The host range of the leaf-galling eriophyid mite *Aceria vitalbae*, a biological control agent for *Clematis vitalba*.

Host range tests were carried out in Serbia for Landcare Research by Dr Biljana Vidovic of the University of Belgrade (Vidovic, 2016, 2017a,b,c).

Introduction

Aceria vitalbae is native to Europe and is found from France to Romania. Its acknowledged host is *Clematis vitalba*, but it has been recorded rarely from *Clematis flammula* (Buhr, 1964).

There is strong evidence mites belonging to the family Eriophyidae that form galls have very narrow host-ranges. Hong et al. (2001) reported that approximately 88% of eriophyid mites in China are found on a single plant species and another 10% on two species within the same genus. Only around 2% were found on plants belonging to more than one genus (Hong et al., 2001). Similarly, Skoracka et al (2010) found that the hosts of 99% of 3,874 eriophyid mites assessed worldwide were restricted to a single family. Some species are free-living on their host plants, sheltering beneath bud scales or in other plant structures. Others are able to induce their host plant to grow complex galls. Gall-forming species rely on the gall to avoid predation, shelter delicate life stages and to build viable populations. Of those eriophyids capable of inducing shelter (such as the gall-forming *Aceria vitalbae*), 72% had a single host (Skoracka et al., 2010),

Eriophyid mites have been used in many biological control programmes against weeds (Smith et al., 2010). Skoracka et al. (2010) and Smith et al. (2010) have pointed out that in several of these projects. eriophyid mites developed on and damaged non-target plants unexpectedly in the laboratory during host-range testing. The field host-range of several of these species has been assessed following release. These confirmed that non-target plants that proved suitable for development under laboratory conditions were much less populated in the field and sustained little or no damage (Smith et al., 2010). This appears to be a particular issue for testing eriophyid species, and may relate to the unnatural physiological state of plant tissues under artificial conditions and the ability of mites to induce galls in such tissue (Smith et al., 2010).

The risk posed by *Aceria vitalbae* to plants in New Zealand was assessed by laboratory experiments. The ability of natural enemies to attack new hosts is strongly influenced by the evolutionary history of those potential hosts. The theory underpinning the prediction of an agent's host range is well developed resulting in an internationally accepted selection protocol. Testing protocols operate on the premise that

the species most likely to be damaged by a control agent are those closely related to the target plant If closely-related species are not susceptible, then less closely-related plants won't be either (Wapshere 1974). Reviews of biological control practice (Briese and Walker, 2002; Sheppard et al., 2005; Paynter et al., 2014) indicate that tests using these protocols area a good predictor of subsequent field host range.

Given the host-use patterns of eriophyid mites, it was concluded that inability of *Aceria vitalbae* to form a gall, or to build a burgeoning population on any test plant was sufficient evidence that it was not a viable host.

Test plant selection

The genus *Clematis* belongs to the sub-family Ranunculoideae of the family Ranunculaceae. There are nine native *Clematis* species, and there are four other native genera in this subfamily in New Zealand (https://floraseries.landcareresearch.co.nz/pages/Taxon.aspx?id=_fcaff98a-fa10-4884-bb07-8054cc16d979&fileName=Flora%201.xml). The genus *Caltha* has two native species, *Anemone* has one, *Myosurus* one, but the genus *Ranunculus* has 43 native species. The phylogenetic study of Cai et al. (2009) recognised distinct groups of related genera (clades) within the sub-family Ranunculoideae. Clade one contains *Clematis* and *Anemone*, while *Ranunculus* and *Myosurus* belong to clade two. Phylogenetically therefore, *Clematis* and *Ranunculus* are not intimately related. *Caltha* is even less related.

Xie et al. (2011) identified 10 recognisable clades within the genus *Clematis*. *Clematis vitalba* was placed in a weak Clade VII. The Australasian species were placed in Clade III. All of the phylogenetic analyses used placed Clade III distantly related to Clade VII. The phylogenetic distinctiveness of the New Zealand native species accords with the earlier sub-generic taxonomy based on morphology, and with the unusual unisexual characteristics of the Australasian species (Xie et al., 2011). The taxonomic position of *C. flammula* varies between studies, but at least one treatment places it in the same sub-genus as *C. vitalba*. However, Xie et al. (2011) place it in Clade V, which may be a neighbor to Clade VII (fig 3 in Xie et al., 2011).

