**Phytomyza vitalbae, Phoma clematidina, and insect–plant pathogen interactions in the biological control of weeds**

R.L. Hill, S.V. Fowler, R. Wittenberg, J. Barton, S. Casonato, A.H. Gourlay and C. Winks

**Summary**

Field observations suggested that the introduced agromyzid fly *Phytomyza vitalbae* facilitated the performance of the coelomycete fungal pathogen *Phoma clematidina* introduced to control *Clematis vitalba* in New Zealand. However, when this was tested in a manipulative experiment, the observed effects could not be reproduced. Conidia did not survive well when sprayed onto flies, flies did not easily transmit the fungus to *C. vitalba* leaves, and the incidence of infection spots was not related to the density of feeding punctures in leaves. Although no synergistic effects were demonstrated in this case, insect–pathogen interactions, especially those mediated through the host plant, are important to many facets of biological control practice. This is discussed with reference to recent literature.

**Keywords:** *Clematis vitalba*, insect–plant pathogen interactions, *Phoma clematidina*, *Phytomyza vitalbae*, tripartite interactions.

**Introduction**

Biological control of weeds is based on the sure knowledge that both pathogens and herbivores can influence the fitness of plants and depress plant populations (McFadyen 1998). We seek suites of control agents that have combined effects that are greater than those of the agents acting alone (Harris 1984). However, recent research suggests that predicting which combinations of agents are likely to generate that effect is difficult, and may be misleading. This is particularly true for interactions between insects and plant pathogens, because entomologists and pathologists tend to work exclusively in their own discipline (Agrios 1980, Connor 1995, Caesar 2000).

Hatcher & Paul (2001) have succinctly reviewed the field of plant pathogen–herbivore interactions. Simple, direct interactions between plant pathogens and insects (such as mycophagy and disease transmission) are well understood (Agrios 1980), as are the direct effects of insects and plant pathogens on plant performance. Very few fungi are dependent on insects for the transmission of their spores, but spores transmitted by insects have a greater chance of reaching a suitable site compared with spores dispersed by water and wind (de Nooij 1988). The reciprocal effects of plants on pathogens and insects through such mechanisms as wound responses, induced resistance, systemic acquired resistance, and hypersensitive reactions are acknowledged, if imperfectly understood (Zidack 1999). However, the potential indirect effects of plant pathogens (especially biotrophs) on insects (and vice versa) mediated through the host plant are often cryptic, poorly understood, but common. Hatcher (1995) identified a range of possible outcomes for such tripartite relationships, and these have considerable relevance for future biological control practice.

This paper describes a manipulative experiment designed to examine some of the interactions between
two biological control agents introduced to New Zealand to attack the invasive weed *Clematis vitalba* L. (old man’s beard). It also explores the importance of plant pathogen–herbivore relationships to the future practice of biological control of weeds.

**Material and methods**

**The hypothesis**

*Clematis vitalba* (Ranunculaceae) grows throughout central and southern Europe, and extends as far as the Caucasus. It was introduced to New Zealand as an ornamental before 1920, and is now naturalized throughout. Vines can climb tall forest trees, forming a dense light-absorbing canopy that suppresses vegetation beneath it. These can become large enough to pull down trees, and also scramble over the ground, suppressing regeneration. Infestations threaten the existence of small forest remnants, and create a nuisance in many other habitats (Hill et al. 2001).

The old man’s beard leaf-mining fly *Phytomyza vitalbae* Kaltenbach (Diptera: Agromyzidae) and the fungus *Phoma clematidina* (Thümen) Boerema were introduced in 1996 (Gourlay et al. 2000). Both agents established and spread quickly (Hill et al. 2001). The speed with which *P. clematidina* dispersed within New Zealand and the co-occurrence of the two agents at new sites suggested that the fungus was carried from place to place by the fly. Before *P. clematidina* was introduced, *P. vitalbae* leaf-mines were usually brown. Following establishment of the fungus, leaf mines were usually black. Although the cause of the discoloration was never formally identified, it was suspected that the new fungus was invading mines. These were commonly surrounded by a yellow halo, a common symptom of infection by plant pathogens (Agrios 1988), suggesting that fungal invasion of leaves could occur from within mines. These observations raised the possibility that the two agents were synergistic in their effects on old man’s beard leaves.

