign against prickly pear. Commonwealth Prickly Pear Board, Brisbane. 177pp.
- Harper, J.L. 1977. The population biology of plants. Academic Press, London and New York. 892pp.
- May, R.M. ed. 1981. Theoretical ecology: principles and applications. Blackwell Scientific Publications, Oxford. 489pp.
- Ricklefs, R.E. 1990. Ecology. Third edition. W.H. Freeman and Company, New York. 896pp.
- Strong, D.R., Lawton, J.H. and Southwood, T.R.E. 1984. Insects on plants: community patterns and mechanisms. Blackwell Scientific Publications, Oxford. 313pp.
- van Driesche, R.G. and Bellows Jr., T.S. 1996. Biological control. Chapman and Hall, New York. 539pp.
- White, G.G. 1981. Current status of prickly pear control by *Cactoblastis cactorum* in Queensland. In: Delfosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds. Brisbane 1980. CSIRO, Australia. 609–616.

Procedures in Biological Control of Weeds

Introduction

Practitioners in biological control of weeds agree that there are a number of steps which should be followed in a biological control program. Initially it is important to have agreement that the target plant is a weed and should be controlled. In the past, many weeds were selected as targets for biological control because mechanical and chemical methods had proven ineffective or too expensive to apply repeatedly. It is now common for biological control to be assessed as an option for weed control in parallel with other control methods. The regulations to import and release biological control agents differ between countries. Some countries have few regulations while others have a system where several departments may have to be convinced that the risks are minimal before a biological control program can proceed.

Following is a summary of the crucial steps in a classical biological control program. Details on each of the major procedures are presented in other sections of these course notes and in Harley and Forno (1992).

Wendy Forno
CSIRO Entomology,
Private Bag 3, Indooroopilly, QLD 4068
Australia

Steps Followed in a Classical Biological Control Program

1. Initiation of a biological control program

- Select the target weed, identify any conflicts of interest as to why it is a weed rather than a useful plant and, if possible, resolve issues before the program commences.
- Review the literature on the target weed to find out all that is known on the biosystematics, distribution, economic importance and management strategies.
- Review the literature on the natural enemies of the plant, their biologies, host range and use as biological control agents against the weed in other countries.
- Compile data.
- Determine whether any other institution worldwide is working or has worked on biological control of the target weed.

2. Approval to work on the weed

- Prepare an application, using data assembled, to seek approval and/or funds from the target country to work on the weed.

3. Foreign exploration (Forno and Purcell this volume)

- If an exploratory phase is necessary, find out the procedures for working in the country/s of the native range of the weed and for exporting insects and pathogens from these countries, and establish

connections with appropriate institutions. Permission to work on and/or export insects and pathogens may require formal linkages with institutions within the country to be explored. It may take more than a year to obtain the necessary documentation to export.

- Establish a base within the native range which is close to an international airport or which has good connections to international services and, if possible, close to institutions which may be of assistance.
- Search for potential control agents through well planned surveys which take account of the distribution and centre of origin of the target weed, closely related plants and seasonal variation in the abundance of natural enemies of the target weed.
- Have specimens determined by specialist taxonomists.
- Prepare an inventory of insects, mites and pathogens that attack the weed.
- Assess those which have potential as biological control agents (Marohasy this volume).

4. Surveys in the introduced range

- Survey the weed in the introduced range to determine the fauna using the plant as a host.
- Establish whether the fauna are native to the target country, whether there are species apparently not native attacking the plant and in particular, check the identity of fungal pathogens and compare with those found in the native range.
- Have specimens determined by specialist taxonomists.
- Compile data.

5. Ecology of the weed and its natural enemies

- Study and, if possible, compare the ecology of the weed in its introduced and native range. This may give some indication as to why it is a weed outside its native range and whether it is a good candidate for biological control.
- Study the ecology of potential biological control agents including their use of related plants. Knowing the ecology of potential agents before introduction to the target country may assist in predicting how and where they should be released to ensure establishment and likelihood of having an impact on the weed.

6. Host specificity studies (Heard this volume)

- Seek approval of the list of plants to be screened to determine the host range of an agent by submitting the list to the regulatory authorities in the target country (Forno and Heard this volume).
- If possible carry out some preliminary host testing of potential biological control agents in the native range. Document the presence, feeding, oviposition and development of these agents on the host and other related plants in the field. This information will assist in the interpretation of laboratory host screening tests.
- Either complete the host testing outside the target country or seek approval to import the agent into an approved quarantine facility for completion of the host screening trials.

7. Approval to import biological control agents (McFadyen this volume)

- Prepare and submit to the regulatory authorities in the target country a report containing all available information on the biology and host range of a particular biological control agent.

- If host screening has been completed outside the target country, then the regulatory authorities may approve importation of an agent and grant approval for release of the agent at the same time. Often the authority will request that the agent be taken through one generation in a quarantine facility to overcome the risk of importation of unwanted contaminant organisms before release.

- If host screening has been partially completed outside the target country, then approval may be given to import the agent into a quarantine facility for completion of the host screening tests; a separate approval will be required for field release of the agent.

- If host screening has not been done outside the target country, then approval may be given to import the agent into an approved quarantine facility for host screening. Again, separate approval must be sought for field release.

- Sometimes an agent has been host screened by another country and then approval may be granted to import the organism either without further testing or with further testing using a much reduced plant list.

8. Importation for release

- Upon importation, each agent is usually reared through at least one generation to eliminate parasitoids and insect pathogens.

- Where the agent is certified as being disease and parasitoid free by the supplier, it may be released in the field but only after transfer from any packaging or plant material which has been imported.

- All imported plant and packaging material must be destroyed, preferably by autoclaving or incineration.

9. Rearing and release (Donnelly this volume; Wright this volume)

- Upon completion of quarantine procedures, compile a report for the regulatory authorities. The report should contain detailed information on the biology and host range of the biological control agent and an assessment of any risk to other flora. After receipt of approval for release, the agent is mass-reared and released in the field.

10. Evaluation (Farrell and Lonsdale this volume)

- Field studies are undertaken to determine establishment, spread and effect of the biological control agent on the weed. Complementary laboratory studies may be undertaken to assist in the interpretation of field data.

11. Distribution (Wright this volume)

- Collaboration with other institutions is often essential to ensure rapid and widespread distribution of agents. Distribution may be from laboratory colonies or from field sites where the agents are abundant.

References

- Harley, K.L.S. and Forno, I.W. 1992. Biological Control of Weeds: a handbook for practitioners and students. Inkata Press, Melbourne & Sydney. 74pp.

Exploration for Agents

When to Explore

Exploration is required to collect, identify and evaluate potential biological control agents. For some weeds, exploration for natural enemies has been very intensive and may be ongoing, whilst for others little or no exploratory work has been done. If exploration has been carried out and potential agents identified, then the cost of the biological control program can be reduced and the introduction of agents hastened by collaborating with the institution which has carried out the exploratory program. If no or insufficient exploratory studies have been carried out and the weed is causing economic losses to primary industry, and/or threatening conservation, then an exploratory program to find natural enemies of the weed which are suitable for introduction may be undertaken. There are recommended basic steps to be followed when commencing an exploratory program.

Knowing the Target Weed

The systematics of the target weed must be well known before commencing surveys. This will prevent wastage of resources resulting from the collection of natural enemies from the wrong target weed.

Wendy Forno and Matthew Purcell
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Published information should be searched using on-line services, abstract journals and CD-ROM, and unpublished information obtained by contacting research and extension departments in areas where the target weed is a problem.

If possible, the population in the introduced range should be compared with the populations in the native range. This can be done using morphological comparisons either alone or in combination with techniques such as electrophoresis or DNA matching.

Geographic Distribution

Herbaria records within the native range are often the best sources of information on the distribution of the target weed. Some herbaria will have records of all specimens in a computer data base allowing easy access to information. In other herbaria, it may be necessary to look at each herbarium specimen of the target weed to get the information on its geographic distribution.

Botanists and amateur botanists can often add to the data obtained from herbaria and can advise on where the centre of origin may occur.

The information obtained from published papers through literature searches, herbaria, and personal communication with botanists should be compiled and mapped.

Ecoclimatic Matching

Where possible, match the climates between the introduced and native range of the weed. The best estimates of the effectiveness of biological control agents are made through observations in situations in the native range ecoclimatically resembling the invaded range (Wapshere 1985). This is possible for some species such as *Mimosa pigra*, where the native range is diverse, but not possible for others such as *Salvinia molesta*, where the native range is very narrow. Computer programs such as CLIMEX may be used to obtain this information. The information is used to decide where to collect a biological control agent as, in some cases, it may be preferable to collect a population of an agent from a climate zone which matches the area where the weed has been introduced. (Maywald and Sutherst this volume).

Capital Resources

Exploration is expensive but costs can be minimised by setting up an exploratory unit either in an existing research institution or in a rented house with garden space for the erection of shade houses, planting of test plants and rearing of insects. The location of the base will depend on whether the weed has a broad geographic range covering several countries or a narrow range within one country. The base need not be permanent and it may be economical to have the exploratory unit based in different countries at different times of the year or for different years.

It is essential to procure transport or to purchase or hire a vehicle for surveying. For aquatic weeds, the acquisition or hire of a boat may be necessary.

Staff

The skills required are those of an entomologist, botanist and mycologist. Staff may be full time or on short-term contracts. They may be locally employed or from an institution in the target country or from institutions in other countries if the skills cannot be obtained in any other way.

It is essential that staff have good liaison with entomologists/botanists/mycologists in the countries of exploration. It may be economical to contract one person. e.g., an entomologist, and subcontract others as required to survey or conduct biological studies on insects and fungal pathogens either on a full- or part-time basis.

Staff should be self reliant, resourceful, good field biologists and have a knowledge of the local language and culture.

Agreements and Infrastructure

On the assumption that permission has been obtained to carry out exploration in a particular country, it is important to maintain good liaison with the regulating bodies within each country. Institutional agreements with existing research organisations may also be formulated for providing administration, facilities (e.g. offices, glasshouses, vehicles etc.) and for employment of staff.

Agreement with institutions such as the International Institute of Entomology, the International Mycological Institute, the Insect Identification and Beneficial Insect Introduction Institute in the USA, and the Australian National Insect Collection to identify insects should be arranged. Specialised taxonomists in some groups are outside these institutions and should be contacted before sending specimens. Some institutions are now charging and it is highly probable that all institutions will charge for identification services in the future. These costs must be anticipated in the budget of the project.

Reference Collection

Accurately identified and labelled voucher specimens of plants and their natural enemies must be assembled as a reference collection located at the exploratory station. Regulations as to where holotypes and paratypes are deposited must be strictly followed.

Where to Explore

Surveys must be carried out over the whole region where the target plant is believed to be native; they should proceed along transects traversing altitude, temperature, rainfall and other ecological gradients. It is hypothesised that the greatest number of natural enemies will occur near the centre of diversification or evolution of the weed genus or subgenus. Some of these are likely to have evolved with the weed and have a host range specific to the weed or to the weed and a few species within the same genus. If the centre of diversification is known, intensive surveys must be made in that region.

Surveys should be designed to take account of season, habitat, soil or water type and follow a predetermined plan. Many natural enemies are seasonal in abundance, even in the tropics, and it is desirable to conduct preliminary surveys at a time when the weed and natural enemies are abundant.

If a population of the weed can be found in the native range which is genetically identical to the population in the target country, then particular attention should be given to this population. If matching populations cannot be found then control agents must be sought from dissimilar populations.

Occasionally, biological control agents are selected from closely related species. Although the chances of success may be reduced, there are some very successful programs where agents have been collected from one plant species and used against another closely related species.

What to Collect During Preliminary Surveys

At each locality, care must be taken to collect from different forms of the target weed, all parts of the plant, and from closely related species growing in the same habitat. Surveying methods are determined by the type and habitat of the target weed. Plants can be searched directly at field sites, or samples can be collected for processing at the field station.

Field-searches are beneficial for obtaining data on the distribution, seasonal abundance, behaviour, and habitat of herbivores, and for collecting large numbers of agents for laboratory studies or exportation. Any evidence of damage on the target plant, including defoliation, gall formation, flower and fruit damage or dead tissue, should be carefully investigated for the causative agents. Potential biological control candidates can be collected directly from the plants by hand or by using an aspirator. The niche and feeding habits of all herbivores should be recorded. Specimens not needed for rearing or laboratory trials can be placed into killing jars then preserved.

Collections of terrestrial weeds can be made using hand pruners or loppers attached to extension poles for taller trees. For aquatics, collections of the target weed can be made from shore by hand, while wading, or by using a rake or grappling hook attached to a rope. In deeper water a boat, surf ski, canoe or equivalent should be used. The plant material should be placed into a labelled collection bag for transportation back to the laboratory. Both plant material and live insect specimens should be protected from extremes in temperature in transit. When temperatures are high, insulated containers with ice bricks or evaporative cooling techniques should be utilised when airconditioning in the vehicle is not available.

Field data sheets should be completed for each collection to record site descriptions (e.g. topography,

vegetation) and details (e.g. name, locality, latitude/longitude), collection date, time, physical parameters (e.g. temperature, humidity, weather), weed parameters (height, flowering, sample weight) and general notes. Each collection should be assigned a number for specimen records and for entry into a database.

Collections processed in the laboratory can be searched by hand or by using insect extraction devices (e.g. berlese funnels). Extraction devices are especially useful for collecting internal feeders such as stem-borers and leaf-miners, and for processing large amounts of plant material. Specimens of the host plant including flowers and fruit should be collected from each region, labelled and stored or sent to specialist botanists for determination.

Immature and mature stages of each insect species should be collected and at least 10 adult specimens should be preserved; if there are different forms (polymorphic species), then specimens of each form should be collected. Where possible, adult specimens should be sent to specialist taxonomists as immature stages are generally difficult/impossible to determine. Label data of other specimens of the same species held in national collections should be requested from taxonomists as they may contain valuable information on hosts, niche, feeding habits and distribution. Pathogenic fungi should be collected, dried and sent for determination.

Immature phytophagous insects should be reared and the hosts of predators and parasitoids noted. Records of the feeding habits and life-histories of each insect should be recorded. Photographic records of specimens can also be useful.

If sufficient resources exist, a laboratory colony of promising biological control agents should be established. These colonies can be used for detailed studies and exportation.

Detailed Studies

Detailed studies are needed to determine which agents have the greatest potential as candidates for introduction against the weed in the introduced range. They can aid in prioritising agents (Marohasy this volume), especially if the list of potential candidates is extensive. Selection is largely on host specificity rather than a prediction of their effectiveness, though there will be a bias towards those agents which have a narrow host range and are very damaging.

Studies should provide information on seasonal abundance, distribution, biology and provide an indication of host range. The latter may be achieved through observation and by screening a few plants closely related to the weedy species.

If sufficient resources exist, field host-testing in the native range of the weed should be undertaken. . Behavioural studies undertaken in natural conditions, minimise restrictions on the testing environment (e.g. cage trails) that reduce the number of stimuli available for agents to find their hosts (Cullen 1990). Such studies should be conducted throughout the year, and involve intensive searching of both the weed species and all other potential hosts along transects or in a defined area where potential agents occur. Other plants which are not present, but are required for host testing, may be placed/planted in the survey areas. The abundance and seasonality of these agents, and the damage they cause, is then determined for each plant species. Field host-testing can eliminate non-specific agents at an early stage, thus reducing unnecessary and time consuming laboratory studies. The effect of the biological control agent on the weed should be documented.

Other studies may include insecticidal exclusion experiments where similar plants of the target weed are divided into two groups under natural conditions. One group is sprayed with insecticide, excluding

insect attack, while the other is untreated and therefore exposed to insect attack. The growth rates of each group are then compared to gauge the impact of insectivorous herbivores.

What to do With the Information

An appropriate data base should be used for the storage and retrieval of field data and determinations of specimens collected. This is a very efficient method of handling large amounts of data and is very portable.

Shipment

It is necessary to carefully prepare insects for shipment to overseas destinations to ensure that they arrive in the best possible condition. Failed shipments result in expensive losses in both time and money.

Permits for shipment/importation of insects must be obtained from the country of export and from the destination country. These permits should be obtained well before the shipment date. The fastest and most direct route (usually by air) must be determined, and the package may be unaccompanied or hand carried. Survival is directly related to the time the agents spend in transit. Avoid stops on route especially those involving transfer of the package to new flights/carriers. It is impossible to guarantee the package will not be subjected to adverse conditions (e.g. temperature extremes) while being held by carriers. A direct route will decrease the chance that the package will be delayed or lost. The consignee in the destination country must be given all details of the consignment.

The life stage shipped should ensure maximum survival of the agents. For example, adult moths are delicate and are susceptible to physical damage, therefore immatures should be shipped. Correct packaging is crucial for the survival of insects and ensures that strict quarantine safeguards are maintained. Packaging techniques vary widely according to the type and habit of the insects. The insects are usually held on host plant material within a gauze or cloth bag tied off with string. Sufficient food must be included to keep the agents alive for the shipment duration. The bags are then placed into sealed plastic containers. Plant material should be held in conditions that prevent plant decay. Some plants, like aquatics, will decay quickly and it is important not to have the plant material too moist. Excess moisture within the containers can be controlled by lining the interior with absorbent paper and/or by ventilation (e.g. gauze covered windows or small ventilation holes). The containers are put into an appropriately sized box (preferably insulated), sealed, then placed into a larger carton surrounded by sufficient packaging so that it is insulated against external temperature and physical damage. Permits are attached to the outside of the package.

References

- Cullen, J.M. 1990. Current problems with host-specificity screening. In: Delfosse, E.S. ed. Proceedings of the VII International Symposium on Biological Control of Weeds, Rome, 1988. Instituto Sperimentale per la Pathologia Vegetale, Rome. 27–36.

- Wapshere, A.J. 1985 Effectiveness of biological control agents for weeds: present quandries. Agriculture, Ecosystems and Environment 13, 261–280.

Prioritising Weed Biological Control Agents

The Need For a Prioritisation Process

All insect species and plant pathogens found attacking a weed in its native range are potential biological control agents. But before the potential of any one species can be realised, living specimens must be shipped, often more than half way around the world, it must be extensively tested for host specificity, successfully reared and eventually, if all obstacles are overcome, mass reared and field released in the new country (Forno this volume). It takes CSIRO from one to six years to process a single insect species (Cullen 1992). Given this enormous investment in research time and resource, potential control agents should be prioritised so that resources can be directed to those agents with greatest potential for successful biological control. It follows that the longer the list of potential agents and the smaller the budget the more critical the prioritisation process, because species given even a medium priority rating on a long list may never be tested and released. And if some of the species given a high priority are technically difficult to test, rear or culture (e.g. Marohasy 1993), the number of potential agents processed is likely to be further reduced because of time and resource constraints. Obviously budgetary and technical considerations are important when prioritising potential agents and should be given formal consideration (Beirne 1985).

Jennifer Marohasy
Department of Entomology
University of Queensland
St Lucia, QLD 4072
Australia

Rating Systems

An extensive scientific literature discusses the attributes a successful insect biological control agents should possess (Harris 1973; Beddington et al. 1978; Goeden 1983; Hokkanen and Pimental 1984; Dennill 1988; Myers et al. 1989; Waage 1990). Harris (1973) was the first to propose a formal system for prioritising insects. This system was based on biological attributes including phenology of attack, number of generations per year, feeding behaviour and size. Each attribute was scored, with high fecundity, for example, being given a higher score than low fecundity. Scores for each attribute were added to give a total score for each species. However, this system and subsequent revisions (Goeden 1983; Hokkanen and Pimental 1984) are rarely used.

It seems that there are no proven scientific principles which can be used to determine in advance which biological control agents are going to establish and become abundant. Cullen (1992) comments that "this is a continual source of frustration and a waste of resources, yet attempts to do better are notoriously difficult and make little progress, to the extent that many workers feel it is not worthwhile, preferring to rely on release of the agent as the only valid test of finding whether it will be successful."

Some Criteria for Prioritisation

Successful biological control agents should fulfil the following three criteria, they must be:

- adequately host specific,
- potentially damaging, and
- able to build up to large populations to realise their potential to damage.

I suggest agents likely to fulfil all three criteria should be given a high priority.

Host Specificity

Detailed host specificity tests (Heard this volume) must be undertaken to ascertain the host range of an insect species or plant pathogen. However, many biological control workers are loath to begin testing species which belong to families and orders which are considered unlikely to be host specific. For example, no mealybugs or scale insects have ever been deliberately introduced to control a non-cactus weed because of doubts about their host specificity. In contrast, Shorthouse (1990, citing Harris 1984) states that approximately 25% of the agents released in Canada by the end of 1983 were gall insects, not because they were thought to necessarily be very effective, but because of their assured high degree of host specificity.

The rationale for consideration of taxa to be host-specific or not has often been based more on precedence and personal bias than science. For example, prior to the well-documented devastation of leucaena by the host specific leucaena psyllid, non-galling psyllids were considered unlikely to have a narrow host plant range (R.E. McFadyen pers. comm. 1992). It is now recognised that most psyllids are very host specific and they are being increasingly used in weed biological control (D. Hollis pers. comm. 1991). As a second example, we would never have known the spectacular control of *Salvinia molesta* or *Eichhornia crassipes* if the status quo had been maintained and fish and snails continued to be trialed as biological control agents because, and I quote Wilson (1964), 'No insects have yet been used for the biological control of aquatic weeds, ... it may be that in the fresh-water environment the relatively small numbers of species of plants and phytophagous insects, and perhaps the domination of this environment by fish, have caused in aquatic phytophagous insects a level of host specialisation much lower than that occurs in the species-rich terrestrial environment'. The origin of this fallacy can be traced to the non-biological control theoretical literature (Brues 1946).

Plant pathogens are usually very host specific but have been used less frequently than insects in weed biological control because of a fear amongst some biologists and bureaucrats that they might 'host shift' and attack non-target plant species (Harris 1973).

Potential to Damage

Are all biological control agents equally capable of damaging their host plant? Intuitively we would answer no, because, for example, a stem borer would surely be more damaging than a leaf miner. However, if the stem borer only feeds in the pith it may do little damage, while the leaf miner may have a significant affect on photosynthesis and may even cause leaf abscission. Harley and Forno (1992) suggest that for a biological control agent to inflict "critical damage" it must either i) attack essential tissues, such as photosynthetic, meristematic or cambium tissues, ii) create an energy imbalance, for example, by stimulating gall production, or iii) have a physiological effect, such as a plant disease increasing stomatal opening with the result that plants become water stressed.

Briese (1993) suggests biological workers should give consideration to the biology and ecology of the target weed in order to discover aspects of its life cycle that either may be exploited by agents to have a significant effect on the plant, or may diminish the impact of the agent on the plant. Marohasy (1995), considering the reproductive biology of acacias, suggests insects which feed within flowers are unlikely to be effective against the weed *Acacia nilotica* because flowers are normally produced in excess with a very large percentage being subsequently lost through abscission. In contrast, flower-galling midges may be very damaging as galled flowers are not shed but develop into galls which act as energy sinks, consuming resources which would otherwise be available for growth and pod maturation (Marohasy 1995). Successful biological control of *A. longifolia* was achieved in South Africa using a wasp which galled flowers (Dennill 1988). The wasp sometimes committed *A. longifolia* to the production of 200% more galls per branch than the normal quota of pods and Dennill (1988) dubbed this phenomena 'forced commitment'. Harris' scoring system (Harris 1973) had suggested galling insects are poor potential biological control agents because they '...have evolved a homeostasis with their host that renders them incapable of inflicting serious damage to it'. It is now recognised that galls can be very damaging, acting as physiological sinks depriving other plant parts of energy and nutrients (Dennill 1988; Shorthouse 1990).

Although potential biological control agents may be shown to have an affect on individual plant performance, it may be an entirely different matter to demonstrate that this 'critical damage' can have an affect on plant population dynamics (Crawley 1989). If the growing season of a plant exceeds the feeding period of an insect or if the plant has food reserves which are inaccessible to the insect, the plant may be able to compensate for any damage. The seed bank in the soil might be so large that even a dramatic reduction in seed production through insect damage will not lead to a reduction in seedling recruitment (Crawley 1989).

Potential to Become Sufficiently Abundant

The potential for an agent to be potentially damaging will only be realised if the agent becomes sufficiently abundant. To effectively prioritise agents we must therefore be able to predict which agents, of those with most potential to damage, are going to become abundant. Obviously many individuals of an insect species which 'eats little' may be more effective than few individuals of an insect species which 'eats much'.

Theoretically the better adapted a biological control agent is to its new environment the more abundant it will become. Insects from similar climates and the same variety or subspecies of plant are thus given a higher priority, because they are more likely to be preadapted to conditions in the new environment (Harley and Forno 1992).

The computer program CLIMEX (Maywald and Sutherst this volume) enables evaluation of climate similarity and dissimilarity between regions and so is a powerful tool in predicting whether potential agents are likely to be limited or favoured by climate. However, factors other than climate may be more significant in some cases (Maywald and Sutherst this volume). For example, a gall fly from the Californian Mediterranean-type climate introduced into climatically dissimilar south eastern Queensland, with predominantly summer rainfall, to control *Baccharis halimifolia* established well and initially became abundant. It now appears parasitism, not climatic dissimilarity, is limiting its abundance (J. Melksham pers. comm.1991).

There is some empirical evidence suggesting potential control agents from the same variety, subspecies or species of plant as the target weed are most likely to establish and become abundant. Recognising that the weedy salvinia in Australia and most other regions was different from the common species of *Salvinia* in South America, and collecting potential control agents only from the species in South America which

matched, were critical to the successful biological control of this water weed (Harley and Forno 1992). Conversely, *Cactoblastis cactorum* is native to South America but was spectacularly successful in Queensland against the north American prickly pear, *Opuntia stricta*, which it had never encountered in South America.

Five of seven insects introduced to control *B. halimifolia* collected from this same plant in north America were successfully reared and field released in southeast Queensland. Only three of fourteen species from *Baccharis* spp., not *B. halimifolia*, were ever successfully reared on *B. halimifolia* (White and Donnelly 1993). However, of the five species that were successfully established from *B. halimifolia* only one is now considered effective and one of the three established from other *Baccharis* spp. is also considered effective (White and Donnelly 1993).

Insect species which have been introduced and become abundant in one region appear to have a high probability of becoming abundant in other regions (Julien 1992). *Crytobagous salviniae* has successfully controlled salvinia in tropical and subtropical Australia, Botswana, India, Papua New Guinea and South Africa. *Cactoblastis cactorum* has successfully controlled *Opuntia stricta, Opuntia ficus-indica* and *Opuntia inermis* where these cactus species occur in Australia, South Africa, Hawaii, New Caledonia and Nevis (Julien 1992).

Technical and Other Considerations

A biological control project requires an infrastructure extending across at least two, usually distant, countries. Potential agents found in one region must be supplied live and in reasonable number to the other region. This can create all sorts of diplomatic, bureaucratic, political and logistic, in addition to technical complications, particularly when a weed's native range, or the distribution of a particularly desirable potential control agent, is restricted to an unfriendly country or a war zone! Even when conditions in the native range are relatively conducive to research, the weed's rarity or inaccessibility, or the time necessary to grow plants of a reasonable size for rearing and testing, may cause all sorts of problems. Because all potential control agents are not equally easy to find, rear, test or package, depending on the available infrastructure, estimated length of the control project and budget, it may be most logical to concentrate on technically 'easy species'.

Insects which can be reared on excised tissue, have a short generation time and mate and oviposit readily in small containers are by far the easiest to rear, test and ship. This would include many species of leaf-feeding and seed-feeding lepidoptera and coleoptera.

A potential agent may be difficult to work with for any number of reasons. The idiosyncrasies of a particular species will often not be evident until after the prioritisation process and work has began. Nevertheless some generalisations can perhaps be made. Insects which require living tissues or organs which are not readily reared on young potted plants of the weed are especially difficult. For example, potted specimens of the tree *Acacia nilotica* will not flower or pod readily and can not be grown to 'full size'. This makes rearing and testing of flower-galling midges and a cerambycid which ring-barks large branches difficult (Marohasy 1993). Many species of fly and some species of lepidoptera and hemiptera will not mate or oviposit readily in standard size cages. Some species of gall midge require a fungal symbiont for gall initiation (Bork and Bissett 1985). Some species of insect may be relatively easy to rear but difficult to transport. For example, psyllids are susceptible to even relatively small fluctuations in relative humidity.

Conclusion

Each weed biological control agent which is eventually mass reared for release represents an enormous investment in research time and resources. Ideally agents should be prioritised in order of greatest potential to control the target weed, and resources not wasted on insects with little or no potential. Despite an extensive scientific literature detailing the attributes a successful agent should possess, in practice there is no useful scientific formula for prioritising agents. However, there is much precedence, and lessons can be learnt from past successes and failures. I believe while we may never be able to predict a success, if we carefully consider the biology and ecology of the target weed in relation to potential control agents (Briese 1993) we can predict failures. Success will be the result of ingenuity, persistence and a little luck.

References

- Beddington, J.R., Free, C.A. and Lawton, J.H. 1978. Characteristics of successful natural enemies in models of biological control of insect pests. Nature 273, 513–519.
- Beirne, B.P. 1985. Avoidable obstacles to colonisation in classical biological control of insects. Canadian Journal of Zoology 63, 743–747.
- Bork, A. and Bissett, J. 1985. Gall midges (Diptera: Cecidomyiidae) are vectors for their fungal symbionts. Symbiosis 1, 185–194.
- Briese, D.T. 1993. The contribution of plant biology and ecology to the biological control of weeds. In: Swarbrick, J.T. ed. Proceedings of the 10th Australian and 14th Asian-Pacific Weed Conference. Brisbane 1993. Weed Society of Queensland. 10–18.
- Brues, C.T. 1946. Insect Dietary: An Account of the Food Habits of Insects. Harvard University Press, Cambridge. 466pp.
- Cullen, J.M. 1992. Prediction and Practice in Biological Control. In: Richardson, R.G., ed. Proceedings of the First International Weed Control Congress. Vol. 2, Monash University, Melbourne. 137–140.
- Crawley, M.J. 1989. Insect herbivores and plant population dynamics. Annual Review of Entomology 34, 531–564.
- Dennill, G.B. 1988. Why a gall former can be a good biocontrol agent: the gall wasp *Trichilogaster acaciaelongifoliae* and the weed *Acacia longifolia.* Ecological Entomology 13, 1–9.
- Goeden, R.D. 1983. Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. Protection Ecology 5, 287–301.

- Harley, K.L.S. and Forno, I.W. 1992. Biological Control of Weeds. A Handbook for Practitioners and Students. Inkata Press, Sydney. 74pp.
- Harris, P. 1973. The selection of effective agents for the biological control of weeds. Canadian Entomologist 105, 1495–1503.
- —1984. Stress as a strategy in the biological control of weeds. In: Papavizas, G.C. ed. Beltsville Symposia in Agricultural Research 5. Biological control in crop protection. Beltsville Agricultural Research Center, Maryland, USDA, 1980. 333–340.
- Hokkanen, H. and Pimental, D. 1984. New approach for selecting biological control agents. Canadian Entomologist 116, 1109–1121.
- Julien, M.H. 1992. Biological Control of Weeds. A World Catalogue of Agents and Their Target Weeds. Third Edition. CAB International, Wallingford. 186pp.
- Marohasy, J.J. 1993. Constraints to the introduction of potentially the most effective control agents for prickly acacia. In: Swarbrick, J.T. ed. Proceedings II, 10th Australian and 14th Asian-Pacific Weed Conference. Brisbane 1993. Weed Society of Queensland. 33–38.
- —1995. Prospects for the biological control of prickly acacia *Acacia nilotica* (L.) Willd. ex Del. (Mimosaceae) in Australia. Plant Protection Quarterly 10, 24–31.
- Myers, J.H., Higgins, C. and Kovacs, E. 1989. How many insect species are necessary for the biological control of insects? Environmental Entomology 18, 541–547.
- Shorthouse, J.D. 1990. Modification of flowerheads of diffuse knapweed by the gall-inducers *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae). In: Delfosse, E.S. ed. Proceedings of the VII International Symposium on Biological Control of Weeds. Rome 1988. Instituto Sperimentale per la Patologia Vegetale, Rome. 221–228.
- Waage, J. 1990. Ecological theory and the selection of biological control agents. In: Mackauer, M., Ehler, L.E. and Roland, J. ed. Critical Issues in Biological Control. Intercept, Andover, Hants. 135–157.
- White, G.G. and Donnelly, G.P. 1993. A review of Queensland Department of Lands research on biological control of weeds. In: Swarbrick, J.T. ed. Proceedings II, 10th Australian and 14th Asian-Pacific Weed Conference. Brisbane 1993. Weed Society of Queensland. 28–33.
- Wilson, F. 1964. The biological control of weeds. Annual Review of Entomology 9, 225–244.

Protocols and Quarantine Procedures for Importation and Release of Biological Control Agents

Introduction

In the early days of weed biological control, up until the 1930s, there were no generally accepted protocols for importing biological control agents into new countries. The introduction of insects and other agents for weed biological control has always been subject to regulation, but these varied greatly from country to country. In the 60 years since then, legislation controlling import and export of live organisms has been passed in many countries, regional quarantine agreements have been drawn up, and there is now a general recognition that it is necessary to have guidelines or protocols covering the deliberate introduction of insects and pathogens into countries as biological control agents.

Legislation and protocols are needed to prevent problems that may arise from uncontrolled release of any live organisms, or biological control agents in particular.The deliberate importation and release of any organism which damages economically important crops, native flora or fauna, or beneficial organisms including other biological control agents should be avoided. Deliberate introduction of a biological control agent with potential for such damage could not only cause economic and environmental harm but also adversely affect the reputation of biological control and so impede future biological control projects. Conflicts of interest within or between countries concerning the pest status of a weed should be resolved prior to release of a biological control agent for the weed. Proper protocols, with provision for open discussion of the proposed introductions, and consideration of all possible viewpoints, can prevent problems arising from conflicts or at least ensure that all potential problems are considered before decisions about import and release are made.

One aspect of protocols is the host-testing methods used to determine the potential host-range of biological control agents (Marohasy this volume; Heard this volume).

The other aspect is the rules or protocols governing the importation and subsequent field release of a new organism into a country. Classical biological control depends on the introduction of exotic insects and pathogens and as such is subject to these legislative controls. Some countries with a long history of weed biological control such as Australia, Canada, and South Africa, have systems in place which have evolved over the years and work more or less well. Other countries, when faced with a request to import an agent for the biological control of a pest, have tended either to permit the import almost without control or else to impose so many conditions and delays that legal importation becomes, in effect, impossible. Internationally accepted guidelines for the importation process can provide the basis for workable protocols in countries without protocols, and in those with highly restrictive protocols.

Rachel McFadyen
Alan Fletcher Research Station
Queensland Department of Natural Resources
PO Box 36, Sherwood, QLD 4075
Australia

Procedures in Australia

The current system in Australia is reviewed in Paton (1995). Two principal Acts apply, the Quarantine Act (Commonwealth of Australia 1985), designed to keep out diseases of humans and agricultural pests, and the Wildlife Protection Act (Commonwealth of Australia 1982), designed to control trade in endangered wildlife.

A third Act, the Biological Control Act (Commonwealth of Australia 1984), was designed to deal with conflicts of interest such as that over the biological control of Patterson's curse, *Echium plantagineum*, which is regarded by some as a weed and by others as a useful plant (Cullen and Delfosse 1985). The Act provides a legal basis for decisions about biological control when conflicts of interest arise, but unfortunately review procedures are protracted and onerous, and the Act has never been used for weed biological control programs.

The Quarantine Act and Wildlife Protection Act are administered by separate Government Departments and a permit is required under both Acts, that is, permission is required from the two Departments. Issue of permits is administered by the Australian Quarantine Inspection Service (AQIS) according to protocols developed over the years, and with the assistance of reviewers in each of the eight States and Territories.

Currently, the two Departments co-operate to issue permits under a single system, described at the internet site www.dpie.gov.au/aqis/homepage/aqishome.html, which works as follows.

- The initial Application, to import an agent into quarantine for detailed testing, is submitted to AQIS in Canberra.
- The application is considered by a small panel of three experts (entomologists) who advise AQIS. At this stage, the main consideration is whether the agent can be safely maintained in the quarantine insectary proposed by the applicant. AQIS have a system of registration of quarantine insectaries, of which there are four in Australia, two in Brisbane, one near Melbourne and one in Canberra.
- The application is approved and AQIS and Wildlife permits to import into quarantine are granted. The agent can now be imported and a colony established in the quarantine insectary.
- Together with the original Application or separately, the applicant submits to AQIS a proposed Test List, a list of the plants on which the agent will be tested. The list is usually based on the phylogeny of the weed (Forno and Heard this volume), and the test methods are not usually specified.
- AQIS sends the proposed Test List to both the Department of Agriculture and the Department of the Environment in each of the six states, to the Federal Departments, to CSIRO, and to various other scientific or technical agencies—21 groups in all.
- In each of these Departments and agencies, the Test List is studied by scientists, who may be botanists, entomologists, conservationists or biological control practitioners, and their comments are sent back to AQIS.
- Any changes, additions, etc. are discussed with the applicant, and an agreed Test List is arrived at. This is then final; applicants can test as many additional plants as they wish, but plants cannot be omitted, nor can other organisations later demand the addition of further plants, without further discussion.
- When testing has been completed, the applicant writes an Application to Release the agent,

detailing the results of the host-testing and all other relevant information, and again submits this to AQIS.

- AQIS sends the Application to Release to the same 21 groups in each State and Territory as before, for their consideration and comment.
- If all the 21 agencies are in favour of the application, both AQIS and Wildlife Australia issue a Release permit. The review process, from submission of the Application to granting the permits, takes 3 to 6 months.
- If any of the 21 agencies have objections to the application, their comments are relayed back to the applicant for reply. Reviewers may be totally opposed to granting the permit, or may ask for extra tests, or more information, etc.
- If most groups are in favour, and one or two are opposed, or require what seem frivolous extra tests, AQIS may grant the permit anyway. However, if there is a serious disagreement, the permit is not granted—AQIS and Wildlife Australia act conservatively if in doubt.
- When there is a real conflict of interest, e.g. where there is likely to be damage to the environment but real benefit to agriculture, the Biological Control Act allows for a public inquiry ending in a decision at Ministerial level.

Features of this system are:

- Wide consultation with Departments of Agriculture and Conservation in all States
- Ability to resolve minor issues easily
- Open process understood by all
- Written applications so that information is permanently on record
- Onus on applicants to put all necessary information into the application.

There are problems of delays, change of personnel in State departments, and in resolving serious conflicts, but in general the system works well, with 57 import and 33 release permits for new biological control agents issued in 1994 (S. Griffin pers.comm. 1995). AQIS permits are valid for two years and for several shipments, which is ideal. Wildlife Australia permits are valid for 6 months and only one shipment, but permits for several shipments may be issued at the same time if required.

Guidelines

In 1989, draft guidelines for biological control projects were jointly commissioned by ACIAR and the South Pacific Commission. These were circulated among biological control scientists, revised and published (Waterhouse 1991).

The International Organization for Biological Control (IOBC) then pressed for worldwide guidelines. These were drawn up by a working group of biological control scientists from all over the world, and are known as the FAO International Code for the Import and Release of Exotic Biological Control Agents (FAO 1995). The FAO Code was approved by all Member States in November 1995, and should now be adopted worldwide. The FAO Code is 14 pages long, but has been summarised in several IOBC newsletters (IOBC/SEARS 1993, IOBC/NRS 1993), as follows.

Articles 1 and 2 define the scope of and terms used in the FAO Code.

Article 3 states three general principles.

- Importation must be made only with the consent of the government of the importing country.

