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## The Biological Control Programme against *Clematis vitalba* in New Zealand

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### Abstract

The biological control of old man's beard (*Clematis vitalba* L.) in New Zealand was initiated in 1989 with a field and a literature survey of insect herbivores in Europe. Agents were selected after host range testing of 40 selected native and exotic plant species. This was conducted in Switzerland and in an insect containment facility in New Zealand. The first agent to be released in New Zealand was the agromyzid fly *Phytomyza vitalbae* Klth. Placed at 33 sites throughout the country since 1996, it is now well established. Mined leaves have been found up to 200 km from release points only 20 months after release. The second agent, the sawfly *Monophadnus spinolae* Klug., was released at only one site and to date no insects have been recovered from this site. A field survey for plant pathogens of *C. vitalba* was carried out in Europe and North America in 1990. Pathogenic fungi were isolated from 57 collections of diseased *C. vitalba* leaves from nine countries. Only two species, *Phoma clematidina* (Thümen) Boerema and *Colletotrichum gloeosporioides* (Penz) Penz and Sacc., were pathogenic, each causing extensive leaf necrosis. A weakly pathogenic form of *P. clematidina* was already present in New Zealand. After host range testing a virulent strain of *P. clematidina* was introduced in 1996. Releases of *P. clematidina* have been made at 23 sites and establishment confirmed at 11 sites. Experiments are underway to determine the impact of the leaf miner and fungus on potted plants of old man's beard, and to investigate potential interactions between the two agents. Five further potential control agents have been assessed and these are discussed.

**Keywords:** biological control, host range testing, *Phytomyza vitalbae*, *Monophadnus spinolae*, *Phoma clematidina*.

In its native range in Europe, old man's beard (*Clematis vitalba* L.; Ranunculaceae) is a common and aggressive vine, which sometimes reaches minor pest status in forests and vineyards in Europe (Buxton 1985), but never forms the large vigorous thickets common in lowland forest fragments in parts of New Zealand. The plant was introduced into New Zealand as an ornamental before 1922 and became naturalized between 1922 and 1935 (Hill *et al.* 1995). The vine climbs forest trees forming a dense canopy, which can reduce healthy native and exotic forest to a low thicket of vines. Old man's beard can attain den-

sities of 7000 stems per hectare and a fresh weight increment of 6.3 kg/m<sup>2</sup>/yr<sup>-1</sup>. Stems can grow an average of 2.3 m in 1 yr, producing 20 new nodes. The plant spreads by seed and adventitious roots (West 1992). West (1992) recorded an average seed-fall of 65 seeds/m<sup>2</sup>/yr<sup>-1</sup> at one site and estimated the life of seed in the soil to be 8–10 yrs. Old man's beard is an environmental weed in New Zealand in remnant native forests, river beds, urban areas, and plantation forests (Hill *et al.* 1995). In New Zealand the Department of Conservation has spent approximately NZ\$225,000 annually on *C. vitalba* control in 9 affected conservancies, amounting to a total expenditure of NZ\$1.01 million between 1989 and 1994 (Hill *et al.* 1994). Over the same period regional authorities had a total nominal expenditure of NZ\$3.8 million. The Department of Conservation expended its money on ameliorating damage in forests, whilst regional authorities mostly spent money and time controlling the weed in riverbank trees and in unoccupied urban land (Hill *et al.* 1994).

The Department of Conservation also spent NZ\$25,400 on research projects, the first of which was a review of the biology of *C. vitalba* (West 1991). Biological control was first suggested in 1984 (Syrett 1984) and an economic evaluation was carried out in 1990 (Greer and Sheppard 1990). This study concluded that funding research on biological control was fully justified. The biological control programme started in 1989/90 with the involvement of several National and Local government agencies.

### Search for potential biological control agents

A search began in Europe in 1985 for invertebrates that could control old man's beard in New Zealand. The preliminary study was done in the UK by Buxton (1985) and then a search in continental Europe by CABI Bioscience staff based at Delémont, Switzerland in 1989/90 (Groppe 1991). These studies found 31 insect, 4 mite and 4 nematode species that were possible biological control agents. Meanwhile, a study conducted in New Zealand over 2 yr at 34 sites concluded that resident natural enemies caused insufficient damage to old man's beard to influence the choice of control agents from Europe (MacFarlane and van den Ende unpublished manuscript).