This hypothesis was reinforced when it was shown that newly emerged flies were capable of transferring *P. clematidina* by walking on a culture on an agar plate and transmitting it to a fresh plate. While short-range transport of the spores was therefore feasible, adult flies appeared to actively avoid the black *Phoma*-infected parts of leaves. The frequency with which flies transmitted the fungus between plants remained unclear (R. Wittenberg, unpublished data). The larvae of *P. vitalbae* produce characteristic mines, but adults can also damage leaflets. Female flies pierce the leaf surface using the ovipositor, and then feed on leaf exudates. Hundreds of feeding punctures can be made in a leaflet, and eggs are laid in just a few of these (R.L. Hill, J. Fröhlich, A.H. Gourlay & C. Winks, unpublished data). Our hypothesis was that feeding punctures formed by adult flies provided a point of entry for the necrotrophic fungus to infect the leaf and/or the mines, either directly via the ovipositor, or indirectly by the fungus invading the wounds. If so, then the damage to the leaf was likely to be greater than if either agent was working alone – an additive or a synergistic effect. To examine the relationship more closely, we designed an experiment to investigate whether the fly was facilitating the performance of the fungus. The aims were to determine:

1. how long *P. clematidina* conidia survived on the bodies of adult flies
2. whether adult flies introduced the fungus into the leaf through penetration by the ovipositor
3. whether feeding punctures on leaves facilitated invasion by water-borne inoculum of *P. clematidina*.

**Methods**

*Clematis vitalba* plants were obtained from two sources. Several hundred seedlings, 5–10 cm tall, were dug from beneath a single *C. vitalba* plant at Kaituna Valley, mid-Canterbury, in mid-December 1999, and were replanted in planter bags (PB3.5). Plants were placed in a shade house, and were ready for use 6 weeks later (batch 1). At this stage, plants bore one pair of fully formed leaves (each with five leaflets), and a second pair of leaves was developing. At the same time, seeds collected from a plant at Lincoln Golf Course, mid-Canterbury, in the previous spring were sown in a seed tray. In late January 2000, seedlings were potted as described above, and were ready for use 6 weeks later (batch 2).

Preliminary experiments established that treatment with 0.5% sodium hypochlorite (NaOCl) successfully stopped infection of *C. vitalba* leaves by *P. clematidina*, but did not prevent infection when conidia were later applied to surface-sterilized leaves. The culture of *P. clematidina* used in these experiments was a subculture of an isolate originally collected from *Clematis ligusticifolius* Nutt. in 1991 in the USA and subsequently released in New Zealand as a biological control agent for *C. vitalba* (A. Spiers, unpublished HortResearch client report 1995). Inoculum was prepared from 15-day-old cultures, grown on 15% V8 agar (made with V8 juice clarified with calcium carbonate) in 9 cm Petri dishes, and incubated at 20°C under white lights with a 12 h photoperiod. A spore suspension was prepared by initially adding 3 mL of sterile distilled water (SDW) to one plate, dislodging spores with a sterile glass rod, filtering through a sterile cell strainer (Falcon, 70 µm nylon, Becton Dickinson, USA), and adding the filtrate to 97 mL of SDW. Conidial density was estimated using a haemocytometer, and the suspension was used to harvest conidia from additional plates until an adequate conidial density was obtained. Suspensions were prepared on three separate occasions.

**Survival of conidia on flies**

Fly pupae of even age were collected from the general culture, soaked in 0.5% NaOCl for 15 min to kill any
Plant-to-plant transmission of Phoma clematidina by Phytomyza vitalbae

Eleven C. vitalba plants (batch 2) were selected for medium size, and convenient leaf size. Four days prior to experimentation, plants were surface-sterilized with 0.5% NaOCl. On each plant, two leaves (each bearing five leaflets) were captured individually in clean glass tubes and killed by narcotizing with CO2. In a laminar flow cabinet, and using sterile techniques, flies were wiped onto potato dextrose agar (Difco Labs, USA) amended with 0.02% streptomycin (Sigma, USA), contained in 9 cm Petri dishes, one fly in each of four quadrants. Flies were incubated at 20°C and 12 h photoperiod. After 10 days, promising cultures were transferred to 15% V8 agar to allow identification of P. clematidina colonies. A further 10 flies were assessed 24 h after conidia were applied, and the remaining five flies were assessed 72 h after application (four sprayed with SDW, and one with the conidial suspension).