- Other countries in the region concerned must be consulted.
- Introductions should only be made when in the public interest (i.e. not just in the interest of a small group).

Article 4 covers procedures for the importation and release of natural enemies, on the following lines.

- Approval for release must be based on information about the agent supplied by the agency proposing the release.
- The host range of any potential agents must be adequately investigated before release.

Article 5 covers methods for shipping natural enemies.

- Only healthy specimens of the desired species should be released: all other imported material must be completely destroyed.
- To ensure this, the organism should be bred through one or more generations in a secure quarantine in the importing country.
- If there is no secure quarantine in the importing country, stocks should be bred through at least one generation in a secure quarantine in another country before shipment to the importing country.

Article 6 covers release and evaluation.

- Records of releases must be kept.
- Evaluations of the impact should be made.

Article 7 deals with the need to create appropriate legislation to govern the importation of natural enemies.

- A special agency should be created to review applications for importation and make risk/benefit judgements.

Article 8 and 9 cover the commercial trade in biopesticides, Article 10 deals with information exchange and the need to keep records and voucher specimens, and Article 11 deals with monitoring observance of the code.

Issues Arising from Procedural Rules

Regional consultation

Australia is the only country which is also a continent, and exotic species that we import are therefore unlikely to spread into other countries (except perhaps to Papua New Guinea). All other countries have to accept that biological agents do not respect political borders, and once established in one country will spread to neighbouring ones, until stopped by a change in climate or an ocean barrier. For example, the moth *Cactoblastis cactorum*, introduced into the Caribbean for the control of several pest cacti, has spread northward naturally or through deliberate introductions, and has now reached Florida in the mainland USA. Here it is damaging endangered species of cactus, and threatening the cactus areas of Mexico and the western USA (Pemberton 1995).

There may be conflicts of interest between different countries regarding release of agents. For example, *Chromolaena* is regarded as a serious weed in most African countries, but some scientists regard it as a useful plant in short-term fallow systems (Herren-Gemmill 1991). Agents established in one country will spread to the others, which may not be desired.

Consultation does not mean that nothing can be imported unless all countries are in agreement; countries remain sovereign and free to make their own decisions. It does however mean that other countries in the area should be informed and their wishes at least taken into account if there is a conflict.

Introductions in the public interest

Introductions should not be made when they favour only one sectional group and will cause harm to other groups in society. For example, a proposed introduction might control a plant which is a weed in plantation crops but is used as a food plant by peasant farmers. Or the agent might severely damage a native plant which is an important larval food for native butterflies. At present, not all countries consider all these aspects before granting permits to import insects.

Host testing and acceptance of testing done elsewhere

Many countries do not accept the results of host-testing done elsewhere and insist all tests are repeated in their country, which is very time-wasting. It is sensible to test plants of importance which have not been tested, and also native plants closely related to the host weed. It is also wise to test local cultivars of any crop plant closely related to the host weed, but otherwise there is no need to repeat tests already done in great detail in other countries. For example, *Uroplata girardi* and *Octotoma championi* for the biological control of lantana, and *Procecidochares alani* for the biological control of mistflower, were imported into Australia on the basis of host-testing done in Hawaii. The only additional testing required before being released was on a few native plants.

Use of pathogens

Most weed biological control programs use insects as biological control agents, and most of the scientists involved are entomologists. This may have resulted in a false belief that plant pathogens are not as safe. Many plant diseases are highly host-specific and make very effective biological control agents, and there is a need to consider them as well. Plant pathologists should therefore be included in the permit approval system as well as entomologists. At present, committees considering applications too often consist of entomologists and weed scientists only.

Quarantine Procedures

Quarantine insectaries

The FAO Code does not specify what is considered a 'secure quarantine'. Even in Australia, with its long history in weed biological control, AQIS has only recently drawn up guidelines for construction of quarantine insectaries for handling biological control agents. Quarantine requirements vary according to the agents to be handled, and considerably greater levels of security are needed for pathogens with minute wind-blown spores than for comparatively large insects. A higher level of security is also required if agents are to be imported directly from field-collected material, which may contain parasites and/or diseases. Better standards are also necessary for quarantines where host-testing is to be carried out, i.e. the agent is to be reared for several generations in the quarantine, and, if found to be unsafe, may be destroyed and never released

Basic requirements for a quarantine used to contain imported agents from overseas are as follows.

- Locked outer doors into an entry chamber. All doors must have good seals; light should not be visible around the door. The outer door should be locked and access restricted to authorised staff.
- An airlock with black internal walls between the entry chamber and the quarantine proper. A light trap for escaped insects should be set into an internal wall, using natural light from the quarantine area.
- A high security laboratory, with double-sealed glass windows. Air conditioning must be protected by a fine gauze mesh and by filters sufficient to remove fine dust particles. These filters must be cleaned inside the quarantine and the dust treated in the steriliser.

- A sink with hot water inside the laboratory. Water from this sink and the insectary should flow either to a septic tank or soil trap, or to a tank which can be boiled or treated with bleach when full. When the soil trap or septic is cleaned, the contents must be sterilised before disposal.

- A steam sterilising unit. Medical units which use the normal power supply are readily available. An incinerator can be used for plant and packaging material, but is not really suitable for insects. If used, it is important to ensure the heat is sufficient to destroy all the rubbish without leaving some unburnt.

- An area suitable for growing plants. This can have a solid roof and glass windows and air-conditioning, in which case artificial lights will be required for plant growth. Or it can be a glasshouse or insectary, in which case all ventilation must be covered with fine stainless steel or bronze gauze or mesh with aperture small enough to prevent escape of organisms held under quarantine. The glass roof must be protected from hail and falling branches, and seals between panes must be secure. Alternatively, the entire internal walls and roof of the structure can be lined with fine metal mesh, forming inner mesh walls and ceiling inside the glass.

Procedures

The essential requirement for quarantine handling of insect colonies is proper training for all staff. Escapes seldom happen because of inadequate facilities, but through staff errors—leaving doors open, throwing out waste without prior autoclaving or other treatment, transferring plants from the quarantine glasshouse to another glasshouse or plant-growing area. It is therefore essential that all staff be properly trained in quarantine procedures. Access to the quarantine must then be strictly limited to the trained staff who should arrange their work schedules to limit the number of entries. Cleaning and routine maintenance should be carried out by the quarantine staff.Access by maintenance technicians should be kept to a minimum, and the technicians involved should be informed of quarantine requirements and procedures.

Waste from all insect rearing activities (plant cuttings, soil, cage debris, etc.) must be placed in clearly labelled bags or bins. These bins must never be emptied without first being treated, in the autoclave or other facility, and this rule must be rigidly enforced. This can be assisted by use of hospital waste bags with a patch or strip which changes colour when sterilised. Dustpans and brushes must be kept in the quarantine for cleaning up, and never removed for use outside.

Laboratory equipment (plastic, glassware, etc.) should be cleaned in hot water and detergent, and treated with a surface sterilant such as bleach or alcohol. Spray bottles with alcohol are very useful for immediate spot treatment of surfaces, and for killing unwanted insects, parasites, etc. Paper towels for wiping up spills, drying surfaces, etc. must be supplied. Cages should be treated with steam, bleach or alcohol after being washed with water.

Plant material used for insect rearing in quarantine should be fumigated or autoclaved before removing from quarantine. Pots and potting mix should be fumigated before removal.

References

- Commonwealth of Australia. 1982. Wildlife Protection (Regulation of Exports and Imports) Act 1982. No. 149 of 1982. Commonwealth Government Printer, Canberra.
- Commonwealth of Australia. 1984. Biological Control Act 1984. No. 139 of 1984. Commonwealth Government Printer, Canberra.
- Commonwealth of Australia. 1985. Quarantine Act 1908. Commonwealth Government Printer, Canberra.
- Cullen, J.M. and Delfosse, E.S. 1985. *Echium plantagineum*: catalyst for conflict and change in Australia. In: Delfosse, E.S. ed. 1985. Proceedings of the VI International Symposium on Biological Control of Weeds, Vancouver, 1984. Agriculture Canada. 249–292.
- FAO 1995. Code of Conduct for the Import and Release of Exotic Biological Control Agents. FAO, Rome. 14 pp.
- Herren-Gemmill, B. 1991 The ecological role of the exotic asteraceaous *Chromolaena odorata* in the bush fallow farming system of West Africa. In: Muniappan, R. and Ferrar, P. ed. Proceedings of the 2nd International Workshop on Biological Control of *Chromolaena odorata*, Bogor. BIOTROP Special Publication 44. 11–22.
- IOBC/ NRS Newsletter. 1993. FAO draft guidelines on biological control importations. Vol 14 No 1, winter 1993. 2–3.
- IOBC /SEARS Newsletter. 1993. FAO draft guidelines on biological control importations. Vol. 1 No. 1, March 1993. 1–3.
- Paton R. 1995. Legislation and its administration in the approval of agents for biological control in Australia. In: Delfosse E.S, Scott R.R. ed. Proceedings of the VIII International Symposium on Biological Control of Weeds. Canterbury, 1992. CSIRO Publishing, Melbourne. 653–658.
- Pemberton RW. 1995. *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States: an immigrant biological control agent or an introduction of the nursery industry? American Entomologist 41, 230–232
- Waterhouse, D. 1991 Guidelines for Biological Control Projects in the Pacific. South Pacific Commission, Information Document No.57. 30pp.

Compiling a Plant List for Testing the Host Range of Agents

Introduction

An important early step in host range testing is to compile a list of plants against which the agent is to be tested. It is impossible to test potential agents against all plant species. The challenge is to compile a list that is short enough to be practicable yet sufficiently comprehensive to be scientifically sound (Wapshere 1974). A scientifically sound list will allow the definition of the host range of the agent and, therefore, will help to determine the risk to economically and environmentally important plants that the biological control agent may pose when released.

The Test Plant List

For each target weed, a basic plant test list should be compiled with the assistance of a taxonomic botanist familiar with the family in which the target weed belongs. Entomologists may suggest additions to the list. Agencies representing conservationists and farmers may add further species to the list. Factors which may assist in the rational selection of these representative species include plant morphology, biochemistry, and geographic distribution.

Wendy Forno and Tim Heard
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Plant lists are:

- different for different target weeds because plants closely related to the target species are at the greatest risk;
- may vary for the testing of different biological control agents against the same weed depending on the mode of feeding and oviposition of the agent or attack by fungi;
- may differ between countries for the testing of the same agent against the same target weed because different countries have different guidelines and have different plant species of economic or conservation importance.

The test list should not be inflexible. There may be sound reasons for modifying the test list during testing as more is learnt about the target weed and the potential agent. Reasons for modification may include:

- if the insect shows some acceptability of a plant species, that is, if oviposition, feeding or development occurs, then relatives of that species should be added to the list.
- if it is found that the agent has particular morphological requirements for development, e.g. thick stems or hard seeds, then a species with thin stems or fleshy seeds may be excluded from the list and more suitable relatives added.

Selecting Plants for Host Testing

Below we provide a set of guidelines for selecting plants for a host test list. We use the weed *Mimosa pigra* (Mimosaceae) to illustrate each of these guidelines. The resulting test list is presented in Table 1.

1. Start with the target species and add varieties of the target weed if any are present in the introduced range.

No varieties of *M. pigra* are known in Australia; the species is very uniform here.

2. Add species from the same genus.

There are no native or economically important species in the genus *Mimosa* in Australia or Southeast Asia but there are two introduced species which have weedy status, *M. pudica* and *M. invisa*. It is important to include these in the list as they may give an indication whether the agent is specific to the species *M. pigra* or to the genus *Mimosa*.

3. Add species from genera in the same tribe.

The genus *Mimosa* belongs to the tribe Mimosae. A representative species of all six other genera in this tribe was included on the list. All species in the genus *Neptunia* are added to the list for two reasons. First, observations in the native range of *Mimosa pigra* in Mexico have shown that the moth *Neurostrota gunniella* (Gracillariidae) is occasionally collected from *Neptunia plena*. Second, during host range testing of other agents for *M. pigra*, several agents showed some acceptance of *Neptunia* for oviposition and feeding.

4. Add species from genera in closely related tribes.

The closely related tribe Acaciae, includes the genus *Acacia*. This genus presents special problems as there are more than 800 native species present in Australia. In a case such as this, the question arises as to how many *Acacia* species should be tested and how does one decide which species should be tested. One approach is to select species from different subgenera according to whether the species occur in the actual or potential range of the weed. For example, Pedley (1978) and Maslin and Pedley (1988) divided the Australian acacias into three subgenera with seven sections in one subgenera. Species in one subgenus and one section of another subgenus are environmentally isolated from the potential range of *M. pigra* and are considered to be of less importance than *Acacia* species within the colonising range of *M. pigra*. The outcome of the selection process was to select a total of 20 species of *Acacia* representing all sections within the subgenera, and within the potential range of *M. pigra* in Australia. The number of species in each group was related to the size of that group, e.g. Juliforae and Phyllodineae are bigger groups that Botrycephalae.

Representative species from the Ingeae, the third tribe in the family were then added to the list.

5. Add species from representative genera in closely related families.

The families Fabaceae and Caesalpiniaceae are very closely related to Mimosaceae. These three families are considered by some to be three subfamilies within the same family. These families contain native species and a number of important introduced pasture and crop plants. Not all can be tested and it is preferable to test a species from each genus rather than several species from a few genera. Also, representatives from each of the tribes in these families were selected.

6. Add species that are chemically similar to the target weed if this information is known.

No evidence was found for including other species under this criterion.

7. Add a selection of plants which are economically important or of interest to conservation in the target country.

These species are usually crop or pasture species which are geographically associated with the target weed but are not closely related taxonomically. They are tested to allay the fears of farmers, conservationists and bureaucrats. It is desirable that the list does not exceed 10 species.

8. Add any host plant records of the biological control agent.

If the potential biological control agent has been collected from any other plant species, or is known from the literature to attack other species, then these species should be added to the list. These additions will normally apply only to specific agents and not to the basic list for the target weed. No examples of such additions to the list are available for *M. pigra.*

9. Add any host plant records of insects in the same genus as the biological control agent.

The Lepidoptera *Ithome* sp. (Cosmopterigidae) is being assessed as a potential agent for *M. pigra.* Its relative, *Ithome lassula* is known to attack *Leucaena leucocephala.* Therefore more species of *Leucaena* will be added to the list.

References

- Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Annals of Applied Biology 77, 201–211.
- Pedley, L. 1978. A revision of *Acacia* Mill. in Queensland. Part 1. Austrobaileya 1, 75–234.
- Maslin, B.R. and Pedley, L. 1988. Patterns of distribution of *Acacia* in Australia.. Australian Journal of Botany 36, 385–393.

Table 1: Basic list of plant species to be tested in Australia to determine the host-specificity of potential biological control agents of *Mimosa pigra*

Family	Tribe	Species	Subgenus	Section
Mimosaceae	Mimoseae	*Mimosa invisa*		
		Mimosa pudica		
		Dichrostachys cinerea		
		Leucaena leucocephala		
		Prosopis glandulosa		
		Adenanthera pavonina		
		Desmanthus virgatus		
		Neptunia dimorphantha		
		Neptunia gracilis		
		Neptunia major		
		Neptunia monosperma		
	Acacieae	*A. farnesiana*	*Acacia*	
		A. deanei	*Phyllodineaes*	Botrycephalae
		A. spectabilis		
		A. aulacocarpa		Juliflorae
		A. concurrens		
		A. gonocarpa		
		A. holosericea		
		A. lysiphloia		
		A. mangium		
		A. plectocarpa		
		A. umbellata		
		A. alleniana		Phyllodineae
		A. conferta		
		A. falcata		
		A. fimbriata		
		A. macradenia		
		A. podalyriifolia		
		A. saligna		
		A. complanata		Plurinerves
		A. simsii		
	Ingeae	*Albizia lebbeck*		
		Archidendron lucyi		
		Cathormion umbellatum		

Family	Tribe	Species	Subgenus	Section
		Calliandra surrimensis		
		Samanea saman		
Caesalpiniaceae	Amherstieae	*Tamarindus indica*		
	Caesalpinieae	*Peltophorum pterocarpum*		
		Erythrophleum chlorostachys		
	Cassieae	*Chamaecrista mimosoides*		
		Senna alata		
	Cercideae	*Piliostigma malabaricum*		
		Lysiphyllum hookeri		
Fabaceae	Abreae	*Abrus precatorius*		
	Aeschynomeneae	*Stylosanthes hamata*		
	Crotalarieae	*Crotalaria mitchelli*		
	Desmodieae	*Desmodium tortuosum*		
	Phaseoleae	*Cajanus cajan*		
		Aeschynomene americana		
		Centrosema pubescens		
		Lablab purpureus		
		Glycine max		
		Macroptilium atropurpureum		
		Phaseolus vulgaris		
		Vigna mungo		
	Robinieae	*Sesbania cannabina*		
	Tephrosieae	*Pongamia pinnata*		
	Trifolieae	*Medicago sativa*		
	Vicieae	*Pisum sativum*		
Anacardiaceae		*Mangifera indica*		
Asteraceae		*Helianthus annuus*		
Cucurbitaceae		*Cucurbita maxima*		
Lauraceae		*Persea americana*		
Malvaceae		*Gossypium hirsutum*		
Myrtaceae		*Eucalyptus miniata*		
Poaceae		*Sorghum vulgare*		
Proteaceae		*Macadamia integrifolia*		
Sapindaceae		*Litchi chinensis*		
Solanaceae		*Lycopersicon esculentum*		

Host Range Testing of Insects

Introduction

The determination of host specificity is fundamental to a biological control of weeds program. No insect should be introduced into a new region before its host range is known. Host range testing determines whether a biological control agent will attack only the target weed or other species, and whether the agent is safe for field release.

Usually preliminary observations on host range are made in the field in the country of origin (Forno this volume). Also the literature is searched for any published accounts of the host range of the potential agent and close relatives. This information needs to be confirmed by more complete host range tests in the laboratory and/or field before the insect is released in a new environment. This lecture covers the design of host range tests drawing on our knowledge of the principles of host specificity, and host plant selection by phytophagous insects. This lecture only covers insects; the methods for screening fungal pathogens are described by Tomley (this volume).

The theory and practice of host specificity testing have been discussed by Cullen (1988), Harley and Forno (1992), Zwölfer and Harris (1971). The technical guidelines in support of the FAO code of conduct for the import and release of biological control agents contain a section on tests for host specificity but this document is still in draft form (see McFadyen this volume).

Tim Heard
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Types of Tests

Types of tests include: oviposition tests, adult feeding tests, and larval development tests. Tests may be done in a quarantine laboratory, or in the native range in the open, or in cages. Oviposition and adult feeding tests may be either choice or no-choice tests. Larval development tests are usually no-choice only. The type of tests done will depend on many factors, particularly the biology of the agent. Usually several types of tests must be done to conclusively demonstrate the host range of an insect.

Oviposition tests

The host range of an insect is the group of plant species on which larval development occurs in nature. In most phytophagous insects the larval food is determined by the ovipositing adult female, not by the larva. Hence, the process of host selection by ovipositing females is used by most biological control workers as the most important indicator of host range.

Oviposition tests determine the behavioural host range of an insect, that is, the host range that occurs as a result of insect behaviour. Larval development tests determine the physiological host range, that is, the range of plants upon which larvae are physiologically capable of developing.

To test this host selection, insects are given access to a range of plant species including the target weed. The adults are later removed and the number of eggs laid on each plant is counted.

Oviposition tests are not possible for many insects which do not express natural host selection behaviour under cage conditions. This may be due to sensitisation, where some attribute of the host, e.g. volatiles, has excited and increased the responsiveness of the insect resulting in it ovipositing on non-hosts or even on cage walls. Sometimes it is possible to evoke natural behaviour by using a larger cage, adding a natural substrate or otherwise making the conditions more natural. If these are unsuccessful, other types of tests are required.

Adult feeding tests

Where both larval and adult feeding seriously damage the host plant, the feeding range of the adults also needs to be determined. Usually this can be done concurrently with the oviposition tests. In this case, the extent of feeding is also evaluated at the end of the test. If the feeding damage is quantified it may be compared to damage on other plants more conclusively and is amenable to statistical analysis. Feeding damage may be measured by counting the feeding scars, counting the structures destroyed, measuring the leaf area destroyed, etc.

Sometimes adult feeding tests are done with newly emerged unfed adults. In this case they are not done concurrently with oviposition tests. This method ensures that the adult food preferences are not induced by early adult experience of feeding on the normal host on which they are reared.

Choice verses no-choice tests

Both oviposition and adults feeding tests may be designed as choice or no-choice tests. In a choice test, a group of insects is allowed access to several plant species simultaneously, including the normal host. In a no-choice situation, the insects are allowed access to only one plant species at any one time.

In both designs, a control plant consisting of the target weed, must be included. This tests whether the insects used were in a suitable condition for oviposition and feeding. It also provides 'baseline' data, or estimates of normal numbers of eggs laid and feeds inflicted by a given number of insects in a given period. Damage to other plant species can then be compared to these baselines.

Choice tests

The advantages of choice tests are that they are more natural (in nature, insects are constantly faced with making choices). Choice tests are also more efficient as several plant species can be processed simultaneously. However, presence of the normal host may influence behaviour on other species.

No-choice tests

No-choice tests are considered more rigorous than choice tests. An insect may not feed or lay eggs in a choice test because it preferred the normal host. Rejection in a no-choice test, however, indicates that the insect will not accept the plant for feeding or laying even though it has no alternative. No-choice tests may follow choice tests for those plant species accepted in choice tests. Alternatively, no-choice tests are done in addition to choice tests, even when no attack occurred in the choice test. A third option is to test some plants using choice tests and others using no-choice. In this case, the plants considered more at risk should be tested using the no-choice method.

No-choice tests may be serial (also called sequential) or parallel. In serial tests a group of insects is alternated between the target weed and test plants. In parallel tests, the group of insects is divided into two subgroups, one of which is placed on the target weed and the other on a test plant.

Starvation tests

These tests are no-choice adult and larvae feeding tests which are continued until the insects die. They ensure that insects will not begin feeding after extended periods of food deprivation.

New test designs

We are currently developing tests that combine the advantages of choice and no-choice tests. One approach is to conduct choice tests but include a limited amount of host plant material. This ensures that the insects consume all the host plant material, or use all available oviposition sites, within the duration of the trial. The insects are then forced to look elsewhere and thus the test becomes similar to a no-choice test. Another approach is to alternate the normal host with a choice of test plant species. The time spent with the normal host gives information on the fecundity of the insects. The test plants are then assessed for their acceptibility in a choice design.

Larval development tests

When oviposition is observed on a test plant, further tests are required. In particular, it is necessary to measure the viability of the eggs, the ability of the plant to support larval development, the mortality of the pupal stage, and the size and fecundity of the resulting adults. These tests determine the physiological host range, that is, the range of plants on which larval development can occur. Larval development tests may be the only viable test for insects which do not show normal oviposition behaviour in cages.

The eggs may be placed on the plant material by the ovipositing adult or they may be manually transferred to the point of feeding. Manual transfer is necesary for some Lepidoptera that randomly lay eggs. The development of the eggs into adults is then monitored.

Normally the larvae are placed on the test plant without previously feeding on any other plant. This is to avoid induced food preference, that is, the phenomenon where larvae that have fed on one plant species will often reject other acceptable host species. Occasionally, however, older larvae are tested. These larvae are partly reared on the normal host and are then transferred to the test species. This tests for the situation where a similar transfer is possible in nature.

Multi-generation tests

When a non-target plant is accepted for oviposition and the plant supports larval development, it is necessary to detemine whether the plant can support populations of the insect indefinitely. For these tests, the insects are bred on the plant in the same way that they are bred on their normal host.

Open-field tests

These tests are conducted in the country where the potential insect agent occurs, i.e. in the country of origin or a country of previous introduction. The control plant and test plants are placed in the field where populations of the insect occur. The test allows the insect to express its natural behaviour. However, it may be difficult to achieve high insect densities. Studies using open field tests are reviewed by Clement and Cristofaro (1995).

General points

All tests should be conducted under optimal conditions for insect development and with sufficient light and nutrients to maintain plant quality. The temperature regime selected for insect development will also be suitable for the host plant.

Tests must be replicated. A minimum of three replications is normal. For choice tests, a different combination of plants species should be used in each replicate. The exposure period should consist of a

minimum of two days as there may be diel rhythms of activity. Also insects may not begin to feed or lay on a less acceptable host until a period of deprivation has occurred.

The Insects Used for Testing

The health and age of the insect culture used for specificity testing must be constantly monitored. The numbers of insects used in each test and the number of tests will vary according to the biology of the insect and the holding capacity of the part of the plant supporting development, e.g. stems, flowers, or leaves. Ideally a different insect culture should be used in each replicate. These cultures may represent insects that are from different localities (as geographic variation in host preference within a species may occur), different ages, different collection dates, etc. The aim is to test insect material that varies genetically, ontogenetically, and physiologically.

The Plants Being Tested

The decision as to which plants are to be tested is discussed elsewhere (Forno & Heard this volume). For efficiency, plant species should be tested in a rational order with the species most at risk tested first. Wapshere (1974) proposed a strategy in which the first step is to test a small group of plants that are very closely related and with morphological and biochemical similarities to the weed. This strategy allows early rejection of agents with a wide host range.

The plant material may be cut or living depending on the plant structure being tested, the size of the plant, the biology of the insect, the duration of the test, etc.

Plant material from different individual plants should be used for each replicate of the host-testing test. This gives more confidence in the results as broader genetic range within the plant species is being tested.

Oviposition and feeding preferences need to be understood and incorporated in designs of host range testing. The material provided from different plant species must be of equal suitability in terms of structure and ontogenetic development and a similar quantity of material must be provided. For example, *Coelocephalapion pigrae* feeds and lays many more eggs into flower buds close to opening than less mature buds, therefore mature buds of all test species must be provided. *Chalcodermus serripes* makes conspicuous feeding scars on pods of its host plant. Studies on the preferences for different plant parts showed that they prefer young leaves for feeding. A young leaf of every test plant species, therefore, had to be included in the tests.

Interpretation of the Results

These tests will provide hard data for decision making. The decision, however, will depend on other factors. If there is a possible danger in introducing an agent, this will have to be balanced against the potential benefits (McFadyen and Heard this volume).

Host Range Testing for Release into a New Country of a Previously Tested Agent

The host range of a monophagous insect is independent of environment. It will remain monophagous in any environment. Therefore, once it has been conclusively proven that an insect is monophagous it is not necessary to test it again. For example, the salvinia weevil *Cyrtobagous salviniae* is known, from laboratory and field studies, to attack only *Salvinia molesta* and other closely related sympatric *Salvinia* species. Other *Salvinia* species occurring elsewhere are not host plants. This insect is suitable for release in any country without further testing. If an insect is oligophagous, it may be suitable for release in one environment but not another. For example, *Cactoblastis cactorum* attacks several species

of Cactaceae. It is suitable for release in Australia where there are no native Cactaceae nor any beneficial introduced species. However, it may not be appropriate to introduce this insect into a country where Cactaceae are ecologically or environmentally important, without careful assessment of the risk.

Case studies of different testing procedures

The following examples of agents tested for control of *Mimosa pigra* illustrate the testing procedures for different agents according to the behaviour of adults, mobility of larvae and availability of the host plant part to be tested and emphasise careful interpretation of results.

1. The stem-boring moth *Carmenta mimosa* Eichlin and Passoa (Lepidoptera: Sesiidae) develops in stems of *M. pigra*. Females are indiscriminate when ovipositing under laboratory conditions, often placing eggs on the frame and gauze of the cage. Choice oviposition tests therefore could not be carried out. Instead larval development tests were done in which eggs or first instar larvae were inserted into stems and development observed. It was shown that although larvae developed beyond the first instar on a few closely related plants, none completed development on any plant other than *M. pigra*. As a result of these tests *C. mimosa* was released in Australia and later in Thailand (Forno et al. 1991).

2. Some tests have to be conducted on portions excised from the whole plant. This was the case with *Coelocephalapion pigrae* (Coleoptera: Apionidae), which develops in the flower-buds of *M. pigra*. The host test list includes many trees which will not produce flower buds when the plant is growing in a pot and the only option was to test flower-buds on cut foliage. Fortunately, *C. pigrae* accepted excised flower-buds of *M. pigra* and fed and reproduced normally. Results of choice tests showed that *C. pigrae* preferred *M. pigra* for oviposition and feeding but occasional oviposition occurred on the weed, *Mimosa pudica,* and the native plant, *Neptunia dimorphantha.* In no-choice tests, the level of oviposition on *M. pudica* and *N. dimorphantha* was greater than in choice tests but was still less than that on *M. pigra.* Development was completed on these three species but survival of immature stages was lower on *M. pudica* and *N. dimorphantha* than on *M. pigra.* This, together with their low acceptance for oviposition, indicates that neither plant could sustain populations of *C. pigrae.* Furthermore, populations of *N. dimorphantha* are disjunct from current and projected populations of *M. pigra.* The results of these tests were accepted by the regulatory authorities and *C.pigrae* was released in Australia in May 1994 (Heard and Forno 1996).

3. *Sibinia fastigiata* (Coleoptera: Curculionidae) oviposits in the pods of its host plant. Adults feed on the nectar and pollen from open flowers and so do not damage the plant. We combined elements of choice and no-choice to design a testing method for this insect. We alternated pods of the normal host with the choice of pods of test plant species. The time spent with the normal host gives information on the fecundity of the insects. The test plants are then assessed for their acceptibility in a choice design. We provided flowers continuously to maintain healthy fecund adults (Heard et al. 1997).

4. Adults of the leaf beetle *Syphrea flavipes* nr. *cardiaca* (Coleoptera: Chrysomelidae) feed on leaves but the larvae feed on roots of the host plant. Adults would not oviposit in the laboratory, therefore oviposition tests could not be done. Larvae developed on excised roots but their survival was poor, therefore larval development tests could not be done. Adults fed on excised leaves in containers and so adult feeding trials could be performed. These trials showed that the adults fed on many species of legumes. Thus we were able to reject this agent before spending resources on developing oviposition and larval development tests.

References

- Clement, S.L. and Cristofaro, M. 1995. Open-field tests in host specificity determination of insects for biological control of weeds. Biocontrol Science and Technology 5, 395–406.
- Cullen, J.M. 1988. Current problems in host-specificity screening. In: Delfosse, E.S. ed. Proceedings of the VIIth International Symposium on Biological Control of Weeds. Rome, 1990. Istituto Sperimentale per la Patologia Vegetale, Rome. 27–36.
- Forno, I.W., Kassulke, R.C. and Day, M.D. 1991. Life-cycle and host testing procedures for *Carmenta mimosa* Eichlin and Passoa (Lepidoptera: Sesiidae), a biological control agent for *Mimosa pigra* L. (Mimosaceae) in Australia. Biological Control 1, 309–315.
- Harley, K.L.S. and Forno, I.W. 1992. Biological Control of Weeds. A Handbook for Practitioners and Students. Inkata Press, Melbourne. 74 pp.
- Heard, T.A. and Forno, I.W. 1996. Host selection and host range of the flower feeding weevil, *Coelocephalapion pigrae*, a potential biological control agent against *Mimosa pigra*. Biological Control 6, 83–95.
- Heard, T.A., Segura, R., Martinez, M. and Forno, I.W. 1997. Biology and host range of the green seed weevil, *Sibinia fastigiata,* for biological control of *Mimosa pigra*. Biocontrol Science and Technology 7, (in press).
- Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Annals of Applied Biology 77, 201–211.
- Zwölfer, H. and Harris, P. 1971. Host specificity testing of insects for biological control of weeds. Annual Review of Entomology 80, 159–178.

Decision Making Based on Host Range Tests

Introduction

The decision to release a potential weed biological control agent into the field is based on judgements about risk of damage to non-target plants of economic or conservation value. These risks should be balanced against the possible benefits of biological control of the target weed.

The risk of damage to non-target plants is assessed from literature searches and field observations in the country of origin or other regions in which the agent has been released (Forno this volume) and from host specificity tests (Heard this volume). The predicted host range of potential biological control agents varies from high specificity, so that only the target weed will be damaged in the field, to broad specificity, so that several non-target plants of economic or conservation value are likely to be seriously affected if the agent was released in the field. Decision making at these extremes is straightforward. If there is no risk to non-target plants then the agent should be released. On the other hand, if several valuable non-target plants are likely to be seriously damaged, the potential agent should not be released.

Between the extremes of possible outcomes of host specificity testing are cases of potential agents which may cause some damage in the field to one or a limited range of valuable non-target plants. In these cases a risk analysis approach balancing the costs (i.e. risk of damage to non-target plants) and benefits (i.e. possibility of control of the target weed) of release of the potential agent is necessary in making the decision to release the agent in the field. Political and social costs need to be considered, and may sometimes outweigh the economic costs. In this paper several case studies from biological control programs in Australia are presented to illustrate this approach.

Rachel McFadyen
Alan Fletcher Research Station
Queensland Department of Natural Resources
PO Box 36, Sherwood, QLD 4075
Australia

Tim Heard
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Zygogramma bicolorata for Control of Parthenium Weed, *Parthenium hysterophorus*

Biology of *Z. bicolorata*

The leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) was imported from Mexico for the biological control of *Parthenium hysterophorus* (Asteraceae: Heliantheae), and released in Australia in 1980 and in India in 1984. The adult beetles feed and oviposit on the leaves; the larvae feed on the young leaves initially and then on larger leaves. Fully-grown larvae pupate in the soil and the new adults emerge a week to 10 days later. There are three to four generations each year if the rainfall is good and there is fresh parthenium present, but if

conditions are dry, adults cease feeding and egg-laying and pass the dry season buried in the soil. Winter is also spent in diapause in the soil, and adults re-emerge after rain in spring to feed and lay again. Feeding by both adults and larvae causes significant damage to the plants, but larval dispersal is limited to 1 to 2 m distance. The larval feeding period is also short, two to four weeks only, while adults live and feed for several months.

Host range of *Z. bicolorata*

Z. bicolorata was known to feed on parthenium and on the closely related plant annual ragweed, *Ambrosia artemisiifolia.* Ragweed and parthenium are in the subtribe Ambrosiinae, and sunflower *Helianthus annuus* is in another subtribe of the same tribe Heliantheae. Other species of *Zygogramma* are pests of sunflower in the USA, but *Z. bicolorata* has never been recorded feeding on sunflower in Mexico or the USA where it is native. Two other species, *Z. disrupta* and *Z. suturalis*, have been introduced into Russia and the Ukraine for the biological control of annual ragweed, resulting in at least partial control of the ragweed and without any reports of damage to sunflower (Julien 1992).

Prior to introduction into Australia and India, detailed hostspecificity testing of *Z. bicolorata* was carried out. Both choice and non-choice tests were undertaken with adults and first instar larvae, on a wide variety of plants in the Asteraceae (McFadyen and McClay 1981). The results were quite clear; when given a choice, adults would neither feed nor lay on any plants except parthenium and ragweed. However, when given no choice, after 1 to 2 days adults will feed on most plants in the tribe Heliantheae, and on some there was extensive feeding and considerable damage. Few eggs were laid on these plants, and larvae did not feed or survive on them.

Decisions based on host specificity testing

As *Z. bicolorata* will not lay and larvae cannot survive on plants other than parthenium and ragweed, and in the native range there is never attack on sunflower in the field, it seemed clear that this beetle was safe to introduce into countries where biological control of parthenium and ragweed is required, even if sunflower is a major crop in the same area. *Z. bicolorata* was therefore released in both India and Australia for the biological control of parthenium.

In Australia, the beetle immediately established and became abundant on annual ragweed in the coastal areas, where no sunflower is grown. In the parthenium area where sunflower is also grown, the beetle did not do well initially and has only become abundant since 1990. Since then, beetle numbers have been enormous in some localities and seasons, but because of drought conditions, little sunflower has been grown in the area and there are no reports of *Z. bicolorata* damaging sunflower. When beetle numbers are very large, related plants such as *Verbesina encelioides* (tribe Heliantheae, subtribe Verbesiniinae) and *Xanthium occidentale* (tribe Heliantheae, subtribe Ambrosiinae) have been extensively damaged in the field but these are both weeds and the damage has not caused any problems.

In India the beetle spread and increased rapidly, building up very large populations on parthenium in the Bangalore area and spreading from there. After a few years, there were reports of serious damage to sunflower crops in the area. In southern India, sunflower is grown in very small fields, only 0.5 hectare in extent, often surrounded by fallow fields or rough pasture infested with dense parthenium. When there were no green leaves on the parthenium, either because it had dried up or because the beetles had eaten all the leaves, adult beetles fed on adjacent sunflower crops causing near-total defoliation of the outer rows. Feeding usually occurred over a 3 to 4 week

period, after which the beetles moved away in search of parthenium or to diapause in the soil. Some eggs were laid on the sunflower, but as the larvae did not survive, this was not significant (Jayanth et al. 1993).

In both Australia and India, feeding on non-host plants only occurred when enormous populations of the beetle, usually of newly-emerged adults, were present in the field, with 30 to 40 adult beetles per parthenium plant and several hundred per m^2. All green parthenium had been eaten and the beetles were starving. These are the conditions prevailing in starvation or no-choice tests, and the beetles reacted in the same way, feeding on and causing heavy damage to normally rejected plants. Field results from Mexico or the USA were not relevant, because these enormous beetle populations have never occurred there. This demonstrates the value of starvation tests on mobile stages of the life cycle, and the problems that may result from too great a reliance on field results in the insect's country of origin.

Impact of the decision

In India, the damage by *Z bicolorata* to sunflower has caused serious questioning of biological control in the media and among scientists. There have been published articles questioning the safety of host-testing and the wisdom of importing exotic insects, even accusations of deliberate sabotage of Indian agriculture. In hindsight, it might have been better not to have released this beetle, even though it is giving good control of parthenium in many areas. The political and social problems that arose when damage was caused to the crops were due in part to the lack of involvement of the growers in the decision to release the agent (Bilston and Norton this volume).

In Australia, there have been no problems so far. In the very different political and agricultural situation in Australia, even if sunflower crops are damaged, there is likely to be less outcry and questioning. Australian sunflower growers are involved in the decisions to release biological control agents through organisations such as the Queensland Graingrowers Association (QGA) or the Farmers Federation (QFF). They have representatives on the Land Protection Board which funds the research, and receive regular reports of ongoing research. Scientists may address meetings of the QGA or QFF to explain current biocontrol research. Farmers thus have an opportunity to express their concerns and to understand the issues, and in general they believe the scientists are doing their best for agriculture.

Euclasta whalleyi for Control of Rubbervine, *Cryptostegia grandiflora*

Rubbervine

Rubbervine, *Cryptostegia grandiflora*, is a very serious weed of pasture and riverine ecosystems in north Queensland which is continuing to spread west towards the Northern Territory and Western Australia. In the open, it forms bushes up to 3 m tall, but also grows as a vine up trees, completely covering them up to 30 m height. Because of its dense foliage, only shed during the dry season, no light reaches the understorey plants which also die. All native vegetation dies in affected riverine ecosystems, and the native animals may also disappear as a consequence. In 1989 rubbervine affected 350 000 km^2 in north Queensland and was rated as Australia's worst environmental weed (Humphreys et al. 1991).

Host range of *E. whalleyi*

Rubbervine is in the family Asclepiadaceae, sub-family Periploicoideae, closely related to the family Apocynaceae. Australia has many native plants in these two families, some of which (*Hoya, Stephanotis*) are also important ornamentals. A biological control program started in 1985, with searches in Madagascar

where the plant is native. Unfortunately, few potential agents were found, and none were host specific to the genus *Cryptostegia.* In tests, two of the species also damaged other native and ornamental plants in the families Apocynaceae or Asclepiadaceae, and were rejected for this reason (McFadyen and Marohasy 1993b).

