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The impact of two introduced biocontrol agents, *Phytomyza vitalbae* and *Phoma clematidina*, on *Clematis vitalba* in New Zealand

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Abstract

Insecticide and fungicide exclusion experiments were performed to determine the impact of two biological control agents, an agromyzid leaf-mining fly *Phytomyza vitalbae* Kaltenbach and a coelomycete fungal pathogen *Phoma clematidina* (Thüm.) Boerema, on the growth and percentage cover of *Clematis vitalba* L. (Ranunculaceae) plants. Both insecticide and fungicide treatments significantly reduced control agent damage to *C. vitalba* leaves over one growing season at Blenheim, New Zealand. However, damage attributable to both agents was rather low and population peaks of both agents occurred in late fall, after the main period of stem growth. There was no significant impact of treatment on growth and only a minor (8–10%), but significant, reduction in percentage cover of *C. vitalba* was recorded. Disease symptoms were generally only expressed late in the growing season, when leaves were senescent, and were correlated with *Py. vitalbae* damage. Therefore, we tentatively conclude that alone, *Ph. clematidina* is insufficiently pathogenic to induce disease symptoms during the main growing season of *C. vitalba*. Selection criteria for any future potential biocontrol pathogen, therefore, need to evaluate inherent epidemiological factors before introduction, to ensure the candidate agent is an aggressive primary pathogen that can exert maximum disease attack on the target plant. Furthermore, the potential of *Py. vitalbae* to exist as an asymptomatic endophyte indicates that extra care may be required when assessing survey results for non-target attack, and when testing candidate pathogen biological control agents for host specificity.
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1. Introduction

Evaluation is arguably the most important phase of biological control because it provides valuable data for decision-making within a particular biological control project, and also contributes generally to ecological theory on biological control and plant–herbivore interactions (Briese, 2004). However, evaluation is not always undertaken to the extent necessary, largely because of a funding-driven emphasis on the finding, testing, and delivery of agents (Briese, 2004). For example, Dhileepan (2003) observed that quantitative data were available for only 38% of target weeds in Australia at an individual plant level and 20% at a plant population level. The corresponding figures for New Zealand are similar (Dr. K. Potter, unpublished data).

Recent concerns about the potential non-target impacts of biological control (e.g., Louda et al., 2003) are resulting in tighter controls over the importation and release of biological control agents worldwide (Sheppard et al., 2003). In New Zealand, the Hazardous Substances and New Organisms (HSNO) Act (1996) requires a rigorous risk analysis to support the potential introduction of biological control agents that not only considers the potential of each proposed agent to attack non-target plant species, but also

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assesses their likely contribution to the biological control of the target weeds (Fowler et al., 2000). Evaluating weed biological control programs is therefore essential to improve our ability to predict the impact and safety of future introductions and, therefore, underpins the continued use of biological control as a tool against invasive alien weeds.

Old man's beard, *Clematis vitalba* L. (Ranunculaceae), a vine that is native to Europe, and extends east to the Caucasus, was introduced to New Zealand as an ornamental before 1920 (Hill et al., 2001). It is now widespread, threatening the existence of many New Zealand native forest remnants (Bungard et al., 1997). Old man's beard kills native trees and shrubs by smothering the canopy with dense foliage which reduces light levels and weighs down and collapses the branches of host trees (Hume et al., 1995).