The effect of Phytomyza vitalbae feeding punctures on the infection rate of Phoma clematidina

Flies less than 2 days old can pierce the epidermis of leaves to feed, but cannot oviposit. All flies were therefore extracted from the bulk culture at 2-day intervals to ensure that no flies exceeded this age. For the 1-day-old damage treatment, insufficient young flies were available, and mixed-age flies from another general culture were used instead. The experiment was evaluated before eggs laid by these flies could hatch and produce mines.

Eighteen plants (batch 1) were selected haphazardly. Twelve were placed in individual acrylic plastic boxes (500 × 300 × 300 mm or 600 × 300 × 300 mm) in a temperature-controlled room set at 19–21°C with a 16 h photoperiod. Five assumed pairs of P. vitalbae (five large flies and five small flies) were added to each box. The remaining six plants were placed in a single box and no flies were added. Flies were removed after 24 h (day 1). On each of the 12 plants exposed to flies, five damaged leaflets were selected and marked (where possible one leaflet per leaf), and the number of feeding punctures was recorded. In some cases, fewer than five leaflets on the plant were damaged. In this case, all damaged leaflets were labelled. We also labelled five leaflets on each of the six plants that were not exposed to flies. Plants were returned to the shade-house and positioned haphazardly. Three further sets of 18 plants were treated for 24 h using the same technique beginning on days 2, 4 and 6. Thus, after 8 days, plants bearing 7-, 5-, 3- and 1-day-old fly damage had been produced. On day 8, six damaged plants from each treatment were sprayed to run-off with sterile water (10–15 mL per plant). The remaining six damaged and the six undamaged plants from each treatment were sprayed to run-off with a suspension of P. clematidina conidia that was adjusted to 1.5 × 10^6 conidia mL⁻¹ (10–15 mL per plant). Three further untreated plants were taken from the shade-house, leaflets were marked,
and the plants were sprayed with sterile water. Each plant was covered with a tall plastic cylinder (200 mm diameter × 300 mm) to ensure free moisture remained on the leaves, and plants were haphazardly placed on a bench in a temperature-controlled room set at 19–21°C.

After 18 h, covers were removed from all 75 plants, and the temperature was reduced to a constant 15°C. After 7 days, marked leaflets were removed from the plants, examined at 10x magnification using transmitted light, and the number of infection sites present (each identified as a dark lesion with a yellow halo) was recorded. The leaflet opposite the marked leaflet (or if this was damaged, the nearest undamaged leaflet on the same leaf) was also removed and assessed.

The data were analyzed by fitting linear mixed-effects models in S-Plus 2000 using function lme and maximum likelihood estimation. The number of spots was taken as the dependent variable, with models including fixed effects for age of feeding damage (1, 3, 5 or 7 days), treatment (flies+water, flies+phoma, no flies+phoma) and damage (damaged leaflet or undamaged leaflet), plus all possible interactions. Plant (1 to 11) and leaf (1 or 2) were included as random effects. Fixed effects were tested by comparing nested models in S-Plus 2000 using function lme and likelihood ratio tests. The dependent variable was square-root transformed prior to analysis to help satisfy model assumptions.

### Results

#### Survival of conidia on flies

No *P. clematidina* colonies were isolated from flies sprayed with water alone. Of the 10 flies plated immediately after conidia were applied, only three yielded *P. clematidina* colonies. No *P. clematidina* colonies were obtained from the 10 flies treated with conidia and plated after 24 h, or the one plated after 72 h.

### Plant-to-plant transmission of *Phoma clematidina* by *Phytomyza vitalbae*

Of the 11 control leaflets painted with the conidial suspension, 9 survived to be assessed. Seven of these developed abundant *P. clematidina* infection spots, indicating that the plants used were susceptible to this fungal isolate. These 7 plants bore 14 sets of leaflets that could be assessed reliably. None of the leaflets to which no flies were added, or flies sprayed with water were added, developed *P. clematidina* infection. Of the 14 leaflets to which flies sprayed with the conidial suspension were added, 4 had no feeding damage, possibly because the flies added were males, or because flies died prematurely. Omitting these, and omitting those plants in which susceptibility to the isolate could not be proven, 10 replicates remained.