The hypothesis adopted for this study was that *A. vitalbae* could form galls and build thriving populations only on *C. vitalba* or closely-related *Clematis* species, and that lack of attack on less-related *Clematis* species was sufficient to circumscribe the host range of the mite. The phylogenetic study of Xie et al (2011) provided a relatedness framework on which to structure this approach. If true, this would also be sufficient evidence that *A. vitalbae* could not attack species of other genera on release in New Zealand.

Fourteen species or hybrids of *Clematis* were tested. The New Zealand native *Clematis* spp. all belong to Clade III (Xie et al. 2011) but for completeness, eight of the nine endemic species were tested (http://www.nzflora.info/search.html?q=clematis). *Clematis cunninghamii* could not be sourced and was not tested. *Clematis marmoraria* is an unusual, reduced species found in limited alpine areas of the Arthur Range in NW Nelson. It was only available as a hybrid with *C. petriei*. Nursery and Garden Industry NZ (now NZ Plant Producers Incorporated) was consulted, and growers recommended ten commercial *Clematis* species that might be tested. Overall, six *Clematis* species hybrids that are exotic to New Zealand were selected to represent commercial interest and to provide representative coverage across the 10 clades described by Xie et al. (2011).

Methods

Three series of tests to determine the host range of *Aceria vitalbae* were conducted at the University of Belgrade in 2016 and 2017 (Vidovic, 2016; 2017a, b, c). Seedlings of native and ornamental *Clematis* spp. were sent from New Zealand (Figure 2), and several ornamental species were sourced locally in Serbia. Seedlings were maintained at 22 ± 2^0C in a 16:8 hour light/dark cycle. A laboratory culture of *Aceria vitalbae* was initiated using mites collected from *Clematis vitalba* near Belgrade. Under a microscope, 20 adult mites were transferred onto two buds of each test or control plant using thin needles, 10 mites per bud. The buds on the plants were examined under a stereomicroscope for the presence of mites after 15 and 30 days (series1) or 60 days (series 2). After 60 days (series 1 and 2) or 120 days (series 3), the plants were harvested and mites were extracted by soaking plants in a detergent mix and then washing over sieves. The presence of mites in the sieve was recorded. The severity of mite damage on each plant was scored as follows:

0 - no damage

1 - Detectable but insignificant damage

2 - Slight deformation of leaves and slight shortening of internodes

3 - Moderate deformation of leaves and moderate shortening of internodes

4 - Severe deformation of leaves and severe shortening of internodes

5 - Deformation of all leaves and shortening of all internodes.

Figure 1. L: *Clematis foetida* seedlings shipped from New Zealand as received in Serbia, R: adult mite transferred to a test plant.

Results

Series 1

Mites transferred onto both controls and test plants were still alive after 15 days. After 30 days, the mites continued to lay eggs and had completed a new generation on *C. vitalba*. Mite colonies survived for 60 days on all *C. vitalba* replicates causing serious leaf deformation (Figure 3). Mite colonies developed only on *Clematis vitalba* controls. No mite survival was record at 30 days on any of the test plants (Table 1). No mites were extracted from any of the test plants at the end of the test and no damage to buds was observed (Figure 4). On the basis of these results (Table 1), the native species *C. foetida, C.paniculata, C. quadribracteolata,* and *C. petriei* were considered to be immune from colonisation by *A. vitalbae* and were not tested further.

Figure 2. Leaves of *Clematis vitalba* control plants after 60 days with galls caused by *Aceria vitalbae*

Figure 3. Test buds of *C. foetida* after 60 days showing no symptoms of *Aceria vitalbae* attack.

Series 2

Two series of tests were conducted in 2017. In the first (series 2), four New Zealand native species (*Clematis marmoraria x petriei, C. marata, C. forsteri, C. afoliata*) and five ornamental species (*C. montana, C. terniflora, C. stans, C. viticella, C.* 'Miss Bateman') were exposed to *Aceria vitalbae.* Plants were examined microscopically after 60 days for the presence of mites and for symptoms of attack. Unlike series 1, mites persisted on test buds on some replicates (10% of plants for *C. marata, C. forsteri, C. terniflora, C. viticella;* 30% of plants for *C. afoliata, C. marmoraria x petriei, C. montana*; 85% for C. stans). The number of mites in affected buds after 60 days exceeded 10, implying that mites reproduced within the buds following initial transfer. However, significant symptoms developed only on *C. stans* (Table 1), where the mean severity score of 3.4 implied moderate to severe leaf deformation and shorting of internodes (Figure 5). There were no formal *C. vitalba* controls established for series 2, but the strong presence of *A. vitalbae* on *C. stans* fulfils this role.