The leaf-feeding moth *E. whalleyi* was the most host specific of the insects found. In both laboratory tests and in the field, it fed and developed on plants in several genera of the subfamily Periplocoidea, but on none outside of this family (McFadyen and Marohasy 1993a). There are only five species in this subfamily in Australia, only one of which is common and found in the same areas as rubbervine. This plant is also a vine, and grows in the same riverine habitats of northern Australia as rubbervine. Where rubbervine invades an area, the native vine is displaced and becomes locally extinct.

Decisions based on host specificity testing

Because of the enormous environmental damage being caused by rubbervine, the lack of other practical control methods or other potential agents, and in view of the fact that the survival of the native vine was severely threatened by the continued spread of rubbervine, the decision was taken to release the moth. The decision process for release of a biological control agent in Australia involves conservation authorities in each State (McFadyen 1998), and in this case the application included letters from the Queensland Department of the Environment strongly supporting the application.

Impact of the decision

Releases were made between 1988 and 1992, and the moth was widespread and causing severe damage to rubbervine by 1995. Larvae have been found feeding on the native vine where this is close to rubbervine, but the moth does not seem to be spreading on the native vine in the absence of rubbervine. It is still too early to judge the final impact of the moth on either rubbervine or the native vine, or whether successful control of rubbervine will allow the native vine to regenerate in the areas where it was displaced.

Neurostrota gunniella for Control of Mimosa, *Mimosa pigra*

Host range of *N. gunniella*

The moth *Neurostrota gunniella* (Gracillariidae) was studied in Mexico and Australian quarantine to determine its suitability for biological control of *Mimosa pigra,* an important weed in Australia. Adults lay eggs on the leaves, the first two larval instars mine the leaves and later instars bore into young stems. *Neptunia plena* is also a host to *N. gunniella* in the native range when this plant occurs among *M. pigra.* No other species, of the 14 legume species surveyed in the field near *M. pigra* infestations, was found to be a host. In laboratory tests, larvae complete development on two species of *Mimosa* (both weeds) and the four native Australian species of *Neptunia.* These six species were the only ones accepted by adults for oviposition in no-choice tests. The duration of larval development did not differ greatly among plant species but larval mortality on *Neptunia* spp. was higher than on *M. pigra.* Damage to *M. pigra* plants was much greater than to *Neptunia* spp. (Davis et al. 1991).

Decisions based on host specificity testing

Although this insect breeds on native *Neptunia* spp., a decision was made to release it in Australia on the following grounds.

- Three species of *Neptunia* occur in different regions or habitats to *M. pigra*; the fourth species does occur in *M. pigra* habitats but was more common elsewhere.

- The damage to *M. pigra* was much greater than to *Neptunia* spp.
- Mortality was higher on *Neptunia* spp. and hence these plants would probably not support high populations.

Since its release in 1989, larvae of *N. gunniella* have caused widespread damage to thin stems of *M. pigra* and adults have distributed widely. A minor level of attack has occurred on *N. gracilis* in the field.

Thailand did not approve release of this insect as laboratory trials in Thailand showed that *N. gunniella* could reproduce on the introduced *Neptunia natans* (L.f.) Druce (= *N. oleracea* Lour.), an important vegetable in Thailand. This species does not occur in Australia and therefore was not tested.

Discussion

These examples demonstrate some of the issues that have to be considered when deciding whether a potential biological control agent should be released or not. The results from host-testing should determine the risk if any to non-target plants, but the decision whether the possible damage outweighs the benefits depends on the importance of the various factors involved. The decision reached will vary in different situations and may not be the same for different countries.

It is important to remember that weed biological control has an excellent safety record, with only eight instances of damage to non-target plants recorded in 100 years of agent introductions (McFadyen 1998). Of these, in five the damage was anticipated but considered not to be important. Two were the result of inadequate host-testing, and in the remaining instance, *Z. bicolorata* on sunflower, the impact of very high populations was underestimated. However, in not one case were there significant economic losses, and the benefits gained from the introductions far outweighed any damage caused.

References

- Davis, D.R., Kassulke, R.C., Gillett, J.D. and Harley, K.L.S. 1991. Systematics, morphology, biology, and host specificity of *Neurostrota gunniella* (Busck) (Lepidoptera: Gracillariidae), an agent for the biological control of *Mimosa pigra* L. Proceedings of the Entomological Society of Washington 93, 16–44.
- Humphries S.E., Groves R.H. and Mitchell D.S. 1991. Plant invasions of Australian ecosystems. A status review and management directions. Kowari 2, 1–134. Canberra: Australian National Parks and Wildlife Service. 188 pp.
- Jayanth K.P., Sukhada Mohandas, Asokan R. and Ganga Visalakshy P.N. 1993. Parthenium pollen induced feeding by *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) on sunflower *(Helianthus annuus)* (Compositae). Bulletin of Entomological Research 83, 595–598.
- Julien M.H. 1992. Biological Control of Weeds. A World Catalogue of Agents and Their Target Weeds. Third Edition. CAB International, Wallingford. 186pp.
- McFadyen, R.E.C. 1998. Biological control of weeds. Annual Review of Entomology 43, 369–393.
- McFadyen R.E.C. and McClay A.S. 1981. Two new insects for the biological control of parthenium weed in Queensland. Proceedings of the 6th Australian Weeds Conference, Gold Coast 1981. pp 145–149.

- McFadyen R.E. and Marohasy J.J. 1993a. A leaf-feeding moth, *Euclasta whalleyi* (Lep. :Pyralidae), for the biological control of *Cryptostegia grandiflora* (Asclepiadaceae) in Queensland, Australia. Entomophaga 35, 431–435.

- —1993b. Biology and host plant range of the soft scale *Steatococcus* new species (Hem.: Margarodidae) for the biological control of the weed *Cryptostegia grandiflora* (Asclepiadaceae). Entomophaga 35, 437–439.

Mass Rearing of Insects for Biological Control of Weeds

Introduction

Mass rearing of insect agents for biological control of weeds involves a choice of appropriate rearing methods. This choice will be influenced by available resources such as money, labour, facilities, essential supplies and transport, and by factors associated with the agent itself.

The literature on mass rearing of insects concentrates mostly on industrial scale rearing used for biological control of insect pests or for sterile insect release programs, and largely ignores biological control of weeds or gives it cursory examination (DeBach 1964; Huffaker and Messenger 1976; Chambers 1977, Mackauer 1972, 1976, 1980; Bigler 1989). This literature is useful for the general principles, particularly in respect to quality control, but there is little or no discussion of technique. Literature reporting on individual weed biological control programs occasionally includes aspects on laboratory or insectary rearing.

Graham Donnelly
Alan Fletcher Research Station
Department of Natural Resources
PO Box 36, Sherwood, QLD 4075
Australia

Options for Mass Rearing

The options for rearing biological control agents for weeds include the following.

- Rearing on plants grown in pots, ant-free trays of soil, or in the ground.
- Rearing on cut foliage or flowers.
- Rearing in cut stem sections.
- Rearing in seeds or fruit.
- Rearing on artificial diets.
- Rearing and collection at nursery sites in the field.

Rearing on growing plants

This technique (Fichera this volume) is useful for most sucking bugs, external leaf-feeders and leaf-miners. The use of growing plants has several advantages over using cut foliage for mass rearing:

- growing plants last much longer than cut foliage;
- insects can be maintained for longer periods without having their food supply changed;
- less handling of immature insects means less chance of injury;
- less labour may be required to grow and maintain plants than to collect and change foliage.

There are economies of scale in growing plants in large trays of soil or in the ground, because less time is required to maintain the plants, but predation by ants is a much more difficult problem than in pots. Plants grown in pots are portable and so can be used much more flexibly in different cages or rearing areas than plants in large trays or the ground.

Rearing on cut foliage

This technique is useful for external leaf feeders but not leaf-miners or most sucking bugs. Its main advantage is to exploit locally growing infestations of the weed and reduce the need for host plant propagation. Cut foliage is brought from the field. The stems must be placed in fresh water or a nutrient solution in suitable containers. It may be necessary to seal around the stems to prevent insects drowning in the liquid. Cut foliage may stay fresh for a few days before being replaced. One disadvantage is the risk of injury to insects through excessive handling during transfer to new foliage.

Rearing on cut flowers

Rearing on cut flowers will probably be the best option for flower feeding insects. Flower feeding insects may need a greater supply of flowers than can be supplied on potted plants. Many perennial plants do not flower readily when grown in pots. Some perennials which are not readily propagated vegetatively take several years to produce flowers. Cut flowers are handled in a similar manner to cut foliage.

Rearing in cut stem sections

This technique is useful for some stem-borers in woody perennial plants. The technique uses much less space and time than growing and maintaining perennial plants of a suitable size. Suitable stems can be obtained from the field and sawn into manageable lengths. The ends of the stem sections can be sealed by dipping briefly into molten wax (Tomley 1990). This allows stem tissues to retain moisture and stay alive while larvae develop. The stems can be exposed to ovipositing adults, or larvae can be introduced into holes bored into the stems. Stems only need to be caged after larvae pupate but before adults emerge.

Rearing in seeds or fruit

Seed feeding insects can be reared in naked seed or in dried fruits such as pods or burrs. The seeds or fruit can be held in cages or even plastic food containers.

Rearing on artificial diets

Diet rearing is sometimes the most productive and economical way to mass rear stem-borers, especially those of woody perennials. Singh (1977) defined an artificial diet as 'an unfamiliar food which has been formulated, synthesised, processed, and/or concocted by man, on which an insect in captivity can develop through all or part of its life cycle'. Smith and Wilson (1995) commented that 'use of artificial diet saves space and handling time, prevents cannibalism of younger larvae by older larvae, and reduces mortality that could occur if transferring larvae from stem to stem.' They added that 'it allows accumulation of larvae, manipulation of development rates using refrigeration to provide synchronous emergenc … and is a suitable medium in which to transport larvae…'.

Various artificial diets can be used to rear weed biological control agents. Wheatgerm-based diets (Adkisson et al. 1960; McMorran 1965), devised for rearing pest Lepidoptera, have been used for rearing stem-boring Lepidoptera for weed biological control. Gardiner (1970) modified McMorran's diet by including dried and ground host-plant material to successfully rear over forty different cerambycid beetle species, although none of these were biological control agents. Another diet for cerambycids was devised by Harley and Willson (1968) for *Plagiohammus spinipennis* an agent for lantana. This did not include dried host-plant material. Tomley (1990) modified the Harley and Willson (1968) diet for the *Baccharis halimifolia* cerambycid *Megacyllene mellyi* by substituting dried and ground *B. halimifolia* wood for the normal cellulose and reducing the water content. For rearing the clear wing moth *Carmenta mimosa*, a

biological control agent for *Mimosa pigra,* Smith and Wilson (1995) modified a diet developed for the cerambycid *Phytoecia coerulescens,* an agent for *Echium plantagineum,* by adding dried and ground *M. pigra* leaves. The *Phytoecia* diet was based on the Harley and Willson (1968) diet and a Shorey and Hale (1965) diet for noctuids containing ground dried beans (Smith and Wilson 1995).

Hygiene is an important aspect of diet rearing. The diet medium should be produced, stored and dispensed under conditions as sterile as possible. Containers should be sterilised before use. Diet medium should be stored under refrigeration. It is important that each larva placed on diet is in an individual container or cell to prevent cannibalism and minimise cross infestation by culture pests. Diet in use can quickly deteriorate due to feeding by the larva, therefore its condition should be monitored frequently. Particular attention should be paid to outbreaks of fungi or mites and to rancid odours. Larvae should be transferred to fresh diet before the old diet breaks down or becomes contaminated. Contaminated containers should be isolated from the others and decontaminated, using, for example, liquid chlorine bleach.

Field collection and nursery sites

Some insects which rapidly establish large populations in the field may be amenable to field collection for release at other locations. The initial sites from which field collection takes place are termed nursery sites. These are discussed further by Harley and Forno (1992) and Wright (this volume). This process is more economical than insectary-based mass rearing if the nursery sites are not remote. An example in recent times is the salvinia weevil *Cyrtobagous salviniae.* Infested salvinia is harvested, bagged and despatched to new release sites. Sometimes sites not specifically set up as nursery sites become collecting sites when agent populations build up locally. Care should be taken so that other unwanted species are not also transferred between sites.

General Techniques in Mass Rearing

Quality control

While the main objective of mass rearing is to produce as many insects as possible for release, the insects produced should be of high quality and their natural attributes should be preserved. The mass-reared insects should be as fit genetically, physically and behaviourally as a natural population. Selection of biological and behavioural characteristics under laboratory rearing conditions may reduce the ability of populations to establish after release into the field. These effects may be minimised by the periodic reintroduction of field collected insects into the laboratory culture once the agents have established in the field. Care should be taken to ensure that the field collected insects are free of parasitoids, ectoparasites and diseases acquired in the field.

Quality control issues such as maintenance of genetic and behavioural fitness are discussed by Boller (1972), Huettel (1976), Chambers (1977), Mackauer (1972, 1976, 1980), Bigler (1989) and Leppla and Fisher (1989).

General hygiene

Insect populations in mass rearing insectaries can be rapidly depleted by outbreaks of disease. Lepidopterous larvae are particularly susceptible to bacterial and viral infections. Entomophagous fungi can affect many insects particularly those which spend part of their life cycle on or in the soil. Allen (1980) discussed insect diseases and their elimination in laboratory and insectary rearing.

Care must be taken to ensure that only healthy insects are used to start colonies. It is prudent to maintain susceptible colonies in as many cages as is practical so that if part of a colony is affected by a disease outbreak, those cages can be isolated. Colonies should be checked regularly for signs of infection. Insects,

host material and soil in infected colonies should be disposed of hygienically and the cages sterilised. Strong bleach is a suitable sterilant.

Insects of many orders are susceptible to microsporidian parasites such as *Nosema*. An apparently healthy colony may be infected by these parasites at sublethal levels. The disease only becomes apparent when otherwise unexplained mortality occurs and insect squashes are microscopically examined by an insect pathologist. Microsporidians are transmitted transovarially so normal hygiene techniques are ineffective in preventing outbreaks, but they are extremely important in preventing spread. Specialised procedures are necessary to ensure colonies are free of microsporidian infection. Dunn and Andres (1980) and Etzel et al. (1981) describe techniques used to eliminate *Nosema* from colonies of insects prior to their use as control agents.

Pest control in food plant material

High quality host plant material to be used as food for the insects has to be available in sufficient quantity to sustain continued production at the desired rate. Plants grown intensively as hosts often become infested by pests such as lepidopterous larvae, aphids, whiteflies, scales, mealy-bugs and two-spotted mites. Where possible, predatory biological control agents such as neuropterans, coccinellids and mites should be used to control these pests. If it becomes necessary to use chemicals, only non-residual chemicals with very short withholding periods should be used.

Facilities for Mass Rearing

Insectaries

Although some agents can be reared in outside cages or in the field, others require the protection of insectaries against predators, parasites, extremes of ambient temperatures or rain to maximise production. Generally, an insectary should be a screened, ant-proof building which allows air flow, contains escaped insects, and keeps predators and parasitoids away from the rearing colony.

Provision of means of control of temperature, photoperiod and, perhaps, humidity allow optimal conditions for the insects to be maintained, thus maximising the numbers of agents produced and avoiding delays due to diapause.

Ant-proofing

Ants are a problem in insect rearing because they:

- colonise the soil in pots, trays and in the ground;
- attack the insects being reared;
- actively culture pest insects such as aphids, scales and mealybugs which make the host plants unsuitable for the agent insects.

Ants can be excluded using water-filled moats around concrete slabs or building piers, or by placing the legs of benches in containers of water.

Host plants should be grown in an area protected from ants to avoid bringing ant-infested pots into the insectary. Ant-infested pots can be disinfested by leaving them submerged in tubs of water for 24 hours. Sufficient detergent or wetting agent should be added to enable the water to penetrate the soil fully and to wet the integument of the ants.

Cages

Cages can be made of the most economical materials available. Wire and fibreglass fly netting, some fabrics and mosquito netting are useful cage materials. Frames are often made of wood, stiff wire, aluminium or steel. Plastic food containers can make ideal small cages. Cages should be designed to suit the host plants, the insects and the health and safety of insectary workers. When plants are going to be in a cage for an extended time the covering should transmit sufficient light for photosynthesis to occur.

In some cases an entire insectary or other structure such as a shadehouse can be used as a large cage.

Influence of Biology and Behaviour of the Insect

Diapause, and the environmental factors inducing hatching and breaking diapause

Some insects enter a period of diapause or aestivation during which development is delayed. These may be obligatory or facultative. Diapause enables insects to survive seasonally unfavourable conditions. With appropriate environmental control facilities it may be possible to artificially prevent diapause occuring or break diapause and thus increase the number of generations.

In the case of the univoltine *Baccharis halimifolia* chrysomelid, *Trirhabda bacharidis,* egg masses are covered in a cement that needs to be weathered off before diapause is broken. In the field this takes most of the year. In the insectary, the egg masses were watered regularly until eggs hatched (W.H. Haseler pers.comm. 1968). A similar technique has been used for breaking egg diapause in the *Acacia nilotica* chrysomelid *Weiseana barkeri,* whose egg masses are cemented together with maternal faeces (Marohasy 1994).

Oviposition sites

Eggs can be laid on plant surfaces, into plant tissues and into crevices in plant surfaces. They can be deposited individually, in small groups or in large masses. It is possible to exploit an insect's specific oviposition requirements to concentrate oviposition and enhance collection of eggs or young larvae. For example, the *Baccharis halimifolia* cerambycid *Megacyllene mellyi* lays eggs individually in fissures in the bark of its host (McFadyen 1983). In insectary rearing of *M. mellyi,* additional crevices were produced on host stem-cuttings by wrapping cloth tape spirally around the cuttings (Tomley 1990). This concentrated the eggs and subsequent young larvae and reduced the quantity of stem material needed and the amount of handling required prior to transfer of larvae to artificial diet. The *Acacia nilotica* chrysomelid *W. barkeri* oviposits egg masses in fissures in the bark of its host. Marohasy (1994) found that adult females of *W. barkeri* oviposit into strips of corrugated cardboard attached to host foliage and used this method to obtain concentrated supplies of eggs for mass rearing.

Pupation sites

If larvae pupate or make cocoons on stems, in the foliage or in leaf litter, the pupae or cocoons can easily be collected and transferred to emergence cages. If they pupate in the soil at the base of plants, they can be reared on potted plants. After pupation of the larvae the plant pots can be retained in emergence cages. Trays of litter, sand or peat can be placed on the floor of a cage to provide additional pupation sites.

Supplementary food requirements for adult insects

The adults of some insects used as biological control agents, including most moths and flies and many beetles, do not feed directly on plant tissues but take pollen and/or nectar from flowers. Others do not feed

at all but may require water. Adult feeding provides energy for movement and other activities, prolongs life, and may increase or sustain egg production. In mass rearing, water is usually supplied through a wick. Sugar and honey solutions supplied through a wick or in saturated cotton-wool can replace nectar and, if available, pollen can be provided. Yeast can be used instead of pollen.

Cannibalism

Some stem-boring larvae eat any other larvae they encounter. If this occurs with a biological contol agent it may be necessary to ensure that larvae occupy separate plant stems during rearing. Only one larva of the cerambycid *Nupserha vexator* survives in a multi-stemmed *Xanthium strumarium* plant after larvae from the branches enter the main stem. In mass rearing this was largely avoided by growing host plants so densely so that only single-stemmed plants were available for the insects.

Aggregation behaviour

Immatures of some insects aggregate. During rearing this may result in rapid consumption of food plants and fouling of the surroundings. With such insects food plants may require frequent replacement and cages will require more frequent cleaning.

Movement in response to stimuli

Behaviour patterns may assist or hinder collection and management during rearing. Such behaviour includes; phototaxis (movement in relation to light), geotaxis (movement in response to gravity), thigmotaxis (movement in response to touch), cryptic behaviour and a tendency to hide.

Collecting methods and traps that exploit these behaviours can be devised. For example, jar type light traps attached to opaque breeding boxes are used to harvest new adults of seed-feeding bruchids. Newly hatched larvae of the lantana noctuid *Neogalea esula* have a tendency to descend in large numbers on silk threads. They were readily collected from oviposition cages for transfer to food plants in development cages. Cryptic insects can be provided with removable trays of suitable material in which to hide.

References

- Adkisson, P.L., Vanderzant, E.S. and Allison, W.E. 1960. A wheat germ medium for rearing the pink bollworm. Journal of Economic Entomology 53, 759–762.
- Allen, G.E. 1980. Impact of diseases on insects and procedures for detecting and eliminating them from cultures prior to release from biological control. In: Delfosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds, Brisbane, 1980. CSIRO, Melbourne. 221–232.
- Bigler, F. 1989. Quality assessment and control in entomophagous insects used for biological control. Journal of Applied Entomology 108, 390–400.
- Boller, E. 1972. Behavioural aspects of mass-rearing of insects. Entomophaga 17, 9–25.
- Chambers, D.L. 1977. Quality control in mass rearing. Annual Review of Entomology 21, 289–308.
- DeBach, P. 1964. Biological Control of Insect Pests and Weeds. Chapman and Hall, London, 844pp.
- Dunn, P.H. and Andres, L.A. 1980. Entomopathogens associated with insects used for biological control of weeds. In: Delfosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds, Brisbane, 1980. CSIRO, Melbourne. 241–246.
- Etzel, L.K., Lewinson, S.O. and Andres, L.A. 1981. Elimination of *Nosema* in *Galeruca rufa*, a potential biological control agent for field bindweed. Environmental Entomology 10, 143–146.
- Gardiner, L.M. 1970. Rearing wood-boring beetles (Cerambycidae) on artificial diet. Canadian Entomologist 102, 113–117.
- Harley, K.L.S. and Forno, I.W. 1992. Biological Control of Weeds: A Handbook for Practitioners and Students. Inkata, Melbourne and Sydney. 74pp.
- Harley, K.L.S. and Willson, B.W. 1968. Propagation of a cerambycid borer on a meridic diet. Canadian Journal of Zoology 46, 1265–1266.
- Heuttel, M.D. 1976. Monitoring the quality of laboratory-reared insects: a biological and behavioral perspective. Environmental Entomology 5, 807–814.
- Huffaker, C.B. and Messenger, P.S. 1976. Theory and Practice of Biological Control. Academic Press, New York and London. 788pp.
- Leppla, N.C. and Fisher, W.R. 1989. Total quality in insect mass production for insect management. Journal of Applied Entomology 108, 425–261.
- Mackauer, M. 1972. Genetic aspects of insect production. Entomophaga 17, 27-48.
- —1976. Genetic problems in the production of biological control agents. Annual Review of Entomology 21, 369-385.
- —1980. Some aspects of quality and quality control of biological control agents during insectary propagation. In: Delfosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds, Brisbane, 1980. CSIRO, Melbourne. 207–220.
- Marohasy, J. 1994. Biology and host specificity of *Weiseana barkeri* (Col.: Chrysomelidae): a biological control agent for *Acacia nilotica* (Mimosaceae). Entomophaga 39, 335–340.
- McFadyen, P.J. 1983. Host specificity and biology of *Megacyllene mellyi* (Col.: Cerambycidae) introduced into Australia for the biological control of *Baccharis halimifolia* (Compositae). Entomophaga 28, 65–72.

- McMorran, A. 1965. A synthetic diet for the spruce budworm, *Choristoneura fimiferana* (Clem.) (Lepidoptera: Tortricidae). Canadian Entomologist 97, 58–62.
- Shorey, H.H. and Hale, R.L. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. Journal of Economic Entomology 58, 522–524.
- Singh, P. 1977. Artificial Diets for Insects, Mites and Spiders. IFI/Plenum Data Company, New York. 594pp.
- Smith, C.S. and Wilson, C.G. 1995. Effect of an artificial diet on *Carmenta mimosa* Eichlin and Passoa (Lepidoptera: Sesiidae), a biological control agent for *Mimosa pigra* L. in Australia. Journal of the Australian Entomological Society 34, 219–220.
- Tomley, A.J. 1990. *Megacyllene mellyi* — a biological control agent for groundsel bush, *Baccharis halimifolia*, in Queensland. In: Heap, J.W. ed. Proceedings of the 9th Australian Weeds Conference, Adelaide, 1990. Crop Science Society of South Australia, Adelaide. pp 513–515.

Distribution of Agents

Introduction

Once a biocontrol agent has been cleared from quarantine and mass rearing has begun, there will probably be considerable pressure to get the agent widely established as fast as possible, but funds are often limited. Even under these circumstances the agent must be distributed efficiently and given the maximum chance to become established. At the same time we need to think about future monitoring work which, in its most basic form, will measure the success of the release program and the overall progress being made towards achieving control of the weed (Farrell and Lonsdale this volume). Achieving wide distribution of the agent is a key to success in a biological control program (Julien this volume).

Distribution of the agent is simply the process of encouraging its widespread establishment. Two important aspects of the distribution process are selection of release sites and the method of shipment and liberation.

Tony Wright
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Selection of Release Sites

Large scale

On the large scale, decisions about where to make releases may be affected by the following.

The areas that have the most serious problems caused by the weed.

Release location is often determined by land holder demand and polical pressures as well as suitablility of climate and other aboitic considerations and logistics (see below).

The areas which are suitable as nursery sites.

Where possible, early releases should be made at sites which can be used as nursery or reservoir sites for later field-collection of the agent. For example, high priority could be given to starting a nursery site located near the project headquarters. Besides being a place to collect extra insects if required, the site will provide some 'insurance' against accidental loss of the mass-rearing colony and can serve as a demonstration and evaluation site.

The areas which have regional officers or other project collaborators.

For obvious reasons, supply of starter colonies to workers who will be helping with rearing and distribution of the agent is recommended at an early stage of the project. Irrespective of whether collaborators intend rearing the agent at nursery sites or in laboratory cages/containers, they should be

provided with written notes on the agent's biology and rearing, collecting and shipping advice. At this stage of the project, those involved in importing and testing the agent will know far more about the agent and how to manage it than most of those involved in later rearing and distribution. We have a responsibility to ensure all, as far as possible, are equally well informed. Apparently obvious and basic details could be included in the information provided, even to the extent of including basic advice on keeping shipping and distribution records.

The areas which have ecoclimatic conditions best suited to the agent.

Knowledge of the weed's distribution, the weed's preferences, discontinuities in its distribution, and geographic barriers to natural spread of the agent should all be considered when selecting release sites. Information gained by matching climates of the agent's host range and the weed's distribution will also help in making decisions on release priorities. Computer programs such as CLIMEX (Maywald and Sutherst this volume) may be of use here.

The ability of the insect to disperse naturally.

The natural ability of the agent to disperse may help hasten its distribution within a region. Prevailing winds aid flying insects and light-bodied weak fliers.

For floating aquatic weeds, movement of plants by water currents down a river system can help agent dispersal. In this case, initial releases of the agent should therefore be made onto weed infestations as high up the river system as possible.

Small scale

On the small-scale, decisions about site selections for releases may be affected by the following.

Easy and safe access

Personal safety of field workers is an important issue. We rarely forget the hazards to workers operating in politically sensitive areas, however dangers present in other situations (e.g. aquatic weeds in deep water, health hazards from land or water pollution, wildlife) are just as real. Failure to identify dangers may lead to tragic and also legal consequences.

The degree of site security and/or freedom from outside interference

It is almost impossible to guarantee the security of a release site, although some sites are obviously more risky than others. Loss of valuable release sites can result from various human activities including herbicide spraying, mechanical clearing and vandalism. Some of these problems may theoretically be avoided by consulting local governments, land-owners and local workers during formulation of plans or later during project operations.

The quality of plants at the site

In many cases, mass rearing of agents will have occurred on ideal plants grown under sheltered conditions, eg glasshouses, and protected from the rigours of other herbivore species and environmental extremes. Sometimes it is possible to identify areas within a weed population where plants appear significantly healthier. Where this is likely to have resulted from better nutrition of the plant, the agents may have a greater chance of establishing.

How progress of the project is to be monitored.

Within this framework, it is obviously best to be able to control, or at least influence, the decisions about which release sites should have highest priority status.

Packaging, Shipment and Liberation

The life-stage of the agent best suited to shipment depends on:

- the biology of the insect,
- its ability to withstand transportation trauma,
- expected travelling time, and
- whether the shipment is a starter colony or for immediate field release.

During collection and packing of shipments, guard against unwanted inclusions such as other plant or insect species (including parasites/parasitoids), soil, seeds etc. Numbers of insects per container obviously vary from case to case, but as a general rule low numbers per container are safer than high numbers. If the container size restricts the number of adults to be sent per shipment, mated females are more valuable to send and release than males.

Safety and security of the shipment may be improved by attaching warning labels on containers and by providing handling and other information as required. Beware of press-fit lids and stoppers which may be dislodged by rough handling or by changes in air pressure.

Heat is perhaps the biggest danger to insects during shipment. If the shipment is not being hand-carried or it will not always be under reliable supervision, provision of extra insulation for the agents may be necessary.

Consignments of agents sent to non-project personnel (who may not keep good records or provide feed-back information) can be accompanied by a consignment sheet for completion and return to the sender (an example of a consignment sheet follows in Appendix 1.). Extra information should be provided with the consignment as appropriate, including handling and release protocols, instructions on providing extra food for trans-shipment or on changing packing material and safety warnings.

Appendix 1.

Biological Control of (weed name)

Consignment Sheet

Insect sp:..... Life stage:.....

Recipient: .....

Carrier: .....

Date consigned: .....

Date released:..... Released by: .....

Condition of insects/material on arrival:
Any evidence of:

- insect mortality?.....
- deterioration of plant material? .....
- over-heating during/after shipment?) .....

Other comments:

Details of Release Site:

- Site Name: .....
- Type (lake, roadside etc):.....
- Location: .....
- Estimated Size:.....
- Percent covered with (weed name): .....
- Other insect agents present?.....

(On reverse, please sketch site to show point of release.)

PLEASE RETURN THIS FORM TO:

(Contact person and address)

Establishment of Agents

Introduction

To achieve control of a target weed using the classical biological control approach, self-sustaining populations of an agent must become established. We can only be confident an agent has become established after monitoring shows not only the insects liberated have survived, but also new generations have appeared.

Under some conditions there may be a lengthy period before we can be confident of establishment. If liberations are performed in a region with extremes of climate, the agent's population must also have survived the less favourable period before we can be confident of agent establishment.

If parasites or predators adapted to the agent are plentiful in the region, the agent may indeed be reproducing on the weed but actually declining in population, ultimately to the point of local extinction (an example is the Malaysian project against *Mikania micrantha* where establishment failed despite much effort and expenditure, presumably because of predators and/or parasitoids). Goeden and Louda (1976) reviewed biocontrol programs whose outcomes were influenced by natural enemies of agents following releases.

Tony Wright
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

Little can be done about unpredictable misfortunes resulting from chance events. Probably every biocontrol worker has experienced losses of valuable agents, or even losses of entire release sites, by fires, floods, human mischief or a host of other causes. Mostly these are not preventable.

However, there is a great number of unfavourable factors or events which potentially can cause havoc to the efficient running of a biological control project and which are indeed preventable or avoidable.

The following is not intended merely to give examples of what should or should not be done, but to also stimulate workers to consider what conditions and threats may be faced by agents following liberation. By anticipating problems and acting to avoid them or at least minimise their effects, the probability of successful establishment is surely enhanced.

Maximising the Chance of Establishment

Biological data

Availability of biological data on the agent is usually of critical importance when planning releases. For example:

- Biological data can indicate the length of the ovipositional period and at which age maximum oviposition occurs. In general, liberation of young adults is preferable to old adults.

- Young healthy insects reared under ideal conditions should be released. Certain species (some weevils are an example) kept confined in large breeding populations or fed low quality plant material under artificial conditions may enter a dispersal phase in which they cease breeding and develop flight muscles. The consequences of liberating non-breeding or delayed-breeding insects are obvious.
- The agent's climatic preferences may make it necessary to adjust the time of release to match the most favourable season.

Choice of release site

Insects survive and breed best when conditions are most favourable. A high degree of variation in quality of sites or suitability of sites for agent population growth is common. Ecoclimatic considerations may also influence site selection. Remember also that, unlike humans, insects prefer to eat food that is good for them or in some way aids their survival.

To maximise the chances of achieving successful establishment, agents are best liberated at sites where plants are known to possess the preferred quality attributes, or, if the plant quality preferences of the agent are unknown, at sites where plants are likely to possess high diversity in plant quality allowing the agents to select the appropriate resource. Given the unpredictability of establishment, release sites should be spread across a range of factors such as soil types, altitude, climate etc.

It is theoretically possible to manipulate plant quality to favour insect attack but so far little work has been done on this. Plant growth regulators, including auxin-class herbicides, are of possible use here. For example, Oka and Pimental (1976) presented several references which reported increases in pest problems on herbicide-treated crops, probably due to consequential increased nitrogen levels in the plants. Improving quality by fertilising the weed with urea is another example and this was used in a program with *Cyrtobagous salviniae* on *Salvinia molesta* reported by Room and Thomas (1985). Where information is known on the agent's preferences, this should be included in release instructions provided to project collaborators.

Numbers of release sites and insects

The more insects available of a particular agent species, the more freedom we have to choose how many and which insects (e.g. age, sex) to release and how many sites to release them at. There are no hard rules here; these aspects are open to argument and mostly depend on the agent's biology and especially on what is possible and practicable. However, given that few sites may be fully secure from disturbance, it follows that the more release sites available the better.

Regarding insect numbers for release, examples exist where an agent has become established following release of surprisingly few insects. Nevertheless it is generally assumed that releases of large numbers should mean that the population is starting out further along the 'lag phase' of its theoretical sigmoidal population growth curve.

Sometimes limited resources make it impracticable to liberate large numbers of insects, for example where weed plants infested with immature stages of the agent are to be planted into existing weed infestations. If numbers of adults per shipment for release must be limited, remember that gravid females are more valuable for release than males or unmated females. However shipping mixed-sex adults has an advantage in that mating may occur during transit.

On the question of releasing one or several agent species against a target weed, there has been much discussion regarding the advantages or disadvantages

of establishing a complex of agents. Arguments against releasing a complex have been put by workers dealing mainly with biocontrol of insect pests, and Huffaker (1978) saw greater benefits in using a complex of agents against weeds than arthropod pests. I think most weed biocontrol workers support the argument of Hassell (1978) that additional agent species will either coexist with the first agent or replace it and whatever the outcome, the equilibrium density of the host will decrease. The campaign against prickly pear in Australia, where 51 species were introduced although only 5 were effective (Wilson 1960), strongly supports the pro-complex theory.

Field cages

A large variety of field cage designs has been used to aid establishment of agents. Cages act to protect the agent, especially immature stages, from predation, parasitism, and weather and to keep the agent above a critical density by preventing them dispersing naturally.

Monitoring for Establishment

Follow-up visits to release sites are always required if establishment is to be verified. Monitoring of the agent population can give information on predation/parasitism, the number of field generations and the number of seasons survived. The agent's life history gained from biological studies may influence the regularity of visits.

'Housekeeping'

Good record keeping is an important part of efficiently managing a biocontrol project. Besides site card files, computer spread-sheets and photographic files, valuable reference and feedback information can be included in consignment sheets returned by collaborators (Wright this volume).

Routine notification of local authorities, project collaborators (and of course funding bodies) of liberations, establishments and other 'milestone' events in the program may be of great help in avoiding problems and preserving good relationships. As mentioned in the taxonomy section (Sands this volume) plant and insect specimens should be provided to relevant authorities, including local museums.

Additional public relations activities may include producing information leaflets and making media releases.

References

- Goeden, R.D. and Louda, S.M. 1976. Biotic interference with insects imported for weed control. Annual Review of Entomology 21, 325–342.
- Hassell, M.P. 1978. The dynamics of arthropod predator-prey systems. Princeton University Press, Princeton, New Jersey and Guildford. 237pp.
- Huffaker, C.B. 1978. An overview of biological control, with particular commentary on biological weed control. In: Freeman, T.E. ed. Proceedings of the IV International Symposium on Biological Control of Weeds, Florida, 1976. University of Florida, Gainesville. pp 3–12.
- Oka, I.N. and Pimental, D. 1976. Herbicide (2,4-D) increases insect and pathogen pests on corn. Science 193, 239–240.
- Room, P.M. and Thomas, P.A. 1985. Nitrogen and establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. Journal of Applied Ecology 22, 139–156.
- Wilson, F. 1960. A review of the biological control of insects and weeds in Australia and Australian New Guinea. Technical Communication No.1 CIBC, 102pp.

Measuring the Impact of Biological Control Agents on Weeds

Introduction

'Success [in weed biological control] can only be claimed when it is shown through suitable evaluation that the agents have caused a decrease in weed density... or have inhibited the spread of the weed....It is erroneous to equate establishment of agents and demonstrable damage with success because the impact of herbivore damage on the dynamics of plants is often not apparent' (Hoffmann 1990), and may not be significant.

This paper provides guidelines for a carefully planned and executed program necessary to demonstrate the impact of biological control agents.

Why Measure Impact?

To gauge ultimate success or failure

Agencies funding biological control of weeds require and deserve an assessment of the impact of their invested funds in reducing the target weed. In broader terms, critical evaluation of the impact of successful releases (see Julien this volume) provides justification for further investment in biological control of weeds.

Grant Farrell and Mark Lonsdale
CSIRO Entomology
GPO Box 1700, Canberra, ACT 2061
Australia

To decide on future directions of the biological control program

On average it takes ten to twelve years for a successful biological control program to reduce populations of the target weed to levels below the noxious threshold (Lawton 1984). In the meantime it is impossible to know whether any progress is being made at all if no follow up studies are undertaken after the release of control agents. The more that is known about the interactions between control agent and target species, the easier it is to make the right decisions about the future directions of the project.

Both *Samea multiplicalis* and *Cyrtobagous salviniae* were released against *Salvinia molesta* in Australia. The relative ineffectiveness of the latter compared with the former was established by studies conducted in Australia and only *C. salviniae* was recommended for introduction to other countries (Room 1986), a considerable saving in time and effort.

The timing and sequence of release of agents can have an impact on the success of those agents. Studies on the weevils *Neochetina eichhorniae* and *N. bruchi* indicate that better control is achieved when both species are released together rather then individually (Harley 1990).

To develop integrated control strategies

Once the interactions between control agent and weed are understood, possibilities for integrated control strategies may become apparent.

The sesiid *Carmenta mimosa* is a stem-borer of the woody weed *Mimosa pigra*. Originally it was thought to be a promising agent and that it would inflict heavy damage on mature trees, perhaps severely enough to cause death. However, recent observations suggest it may, in the immediate future, be more useful as an agent to use in conjunction with fire to control seedling regrowth (G.S. Farrell, unpublished data).

Outbreaks of St John's wort, *Hypericum perforatum*, are successfully controlled by two chrysomelids (*Chrysolina quadrigemina* and *C. hyperici*) in Australia and California (DeBach 1964). *H. perforatum* growing in shady habitats was found to escape from *Chrysolina* spp. and these habitats provide refugia from which the weed may repeatedly reinvade the rest of its habitat (Lawton 1984). Finding alternative methods to control *H. perforatum* in these shady habitats obviously is important to the overall control of the weed.

To develop the theory and practice of weed biological control

Better biological control can only come from knowing more about why projects succeed or fail. Prediction of success (Julien this volume) and, therefore, prioritisation of potential biological control agents (Marohasy this volume) are notoriously difficult. By and large, the critical dynamic processes that result in control of weeds by herbivores are not understood (Lawton 1984), and there is a paucity of data in this area.

