How Specific is Specific Enough? - Case Studies of Three Rust Species under Evaluation for Weed Biological Control in Australia

M. K. Seier[1], C. A. Ellison[1], G. Cortat[2], M. Day[3] and K. Dhileepan[3]

[1]CABI Europe - UK, Bakeham Lane, Egham, Surrey, TW20 9TY, UK
m.seier@cabi.org _c.ellison@cabi.org
[2]CABI Europe - Switzerland, Rue des Grillons 1, CH-2800 Delémont, Switzerland
g.cortat@cabi.org
[3]Department of Employment, Economic Development and Innovation, Biosecurity Queensland, Ecosciences Precinct, GPO Box 267, Brisbane QLD 4001, Australia
Michael.Day@deedi.qld.gov.au Kunjithapatham.Dhileepan@deedi.qld.gov.au

Abstract

Host specificity is one, if not the most critical attribute for any biological control agent to be considered for introduction into a new environment. Nevertheless, specificity is not always an absolute measure. The acceptability of any potential non-target effects will depend on a number of factors such as the extent of the non-target damage, the status of the affected species and the inherent characteristics of the receiving ecosystem; and, any decision for introduction will have to be based upon an encompassing risk – benefit analysis. The concept of "acceptable levels of host specificity" is illustrated using the case studies of three rust species which are currently under evaluation as potential classical biological control (CBC) agents for Australia: *Puccinia lantanae* Farlow from Peru for lantana, *Lantana camara* Linnaeus; *Phakopsora jatrophicola* Cummins from Mexico for bellyache bush, *Jatropha gossypifolia* Linnaeus; and *Ravenelia acaciae-arabicae* Mundkur & Thirumalachar from India for prickly acacia, *Acacia nilotica* ssp. *indica* (Linnaeus) Wildenow ex Delile. Based on the research conducted to date, the risks associated with the potential release of each individual pathogen as a CBC agent for Australia and, in the case of *P. lantanae* also for New Zealand and South Africa, are assessed. Finally, comparisons are drawn with the risk evaluation undertaken for two other rust pathogens, Maravalia cryptostegiae (Cummins) Y. Ono and *Puccinia xanthii* Schweinitz var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero, previously introduced for weed control into Australia.

Introduction

For a natural enemy to be employed as a classical biological control (CBC) agent against an invasive alien weed, it is essential to elucidate its potential host-range prior to consideration for release. Specificity testing of both arthropods and pathogens has routinely been undertaken according to the centrifugal phylogenetic procedure (Wapshere, 1974), and constitutes a major component in a CBC program. More recently, Briese (2003) suggested that the degree of phylogenetic separation between test species and the target weed should also be taken into account when compiling test lists. Standard host-range testing of fungal pathogens is conducted under optimum ambient conditions for host infection and disease development with respect to temperature and the length of the initial dew period, and by applying high inoculum loads of the infective spore stage. The aim of such an experimental set-up is to

establish the fundamental host range of a candidate agent as the widest possible range of hosts that a pathogen could infect and utilize to complete its life cycle. Based on results of this experimental host-range screening, predictions can be made about the anticipated field host-specificity or ecological host range of a pathogen. Frequently, these have been shown to be narrower than fundamental host ranges of CBC agents, as optimum experimental conditions can create so-called "false positives" (Bruckart et al., 1985; Evans et al., 2001).

Risk analyses which are undertaken before the introduction of an agent into a new environment are based largely, but not exclusively, on the data generated during host-range testing. These analyses aim to weigh the perceived risks associated with such introductions, i.e. to the environment, the economy and/or the cultural background of the geographic region, against the anticipated benefits or positive impacts the agent will exert i.e. by controlling the target weed. Equally, the risks posed through the introduction of a CBC agent will need to be balanced against the current and future environmental and/or economic consequences due to the invasive weed, or the cost and impact of management with conventional control methods, such as herbicide applications (Harris, 1990; McFadyen, 1998). A risk assessment of a fungal pathogen needs to be based on its predicted ecological host range rather than its fundamental host range, by asking: "How likely is any non-target to come under attack in a field situation?". Fungal pathogens which exhibit a fundamental host range limited exclusively to the target weed can easily be evaluated. More commonly encountered, however, are cases where pathogens have the potential to damage a limited number of non-target species under optimum conditions for infection and disease expression. In those cases, the "status" of a potentially affected non-target species becomes relevant i.e. whether the attacked plant species is native, naturalized or introduced; whether it is of ecological, economic and/or cultural importance; whether it occurs geographically separated from the target weed or grows sympatrically. Furthermore, the extent of the anticipated impact on a non-target species will be of importance; the likelihood that exposure to infective propagules (e.g. airborne spores) will occur in the field and that conditions for infection and disease development will be met given prevailing geographic and climatic conditions, also needs to be considered. For example, an endemic plant species, of high biodiversity importance, growing in close proximity to stands of the targeted invasive weeds, will raise more concern if damaged by a newly introduced CBC agent, than a non-native species of minor economic value, cultivated in a separated geographic region with different climatic conditions.