Only one of these (10%) developed *P. clematidina* infection. This leaflet carried 510 *P. vitalbae* feeding punctures, the second-most damaged of all of the leaflets.

#### Effect of *Phytomyza vitalbae* feeding punctures on the infection rate of *Phoma clematidina*

Few infection spots appeared on leaves not sprayed with *P. clematidina* (Table 1), and these were probably attributable to other micro-organisms. There was no feeding damage on leaves not exposed to *P. vitalbae*. Leaf infection spots typical of *P. clematidina* were observed on leaves sprayed with the conidial suspension, whether damaged by adult flies or not (Table 1), indicating that the conidial suspension was capable of inducing disease symptoms.

Microscopic examination of the leaves revealed that infection spots occurred apparently randomly across the leaf surface. Fungal invasion of the leaf lamina appeared to be independent of the position of feeding punctures, and there was no evidence of invasion of feeding puncture margins by *P. clematidina*.

### Table 1. Mean number (±SE) of *Phoma clematidina* infection spots observed per leaflet when *Clematis vitalba* seedlings were exposed to (1) both *Phytomyza vitalbae* adult feeding damage of different ages and *P. clematidina*, (2) *P. vitalbae* alone, (3) *P. clematidina* alone. Control leaflets were not exposed to feeding damage by *P. vitalbae* adults.

<table>
<thead>
<tr>
<th>Plant-to-plant transmission of <em>Phoma clematidina</em> by <em>Phytomyza vitalbae</em></th>
<th>Effect of <em>Phytomyza vitalbae</em> feeding punctures on the infection rate of <em>Phoma clematidina</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding punctures</td>
<td>Spots on damaged leaflets</td>
<td>Spots on control leaflets</td>
</tr>
<tr>
<td>7-day-old</td>
<td>81.2 ± 12.2</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>damage</td>
<td>n = 25</td>
<td>n = 25</td>
</tr>
<tr>
<td>5-day-old</td>
<td>86.2 ± 12.2</td>
<td>7.1 ± 1.7</td>
</tr>
<tr>
<td>damage</td>
<td>n = 25</td>
<td>n = 25</td>
</tr>
<tr>
<td>3-day-old</td>
<td>54.1 ± 10.1</td>
<td>5.5 ± 1.5</td>
</tr>
<tr>
<td>damage</td>
<td>n = 28</td>
<td>n = 28</td>
</tr>
<tr>
<td>1-day-old</td>
<td>121.8 ± 13.1</td>
<td>7.8 ± 2.1</td>
</tr>
<tr>
<td>damage</td>
<td>n = 25</td>
<td>n = 25</td>
</tr>
</tbody>
</table>
As no damage was possible on plants not exposed to flies, observations from plants treated with ‘no flies+phoma’ were omitted from models that included damage as an effect. Comparing the model with damage (and its interactions with other factors) with the model with day and treatment effects only shows no evidence that numbers of spots differed between damaged and undamaged leaflets ($\chi^2_8 = 9.92, P = 0.271$). This suggests that the presence of punctures does not improve the chance of infection. Replacing the factor damage with the number of punctures gave similar results, with no evidence that greater numbers of punctures lead to more spots ($\chi^2_8 = 13.0, P = 0.111$).

To test for treatment and age of damage effects we included all observations. There was very strong evidence that number of spots differed between treatments, and that the size of these differences depended on the number of days since flies were put in the boxes ($\chi^2_6 = 38.3, P < 0.0001$). Numbers of spots were consistently higher on the plants treated with $P. clematidina$ than on the water-treated plants, on which few spots were found. For plants treated with $P. clematidina$, mean spot numbers were not significantly different between the with- and without-fly treatments for day $= 1, 3$ or $7$ ($P > 0.2$ for all three days). However, spot counts were significantly lower for the “no flies + Phoma” treatment than the “flies + Phoma” treatment on day $= 5$ ($P = 0.004$).