Series 3

The experiments completed in series 2 indicated that small populations of *Aceria vitalbae* could persist in the buds of several test plants for over 60 days without causing the leaf galls that typically developed on *C. vitalba*. A further series of tests examined the longer term viability of these populations and the consequences of longer-term infestation for plant health. In this case, two buds were again infected with 10 mites. At 60 days the buds that had been infested were examined carefully, without damaging the infested buds. At 120 days all buds on the test plants were destructively sampled. The symptoms associated with each bud were assessed and the level of mite infestation was assessed. The *Clematis* species tested were the same as those tested in series 2, where these were available. The only *Clematis montana* plants available were hybrids. A *Ranunculus* species was also selected for testing but all plants died before the 60 day assessment (but not as a result of mite infestation).

All of the *Clematis vitalba* buds initially infested with mites survived to 60 days. All had developed leaf galls and the mean damage score was 5. There were more than 50 mites observed in every gall. Twelve of the 16 *C. stans* buds initially infested with mites remained at 60 days (Table 1). One bud contained about 50 mites, but the other 11 buds each contained approximately 10 mites. The leaves growing from the buds were twisted, and growth was reduced (Figure 6)

Figure 6. Damage to *Clematis stans* buds at 60 days when initially infested with 10 mites.

Four of the 18 *C. marmoraria* x *petriei* buds initially infested with mites contained mites after 60 days. The estimated population was 10-20 mites per bud. Infestation had stopped shoot elongation. Similarly, Three of 20 initially infested *C. marata* buds had mites at 60 days, but there were fewer than 10 mites per bud. Infestation had slowed development (Figure 7).

There was no observable damage to other test plants.

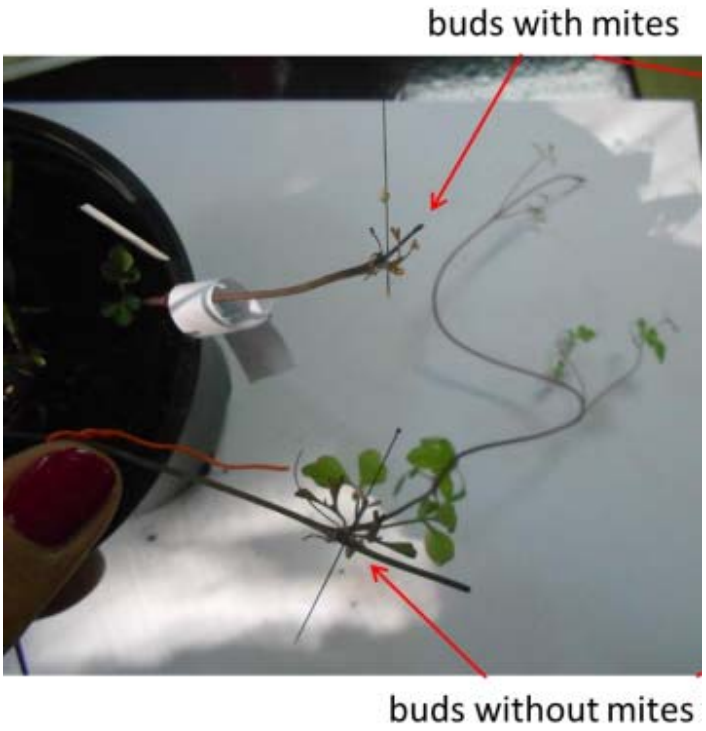

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Figure 7. Retarded growth of one *C. marata* bud 60 days after application of mites.

After 120 days all of the buds/growing points on the test plants and on controls were destructively sampled and the number of mites present per bud was estimated. Every bud on the six control plants was infested, and the estimated number of mites per bud was 20-50. Every bud exhibited leaf gall symptoms (Table 1).

Six plants of *C. stans* survived to 120 days and carried a total of 84 buds. All plants had mites but only 25 of the total 84 buds were infested, and the mean number of mites per bud was 3.9. The damage recorded at 60 days was no longer visible on test plants. Similarly, mites persisted on three of the native *Clematis* species, but the number of mites per bud had fallen to very low levels (Table 1). The proportion of buds infested was also low: 5 of 102 buds for *C. marmoraria x petriei;* 29 of 241 for *C. marata*, and a single mite was found on *C. afoliata.*

Figure 5. Damage to *Clematis stans* by *Aceria vitalbae* A. infected, with symptoms, B. Healthy