Much of our understanding of insect plant dynamics has come from, and is being driven by, biological control of both weeds and herbivorous insect pests. Studies on the spatial scale of weed control programs are beyond the scope of most individual or academic ecologists, and so offer a unique opportunity for the analysis of insect plant dynamics in what are effectively large-scale ecological experiments.

Phases of Assessment of Control Agent Impact

There are three phases in evaluating the success of a biological control program. First, before release of the agents, the status of the weed should be documented to provide a baseline for later comparison. Second, following release of the agents, establishment and spread of the agent population should be monitored. Third, once establishment has been confirmed, data on populations of the weed and agents should be compared to the pre-release baseline data to assess impact of the agents.

Baseline studies

Baseline measurements of the population density and growth characteristics of the weed are necessary for later quantitative evaluation of the effects of control agents, and to understand the biology and ecology of the weed as a basis for prioritisation of potential biological control agents.

Monitoring establishment and spread

Once an agent has been released, its establishment and spread should be monitored (Wright this volume). Field release sites should be regularly monitored for the presence of the agent, evidence of breeding and changes in population density. Plans for determining spread away from the initial release sites should be made well in advance of their being needed.

Deciding when a released population is established depends very much on the phenology of the species. As a minimum, control agent populations should have passed through several generations and densities should be generally increasing or stable. Establishment of multivoltine species can be claimed if a population has survived and bred at a site for over twelve months. Establishment of univoltine species cannot be claimed until several years after release.

Monitoring rates of spread of agents should be done concurrently with monitoring for establishment. Fixed transects can be useful for initial indications of migration. For species with high dispersal capabilities, or once the immediate available habitat around a release site has been invaded, it is necessary to establish observation sites radiating from the release site. These sites should be visited at regular intervals and inspected for evidence of the agent. The distance of such sites from the release point will depend on the agent species.

Migration will not necessarily be uniform or linear over time. Although some species begin dispersing from the moment of release, others build up to locally high densities before dispersing long distances in search of host plants unaffected by their own species.

Evaluation of impact

Once establishment of populations of a control agent beyond the general area of its release has been confirmed, resources should be directed to measuring impact on the weed.

Studies of the effects of herbivorous insects on plants usually only consider losses to photosynthetic capacity or seed production (Lonsdale et al. 1995). It is one thing to measure the effect of a herbivorous insect on plant performance, but it is another to demonstrate that the herbivory is affecting the plant population dynamics (Crawley 1989). For example, the bud-feeding weevil *Trichapion lativentre* reduced seed production in the weed *Sesbania punicea* by more then 98%, but failed to cause a corresponding decline in the density of mature plants because the seed loss only removed plants that would have died from competition anyway (Hoffmann and Moran 1991). This example demonstrates that simple measures of damage alone may not be sufficient to indicate the success or otherwise of an agent.

The range of approaches that may be taken to determine the impact of biological control agents on the target weed includes experimental manipulation, correlation, or before-and-after photography. The principles of experimental design are applicable to some degree regardless of the approach used.

Principles of Experimental Design

Experiments are used to provide answers to specific questions about nature. These questions are stated as hypotheses. The simplest way to think about hypotheses is as a summary of the experimenter's idea on how they think nature works. Hypotheses are usually stated in terms of the expected effect on one factor of some action or some modification of another factor. This action or modification, carried out in an experiment, is called a treatment. For example, the hypothesis may be that feeding by an insect reduces seed output of a plant. The hypothesis is tested in an experiment in which two or more treatments, for example, various densities of an insect, are assigned at random to experimental units, such as, individual plants. The effects of the treatments (insect densities in the example) on the experimental units (individual plants) are measured (number of seeds per plant counted) and compared, usually statistically. Hurlbert (1984) and Hairston (1989) provide a general discussion of experimentation in ecology. A clear statement of the hypothesis at the outset is invaluable in designing the experiment, analysing the results, and in writing it up, because it guides one through the maze of complications and subsidiary questions that arise in any field research program.

Hypothesis generation

The hypothesis forms the foundation on which the experimental procedure is built. Knowledge of the ecology of the agents and the weed is necessary so that testable hypotheses are formulated, appropriate

treatments are applied to the plants, and that efficacy is measured in such a way as to test the hypothesis. There is no point having a carefully thought out and rigorously executed experimental design if the hypothesis tested was of no value or, worse, not actually testable.

The measure of control agent efficacy must be appropriate for the plant in question. For example, in a woody weed with a seed bank any damage to the plant tissue is probably irrelevant at the level of the weed population unless it impacts on either plant longevity or on seed production or viability. In contrast, for water weeds that mainly reproduce vegetatively, damage to the growing tips of the plant may be critical. As stated previously, effects on population dynamics of the weed population must be assessed rather than simple measures of damage alone.

Allowing for heterogeneity

Environmental variation, which occurs in most habitats, must be taken into account in the experimental design. Under field conditions, it is usually impossible to exert control over the large numbers of independent variables which introduce heterogeneity into the experiment. Such heterogeneity is usually important because researchers are interested in testing the predictive power of the hypotheses on the overall weed population, not just a homogeneous subset of individuals.

Spatial arrangement of the experiment is important. It is not valid to have some of the treatments at one site and the rest somewhere else. Clearly, if treatments are carried out at different sites then it is impossible to know whether any differences are due to the treatments themselves or to the differences between sites.

All treatments should be applied across the same range of heterogeneity. Successful experimentation involves accounting for as much variation as possible, leaving the minimum unexplained. Variation exists in every system, even before we apply treatments, particularly in field experiments. Experimental blocking is a very efficient way of accounting for many kinds of spatial, temporal and operator variation. For example, if you suspect that there is an environmental gradient across the site, (say in slope, hydrology, nutrients, or shade), then blocks should be laid out along the supposed gradient. Even if spatial differences are not obvious, they always exist and it is therefore still useful to set up the experiment in blocks. The blocks should all be large enough to contain at least one repetition of every treatment. Blocks should be as compact (i.e. square) as possible, but if rectangular, the long axis should be at right angles to the gradient to minimise the within-block variation. The plots for the different treatments should be placed as far as possible side by side, along an axis at right angles to the gradient. Treatments can then be allocated at random to plots within each block. Such an experiment, called a randomised block design, can then be analysed by analysis of variance. The effects of environmental heterogeneity can be removed from the overall variation as the block factor when carrying out the analysis (see, e.g. Snedecor and Cochran 1980 Chapter 14).

Blocks can be used to allow for temporal and operator associated variation as well as spatial variation. If all replicates cannot be applied at the same time, complete blocks should be set up together to avoid variation due to time between treatments. If each operator works in the same block on each sample occasion, systematic variation between operators in the way they measure or apply the treatment will be added to differences between blocks rather than differences between treatments.

Within a site, recognition of micro-habitats is important. For example, plants standing in free water will have different properties from those not

submerged. Also, isolated plants have a very different growth habit from plants within a stand. By using blocking, or by pairing plants within a micro-habitat, heterogeneity between treatments is reduced and the experiment can include a representative array of plants on which to test the hypothesis.

If environmental variability is high, the number of replicates (i.e. blocks in a randomised block design) should be increased so that treatment effects will still be detectable against the heterogeneity. It is possible, and very good practice, to determine the minimum number of replicates required for an effective experiment before setting it up. This requires (i) some idea of the variation inherent in the material (the coefficient of variation or CV) and (ii) an idea of how big a difference between means one is interested in detecting. We can then use tables (available in Cochran and Cox 1957), or statistical software (e.g. Sigmastat from Jandel Scientific) that will estimate how many replicates are required to obtain a statistically significant result. As the CV increases, the required number of replicates increases. Similarly, if we need to detect very small differences between treatments, this will also require more replicates. The CV could be estimated from preliminary studies on the organism in question, or from the literature, or from previous experience with similar organisms. The size of the difference we need to detect can be a subjective decision, but is better if arrived at objectively. For example, if there is information on the economic threshold of the weed (i.e. to what density it must be depressed in order for satisfactory control to be achieved), we could use this to establish the size of the difference to be detected. Clearly, to estimate the number of replicates required, we may have to make some assumptions. Provided these assumptions are clearly stated, however, this is far better than simply a stab in the dark that might result in too few replicates or too many being set up. In either case resources are wasted.

Covariance analysis is another technique used to reduce experimental error and increase sensitivity in designed experiments. Essentially, one measures the background variation for the variable in question in order to remove it from the analysis so that one is left only with the difference due to the treatment. For example, individual shrubs vary widely in seed production. If we are to apply an insecticide treatment to twenty shrubs that produce seeds annually, with twenty as controls, a covariance design would involve measuring the seed production for all forty shrubs in the year before the application of the treatment. One then uses the previous year's seed production as a covariate in the analysis. If we do not have the resources to measure this in the year before, we can at least measure the size of the shrub in the year of treatment, because this will explain much of the variation in seed output, irrespective of whether the plant is a control or treatment plant.

There are situations where replication is simply not possible. Perhaps we can only afford one treatment lake to receive biological control agents and one control lake for comparison. In this situation, Hurlbert (1984) recommends that we avoid inferential statistics like ANOVA, *t*-tests and χ^2 (chi-square) tests and instead simply present the means and the variation around the means for the two systems. Inferential statistics are not applicable and would not make the results any clearer. One can merely draw qualified conclusions about the treatment in question, but there is no true replication so no *P* value can be calculated.

Controls for comparison

The use of controls is fundamental to experimental design. Individuals and populations will change during the course of an experiment even in the absence of treatments. Untreated controls allow this background change to be measured and separated from the effects of treatments.

The location of controls is important. Controls should encompass the same range of environmental variation as the treatment sites. Obviously, we should not select low weed density sites to receive the agents and high density sites to be left as controls—we should have some low and high density treatment plots, and some low and high density controls. Furthermore, control and treatment sites should be well interspersed, although care is needed to prevent drift (insecticides) or dispersal (biological control agents) of applied treatments into controls.

Sampling methods

Experimental units are often too large to be measured completely. A sample of part of the experimental unit may be used as the basis for measurement. Care must be taken to ensure that the sample is representative of the whole experimental unit.

Details of how to sample, what to sample and when to sample are very much specific to each weed-control agent combination. For development of sampling techniques for control agents, see Southwood (1978) or other general textbooks on ecological techniques. Preliminary sampling of the weed population and control agent population should be carried out for at least a year and at several sites to determine seasonal and spatial variation in the populations. The preliminary sampling should be carried out as part of pre-release studies of the weed and the study of the establishment and spread of the agent. Information gained from this work can be used to develop the sampling program and contribute to the timing of samples and location of sample sites. Elaborate or overly complicated sampling designs should be avoided as the time and labour required may conflict with the need to sample large numbers of sites over a wide area.

Quadrats of varying sizes are used for counting plants or insects per unit of ground area, or for measuring biomass. The choice of quadrat size depends on the vegetation to be studied and the question to be asked, but a good rule of thumb is that, for square quadrats, the length of a side should not be less than the height of the plant. See Krebs (1989, pp. 64–72) for a discussion of quadrat size.

Litter trays (Farrell et al. 1992) provide an estimate of production per unit area of seed output, leaf fall or biomass. They can be emptied as often as required; more frequent emptying gives a more detailed time course (e.g. Lonsdale 1988).

Surface samples using vacuum cleaners and soil cores using soil augers allow one to estimate the soil seed population. Monitoring soil seed banks can be very time consuming, particularly if the soil is heavy, and it is important to ask whether it is actually necessary to study seeds in the soil at all before embarking on a major project. It may be that a sufficient understanding of the plant's demography can be obtained by simply monitoring input (seed fall) and output (seedling emergence), both of which involve rather less earth-moving than do soil cores. Such information can be made more complete by using seed bags for burying known numbers of seeds in the soil to investigate their longevity. Bags are retrieved at intervals and the seeds taken out for germination tests. A rate of decline can then be calculated by regression techniques (see e.g. Lonsdale et al. 1988).

Using an appropriate statistical test on the data

During design of an experiment books on statistical design or, better still, a statistician should be consulted to ensure that statistical tests can be applied validly to the resulting data to test the hypothesis.

Experimental Manipulation

This approach is by far the most rigorous for exploring natural phenomena and is the preferred method of analysis for investigating the impact of biological control agents.

The advantage of experimental manipulation is that:

- it allows rigorous statistical analysis, and so yields more reliable information than other methods.

The disadvantage of experimental manipulation is that:

- costs in time and resources are higher than for other methods.

However, against this must be weighed the long-term costs, implicit in other approaches, of never having a definitive understanding of the workings of the system under study.

The basic requirement is to be able to apply biological control agents to weeds in such a way that their effects can be quantified. At the simplest level the hypothesis is that biological control agents will reduce the impact of the weed. The rates of change of population density, or in growth rate and/or survival of the weed, are compared between the treatment, in which the control agent is present on the weed, and the control, from which the control agent is absent. There are three basic methods to ensure that control agents are excluded from the experimental units to be used as controls: exclusion by insecticides or cages, or controlled releases.

Exclusion by insecticides

Insects can be excluded from control plants by spraying them at regular intervals with insecticide or by sprinkling granular formulations of systemic insecticides on the soil.

The advantages of exclusion by insecticides are that:

- controls and treatments can be at the same site;
- it can be used when the biological control agent is already widespread.

The disadvantages of exclusion by insecticides are that:

- achieving reliable and uniform exclusion is difficult;
- spray drift or movement of granules by animals or water may affect nearby controls;
- some insecticides have detrimental side effects on the plants;
- all insects feeding on the weed, not just the biological control agent, are affected;
- uptake of residues by commercial plants or livestock may make them unsuitable for markets if acceptable limits for those residues have not been approved.

To allow for the first and second possibilities, densities of biological control agents on treated and untreated plants must be sampled.

The approach is perhaps best suited to small-scale experiments because of drift and residue problems. This approach is not widely used because of the practical difficulties, but should be attempted more often.

All recommended precautions, such as wearing protective clothing and a mask, washing hands after handling, etc. should be followed when using insecticides.

An insecticidal exclusion experiment was used by Lonsdale et al. (1995) to investigate the effects of a biological control agent, the chrysomelid *Calligrapha pantherina,* on the malvaceous annual tropical weed *Sida acuta.* Twenty permanent 1 m^2 quadrats were marked out amongst healthy flowering stands of *S. acuta* in a heavily infested pasture. Half of the quadrats were randomly allocated an application of the systemic, granular insecticide carbofuran which was applied to the soil. Beetles were released at one end of the paddock and the resulting population allowed to expand over the quadrats. At the end of

flowering all vegetation in the quadrats was clipped at ground level and the *S. acuta* separated out. A number of plant demographic characters were measured from these samples. The most dramatic impact on the plant was the reduction in seed production caused by the action of the beetle (Fig. 1).

Exclusion by cages

Insects can be excluded from control plants by caging them before the agent establishes on those plants, or by spraying them with a non-residual insecticide after they have been caged. The reverse approach may also be made of adding insects to some cages but not to control cages. Cages are usually made with a metal or timber frame covered by gauze with apertures small enough to prevent entry by the agent. Particular attention must be paid to sealing joins in the gauze, the junction between the cage base and the soil, and the entry point allowing access for sampling.

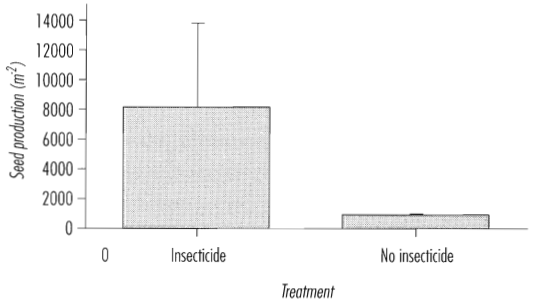


Figure 1. Use of insecticide exclusion in an experiment to measure impact of a control agent. Effect of **Calligrapha pantherina** *on* **Sida acuta** *populations in 1990. Values are geometric means with 95% confidence limits (see Lonsdale et al. 1995)*

The advantages of exclusion by cages are that:

- controls and treatments can be at the same site;
- it can be used when the biological control agent is already widespread.

The disadvantages of exclusion by cages are that:

- cages are susceptible to damage by wind, livestock and vandals;
- cages can significantly modify plant growth;
- insect behaviour and population growth can be significantly altered by confinement in the cage, and protection from predators, parasitoids and weather;
- experiments are restricted to the spatial scale of the cages.

Controlled releases

Release of agents at some sites, but not others, allows the impact of the agents to be assessed by comparing performance of plants at the release and no-release (control) sites.

The advantages of controlled releases are that:

- results may be applicable at a landscape scale.

The disadvantages of controlled releases are that:

- rapid migration by the agents may result in invasion of controls;
- geographical constraints can make statistically valid allocation of release and control sites extremely difficult, if not impossible.

Political support for this method may be necessary to withstand pressures for early release of the agents at all sites, including the control areas.

Correlation

At its simplest, correlation involves collecting data on insect density and some measure of insect impact on the weed. This is done at several sites where insect density varies by at least an order of magnitude. The data are then tested by correlation analysis to see if there is any relationship between changes in insect density and the measure of impact. Where the relationship is significant, a regression line may be fitted so that the effect may be quantified. By applying the fundamentals of experimental design to setting up the sites, and if pre-release studies are used to predict the types of impact to look for, clear results can be obtained. However, the method is still reliant on correlation, so a causal relationship cannot be strongly claimed for any relationship observed. Alternative explanations of the observed phenomena, other than effects of the biological control agent on the weed, are possible. This does not mean that conclusions based on the method are wrong, just that there is little assurance that they are right (Hairston 1989).

Correlation works best when permanent sites are established and trends over time can then be studied. Site selection should be approached with the same rigour as if designing an experiment. The number of sites and their geographical distribution will be determined by the characteristics of the system under study. The aim should be to get as representative a sample of weed habitats as possible and, importantly, a range of control agent densities.

The advantages of correlation are that it:

- avoids difficulties of exclusion experiments;
- can be used at scales from fine to large.

The disadvantages of correlation are that:

- it is not rigorous—correlation does not necessarily imply causation;
- densities of agents at a site may vary over the period of measurement, so requiring decisions about what density should be used in the analysis.

Correlation was used to determine the impact of the tip-boring moth *Neurostrota gunniella* on the weed *Mimosa pigra* in the north of Australia. Fifteen sites were established on the lower Adelaide River flood plain in the Northern Territory. Sites were a minimum of 500 m apart and clusters of sites were up to several km apart. At each site 20 litter trays were erected to measure the seed output of mimosa. The number and design of these trays had been determined by previous work (Lonsdale 1988 and unpublished data, see Farrell et al. 1992 for tray design). Trays were placed out in November before seeding and collected around the end of seeding in July. At each site insect density was measured by randomly collecting 100 branch tips, 50 cm long, from trees over and near the litter trays. A preliminary sampling program had established that the later instar larvae of *N. gunniella* do the majority of the damage to the plant, and these larvae occur in the upper 20–50 cm of the branches. Branch samples were returned to the laboratory where they were dissected and the number of larvae counted. Larval density per site was then plotted against seed density. Figure 2 shows the data for the first year of the program. Analysis of the data showed a negative correlation between larval density and seed density. At the highest densities, *N. gunniella* apparently suppressed seed production by up to 60%. This was not in itself sufficient to impact on the demographics of the weed, however, and no changes in plant density occurred.

Before-and-after Photography

If resources for detailed, long-term monitoring are not available then a simple alternative is to use a series of either true colour or infrared colour photographs taken routinely from a number of fixed points to track changes in weed density.

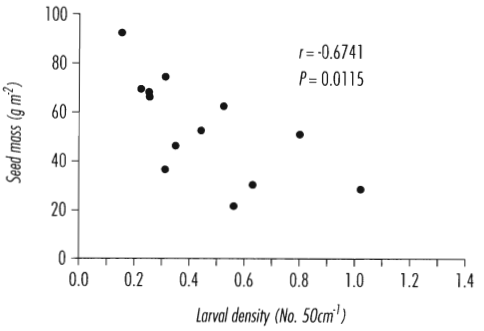


Figure 2. Use of correlation to measure impact of a control agent. Relationship between larval density of **Neurostrota gunniella** *and seed output of* **Mimosa pigra.** *on the Adelaide River, northern Australia in the 1990/91 wet season.*

As with correlation the application of basic experimental design principles greatly improves accuracy and reliability.

Sites should be chosen to provide the best chance of documenting changes in the weed density or cover. Photo points must be marked exactly and clearly and be easily found even after several years. Natural landmarks should be included within the photo to aid accuracy. A copy of the initial photo should always be carried to each site for reference. If funds are available and the service regular enough, aerial photographs can provide very accurate and reproducible images for this type of monitoring. A number of different film types can be used, but one advantage of colour infrared images is that stressed and dying vegetation appears as different tones to healthy vegetation.

Frequency of photographs should be related to the seasonality of damage caused by the control agent. For annual weeds a single image per site per year may suffice. For perennial plants or in tropical environments a much more frequent rate of photographs may be needed.

The advantages of before-and-after photography are that it:

- is cheap;
- is quick;
- can be used at fine and large scales;
- can provide strong evidence for non-scientists of the impact of control agents on the target weed.

The disadvantages of the method are that it:

- does not provide data for quantitative analysis, although this could be partly remedied, e.g. by using photos to estimate cover value;
- is insensitive to small changes in weed density;
- does not provide information on the control agent, although this can be overcome by combining with ground monitoring of the agent;
- fails to provide any explanation of why or how an agent has succeeded or failed.

Before-and-after photographs for biological control of cactus, alligator weed and salvinia in Julien (this volume) show that this technique clearly demonstrates the impact of successful biological control.

Modelling

Models are a statement in precise mathematical terms of an hypothesis about the way a system functions. The hypothesis can be tested, or validated, by comparing model predictions against observations in the field under the same set of conditions as those specified in the model. Validation of the model under

a range of conditions increases confidence that the hypothesised mechanisms described in the model are correct. The validation procedure is not proof of a causal relationship, i.e. that the mechanisms in the model are the same as those in nature, but as its predictions are corroborated under differing circumstances, it becomes more and more probable that we have understood the system through our model. A very powerful approach is to use modelling in conjunction with experimentation. The model provides a machine for hypothesis generation, and a framework for hypothesis testing, that allows the experimental work to be logically structured.

It has been said that a model should be as simple as possible, but not too simple. We can start with something simple and refine it or make it more complex as evidence of its inadequacies become apparent. A model may be a complex mathematical structure requiring a computer to run it, or as little as a set of related regression curves, or even simply a set of verbal rules which lead to a range of predictions. It is never too soon to formalise what we know, or what we think we know. A model can be used to predict the likely impact of biological control agents and other control measures on the weed population under various scenarios. By rapidly testing a wide range of scenarios or hypotheses about the way in which the weed population and biological control agents could interact, the critical aspects of these interactions that are likely to determine the success or failure of the project can be identified for field testing and observation.

A number of general models for different types of plant populations have been developed; for example the Watkinson model for annual plants (Watkinson 1980), and the Leslie matrix models (Begon and Mortimer 1986; Leslie 1945) for plants with age specific fecundity and mortality (i.e. perennials). These basic models can serve as a starting point for development of a model for a particular species; for example, see Watkinson et al. 1989; Hoffmann 1990 and Lonsdale et al. 1995.

Knowledge of the biology of the biological control agent and how control agents impact on the weed is required; the better the knowledge of the demographics of the weed the better the model. Such information can be gathered during initial laboratory and field testing in the country of collection, and in laboratory studies while the potential agent is being assessed for suitability to release. It may be necessary to undertake small-scale experiments in the laboratory or in the field after initial release of the agent to obtain the information required.

Advantages of the use of modelling are that it can:

- be used to make predictions far into the future;
- predict when the maximum impact of the biological control agent will be reached;
- be used at scales from fine to large, depending on the model.

Disadvantages of the use of modelling are that it:

- is not as rigorous as experimentation;
- may require computer skills;
- may require suitable computer equipment;
- requires at least some plant data;
- can be susceptible to errors in specification of parameters and functions, and in assumptions which may or may not be stated.

A matrix model that incorporates density-dependent fecundity, survival and growth has been developed for *Mimosa pigra* (M. Lonsdale and G.S. Farrell unpublished data). The model has been used to

predict what happens to *M. pigra*, a weed with a large seed bank, when canopy plants are killed but the seed bank is left untouched. *M. pigra* has a seed bank in the order of 10^4 seeds m^{-2} and adult stand densities around 1 plant m^{-2}. Figure 3 shows the result of model simulation of canopy removal, but with no impact on the seed bank. The role the seed bank plays in the weediness of this plant is apparent. Within two years a dense thicket has redeveloped and equilibrium has been reached within 4–5 years.

What happens if reductions to seed output are also modelled? An order of magnitude change to seed production reduces the seed bank, but only by a third. Reductions of seed output to one thousandth of the normal amount results in extinction. But with reduction even down to 0.003 of the normal output, mature stand density is still within the range seen in normal thickets and there is enough plasticity in plant growth form for mature plants to maintain a closed thicket down to around 0.1 plants m^{-2}. Massive reductions in seed output are required to impact on the weed.

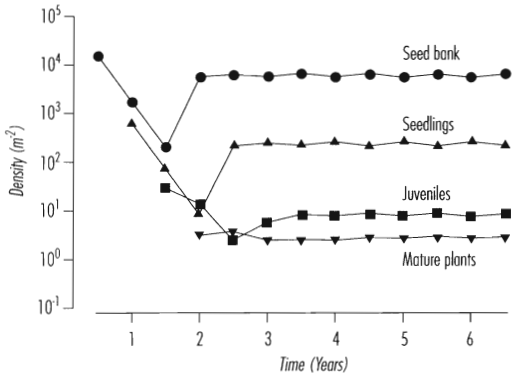


Figure 3. Recovery of a **Mimosa pigra** *population after a major disturbance has killed the mature stand (year = 0). At first, the seed bank becomes depleted as seeds germinate, but a proportion of these survive through the seedling stage to produce seeds as juveniles and adults. Within 2–3 years, a closed stand has returned.*

Conclusions

Monitoring weed populations and the impact of biological control agents on those populations is an essential component of a biological control program.

Data from monitoring should be an integral part of any particular program by guiding prioritisation of potential agents, and guiding decisions on further releases of the same agent or the need for alternative agents or alternative control measures. In the longer term, monitoring provides a basis for evaluating success or failure and possible explanations for those outcomes. Data demonstrating success is necessary to convince governments and funding agencies that biological control is a valid approach to weed control, and that funding should be made available for control of other weeds.

Within the resources available, biological control workers should be as rigorous and quantitative (i.e. scientific) as possible. Some workers have described biological control of weeds as more a craft than a science. While this seems a harsh judgement, biological control must certainly become more exacting if it is to mature as a science. Further data from rigorous case studies will form a basis for further development of the theoretical basis for biological control of weeds.

References

- Begon, M. and Mortimer, M. 1986. Population Ecology. A Unified Study of Animals and Plants. 2nd Ed. Blackwell Scientific Publications, London. 220pp.
- Cochran, W.G. and Cox, G.M. 1957. Experimental Designs. Wiley, New York, 611p.
- Crawley, M.J. 1989. Insect herbivores and plant population dynamics. Annual Review of Entomology 34, 531–564.
- DeBach, P. 1964. Biological Control of Insect Pests and Weeds. Reinhold, New York. 844pp.
- Farrell, G.S., Wilson, C.G. and Napompeth, B. 1992. Monitoring the biological control of *Mimosa pigra*. In: Harley, K.L.S. ed. A Guide to the Management of *Mimosa pigra*. CSIRO, Canberra. pp 63–83.
- Harley, K.L.S. 1990. The role of biological control in the management of water hyacinth, *Eichhornia crassipes*. Biocontrol News and Information 11, 11–22.
- Hairston, N.G. Sr. 1989. Ecological Experiments Purpose, Design and Execution. Cambridge Studies in Ecology. Cambridge University Press, Cambridge. 370pp.
- Hoffmann, J.H. 1990. Interactions between three weevil species in the biocontrol of *Sesbania punicea* (Fabaceae): the role of simulation models in evaluation. Agriculture, Ecosystems and Environment 32, 77–87.
- Hoffmann, J.H. and Moran, V.C. 1991. Biocontrol of a perennial legume, *Sesbania punicea* using a florivorous weevil, *Trichapion lativentre*: weed population dynamics with a scarcity of seeds. Oecologia 88, 574–576.
- Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54, 187–211.
- Krebs, C.J. 1989. Ecological Methodology. Harper Collins, New York. 654pp.
- Lawton, J.H. 1984. Ecological theory and choice of biological control agents. In: Delfosse, E.S. ed. Proceedings of the VI International Symposium on Biological Control of Weeds, Vancouver, 1984, Canadian Government Publication Centre, Ottawa. 13–26.
- Leslie, P.H. 1945. On the use of matrices in certain population mathematics. Biometrica 33, 183–212.
- Lonsdale, W.M. 1988. Litterfall in an Australian population of *Mimosa pigra*, an invasive tropical shrub. Journal of Tropical Ecology 4, 381–392.
- Lonsdale, W.M., Farrell, G. and Wilson, C.G. 1995. Biological control of a tropical weed: a population model and experiment for *Sida acuta*. Journal of Applied Ecology 32, 391–399.
- Lonsdale, W.M., Harley, K.L.S. and Gillett, J.D. 1988. Seed bank dynamics in *Mimosa pigra*, an invasive tropical shrub. Journal of Applied Ecology 25, 963–976.

- Room, PM. 1986 Biological control is solving the world's *Salvinia molesta* problems. In: 7th International Symposium on Aquatic Weeds, Loughborough, 1986, European Weeds Research Society, Loughborough. 271–276.
- Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 7th Edn. Iowa Sate University Press, Ames, USA. 506pp.
- Southwood, T.R.E. 1978. Ecological Methods. 2nd Edn. Chapman and Hall. London. 524pp.
- Watkinson, A.R. 1980. Density-dependence in single species populations of plants. Journal of Theoretical Biology 84, 345–357.
- Watkinson, A.R., Lonsdale, W.M. and Andrew, M.H. 1989. Modelling the population dynamics of an annual plant *Sorghum intrans* in the wet-dry tropics. Journal of Ecology 77, 162–181.

Plant Pathogens as Classical Biological Control Agents

Introduction

Plant pathogens are used in non-classical inundative releases of mycoherbicides, as discussed by Auld (this volume), and in classical biological control of weeds, as discussed in this paper.

Of all pathogens, fungi have received most attention for the biological control of weeds because they are the most common class of pathogens attacking higher plants and are the easiest pathogens on which to work. Of the 80 or so species or strains of pathogens being studied as potential weed biological control agents in the early 1980s, about 70 were fungi and the remainder were nematodes, viruses or bacteria (Templeton 1982). Of the 67 projects involving fungi, 38 involved Deuteromycetes, mainly for inundative release, 18 involved the Basidiomycetes, seven involved Ascomycetes, and four involved Phycomycetes (Templeton 1982). The rust fungi (Basidiomycetes) have been the most common candidates for classical biological control, perhaps because of their high degree of host specificity. Pathogenic fungi deliberately introduced into various countries as classical biological control agents of weeds are listed in Table 1

Allan Tomley
Alan Fletcher Research Station
Queensland Department of Natural Resources
PO Box 36, Sherwood, QLD 4075
Australia

Procedures For Pathogens in Classical Biological Control of Weeds

Full-scale testing of exotic pathogens in Australia has not been allowed. To avoid the risk of escape of potentially harmful pathogens from quarantine, all testing has been done off-shore. Three laboratories that specialise in this work are:

- CSIRO Montpellier, France,
- CAB International Institute of Biological Control, United Kingdom, and
- IFAS Plant Pathology Department, University of Florida, USA.

The process for use of pathogens in classical biological control of weeds is similar to that for insects (Forno this volume). However, there are differences in host testing procedures and in information required on the taxonomy and biology. The four critical areas to consider when assessing a candidate pathogen are:

- taxonomy,
- life-cycle,
- pathogenicity testing, and
- host specificity.

Taxonomy

The taxonomy of fungi is not always clear, so taxonomic studies may be necessary. Specimens of potential biological control agents should be lodged

Table 1. Pathogenic fungi introduced to various countries as classical biological control agents of weeds (based partly on Julien 1992).

Pathogen	Target weed	Target country	Year of introduction
Colletotrichum gloeosposoides	*Clidemia hirta*	Hawaii	1986
Diabole cubensis	*Mimosa pigra*	Australia	1995
Entyloma compositarum	*Ageratina riparia*	South Africa	1989
Maravalia cryptostegiae	*Cryptostegia grandiflora*	Australia	1993
Phaeoramularia sp.	*Ageratina adenophora*	South Africa	1988
Phragmidium violacaum	*Rubus procerus*	Chile	1973
		Australia	1991
Puccinia abrupta var. *partheniicola*	*Parthenium hysterophorus*	Australia	1991
Puccinia carduorum	*Carduus tenuiflorus*	USA	1987
Puccinia chondrillina	*Chondrilla juncea*	Australia	1991
Puccinia evadens	*Baccharis halimifolia*	Australia	1996
Sphaerulina mimosae-pigrae	*Mimosa pigra*	Australia	1994
Uromyces galegae	*Galega officinalis*	Chile	1973
Uromyces heliotropii	*Heliotropium europaeum*	Australia	1992
Uromycladium tepperanium	*Acacia saligna*	South Africa	1987

with a reliable herbarium for identification, e.g. the CAB International Institute of Mycology in the United Kingdom.

Many fungi are pleomorphic, that is a species may produce several spore types which may be present at different times. The spores can be the result of sexual or asexual propagation. The state characterised by sexual spores is called the perfect state or teleomorph, and the state characterised by the asexual spores is called the imperfect state or anamorph. Under the International Code of Botanical Nomenclature, it is permissible to treat each of the states as separate species. However, once it has been established that both states are of the one fungus, the name accepted for the perfect state (teleomorph) takes precedence (Hawksworth et al. 1983).

Life-cycles

The full life-cycle of the candidate pathogen should be determined so that the host of each spore type can be accounted for. If there are missing links, these need to be found, or a sound theory explaining why various stages are not present needs to be formulated.

The rust fungi can have up to five different spore stages (Fig. 1). An individual life-cycle may have all or only some of these. For example, the rust

Phragmidium violaceaum has all spore forms, but *Puccinia xanthii* has only teliospores and basidiospores.

While some rusts do have a full life-cycle, all stages may not be present in the field. Only the uredinial and telial stages of *Puccinia abrupta* var. *partheniicola* have been found in the field in Mexico. While the teliospores are functional, germination has only been observed in the laboratory after dormancy had been broken by chemical treatment. The rust appears to cycle in the field by the urediniospore stage only. These spores have the ability to remain dormant over winter while retaining their viability.

Pathogenicity testing

Pathogenicity tests are aimed at selecting the most effective strain of the pathogen for the biotype(s) of the target weed and the environmental conditions under which the weed grows. Specimens of the target weed, grown from seed collected from the target area, are inoculated under the target environmental conditions with the strains of the candidate pathogen that have been collected from different parts of its native range. Pathogenicity is then assessed from symptoms exhibited by the target weed and microscopic examination as for host specificity testing.

Host specificity

As for insects (Heard this volume), preliminary information about the host specificity of a potential agent pathogen in its country of origin might be obtained by searching the literature, particularly crop protection literature, checking herbarium collections and records, and checking with workers in agriculture to determine whether the pathogen is a known pest of crops.

In the absence of other preliminary information, host range tests against a small selection of plants, usually closely related to the target weed, should be done in the country of origin. This will determine whether further, more detailed, testing is warranted.

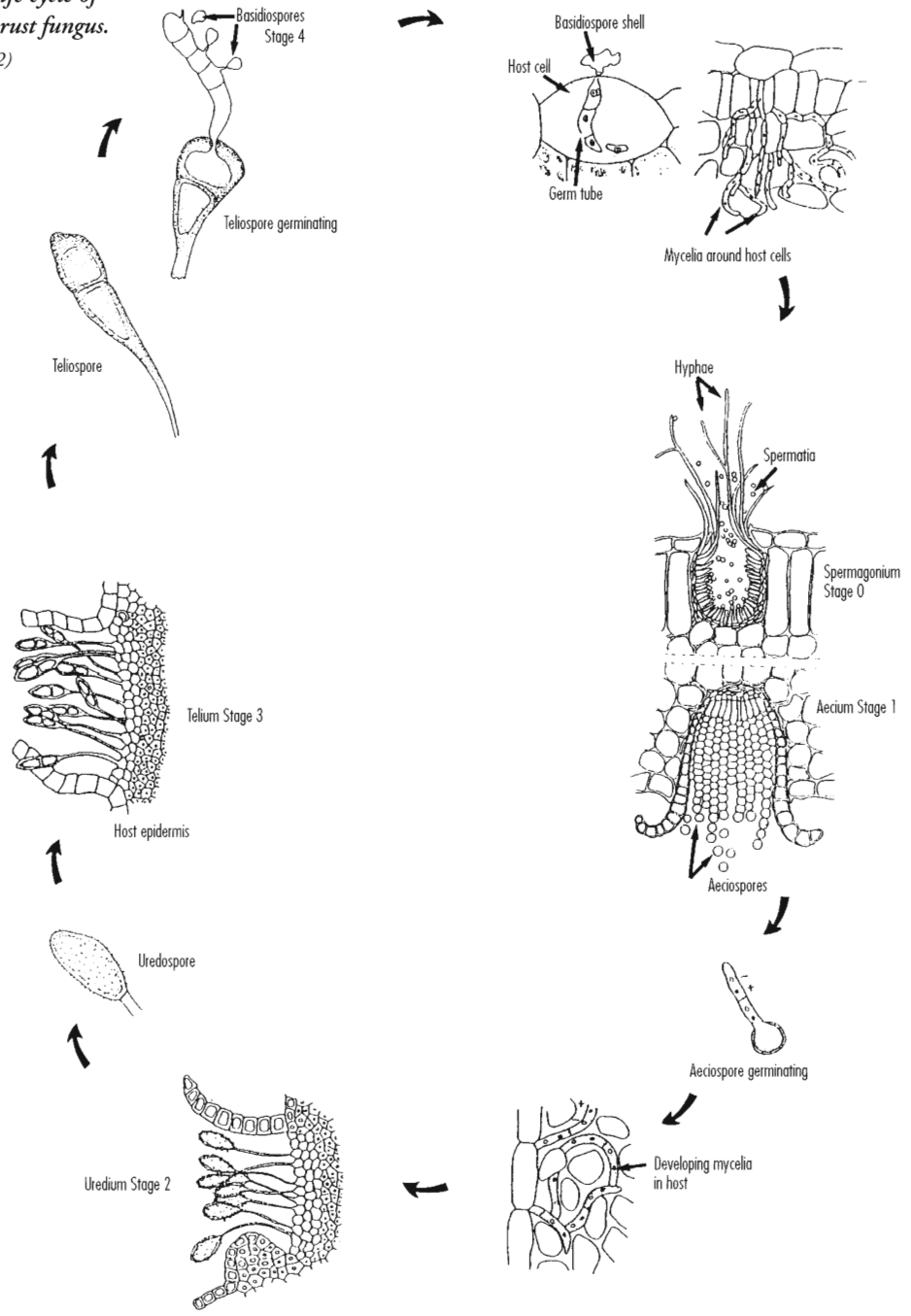
Supply of test plants

Since testing for Australian weeds is carried out overseas, it is necessary to provide the laboratory carrying out the work with test plants. Where possible test plant seed is sent to the country where the testing is to be carried out. This is the easiest and cheapest method as there are usually fewer problems with quarantine requirements. However, in some cases it is necessary to grow the plants before despatch to the testing agency. In this case, the plants must be able to withstand reasonably rough handling, including being turned upside down, and exposure to extremes of temperature. A packaging method for sending plants overseas which has proven to be reliable involves sealing each plant in its own container which is lined with absorbent paper to prevent sweating and breakdown of the foliage in transit, and tightly packing the individual containers into a polystyrene box which is protected by an outer layer of strawboard.