Discussion

Even though flies were treated with a dense suspension of $P. clematidina$ conidia, and were rolled onto a substrate conducive to spore germination, the fungus could only be isolated from flies 60 min after treatment. Even then, only three of the 10 flies tested yielded colonies. It is not known if this apparently low infectivity is a result of preening by adult flies, death of conidia on flies, or a methodological difficulty in recovering the fungus, but the results suggest that transport of conidia between plants by flies does not have a high probability of success. If conidia are as short-lived as this experiment suggests, then long-distance transport is particularly unlikely.

Similarly, adult flies sprayed with a dense suspension of $P. clematidina$ conidia showed limited ability to transmit the disease directly to a leaf through adult feeding or oviposition. Many of the treated leaves could not be scored due to lack of leaf damage or lack of infection in positive controls, but only one of the 11 remaining replicates developed disease symptoms. Again, the probability of flies contributing significantly to the incidence of disease appears low. However, this frequency of facilitation may be sufficient to explain the field observations of $P. clematidina$ invading leaves from mines.

The third experiment sought to determine whether feeding punctures created by $P. vitalbae$ predisposed leaflets to infection by waterborne spores of $P. clematidina$. Haloes were sometimes observed around feeding punctures but these did not develop disease symptoms. Portions of leaves that were heavily punctured occasionally shrivelled and turned black, but this was never associated with typical disease symptoms or with a halo around the necrosis. In all of the leaflets examined, there were no cases where black infection sites were associated with feeding punctures. Invasion seemed to occur successfully in the absence of flies, directly through the leaf surface, often at depressions in the leaf or petiole where free water might accumulate. Given these observations, it is not surprising that statistical analysis was unable to detect any significant relationship between the number of feeding punctures per leaflet and the number of infection spots present, irrespective of age. There was a slight indication in the 1-day-old damaged plants that heavy adult feeding might reduce infection by $P. clematidina$. If this is true, the mechanism may be mechanical, as feeding of this intensity reduced the amount of leaf lamina available for fungal invasion, or it may be a resistance response. These three experiments provide complementary evidence that if there is any behavioural synergy between these agents, it is minor. Infection by pathogens does not necessarily cause disease every time, in all plant parts, or in all plant ages (Barbosa 1991). The amount of disease could also be dependent on the age of the plant and/or the leaf age (Barbosa 1991). This may have been a possible cause for the lack of visible infection noted around the wounding sites in $C. vitalba$.

The concept of three-way complex interactions between plants, plant pathogens and insects is well established, and is depicted simplistically in Figure 1. Interactions between insects and fungi can be direct (mycophagy, spore dispersal), as can interactions between plants and either insects or fungi (e.g. infection, phytophagy, defoliation, plant resistance to insects, plant resistance to fungi). However, the presence of insect damage can influence the performance of fungi (and vice versa) indirectly through host-plant responses. Of particular importance for biological control is the concept of cross-resistance, where resistance to pathogen infection induced in the host plant by a pathogen can confer incidental resistance to a herbivore (and vice versa). The presence of insects or pathogens can also alter nutrient fluxes within the plant, and these can influence the performance of other organisms either positively or negatively. The array of possible outcomes from indirect fungus–insect interactions range from synergistic effects, where the impact on a plant variable is significantly greater than that obtained from either species alone, to inhibitory, where a plant variable is affected less than by the weaker of the two agents alone (Hatcher 1995). Hatcher & Paul (2001) provide a range of examples relevant to biological control of weeds that demonstrate these effects. Small-scale experiments to assess the interactions...
between *Rumex* spp., the rust *Uromyces rumicis*, and the leaf-feeding chrysomelid beetle *Gastrophysa viridula* indicated reciprocal negative effects between the insect and the pathogen. It was predicted that the combined effects over the life of the plant could be inhibitory. However, in longer trials, effects varied, ranging from inhibitory in one case, to additive in another. Explanations for this variation included behavioural changes by the beetle to select plant material that was not infected, and increased consumption by the beetle in response to reduced foliage quality (see Hatcher & Paul 2001). Hatcher & Paul (2000) have also shown that the impact of *G. viridula* on infection of *Rumex obtusifolia* can be systemic, conferring protection from fungal attack on leaves not attacked by the beetle.

