The host ranges of two potential biological control agents for Sydney golden wattle, *Acacia longifolia*.

This is a brief summary of published information regarding the host ranges of *Melanterius ventralis* Lea (Curculionidae) and *Trichilogaster acaciaelongifoliae* Froggatt (Pteromalidae). It includes information about the hosts of these insects in their native range as well the conclusions from experiments designed to define the fundamental host ranges of the two agents. This report concludes that there is sufficient knowledge to predict their likely host range in New Zealand without additional testing.

Host range of *Melanterius ventralis*

Biology

Melanterius ventralis Lea (Coleoptera: Curculionidae) is a weevil that lays eggs into the young pods of Sydney golden wattle. Hatching larvae burrow into the seed and each larva destroys a single seed. It was released in South Africa in 1985, and now causes 'extensive' damage to the seed production capacity of its host plant (Impson et al., 2011).

Host range tests

Melanterius ventralis was introduced from Australia following host range tests. The ability of adult weevils to feed on, and to lay eggs on the pods of twenty-seven test plant species was tested in field trials. Insects were contained in cloth sleeves (Donnelly, 1992). Only plants from the family leguminosae were tested, including Australian *Acacia* species, African *Vachelia* species (formerly Acacia) other legume shrubs, and six commercially important legumes such as peas, beans and lucerne. Gauze sleeves which were tied onto branches or plants bearing young developing pods. Ten newly mated adults were introduced into each sleeve. This was replicated 3 times for each test plant and for *Acacia longifolia* controls. The sleeves were checked at least once per week for as long as pods continued to develop in sleeves to ensure that the pods were developing normally and that *Melanterius* adults had survived the duration of seed development. Where weevil adults died prematurely, or where pods failed to develop within the sleeve the replicate was repeated. Pods were harvested once ripe and returned to the laboratory. Each pod was examined for the presence of adult feeding damage and evidence for oviposition. Where eggs had been laid, the ability of larvae to complete development was assessed.

Adults fed on the pods and seeds of *Acacia longifolia* controls, and larvae successfully completed development on the pods. There was no evidence of adult feeding or oviposition on the pods or seeds of related acacias, or on any other legume tested (Table 1). As no eggs were laid, no larval development was observed on any species other that *Acacia longifolia.*

Conclusions

Donnelly (1992) also tested two other *Melanterius* spp. using the same technique. The host range of these species was also restricted, but *M. acaciae* attacked several species closely-related to the target host (*A. melanoxylon*) and *M. servulus* had a relatively wide host range within the invasive Australian *Acacia* species present in South Africa. The field records of host plant use by all three species in the decades following release in South Africa indicates that these tests successfully predicted field host range (F. Impson, ARC South Africa, pers. comm).

There are no native species in New Zealand in the same sub-family as *A. longifolia.* Given that the weevil did not attack any species other than *A. longifolia* in tests this species poses no threat to native legumes in New Zealand.

Acacia melanoxylon, or Tasmanian blackwood, is valued as a timber tree in New Zealand. This species was not a susceptible host in tests and has not been a field host in South Africa.

Other *Acacia* species may have limited value as amenity plants in New Zealand, and some are acknowledged weeds. Adult weevils feed on pollen and larvae feed on seeds in pods. The impact of the weevil on the amenity value of these plants would be negligible. Further, *Melanterius ventralis* did not attack *A. cyclops* or *A. melanoxylon* in tests. These species belong to the *cognata* subclade of mimosoid clade D (Figure 1; Kleinjan & Hoffman, 2011), the sister to the *longifolia* subclade. This indicates that *M. ventralis* is specific to species in the *longifolia* subclade, and risk of seed attack outside this taxon is negligible.