Thus, infection of a non-target species under controlled or artificial conditions does not necessarily preclude the introduction of the agent. The following case studies of rust species undergoing, or having undergone evaluation as CBC agents for Australia - and for one case also for New Zealand and South Africa - illustrate the concept of "acceptable level of host specificity" and highlight how this depends on specific circumstances.

Case studies

Rust pathogens currently undergoing evaluation as potential biocontrol agents

Phakopsora jatrophicola for control of bellyache bush, Jatropha gossypifolia

Jatropha gossypifolia L. (Euphorbiaceae), native to the Caribbean rim and its islands, is an invasive weed in Queensland (QLD), as well as in Western Australia (WA) and the Northern Territory (NT) in Australia (Bebawi et al., 2007). In the native range, the macrocyclic rust *Phakopsora jatrophicola* Cummins is a widespread and damaging pathogen of bellyache bush, as well as of other selected *Jatropha* species, i.e. *Jatropha curcas* Linnaeus and *J. integerrima* Jaquin (Leahy, 2004; Farr and Rossman, 2011). Since 2008, the rust has been evaluated as a potential biocontrol agent for Australia under quarantine conditions at CABI UK (CABI Europe - UK), using a urediniospore accession ex *J. gossypifolia* collected in the Mexican State of Veracruz (IMI 397220). Following confirmation that all major Australian biotypes of bellyache bush tested were susceptible to this rust accession, preliminary host range testing commenced and included the biofuel species *J. curcas*, the ornamental species *J. multifida* Linnaeus, *J. integerrima* and *J. podagrica* Hooker,

as well as rubber (*Hevea brasiliensis* (Willdenow ex A. Jussieu) Müller Argoviensis and cassava (*Manihot esculentum* Crantz) as economically important members of the *Euphorbiacae*. Results showed *J. multifida* to be 'fully susceptible' (consistent sporulation) and *J. curcas* to be 'partially susceptible' (restricted sporulation) to the rust accession. *Jatropha integerrima* was classed as 'resistant' (macroscopic necrotic symptoms, but no sporulation) or 'partially susceptible', depending on biotype, while *J. podagrica* was rated as 'resistant'. Both rubber and cassava were immune (no symptoms).

Despite *P. jatrophicola* being able to attack three non-target *Jatropha* species, full host-range testing of the rust was proposed, endorsed by the Queensland government, based on the following considerations. The genus *Jatropha* is introduced to Australia and *J. integerrima*, *J. multifida* and *J. podagrica* are ornamentals of minor importance. The important crop plants rubber and cassava are not susceptible. Infection of *J. curcas*, as having potential economic value as a biofuel crop, could cause concern; however, this species is a declared weed in QLD, NT and WA as well as an approved target for biocontrol nominated by the NT government. Importation of *J. curcas* into Australia is now prohibited by the Australian Quarantine and Inspection Service (AQIS). The species is currently not cultivated in Australia and, in view of its weed status, it is unlikely that *J. curcas* would become a major biofuel crop in Australia in the future.

While *P. jatrophicola* is associated with different *Jatropha* species in the centre of origin, host-specificity studies indicated the existence of host-specialized accessions within this rust species. *Jatropha curcas,* a reported field host of the rust, was only partially susceptible towards the accession IMI 397220 ex *J. gossypiifolia*. Similarly, cross-infectivity studies using a Mexican rust accession of *P. jatrophicola* ex *J. curcas* (IMI 397097) against *J. gossypiifolia* caused limited sporulation on the latter host. Based on these observations, it was considered prudent to survey a range of different geographic locations for accessions of *P. jatrophicola* ex *J. gossypiifolia* potentially less virulent to *J. curcas.* A rust accession ex *J. gossypiifolia* from Trinidad (IMI 397 973), selected due to comparatively low virulence to *J. curcas,* is currently undergoing full host-range testing.