Hatcher & Paul (2001) provide other examples relevant to biological control of weeds that demonstrate the real but complex nature of these interactions. For example, the effect of the weevil *Perapion antiquum* and the fungus *Phomopsis emicis* on the accumulated dry weight of *Emex australis* was equivalent to one of the agents working alone, but the effect on stem length and fruit weight was inhibitory. In another case, the combined effect of three beetles with the rust *Puccinia carduorum* on the performance of *Carduus thoermeri* was considered to be universally positive.

There are many other examples available in the literature. For example, de Nooij (1988) showed that the weevil *Ceutorhynchidius troglodytes* provided an entry wound for the pathogenic fungus *Phomopsis subordinaria* in the plant tissue of *Plantago lanceolata*. The weevils were indispensable for the infection process to occur, with no infection occurring in the absence of the weevil. However, wounding of the stalk did not always result in penetration of the pathogen. In another example, Connor *et al.* (2000) found that there were no significant combined effects of *Platyrepia virginalis* and the fungus *Phoma pomorum* on *Cynoglossum officinale* (houndstongue) in laboratory studies, and that larvae appeared to avoid damaged leaves. On the other hand, Teshler *et al.* (1996) proposed a synergistic interaction between an insect and pathogen feeding on *Ambrosia artemisiifolia*.

Effects are not restricted to root (Caesar 2000) or foliage organisms. In the field, the gall wasp *Dryocosmus dubiosus* experiences significant mortality due to a fungus. Galls with heavy fungal infection generally did not contain living larvae compared with galls without the fungus (Taper *et al.* 1986). Similar studies have begun to examine the tripartite interaction between the white smut *Entyloma ageratinae*, the gall fly *Procecidochares alani*, and the weed *Ageratina riparia* (mist flower) (S. Casonato, unpublished data, Fröhlich *et al.* 2000).

One system that Hatcher & Paul (2001) did not review is the recent research into the relationships between the thistle *Cirsium arvense*, the biotrophic rust *Puccinia punctiformis*, and the insect fauna that attacks the thistle in Europe. Friedli & Bacher (2001a,b) claimed a mutualistic interaction between *Apion onopordi* (Curculionidae) and *P. punctiformis* on *C. arvense*. The weevil benefited the rust fungus by transmitting urediniospores in the process of oviposition, increasing the incidence of rust-infected stems in the following year. The rust benefited the weevil because adults emerging from rust-infected stems were significantly larger than those developing in healthy stems. Bacher *et al.* (2002) have expanded this research. However, this mutually positive relationship does not hold with all insects that feed on *C. arvense*. Kluth *et al.* (2001) found that while larvae of *A. onopordi* were more abundant in infected stems, several other endophages preferred uninfected stems. The incidence of

![Figure 1](image-url)  
**Figure 1.** The tripartite relationship between insects, plant pathogens and their host plants.
ectophages on stems appeared unaffected by rust infection. However, on the same host plant, Krue (2002) showed that the chrysomelid beetle *Cassida rubiginosa* consumed more, developed faster, survived better and was larger when fed on healthy leaves rather than leaves from plants systemically infected by the necrotrophic fungus *Phoma destructiva*. In thistle populations where both pathogens are present, the potential interactions are likely to be complex and variable (see also Kok & Abad 1994).

The complexity of interactions presented here, and by Hatcher & Paul (2001), shows that insects can change plant conditions to the advantage or detriment of fungi, and vice versa (Carruthers et al. 1986). As a result, the impact on host-plant performance can range from synergistic to inhibitory. In fact, real-world situations would include plant–plant interactions such as competition and environmental variables that can induce plant stress (such as drought and soil type), and pathogen–pathogen and insect–insect interactions. Even with pathogen–insect interactions that are synergistic, the effect of the interaction is dependent on various circumstances and may be reliant on the biocontrol agents “attacking” the plant at critical times. It is clear that the host plant should be taken into account when considering insect–fungal interactions. However, Hatcher & Paul (2001) observed that while experimental studies of plant pathogen–insect interactions exist, field studies that might shed light on such complex interactions are rare.

What does this mean for day-to-day practice of biological control of weeds? There have been repeated calls for pathologists and entomologists to work together to gain a better understanding of the nature of relationships and how they can be used to improve levels of control (e.g. Cullen 1996, Caesar 2000, Kremer 2000). However, plant pathogen–insect interactions have relevance in almost every stage of the biocontrol process, not just efficacy.