Figure 1. Relationships between clades within the genus *Acacia* (from Kleinjan & Hoffman, 2013)

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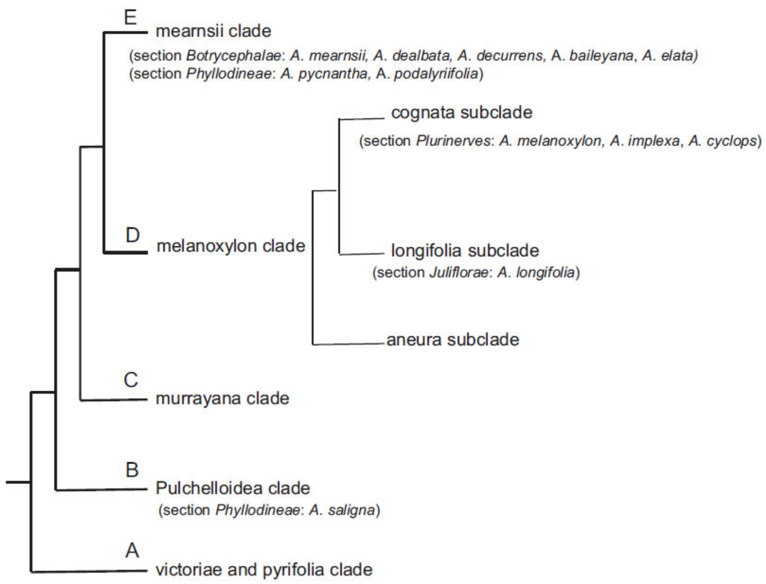

Fig. 2. Schematic representation (modified from Miller et al., 2011) of relationships within *Acacia* s.s.. Species classified as invasive weeds in South Africa are listed in parentheses in their relative positions. Note: Four broad groups were recognized within clade D by Miller et al. (2011), however, pending further sampling and improved resolution, only the three principal monophyletic subclades are included here. They are referred to as the cognata, longifolia and aneura subclades respectively. The cognata subclade includes Groups D(i), D(ii) and some additional lineages, the longifolia subclade is equivalent to group D(iii) and the aneura subclade equivalent to group D(iv) (see Miller et al., 2011).

The risk posed to environmental and economic values in New Zealand by *Melanterius ventralis* can be regarded as negligible on the evidence of previous host range tests, extensive field records in South Africa and the taxonomic position of *Acacia* species. No further host range testing is required.

Table 1. Feeding, oviposition and development of *Melanterius ventralis* on a range of legume species revealed by field host range tests conducted in South Africa (after Donnelly, 1992). (+++ = heavy feeding or oviposition, or full larval development, ++ = moderate feeding, + = 1 or 2 probe marks on pod wall, rarely penetrating seed, nil = no activity, – = no activity as no eggs were laid).

Test Species	**Subfamily**	**Tribe**	**Adult feeding**	**Oviposition**	**Larval development**
Acacia longifolia (control)	Mimosoideae	Mimosoids	+++	+++	+++
Acacia melanoxylon	Mimosoideae	Mimosoids	nil	nil	–
Acacia saligna	Mimosoideae	Mimosoids	nil	nil	–
Acacia baileyana	Mimosoideae	Mimosoids	nil	nil	–
Acacia cultriformis	Mimosoideae	Mimosoids	nil	nil	–
Acacia cyclops	Mimosoideae	Mimosoids	nil	nil	–
Acacia elata	Mimosoideae	Mimosoids	nil	nil	–
Acacia mearnsii	Mimosoideae	Mimosoids	nil	nil	–
Acacia pycnantha	Mimosoideae	Mimosoids	nil	nil	–
Paraserianthes lophantha	Mimosoideae	Mimosoids	nil	nil	–
Vachelia ataxacantha	Mimosoideae	Mimosoids	nil	nil	–
Vachelia hebeclada	Mimosoideae	Mimosoids	nil	nil	–
Vachelia karroo	Mimosoideae	Mimosoids	nil	nil	–
Vachelia robusta	Mimosoideae	Mimosoids	nil	nil	–
Vach. schweinfurthii	Mimosoideae	Mimosoids	nil	nil	–
Vachelia sieberana	Mimosoideae	Mimosoids	nil	nil	–
Vachelia divaricata	Mimosoideae	Mimosoids	nil	nil	–
Vachelia capensis	Mimosoideae	Mimosoids	nil	nil	–
Schotia brachipetala	Detarioideae	Schotiae	nil	nil	–
Aspalathus divaricata	Papilionoideae	Crotylarieae	nil	nil	–
Crotalaria capensis	Papilionoideae	Crotylarieae	nil	nil	–
Podalyria calyptrata	Papilionoideae	Podalyrieae	nil	nil	–
Virgilia oroboides	Papilionoideae	Podalyrieae	nil	nil	–
Tephrosia grandiflora	Papilionoideae	Millettiae	nil	nil	–
Phaseolus vulgaris	Papilionoideae	Phaseoleae	nil	nil	–
Pisum sativum	Papilionoideae	Fabeae	nil	nil	–
Vicia sp.	Papilionoideae	Fabeae	nil	nil	–
Lupinus angustifolius	Papilionoideae	Genisteae	nil	nil	–
Lupinus albus	Papilionoideae	Genisteae	nil	nil	–
Medicago sativa	Papilionoideae	Trifolieae	nil	nil	–