Ravenelia acaciae-arabicae for control of prickly acacia, Acacia nilotica ssp. indica

Prickly acacia, a member of the Leguminosae - Mimosoideae, is a major invader of arid and semi-arid land in QLD. Survey work conducted in the Indian native range of this weed species found the macrocyclic galling rust species *Ravenelia acaciae-arabicae* Mundkur & Thirumalacha to be virulent to *A. nilotica* ssp. *indica* (Linnaeus) Delile, while not attacking any other subspecies or other *Acacia* species (Dhileepan et al., 2010). Based on the damage inflicted on its host and its apparent field host-range, the rust was selected for further evaluation as a potential biocontrol agent. Preliminary host-range testing using a urediniospore accession of *R. acaciae-arabicae* from Tamil Nadu, India (IMI 398973) against 17 selected *Acacia* species commenced under quarantine conditions at CABI UK in June 2010. Susceptibility of the assessed *Acacia* species towards the rust was variable. Of those tested, 16 species showed macroscopic symptoms ranging from mild to severe leaf chlorosis and/or necrosis, sometimes accompanied by strong polyphenolic plant reactions. Critically, the rust was able to sporulate with viable, infective urediniospores on the Queensland native species, *Acacia sutherlandii* (F. Mueller) F. Mueller. Although sporulation on *A. sutherlandii* was always accompanied by dark necrotic lesions, indicating that this non-target species is not a natural host, its susceptibility is, nevertheless, cause for concern.

The risks posed by *R. acaciae-arabicae* to the important Australian native genus *Acacia,* particularly *A. sutherlandii* that grows sympatrically with the target weed prickly acacia in the field, was considered unacceptably high, and further assessments were put on hold. Instead, the focus has shifted towards a second rust species, *Ravenelia evansii* Sydow & P. Sydow, associated with prickly acacia in its native range, but predominantly present in north-western parts of India (Dhileepan et al., 2010; Shivas et al., 2011). The susceptibility of *A. sutherlandii* to this rust species is currently being evaluated.

Puccinia lantanae for control of lantana, Lantana camara

Lantana (Verbenaceae), native to Central and South America, is one of the most widespread invasive plant species and has been a target for CBC for over a century (Day et al. 2003). In Australia, 31 biological control agents have been introduced, including the highly host specific rust *Prospodium tuberculatum* (Spegazzini) Arthur in 2001 (Ellison et al., 2006; Thomas et al., 2006). A second rust species, the microcyclic rust *Puccinia lantanae* Farlow, is currently being screened in quarantine at CABI UK for potential introduction into Australia, New Zealand and South Africa. A damaging rust accession from the upper Amazon in Peru (IMI 398849), causing not only the typical leaf infection but also infection of the petioles, stems, and systemic infection of meristems, was assessed for its infectivity and virulence towards 30 weedy lantana forms from Australia, five from New Zealand and six from South Africa. A qualitative scoring system was used, based on the number and size of telial pustules formed after inoculation with a standardized spore dose. Of those screened, 18 forms were found to be fully susceptible (with examples from all three target countries); three as moderately susceptible and six as weakly susceptible (supporting limited telia formation); and 14 were rated as immune or resistant (unable to complete life cycle).

Host-range testing of the rust for Australia focused on plant species in the Verbenaceae. None of the non-target species were found to be fully susceptible under optimum experimental conditions. However, one species (*Lippia alba* [P. Miller] N.E. Brown) was weakly susceptible and two (*Phyla canescens* [Kunth] Moldenke and *Verbena officinalis* Linnaeus [var. *africana* and var. *gaudichaudii*]) were weakly to moderately susceptible. Only the infection of the two varieties of *V. officinalis* is considered relevant, since these two varieties are regarded as native to Australia and occur sympatrically with lantana; the other two susceptible species are introduced weeds. It was not possible to maintain a culture of the rust on these non-target species and no infection was achieved when a low concentration of spores was applied. In addition, there was variation in the susceptibility of individual *V. officinalis* test plants, suggesting that in the field, natural resistance within wild populations is likely to mitigate any potential impact of the rust. For New Zealand, where there are no native species within the plant family Verbenaceae, host-specificity testing has focused on native non-target species belonging to the closely related families Lamiaceae and Bignoniaceae, as well as other families in the Lamiales. All of the test species were classed as immune or resistant. Host-range testing undertaken for South Africa is still on-going. Encouragingly, the native species *Lantana rugosa* Thunberg and three *Lippia* species potentially the most at risk, are rated as resistant to the rust.

