The prospect of biological control of *Mimosa pigra* with fungal pathogens in Australia

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Abstract

During surveys in Central and South America two fungal pathogens were identified as high-potential classical biological control agents for *Mimosa pigra* L. in Australia. The first, a wet-season fungus *Phloeospora mimosae-pigrae* H.C. Evans & G. Carrión, was released in Australia in 1994 and the second, the dry-season rust *Diabole cubensis* (Arthur & J.R. Johnst.), in 1996. Field observations in Central and South America and pre-release evaluations in England indicated promising impacts by the fungal pathogens as classical biological control agents, but both failed to establish long-term in Australia.

The coelomycete anamorph (asexual form), *P. mimosae-pigrae*, was successfully mass-cultured in liquid medium and a standardised culturing and application protocol was developed and tested for large-scale field applications during the wet seasons from 1996 to 1998. The fungal pathogen established temporarily in the field and caused a considerable reduction in growth of mimosa seedlings. However, mature plants were less affected by *P. mimosae-pigrae* due to premature leaf-drop of diseased plant tissue. In addition, the fungal pathogen failed to develop its sexual form (teleomorph) in Australia, which is assumed vital for its survival. Post-release evaluation concluded that while *P. mimosae-pigrae* was unable to self perpetuate under the conditions in Australia, and thus was not suitable as a classical biological control agent, it has suitable characteristics and potential to be developed and used as a mycoherbicide for the control of mimosa.

The environmental conditions in northern Australia also appeared to prohibit the long-term establishment of the dry-season rust *D. cubensis*. Mass-production of spores of the rust was labour intensive, yields were inconsistent and spores rapidly lost their viability. The rust caused disease symptoms on plants of mimosa after field inoculations but failed to spread and reinfect new growth of plants.

***Keywords*:** mimosa, biological control, pathogens.

Introduction

The giant sensitive plant, *Mimosa pigra* L. (Mimosaceae), is an invasive noxious weed that has reached infestation levels in Southeast Asia and northern Australia beyond effective control by chemical and mechanical means. These methods provide only short-term solutions to this serious problem (Braithwaite *et al*. 1989). Biological control, through the introduction of natural enemies, is considered the most efficient and environmentally acceptable approach for the long-term control of mimosa in Australia (Lonsdale *et al*. 1988). A biological control program was initiated in 1979 and initially focused on insect species

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(Forno 1992). Eleven insect species, including seed-feeding bruchids, flower- and leaf-feeding beetles and weevils, and a root-feeding beetle have already been introduced, contributing towards a desired balance between mimosa and its natural enemies in northern Australia (Heard and Segura 2004). The approach of using plant pathogens for the control of mimosa was included into the program in 1988, when searches for fungal pathogens were undertaken in Central and South America, the centre of origin of mimosa (Evans *et al*. 1993, Evans *et al*. 1995). The search for fungal pathogens resulted in the recording of a range of obligate or co-evolved fungal pathogens on mimosa (Evans *et al*. 1995). Two of these were selected as biological control candidates and were introduced into Australia in 1995 and 1996 (Hoskins and Rea 1999). Evaluations undertaken are discussed in relation to classical biological control and the prospect of these two fungal pathogens as biological control agents of mimosa in Australia.

Two strategies in the use of pathogens

The two distinct approaches of classical biological control and inundative control have been adopted for the use of plant pathogens as biological control agents of exotic weeds. Their main criteria will be briefly described. More detailed reviews of the two strategies have been recently conducted by Evans (2002) and Charudattan and Dinoor (2000).

Classical biological control

This approach has been developed and refined by entomologists over the last century and is based on a fairly simple concept. Classical biological control of exotic or alien weeds includes the importation of co-evolved, highly specific natural enemies from the weed's native geographic range. This process involves travelling to the country or area from which a newly introduced weed originated and importing plant pathogens that appear to impact most on the target weed. The aim of classical biological control is to redress the imbalance between natural enemies and a plant that was introduced to a new location and became invasive partly due to the lack of natural enemies. Classical biological control is a slow process and thus has not been widely used as an integrated part of weed-management plans in intensively managed crops due to their short cropping season. Disruptions associated with cropping practices or through the use of chemicals can have adverse effects on the performance and establishment of classical biological-control agents.

Inundative biological control

The inundative or bioherbicide approach is a method of exploiting and increasing the population of an indigenous fungus for management of endemic weeds through mass production, formulation and seasonal application of the fungal pathogen. This method relies upon continual human management and does not provide a permanent solution, which the classical approach may provide.

Selecting fungal pathogens

Fungal pathogens of mimosa

The program adopted for mimosa control in northern Australia is defined as a classical biological control program and research was carried out according to the essential steps detailed in Wapshere (1989), Harley and Forno (1992) and Forno (1992). Two fungal pathogens were selected on the basis of their host-specificity and significant host damage as potential biological control agents of mimosa in Australia.