Host range of *Trichilogaster acaciaelongifoliae*

Biology

Trichilogaster acaciaelongifoliae (Froggatt) (Hymenoptera: Pteromalidae) is a minute wasp that lays eggs in the reproductive (and sometimes vegetative) buds of *Acacia longifolia*. The wasp induces galls, effectively eliminating seed production on affected racemes. The galls can be large and act as energy sinks on the plants. When galling is heavy this can lead to mortality of branches or whole plants, especially under stresses such as drought. It was introduced from Australia and first released in South Africa in 1982. In Australia, Noble (1940) reared *Trichilogaster acaciaelongifoliae* from *Acacia* *longifolia*, *A. longifolia* subspecies *sophorae* and its sister species, *A. floribunda*. These species belong to the longifolia subclade of mimosoid Clade E (Kleinjan & Hoffman, 2013, a, b).

Host range tests in South Africa

Kleinjan and Hoffman (2013 a, b) have summarised the results of tests conducted in the 1980s to define the host range of the gall wasp prior to release in South Africa. Nine African species in the Vachelia and Senegalia groups (formerly *Acacia*) were exposed to the gall wasp in various tests. No galls developed in flower buds or vegetative buds. Thirteen *Acacia* species of Australian origin were tested. Results largely confirmed field observations in Australia, although *A. floribunda* was not a host in choice tests even though it was a known host in Australia. This negative test result reinforces modern practice that no choice tests are the best indicator of fundamental host range. It is a field host in South Africa (Kleinjan & Hoffman, 2013).

Similarly, galls did not develop on *Acacia melanoxylon* in the choice tests, although ovipositional probing by adults did occur. *Acacia melanoxylon* belongs to the *cognata* subclade of mimosoid Clade D (Figure 1). Following the establishment of *T. acaciaelongifoliae* in South Africa, stunted gall symptoms were encountered on *A. melanoxylon* in the field. Marchante et al. (2011) subsequently showed that oviposition does occur on this species under no-choice conditions.

Paraserianthes lophantha was not tested but *T. acaciaelongifoliae* spillover gall symptoms were encountered on this species in the field subsequent to its establishment.

Host range tests in Portugal

Marchante et al. (2011) report the risk assessments undertaken before *T. acaciaelongifoliae* was approved for release in Portugal. Forty plant species were selected for testing to assess the risk posed to non-target plants in the EU. Wasps were imported from South Africa, and the tests were conducted in Containment in Coimbra, Portugal (Marchante et al., 2011). Thirty of the species tested belonged to families outside the Leguminosae.