Methodology

Host specificity testing involves assessing the response to infection by the test plants at the cellular level. Plants are inoculated in batches of four or five species along with the target weed as a control. Inoculation of each test species should be replicated three or four times. Each replicate is valid only if infection of the control plant is normal. The plants should be inoculated and incubated under ideal conditions for the development of the particular pathogen, and maintained for twice the length of the latent period for the pathogen on its natural host to allow complete development, eg the production of urediniospores. Pathogen development should be observed both macroscopically and microscopically. In the latter case, sample sections of the leaves are examined using techniques such as whole leaf clearing and staining technique (Bruzzese and Hasan 1983), and scanning

Figure 1. Stages in the life cycle of amacro-cyclic rust fungus.
(Alexopoulis 1962)



electromicrographs. Processes examined include: the fate of the spores on the leaf surface, development of infection hyphae, appressoria, penetrant hyphae and haustoria and reaction of the test plant at both organ and cell level, for example, deposition of callous tissue, necrosis of cells to form a barrier, presence of polyphenols, chlorosis and leaf abnormalities (tumefactions).

The methods used for host specificity testing of a rust for rubber vine (H. Evans pers. comm. 1992) are summarised below as an example of appropriate methods for pathogens.

Batches of 4–5 test species were screened at any one time. Three to four plants were included for each species and only vigorously growing (flushing) plants were used. A range of leaf types (young to senescent) were inoculated by brushing their lower leaf surfaces with a dense urediniospore suspension ('saturation' inoculum, ca 5×10^6 spores/mL) in sterile distilled water and 0.01% Tween 20, using a fine camel hair brush. In the case of aquatic plants (with stomata only on the upper leaf surface) and leafless, fleshy asclepiads, the sites of inoculation were modified accordingly.

Rubber vine plants (*C. grandiflora, C. madagascariensis* var. *madagascariensis* and var. *glaberrima*) inoculated with a spore concentration of 1.5×10^6 to avoid defoliation, were used as controls for each test. Plants were maintained for 24 hours at previously determined optimum conditions for rust infection (ca. 23°C, 100% RH). Inoculated plants were then transferred to a greenhouse chamber at 25°C and 50–60% RH during 12-hours light, and 20°C and 60–80% RH during the night.

Plants were observed over a three-week period, i.e. more than double the latent period for sporulation of the rust on control rubber vine plants. Samples of all inoculated leaves were removed at 10 and 21 days for clearing and staining (Bruzzese and Hasan 1983).

Samples from fleshy leaves and stems were cut longitudinally to facilitate subsequent microscopic examination of the inoculated area. In addition to the light microscope examination, all inoculated material was inspected for external symptoms of infection using a stereoscopic microscope.

Each test species was screened on at least two separate occasions, and any plant showing an unusual macro- or micro-reaction to the rust was investigated further. Symptomatology was assessed according to 15 categories (Table 2) and the susceptibility to infection of each test species was rated based on systems devised by Kochman and Goulter (1983) and Bruzzese and Hasan (1983) (Table 3).

Assessment categories within any one test species were, for the most part, consistent. However, variable reactions occurred occasionally between replications, although most frequently within the same treatment. Leaf age was the main factor governing variation in susceptibility ratings and the full range of reactions are shown in Table 3. Weak or non-dominant reactions are represented by parentheses.

Results of the host range tests for plants within the subfamilies Periplocoideae and Secamonidea are shown in Table 4 as examples.

All species and varieties within the genus *Cryptostegia* were highly susceptible to infection by *Maravalia cryptostegiae*. The only other species exhibiting susceptibility to *M. cryptostegiae* were the Madagascan *Gonocrypta grevei* and the Australian *Cryptolepis grayi*. Further testing of *M. cryptostegiae* types against *G. grevei* indicated that there are at least two distinct physiological races or pathotypes of *M. cryptostegiae*, one adapted to *Cryptostegia*, the other to *Gonocrypta*. *Cryptolepis grayi* showed varying levels of susceptibility to the rust, ranging from resistant to moderately susceptible, and the appearance of fertile pustules on one plant of the first test run warranted further

Table 2. Assessment categories for macro-and microsymptoms of pathogen infection.

Category	Symptoms
0	spore lysis, low (<10%) or no germination
1	spore germination (>20%)
2	abnormal germ-tubes
3	abnormal appressorial development, invariably non-stomatal
4	normal appressional development, invariably over stomata
5	collapsed appressoria, no penetration
6	penetrant hypha with or without evident substomatal vesicle
7	necrosis of penetrant hypha, heavy staining (polyphenol) around and beneath stomata
8	short internal hyphae only, no haustorial mother cells/haustoria
9	collapsed or necrosed internal hyphae, callose or polyphenols present
10	longer internal hyphae, haustorial mother cells and haustoria
11	hyphal collapse, host cell plasmolysis and/or callosed haustoria
12	extensive internal hyphal network, initiation of sorus formation
13	external symptoms; chlorosis or reddening; leaf abnormalities (tumefactions)
14	restricted sporulation (<1 pustule/cm^2)
15	abundant sporulation (>15 pustules/cm^2)

investigation. Fertile pustules were observed in 3 of 14 plants in further tests, despite development of host cell plasmolysis and haustorial inhibition which restricted development of sporogenous tissues by the rust. Results of the screening suggest that *Cryptolepis*, like *Gonocrypta*, is a generic host of *M. cryptostegiae*. Decisions based on these tests are discussed by McFadyen and Heard (this volume).

Importation

Once approval to import the pathogen is given, it is transported in a double metal container. Under normal circumstances, prior to release in the field, the pathogen is reared through one or more generations in quarantine, in the recipient country, to make sure that it is not contaminated with other fungi, particularly parasites, such as *Darluca* sp. In some circumstances, this step may be waived if the exporting organisation guarantees that the culture is pure.

Mass Production of Inoculum

Large amounts of spores are produced, using a suitable culture technique, for release in the field. The technique may involve culture on the weed host or

Table 3. Susceptibility ratings used for assessing the reactions of rubber vine and other test plants to the rubber vine rust

Score	Rating	Macro/microsymptoms
0	Immune (I)	No visible symptoms; no stomatal penetration
1	Highly resistant (HR)	Visible symptoms: chlorosis, flecking or general discoloration; hypersensitive reaction at the stomatal or substomatal level
2	Highly resistant	Development of internal hyphae but restricted by production of callose or polyphenols
3	Highly resistant	Internal hyphae with more extensive branching producing haustorial mother cells but aborted at cellular level
4	Highly resistant	Development of hyphal network; haustoria abundant but invariably non-functional (collapsed or callose ring), with or without host cell plasmolysis. No visible symptoms
5	Resistant (R)	Hyphal network extensive; initiation of sori, non-eruptive or eruptive and appearing as swellings or blisters on leaf surface, abortive, no sporulation. Host cell plasmolysis and/or haustorial collapse. Macrosymptoms generally present: chlorotic spots
6	Resistant	Eruptive sori, usually small in size; sporulation restricted (few pustules/leaf) and delayed; evidence of mainly collapsed-callosed haustoria. Macrosymptoms generally present: widespread chlorosis, leaf distortion
7	Partially resistant (moderately susceptible) (PR)	As above, but pustules larger and more abundant but still less than 1/cm^2
8	Highly susceptible (HS)	Numerous pustules (>15/cm^2), abundant sporulation; majority of haustoria healthy. Typically chlorotic then necrotic leaves; but premature leaf fall not evident
9	Highly susceptible	As above, but premature leaf fall common; with or without chlorosis or reddening (anthocyanin production)

use of artificial media (Auld this volume). Depending on circumstance these spores might be used in the fresh state or collected and stored for a period prior to release. Drying and refrigeration at 4°C or in liquid nitrogen is a suitable storage method.

Field Release

For successful establishment in the field, the requirements of the particular pathogen with respect to temperature, humidity/dew period and other environmental factors must be considered. Infection of plants may not be achieved if environmental conditions at the time of release are not suitable.

Table 4. Symptoms and susceptibility ratings of test plants within the subfamilies Periplocoideae and Secamonideae

Taxa	Macro/micro symptoms																Ratings
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Subfamily Periplocoideae																	
Cryptostegia grandiflora	-	+	-	-	+	-	+	-	-	-	+	-	+	+	-	+	9 HS
C. madagascariensis																	
var. *madagascariensis*	-	+	-	-	+	-	+	-	-	-	+	-	+	+	-	+	9 HS
var. *glaberrima*	-	+	-	-	+	-	+	-	-	-	+	-	+	+	-	+	8 HS
var. *septentrionalis*	-	+	-	-	+	-	+	-	-	-	+	-	+	+	-	+	8 HS
Gonocrypta grevei	-	+	-	-	+	-	+	-	-	-	+	(+)	+	(+)	+	(+)	5,6,7,8 P, HS,HR
Pentopetia androsaemifolia	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	2 HR
Gymnabthera fruticosa	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	2 HR
G. nitida	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	2 HR
Finlaysonia obovata	-	+	-	(+)	+	-	+	-	-	-	+	+	(+)	(+)	-	-	5 R
Cryptolepis grayi	-	+	-	-	+	-	+	-	-	-	+	(+)	+	(+)	(+)	-	5,6,7 P,PR
C. albicans	-	+	-	-	+	-	+	-	+	+	(+)	(+)	(+)	(+)	-	-	4,4 HR,R
Subfamily Secamoneoideae																	
Secamone elliptica	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	2 HR

Note. Parenthesis indicate variable but replicable symptoms between individual plants in a test.

Spores may be released in the field as water- or oil-based suspensions, sprayed or brushed onto the leaves or stems, or in the dry form, diluted if necessary with a powder such as talc. Alternatively, infected potted plants may be set out in the field amongst the plants to be infected. For short-lived spores this latter method allows a bigger window of opportunity for new infections to occur, as fresh spores are produced constantly so increasing the chance of coinciding with suitable environmental conditions. Where dew is unlikely to form naturally to allow infection, it may be artificially induced by enclosing foliage in plastic bags at night.

Integration With Other Control Methods

It is unlikely that the release of one biological control agent will provide acceptable control at all sites in all seasons. Further studies will be required if the agent becomes widely established to understand its interaction with other biological control agents already present and also how chemical, mechanical or ecological methods of control could best be used to enhance the overall controlling effect (Adkins this volume; Farrell and Lonsdale this volume).

References

- Alexopoulis, C.J. 1962. Introductory mycology. 2nd edn. John Wiley and Sons, London and New York. 613pp.
- Bruzzese, E. and Hasan, S. 1983. A whole leaf clearing and staining technique for host specificity studies of rust fungi. Plant Pathology 32, 335–338.
- Hawksworth, D.L., Sutton, B.C. and Ainsworth G.C. 1983. Dictionary Of The Fungi. Seventh Edition. , CAB, Slough, England. 445pp.
- Julien, M.H. 1992. Biological Control of Weeds. Third Edition. CAB International, Wallingford. 186pp.
- Kochman, J.K. and Goulter, K.C. 1983. Reaction types produced by the rust fungus (*Puccinia helianthii* Schw.). Sunflower, August 1983. 3–4.
- Templeton, G. E. 1982. Status of weed control with plant pathogens. In: Charudattan, R. and Walker, H.L. eds. Biological Control of Weeds With Plant Pathogens. John Wiley & Sons, New York. 29–44.

Bioherbicides

Introduction

The notion of using plant diseases to control troublesome plants is an appealing one. Fungi have been used in attempts to control weeds in various parts of the world since the 1950s. Initially researchers concentrated on native or naturalised fungi, spreading them on to target weeds by various means. Results were generally unpredictable, varying with time and location. One fungus to achieve success was the persimmon wilt fungus, *Acremonium diospyri*, which although not commercially available, has been used since 1960 to control persimmon trees in Oklahoma rangelands. The fungus is provided free to local landholders by a benevolent foundation. Suspensions of conidia provided in plastic 'squirt' bottles are applied to wounds made in the trees with a hand axe (G.E. Templeton pers. comm. 1988).

A step forward was made in the Peoples Republic of China in 1963. A *forma specialis* of the fungus *Colletotrichum gloeosporioides* was developed by simple fermentation procedures into a product, 'Lu-bao No. 1', used to control the parasitic weed dodder (*Cuscuta* spp.), in soybeans. It was applied in inundative doses of spores to create an artificial and localised epidemic. Although the original strain of this fungus has been replaced, the daughter product 'Lu-bao No. 2', is still used. It is applied as a liquid suspension of spores like a conventional herbicide (Y.H. Li pers. comm. 1987). This type of product has become known as a 'mycoherbicide' although the term bioherbicide is now generally used to cover the possibility of using other microorganisms.

In the 1970s a number of research labs in the USA concentrated on developing pathogens which were already present in that country. This led to the release of two commercial bioherbicides in the early 1980s. DeVine® was produced in a cooperative venture between the Florida Department of Agriculture and Consumer Services and Abbott Laboratories (Ridings 1986). DeVine® is a formulation of chlamydospores of *Phytophthora palmivora* used to control strangler vine (*Morrenia odorata*) a weed of citrus groves in Florida. Although mass production of chlamydospores by fermentation was possible, long shelf life for the product could not be achieved. DeVine® is handled like fresh milk through its distribution system; it has an expiry date of 6 weeks (Kenney 1986). It is feasible to distribute and market this relatively labile product because of a limited target area.

The second product, Collego™, was developed as collaborative effort between a group at the University of Arkansas led by Professor George Templeton, the US Department of Agriculture and the Upjohn Company. Collego® is a formulation of the pathogen *Colletotrichum gloeosporioides* f.sp. *aeschynomene* which is used to control northern jointvetch (*Aeschynomene virginica*), a weed of rice. Although production of spores by submerged fermentation and drying spores for shelf life was relatively easily achieved on a small

Bruce A. Auld
Agricultural Research & Veterinary Centre
Forest Road, Orange, NSW 2800
Australia

scale for research purposes, improvements were required for commercial production. Research was required on stock culture and media, fermentation conditions, as well as fermentor impeller designs and baffling (Bowers 1986).

The development of Collego® and DeVine® stimulated widespread research interest in mycoherbicides. The result of this activity led to several potential new products. One of these was registered in Canada in 1992: BIOMAL™ is a bioherbicide for control of round-leaved mallow (*Malva pusilla*) in wheat (*Triticum aestivum*) and lentils (*Lens culinaris*) in Manitoba and Saskatchewan, Canada, and northern wheat-producing areas in the USA. It is a selected strain of *Colletotrichum gloeosporioides* f.sp. *malvae* applied in spore suspensions containing 2×10^9 spores/L at the rate of 3×10^2 L/ha. Control of round-leaved mallow has been achieved in field tests (Makowski and Mortensen 1989).

A bioherbicide for control of American blackcherry (*Prunus serotina*) in pine forests in The Netherlands has been developed with a strain of the fungal pathogen *Chondrostereum purpureum* (Scheepens 1980). Like the persimmon wilt disease, it requires wound inoculation to initiate the disease development. Weed trees are cut mechanically, and the cut surfaces of the stumps are painted or sprayed with mycelial fragments in agar suspensions in the same manner as the 'cut-stump' herbicidal control method. This idea is currently being investigated in woody weed control in British Columbian forests in Canada.

Another approach combining an insect biocontrol agent and a fungus has been used in an attempt to control the aquatic weed water hyacinth (*Eichhornia crassipes*) in Florida USA, by Professor Charudattan. Spores of the fungus *Cercospora rodmanii* are sprayed in small areas to create 'hot spots' of infection. Two previously imported weevils, which attack the inflated petioles of water hyacinth, spread the fungal spores as they move from plant to plant.

Outline of a Bioherbicide Research Program

In our research at the Agricultural Research and Veterinary Centre, Orange, the current emphasis is the control of the widespread weed Bathurst burr (*Xanthium spinosum*) by the fungus, *Colletotrichum orbiculare* (Auld et al. 1988). The work has included discovering the most strongly pathogenic isolates, defining optimal conditions for disease development (McRae and Auld 1988; Auld et al. 1990a), fermentation and formulation research and field tests (Auld et al. 1990b). This research project illustrates the work that is typically involved in a bioherbicide project; the steps are summarised in Table 1.

Amongst the problems that may arise in a bioherbicide research program, two areas often provide difficulties. The first is mass production of fungi (Auld this volume). The other is the need to overcome or reduce a requirement for dew which many fungi have. A good deal of research examining formulation has gone into this area recently.

Formulation of Bioherbicides and Bioinsecticides

Formulation has two main aims: to provide an economical and easily useable form of the active ingredient with long shelf life, and, if possible, to enhance the effectiveness of active ingredient. In many circumstances an aim will also be to minimise the quantity of water required.

An active ingredient may be applied in the dry state as dust or granules or as a liquid or in the presence of liquid. This may be as a wettable powder, in oil, as aqueous concentrate, or emulsifiable concentrate. These concentrates are diluted by the addition of water. Other techniques such as microencapsulation will not be dealt with here, although they may have scope in the application of biological control agents.

Table 1. Outline of a commercial bioherbicide research program

1. Define important target weeds. Factors to consider:
   - Value of infested crop.
   - Availability of other control measures and their costs including external costs (Auld et al. 1987).
   - Special circumstances which favour the use of biological control, e.g., a vineweed on the crop.
2. Reduce list (1) to a workable number (three species or less).
3. Survey the weed(s) for pathogens over its range and growth season.
4. Carry out Koch's Postulates test with a small number of isolates of each pathogen.
5. Test a range of media for suitability for spore production.
6. Examine the effect of increasing inoculum concentration on the hosts.
7. Identify promising pathogens.
8. Undertake a literature search for (i) host range, (ii) anything else—e.g., suitable growth media.
9. Reduce program to one weed and one or two pathogens.
10. Screen a range of isolates for efficacy.
11. Carry out a host range test with a small group of selected isolates.
12. If any reaction in any useful plant species, test a range of cultivars.
13. Define optimal environmental conditions for infection and disease development.
14. Examine the possibility of mass production of fungus—e.g., submerged shake flask culture.
15. Carry out preliminary field tests. Small-scale tests may be done earlier.
16. Further laboratory experiments may be needed to help interpret field results.

Given that a biological control fungus can be produced en masse and dried, it can be applied as dry material or formulated as a wettable powder. In both cases clays such as kaolin, silica gel or diatomaceous earths can be used as fillers or carriers. Dusts are prepared so that particle sizes are between 3 and 50 μm in diameter; dusts are, however, vulnerable to wind. Granular formulations are less so, being mostly within the range of 0.3 to 1.0 mm diameter. Granules have been used for mycoherbicide applications using sodium alginate pellets (Walker and Connick 1983) and a pasta like process (Connick et al. 1991). Wettable powders may contain dispersing or suspending agents as well as inert fillers and wetting agents. Sodium alginate at 0.2–0.5% of final volume, for instance, will help keep some spore/clay powders in suspension. Wettable powders have been the most common form of microbial formulation. They have advantages for storage and transport, as well as minimal interaction between spores and other

components. Moreover, given that most fungi used for plant and insect control require free water or very high humidity for infection, the provision of water at application is a logical tactic.

Because many plant pathogenic fungi have a requirement for free water (or dew period) for infection, recent efforts by many mycoherbicide researchers have been directed towards overcoming this dew requirement via formulation; in particular, formulating fungal spores within the aqueous phase of an invert emulsion in oil (or oil mixtures) (Quimby et al. 1989). Although the technique has been shown to overcome the need for dew in some fungi there are disadvantages with the method: the amount of oil required adds to the cost of the product, and application of the viscous material may be difficult. Air-assist nozzles (McWhorter et al.1988) have been used to spray inert emulsions, however some recent formulations do not require special equipment (Yang et al. 1993).

The use of non-aqueous carriers such as oil-based suspensions has been investigated by some workers (Agudelo and Falcon 1983) and may show promise for low volume applications of the insect pathogen *B. bassiana* (Prior et al. 1988) and enhance some bioherbicides' performances (Boyette et al. 1991; Boyette 1994).

The sensitivity of spores to any ingredient will override other considerations and viability tests must be made continually as a formulation is developed. For instance, Soper and Ward (1981) report variation in the tolerance of the insect pathogen *Metarhizium anisopliae* to various kaolins.

Another approach to address the dew or free-water requirement of bioherbicides is to use them when rain or dew is expected or in association with irrigation. Unfortunately in many areas this may not be possible and conditions favourable for fungal growth may not even occur at all when the weeds have to be killed. But in tropical areas where humidity is high, rainfall predictable and irrigation common, bioherbicides may have a very useful role.

Application

Because of their small size, fungal spores can usually be applied in suspension with conventional equipment. Matthews (1983, 1985) has discussed some particular problems and requirements for spray application in the tropics and developing regions. Obviously equipment will need to be free of residues of fungicides and any other harmful chemicals. Notwithstanding this there may be scope for the addition of low concentrations of conventional herbicides to increase the efficacy of a fungus.

The range of techniques available for application therefore is as broad as those available for conventional pesticides and herbicides. These include high volume (about 1000 L/ha), medium (350 L/ha), low to very low volumes (3–150 L/ha), ultra low volumes (0.5–3 L/ha), controlled droplet application and electrostatic spraying. With medium and lower volumes, high powered fans are usually required; with ultra low volumes centrifugal energy may be employed (e.g. Symmons et al. 1989).

International Bioherbicide Group

An International Bioherbicide Group (IBG) was formed in 1992. Anyone interested in doing research with bioherbicides can receive our newsletter, 'IBG News'. They should send their name, address and area of interest to me.

References

- Agudelo, F. and Falcon, L.A. 1983. Mass production, infectivity, and field application studies with the entomogenous fungus *Paecilomyces farinosus.* Journal of Invertebrate Pathology 42, 124–132.
- Auld, B.A., Menz, K.M. and Tisdell, C.A. 1987. Weed Control Economics. Academic Press, London.
- Auld, B.A., McRae, C.F. and Say, M.M. 1988. Possible control of *Xanthium spinosum* by a fungus. Agriculture, Ecosystems and Environment 21, 219–233.
- Auld, B.A., Say, M.M. and Millar, G.D. 1990a. Influence of potential stress factors on anthracnose development on *Xanthium spinosum.* Journal of Applied Ecology 27, 513–519.
- Auld, B.A., Say, M.M., Ridings, H.I. and Andrews, J. 1990b. Field applications of *Colletotrichum orbiculare* to control *Xanthium spinosum.* Agriculture, Ecosystems and Environment 32, 315–323.
- Bowers, R.C. 1986. Commercialization of Collego™—an industrialist's view. Weed Science 34 (Suppl. 1), 24–25.
- Boyette, C.D. 1994. Unrefined corn oil improves the mycoherbicidal activity of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. Weed Technology 8, 526–529.
- Boyette, C.D., Quimby, P.C., Connick, W.J. Jr., Daigle, D.O. and Fulgham, F.E. 1991. Progress in the production, formulation and application of mycoherbicides, In: TeBeest, D.P. ed.. Microbial Control of Weeds. Routledge, Chapman and Hall, New York, 209–222.
- Connick, W.J. Jr., Boyette, C.D. and McAlpine, J.R. 1991. Formulation of mycoherbicides using a pasta-like process. Biological Control 1, 281–287.
- Kenney, D.S. 1986. DeVine®—The way it was developed—an industrialist's view. Weed Science 34 (Suppl 1), 15–16.
- McRae, C.F. and Auld, B.A. 1988. The influence of environmental factors on anthracnose of *Xanthium spinosum.* Phytopathology 78, 1182–1186.
- McWhorter, C.G., Fulgham, F.E. and Barrantine, W.L. 1988. An air assist spray nozzle for applying herbicides in ultra low volume. Weed Science 36, 118–121.
- Makowski, R.M.D. and Mortensen, K. 1989. *Colletotrichum gloeosporioides* f.sp. *malvae* a bioherbicide for round-leaved mallow (*Malva pusilla*): conditions for successful control in the field. In: Delfosse, E.S. ed. Proceedings VII International Symposium on the Biological Control of Weeds, Rome, 1988. Instituto Sperimentale per la Patologia Vegetale, Rome. 513–522.
- Matthews, G.A. 1983. Pesticide application technology. In: Proceedings of the Seminar 16 Nov. 1982 Serdang, Malaysia, MAPPS, Kuala Lumpur, 163–170.
- —1985. Appropriate spraying technology for third world farmers. World Crops 37, 84–85.
- Prior, C., Jollands, P. and Le Patourel, G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curcuilionidae). Journal of Invertebrate Pathology 52, 66–72.

- Quimby, P.C., Fulgham, F.E., Boyette, C.D. and Connick, W.J. Jr. 1989. An invert emulsion replaces dew in biocontrol of sicklepod—a preliminary study. Pesticide Formulations and Application Systems 8, 264–270.
- Ridings, W.H. 1986. Biological control of strangler vine in citrus—a researcher's view. Weed Science 34 (Suppl. 1), 31–32.
- Scheepens, P.C. 1980. Bestrijding van de Amerikaanse vogelkers met pathogene schimmels, een perspectief. Verslag 29, CABO, Wageningen, The Netherlands, 14pp.
- Soper, R.S. and Ward, M.G. 1981. Production, formulation, and application of fungi for insect control. In: Papavizas, G.E. ed., Biological Control in Crop Production. Allanheld and Osmun, Totowa, 161–180.
- Symmons, P.M., Boase, C.J., Clayton, J.S. and Gorta, M. 1989. Controlling desert locust nymphs with bendiocarb applied by a vehicle-mounted spinning disc sprayer. Crop Protection 8, 324–331.
- Walker, H.L. and Connick, W.J., Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Science 31, 333–338.
- Yang, S.-M., Johnson, D.R., Dowler, W.M. and Connick, W.J. Jr. 1993. Infection of leafy spurge by *Alternaria alternata* and *A. angustiovoidea* in the absence of dew. Phytopathology 83, 953–958.

Mass Production of Fungi for Biopesticides

Introduction

Mass production methods for fungi as biological control agents are the same whether the target is a weed, an insect pest or another disease. Therefore in this chapter I will discuss techniques that have been used for plant pathogenic fungi as well as for insect pathogens and other plant pathogens.

Production of many fungi for biological pest control may often be relatively easily achieved in quantities suitable for laboratory and glasshouse research and even small-scale field trials. However, if large-scale practical use is ultimately intended, mass production techniques for the fungus must be developed. The purpose of this chapter is to introduce methods of mass production which can be used and indicate where they can be adapted to a pilot scale in a non-specialised laboratory.

Submerged Culture Fermentation

Many filamentous fungi sporulate in submerged culture (Vézina et al. 1965). In the industrialised world at least, liquid fermentation provides the simplest and most economical way to produce large numbers of fungal spores. This is because existing equipment can be used without modification. The commercial biological control agents for weed control, Collego™ and Devine® are produced by this method (see Churchill 1982; Stowell 1991). Mycotal® for aphid control and Vertalec® for whitefly, comprising *Verticillium lecanii* as blastospores (Latge et al. 1986), and preparations of some strains of *Beauveria bassiana* for insect control (Thomas et al. 1987) also use this method.

Although a bioreactor (fermentor) would usually be used in industrial submerged culture, it may be possible to produce propagules in flask culture. Oxygen mass transfer is a major problem for aerobic processes as the solubility of oxygen in water is only about 6ppm. Oxygen transfer can be increased by minimising boundary layer resistance and maximising surface area for transfer. This is achieved by agitation.

The type of agitation may be important and the air/liquid ratio as well as the surface contact area may need to be high. Conical flasks of 50–2000 mL may be used. The medium being used is sterilised with the flask, relying on a porous closure, like cotton wool, to act as a depth filter. Flasks with side indentations to act as baffles will encourage greater turbulence and aeration. Reciprocating shakers are inferior to orbital shakers which can give high oxygen transfer rates because of the trailing of the liquid around the flask (Fig. 1) allowing greater area of liquid/air contact. Reciprocating shakers tend to splash the closure increasing the danger of contamination. Sophisticated shakers in which temperature and light are controlled may be necessary.

Bruce A. Auld
Agricultural Research & Veterinary Centre
Forest Road, Orange, NSW 2800
Australia

Another method is to bubble compressed air into flasks to provide oxygen and (limited) agitation (e.g. see Papavizas et al. 1984). However a pilot fermentor allows greater oxygen input and control as well as control of temperature, pH, agitation and foaming. The speed and diameter of the impellor has a big influence on oxygen transfer. The diameter of the impellor should be about 1/3 the diameter of the reactor. Baffle plates assist turbulence, breaking up the liquid; they should be about 1/10 the diameter of the reactor. However they may be a nuisance with some filamentous fungi. Standard industrial bioreactors are from 1 000 L to 800 000 L. Smaller pilot bioreactors cannot mimic exactly what larger bioreactors will do. For instance there is a marked increase in shear at impellor tips but longer mixing times as volume increases. The minimum size of pilot bioreactors generally available is 1 litre.

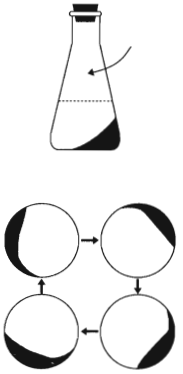


Figure 1. The movement of liquid (shaded) in an orbital shaker as seen from the side (above) and above (below) as the flask is moved by the shaker. This movement maximises the area of liquid/air contact for small volumes of liquid.

Bioreactor operation may be batch culture, semi-batch or continuous culture (e.g. see Trinci and Wiebe 1990). Batch culture is the simplest procedure with less likelihood of contamination. The output of a product such as fungal spores generally follows a sigmoidal curve with lag, exponential, stationary, and perhaps decline, phases (Fig. 2). The growth of product can be affected by substrate limitation and inhibition, product inhibition as well as temperature and pH.

If the production of a given fungus has a long lag phase in batch culture it may be worth investigating semi- (or fed) batch culture to shorten the lag period.

Possibly one of the greatest problems encountered with fermentation will be contamination. Sterilisation is a vital part of the process but the degree of 'sterility' required is a function of the end use.

Media for growth of the biological control organism should be as simple as possible utilising a standard set of inorganic salts and sources of carbon and nitrogen. Production of spores in the fermentor may be

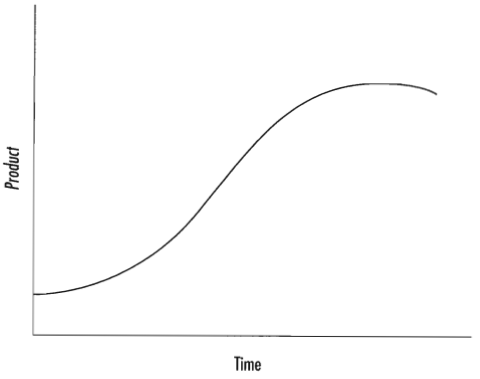


Figure 2. Typical production curve for a fermentation run with time. Note: lag, exponential and 'stationary' phases.

enhanced by changes in media components or simply by diluting the medium (Auld et al. 1990). Production may also be increased by beginning the fermentation process with increasing volume or concentration of starter culture. Latge and Moletta (1988) provide an extended treatment of the production of entomopathogenic fungi in submerged culture and Stowell (1991) gives examples of mycoherbicide production.

Within the bioreactor fungal growth may take the following physical forms: small discrete cells; small compact pellets; larger floccose pellets; or a filamentous form (Solomons 1980).

The method of reproduction in submerged culture may differ morphologically and physiologically from in vivo production. In the production of Collego™ only about 8–10% of the spores produced are normal conidia, most of them are fission spores (Churchill 1982).

Some fungi which do not sporulate in submerged culture may produce mycelium which can be dried (Pereira and Roberts 1990) and applied as fragments or pellets in the field; among them is *Metarhizium anisopliae* produced by the Bayer Company as BIO 1020®. Such fungi may sporulate following dew (Roberts and Wraight 1986; Robmach et al. 1986; Roberts et al. 1987). Walker and Connick (1983) describe the production of sodium alginate pellets of dried mycelium for a mycoherbicide.

It may be necessary to screen isolates for spore productivity under fermentation conditions as well as the virulence of the spores produced. The most virulent isolates may not be the most productive in vitro.

In some cases it may be more appropriate to produce survival propagules rather than infective propagules. Chlamydospores of *Trichoderma* and *Gliocladium* for instance were more effective in field use than conidia (Lewis and Papavizas 1984; Papavizas et al. 1984).

Jackson et al. (1996) reviewed several years recent work on fermentation of the bioherbicide fungus, *Colletotrichum truncatum.*

Solid Substrate Fermentation

Solid substrate fermentation has been widely used to produce fermented foods in China, Japan and South-east Asia (Wood and Yong Fook Min 1975). Media may contain some liquid; the 'solid' substrate itself may be relatively inert (eg. paper, wood, vermiculite), allowing for use of defined nutrient levels. The fungus *Sporidesmium sclerotivorum* used against the lettuce pathogen *Sclerotinia minor* is grown on vermiculite moistened with liquid medium (Adams and Ayres 1982). On the other hand some nutritive solid substrates may be available locally at low costs (eg. rice husks, coffee pulp, sorghum grain, straw, groundnut shells). Particle size, moisture content and temperature may all need to be controlled for successful production. Equipment used may be bags, trays or rotating drums.

Industrial submerged culture fermentation production requires considerable capital investment. Production on solid media may be relatively costly in terms of labour and materials in the western industrial environment but not necessarily so where labour is less costly and suitable raw material is freely available in developing countries.

Beauveria bassiana has been produced on solid substrates such as heat sterilised grains in Russia and the Peoples Republic of China. In the latter system 500 mL flasks of substrate are used to 'seed' 5 kg lots of steamed grain which is mixed with ten times the amount of wheat bran for fermentation in flat trays or in outdoor pits (Bartlett and Jaronski 1988). The

fungus is produced in liquid surface culture in large inflated plastic bags in Czechoslovakia (Kybal and Vilcek 1976; Samsinakova et al. 1981). Solid substrate fermentation is an alternative for fungi which will not sporulate in submerged culture. Goettel (1984) has also described a technique for producing fungi using cellophane sheets on bran in autoclave bags. Abbott Laboratories in the USA, have made commercial scale up tests for the fungus, using solid substrate to produce either a wettable powder or granular formulation (Bartlett and Jaronski 1988). R.J. Milner (pers. comm. 1991) has developed a solid substrate fermentation system for *M. anisopliae* using rice, inoculating with conidia and harvesting conidia by a washing technique (Australian Patent PK3451/90).

Two-phase Systems

A two-phase system has been used for *B. bassiana* and *M. anisopliae* production in Russia, where mycelium produced in deep tank fermentation is allowed to sporulate in shallow open trays (Roberts and Yendol 1981; Goral and Lappa 1973). Walker and Riley (1982) described a similar preparation method for *Alternaria cassiae* for control of the weed *Cassia obtusifolia*. In Brazil *M. anisopliae* is produced on autoclaved rice or wheat bran in autoclavable plastic bags following inoculation with blastospores produced in liquid shake culture (Aquino et al. 1975, 1977).

Recovery of Product and Storage

Recovery of spores from bioreactors may be a problem with filamentous fungi, requiring large centrifuges to spin off spores. Filtration methods often leave a large number of spores behind in the mycelial mass if the latter is formed during fermentation.

Following recovery of spores from a production process it is usually necessary to dry them for long-term storage. It is not always possible to do this and retain viability of the organism. It is for this reason that the mycoherbicide Devine® is sold in liquid form like fresh milk. Drying should be done as quickly as possible under 'clean' conditions to prevent bacterial contamination. Inert materials such as diatomaceous earth, silica gel or clay may be suitable to hasten drying and to act as carriers.

Production Capacity

In a non-specialised fermentation laboratory, the demonstration that sporulation of a given fungus occurs in submerged culture may be an adequate goal. Cooperation of fermentation specialists could then be sought. However, ultimately, the maximum production per unit volume of fermentation liquor must be established and this related, as bioreactor capacity (time × volume) to concentrations per unit volume required for field use.

Bartlett and Jaronski (1988) cite typical rates of *Beauveria* conidia per hectare are about 1×10^{14}; yields for *Beauveria* conidia obtainable from liquid surface culture are 1×10^{14} m^2, submerged liquid fermentation 3×10^{11} per litre and 7×10^{12} per kg for solid substrate fermentation. Thus if the current submerged liquid production technology were to be used, a fermentation capacity of over 300 litres is required for each hectare treated. Apart from research on maximising production per unit volume or area, improved formulation and application techniques may reduce the density of spores required in the field. Given that there will ultimately be physical limitations to the amount of spores that can be produced in a given area or volume, work on improving application may be required in parallel with production research.

Developing Countries

As previously stated mass production by fermentation in large submerged culture bioreactors requires considerable capital investment and may not be appropriate in many developing countries. However, many fungi will grow and sporulate on simple solid substrates using basic equipment which could be operated by people with limited training. The need for mass production of fungi should not be seen as a barrier to their use as inundative biological control agents (mycoherbicides or bioherbicides and other biocides). Local mass production could take place with very limited facilities near the end-use field site. This would mean that, at any one location, production could be linked to local needs and would not require vast amounts of fungi to be produced for sending elsewhere.

References

- Adams, P.B. and Ayers, W.A. 1982. Biological control of Sclerotinia lettuce drop in the field by *Sporidesmium sclerotiorum*. Phytopathology 72, 485–488.
- Aquino, de L.M.N., Cavalcanti, V.A., Sena, R.C. and Queiroz, G.F. 1975. A new technique for the production of the fungus *Metarhizium anisopliae*. Boletim Tecnico CODECAP 4, 1–31.
- Aquino, de L.M.N., Vital, A.F., Calvalcanti, V.A. and Nascimento, M.G. 1977. Culture of *Metarhizium anisopliae* (Metchn) Sorokin in polypropylene bags. Boletim Tecnico CODECAP 5, 7–11.
- Auld, B.A., McRae, C.F. and Say, M.M. 1990. Potential for *Xanthium spinosum* control by *Colletotrichum orbiculare* as a mycoherbicide. In: Delfosse, E.S. ed. Proceedings of the VII International Symposium on Biological Control of Weeds, Rome, 1988. Instituto Sperimentale per la Patologia Vegetale, Rome. 435–443.
- Bartlett, M.C. and Jaronski, S.T. 1988. Mass production of entomogenous fungi for biological control of insects. In: Fungi in Biological Control Systems, Burge, M.N. ed. Manchester University Press, Manchester, 61–85.
- Churchill, B.W. 1982. Mass production of microorganisms for biological control, In: Biological Control of Weeds with Plant Pathogens. Charudattan, R. and Walker, H.L. ed. Wiley, New York, 134–156.
- Goettel, M.S. 1984. A simple method for mass culturing entomopathogenic Hyphomycete fungi. Journal of Microbiological Methods 3, 15–20.
- Goral, V.M. and Lappa, N.V. 1973. In-depth and surface culture of the green muscardine fungus. Zashchita Rastyeni 1, 19.
- Jackson, M.A., Schisler, D.A., Slininger, P.J., Boyette, C.D., Silman, R.W. and Bothast, R.J. 1996. Fermentation strategies for improving the fitness of a bioherbicde. Weed Technology 10, 645–650.
- Kybal, J. and Vilcek, V. 1976. A simple device for stationary cultivation of microorganisms. Biotechnology and Bioengineering 18, 1713–1718.
- Latge, J.-P. and Moletta, R. 1988. Biotechnology, In: Atlas of Entomopathogenic Fungi, Sampson, R.A., Evans, H.C. and Latge, J.-P. eds. Springer-Verlag, Berlin. 152-164.
- Latge, J.-P., Hall, R.A., Cabrera Cabrera, R.I. and Kerwin, J.C. 1986. Liquid fermentation of entomogenous fungi, In: Fundamental and Applied Aspects of Invertebrate Pathology, Sampson, T.A., Vlak, J.M. and Peters, D. ed. 4th International Colloquium of Invertebrate Pathology, Wageningen. 603–606.