Given the variation in susceptibility of weedy lantana forms and the results of the host-range testing, the position on risks associated with a potential introduction of *P. lantanae* versus the anticipated benefits is likely to differ between the three countries concerned. For New Zealand, no apparent hurdles concerning introduction of the rust are anticipated and an application to release this rust is under preparation by Landcare Research (L. Hayes, pers. comm.). The same may apply to South Africa, providing none of the non-target species still to be assessed are attacked by the rust. Australia, however, has to deal with a more complex risk analysis, given the infection of the two native varieties of *V. officinalis*. The risk, albeit low, posed to this non-target species needs to be weighed against the likely impact the rust would have on lantana, given the resistance expressed by some of the forms. Further work under quarantine in planned, particularly to assess the impact of the rust on the growth of *V. officinalis*, prior to the preparation of an application to AQIS for its introduction.

Rust pathogens previously evaluated and introduced into Australia

A number of fungal pathogens considered as safe, based on a risk analysis have previously been introduced as CBC agents for weed biological control into Australia (Julien and Griffiths, 1998). Despite known non-target effects, the rubber-vine rust, *Maravalia cryptostegiae* (Cummins) Y. Ono, and the parthenium summer rust, *Puccinia xanthii* Schweinitz var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero, were regarded as having an "acceptable level of host specificity" to be utilized as biocontrol agents in Australia.

Maravalia cryptostegiae for control of rubber-vine

Rubber-vine *Cryptostegia grandiflora* (Roxburgh ex. R. Brown) R. Brown, a Madagascan endemic belonging to the Apocynaceae - Asclepiadoideae, has been described as "the biggest single threat to natural ecosystems in tropical Australia" (McFadyen and Harvey, 1990). As part of a CBC program, the rust species *M. cryptostegiae* (Roxburgh ex R. Brown) R. Brown), infecting the weed in its native range, was evaluated as a promising biocontrol agent during a five-year study. Under optimum ambient conditions for infection and disease development, and by applying high urediniospore inoculum loads, the rust was able to infect and produce limited sporulation on the endangered Australian-native asclepiad species, *Cryptolepis grayi* P.I. Forster (Evans and Tomley, 1994). However, when simulating more realistic field conditions for infection, by using a wind tunnel for urediniospore dispersal, *C. grayi* showed no rust sporulation (Evans and Tomley, 1996). Based on these results, it was concluded that the fundamental host range of *M. cryptostegiae* is wider than the anticipated ecological host range and, thus, that *C. grayi* is unlikely to come under attack in the field. Furthermore, the habitats of this Australian endemic and of the target weed rubber-vine have no geographic overlap, while the ecosystem which harbors *C. grayi* is itself under threat and likely to disappear in the foreseeable future (Evans, 2000). Weighing these considerations against the threat posed by the invasive rubber-vine to entire tropical ecosystems, the risk presented by the rust to *C. grayi* was considered acceptable and importation of the pathogen was approved by AQIS in 1994. Since its release, *M. cryptostegiae* (IMI 331455) has not been reported to attack any non-target species (Barton, 2004) and the cost-benefit ratio for agriculture in QLD has been calculated as 108:1, with an accrued benefit of AUS$ 232.5 million up to 2004 (Page and Lacey, 2006). Saving Australian ecosystems from rubber-vine will be priceless.