Small shoots bearing reproductive buds were placed in a 5-cm tall petri dish with a single female. Each plant was tested nine times. After the wasp died (2-3 days), the buds were dissected, and the number of eggs laid was recorded. Wasps laid eggs on several plants. Their acceptability as hosts was further examined by containing females on potted plants and maintaining those plants to check whether galls formed.

The behaviour of individual wasps was observed. Females were observed to spend only 3% of their time on buds (Marchante et al., 2011). This seems a small proportion and may have been influenced by experimental conditions. The buds of most test plant were not visited at all, however, wasps were observed to probe in the buds of several plant species other than mimosoid species (Table 2., Marchante et al., 2011) and this was confirmed by later dissections.

Acacia longifolia controls proved to be the most acceptable hosts in Petri dish tests. As expected, eggs were also laid on *A. melanoxylon*, a species closely related to *A. longifolia* in mimosoid clade D and a known host in South Africa. No eggs were detected in peas or beans or medics, nor in three legume shrubs native to Europe that belong to the tribe Genisteae. However, eggs were consistently (4 of 9 tests) laid on the buds of a fourth species in this tribe, *Cytisus striatus*. While 31.8% of all buds in controls were found to contain eggs, 21.8% of *C. striatus* buds were attacked. Further, 4.3% of *Vitis vinifera* (grape) buds in two tests contained eggs, these were laid on the outside rather than within bud tissue.

Galls formed on potted *A. longifolia* plants but did not form on *C. striatus* or *V. vinifera* plants (though the level of gall formation on controls was not high (see section 3.3, Marchante et al., 2011)). *Vitis vinifera* was surveyed in Australia and South Africa but this species is clearly not a host in either country. *C. striatus* does not grow in Australia and South Africa, so *Genista monspessulana* and *Spartium junceum*, two related species in the tribe Genisteae were surveyed instead. Neither was a host for *T. acaciaelongifoliae* (Figure 2, Marchante et al., 2011).

Figure 2. Abundance of galls on selected plants in their native range (from Marchante et al. 2011)

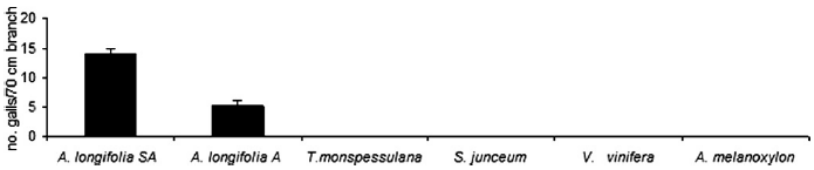

Fig. 5. The abundance of galls of *Trichilogaster acaciaelongifoliae*, on the terminal 70 cm of branches, on five plant species including the target, *Acacia longifolia*, in South Africa (SA) (Western Cape) and Australia (A) (New South Wales). The non-target species included two species (*Vitis vinifera* and *Acacia melanoxylon*) where the wasps laid eggs during no-choice tests, and two species closely related to *Cytisus striatus*. Non-target species were sampled in SA and A, except for *Spartium junceum*.

Conclusions

Evidence for the high degree of host specificity of the gall wasp be inferred from these sources:

- Knowledge of the hosts of *Trichilogaster* species (Kleinjan & Hoffman, 2013)
- Host surveys in the native range in Australia (Noble, 1940)
- Host records in the adventive range (
- Results of host range tests conducted in the 1980s (van den Berg, 1980 in Kleinjan & Hoffman, 2013)
- Results of host range tests completed before release in Portugal (Marchante et al., 2011)

This report concludes that the host range of *T. acaciaelongifoliae* is restricted to *Acacia* species belonging to Clade D of the mimosoid tribe. Its primary hosts belong to the longifolia subclade, but the consistent observation of inferior gall formation on *A. melanoxylon* suggests that species of the cognata subclade (and the aneura subclade?) might also fall into the fundamental host range of the gall wasp. Observations of galls on *Paraserianthes lophantha* in South Africa remain anomalous.