Two biological control agents, an agromyzid fly *Phytomyza vitalbae* Kaltenbach, and a coelomycete fungal pathogen *Phoma clematidina* (Thüm.) Boerema, were released against *C. vitalba* in 1996 (Gourlay and Wittenberg, 2000). The larvae of *Py. vitalbae* mine *C. vitalba* leaves, reducing photosynthetic area and inducing leaf senescence (Hill et al., 2001), whereas *Ph. clematidina* infection was recorded to cause leaf spotting and vine wilting (Spiers, 1995). Both agents established rapidly and spread (Hill et al., 2001) and the speed with which *Ph. clematidina* dispersed within New Zealand and the co-occurrence of the two agents at new sites raised the possibility that the two agents were synergistic in their effects on *C. vitalba* leaves (Hill et al., 2004). However, experimental work by Hill et al. (2004) indicated that *Py. vitalbae* is unlikely to be a good vector of *Ph. clematidina*, and that feeding damage by adult flies did not enhance fungal establishment. Nevertheless, Hill et al. (2004) noted that before *Ph. clematidina* was introduced, *Py. vitalbae* leaf mines were usually brown, yet 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 *Ph. clematidina* was invading larval leaf mines.

Surveys to investigate potential non-target impacts of *Py. vitalbae* and *Ph. clematidina* are ongoing, although preliminary results have been published for *Py. vitalbae* (Paynter et al., 2004). This experiment was, therefore, designed to (a) quantify the impact of *Py. vitalbae* and *Ph. clematidina*, both separately and in combination on *C. vitalba* growth, and (b) determine whether there is any evidence for competition or synergy between *Py. vitalbae* and *Ph. clematidina*.

2. Materials and methods

2.1. Field site

Clematis vitalba vines were located near Blenheim in a wasteland area alongside the Wairau River (41°52′E, 173°73′N) in the South Island of New Zealand. Semi-deciduous *C. vitalba* vines were growing under a largely exotic partial canopy of poplar (*Populus*) and wattle (*Acacia*) trees. This site was selected due to the presence of an abundance of discretely growing vines, to which treatments could be allocated, and because both biological control agents were known to be well established. Forty discrete mature *C. vitalba* vines were selected and randomly assigned to one of four treatments (10 vines per treatment), which were applied every 6 weeks from 15 September 2003 until 22 April 2004 as follows:

1. A control treatment: water only, sprayed to run off.
2. A fungicide treatment: Carbendazim (methyl-2-benzimidazol carbamate); 1 g each of BavistinDF (BASF New Zealand, Auckland, New Zealand) and Captan (Nufarm, Auckland, New Zealand) mixed with 1 L of water and sprayed to run off.
3. An insecticide: treatment 200 g/L azinphos-methyl (Gusathion, Bayer New Zealand, Auckland, New Zealand)—1 g/L sprayed to run off.
4. A combined insecticide plus fungicide treatment: plants initially treated with fungicide, according to treatment 2, then allowed to dry before being treated with insecticide, as for treatment 3.

Work was also conducted at a second field site at the Mangawhararikí River, near Mangaweka (39°48′S, 175°49′E) in the North Island of New Zealand. However, this field site was destroyed by floods and subsequent landslides in February 2004.

2.2. Data collection

2.2.1. Stem growth

Six randomly selected shoots on each vine were marked on 15 September 2003 and were subsequently measured (including lateral growth if lateral shoots appeared) every 6 weeks from 5 November 2003 until 8 March 2004. Difficulty in relocating many of the shoots originally tagged on 15 September resulted in new shoots being tagged on March 8 and measured until 3 June.

2.2.2. Percentage cover

A 50×50 cm permanent quadrat was set up for each vine. On 27 January, 22 April, and 3 June 2004, a close-up photograph of the foliage growing within each quadrat was taken for analysis of percentage cover of *C. vitalba* and incidence of fungal and insect damage. This was done using Digital Sampling Method, Version 1.00 (Landcare Research, New Zealand) as follows: 100 points on each photograph were randomly generated and each was scored, according to whether it scored positive for *C. vitalba* vegetation (and if the control agents were present or absent), competing vegetation or litter. *C. vitalba* percentage cover was then calculated as the sum of randomly generated points that scored positive for *C. vitalba* vegetation. In addition, on 22 April 2004, the degree to which each vine was shaded by the canopy was estimated by photographing the canopy directly above each adult vine and using Digital Sampling Method to estimate 'canopy gap', defined as the proportion of the photograph that contained clear sky.

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