Phloeospora mimosae-pigrae

The holomorph *Sphaerulina mimosae-pigrae*/*Phloeospora mimosae-pigrae* H.C. Evans & G. Carrión was described by Evans *et al*. (1993). Field surveys in Central and South America indicated that the coelomycete anamorph, *Phloeospora mimosae-pigrae*, is a widespread pathogen, specific to mimosa and active mainly during the wet season (Evans *et al*. 1993). This pathogen caused extensive cankering of leaf rachises, branches and stems, followed by progressive dieback and plant decline. The teleomorph *Sphaerulina mimosae-pigrae* occurred regularly in the field in Central and South America and was seen as an important stage in the life cycle of the fungal pathogen, particularly in regard to its potential as a classical biological control agent, due to the ability of long-distance spread through aerially dispersed dry spores. Coelomycetes, especially those with slime spores such as the anamorph *P. mimosae-pigrae*, only spread short-distance by rain-splash or run-off and are more often considered for development as a bioherbicide due to their poor dispersal mechanisms (Charudattan and Dinoor 2000). However, the presence of both the anamorph and teleomorph was seen as a highly desirable characteristic for a classical biological-control candidate.

Diabole cubensis

The rust *Diabole cubensis* (Arthur & J.R. Johnst.) was described by Arthur (1922). The selection of *D. cubensis* as a classical biological control agent

was based on field observations in Central and South America where the rust caused significant damage on mimosa, specifically severe leaf chlorosis and subsequent defoliation. An additional important selection criterion was the host-specificity of the rust to mimosa (Evans *et al*. 1995). Traditionally, rusts are considered as classical biological control agents due to their highly efficient spore dispersal mechanism (Evans and Ellison 1990). In Central and South America, the rust occurred over an altitude range from sea level up to 720 metres and was most abundant in the field during the dry season (Seier 1998). Thus, the rust was expected to complement the biological control activity of the wet-season fungal pathogen *P. mimosae-pigrae*.

Pre-release evaluation

Field observations in Central and South America provided the initial information on *P. mimosae-pigrae* and *D. cubensis*. However, to make a scientific assessment, particularly a pest risk assessment, prior the introduction of both fungal pathogens into Australia, detailed biology and host-range studies were carried out at CABI Bioscience (Silwood Park, UK) (Seier 1998, Evans 2000).

Development of disease

Phloeospora mimosae-pigrae

Optimum temperature conditions for the germination of *P. mimosae-pigrae* were 20 to 25°C with a relative humidity of 70 to 100% for a period of 24 hours. The fungal pathogen exhibited a two-phase disease process on mimosa: an initial symptomless period of approximately two weeks when the fungus established within the host, and a destructive phase during which *P. mimosae-pigrae* destroyed and sporulated on infected plant tissue (Seier 1998). *Phloeospora mimosae-pigrae* consisted of two different strains: a leaf-spot strain and a rachis strain. Pre-release evaluations focused on the rachis strain due to its more severe damage. Seier (1998) also noticed that the teleomorph, *S. mimosae-pigrae*, never developed during pre-release evaluations.

Diabole cubensis

The obligate rust *D. cubensis* showed typical characteristics of a biotroph host–pathogen relationship, causing chlorosis but no necrosis of colonised tissue. With an incubation period of 24 hours at a temperature range of 21 to 23°C and a relative humidity of 60 to 80%, disease symptoms, in the form of chlorotic leaf-spots, developed after a symptomless period of *ca.* two weeks. Subsequently, the rust sporulated on these leaf spots and telia containing teliospores formed. At a later stage, *ca.* five weeks after inoculation, pycnia developed on inoculated pinnules, which were believed to play a role in the formation of a second development of telia (Seier 1998).

Host-specificity studies

Host-range testing was carried out using a wide range of plant species, both native and introduced to Australia. The Australian Quarantine and Inspection Service (AQIS) approved the plants included in the host-range testing. A total of 94 plants was tested (Seier 1998). The studies by Seier (1998) confirmed the host-specificity of both fungal pathogens to mimosa, as previously assessed by field observations in Central and South America (Evans *et al.* 1993, Evans *et al.* 1995)

Post-release evaluation

Post-release evaluations of the fungal pathogens in Australia provided information on their ability to infect and spread on mimosa plants in their exotic range and thus concluded the assessment of the prospect of the fungal pathogens as classical biological control agents in Australia.

Culturing and application ability

Phloeospora mimosae-pigrae

The strain deposited as a reference collection in liquid nitrogen at the International Mycological Institute (IMI) as IMI 340370 was originally imported into Australia in 1993. Releases on a broad scale commenced in 1995. Using a combination of classical and inundative approaches, a culturing and application technique was developed (Hennecke *et al*. 2001). The pathogen was mass-produced in a V-8 juice (a commercially available vegetable juice) liquid culture achieving up to 360 litres of conidia suspension per batch and allowing broad-scale field applications during the wet-seasons from 1996 to 1998. Medium-scale field applications were carried out using motorised backpack sprayers and large-scale aerial applications were carried out by helicopter. Even though the ability of a fungal pathogen to be easily mass-produced in an artificial medium is not a high priority for a classical biological control agent, it can enhance the broad-scale establishment of a fungal pathogen that is seasonally limited. This was particularly an advantage in the case of *P. mimosae-pigrae*, which appeared to be active only during the wet season. Additionally, the ability to be aerially applied by helicopter allowed access to remote infestations of mimosa that were inaccessible by land.