#### Groppe (1991) listed the following species as potential agents:

Order : Family	Species	Attacks	
LEPIDOPTERA : Geometridae	<i>Eupithecia haworthiata</i> (Doubl.)	Buds, flowers	
	<i>Horisme corticata</i> (Schiff.)	Leaves	
	<i>Horisme vitalbata</i> (Schiff.)	Leaves	
	<i>Melanthia procellata</i> (Schiff.)	Leaves	
	Thyrididae	<i>Thyris fenestrella</i> (Scop.)	Leaves
DIPTERA : Cecidomyiidae	<i>Contarinia</i> sp.	Buds, flowers	
	Agromyzidae	<i>Phytomyza fulgens</i> (Hd.)	Leaf miner
	<i>Phytomyza vitalbae</i> (Kltb.)	Leaf miner	
	<i>Phytomyza kaltenbachi</i> (Hd.)	Leaf miner	
	<i>Phytomyza clematidis</i> (Watt.)	Leaf miner	
HYMENOPTERA : Tenthredinidae	<i>Monophadnus spinolae</i> (Klug.)	Leaves	
	<i>Eurhadinoceraea ventralis</i> (Pz.)	Petioles, leaves	

Order : Family	Species	Attacks	
COLEOPTERA :	Scolytidae	<i>Xylocleptes bispinus</i> (Duftschmid)	Stems
		<i>Xylocleptes biuncus</i> (Reitter)	Stems
	Cucujidae	<i>Laemophloeus clematidis</i> (Er.)	Stems
ACARINA :	Eriophyiidae	<i>Aceria vitalbae</i> (Can.)	Shoots, leaves

The agent that promised most in terms of its potential to damage old man's beard plants was the bark beetle *Xylocleptes bispinus*. Five of the agents (a leaf miner, 3 moths, and a sawfly) were foliage feeders. Preliminary host specificity of these agents to 40 species of plants, including 8 *Clematis* sp. native to New Zealand, ornamental *Clematis* sp. and other species within the Ranunculaceae, was tested in Europe. These tests showed that *Phytomyza vitalbae* and *Monophadnus spinolae* were host specific enough to introduce to New Zealand. Host specificity tests on *Thyris fenestrella*, *Xylocleptes bispinus*, *Horisme vitalbata*, and *Melanthia procellata*, were inconclusive.

The status of these species is discussed below.

### Pathogens as potential biological control agents

Spiers (1991) completed a survey of pathogens affecting old man's beard in Continental Europe, England, and North America. He collected leaves of *Clematis* spp. that showed disease symptoms. The pathogens associated with those symptoms were isolated and identified. These included:

*Botrytis cinerea* Pers. (anamorphic Sclerotiniaceae), *Ramularia* sp., *Septoria* sp. (anamorphic Dothideaceae), *Cercospora* sp. (anamorphic Mycosphaerellaceae), *Phoma clematidina* (Thümen) Boerema (anamorphic Pleosporaceae), *Colletotrichum actuatum* Simmonds ex Simmonds, and *Colletotrichum gloeosporioides* (Penz) Penz and Sacc. (anamorphic Phyllachoraceae).

All fungi were cultured and screened for aggressiveness towards *C. vitalba* leaves and 2 species were found to be pathogenic: *P. clematidina* and *Colletotrichum gloeosporioides*. The others were either not pathogenic (*Ramularia* sp., *Septoria* sp., *Cercospora* sp., *Colletotrichum actuatum*), or not host specific (*B. cinerea*). After extensive laboratory tests a single isolate of *P. clematidina* from *C. ligusticifolia* Torrey and Gray, that was collected in Washington State, USA, was selected for release in New Zealand (Spiers 1992). This pathogen was found to have caused severe defoliation of *C. vitalba* in parts of Europe (A.J.S., unpublished data). The new strain of *P. clematidina* was released in New Zealand in 1996. *Colletotrichum gloeosporioides* was also found to be pathogenic to New Zealand plants of *C. vitalba*, but was rejected as a potential biological control agent because it attacked some native *Clematis* species (Spiers 1994).