Clade	Species	Test Series	Plants examined	% of plants with mites at 60 days	Mean symptom score at 60 days	Est. mites per bud at 60 days	Plants examined at 120 days	% plants with mites at 120 days	Mean symptom score at 120 days	Mean estimated mites per bud (total buds examined)
Clade VII	*Clematis vitalba*	1	10	100%	0	20-50				
		2	no control	-	-	-				
		3	6	100%	5	>50	6	100%	5	35 (58)
Clade VII	*C. stans*	2	7	85%	3.4	-				
		3	8	75%	3.0	10-50	6	100%	0	3.9 (84)
Clade VI	*C. viticella*	2	10	10%	0	-				
Clade V	*C. terniflora*	2	6	17%	0.2	-				
Clade X	*C. montana*	2	10	30%	0.3	-				
Clade XxVI	*C. montana x viticella*	3	8	0%	0	0	-	-	-	-
Clade III	*C. cunninghamii**	No tests								
Clade III	*C. afoliata**	1	10	0%	0	0				
		2	15	20%	0					
		3	8	0%	0	0	7	14.20%	0	0.01 (49)
Clade III	*C. foetida **	1	10	0%	0	0				
Clade III	*C. forsteri**	2	10	10%	0					
		3	1	0%	0	0	-	-	-	-
Clade III	*C. marata**	2	10	10%	0					
		3	10	30%	0.9	c.10	8	37.50%	0	0.9 (241)
Clade III	*C. marmoraria x petriei**	2	10	30%	0					
		3	9	22%	0.2	10-20	6	33.00%	0	0.6 (102)
Clade III	*C. paniculata**	1	10	0%	0	0				
Clade III	*C. petriei**	1	10	0%	0	0				
Clade III	*C. quadribracteolata**	1	10	0%	0	0				
Clade II?	*C.* 'Miss Bateman'	2	10	0%	0					
other	*Ranunculus* sp	3	0	-	-			-	-	

Table 1. Combined results of three series of experiments testing the susceptibility of *Clematis* species to *Aceria vitalbae*

Discussion

Clematis vitalba is the acknowledged host of *Aceria vitalbae* in its native range, but there are several field records of leaf-galls on *C. flammula* which appear to be genuine. *A. vitalbae* is therefore physiologically capable of inducing occasional galls on at least one other *Clematis* species and cannot be considered a strictly monophagous eriophyid in Europe. On the other hand, *Clematis* species are widely cultivated as ornamental species throughout the native range of the mite, yet a search of the web reveals no records of galls forming on other *Clematis* species growing in a horticultural context. The same is true of *Anemone* spp. and *Ranunculus* spp., related ornamentals which have representatives in the New Zealand flora. The host range of *A. vitalbae* in Europe is therefore very narrow, and essentially restricted to *C. vitalba.*

The narrow host range suggested by European field records has been confirmed by the laboratory tests. Mites transferred to buds of *C. vitalba* controls all produced leaf deformations and high populations of mites. After 120 days in series 3, mites had spread and damaged all 58 buds on the control plants. The only other test plant that produced significant leaf damage was *Clematis stans,* which is closely related to *C. vitalba* in Clade VII (Xie et al. 2011). However, by 120 days in series 3, mites were present in 30% of *C. stans* buds and the mean number of mites per bud was only 3.9. No damage was discernible. Damage on all other plants over all tests was barely detectable and scored an average of less than 1. A feature of the tests was the ability of mites to persist (and presumably reproduce) on test plants, even in the absence of galls. However, in all cases numbers declined over time and populations were not viable in the long-term. This was particularly evident for the native *Clematis* species which were either not colonised at all, or attracted low, undamaging mite numbers that declined over time. It is interesting to note that *C. stans* is cultivated as an ornamental in the native range of *A. vitalbae* but has not been recorded as a host.

Eriophyid mites disperse passively on the breeze, drifting onto potential host plants. The inoculum used in these tests was 20 adult mites per bud. This is a greater colonisation pressure than would occur naturally through aerial drift. The natural frequency of colonisation is likely to be lower than that induced in these laboratory tests, except perhaps in the immediate vicinity of old man's beard plants.

On the basis of these results, and in light of the host plant records in Europe:

- *A. vitalbae* is expected to effectively colonise only *C. vitalba* in New Zealand,
- Occasional galls might be expected on exotic non-target *Clematis* species especially those related to *Clematis vitalba* (Clade VII; Xie et al., 2011).
- Given the lack of colonization success and gall formation demonstrated in these tests the risk to native *Clematis* species is considered to be insignificant.

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