- Lewis, J.A. and Papavizas, G.C. 1984. A new approach to stimulate population proliferation of *Trichoderma* spp. and other potential biological control fungi introduced into natural soils. Phytopathology 74, 1240–1244.
- Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle-Ristaino, J. 1984. Liquid fermentation technology for experimental production of biological control fungi. Phytopathology 74, 1171–1175.
- Peireira, R.M. and Roberts, D.W. 1990. Dry mycelium preparations of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Journal of Invertebrate Pathology 56, 39–46.
- Roberts, D.W., Dunn, H.M., Ramsay, G., Sweeney, A.W., Dunn, N.W., McCabe, B. and Soper, R.S. 1987. A procedure for preservation of the mosquito pathogen. *Culicinomyces clavisporus*. Applied Microbiology and Biotechnology, 26, 186–188.
- Roberts, D.W. and Wraight, S.P. 1986. Current status on the use of insect pathogens as biological control agents in agriculture: fungi. In: Fundamental and Applied Aspects of Invertebrate Pathology, Sampson, T.A., Vlak, J.M. and Peters, D. ed. 4th International Colloquium of Invertebrate Pathology, Wageningen. 510–513.
- Roberts, D.W. and Yendol, W. 1981. Use of fungi for microbial control of insects. In: Microbial Control of Insects and Mites, Burges, H.D. and Hussey, N.W. ed. Academic Press, New York. 125–149.
- Rombach, M.C., Aguda, R.M. and Roberts, D.W. 1986. Biological control of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) with dry mycelium applications of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Philippine Entomologist 6, 613–619.
- Samsinakova, A., Kalalova, S., Vlcek, V. and Kybal, J. 1981. Mass production of *Beauveria bassiana* for regulation of *Leptinotarsa decemlineata* populations. Journal of Invertebrate Pathology 38, 169–174.
- Solomons, G.L. 1980. Fermenter design and fungal growth. In: Fungal Biotechnology; Smith, J.E., Berry, D.R. and Kristiansen, B. ed. Academic Press, London. 55–80.
- Stowell, L.J. 1991. Submerged fermentation of biological herbicides. In: Microbial Control of Weeds. Te Beest, D.O. ed. Routledge, Chapman & Hall, New York. 225–261.
- Thomas, K.C., Khachatourians, G.G. and Ingleden, W.M. 1987. Production and properties of *Beauveria bassiania* conidia cultivated in submerged culture. Canadian Journal of Microbiology, 33, 12–20.
- Trinci, A.P.J. and Wiebe, M.G. 1990. Production of fungal biomass and fungal spores in continuous and fed-batch culture. Aspects of Applied Biology 24, 255–261.
- Vezina, C., Singh, K. and Shegal, S.N. 1965. Sporulation of filamentous fungi in submerged culture. Mycologia 57, 722–736.
- Walker, H.L. and Connick, W.J. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Science 31, 333–338.
- Walker, H.L. and Riley, J.A. 198 Evaluation of *Alternaria cassiae* for the biological control of sicklepod (*Cassia obtusifolia*). Weed Science 30, 651–654.
- Wood, B.J.B. and Yong Fook Min 1975. Oriental food fermentations. In: The Filamentous Fungi Volume 1. Industrial Mycology. Smith, J.E. and Berry, D.R. ed. Edward Arnold, London. 265–280.

Plant Propagation and Culture

Introduction

Plants have five basic requirements for healthy growth: light, air, water, heat, and nutrition. Healthy growth will be sustained as long as these requirements approach optimal levels and the plants are free from pest animals and diseases. Most plants also require anchorage in some form of substrate. In most cases, this substrate also serves as the plant's primary source of moisture and nutrition.

A vast literature covers every detail of plant culture from the broadest to the most specific aspects. This paper provides guidelines for routine aspects of plant culture including problems that are likely to be encountered and their rectification. Topics covered are:

- specific requirements
- light
- air
- temperature
- moisture
- potting mixtures
- nutrients
- containers
- hygiene
- propagation / growing areas
- propagation by seed
- vegetative propagation

For the most part, this paper refers to container-grown plants, although some information may be extrapolated to field culture.

Gio. W. Fichera
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia.

Specific Requirements

The first consideration in growing any plant species is to determine if the species has specific requirements. This may include questions such as:

- Is the plant a water lover?
- Is it tropical or temperate in its temperature needs?
- Has it evolved on highly fertile heavy soils or those that are very sandy and poor in nutrition?
- Is it a plant that grows in exposed sunny places or does it grow predominantly in the shade of other plants?

The answers to such questions will indicate the conditions that should be provided.

When growing the many plant species on a test list it is not practical to duplicate specific optimal requirements for each species. It is usually only

practical to provide one or a few sets of growing conditions that will serve all of the plant species. Fortunately, most species will tolerate and grow well in a range of conditions. Relatively few species are constrained by a narrow range of requirements.

Light

Light has many effects on plant development. For the most part, plants must make do with the natural sunlight received daily. However, by manipulating light intensity, duration, colour spectrum and so on, plant growth can be modified for specific needs. This may be necessary to prolong flowering, to allow year-round rearing and assessment of flower, pod and seed feeding insect agents, or to prolong or promote vegetative growth by inhibiting flowering or leaf-fall for young shoot feeders. For example, with the shortening day length as winter approaches, plants of the pantropical *Sida acuta* are stimulated into flower and fruit production with such an intensity that vegetative growth ceases. Leaf size decreases to less than half that produced during summer months. This presented a problem when rearing a biological control agent, the voracious leaf feeding beetle *Calligrapha pantherina*. By supplementing artificial lighting from the beginning of autumn to maintain 14 hours of light each day, flowering was delayed, plants continued to grow vegetatively, and there was little reduction in leaf size until after mid winter.

Shading may be important. For example, young new growth of *Theobroma cacao* (cocoa) develops brown burn lesions along the margins when exposed to full sunlight. Sprouting seed and emerging seedlings of many plant species are damaged by direct sunlight, so shading is required for establishment. Conversely, shading of species requiring strong light causes etiolation (unnatural stem elongation) and increased susceptibility to breaking and disease.

Air

Air supplies plants with carbon dioxide (CO_2) and oxygen (O_2). Through the process of photosynthesis, plants utilise light to synthesise carbohydrates (food) from CO_2 absorbed through pores (stomata) in the leaves, and water. CO_2 is sufficiently abundant in the atmosphere for normal growth of plants. CO_2 enrichment of the atmosphere surrounding plants, combined with optimisation of other plant requirements, has been used in the plant nursery industry to increase plant quality and growth rate. However, this technique is unlikely to be useful for growing test plants.

Through the process of respiration, O_2 is combined with carbohydrates and nutrients to produce new cells and repair damaged ones, thus enabling plants to grow. Oxygen is absorbed through most plant parts and is critical to all tissue, particularly plant roots. Deficiency of O_2 in roots is caused by the following factors:

- waterlogging, where all pores in the medium are filled with water for prolonged periods;
- compacted potting mix or potting mix with a high proportion of very fine particles constituting a physical barrier to O_2 diffusion into the mix;
- organic decomposition in a potting mix that is high in organic matter producing methane which fills pore spaces preventing O_2 entry;
- high microbial activity in the potting mix producing high levels of CO_2 which replaces the air in pores.

In severe cases of O_2 deficiency roots die, resulting in death of the plants. Most plants under stress probably have some ability to translocate O_2, but growth is less than if O_2 was absorbed directly by the roots from air in the potting mix (Handreck and Black 1984).

Aquatic plants are able to supply their roots with O_2 brought down from their leaves through special stem cells (Winterton this volume).

Temperature

For most species the optimum temperature for rapid root and shoot growth is in the range 15–30°C. Generally species from temperate areas tend towards the lower end of the range, while tropical species grow best at around 30°C. The precise temperature for optimal growth varies with plant species and variety, moisture content of the medium, light intensity and nutrient supply.

Both high and low temperature can inhibit plant growth and, at the extremes, kill plants. The temperature limits to growth vary with specific requirements of different plant species. Generally, the lower limit for root growth is about 7°C for temperate species and 12–16°C for tropical species. Root growth is reduced at temperatures above 35–40°C. Roots may be damaged or killed by high temperatures that may be reached in pots in hot weather. Roots are killed in a few minutes at 60°C, in about four hours at 50–55°C, or if exposed for more than four hours at 40–45°C, but they may regenerate if exposed for less than four hours at 40–45°C (Handreck and Black 1984). Roots damaged or killed by high temperature are susceptible to attack by pathogens.

Temperature may affect growth indirectly by influencing fertilizer release rates and microbial activity in the potting mix.

Moisture

Watering is usually the most easily satisfied requirement of plants, but moisture stress through over- or under-watering is very common. When little is known about the water needs of a plant species, some insight may be gained from the species' characteristics (leafiness, height and spread, annual, deciduous or perennial). Knowledge of the natural habitat might also provide useful information, e.g. soil type and whether the plant occurs in wet or dry areas, etc. Seasonal differences must also be considered; the water needs of a deciduous plant are less during cool and/or dry periods, when it is leafless and dormant, compared to when it is actively growing with a full canopy.

Cultured plants are often watered too much. Excessive watering causes depletion of nutrients through leaching in open, highly porous potting mixes, or waterlogging of less porous potting mixes. Over-watering can result when the watering regime is not adjusted with the changing seasons. Holding plant pots in drip trays that are constantly kept filled with water may also lead to waterlogging.

Reducing the volume of water being applied to the pot is the obvious solution to over-watering and waterlogging. Removing drip trays may overcome waterlogging problems. Re-potting the plants into a more open and better aerated potting mix should rectify waterlogging problems, but can lead to increased leaching of nutrients if too much water is applied. Leaching problems may be reduced by reducing the porosity of the mix (but balance this against the possibility of increased waterlogging), by increasing the humus or colloid content of the mix to provide better nutrient retentive properties, or by applying fertilizer at more frequent intervals.

Waterlogging causes direct physical damage to roots, and indirect effects on plants through increased susceptibility to diseases, production of organic toxins such as methane and organic acids, increased salinity and conversion of soluble nitrogen to forms not useable by plants. Toxicity of some elements, such as iron and manganese, increases greatly under very wet conditions. Through evaporation, dissolved salts from

fertilizers may accumulate at the surface of waterlogged potting mix as white powder which may be injurious to the plant, possibly resulting in death.

Waterlogged plants exhibit wilting symptoms similar to plants suffering from lack of water or root rot diseases. Leaves often exude water from the tips and margins, where salts accumulate as white deposits and cause brown to black necrotic patches. A further indication of waterlogging is a pungent odour from the root ball, like that of rotting vegetation, when the pot is removed.

Healthy plant roots are normally white in colour with numerous lateral branches and a proliferation of root hairs just behind the growing tips. Under waterlogged conditions, roots are sparse, brown to black in colour, and tips, lacking root hairs, are in various stages of decomposition, or in severe cases, missing. Adventitious roots may begin to develop on the stem at or just above the surface of the mix. The effects of waterlogging manifest faster and are more severe at high temperatures, and in summer the effects may become rapidly lethal to some plant species (Handreck and Black 1984).

Too little water causes wilting, leaf abscission, flower abortion and withering or loss of fruits. If plants have not dried out completely they will regenerate if watered.

Water quality is important. Water with pH too high or too low, or with excessive levels of dissolved salts or sodium causes severe damage to plants. Water drawn from dams, creeks, and particularly bores should be tested to determine its suitability for use. For example, hard water contains calcium, magnesium, sodium bicarbonate and carbonates which can make growing media more alkaline, that is, raise the pH. Yellowing due to iron chlorosis is a common first symptom that something is probably wrong with the pH of the medium. Excessive watering with soft water, that is, water containing very little or no carbonates, calcium, etc. can, through leaching, strip elements from the potting mix causing it to become more acidic. In regions where good quality water is scarce, treated sewage effluent water may be used for plants. However, this water may also raise the pH of the potting mix.

Expensive water processing equipment can be used to remove the compounds that make water hard. However, the cheapest approach to dealing with water quality problems is to be aware that the water being used is imperfect. It is then necessary to remain vigilant for signs of plant nutritional deficiencies or toxicities that indicate problems due to changing pH.

Potting Mixture

The composition of the potting mixture has a major impact on plant growth. Plants derive anchorage, water, nutrients and air from the potting mix. A potting mix should be formulated from components that will provide the properties desired for the plant species to be grown.

Points to consider when preparing potting mix are:

- the components that make up the mix;
- total water holding capacity and permeability;
- aeration, compaction and root penetration;
- cation exchange capacity;
- buffer capacity;
- pH.

Potting mix components

The choice of components is influenced by their availability and cost. Components may include composted hardwood sawdust, composted milled pine bark, milled coconut coir (coco peat), sphagnum peat

moss, perlite, coarse grade vermiculite, coarse sharp freshwater washed sand, soil, rice hulls, and peanut/groundnut shells.

Composition of the medium, that is, the ratio of the components, is largely determined by requirements of the group of plants to be grown. A potting mix requires components that retain ample available water and nutrients: such as peat, coco peat, sawdust, vermiculite, or soil; provide good drainage, aeration, and friability, such as perlite, rice hulls, peanut shells, or pine-bark; and provide stability against top heavy plants falling over, such as sand, gravel, and soil. In addition, at least one of the components should provide a good buffer capacity and cation exchange capacity.

What may be a very good quality soil rarely serves as a good potting mix on its own, and usually suffers from poor porosity and aeration, compaction and difficulty in re-wetting.

Total water holding capacity (TWHC) and permeability

This is the total volume of water held in a saturated potting mixture after it has been allowed to freely drain for about 24 hours. This may be referred to as the potting mix's container capacity. Water held with little force in the larger pores is either readily available to plant roots or evaporates at the surface. This water is replaced by that held with stronger forces in smaller pores and on particles. As the potting mix dries, the water remaining is that held most strongly. A point is reached at which none of the remaining water is available to plants. When no water is available, plants wilt. The point at which plants have sustained irreversible root damage, and beyond which they fail to recover, is referred to as the permanent wilting point. The difference in water volume between TWHC and the permanent wilting point is known as available water. It follows, then, that a potting mix with good aeration will have a larger proportion of large pores, holding more water that is readily accessible for plants, compared with a potting mix with predominantly minute particle size, which may have a greater TWHC but not necessarily more available water.

Permeability of water into open mixes is generally good. Reduced permeability, caused by water-repelling (hydrophobic) fine organic particles or very fine sand grains, can result if mixes are allowed to dry, i.e. have low available water. Incorporation of some coarser material into the medium counteracts this problem.

Aeration, compaction and root penetration

These three factors and permeability are closely related. A compacted, hard potting mix will usually have poor aeration, present a physical barrier to root penetration and is subject to waterlogging. Although water holding capacity can be quite high, permeability into the mix is usually impeded should the mix dry.

pH

pH is a measure of the acidity or concentration of H^+ ions in a potting mix. The higher this concentration, the more acid or lower the pH. Very high or very low pH directly damages delicate plant roots. Indirect effects of low or high pH include:

- reduced availability of certain elements due to chemical changes;
- increased availability of other elements, e.g. manganese, iron and zinc, when pH is low, or phosphorus when pH is high, to such levels that they become toxic to plants. At high levels these elements can also interfere with the uptake of other elements, e.g. copper deficiencies can be caused by high levels of iron, zinc, manganese and phosphorus;

- reduced proliferation of desirable microorganisms, e.g. symbiotic bacteria (*Rhizobium* spp.) in roots of leguminous plants, while promoting undesirable microorganisms, e.g. the fungi *Pithium* spp. that cause damping-off of seedlings, and *Fusarium* spp. that are responsible for wilts in numerous plant species.

The optimum pH range, which maximises nutrient availability to plants, is 5.0 to 6.0 for organic soils and potting mixes (>10% organic matter content), and 5.5 to 6.5 for mineral soils and potting mixes (Handreck and Black 1984).

Cation exchange capacity

Cation exchange capacity is a measure of a potting mix's ability to retain positively charged ions, i.e. cations. Cations such as K^+, Ca^{2+}, Mg^{2+}, NH_4^+, Mn^{2+}, Fe^{2+} and Cu^{2+} are all key elements needed for healthy plant growth. These cations are loosely held on the negatively charged surface of colloids comprising the humus (fine organic matter), or clay (soil) components of the mix. Plant roots release H^+ cations which exchange with the other cations held on the colloid surface close to the roots. These freed cations are then taken up by the roots. Mixes with a high cation exchange capacity will also have the greatest capacity to resist or buffer the acidifying effect of fertilizers.

Buffer capacity

Buffer capacity of a potting mix is its ability to resist a rapid change in pH, especially after fertilizers are added. Potting mixes with a high cation exchange capacity, which is provided by the inclusion of fine organic matter or clay in the mix, have good buffer capacity, i.e, they resist changes in pH when fertilizers are added.

When fertilizers such as ammonium phosphate or ammonium sulphate are added to a potting mix, phosphoric and sulfuric acids can be produced, which in the absence of good buffer capacity, could lower the pH of the mix to levels potentially unsuitable for healthy plant growth. In potting mixes with good buffer capacity, the reactions of fertilizer with water still produce H^+ ions, but these are largely taken out of solution through colloidal exchange and hence pH changes are minimised. See Handreck and Black (1984).

Materials with relatively high buffer capacity include; exfoliated vermiculite, most peat mosses, fine lignite particles and illite clay. Those with poor buffer capacity include; pine bark, perlite, quartz sand and many commercial potting mixes (Handreck and Black 1984).

Examples of potting mixes

An example of a potting mix used in CSIRO glasshouses at Long Pocket Laboratories is as follows:

- two parts New Zealand sphagnum peat moss,
- two parts coarse grade vermiculite,
- two parts perlite,
- 1.5 parts coarse sharp freshwater washed sand, and
- added lime to adjust the peat's low pH of about 4 to a pH of about 5.0–6.0.

This medium has good water permeability and retention, good aeration and friability, very good buffer capacity and cation exchange capacity, and provides good ballast. However the cost of $189 Australian dollars per m^3, in 1995, is a significant drawback so this mix is used for propagation only.

A commercial nursery potting mix ($48/$m^3$), comprising of composted hardwood sawdust, milled pine-bark, and coarse sharp sand, is used for all other

general purpose potting. To suit the specific needs of a number of the more temperamental species, vermiculite, perlite, and more sand is added for a final cost of $A111/m^3$. This cost is further offset by recycling 70% of the mix.

Nutrients

Today many fertilizer formulations exist to cater for every aspect of plant nutrition. These include fertilizers for field crops, gardens and nurseries. There are slow release, liquid foliar feed, and hydroponic feed formulations.

The following are points to consider when selecting fertilizers.

- A complement of all essential elements must be supplied to plants either as a single formulation or as a combination of a few formulations.
- Manufacturer's recommended application rates should be followed; use of higher rates may cause severe damage or death due to salt burns.
- The same mix and fertilizer rates should not be used for both seedlings and larger plants because they do not have the same nutritional needs.
- The performance of any fertilizer (release mode and rates at varying temperature and moisture levels), durability (i.e. whether it will be leached away with the first irrigation or rain), and pH, should be observed, and actions implemented to counter any long term detrimental effects. Alternatively, a more appropriate formulation should be selected.
- There is a reduced fertilizer need when plant growth is low during dry seasons and winter.
- Requirements vary between species. For example, annual cereals have very different nutritional requirements than perennial trees.
- Fertilizer(s) should provide a relatively constant nutrient supply rather than fluctuating high and low levels. Combinations of slow-release granular formulations and immediately available liquid fertilizers may provide the constant nutrient supply.
- Liquid fertilizer can produce a rapid growth response in plants with reduced vigour, or rapidly correct a nutrient deficiency. However, liquid fertilizers are readily leached from the medium.
- The formulation should be easy to apply.
- Cost per unit should be considered in terms of longevity, application rates, time and labour cost for application. A cheap fertilizer, which may need to be applied in large amounts and frequently, may cost more than an expensive formulation requiring only a single, small or annual application.
- Prepared potting mix containing slow-release fertilizer should not be steam sterilised at 100°C as such treatment can destroy some or all of the slow-release properties of the fertilizers and may lead to rapid release of nutrient salts that may injure or kill plants. Pasteurising mixes at 60–65°C for 30 minutes does not release toxic levels of nutrient salts.
- Soluble nutrients are continuously released from coated fertilizers during storage of moist mixes, causing the salinity of the mix to increase to damaging levels. Mixes containing coated fertilizers should be used within a couple of days of preparation, otherwise leaching (over watering) to remove the salts, and nutrients, should be carried out immediately after potting (Handreck and Black 1984).
- Fertilizer should be well mixed into the potting mix whenever possible, rather than applied to the surface. Release rates are more consistent in the

mix because temperature and moisture fluctuate less than on the surface. Fertilizer applied to the surface may be lost by splashing water, wind toppling plants, pots being weeded, or accidental bumping or dropping the pot. Nutrients applied to the surface are dependent on water permeating from the surface to wash them into the root-zone, whereas those mixed into the potting mix are released where they are absorbed.

- Fertilizer residues, particularly from slow-release formulations, are likely to be present in varying concentrations in any recycled potting mix. To avoid injury from the accumulated salts, their concentration should be measured as the total soluble salts or total dissolved salts extracted in solution from a known volume of media. Alternatively, the electrical conductivity of the solution should be measured. Handreck and Black (1984) state that approximate maximum allowable electrical conductivity for saturation extract of growing media is 1.4 deciSiemens per metre (dS/m) for sensitive plants and 2.5 dS/m for moderately tolerant plants. Above these levels deleterious effects on plant growth may occur. Alternatively, before the recycled mix is used, a plant known to be sensitive to salt levels should be planted in the recycled mix and observed for symptoms of high salt levels. Excess salts can be leached from the mix by thoroughly flushing with water, or by combining the recycled mix with new mix to which no fertilizer has been added.

Containers

Unlike the heavy, fragile and poorly drained clay pots, today's light-weight, tough, durable, inexpensive, readily available, plastic pots and bags, with ample drainage holes and some with root trainers, have revolutionised the bulk production and handling of container grown plants. They cater for almost every application and are available in many sizes and varied shapes.

When choosing pots or bags:

- select pots of suitable size for the species to be grown and length of time they will spend in that container, e.g. greater container depth and size is needed for long-term, deep tap-rooted species;
- avoid containers with large overhanging rims, as this area can harbour difficult to access pests such as mealy-bugs and mites;
- try to work with pots of uniform size, as this will maximise the use of space in growing areas.

Holding advanced seedling stocks in small 50 mm or 75 mm pots (grow-tubes), saves space. The seedlings can be potted to larger containers as required.

Hygiene

Hygiene is very important at all stages of plant culture. Contamination by pathogens and/or insects at the propagation or young plant stage is likely to be passed on at each level of plant advancement, with an escalation of problems over time.

Use clean and sterile utensils and materials and always have clean working areas, containers, hands and clothing. When work has been completed, clean up the work area and equipment. Sterilise equipment (secateurs, knives, propagation containers and work areas) with a sterilising compound such as, 100% alcohol or a chlorine-based solution, and store equipment in a clean location. Do not use chlorinated products near insect colonies as minute doses may be deadly to them. Discard rubbish, particularly plant material and potting mix contaminated with insect pests, diseases or quarantined seed, into proper disposal units, not on the floor or benches.

Recycled potting mix, that has not been treated, should only be used for larger plants as these are usually more resilient to harmful microorganisms that

may be present in the mix. New potting mix should be used when propagating seeds and cuttings and potting germinated seed. Alternatively, recycled mix may be used if it has been properly heat treated e.g. pasteurised at 60°C for 30 minutes. If pasteurisation is not possible and the recycled mix is suspected of harbouring harmful microorganism, it should be treated with a suitable broad spectrum chemical pesticide (fumigant or drench) that is known to destroy the suspected pest.

Propagation/Growing Areas

Growing areas may be outdoors (in containers or in-ground), in shade houses, polythene houses, polycarbonate or glasshouses. Where possible they should be automatically irrigated to reduce labour costs, and to provide consistency in watering. All areas should be clean and allow free drainage of excess water and should meet the requirements of the plant species being grown.

Ideally propagation areas generally consist of a bed or bench supplied with heating and misting facilities. Seedlings and cuttings of many plant species develop more quickly when supplied with heat in the root region (bottom heat), thus minimising the time when the material is most vulnerable to pathogen attack and desiccation. Propagation areas should be away from general growing areas to minimise transfer of pests.

Propagation by Seed

Variation in seed size, shape and hardness should be considered when deciding on depth of sowing and how the seed is treated before sowing, in order to maximise germination.

While many seed varieties germinate rapidly when sown, others have a dormancy period, caused by physical (hard seed coat), chemical (inhibitors) or temperature sensitive (vernalisation or fire) barriers. Simple techniques have been developed to break these barriers. Physical barriers may be overcome by damaging the hard seed coat to allow water access into the seed, for example, by cutting the seed coat with a sharp tool, rubbing with an abrasive material, or immersion in boiling water, or for large seeds, such as macadamia nuts, cracking in a vice. The inhibitors providing chemical barriers are mostly in the soft flesh surrounding the seed. Removal of the flesh and thorough washing is generally enough to promote germination. Some flesh is pasty and very adhesive and may require soaking in a warm-water bath until it is dissolved. Seed from species in areas with seasonal cold or fire periods often have temperature sensitive barriers. Dormancy is probably controlled by chemical or physical barriers which are destroyed when subject to extreme cold (stone and pome fruit, etc.), or dry heat (many Australian native species of *Banksia, Acacia* and *Eucalyptus*).

Vegetative Propagation

Vegetative propagation has advantages over propagation from seed for the following reasons.

- Genetically identical material can be taken from individual specimens within a species or from specific segments of a particular specimen for which significant variability is probable if grown from seed.
- Hybrid species for which seed does not breed true can be replicated.
- Species for which vegetative material is abundant, but seed is very difficult to acquire, or extremely difficult to germinate and culture, can be grown.
- Cuttings from mature plants will often flower and fruit in a much shorter time than seedlings.

There are many types of vegetative propagation including tissue culture, cuttings, layering, marcotting, grafting, rhizomes, stolons, division, etc. Of these, cuttings (leaf, tip, stem, roots), are most commonly used.

Irrespective of the propagative form selected, factors to be considered include the following.

- High levels of hygiene are required.
- Turgidity and freshness of material for propagation must be maintained and the material planted as soon as possible after collection, as this will enhance the chances of root development and establishment.
- Which and when plant material is taken for propagation will be determined by the species and the intended use of the material. The material may be used to; boost plant numbers quickly, propagate identical specimens, rejuvenate old woody stock lacking in vigour, or produce mature, flowering, miniature specimens of large-growing species.
- A root promoting hormone such as indole acetic acid, indole butyric acid or naphthalene acetic acid should be used. These are available in liquid or powder and in varying strengths for specific purposes.
- Use bottom heat, which speeds callous formation and root development and so reduces chances of pathogen infection.

Seasonal changes can affect the root development capacity of cutting material. Different material may be needed at different times to achieve success. For example, young tips may be used during active growth periods or semi-hard greenwood or hardwood during dormant times. Cuttings of many species generally behave very poorly or fail completely if taken when specimens are in a reproductive phase of growth.

Conclusion

A broad and superficial overview of culturing potted plants has been presented. However, it should not be taken as the complete picture in the production of healthy plants. One area not covered, but of critical importance, is pest management. Any successful plant growing program must incorporate strategies for pest management. These should include; hygiene management, isolation of infested plants, use of biological controls when available and the judicious use of pesticides. It is strongly recommended that further reading dealing with the topics above be pursued. The book by Handreck and Black (1984) is an excellent starting and general reference.

Reference

- Handreck, K.A. and Black, N.D. 1984. Growing media for ornamental plants and turf. New South Wales University Press, Kensington, Australia. 401pp.

Aquatic Plant Cultivation

Introduction

Plant growth is dependent upon several essential factors which govern the plant's growth rate directly and indirectly, according to their levels and availability to the plant. They are divided as follows:

Primary

- temperature
- light
- nutrients

Secondary

- substrate nature
- water chemistry
- pests/disease
- competition

Primary factors directly affect the mechanisms of plant growth. One or more secondary factors indirectly affect or moderate these growth mechanisms by affecting:

- plant health
- plant physiology
- availability of primary factors

Shaun Winterton
Entomology Department, University of Queensland, QLD 4072
Australia

The interrelationships between primary and secondary factors are displayed in Figure 1.

Availability of primary factors may be affected, for example, by reduction of available light or nutrients through competition from other plants or algae, or by water chemistry limiting the availability of micro-nutrients to the plant via oxidation of trace metals into insoluble precipitates.

Plant growth can be defined using the Liebig 'Law of the Minimum' which states that: 'The growth of a plant is dependant upon the foodstuff presented to it in minimum quantities...' (Salisbury and Ross 1992), i.e. a limiting factor. Therefore, we can suppose that if one or more factor is limiting, then growth rate will be dependant upon the level of that factor(s), irrespective if all other growth factors are optimal.

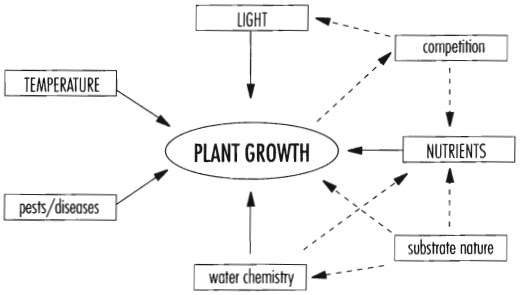


Figure 1. Interrelationships between primary (solid lines) and secondary (dashed lines) factors on plant growth.

The following is an outline of those factors that should be addressed when cultivating aquatic macrophytes. Emphasis and specific examples will mainly deal with floating and submerged weeds of economic importance, such as: *Eichhornia crassipes* Solms., *Salvinia molesta* Mitchell, *Pistia statiotes* L., *Elodea canadensis* (Michx.) Planch. and *Myriophyllum* species.

Primary Factors

Temperature

Temperature requirements for plants vary according to species. Generally, tropical and subtropical aquatic plants grow well at temperatures between 22 and 28°C, with growth rate slowed at lower temperatures. The optimal temperature range for a particular species is indicative of the plants native region; those species native to tropical areas may have relatively narrow temperature range while those from temperate areas have a much broader range. Moreover, temperate plants have a much lower temperature threshold for growth than plants from warmer regions. Such temperate weeds include *Elodea canadensis* which is a major weed in cooler southeastern Australia but is rarely found in the warmer regions of the north (Bowmer et al. 1995). Emergent and floating plants growing outdoors in temperate regions may suffer frost damage where frost occurs. For example, water hyacinth often suffers mortality of leaves high in the stand canopy when exposed to frosts (Center and Spencer 1981). The optimum temperature range for water hyacinth is 25 to 27°C, with growth ceasing when water temperature is below 10 and above 40°C (Gopal 1987).

Some sensitive, tropical species may be prone to rot (leaf shedding) if subjected to large diurnal changes in water temperature or when plants are transferred from warm to cold water too quickly without gradual acclimatisation.

Light

Adequate lighting is essential for plant growth. The amount of light needed depends on the requirements of the plant and varies with different species. Many weed species, particularly floating and emergent forms, grow well under a range of natural light levels but usually grow best in full sunlight. The amount of light and the spectrum received by the plant will often dictate the growth form. Water hyacinth development is related to the light intensity and the red/far-red wavelength ratio (z) (Méthy et al. 1990). Low light intensity and low z independently reduce growth rate and ramet production and increase petiole length and lamina area (Méthy et al. 1990; Méthy and Roy 1993).

Ideally, natural light is the best and least expensive form of lighting, particularly in tropical and sub-tropical regions where day length is long all year round. In this case most plants can be simply grown outdoors in pools or dams in full sunlight, or covered with shade-cloth to regulate light levels. In temperate regions supplementary artificial lighting may be necessary in winter when day length is shorter. Forms of artificial lighting used vary with the amount of light required and setting up and running costs. Fluorescent tubes are the cheapest form with a wide variety of different tubes available depending on the wavelength required. 'Horticultural' or 'Growth' tubes are the most common type, designed specifically for growing plants. Other, more expensive lighting types, with greater light output per unit area, are metal halide and high density sodium lamps.

Nutrients and fertilizers

There are 13 elements believed essential for plant growth (Table 1). They are divided into macronutrients and micronutrients depending on the relative optimum concentrations of each required by the plant. Generally nutrient solutions used for growing terrestrial plants hydroponically are suitable

also for growing aquatic plants although in a much diluted form (generally 10%). The concentrations of various nutrients in a fertilizer will vary depending on the species being cultivated, but most weed species will grow well with high levels of nutrients added. Table 1 lists concentration ranges of essential elements in hydroponic solutions. This list may be used as a base from which specialised recipes for particular species may be developed by experimentation. Very good recipes for nutrient solutions for hydroponic culture may also be found in Salisbury and Ross (1992) and Handreck and Black (1989).

Table 1. Acceptable ranges of nutrients in hydroponic cultures (Handreck and Black 1989)

Element		Concentration range ppm
Nitrogen	Nitrate	70–200
	Ammonium	0–31
Phosphorus		15–60
Potassium		200–400
Calcium		150–300
Magnesium		25–60
Sulfur		<50
Chloride		<350
Iron		0.5–14
Manganese		0.2–1.0
Boron		0.2–0.5
Zinc		0.04–0.2
Copper		0.02–0.1
Molybdenum		0.03–0.08

Most submerged aquatic plants absorb nutrients in a water soluble form either (i) directly through the surface of the leaves from the surrounding water, and/or (ii) from the substrate through their roots. Typically, submerged rosette-type plants (e.g. *Sagittaria*, *Vallisneria*, *Blyxa*) absorb most of their nutrients from the substrate, whereas elodeid-type plants (e.g. *Elodea*, *Lagarosiphon*, *Myriophyllum*, *Egeria*, *Ceratophyllum*) absorb a significant amount (although not all) of their mineral nutrient needs from the surrounding water (Barko et al. 1991). Therefore, for optimum growth, nutrients must be available to the plant in the water as well as in the substrate. Floating plants, with only their roots in water, do not require a fertilised substrate. They may be grown in simple plastic-lined pools filled with fertilizer added directly to the water. Conversely, emergent plants (e.g. *Typha*, *Monochoria*, reeds and sedges) and water lilies (*Nymphaea* spp.) derive all of their mineral nutrients from the substrate and require a well fertilised substrate.

Generally, the substrate is the primary source of phosphorus, iron, manganese and most micronutrients, while the open water is the primary source of calcium, magnesium, sodium, potassium, sulfate, and chloride (Barko et al. 1991), although varying levels of each can be found in both media. Nitrogen is found in a number of forms (nitrate (NO_3^-) and ammonium (NH_4^+) the most common for plant uptake) in both the sediment and open water.

Increasing levels of nutrients, in particular nitrogen and phosphorus, result in increased growth in most aquatic plants. There are many accounts in the literature of the effect of nutrient additions to the growth of nuisance aquatic plants, including: *Eichhornia crassipes* (Reddy et al. 1989; Reddy et al. 1990; Aoyama and Nishizaki 1993), *Trapa natans* (Tsuchiya and Iwaki 1983; Tsuchiya and Iwakuma 1993), *Azolla* spp. (Subudhi and Watanabe 1981;

Cary and Weerts 1992), *Elodea canadensis* (Madsen and Baattrup-pedersen 1995), *Salvinia molesta* (Room 1985; Room and Thomas 1986; Forno and Semple 1987) and *Myriophyllum* spp. (Barko 1983; Chambers and Kalff 1985).

The composition of nutrient fertilizers for aquatic plants and their method of application to plants in an aquatic medium is quite different to those for terrestrial plants. The behaviour of nutrients in water and the chemical form in which they are found is very different to a soil medium Water is generally an oxidising environment (i.e. oxygen rich) and elements such a iron (Fe), magnesium (Mg), phosphorus (P), etc. readily react with oxygen to form insoluble compounds (precipitates) which the plant cannot absorb. To keep these elements in solution longer and thus available to the plant longer, compounds called chelates must be added. The most common form used is Ethylene-diamine-tetra acetic acid (EDTA). In the substrate where oxygen tensions are low iron is mostly in its soluble form and therefore plants rooted in the substrate rarely need treatments of iron fertilizer to the water column.

Problems may occur when there is a deficiency or overdose (toxicity) in one or more nutrients. Deficiencies in particular nutrients usually manifest as characteristic external symptoms which may include any combination of:

- stunting,
- leaf or petiole discolouring (mottling, striations),
- premature leaf death,
- poor root systems.

A general diagnostic key to the identification of particular deficiency symptoms is presented in Salisbury and Ross (1992), with a more specific key to water hyacinth deficiency symptoms presented in Newman and Haller (1988).

Secondary Factors

Substrate nature

Characteristics of the substrate are particularly important for plants with roots embedded in the substrate. The substrate will affect the availability of nutrients and the ability of the roots to take up those nutrients. Characteristics which will have the greatest effect on plant growth are:

- pH,
- substrate temperature relative to water temperature,
- substrate depth,
- grade/size of particles,
- substrate reactivity (chemical stability),
- oxygen reduction potential (O.R.P) (i.e. anaerobic versus aerobic).

Water chemistry

The chemical make-up of the water will influence the availability of plant nutrients in it. As for the substrate factors such as pH and dissolved oxygen concentration will affect the availability of nutrients. Water alkalinity (i.e. carbonate content of the water) will dramatically influence the level of dissolved carbon dioxide in the water. Fortunately, most plants (particularly weed species) will tolerate a wide range of water conditions, therefore there is little use spending valuable time and resources attempting to change or condition the water to suit the plant. The emphasis should be put on preventing large fluctuations in water chemistry, which can have greater effects on plant growth.

Pests/disease

Various phytophagous arthropods and disease-causing pathogens affect an aquatic plant culture over time. The most commonly encountered pests are aphids,

spider mite and lepidopteran (caterpillar) larvae. All three may seriously damage emergent and floating parts of plants. Larvae of the aquatic caterpillar species (*Nymphula* and *Paraponyx* spp.) may cause severe damage to cultures of submerged plants.

Once a biological control agent has been successfully established in a region it is sometimes difficult to maintain an insect-free culture of the target plant. Effective control of insects is achieved with non-residual pyrethrin based insecticide spray, while mites are controlled with miticides. All plants suspected of being contaminated with insect or disease organisms (pathogens) must be isolated from the culture immediately and either treated chemically with appropriate sprays or destroyed. New plants from the field should also be quarantined to prevent introduction of insect and disease organisms to the culture.

Competition

As plants grow and multiply they will consequently begin to compete for resources (e.g. light, nutrients), and thus growth rate will be reduced. Periodic thinning or harvesting is required to reduce competition, thereby ensuring that there are adequate levels of all essential factors per plant. Periodic thinning is particularly important for water hyacinth which displays density dependant mortality (i.e 'self-thinning') at very high densities. Algae (planktonic and benthic) will often compete successfully for light and nutrients with higher aquatic plants, particularly in water with a high nutrient level. Therefore, good algal control is essential and acheived by; avoiding over-fertilising ponds, ensuring that ponds are fully stocked with aquatic plants to reduce the amount of open water available for algae growth and using shade cloth/covers. Algacides should be used only as a last resort, because, depending on their concentration, they damage not only the algae but also the culture plants.

General Guidelines to Follow

- Stability is the main aim of good plant growth, drastic fluctuations in light, temperature, nutrients, etc., will have negative and possibly lethal effects on plant growth.
- Do not over fertilise; fertilise often, with small amounts of fertilizer and look out for deficiencies in particular nutrients.
- Regular, daily monitoring of plant growth and health is essential.
- Periodically remove excess plants to maintain the culture in a state of active growth by reducing competition from other plants.
- Regularly check for pests and disease; isolate or destroy infected plants.
- Monitor water temperature and heat if required.