Consistent use of *C. striatus* in oviposition tests also remains anomalous. Three other members of the tribe Genisteae did not receive eggs in tests, and two others were not field hosts in Australia. No galls formed in potted *C. striatus* plants. All species of the tribe are regarded either as weeds or potential weeds in New Zealand (including gorse and broom), with the possible exception of tree lucerne. *C. striatus* is not naturalised in New Zealand. It seems likely that the observation of eggs deposited on *C. striatus* buds in Petri dish tests was a laboratory artifact. Even if galls did form on the flower buds of these sub-optimal species, it is unlikely that these could develop sufficiently well to cause vegetative damage to growing plants.

Trichilogaster acaciaelongifoliae galls are not thought to mar the value of ornamental wattles in Australia or South Africa other than *A. longifolia* and *A. floribunda*.

The New Zealand flora records four genera of indigenous legumes growing on the mainland; *Sophora* (13 spp.), *Carmichaelia* (27 spp), *Montigena* (I sp.), and *Clianthus* (2 spp.) (http://www.nzflora.info/). None of these species has been tested. These genera belong to the Papilionoid clade of the Leguminosae. It is considered highly unlikely that these could be potential hosts of *T. acaciaelongifoliae* because:

1. All *Trichilogaster* species have a narrow host range, and are associated strictly with *Acacia* species (Kleinjan & Hoffman, 2013)
2. In its native range *T. acaciaelongifoliae* galls have only been recorded from *A. longifolia* and *A. floribunda* (e.g. Noble 1940). There are no records from any papilionoid species in Australia.
3. In its adventive range, spillover gall production has been observed on two further mimosoid species, *A. melanoxylon* and *P. lophantha*, but both are sub-optimal hosts and galls form only sporadically with negligible effects (EFSA, 2015)
4. In tests prior to introduction to South Africa or Portugal, no galls were observed on any plants outside the genus *Acacia*

There is sufficient existing evidence to conclude, without additional testing, that *T. acaciaelongifoliae* would pose negligible risk to non-target native, economic or ornamental legume species in New Zealand.

References

Donnelly, D. (1992) The potential host range of three seed-feeding *Melanterius* spp. (Curculionidae), candidates for the biological control of Australian *Acacia* spp. and *Paraserianthes* (Albizia) *lophantha* in South Africa. *Phytophylactica* 24: 163-167.

EFSA Panel on Plant Health (2015) Risk to plant health in the EU territory of the intentional release of the bud-galling wasp *Trichilogaster acaciaelongifoliae* for the control of the invasive alien plant *Acacia longifolia*1 EFSA Journal 13 (4): 4079.

Hill, R.L. (2005) Prospects for the biological control of Sydney golden wattle,*Acacia longifolia,* using *Trichilogaster acaciaelongifoliae* and *Melanterius ventralis*. Unpublished Landcare Research Contract Report LC0506/009, 22 p.

Impson, F.A.C., Kleinjan, C.A., Hoffman, J.H., Post, J.A., Wood, A.R. (2011) Biological control of Australian *Acacia* species and *Paraserianthes lophantha* (Willd.) Nielsen (Mimosacae) in South Africa. *African Entomology* 19: 186-207.

Kleinjan, C.A., Hoffman, J.H. (2013a) Advances in clarifying the phylogenetic relationships of *Acacias*: relevance for biological control. *Acta Oecologica* 48: 21-29.

Kleinjan, C.A., Hoffman, J.H. (2013a) Supplementary information, Appendic A https://www.sciencedirect.com/science/article/pii/S1146609X13000064?via%3Dihub

Marchante, H., Freitas, H., Hoffman, J.H. (2011) Assessing the suitability of a well-known bud-galling wasp, *Trichilogaster acaciaelongifoliae,* for biological control of *Acacia longifolia* in Portugal. *Biological Control* 56: 193-201.