The host-range of *Grypus equiseti* (F.) (Erirhinidae), a potential control agent for field horsetail *Equisetum arvense* L.

Test plant selection

A centrifugal phylogenetic method (Wapshere 1974) has long been used to determine the host-range of a potential biological control agent by sequentially testing plant taxa most closely related to the target weed followed by increasingly distantly related taxa until the host-range has been circumscribed. This approach is supported by recent advances in molecular techniques: host-shifts in lineages of specialist phytophagous insects are strongly linked to the evolution of host-plant lineages, and in particular plant chemistry. Such insects show a strong phylogenetic conservatism of host associations (see Briese 1996; Briese & Walker 2002). This pattern of strong phylogenetic conservatism in diet suggests the non-target plants of greatest risk are those closely related to known hosts (Futuyma 2000), and this has been validated by recent reviews of non-target attack by insect (Pemberton 2000; Briese & Walker 2002; Louda et al. 2003; Paynter et al. 2004) and fungal (Barton (nee Frohlich) 2004) weed biological control agents.

The horsetails are an ancient taxon. Pryer et al. (2004) estimated that they diverged by the end of the Devonian (c. 354 million years ago); an estimate supported by the presence of fossil relatives of horsetails dating back to the late Devonian. Recent molecular analyses place the horsetails within the fern phylum (Pteridophyta), with the most closely related New Zealand native plants to *Equisetum* currently believed to be the Marattioid ferns (Pryer et al. 2004; Wikstrom & Pryer 2005; Schuettpelz et al. 2006). Uncertainty remains regarding the exact relationships among horsetail, marattioid, and leptosporangiate ferns. However, the horsetails are in a different class (Equisetopsida) and are only tenuously related to true ferns (Filicopsida, Polypodiopsida), and other fern allies (Lycopodiopsida, Psilotopsida) http://nzflora.landcareresearch.co.nz/default.aspx?selected=NameDetails&NameId=A8AF1C5B-67B3-4B26-B9CB-0A6970A4D29A&StateId=&Sort=0&TabNum=2

Paynter and Barton (2008) provide the rationale for selecting test plants for this project. No *Equisetum* species are native to New Zealand, and there are no native genera belonging to this class in the native flora. All exotic *Equisetum* species present in New Zealand are considered to be unwanted organisms and cannot be propagated, sold or distributed (NPPA). Due to the extreme taxonomic isolation of *Equisetum arvense* from native or valued exotics in New Zealand, a short test plant list was compiled that was adequate to demonstrate the host-range and, therefore, environmental safety of candidate biocontrol agents (Paynter and Barton 2008). *E. hyemale* was available and was tested. *Ptisana salicina* (formerly Marattia) was chosen to represent the marattioid ferns (it is the only NZ representative of the Marattiaceae). One species *Todea* and two species of *Leptopteris* are the only native osmundaceous ferns. Two genera of whisk ferns and two genera of ophioglossoid ferns are native to New Zealand. The species selected to represent these native taxa are listed in Table 1. The New Zealand Plantfinder (http://www.plantfinder.co.nz/, accessed 21/2/2008), indicated that no additional exotic Marattioid, Ophioglossoid, Whisk or Osmundaceous ferns are sold commercially in New Zealand. (Paynter and Barton 2008).

Phylum	Class	Order	Family	Species
Pteridophyta	Equisetopsida	Equisetales	Equisetaceae	*Equisetum arvense* *E. hyemale*
	Marattiopsida	Marattiales	Marattiaceae	*Ptisana salicina*
	Polypodiopsida	Osmundales	Osmundaceae	*Todea barbara* *Leptopteris hymenophylloides*
	Psilotopsida	Psilotales	Psilotaceae	*Tmesipteris elongata*
		Ophioglossales	Ophioglossaceae	*Ophioglossum coriaceum* *O. petiolatum*

Table 1. Test plants selected to represent the New Zealand Flora of pteridophytes.

Methods

ADULT FEEDING AND OVIPOSITION

In 2014, preliminary tests were performed using weevils imported into containment from England and in 2015 larger scale tests were performed using weevils that were the progeny of beetles imported from England.