References

- Aoyama, I. and Nishizaki, H. 1993. Uptake of nitrogen and phosphate, and water purification by water hyacinth *Eichhornia crassipes* (Mart.) Solms. Water Science and Technology 28, 47–53.
- Barko, J.W. 1983. The growth of *Myriophyllum spicatum* L. in relation to selected characteristics of sediment and solution. Aquatic Botany 15, 91–103.
- Barko, J.W., Gunnison, D. and Carpenter, S.R. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquatic Botany 41, 41–65.
- Bowmer, K.H., Jacobs, S.W.L. and Sainty, G.R. 1995. Identification, biology and management of *Elodea canadensis*, Hydrocharitaceae. Journal of Aquatic Plant Management 33, 13–19.

- Cary, P.R. and Weerts, P.G.J. 1992. Growth and nutrient composition of *Azolla pinnata* R. Brown and *Azolla filiculoides* Lamarck as affected by water temperature, nitrogen and phosphorus supply, light intensity and pH. Aquatic Botany 43, 163–180.
- Chambers, P.A. and Kalff, J. 1985. The influence of sediment composition and irradiance on the growth and morphology of *Myriophyllum spicatum* L. Aquatic Botany 22, 253–263.
- Center, T.D. and Spencer, N.R. 1981. The phenology and growth of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in a eutrophic north-central Florida lake. Aquatic Botany 10, 1–32.
- Forno, I.W. and Semple, J.L. 1987. Response of *Salvinia molesta* to insect damage: changes in nitrogen, phosphorus and potassium content. Oecologia 73, 71–74.
- Gopal, B. 1987 Aquatic Plant Studies 1: Water Hyacinth. Elsevier Amsterdam 471pp.
- Handreck, K.A. and Black, N.D. 1989. Growing Media for Ornamental Plants and Turf. 2nd edn. New South Wales University Press, Australia. 401pp.
- Madsen, T.V. and Baattrup-Pedersen. 1995. Regulation of growth and photosynthetic performance in *Elodea canadensis* in response to inorganic nitrogen. Functional Ecology 9, 239–247.
- Méthy, M., Alpert, P. and Roy, J. 1990. Effects of light quality and quantity on growth of the clonal plant *Eichhornia crassipes*. Oecologia 84, 265–271.
- Méthy, M. and Roy, J. 1993. Morphogenetic changes induced by a low red: far-red ratio and their growth consequences in water hyacinth (*Eichhornia crassipes*). Journal of Experimental Botany 44, 1275–1280.
- Newman, S. and Haller, W.T. 1988. Mineral deficiency symptoms of water hyacinth. Journal of Aquatic Plant Management 26, 55–58.
- Reddy, K.R., Agami, M. and Tucker, J.C. 1989. Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. Aquatic Botany 36, 33–43.
- —1990. Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. Aquatic Botany 37, 355–365.
- Room, P.M. 1985. Equations relating growth and uptake of nitrogen by *Salvinia molesta* to temperature and the availability of nitrogen. Aquatic Botany 24, 43–59.
- Room, P.M. and Thomas, P. A. 1986. Population growth of the floating weed *Salivinia molesta*: field observations and a global model based on temperature and nitrogen. Journal of Applied Ecology 23, 1013–1028.
- Salisbury, R.B. and Ross, C.W. 1992. Plant Physiology. 4th edn. Wadsworth Publishing Co. California USA. 682pp.
- Subudhi, B.P.R. and Watanabe, I. 1981. Differential phosphorus requirements of *Azolla* species and strains in phosphorus-limited continuous culture. Soil Science and Plant Nutrition 27(2), 237–247.
- Tsuchiya, T. and Iwaki, H. 1983. Biomass and net primary production of a floating-leaved plant, *Trapa natans*, community in Lake Kasumigaura, Japan. Japanese Journal of Ecology 33, 47–54.
- Tsuchiya, T. and Iwakuma, T. 1993. Growth and leaf life-span of a floating-leaved plant, *Trapa natans* L., as influenced by nitrogen flux. Aquatic Botany 46, 317–324.

The Importance of Taxonomy in Biological Control Studies

Introduction

The buzzword biodiversity has gained popularity, focussing on the spectrum in species of animals and plants and their importance in stable environments. The book, *The Diversity of Life*, by E.O. Wilson (1992) has attracted international attention and stimulated renewed awareness of the fragmented knowledge in the identity of living organisms. Taxonomy is the 'theory and practice of classifying organisms' (Mayr 1969). Taxonomy provides a framework for classification of individuals and/or populations of organisms according to their similarities. The basis for studies of biodiversity is therefore sound taxonomy.

Biological control seeks to reduce by biological means the abundance of weeds, usually exotic. Since most plants are stabilised in their native range by competition and natural enemies as components of overall diversity, biological control of any weed species is most likely to succeed if programs commence by understanding the plant in its natural environment. Knowledge of the identities of the plant, its relatives and the herbivores and pathogens associated with a plant, is essential for such projects but the biological control worker is often faced with a scarcity of this information.

Don Sands
CSIRO Entomology
Private Bag 3, Indooroopilly, QLD 4068
Australia

For new biological control projects the identity of a target weed may not be well defined, its native distribution poorly known and potential agents not identified or properly documented. Identification of a weed is necessary at all taxonomic levels to establish its origin, distribution (indigenous and exotic), relationships with other plants (especially for specificity testing with agents) or uses. Accurate identification is also needed to enable decisions relating to searching for, or selecting, new agents for further testing. A weed at first thought to be one taxon, is sometimes found to be a complex of closely-related taxa.

At an early stage in biological control projects, taxonomic studies are also required for natural enemies associated with the target weed or related plants, and to correlate identities of the natural enemies with their biological characteristics (biosystematics). However, it is doubtful if accurate predictions can be made as to whether an agent will be effective, even when the nature of the agent–plant interaction has been studied (Marohasy this volume). Sometimes closely-related agents have an entirely different capacity to achieve biological control of a particular weed, for example the weevils used for control of salvinia (Sands and Schotz 1985). Further, an agent may be capable of achieving control of a particular form of weed but ineffective in controlling closely-related taxa (subspecies, varieties, forms) of the same plant species, though they can develop on them. Recognition of these different plant taxa in the native range may assist in the selection of biotypes of agent species (i.e. morphologically indistinguishable

populations but with different biological characteristics) better adapted to each of the forms of weeds (Sands and Harley 1981), a not uncommon situation (e.g. different agents are better adapted to different varieties of lantana). Occasionally it may be necessary to assess the host interactions of biotypes of agents as if they were different species.

If the taxonomy of a weed can be first addressed at the exploratory stage, then the more complex process of selecting effective agents will be facilitated. Identifying herbivores associated with a weed in the native and exotic range requires experts for each potential agent group (usually for an insect family). Quite often the taxa are more complex than at first thought by specialists. Moreover, agents are often located and sometimes successfully utilised before their identity has been established. The discoveries of new agents and their biology by biological control workers have frequently led to the description of new species by taxonomists.

Only those classification procedures and taxonomic complexities most commonly encountered by biological control workers on weeds are discussed below.

The Plant Kingdom

Levels of classification

Divisions

Thallophytes

Algae
- Cyanophyta (blue-green algae)
- Euglenophyta (euglenoids)
- Chlorophyta (green algae
- Chrysophyta (yellow-green algae and diatoms)
- Phaeophyta (brown algae)
- Pyrrophyta (dinoflagellates)
- Rhodophyta (red algae)

Fungi
- Schizomycophyta (slime moulds)
- Eumycophyta (true fungi)

  Classes

  - Phycomycetes, Ascomycetes, Basidomycetes (Lichens)
  - Deuteromycetes (fungi imperfecti)

Bryophytes
- Bryophyta (mosses, liverwarts)

  Classes

  - Musci, Hepaticae, Anthocerotae

Tracheophytes
- Psilophyta (fork ferns)
- Lycophyta (clubmosses)
- Sphenophyta (horsetails)
- Polypodiophyta (ferns and fern allies)

Seed-bearing plants

Pinophyta (conifers and cycads)

Magnoliophyta (or Angiospermae) (flowering plants)

Class
Subclass
Order
Suborder
Tribe
Subtribe
Family
Genus
species
subspecies
variety
form

Using the lower levels of classification

There are several differences in the application of scientific names and authors applied to the taxonomy of plants when compared with animals. Only the differences that commonly cause confusion for biological control workers are discussed.

For plants, as well as subspecies, varieties and forms have status as scientific names at lower than species level. Each has an author, placed in brackets if the status of genus or species is recombined. The name of a reviser is also included following the author (then placed in brackets). The name of a subsequent reviser replaces an earlier reviser.

Preserving and identifying plant specimens

Plant specimens are best preserved by pressing flat using a plant press. When dry, pressed specimens can be held in cardboard containers with naphthalene flakes as a preservative. All information relating to locality, date, collector, altitude (if known) should be placed with the specimen and accompanied by a specimen number (see accession register). Flowers, seeds too large to press or other parts of the plant can be preserved in 70% alcohol.

Identification can usually be carried out by botanists attached to a national herbarium but it is sometimes necessary to send specimens overseas to specialists in a particular plant group.

Field-collected plant material and associated organisms

Developing a herbivore profile for a weed

Prior to release of exotic agents it is most important to document the indigenous herbivores that feed on weeds. This enables plant damage to be associated with species already present, to measure their impact before liberation of exotic agents, and to avoid attributing damage by indigenous species to exotic agents after their release (Farrell and Lonsdale this volume).

The accession register

Whenever plant material is collected from the field a record should be made in an accession register. The best method consists of using a lettering system followed by numerals as follows:

The Accession Reference No.

Use two appropriate letters to represent each different locality;

Use numbers to represent different sites, or plant species, or dates of collection.

The accession register provides a record for all collections of plant specimens from the field, associated insects, fungi and other organisms, with a cross reference to specimens (e.g. vouchers) and any other information relating to a plant specimen. The accession number can easily be attached to insect specimens while they await formal labelling.

Examples:

MC 44 represents Mt Coot-tha, 18 April 1993, collected by D.P.A. Sands, specimen of *Stephania japonica* on which larvae of *Eudocima salaminia* were present.

MC 45 represents same locality, date and collector but the plant was *Sarcopetalum harveyanum* with no insects present.

MC 46 was the same as MC 44 but collected 5 days later.

The Animal Kingdom

Levels of classification

Phylum: Arthropoda

Superclass: Hexapoda

Class and Order: (Collembola, Protura, Diplura)

Class: Insecta

Subclass
Superorder
Order
Suborder
Superfamily
Family
Subfamily
Tribe
Subtribe
Genus
Subgenus
species
subspecies

Taxonomy of insects

In biological control programmes it is important to use the most accurate names for the genus and species of agents, if names are available. If the species is undescribed or cannot be identified, it should be given a convenient reference number. Avoid referring to an unidentified organism as species near so-and-so in documents, since it is common practice for the name of the related species to be incorrectly applied to the unidentified taxon in the literature at a later date.

The genus (first letter in capital) and species (first letter in lower case) are always written in *italics* or underlined. Subgenera or species groups are sometimes used in classification of closely related species but their use is not necessary for biological control workers. When referring to a species in a document, the genus name may be abbreviated to the first letter followed by a full stop after its first usage in full, provided no other taxon referred to in the document can be confused with it (i.e. by having the same species name).

For rules relating to the application and use of scientific names in taxonomy, refer to the International Code of Zoological Nomenclature (Ride et al. 1985). It is updated from time to time by the International Commission on Zoological Nomenclature.

The following guide notes address the most common questions relating to taxonomy raised by workers in biological control.

The trinomial system of zoological nomenclature

Formal categories

Genus
Used as a group for the most closely-related species.

Species
For the majority of distinguishable insect taxa a binomial system of formal names is applied. The species (abbreviated sp. or plural spp.) name is always accompanied by the genus in documents. Sibling species are morphologically similar species that have been shown to represent distinct, reproductively-isolated taxa. Their formal description is often avoided due to the difficulty of separating preserved specimens from other similar species.

Subspecies
The trinomial system may be applied to geographically distinguishable populations of a species, when subspecies (abbreviated ssp.) are described. A subspecific name is added after the species name (+ author's name for the new subspecies name when first used). The original species is referred to as the nominotypical subspecies. The species name is repeated to indicate a trinomial status followed by that author's name.

Informal categories

Clines
A cline is a gradient in a measurable character within a species. Sometimes populations within a cline are given subspecific, trinomial names. The term is most commonly applied to geographical variation when populations interbreed with neighbouring populations and show a gradual change, rather than an abrupt one.

Biotypes
Biotypes have no formal taxonomic status but they may be significant in biological control studies. The term refers to populations of a species which although morphologically indistinguishable, exhibit different biological characteristics. Quite often subsequent detailed studies reveal morphological differences in populations which enable their description as a separate species or subspecies.

An 'author' of a taxon and how is it applied

The author of a taxon is the family name of the person that first described the taxon. The authors' name is used (with first letter upper case in standard type) following the scientific name when first referred to in a document. The author's name is placed in brackets if, after taxonomic revision, the species is placed in a genus different from the one used by the author for description of the species or subspecies.

Type specimens

'Types' are those specimens that are used ('designated' as holotype and paratypes) by an author for the description of a new taxon, or when a single specimen (lectotype) is selected by a subsequent author from a series (of syntypes) used by the original author to describe a taxon (the remainder are designated paralectotypes). In special circumstances if a specimen is needed to fix the identity of a species, as a basis for distinguishing a species (e.g. when types are lost), a neotype may be designated by a subsequent author to define the identity of a species only known from the literature. An allotype is sometimes designated as the opposite sex to the holotype and it has the same status as a paratype.

Dates of publication for scientific names and synonyms

All scientific workers are familiar with, and frustrated by the changes that occur in the use of scientific names. The date for first publication of the description of a taxon is important in deciding on the validity of scientific names. Changes in names usually result: (i) from discovery of a valid, published name for a taxon which pre-dates a name (which becomes a synonym) in common usage. The earlier name, if valid, is said to have priority; (ii) when the description of a new genus results in a new combination for a species better known in another genus, and (iii) when a name used for a taxon has been applied incorrectly and belongs to a different species.

Sympatric and allopatric distribution of taxa

When two taxa breed in the same geographical area without hybridising their distribution is said to be sympatric. Conversely, when their breeding populations are geographically separated their distribution is said to be allopatric.

Guide to Identifying and Labelling Insect Specimens

The importance of voucher specimens

Voucher specimens are those that have been examined by a specialist, determined as a certain taxon and labelled accordingly. The full scientific name with author should always be placed on a label for voucher specimens. For biological control workers, it is desirable to provide a specialist with a good series of specimens (so that they will recognise a mixture if collected), to ensure that they are all individually properly labelled and that they have a reference number attached to each specimen. A mixture of sexes should be provided when available and the specialist asked to return labelled representatives (if sufficient are available) so that they can be used for further reference.

The biological control worker can then make provisional identifications based on a comparison of specimens with voucher specimens. However, these determinations should never be regarded as authoritative without further reference to a specialist. A most useful guide can be found in Dahms et al. (1994).

Voucher specimens should be retained from:

1) original consignments (even if damaged),
2) representatives of a species first released in the field, and
3) specimens recovered after establishment is confirmed.

Whenever possible, voucher specimens of exotic taxa proposed for import should be lodged with an approved institution, prior to an application for a permit to import.

The identification register

Maintaining the identification register forms an essential part of all biological control projects. It provides a record for all arthropod specimens submitted to taxonomists or organisations when identification is needed or requires confirmation. A registration number should be entered in the register corresponding with a number attached to every specimen (or enclosed with specimens if held in containers of preservative).

Methods for labelling insect specimens

Labels should be attached to the pin holding a dried specimen and written with water proof ink unless printed by an acceptable method. For specimens held in liquid preservative the label should be placed in the same tube with the specimen. Labels written in ink are prone to dissolve in liquid preservatives. It is better to use pencil.

Information on the label should include:

Essential

Locality (Country, State and site names), date, collector.

Desirable

Latitude and longitude, altitude, host (if confirmed) plant, habitat, substrate.

Methods for Packing Preserved Specimens for Consignment

Pinned specimens should be pressed firmly into the cork or foam plastic substrate in the specimen container. Cross pins for large specimens or pins placed each side of the abdomen for small specimens should be used to prevent side movement. If there is any doubt about the cleanliness or presence of living organisms (especially psocids, beetles, mites) then the container should be placed in the deep freeze prior to packing. Similarly, if there is a possibility that specimens received are carrying living pests it is wise to fumigate or at the very least, place in deep freeze for at least 24 hours. Take care when thawing as frozen insects are very brittle.

When consigning specimens it is best to place the closed specimen box in a plastic bag containing a little powdered naphthalene as preservative. Select a larger container, preferably rigid cardboard, with sufficient volume to hold a loose packing (shredded paper, foam etc.) material, 2–4 cm thick surrounding the bag containing the specimen box. Fill the cardboard box completely but not firmly, with packing material before sealing with adhesive tape. Containers holding specimens should always be labelled 'FRAGILE'.

Tubes containing specimens are best held in a plastic foam block, excavated to hold the specimen tube. The block with enclosed tube should then be placed in a firm container surrounded by loose packing.

Legal Restrictions for Export and Import of Living and Dead Specimens

Certain insects are protected by an international agreement on wildlife trade, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). There is a requirement for most countries, quite separate from restrictions imposed by national or state quarantine and national parks authorities, to declare that a consignment containing any living or dead insect does not contain prohibited species. Containers with living insects should be also labelled 'living beneficial insects' and 'open only in presence of consignee'.

In many countries all native or exotic insects, or some species (live and/or dead) are restricted exports. Export permits are required before air freight or postal carriers are permitted to accept international containers of insect specimens. Copies of the permits should always accompany a consignment and be easily accessed without having to open the container.

Preservation and Mounting of Insect Specimens

The identification of indigenous insects and potential agents associated with a weed requires careful preparation of specimens for identification by specialist taxonomists.

Specimens mounted by pinning and drying

Kill the specimen using ethyl acetate, cyanide, deep freeze, or saturated oxalic acid injection, maintain humidity for a maximum period of 24 hours until pinned or glued on card, then air dry.

- Adults of:
  Lepidoptera (moths); Coleoptera (beetles); Diptera (flies), Hemiptera (bugs); Orthoptera (grasshoppers, crickets); Mantodea (mantids and mantispids); Blattodea (cockroaches).

All mounted specimens should be stored with powdered naphthalene.

Specimens collected directly into alcohol (75%)

- Adults of:
  Arachnida (spiders); Dermaptera (earwigs); Thysanoptera (thrips); Strepsiptera.
- Immature stages of:
  Hemiptera (bugs, leafhoppers, scale insects etc.); Phasmatodea (stick insects); Orthoptera (crickets, grasshoppers); Blattodea (cockroaches).
- All stages for-:
  Siphonaptera (fleas).

Specimens collected into fixative then preserved in alcohol (75%)

Specimens should be placed in a fixative such as KAA or Carnoys fluid for 1 hour before being transferred to alcohol.

- Immature stages:
  Lepidoptera (moths, butterflies); Hymenoptera (wasps, bees, ants); small Diptera (flies); Neuroptera (lacewings).

Drop into hot water (90–95°C) for 2 minutes, then preserve in alcohol (75%)

- Larvae of large Diptera (flies)

Slide mounts

After using a fixative, slide mount: insect genitalia and wings for venation studies for all groups; adults of Thysanoptera (thrips), Strepsiptera, some Hymenoptera (small wasps). After maceration, slide mount: Coccidae (scale insects, mealybugs).

References

- Dahms, E.C., Monteith, G. and Monteith, S. 1994. Collecting, preserving and classifying insects. Queensland Museum, Brisbane. 28pp.
- Mayr, E. 1969. Principles of Systematic Zoology. Mc Graw-Hill, New York. 428pp.
- Ride, W.D., Sabrosky, C.W., Bernardi, G. and Melville, R.V. ed. 1985. International Code of Zoological Nomenclature. 3rd edn, London, International Trust for Zoological Nomenclature in Assoc. British Museum (Natural History). 338pp.
- Sands, D.P.A. and Harley, K.L.S. 1981. Importance of geographic variation in agents selected for biological control of weeds. In: Delfosse, E.S. ed. Proceedings of the V International Symposium on Biological Control of Weeds, Brisbane, 1980. CSIRO, Melbourne. 81–89.
- Sands, D.P.A. and Schotz, M. 1985. Control or no control: a comparison of the feeding strategies of two salvinia weevils. In: Delfosse, E.S. ed. Proceedings of the VI International Symposium on Biological Control of Weeds. Vancouver, 1984. Agriculture Canada. 551–556.
- Wilson, E.O. 1992. The Diversity of Life. Penguin Press. 424pp.

Climate Matching Using the CLIMEX Program

Introduction

The distribution and abundance of plants and animals, particularly poikilothermic animals that have a body temperature that fluctuates with the temperature of the environment, are influenced by climate (Andrewartha and Birch 1954). Introduced pests generally proliferate in regions with a climate similar to or more suitable than that of the region of origin. The better adapted a potential biological control agent is to the climate of the region of introduction, the greater the chance that it will establish and become abundant (Marohasy this volume). Matching the climate of the region of introduction with that of the region of origin allows the potential geographic range of an invading species to be predicted. Conversely, exploration for potential agents in the region of origin can be guided by matching the climate with that of the region of introduction.

Estimating potential geographic distributions without computers would be impractical. Computer modelling programs can be used as tools in predicting the spread of a weed as a basis for economic evaluation of impact of the weed (Adamson this volume). One such program is the CLIMEX model. While CLIMEX cannot estimate the impact that a biological control agent will have on a weed species, it can help to identify those agents which have a similar potential distribution to the target weed. Agents whose potential distribution matches poorly with the distribution of the weed can then be given lower priority in the biological control investigation, saving time and resources.

Gunter Maywald and Robert Sutherst
CSIRO Entomology
Cooperative Research Centre for Tropical Pest Management
Gehrmann Laboratories, University of Queensland, QLD 4072
Australia

An Overview of CLIMEX

CLIMEX is a dynamic simulation model which manipulates available meteorological data to estimate an animal or plant's geographic distribution and relative abundance as determined by climate.

CLIMEX is applied to different species by selecting the values of a series of parameters which describe the species' response to temperature and moisture. An annual population 'Growth Index' (GI_A) describes the potential for growth of a population during the favourable season and four stress indices (Cold, Hot, Wet and Dry) describe the probability of the population surviving through the unfavourable season. The GI_A and Stress Indices are combined into an 'Ecoclimatic Index' (EI) to give an overall measure of favourableness of the location or year for permanent occupation by the target species. The results are presented as tables, graphs or maps.

A species' climatic requirements are inferred from its known geographical distribution (its native range or in another location where it is has been introduced), relative abundance and seasonal phenology. Some life cycle data, such as developmental threshold temperatures, can be used to fine tune or interpret the

CLIMEX parameter values. Initial estimates of parameter values are refined by comparing predictions with the known presence or absence, or preferably relative abundance, of the species in each location. Once parameter values have been estimated, CLIMEX can be used to make predictions for other, independent locations from a database of meteorological data from nearly 3000 locations worldwide.

CLIMEX assumes that the distribution of a species is solely determined by climate. This 'potential' distribution is often modified by physical and biological factors such as soil type, microclimatic factors, topography, food quality and availability, parasites, predators and pathogens. The impact of these modifiers must be considered when making assessments of CLIMEX predictions. Past experience indicates that effects are marginal compared to climate in the majority of cases.

CLIMEX assumes that animal and plant populations experience a season which is favourable for population growth and one that is unfavourable and may jeopardise its persistence in a given area. In practice exceptions do occur, with annual plants for example, frequently not being limited by the conditions during the period that is unfavourable for growth because they are in the seed stage. There are also small areas of the world, in East Africa for example, where there are two seasons of favourable and unfavourable conditions each year associated with bimodal rainfall distributions. The former case can be handled with CLIMEX, but the latter is not easily included at present.

Systems Requirements

CLIMEX (Windows Version 1.0) requires an IBM compatible 486 computer or higher running Microsoft Windows 3.1. As a minimum standard, a 486 66MHz PC and a VGA or SVGA monitor are required. Fully installed, CLIMEX requires approximately 7 Megabytes of space on the hard disk.

The Indices

The CLIMEX model uses growth-related indices and stress indices to estimate the potential for growth and survival of a population of a species at a given location, and integrates the indices into the Ecoclimatic Index. Understanding these indices makes their use and interpretation of CLIMEX easier and removes some of the guesswork from prediction and analysis (Sutherst et al.1995).

Growth-related indices relating to seasonal activity patterns and relative abundance are:

- Annual Growth Index (GI_A)
- Weekly Growth Index (GI_W)
- Temperature Index (TI)
- Moisture Index (MI)
- Diapause Index (DI)
- Light Index (LI).

These indices are estimated weekly, except for the Annual Growth Index.

Annual stress indices, relating to conditions during the unfavourable season that limit the geographical distribution, are:

- Cold Stress (CS)
- Heat Stress (HS)
- Dry Stress (DS)
- Wet Stress (WS)
- Stress Interactions (Cold/Wet, Cold/Dry, Hot/Wet, Hot/Dry)

Ecoclimatic Index (EI)

The Ecoclimatic Index (EI), scaled between 0 and 100, integrates growth and stress indices on an annual basis to give a single measure of the potential of a given location to support permanent populations of a species at various levels of abundance (Sutherst and Maywald 1985). The index provides simple, concise information on which to base policy decisions.

EI is derived as the annual mean of weekly values of GI (this determines the potential for the population to increase) reduced by the stress indices:

$$EI = GI_A \times SI \times SX$$

where:

$$GI_A = 100 \left(\sum_{W=1}^{52} GI_w \right) / 52 ,$$

$$GI_W = TI \times MI \times DI \times LI ,$$

$$SI = (1–CS/100)(1–DS/100)(1–HS/100)(1–WS/100),$$

and

$$SX = (1–CDX/100)(1–CWX/100)(1–HDX/100)(1–HWX/100)$$

Major Functions

CLIMEX may be used to predict relative abundance of a species in different places (Compare Locations function), based on long-term averages of meteorological data, and to predict relative abundance of a species in the same place under climatic data in different years (Compare Years function).

The 'Match Climates' function of CLIMEX is used to search the meteorological database for locations with climates similar to that of a nominated location. The level of similarity is given by the 'Match Index' with a range from 0 to 100, which is the product of five component indices, I_{tmax}, I_{tmin}, I_{rtot}, I_{hum} and I_{rpat} (maximum temperature, minimum temperature, total rainfall, relative humidity and rainfall pattern, respectively). Each of these indices range between 0 and 1, with a value of 1 indicating an exact match in the corresponding variable. All five indices can be weighted so that any climate match can have more (or less) emphasis on one or more indices. The default setting is for each of the indices to be weighted evenly, except for relative humidity which is not used.

Results from the three functions can be displayed as a map, table or graph.

An Example: Alligator Weed and a Biological Control Agent (based on Julien et al. 1995)

Alligator weed, *Alternanthera philoxeroides*, invades a range of habitats from dry terrestrial to aquatic, where it may be rooted into the bank or substrate beneath shallow water or may form independent free-floating mats. It is a northeast Argentinian species that has invaded the Americas, Asia and Australia and some of the adjacent island countries. In USA and China alligator weed has reached the limits of its distribution, but is expanding its distribution in Australia. To assist planning for surveillance, quarantine and control, it would be useful to predict areas that would support growth of this weed in Australia, and elsewhere.

A flea-beetle, *Agasicles hygrophila*, was introduced to USA in the 1960s and achieved control of the weed in the southern portion of its range. Following introduction to Australia, the flea-beetle achieved control of aquatic infestations of the weed. Again, to assist with weed management it would be useful to use current knowledge to predict where this biological control agent could be successful.

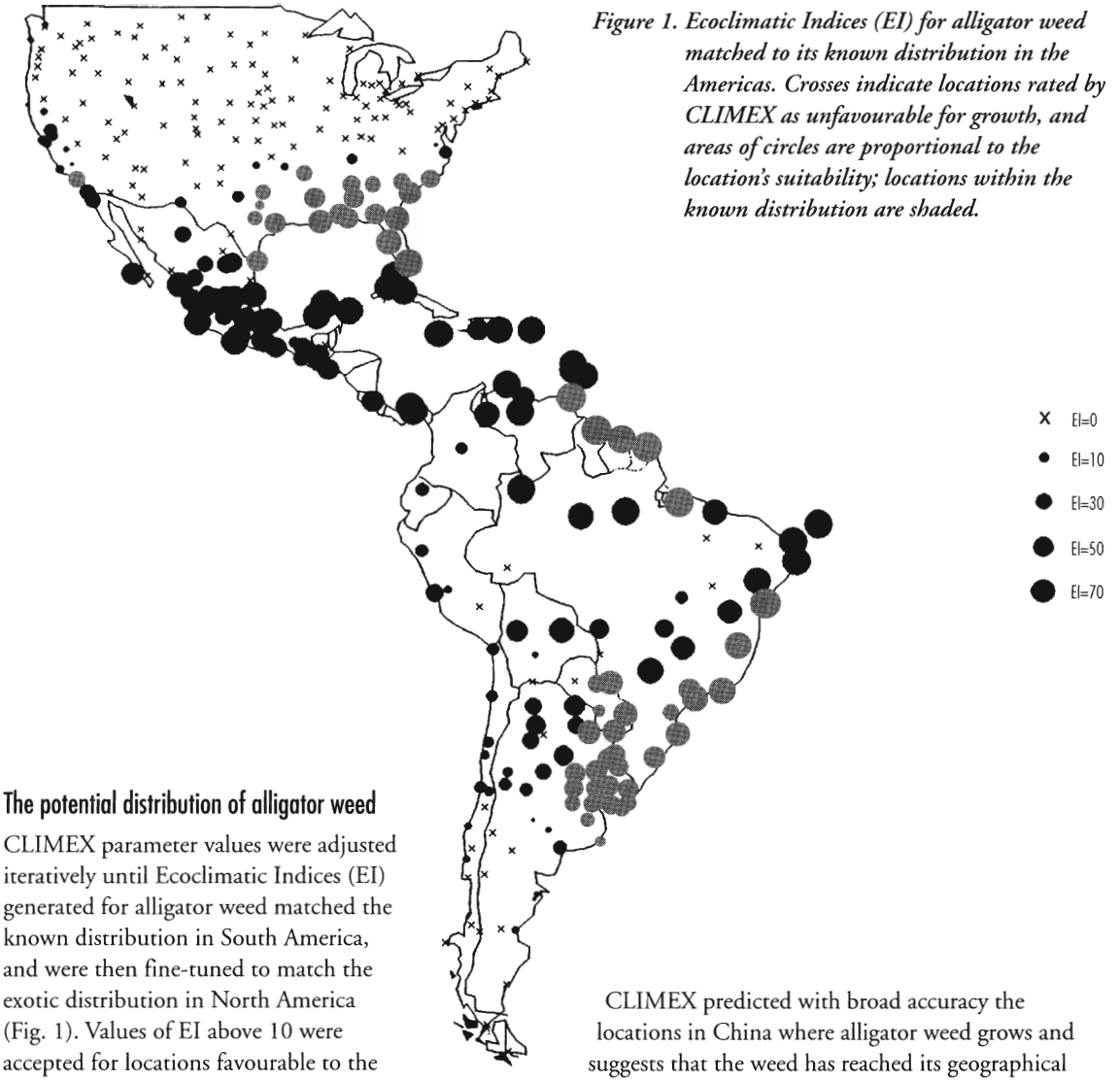


Figure 1. Ecoclimatic Indices (EI) for alligator weed matched to its known distribution in the Americas. Crosses indicate locations rated by CLIMEX as unfavourable for growth, and areas of circles are proportional to the location's suitability; locations within the known distribution are shaded.

The potential distribution of alligator weed

CLIMEX parameter values were adjusted iteratively until Ecoclimatic Indices (EI) generated for alligator weed matched the known distribution in South America, and were then fine-tuned to match the exotic distribution in North America (Fig. 1). Values of EI above 10 were accepted for locations favourable to the growth of alligator weed. Moisture stress indices were not used because of growth of the weed in aquatic, swampy and high water table habitats. The parameter values were then applied to predictions of potential distribution in Asia (Fig. 2), Australia (Fig. 3), and Africa and Europe (Fig. 4).

CLIMEX predicted with broad accuracy the locations in China where alligator weed grows and suggests that the weed has reached its geographical limit in that country (Fig. 2), thus validating the CLIMEX model of the species. Large parts of Australia (Fig. 3) and Africa (Fig. 4) where alligator weed is not yet present are favourable for growth of the weed, and so are at risk should the weed be introduced.

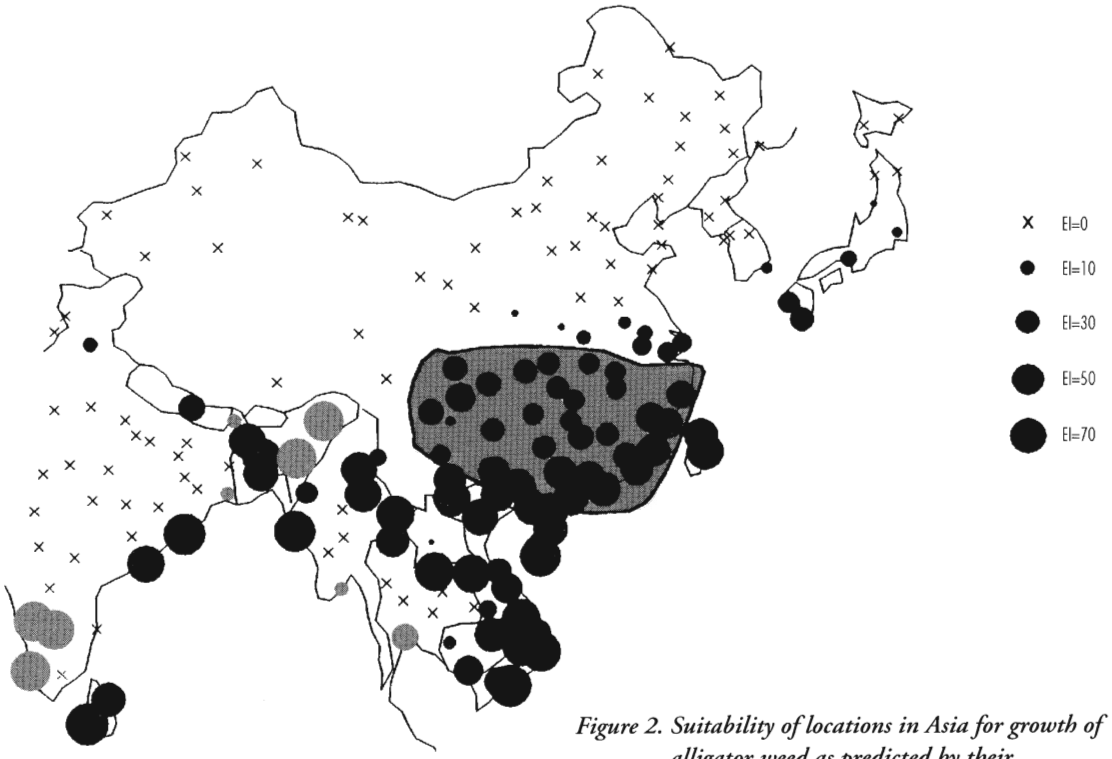


Figure 2. Suitability of locations in Asia for growth of alligator weed as predicted by their Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable, and areas of circles are proportional to the suitability of the locations growth; locations within the known distribution are hatched shaded.

The potential distribution of the alligator weed flea-beetle

As for the plant, CLIMEX parameter values were adjusted iteratively until Ecoclimatic Indices (EI) generated for the flea-beetle matched the known distribution in South America (Fig. 5). Comparison of predictions for North America based on these parameters (Fig. 5) with knowledge of localities where the flea-beetle effectively controlled the weed suggested that locations with an EI between 0 and 25 may support populations of the beetle, but the effects of climate limit population increase and prevent control of the weed. The potential distribution of the flea-beetle and areas of likely success in controlling alligator weed, i.e. where the EI exceeded 25, were predicted for Asia (Fig. 6), Australia (Fig. 7), and Africa and Europe (Fig. 8).

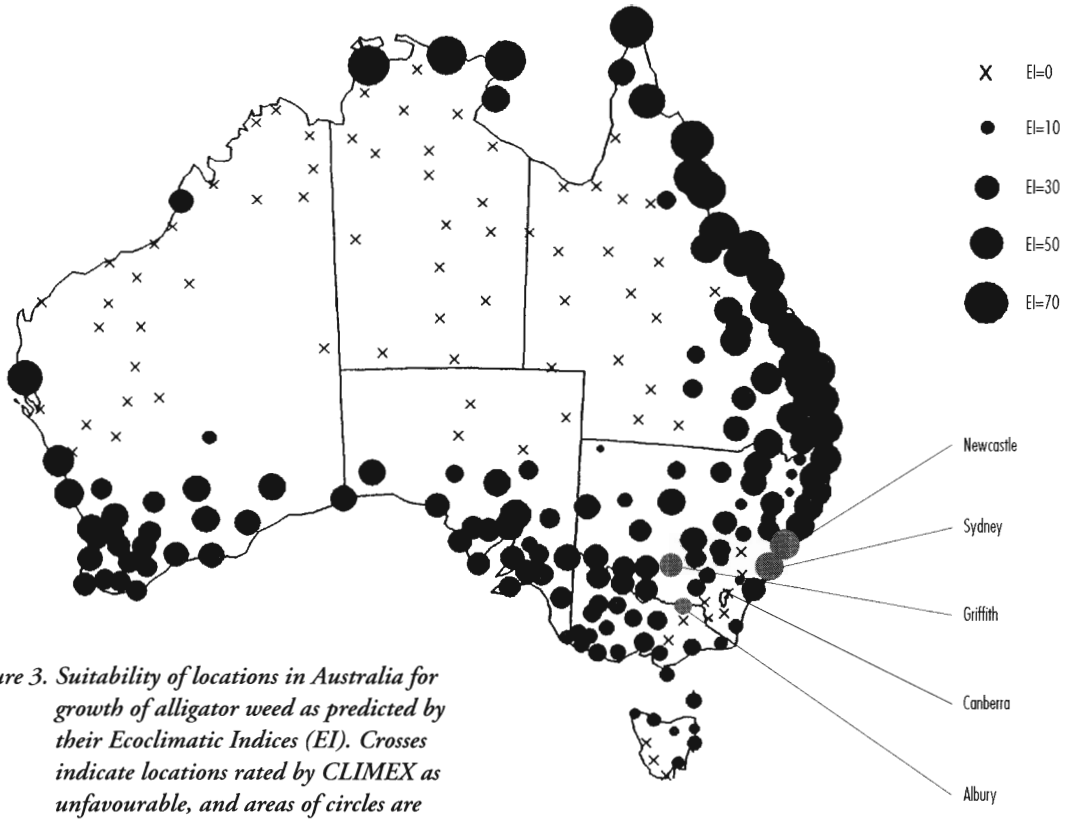


Figure 3. Suitability of locations in Australia for growth of alligator weed as predicted by their Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable, and areas of circles are proportional to the suitability of the locations growth; locations within the known distribution are shaded.

Comparison of the potential distributions for alligator weed and the flea-beetle indicate that the beetle has a more restricted distribution than the weed, and is likely to be successful in controlling the weed over only part of the potential range of the weed. In Australia the beetle is likely to be successful in controlling infestations of alligator weed if they develop in temperate and subtropical coastal areas, but is unlikely to be effective in the southern cooler temperate areas, in the most northern coastal tropics or in inland river systems where alligator weed poses a threat.