### Biological Control Agents Released

#### *Phytomyza vitalbae* (Diptera Agromyzidae)

Old man's beard leaf miner (*P. vitalbae*) is widespread and common in Central Europe and Great Britain (Spencer 1976). Heavy mining deforms leaves (Hering 1957) and reduces their photosynthetic area (Hill *et al.* 1995). The larvae feed between June and October and can produce up to 9 mines per leaflet (Wittenberg and Schroeder 1993).

Adult female flies cause damage by puncturing leaves with their ovipositors to feed on leaf exudates. The biological characteristics of *P. vitalbae* have been reported by Wittenberg and Groppe (1992), Wittenberg and Schroeder (1993), Wittenberg (1994, 1995), Wittenberg *et al.* (1996). The fly is multivoltine, with a fecundity of 521 eggs. These life history characteristics suggest that *P. vitalbae* has the capacity to produce larger populations in New Zealand than it does in Europe. Three solitary larval ectoparasitoids of the leaf miner have been identified in New Zealand: *Diglyphus isaea*, *Pnigalio soemius* (Dr J. Berry, Landcare Research personal communication), and the most common species found, *Diacris* sp. It is not known whether the level of parasitism is a key factor in the population dynamics of the fly, but it seems unlikely that the fly is parasitoid-limited (R.L.H., unpublished data).

Host specificity studies of *P. vitalbae* were conducted at Delémont initially, and later in secure containment in New Zealand. Some 23,000 eggs and 322,000 feeding punctures were counted, feeding was found to be restricted to vines belonging to the family Ranunculaceae and to species within the tribes Ranunculaceae and Anemoneae (Hill *et al.* 1995). Oviposition occurred on European species of *Clematis viticella* L., *C. tangutica* (Maxim.) Korsh., *C. montana* DC., *C. jackmanii* Van Houtte, and *C. orientalis* (Maxim.) Korsh. and on the New Zealand species of *C. marata* J. Armstr., *C. cunninghamii* Turcz., and *C. foetida* Raoul. (Hill *et al.* 1995). Occasional mines were observed on other European *Clematis* spp. in natural populations in Switzerland (Hill *et al.* 1995). From field observations, and further tests in outdoor cages and in secure containment in New Zealand, it was considered that the risk of damage to all of the New Zealand native *Clematis* species was low. Application for release of *P. vitalbae* into New Zealand was made in 1996. In the application it was made clear that release of *P. vitalbae* could lead to incidental damage to non-target *Clematis* species. Permission to release the insect was granted nonetheless. Since 1996, 42 releases have been made at 33 sites. Monitoring confirms that establishment has occurred at 24 sites and in some areas mined leaves have been found up to 200 km from the release point after just 20 mo.

### ***Monophadnus spinolae* (Hymenoptera Tenthredinidae)**

The sawfly *M. spinolae*, is bivoltine in Europe, and might be trivoltine in parts of New Zealand. Under captive breeding conditions, 3 generations have been observed and females were found to survive for up to 3 wk and produce up to 68 larvae. Eggs are laid singly into leaves and after about 23 d, larvae emerge and consume several leaves during development (Wittenberg 1994). Larvae have the unusual habit of destroying the vascular system of the petiole before feeding on the leaf (Wittenberg and Schroeder 1993), thus ensuring that each larva will destroy at least 1 leaf. The last instar larvae do not feed but drop to the ground to pupate in a cocoon in the soil. Host range testing of *M. spinolae* began in 1992 at Delémont, and was completed in 1995. Seeds and cuttings of 43 New Zealand plant species were collected, sent to Switzerland, and established in the experimental garden at Delémont. The first tests used field-collected larvae ranging in age from 1<sup>st</sup> to 5<sup>th</sup> instar. Although 80% of *Clematis alpina* (P. Miller), 30% of *C. foetida*, and 10% of *C. jackmanii* leaves were attacked, no LI or LII larvae survived to maturity (Wittenberg and Hill 1996). Similar tests in 1993 and 1994 showed substantial feeding on *C. foetida*, *C. cunninghamii*, and *C. alpina* leaves (Wittenberg 1994). When these 5 species were retested in no-choice tests using newly emerged larvae rather than field-collected larvae, all died without feeding within 3 d (Wittenberg and Hill 1996). Application to import and

release *M. spinolae* was made in 1996 and in 1998 a single release of 100 eggs, larvae, and pupae was made at Kaituna Valley, Canterbury. The release site has been monitored for 1 year, but neither larvae nor adults have been observed. Mass rearing of the sawfly has proven difficult due to a 20:1, M:F sex ratio, asynchronous emergence, and poor larval survival. Nevertheless further releases are planned in 1999.