In each replicate one adult weevil was transferred to cut plant material in a Petri dish. In 2014, leaves and/or stems of either *Equisetum arvense, Ptisana* (Syn. *Marattia*) *salicina, Todea barbara, Leptopteris hymenophylloides, Tmesipteris elongata* or *Ophioglossum coriaceum*. In 2015 *Equisetum hyemale* and *Ophioglossum petiolatum* were also included in the tests. Each Petri dish was checked every 2-3 days when feeding damage was recorded and any eggs present were counted. Foliage was then replaced with fresh material. Feeding damage was scored as follows: 0 = none; 0.1 = trace; 1 = minor damage (single taste / little frass), 2 = some damage (< 1/2 eaten / some frass), 3 = significant damage (>1/2 eaten / significant frass everywhere). After 6 to 9 days the test was terminated and the mean damage score was calculated for each beetle and the cumulative number of eggs laid was recorded.

LARVAL DEVELOPMENT

Larval tests were done using the F1 progeny of beetles imported from England in 2014. For each replicate, one larva was transferred to cut plant material in a Petri dish (leaves and/or stems of the following test plants: *Equisetum arvense, Equisetum hyemale, Ptisana* (Syn. *Marattia*) *salicina, Todea barbara, Leptopteris hymenophylloides, Tmesipteris elongata, Ophioglossum coriaceum* or *Ophioglossum petiolatum*). The larvae were checked periodically, moistened and plant material renewed as necessary for up to 236 days when all larva had either pupated, or become moribund or died. Thirty-five replicates were performed with *Equisetum arvense* and 25 replicates were performed with *Leptopteris hymenophylloides*. Twenty replicates were performed with the remaining test plant species listed above.

Analysis

ADULT FEEDING AND OVIPOSITION

Analyses were performed using the R statistical package (R Core Team and contributors worldwide). Preliminary investigation of the data indicated that the data were not normal and

lacked homogeneity of variances between treatments. Non-parametric statistics were therefore used to analyse the data.

Kruskal-Wallis rank sum tests was performed to investigate if (1) the feeding score varied according to plant species, where the feeding score was declared as the response variable; and (2) the number of eggs varied according to plant species, where the number of eggs was declared as the response variable. For both analyses treatment was treated as a factor, with levels corresponding to each plant species.

Two separate analyses were performed for the tests conducted in 2014 and 2015.

LARVAL DEVELOPMENT

Analyses were done using the Genstat statistical package (VSN International Ltd) using the Generalized Linear Models option specifying Binomial Errors and a logit link. A binary dependent variable was used (where 0 = larva did not survive to pupation and 1 = larva did survive to pupation) and all elements of the denominator array were set to 1. Treatment was declared as a factor, with levels corresponding to each plant species.

Results

ADULT FEEDING AND OVIPOSITION 2014

Feeding damage score varied significantly according to treatment (Fig 1a; Kruskal-Wallis chi-squared = 28.1036, df = 5, p-value = 3.474e-05) as did the mean number of eggs laid (Fig 1b; Kruskal-Wallis chi-squared = 20.0605, df = 5, p-value = 0.001217). Adult feeding was almost entirely confined to *E. arvense.* The number of eggs laid was much higher in Petri dishes containing *E. arvense* compared to all other test plants. Moreover, with the exception of the *E. arvense* controls, very few eggs were laid on the test plants and beetles generally oviposited on the dish or filter paper, which suggests that in no-choice conditions beetles were “dumping” eggs randomly in the absence of their preferred host plant. When a second analysis was performed counting only eggs laid on plants, the test result was even more clear-cut (Fig 1c; Kruskal-Wallis chi-squared = 24.13, df = 5, p-value = 0.000205)