Puccinia xanthii var. parthenii-hysterophorae for control of parthenium weed

Following the introduction of the parthenium winter rust, *Puccinia abrupta* var. *partheniicola* (H.S. Jackson) Parmelee into Australia (Dhileepan and McFadyen 1997), a second microcyclic rust species, *Puccinia xanthii* var. *parthenii-hysterophorae* (Roxburgh ex R. Brown) R. Brown) (formerly *P. melampodii* Dietel & Holway) or parthenium summer rust, was evaluated as a complement for control of the highly invasive and allergenic weed parthenium, *Parthenium hysterophorus* Linnaeus (Asteraceae), in the more tropical regions of QLD. Comprehensive host-range testing of 80 non-target species under optimum conditions, showed that the asteraceous Australian-native species *Flaveria australasica* Hooker, as well as one commercial variety of *Zinnia elegans* Jacquin, were highly susceptible, supporting abundant sporulation of the summer rust. *Helianthus argophyllus* (D.C. Eaton) Torrey & A. Gray, *Parthenium confertum* A. Gray and two commercial varieties of *Calendula officinalis* Linnaeus proved to be moderately susceptible showing restricted sporulation (Seier et al., 1997). Subsequent wind-tunnel experiments conducted with *H. argophyllus*, and one susceptible variety of both *Z. elegans* and *C. officinalis,* resulted in restricted infection on *C. officinalis* only (Seier et al., 1997). As previously done for the rubber-vine rust, the risk analysis for *P. xanthii* var. *parthenii-hysterophorae* was based on its apparent ecological host range as established in the wind-tunnel rather than its fundamental host range. Of further consideration was the fact that the susceptibility of crop and ornamental species to the rust was highly dependent on the variety, an observation which had already been documented by Morin et al. (1993) for an accession of *Puccinia xanthii* Schweinitz attacking the invasive weed *Xanthium strumarium* Linnaeus (Bathurst burr). This latter accession had previously been introduced into Australia by accident and had subsequently been noted to attack some cultivars of sunflower as well as *F. australasica* in the field (Alcorn and Kochman, 1976). Commercial damage to Australian sunflower crops, however, has not been recorded (J.K. Kochman, pers. comm.) and infection rates on *F. australasica* are generally low without any apparent impact on this species (K. Dhileepan, unpublished data). Based on the results of the host-range testing for the parthenium summer rust, and the "unintentional" field experience with the accidentally introduced *P. xanthii* accession attacking Bathurst burr, *P. xanthii* var. *parthenii-hysterophorae*

(IMI 379934), then named *P. melampodii*, was approved for introduction into Australia in 1999. To date, no non-target effects have been reported (K. Dhileepan, unpublished data).

Conclusions

The case studies presented here highlight the lack of an absolute measure for the required host specificity of a CBC agent, and raise the concept of an "acceptable level of host specificity", which is very much dependent on a variety of factors unique to each case. The status of, or the value put upon, a non-target species potentially under attack by a biocontrol agent, is of high relevance as illustrated in the case of *Phakopsora jatrophicola*. Australian authorities regard the risk posed to *Jatropha curcas* - a declared weed species in the country - and two ornamental *Jatropha* species of minor economic importance, as less critical when compared to the potential positive impact the rust may have on the invasive bellyache bush populations. Conversely, the anticipated damage to *Acacia sutherlandii* - a native species growing sympatrically with the target weed - by the rust *Ravenelia acaciae-arabicae*, is unlikely to be acceptable regardless of any potential benefit of the CBC agent. A risk analysis may also conclude that the level of damage to a desirable non-target is acceptable. For example, AQIS approved the introduction of the parthenium summer rust, despite the attack of one variety of *Calendula officinalis*. However, India, where *C. officinalis* is a highly valued species due to its cultural importance, is likely to view this differently. The importation of *Puccinia xanthii* var. *parthenii-hysterophorae* is unlikely to be endorsed, therefore, although *Parthenium hysterophorus* is an equally problematic weed as in Australia (Evans, 2000). The importance of geographic separation is demonstrated for the case of the rubber-vine rust. The introduction of the pathogen was approved despite potential damage to the endemic *Cryptolepis grayi*, partly because the target weed rubber-vine and the non-target have no overlap in their geographic ranges. The benefit to Australian ecosystems affected by invasive rubber-vine was thus considered to outweigh the risk to an Australian endemic species.

It can be critical for a risk analysis to predict the ecological host range of a CBC agent as accurately as possible. This was demonstrated with rubber-vine rust and the parthenium summer rust, where wind-tunnel experiments were conducted to provide more realistic field conditions for spore dispersal and infection. Inherent characteristics of the receiving environment, such as climatic conditions, can further narrow the ecological host range of an agent and need to be considered.

Overall, the benefits of the introduction of a CBC agent have to significantly outweigh the associated risk to justify its introduction into a new environment. It is the aim of the risk analysis to establish this, and the decision is case specific. In the example of the lantana rust, from an Australian perspective, the fact that *Puccinia lantanae* only infects some of the important weedy lantana varieties, may influence the decision to introduce the rust, given its ability to cause limited sporulation on the two native varieties of *Verbena officinalis*. Conversely, for New Zealand and South Africa, where there is no apparent risk to non-target species, it is likely that the rust will be released despite it only attacking some of the weedy forms in these countries.

Acknowledgements

Funding for the studies conducted was provided by the Queensland Government, Australia; Meat and Livestock Australia; Landcare Research, New Zealand; and the Agricultural Research Council Plant Protection Research Institute, South Africa. We would like to thank Harry C. Evans for his constructive comments on the manuscript.

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