### ***Phoma clematidina* (anamorphic Pleosporaceae)**

*Phoma clematidina* causes leaf and stem necrosis, girdling the stem in some cases and causing wilting, premature defoliation, and reduced vigour (Spiers 1995). Wilting induced by this pathogen has caused up to 50% loss amongst *Clematis* hybrids in some nurseries in Europe (Spiers 1992). Initial infection of leaves or stems in spring is caused by conidia released from pycnidia formed in lesions on overwintering leaves and dead stems. These conidia are dispersed by rain-splash (Spiers 1994). The severity of infection is governed by the amount of rainfall during spring and early summer. During collection trips (in Europe) this fungus was observed to cause severe defoliation of *C. vitalba*, sufficient to weaken the plant, but not kill it (Spiers 1998). *Phoma clematidina* infection can be assisted by wounding of the leaves and stems although this is not essential for infection to occur (Spiers 1992). The isolate recently released into New Zealand was specifically selected for its high pathogenicity and did not need wounding of the leaves or stems to facilitate infection, although wounding by an insect biological control agent should increase the damage to *C. vitalba* (Spiers 1994). Before host testing could start, an isolate of *P. clematidina* had to be identified that was pathogenic to expanding and fully expanded leaves of New Zealand collections of *C. vitalba*. This was done by placing leaves on capillary matting in plastic humidity chambers and atomizing on a spore suspension (Spiers 1994). To facilitate the spread of *P. clematidina*, conidia were washed from agar plates with distilled water and diluted to give 100,000 conidia/ml (Spiers 1994). This suspension was atomized onto both surfaces of detached, expanding, and fully expanded leaves of test plants. *Phoma clematidina* formed small lesions on petioles of *Clematis paniculata* J. G. Gmel., and *C. quadibracteolata* Colenso, and leaf spots on *C. montana* DC. (Spiers 1994). Necrotic spots were also formed on 3 weed *Ranunculus* species (Spiers 1994). Further tests were completed on endemic *Clematis* and *Ranunculus* species. Detached leaves, stems, and whole plants of 16 representatives of the genus *Ranunculus* and 16 endemic species of *Clematis* were tested. Minor spotting occurred on leaves of some plants but no pycnidia were formed on any species except *C. vitalba*. An application for release of the fungus was made in 1996 and approved. The new virulent strain of *P. clematidina* has now been released at 23 sites and has spread rapidly throughout the country. The fungus is well established at 11 sites and causing extensive leaf necrosis, leaf fall, and stem dieback at several of these.

### ***Xylocleptes bispinus* (Coleoptera Scolytidae)**

Of all the potential control agents available in Europe for old man's beard control, *X. bispinus* is the most damaging. This beetle attacks young, woody stems, which often die as a result, and is capable of killing significant portions of the plant. Old man's beard stems in Europe rarely exceed 3 cm in diameter, and this may be the result of *X. bispinus* attack (Wittenberg and Schroeder 1993). Mature male bark beetles emerge from an old vine and fly at dusk to locate a new vine suitable for breeding (Wittenberg and Schroeder 1993). Males attract females using pheromones and then excavate a mating chamber at a

node in a *C. vitalba* vine. After mating the female makes several egg niches near the mating chamber, into which she will lay an average of 21 eggs. After oviposition has been completed, the female mines a spiral tunnel at the end of the mating chamber, sometimes cutting off the water and nutrient supply to the rest of the plant. The eggs are laid close to each other below the bark and are covered by bore dust. The apodous larvae are entirely white except for their dark brown mandibles. They pass through 3 instars during the 50 or so days it takes for them to mature. During this time the larva moves along the spiral tunnel made by the female and begins feeding on living tissue just under the bark. Mature larvae then close the hole that points towards the tunnel exit with a hard lid of bore dust and excavate pupal chambers in the wood of the stem. Complete development takes some 59 d and adults overwinter inside the old stems (Wittenberg and Schroeder 1993).