Diabole cubensis

The rust strain IMI 368060 ex mimosa Veracruz, Mexico was introduced into Australia in 1996. This obligate rust, which could not be artificially cultured, was maintained and cultured through propagation on seedlings of mimosa in the greenhouse. The removal and transfer of teliospores to uninfected plants by a fine camelhair brush proved to be extremely labour intensive. The specific temperature and humidity requirements for the incubation of the rust were provided through a controlled incubation chamber, allowing the production of teliospores all year (Seier 1998). However, the development of symptoms and therefore sporulation of the rust was inconsistent during the year, decreasing during the peak of the wet-season. Excess teliospores not used for maintaining the production-culture were stored in sterile cryogenic vials in liquid nitrogen as pure dry spores for field releases in the dry-season. The viability of teliospores was retained through quick transfer of teliospores into storage in liquid nitrogen; viability rapidly decreased when spores were left detached from plant tissue without correct storage. Field applications of *D. cubensis* were carried out during the northern Australian dry-seasons from 1996 to 1999 when ambient temperature and humidity requirements for the infection of *D. cubensis* were met. Rust suspensions of 1×10^6 spores mL^{-1} were applied using a hand sprayer.

Post-release population viability

Phloeospora mimosae-pigrae

The coelomycete was able to infect and develop under field conditions in Australia. The main requirement for infection for this pathogen in the field was the availability of 70–100% relative humidity during the incubation period of 24 hours. This limited the active period of *P. mimosae-pigrae* from *ca.* October to April, depending on the variable length of the annual wet-season. Visible symptoms under optimum conditions developed approximately two to three weeks after inoculation, and symptoms persisted on the plant for up to eight weeks after inoculation. The pathogen caused extensive chlorosis on pinnules and rachises and led to premature leaf-drop of diseased tissue. On young seedlings, *P. mimosae-pigrae* resulted in a biomass reduction of up to 48% and appeared to have no effect on the performance of other biological control insects feeding on the same plant part of mimosa (Paynter and Hennecke 2001). However, the developed anamorph *P. mimosae-pigrae* was not able to spread short-distances to neighbouring plant tissues by rain-splash. Run-off also had no effect on dispersal because no significant leaf growth occurred below the diseased area of plants. The teleomorph *S. mimosae-pigrae* did not develop in Australia, prohibiting the long-distance spread of the fungal pathogen and reducing its ability to survive the non-active period of *P. mimosae-pigrae*, the dry-season. This fungal pathogen appears not to have established in Australia.

Diabole cubensis

Field applications were carried out between May and September during the dry-season and resulted in chlorotic leaf-spots *ca.* two weeks after inoculation under optimum conditions. Subsequently, telia were visible on inoculated leaves of mimosa plants. Sporulation of *D. cubensis* occurred on inoculated leaves of mimosa of all ages and telia were visible on pinnules and rachises. Diseased leaf tissue dropped approximately four weeks after inoculation, and nine weeks after inoculation no diseased leaves remained on the inoculated plants. Due to the rapid decrease in the viability of spores, most of the rust was lost when diseased leaves dropped to the ground. Leaf-drop also occurred in response to water stress during the dry-season and to attack by other biological control insects (Paynter and Hennecke 2001). A combination of diseased material dropping off the plant, the disease attacking older leaves that were lower in the canopy, and the lack of wind dispersal from the lower canopy to other plant parts contributed to unsuccessful long-term establishment of *D. cubensis* in Australia.

Conclusion

Both pathogens infected mimosa and developed disease symptoms in the short-term but failed to establish long-term in Australia. The coelomycete *P. mimosae-pigrae* has potential as an inundative biological control agent, considering the significant short-term damage caused by this pathogen on mimosa seedlings. Investigations into formulation and storage of *P. mimosae-pigrae* and analysis of the cost versus efficiency of a potential bioherbicide are required to justify further development of a bioherbicide for this weed.

The ecology of mimosa in Australia is well documented, but there is a general lack of information about some aspects of the ecology of mimosa in the centre of origin, such as growth rate (Lonsdale *et al.* 1988, Lonsdale 1993). Single observations in Mexico in 2000 found *P. mimosae-pigrae* was visible only on small, up to *ca.* 80 cm tall mimosa plants, which existed amongst other vegetation. At a different field site, a few kilometres from the first site, the fungus was not visible

on taller, more widely spaced mimosa plants of *ca.* three metres height with more sparse leaves, similar to the phenotype of the majority of mimosa stands in Australia. This single observation suggests that phenotype, or microclimates relating to phenotype, may be important for this pathogen's survival. This suggests that selection of pathogens as potential biological control agents may be improved with a better understanding of the ecology of the target plant in both the centre of origin and in its exotic range. Despite the failure of *P. mimosae-pigrae* and *D. cubensis* to establish in Australia, both were able to infect and damage mimosa plants under ambient conditions. If, as a result of ongoing attempts to control mimosa, the phenotype and hence microclimate around the weed changes, these pathogens could be considered for reintroduction.

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