Control of alligator weed

On the basis of CLIMEX predictions, it is obvious that alternatives to the flea-beetle will be needed for control of alligator weed over much of its potential range.

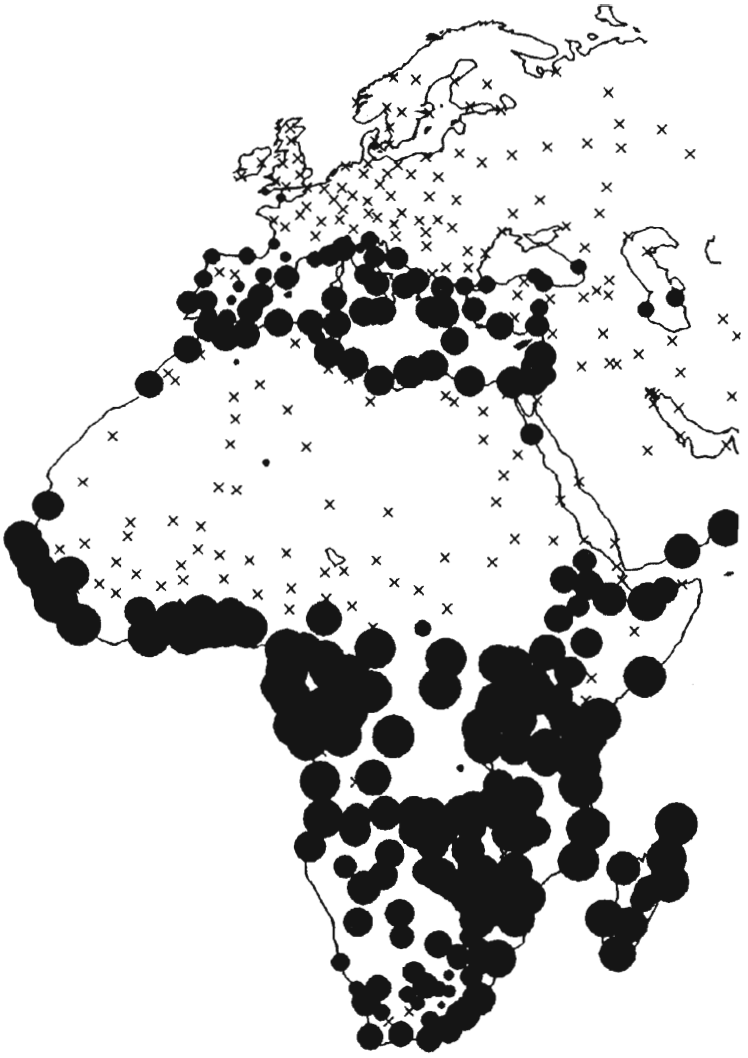


Figure 4. Suitability of locations in Africa and Europe for growth of alligator weed as predicted by their Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable, and areas of circles are proportional to the suitability of the locations.

Figure 5. Ecoclimatic Indices (EI) matched to the known distribution of the alligator weed flea-beetle in the Americas. Crosses indicate locations rated by CLIMEX as unfavourable for the flea-beetle, and the areas of circles are proportional to the suitability for the flea-beetle; shaded circles (EI<25) indicate locations where the flea-beetle may establish but not exert control over alligator weed. Stars indicate localities where the flea-beetle has been found but climatic data are not available.

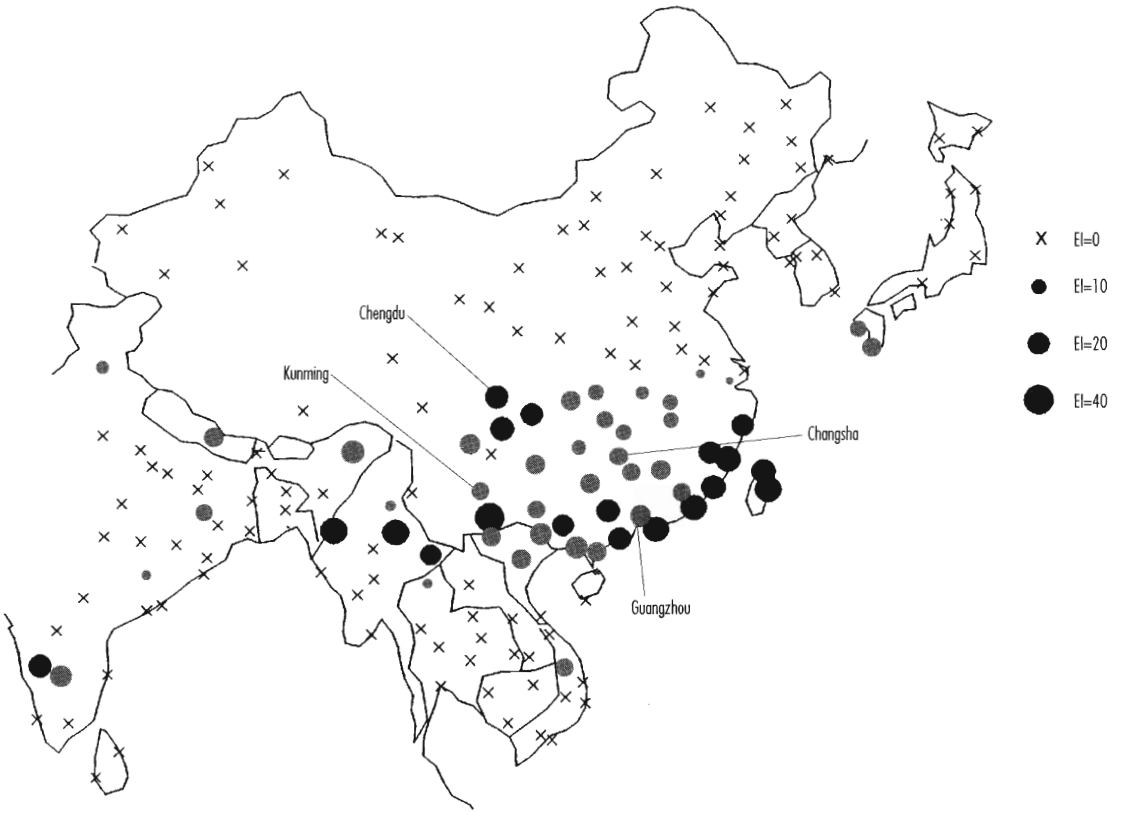


Figure 6. Suitability of locations in Asia for the alligator weed flea-beetle predicted by Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable for the flea-beetle, and the areas of circles are proportional to the suitability for the flea-beetle; shaded circles (EI<25) indicate locations where the flea-beetle may establish but not exert control over alligator weed. Release sites up to 1995 are named.

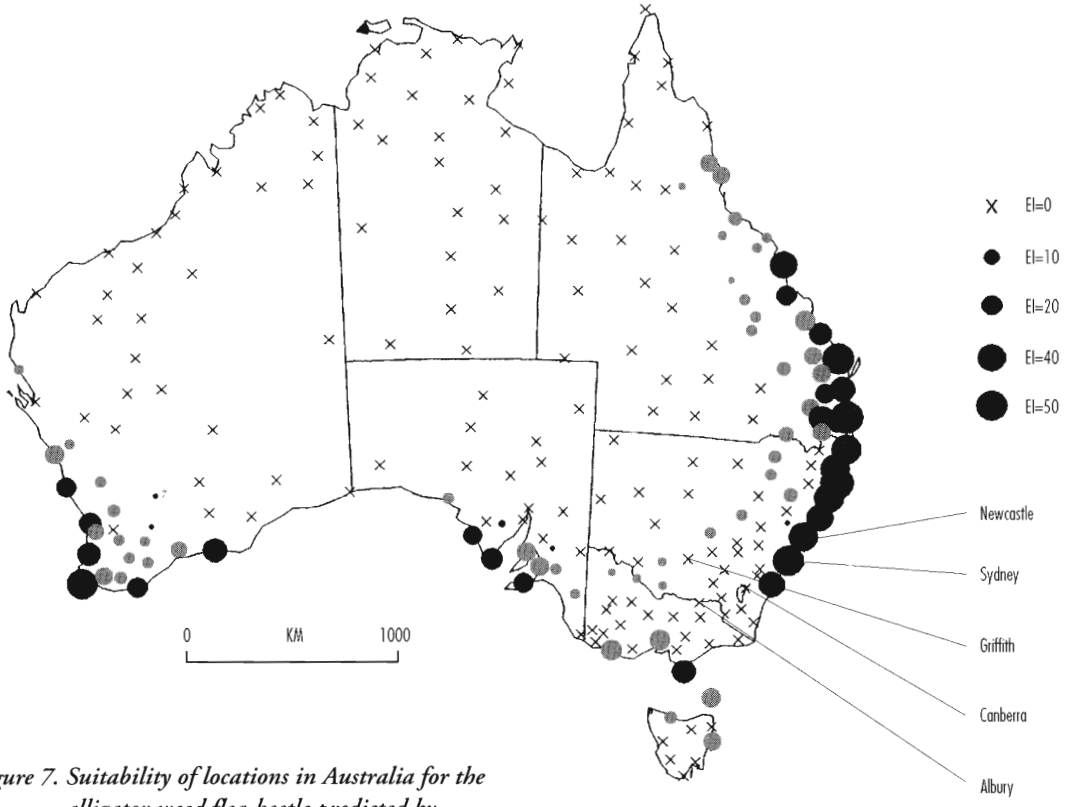


Figure 7. Suitability of locations in Australia for the alligator weed flea-beetle predicted by Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable for the flea-beetle, and the areas of circles are proportional to the suitability for the flea-beetle; shaded circles (EI<25) indicate locations where the flea-beetle may establish but not exert control over alligator weed. Alligator weed infestations up to 1995 are named.

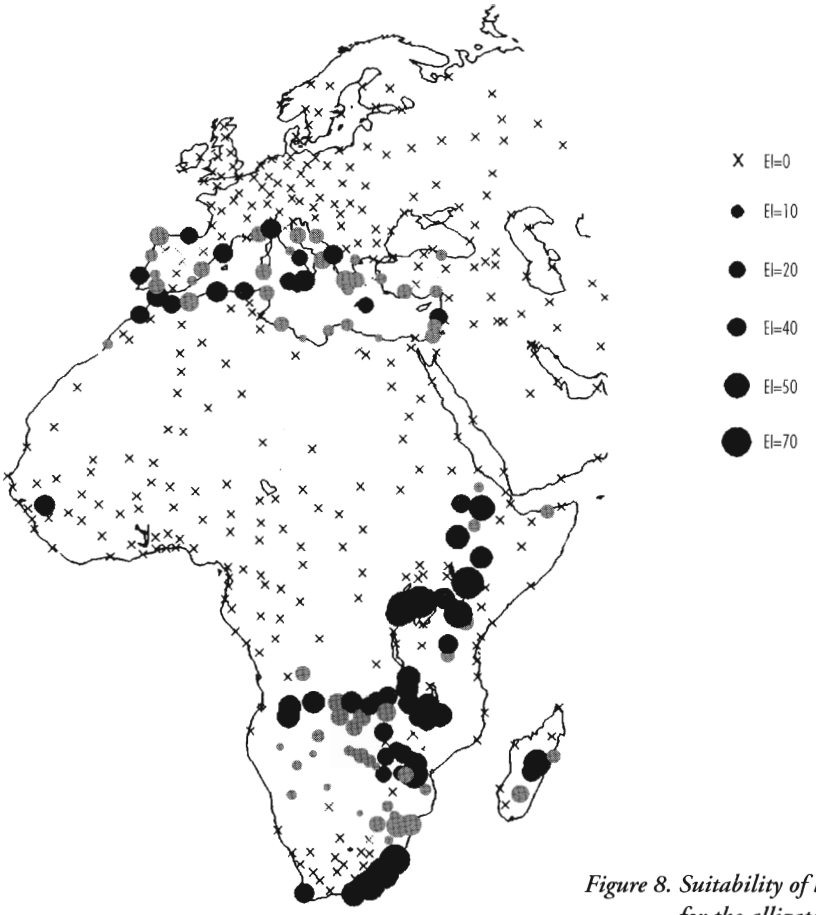


Figure 8. Suitability of locations in Africa and Europe for the alligator weed flea-beetle predicted by Ecoclimatic Indices (EI). Crosses indicate locations rated by CLIMEX as unfavourable for the flea-beetle, and the areas of circles are proportional to the suitability for the flea-beetle; shaded circles (EI<25) indicate locations where the flea-beetle may establish but not exert control over alligator weed.

References

- Andrewartha, H.G. and Birch, L.E. 1954. The Distribution and Abundance of Animals. University of Chigago Press, Chigago. 782pp.
- Julien, M.H., Skarratt, B. and Maywald, G.F. 1995. Potential geographical distribution of alligator weed and its biological control by *Agasicles hygrophila*. Journal of Aquatic Plant Management 33, 55–60.
- Sutherst, R.W. and Maywald, G.F. 1985. A computerised system for matching climates in ecology. Agricultural Ecosystems and Environment 13, 281–299.
- Sutherst, R.W., Maywald, G.F. and Skarratt, D.B. 1995. Predicting insect distributions in a changed climate. In: Harrington, R. and Stork, N.E. ed. Insects in a Changing Environment. Academic Press, London. 59–91.

A Participatory Approach to Biological Control of Weeds

Introduction

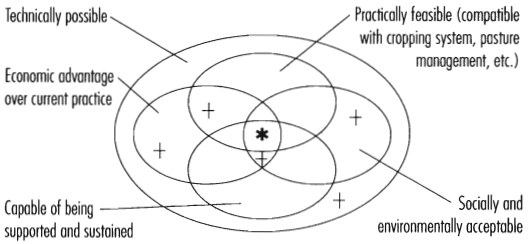
Classical biological control is traditionally seen as not requiring significant participation of landholders either in the development or implementation phases of a project. One of the main advantages of classical biological control is that, once established, populations of most successful biological control agents increase and spread with little intervention. However, biological control practitioners have become increasingly aware of the need to account for, and take appropriate action with respect to, a broad range of issues that can affect initiation and progress in a biological control project.

Clearly, some consideration needs to be given to socioeconomic, farming and cropping systems if these broader issues are to be addressed.

Key Factors Affecting Success of Biological Control of Weeds

There are obviously important biological and procedural factors that influence the success of a weed biological control project (Marohasy this volume; Julien this volume). The most important of these are that the agent to be introduced can be shown to cause significant damage or mortality to the target pest and to be specific to that pest and not adversely affect other non-target organisms. However, as shown in Figure 1, there are likely to be other conditions that need to be met for successful biological control, particularly as part of an integrated weed management program. These could include whether the existing land management strategy will encourage and not hinder the establishment and spread of the biological control agent, whether the biological control strategy will be more cost effective than the practices which farmers are presently using, and whether there is a support system that will monitor and adjust the biological control strategy as conditions change. If weed biological control strategies are to be successful, then all of these factors may need to be met. Failure can be due to not meeting just one of these factors.

Figure 1. Of all possible weed management strategies (+), there may be only one (*) that falls within the bounds of all conditions required of the strategy



Larissa Bilston and Geoff Norton
Cooperative Research Centre for Tropical Pest Management
Gehrmann Laboratories, University of Queensland
QLD 4072
Australia

If we look at potential weed biological control projects in the context of Figure 1, there are two ways in which we can attempt to increase the chances of success. First, we need to make sure that the project proposal is drafted to best meet the conditions identified in Figure 1, and is thus likely to be supported by all stakeholders. Second, we can work on reducing the constraints to the successful establishment and spread of biological control agents, through modifying land management practices, for example, and helping to integrate biological control with other practices.

While descriptive and quantitative systems analysis techniques, including computer modelling (Farrell and Lonsdale this volume) could be of value at a later stage (Norton and Mumford 1993), we recommend that the broader issues that might influence success of biological control are best dealt with initially through a participatory process. As described below, this involves a participatory problem specification and planning workshop, the development of appropriate action plans and setting up the basis for on-going participation in implementation.

Several types of participatory processes could be used to allow a wide range of issues to be raised and discussed relatively quickly, including rapid rural appraisal, participatory rural appraisal, focus groups, and various types of structured workshops. The common themes of these approaches are qualitative data gathering, active participation of those who have an interest in the outcome of research, and facilitating responsiveness of research to both on- and off- farm decision-makers (Foster et al. 1995).

The participatory approach to weed biological control that is being advocated in this paper can be viewed as consisting of two inter-related elements—participatory planning and participatory implementation.

Participatory Planning: Problem Specification Workshops

A problem specification and planning workshop to address weed biological control issues can help stakeholders examine options and identify constraints that need to be overcome to increase the likelihood of success.

A major feature of these participatory workshops is the active involvement of key players in setting priorities and designing strategies to address the priorities. Workshops in weed biological control are likely to involve producers, extension agents, local government officers, research scientists, policy makers, quarantine officers and other key players.

The output of these workshops is likely to include the following:

- an independent analysis of the biological control problem
- structured information exchange on issues determined by workshop objectives
- development of an integrated strategy involving recommendations on key research and implementation strategies, as derived from objective problem analysis. The areas frequently covered include:
  - applied research strategies;
  - advisory/implementation proposals;
  - policy recommendations; and
  - communication, education and training activities.
- ownership of this strategy by key players
- written summary of workshop results
- increased collaboration between scientists.

Workshop process

The workshop process is best described by an example, specifically the workshop held in Rockhampton, Queensland, in October 1993, focussing on parthenium, *Parthenium histerophorus.* Parthenium has been recognised as a major pasture weed in Queensland since 1975. The Queensland Department of Natural Resources (QDNR) has responsibility for policy development and implementation of control measures for exotic weeds, such as parthenium, in Queensland. A roadside spraying program was initiated in 1976 and the biological control program in 1977; both are still in operation. Spraying of roadsides and isolated infestations was seen as a stop-gap measure until successful biological control could be developed. Control using herbicides was not economically feasible except over small areas and for the short term.

Options for the long-term management and control of parthenium had not been formally reviewed since 1975. Biological control had been partially successful but the level of control was inadequate. Research into biological control was running out of 'easy' options and there was a need to assess progress to date and to determine future directions and funding needs.

Participant selection

The outcomes of each workshop are heavily influenced by the *individuals* who participate. It is important to invite stakeholders with diverse views, knowledge and background in order to gain a broad perspective of pest management issues. As a rule of thumb the composition of participants should be one-third growers, one-third scientists and extension officers,-and the final third represent chemical companies, consultants, industry and funding bodies and other relevant stakeholders. Also, within each of those categories, it is advisable to have individuals who cover the range of:

- farm sizes;
- attitudes towards IPM;
- gender;
- relevant technical fields; and
- involved companies or organisations.

Wider industry participation and support of workshop outcomes is possible if industry bodies and commercial companies are asked to select their own representatives according to the above criteria. Invited participants to the parthenium workshop included representatives from all groups involved in the parthenium problem: graziers, farmers, local government, state government, policy makers, operations staff, extension officers, scientists from the fields of pasture management, weed ecology and biological control, and research funding bodies. Participants were predominantly from Queensland, but some participants from the neighbouring state of New South Wales were included.

Workshop objectives

The objectives discussed and accepted at the beginning of the workshop were:

- to review the parthenium problem;
- to define the management/control options now available; and
- to make recommendations on policy, training, research and implementation of management/control options to improve the management of parthenium.

Objectives implicit in the workshop process were exchange of information and production of a report on the workshop and its findings.

Having brought the stakeholders together for a two-day workshop we then implemented the workshop in a structured manner as shown in Figure 2. It consisted of three stages: problem specification, the identification of major opportunities and constraints, and needs analysis as a basis for the development of action plans for future implementation. To facilitate this process we used a number of techniques that we have found to be extremely useful in encouraging group activity and focusing the groups' attention.

Stage 1—problem specification

The initial participatory analysis of the problem provides a sound basis for identifying the major factors that need to be investigated further if practical improvements in weed management are to be achieved. This sets an agreed context in which the overall strategy can be designed. Thus, at the end of the workshop, a clear statement of the problem can be made, the most likely means of achieving improved weed management identified, and recommendations developed on the research, extension, training, and policy actions required to increase the chances of successful weed management being achieved.

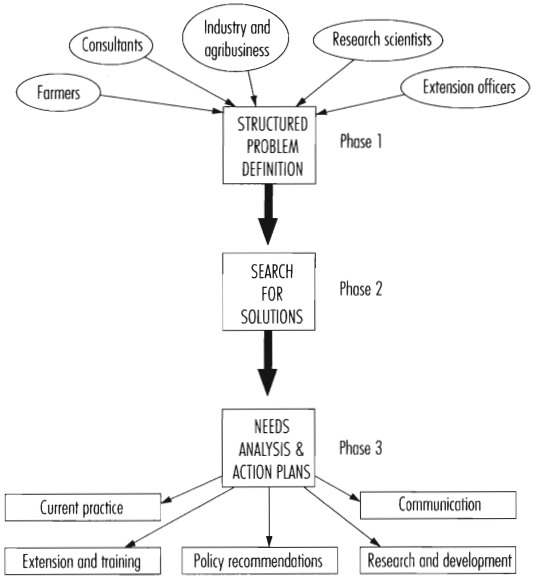


Figure 2. The problem specification workshop process.

In the parthenium workshop, historical profiles were used in the initial problem specification phase, to help understand the major factors that have influenced the problem to date and how these factors have changed over the past ten or twenty years. This not only helped all the group to understand the complex of factors surrounding the particular problem but also provided a basis for thinking about the changes that are likely to happen in the next three to five years that will have an influence on the options and the feasibility of improved weed management in the future.

The following are the main points arising from the parthenium historical profiles.

- The area infested by parthenium increased markedly during the wet years of 1975–76. Future spread will be limited by suitable soil types and climate.
- The major factors considered to have affected the rate of spread were stocking pressure, seasons, recognition of and subsequent response to the problem, biological control, floods, land clearing, farming, landholder cash flow/ terms of trade, exploration and infrastructure development.
- Changes in land use have influenced the problem status of parthenium.
- Parthenium has been spread by:
  - movement of machinery and vehicles;
  - stock movement, particularly for agistment during drought;

- stock feed; and
- contaminated seed.

- As parthenium spreads into urban coastal areas, human health problems (respiratory and dermatitis) will increase.
- Government funding for parthenium control is likely to be restricted in future.
- Stocking rate and income have been reduced because of parthenium.

Stage 2—the search for solutions

Participants at the parthenium workshop identified options and opportunities for improving parthenium weed management, and constraints to improvement. Three groups addressed opportunities and constraints in the areas of:

- policy options for management of parthenium;
- options for management of parthenium on grazing properties; and
- options for management of parthenium on cropping properties.

Each group listed all possible options for improved management with factors affecting the performance of that option and a rating of feasibility and acceptance. Priorities were assigned to each of the options and the high priority options were carried forward to the next stage.

An alternative approach to identification and exploration of opportunities and constraints used in other workshops is a pin-boarding technique. Here each participant records their ideas concerning the opportunities and the constraints for improving weed management, writing one idea per card. The participants then split into groups to pin their cards on to a board and arrange them according to their similarity. The groups then write a header card for each of the clusters of cards that succinctly describes the essential ideas contained in that cluster of cards. The key issues covered by the header cards are reported to the whole group, providing a basis for the workshop participants to decide which key issues they would consider during the third phase of the workshop.

Stage 3—needs analysis and action plans

During the final phase of the workshop, participants worked in small groups, each group focussing on a key issue. They discussed what needed to be done to improve the current situation, and then developed action plans detailing objectives, what has to be done by when and who will take responsibility for implementing the plans.

In the parthenium workshop, participants formed five groups to examine policy, research, extension and training needs for the priority options identified in the previous session. Options for management in areas of isolated infestations were separated into two sets, eradication and prevention of spread. Education was identified as an important option by all groups during the previous session, so it was separated as a specific topic to avoid duplication of effort. Major changes in management were identified as necessary by the pastures group, so this was retained as a topic. The cropping group had identified education as the major factor needed for improved management, so cropping was not addressed specifically. Given the key role identified for biological control and uncertainty about future directions in this area, one group was assigned this issue. Thus the topics for the five groups were:

- prevention of spread;
- eradication of isolated infestations;
- education on all aspects of the problem and solutions;

- management/ control in pastures; and
- biological control.

Key action plans from the parthenium workshop

While all the action plans developed during the workshop were regarded as important, limited resources were likely to constrain implementation of all plans. The top priority action plans from each section to be addressed as quickly as possible were:

- prevent spread by livestock;
- continue roadside inspection and treatment;
- increase ability of the community to identify parthenium, and to increase knowledge of eradication procedures;
- develop state and transition models of vegetation change for all at-risk areas defined by land types;
- define and refine opportunities and strategies for use of herbicides;
- define the biology and ecology of parthenium, and evaluate effects of established biological control agents;
- review untested biological control agents and complete testing and field release of suitable agents; and
- evaluate costs and benefits of eradication of isolated infestations and issuing free herbicide by government.

Action plans from the parthenium workshop relating to biological control

Action:	Ecological studies to improve understanding of parthenium, and to evaluate effects of established agents
Who:	Centre for Tropical Pest Management—doctoral student
When:	3-year study
Cost:	$ 350,000
Funding:	Rural Lands Protection Board, Meat Research Corporation, Grains Research and Development Corporation, Integrated Catchment Management groups (e.g. Murray-Darling)

Action:	Review known untested agents and complete testing and release of suitable agents
Who:	QDNR, International Institute for Biological Control for pathogens
When:	4-year investigation
Cost:	$750,000
Funding:	Rural Lands Protection Board, Meat Research Corporation, Grains Research and Development Corporation

Action:	Explore for agents in Bolivia, an area that has not been investigated, and explore further for plant pathogenic agents throughout the native range of parthenium
Who:	Under contract to QDNR
When:	2-year part-time survey
Cost:	$500,000
Funding:	Rural Lands Protection Board, Meat Research Corporation, Grains Research and Development Corporation

For optimal long-term application of biological control, priorities for the three action plans were nominated in the order listed, i.e. top priority was assessment of efficacy of established agents. However, if a short-term approach were adopted the review of untested agents would be nominated as top priority.

Participatory Implementation

Experience has shown that workshop follow-up greatly enhances the workshop's impact on improving pest management. In-depth case studies of problem specification workshops were conducted (L. Aitken pers. comm. 1995) to evaluate their impacts. Workshop participants placed high value on:

- their involvement in defining pest problems with other stakeholders;
- the way the workshop acted as a forum for drawing information together and creating awareness of the depth and breadth of the problem; and
- the exchange of viewpoints from a wide range of people.

The study also found that action plans tend to be implemented, but often become altered and/or absorbed into institutional activity. Whilst this interaction is effective, efficient, and demonstrates its relevance, it tends to obscure the workshop outcome from the view of many stakeholders. As a result, workshop participants felt a need for improved communication.

The study also showed that the level of extra communication required depended on existing networks between the stakeholders concerned. For instance, parthenium weed stakeholders felt better informed about workshop outcomes after the parthenium workshop than tomato pest management stakeholders were after a tomato workshop. In the case of parthenium, collaboration and communication existed between researchers and Landcare groups prior to the workshop, and continued afterwards in the participatory implementation of action plans. One Landcare group in a badly infested area reformed to become the Parthenium Action Group. They secured funds for a full-time coordinator to manage their biological agent rearing, release and exchange program, hiring out spray equipment, community education and communication amongst stakeholders. The group maintains strong links with researchers and extension officers from universities and state departments of agriculture and natural resources.

In comparision, general communication amongst members of the tomato industry seemed to be weaker, informal and less frequent. Many tomato workshop participants were not aware of the considerable progress made on action plans since the workshop, and some had concluded that very little had happened. Since the study, the tomato industry has begun publishing a monthly newsletter, including a section on pest management, for general distribution.

Whilst stakeholders enjoy participating in planning better pest management strategies, and some become involved in implementing action plans, many do not have the time to contribute on an ongoing basis. However, having donated two days of their time to participate in the workshop, they value follow-up communication. As stakeholders, they have a vested interest in the outcomes of the workshop and naturally want to be informed of progress. Thus, the final stage in a workshop is to plan a follow-up and communication strategy.

It is vital that someone at each workshop agrees to be the 'champion' for each action plan, and takes responsibility for following it up after the workshop.

Other aspects of a follow-up strategy which work well include the following.

- Electing/nominating an action plan coordinator to keep regular contact with action plan champions to see if progress has been made or assistance required.
- Establishing working parties for each action plan, with one person from each working party joining a steering committee to maximize the impact of the workshop.
- Communicating the proceedings of the workshop to participants and other stakeholders as soon as possible after the workshop. A summary of action plans should be distributed within a few weeks of the workshop, and a full report prepared and distributed within a few months.
- Communicating action plan progress to participants and the broader industry. This can take the form of a newsletter, or information included in existing industry magazines. It could also be in the form of follow-up workshops or update meetings.

Conclusions

Involvement of a wide range of people affected by a pest management problem is necessary to develop a clear and thorough understanding of the problem, and make relevant and realistic action plans for improvement. Problem specification workshops have been shown to be an effective tool for involving stakeholders in this process. However, on-going communication and coordination of action plans towards the common goal is required to optimise the impact of the joint planning process.

References

- Foster, J., Norton, G. and Brough, E. 1995. The role of problem specification workshops in extension: an IPM example. Journal of Extension 33. Extension Journal, Inc. gopher://joe.org/00/joe/1995august/a1.
- Norton, G.A. and Mumford, J.D. ed. 1993. Decision Tools for Pest Management. CAB International, Wallingford, U.K. 279 pp.

Benefit Cost Analysis for Biological Control Projects

Introduction

Benefit cost analysis (BCA) is now used by all major international research and development funding agencies as a decision making tool in the allocation of financial resources amongst competing project proposals.

However, BCA has limitations when attempting to evaluate biological control projects. Therefore, it is important to understand the BCA process so that the results of the analysis can be questioned and/or defended. This is important because it may mean the difference between funding or not funding a project.

Benefit Cost Analysis

Definition of benefit cost analysis

BCA is based on three key concepts as follows.

1. Opportunity cost, i.e. the return that is foregone because the next best alternative investment was not chosen, e.g., if research into herbicides was the preferred option for investment in weed management, then the opportunity cost may be the return foregone if the same amount had been invested in biological control research.
2. Willingness to pay (WTP), i.e. what a person would be willing to pay in order to purchase, or consume, a set quantity of a good or service, e.g. how much would a person be willing to pay to clear a weed infestation from an area of land.
3. The 'cost benefit rule', i.e. 'A project is acceptable where, subject to budget constraints and certain other conditions, net social benefits valued according to the opportunity cost and willingness to pay principles are positive rather than negative.' (Australian Department of Finance 1991, p ix).

BCA attempts to place a monetary value on all aspects of any project. In doing so, results of the BCA analysis may be influenced by definition of the scope of the analysis, and by methods used in the analysis. Subjective decisions must be made at various levels in BCA including the following.

- Scope of the project definition. Did the BCA focus on one group, several groups or look at society as a whole, and have all of the direct and indirect costs and benefits been taken into account?
- Method(s) used to value the costs and benefits.
- Working assumptions made during the BCA.
- The discount rate.
- Ranking projects.
- Sensitivity tests. Were sensitivity tests performed to determine the variables which most influence the rankings of the projects (see Perkins 1994).

David Adamson
Cooperative Research Centre for Tropical Pest Management
Gehrmann Laboratories, University of Queensland, QLD 4072
Australia

- Uncertainty. Clark (1991) and Gittinger (1982) provide an explanation of how risk, adoption rates, probability of success, inter- and intra-generational concerns, etc. can influence a BCA.

Because of the subjectivity of decisions at various levels in BCA, the outcome of BCA can be manipulated by:

- modifying results to make them acceptable to the decision makers by altering such things as assumptions, discount rates, and risk preference;
- trivialising an issue, particularly environmental and social issues, so that many costs and benefits associated with that issue are ignored or regarded as externalities, as explained below.

Checklists of factors that should be considered when analysing the results from BCA are presented below for some of these major issues.

Assumptions

Assumptions are usually the weakest point of any BCA since they are often subjective in nature. Economists commonly have little idea about basic biology, so assumptions made about the ecosystem could and oversimplify the true nature of the problem and must be examined. Biologically and ecologically significant factors may be excluded from the analysis because they are not perceived as having a direct effect on costs and benefits.

When examining assumptions in a BCA:

- check that all assumptions are clearly stated;
- determine if the analysis is too narrow in its approach; and
- check that assumptions are supported by theoretical, experimental, observational or anecdotal evidence.

Data

When examining data used in a BCA, check:

- that the data source of the is stated;
- for discrepancies introduced by using multiple data sources;
- that socioeconomic factors, climate anomalies and associated biological events that may affect the data were considered;
- whether the data are based on one year, and thus subject to particular events in that year, or on several years observations; and
- that statistical methodology was acceptable, perhaps with the assistance of a statistician or econometrician.

Externalities

A variable can be included in calculations in a BCA only if a monetary value can be assigned to it. An externality exists when there are costs or benefits associated with an action for which a monetary value is not readily assigned. Externalities are often referred to as 'non-marketed goods or services', as opposed to 'marketed goods' such as a drum of insecticide which can be purchased at a price determined by the market place. Determining the monetary value of an externality may be difficult because the factor being considered is not 'marketable' (e.g. maintenance of biodiversity) or because links between the project actions and that factor are unpredictable (e.g. reductions in health problems if successful biological control reduces pesticide application).

It is important to realise that although externalities are very difficult to evaluate, their inclusion or exclusion could radically alter the results of a BCA. Therefore it is important to check that all possible externalities are listed.

For a biological control project the following externalities could be considered:

- reduced pesticide application following successful biological control could be accompanied by:
  - reduced health problems due to pesticides,
  - increased populations of beneficial organisms previously affected by pesticides in agricultural areas,
  - reduced effects of pesticide spray drift and residues in run-off into watercourses and conservation areas,
  - reduced risk of pesticide residues in produce,
  - possible appearance of other pests previously controlled by the pesticide;
- improved livestock weight gain or crop production following improved control of the target pest;
- reduced health complications caused by an allergy to the target pest;
- preventing the income gap (social equity) between the rural and urban areas from increasing as rapidly by reducing operating costs in the rural areas.
- improved understanding by farmers of the agro-ecosystem through the use of biological control techniques.
- reduced competition between the pest and native flora and/or fauna.

Efforts are sometimes made to evaluate non-marketed goods and/or services for inclusion in BCA, and this is covered in the following sections.

Resource evaluation techniques

Some of the major techniques available for valuing unpriced, non-market resources are; surveys, contingency valuation, hedonic pricing, and the travel cost method. This area of BCA is exceptionally difficult, and there are problems with all techniques.

Survey

A survey is the easiest way to collect data. Some aspects of surveys that should be examined include:

- number of respondents in the survey, which should usually exceed 30;
- how recent, and therefore how relevant, was the survey;
- inclusion of all relevant issues;
- validity of design and statistical analysis.

Contingency valuation (CV)

Briefly, CV attempts to place a value on environmental issues, usually by surveying willingness to pay (WTP) and willingness to accept (WTA), which is what a person would be willing to accept as payment, or pay for selling, or buying, a good or service. CV is a highly contentious issue in BCA. When analysing results from CV, check that:

- the tendency of people to overestimate money received (WTA) and underestimate money paid (WTP) has been considered;
- respondents had a full understanding of the issues about which they were surveyed.

Hedonic pricing (HP)

Briefly, HP attempts to establish a relationship between the non-market resource (e.g. the environment) and a market situation (e.g. land values and the housing market). HP relies on very restrictive assumptions, such as a fully independent market free from government intervention. When analysing results from HP, consult an econometrician for advice on the choice of functional form, and checking for omitted variable bias, and multicolinearity in the data set.

Travel cost method (TCM)

TCM attempts to determine the value of a recreational site by using the entrance cost, travel costs, the number of times the recreational site is visited, and time costs. Survey work is required for this technique. Analysis of the survey data should include consideration of:

- one-off versus repeat visits;
- holiday makers versus residents;
- reasons for the visit;
- method of calculation of travelling costs; and
- the problems of estimating WTA versus WTP.

Discount rates

Discount rates are based on the assumption that people prefer to consume now rather than later. The most commonly used discount rates in Australia are 5%, 8% or 10%. The chosen discount rate will influence the results as follows.

- High discount rates are biased against projects with long-term benefits.
- High discount rates minimise the apparent impact of long-term environmental damage.
- High discount rates minimise intergenerational concerns, but may maximise intragenerational concerns with equity at the current time.

Free rider problem

The free rider problem occurs when those paying for use of a resource do not have exclusive use of the resource and property rights cannot be protected. Classical biological control provides an example of the free rider problem: biological control agents supplied may rapidly spread onto adjoining properties, so that eventually everyone will receive the biological control agent resource whether they want it or not. Given that those who want the biological control agent sufficiently to pay for it and those who would not pay, the free riders, will both receive the benefits of the agent, and it may be difficult to charge for use of the biological control agent.

The free rider problem can discriminate against classical biological control projects because there may be no realised (monetary) benefit from investment in research into biological control.

Risk

Techniques such as sensitivity analysis should be used in BCA to take into account risks of failure to find, import and release agents which successfully control the target pest.

Ranking projects

There are three criteria for ranking projects and it is important to know what they are:

- net present value (NPV); i.e. the projects value after all discounted costs and benefits have been calculated. Any project with an NPV greater than zero is profitable;
- benefit cost ration (B/C ratio); i.e. the return on each dollar invested in the project. So for every dollar invested you receive x mumber of dollars in return. Any project with a B/C ratio greater than 1 is profitable.
- internal rate of return (IRR); i.e. the level at which the discount rate will make the NPV equal to zero. Any IRR greater than the discount rate used should be accepted.

The following table represents a hypothetical situation where the differences in NPV, B/C ratio and IRR are presented. In this situation may be best to choose project B because it is ranked first in NPV and IRR, even though it does not have the best B/C ratio. However, this is subjective comment and perhaps project C should be chosen because it has a greater return per dollar invested (i.e. B/C ratio).

Project	NPV	B/C ratio	IRR
A	$10,000	2:1	18%
B	$11,000	3:1	19%
C	$9,000	5:1	17%

Conclusion

Tisdell (1991) pointed out that economic 'values can only be a partial guide to social decision-making'. Thus BCA should be only part of the decision making process. Factors that should also be considered, including some already mentioned, are:

- who does the project benefit;
- how long until the project delivers returns and for how long;
- does the project provide a short- or long-term solution to the problem;
- has a project been discriminated against just because the benefits are in the future (i.e. intergenerational concern);
- has the social equity question (i.e. intergenerational concern) been addressed;
- is it a vote winning or vote losing strategy (i.e. is the policy politically acceptable);
- are there direct financial returns to the investment body;
- does the project make good business sense (i.e. accountability);
- does society consider that this pest needs to be controlled;
- availability of resources (financial, structural, political);
- the ease of use of each project output;
- the probability of success of the project;
- the return from primary research versus full investment.

Provided that BCA is applied competently and impartially, results of BCA are a useful component in the decision making process.

References

- Australian Department of Finance 1991. Handbook of Cost-Benefit Analysis. AGPS, Canberra. 141pp.
- Clark, C. 1991. Economic bias against sustainable development. In: Constanza, R. ed. Ecological Economics: the Science and Management of Sustainability. Columbia University Press, New York. 319–330.
- Gittinger, J.P. 1982. Economic Analysis of Agricultural Projects. 2nd edn. The John Hopkins University Press, USA. 505pp.
- Perkins, F. 1994. Practical Cost Benefit Analysis: Basic Concepts and Applications. MacMillan Education Australia Pty Ltd, Hong Kong. 369pp.
- Tisdell, C.A. 1991. Economics of Environmental Conservation: Economics for Environmental and Ecological Management. Elsevier, USA. 186pp.