Host specificity tests were carried out on stem material of native *Clematis* species that was field-collected in New Zealand and sent to Switzerland. This was done because imported plants grew poorly in the gardens at Delémont and were unable to produce stems of more than 3 mm in diameter for feeding experiments (Wittenberg and Schroeder 1993). Oviposition occurred on 8 New Zealand native and 9 ornamental *Clematis* species (Wittenberg 1994). *Xylocleptes bispinus* larvae were able to develop, to some degree, on 6 of the 8 native *Clematis* species and all 9 ornamental *Clematis* species on which oviposition occurred. However, these tests were carried out on cut shoot material and this may be more susceptible to attack than the whole plant (Wittenberg 1994, 1995, Wittenberg *et al.* 1996). These results suggest that *X. bispinus* has the potential to develop on some other *Clematis* species, although it has a clear preference for close relatives of *C. vitalba*. Literature records in Europe also suggest that this species is not entirely host specific. Because of its complex behaviour pattern and the size of the test plants exposed in confinement, Wittenberg (1995) considered it was not possible for *X. bispinus* to express normal host selection in laboratory tests on cut stem material. Large-scale field-cage tests using whole plants are needed to allow the likely behaviour of *X. bispinus* females in the field to be assessed. Such tests are not planned at present.

### ***Thyris fenestrella* (Lepidoptera Thyrididae)**

This unusual species belongs to a moth family of predominantly tropical and subtropical distribution. Its larvae feed on foliage and the adult females, which prefer to fly and oviposit on warm, sunny days, are nectar feeders with a recorded fecundity of between 30 and 80 eggs. Oviposition tests on this species were conducted in large tents in the CABI gardens. It was assumed that the tents would allow the females to express their normal behaviour patterns, but they showed no interest in the plants and were intent only on escape. Just 1 of the 2 females oviposited on 1 *C. jackmanii* plant, therefore these results were considered to be inconclusive. Transfer tests using field collected LII and LIII larvae showed the species capable of completing development on 12 of the 18 *Clematis* species presented to them. Most of these were European species of *Clematis* but 2, *C. forsteri* J. G. Gmel., and *C. afoliata* Buchanan, were native to New Zealand (Wittenberg and Häfliger 1997). This agent requires further testing.

### ***Horisme vitalbata* (Lepidoptera Geometridae)**

This foliage-feeding geometrid moth proved difficult to find in the field in Europe. Only 3 females and 1 male were collected from the field, for oviposition tests and to start a laboratory colony for larval feeding tests. Preliminary feeding tests using newly

emerged larvae of *H. vitalbata* indicated a relatively wide host range. Not only were most of the 20 *Clematis* species presented attacked, but larvae also fed on *Ranunculus* spp. and *Aconitum* spp. Larval feeding showed a wide host range, but in oviposition tests carried out in a field cage, with 18 test plants plus *C. vitalba*, eggs were only found on *C. vitalba* (11 eggs, n=1), (Wittenberg *et al.* 1996; Wittenberg and Häfliger 1997). These host specificity tests proved to be inconclusive and as adult moths were short-lived, there was no further testing. This species will not be considered for importation as a possible control agent until the wide larval feeding range demonstrated in the field is further tested in the laboratory.

### ***Melanthia procellata* (Lepidoptera Geometridae)**

Adults of this leaf-feeding geometrid moth were collected by light trapping. The moths were used in choice oviposition tests in field cages in Switzerland. Although *C. vitalba* was the preferred plant, oviposition occurred on 9 of the other 18 *Clematis* species tested, including 3 New Zealand natives. Larval feeding tests using mature larvae, which are less specific than younger instars, showed feeding occurred on all but 2 of the species on which eggs were laid (Wittenberg and Häfliger 1997). Feeding tests using 1<sup>st</sup> instar larvae are needed to confirm the host specificity of this species, but are not planned at present. Although Blaschke (1914) recorded *C. vitalba* as the preferred host of *M. procellata*, this species is not sufficiently host specific for introduction into New Zealand as a biological control agent for *C. vitalba*.