The legislation under which biological control agents are introduced into New Zealand requires the importer to identify and assess all reasonable and foreseeable risks associated with the proposed control agent (A. Sheppard, unpublished data). As tripartite relationships become more widely known, it is likely that risk assessment of such potential interactions will be required, however difficult or impractical that might be.

Wilding conifers are becoming a major threat to environmental and ecological values in southern hemisphere countries. Biological control of cones and seeds seems to be a logical approach to reducing the rate of spread, but cone-feeding insects may spread the devastating pine pitch canker, *Fusarium subglutinans* f.sp. *pini* (Hoover et al. 1996). Assessment of the risk posed by the introduction of new cone-feeding insects is under way in South Africa (Moran et al. 2000) and is beginning in New Zealand (even though the disease does not exist here).

It is conceivable that host-range testing of control agents in the country of origin could be compromised by plant pathogen–insect interactions. If tests are conducted on test-plant material in which changes have been initiated by fungal infection or insect attack, there is the possibility of overpredicting or underpredicting host range if a strong interaction occurs. Researchers could minimize this risk by ensuring that test material is obtained from plants that are free of fungal or insect damage.

The successful control of *Eichhornia crassipes* in several countries has been enhanced by the infection of insect-damaged plants by indigenous micro-organisms acting as secondary invaders (Charudattan 1986). While the likelihood of such an interaction might have been predicted before release of the introduced herbivores (Hatcher & Paul 2001), the resident organism likely to cause the rots could not. The contribution of indigenous pathogens to the successful control of *Opuntia inermis* in Australia is a similar case (Martin & Dale 2001).

The establishment success of a pathogen or insect could be affected either by unpredicted post-release inhibitory interactions with resident organisms, or by the omission of a necessary relationship from the country of origin. At present, agents are selected for complementary modes of action, minimizing competition for resources, and separating agents spatially and temporally (e.g. Morin et al. 1997, Fröhlich et al. 2000, Hill & Gourlay 2002). Increased knowledge about the tripartite interactions and the risks of cross-resistance could allow selection of agents that will not interfere with each other on release (Zidack 1999), and there is the prospect of “designing” synergistic combinations of control agents. It may be important to introduce agents in the correct order to maximize the likelihood of establishment. Hence tripartite interaction studies could potentially increase the success of biological control programs by introducing synergistic agents, rather than those that are inhibitory or equivalent (Hatcher 1995). There is also potential to reduce costs by introducing agents that appear to complement each other in their effect on the target weed.

Sheppard (2003), McEvoy & Coombs (1999) and others advocate selection of only those control agents that have demonstrated efficacy in pre-release evaluation in their home range. The predicted efficacy of an agent may be underestimated or overestimated if strong, but cryptic interactions are acting in the country of origin. The corollary is that an agent may behave differently when introduced to a new range without the tripartite relationship that influenced its performance in the home range. If a target weed accumulates pathogens and phytophagous insects in its new range, new tripartite relationships could change the expected performance of the control agent following release.

A long-time tenet of biological control of weeds has been that increasing “cumulative stress” on weeds by
serial introduction of control agents will increase the level of control (Harris 1984). As Hatcher & Paul (2001) observed, “one clear message from studies of pathogen–herbivore interactions is that ‘more species’ does not necessarily equal ‘greater stress’ due to potential negative interactions between organisms”. On the other hand, studies to date suggest that few tripartite relationships have proven strictly inhibitory. Infection or infestation rates vary between sites, between plants, and even within plants (Hatcher & Paul 2001). Even if an inhibitory interaction between potential control agents did exist, it is likely that the inhibition would only be expressed in part of the potential range of each agent. Hatcher & Paul (2001) have pointed out that there are too few studies available upon which to generalize the importance of tripartite interactions for biological control success. As shown here, this applies to other facets of biological control practice as well. All we know is that a wide range of potential interactions exist. This leaves biological control researchers with a familiar conundrum – whether to invest in detailed research to reveal those tripartite relationships before introduction, or to “suck it and see”. There appear to be both future opportunities and risks for biological control of weeds in this under-researched field.

Acknowledgements

We thank Alison Gianotti for her help with the experiment, and Ray Webster for the statistical analysis.

References