### ***Colletotrichum gloeosporioides* (anamorphic Phyllachoraceae)**

*Colletotrichum gloeosporioides* was consistently isolated from European collections of *C. vitalba* leaves. Isolates were pathogenic to New Zealand *C. vitalba*, causing rapid and extensive necrosis. Other forms of *C. gloeosporioides* have been used in biological control. For example *C. gloeosporioides* f.sp. *aeschynomene* TeBeest was used to control a vetch (*Aeschynomene virginica*, L.) in rice and soybean fields in Arkansas, where it was marketed as a mycoherbicide "COLLEGO<sup>®</sup>" (Spiers 1992). *Lupinus* species have little resistance to *Colletotrichum* (Spiers 1992). Lupins are valued for their ability to fix nitrogen amongst conifers planted in sand dunes in coastal areas of northern New Zealand (Spiers 1992). Consequently *C. gloeosporioides* was rejected as a possible biological control for old man's beard at least until a strain specific to *C. vitalba* can be found.

## **Discussion**

Old man's beard is a fast-growing and vigorous environmental weed in New Zealand. The adverse effect it has in lowland forests can mainly be attributed to the high biomass of the vine in the tree tops, and the speed at which this accumulates. Controlling the impact of such an aggressive plant using classical biological control is a challenge because of its ability to produce so many wind-born seeds and a high biomass.

Three control agents of *C. vitalba* have been released in New Zealand so far. *Phytomyza vitalbae* has established widely and has dispersed from release points remarkably quickly forming new outbreaks. Populations are building rapidly, and *C. vitalba* foliage can contain up to 4 mines per leaflet in some areas. Laboratory experiments are underway to predict what level of mining will be necessary to reduce growth rates of old man's beard. Initial results demonstrate large variations in phenology in relation to leaf miner density. At some field sites the density of mines peaks in autumn, late in the grow-

ing season, and is therefore unlikely to affect annual plant growth. At others, flies are abundant in early spring, when plant growth is fastest and therefore may have a greater impact on plant growth. It was predicted that incidental damage to other *Clematis* spp. was likely because *P. vitalbae* is not entirely host specific. Systematic searches are underway to identify cases where mines have been found on non-target plants, to ensure the correct identification of the leaf miners present, and to measure the density of mining on both old man's beard and the non-target plants. Leaf miners have been found at 1 isolated site on non-target, exotic *Clematis* species. Establishment and spread of *P. vitalbae* and *P. clematidina* on *C. vitalba* in New Zealand are being monitored. Diseased leaves of *C. vitalba* found at new sites are often associated with mined leaves, suggesting that the fly may facilitate the establishment of the fungus, either by providing entry points on leaves or by acting as a vector for *P. clematidina* (R.W., unpublished data).

Laboratory experiments are planned to determine the relative importance of the 2 agents in reducing the growth rate of old man's beard. A further importation into containment in New Zealand of *Monophadnus spinolae* will be made in 1999 to augment breeding stocks. Emerging adults will be released from quarantine to start a colony, and widespread releases in the coming summer season are planned.

*Xylocleptes bispinus* can kill sections of the plant by ring-barking stems. It remains the agent most likely to have a major impact on old man's beard infestations in New Zealand. However, it has been recorded attacking other *Clematis* spp. in Europe, and this alone may eliminate *X. bispinus* as a potential agent in New Zealand, where several endemic *Clematis* spp. occur. Results of host specificity tests conducted to date have not been sufficiently robust to determine this fully. Tests using whole plants will be logistically difficult, but are necessary to complete these studies.

Other biological control agents that show promise but require further work include: *Colletotrichum gloeosporioides*, which could prove effective if a strain specific to *C. vitalba* can be found, and *Thyris fenestrella*, which requires further feeding tests using LI larvae and oviposition tests in small cages indoors (Wittenberg and Häfliger 1997).

Further overseas studies of new agents for old man's beard have been suspended until the impact of the first agents can be assessed.

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