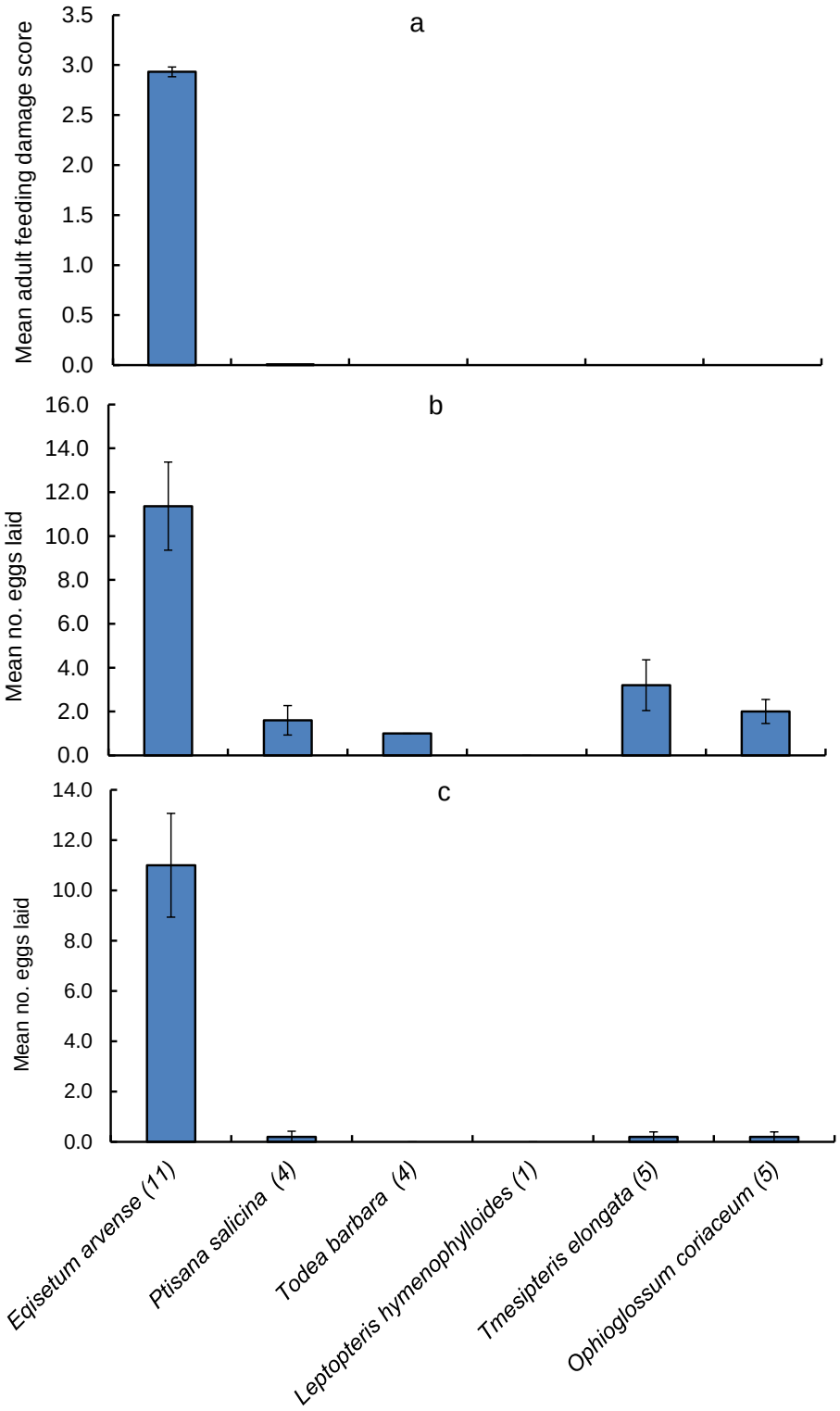

Fig 1. Results of the preliminary tests on adult *Grypus equiseti* conducted in 2014 (see text for details). (a) Mean feeding damage score ± SEM; (b) mean ± SEM number of eggs laid in Petri dishes containing *Equisetum arvense* and a range of test plants and (c) mean ± SEM number of eggs laid in Petri dishes containing *Equisetum arvense* and a range of test plants excluding eggs not laid on plant material The numbers in brackets above each plant species indicate the number of replicates performed.

2015

The results in 2015 were similar to 2014; feeding damage score varied significantly according to treatment (Fig 2a; Kruskal-Wallis chi-squared = 168.20, df = 7, $P < 0.001$) as did the mean number of eggs laid (Fig 2b; Kruskal-Wallis chi-squared = 23.33, df = 7, $P < 0.01$). Apart from relatively trivial feeding on *O. petiolatum* and *Ptisana salicina*, feeding was confined to the two *Equisetum* spp.

As in 2014, with the exception of the *E. arvense* controls, very few eggs were laid on the test plants and beetles generally oviposited on the dish or filter paper, which suggests that in no-choice conditions beetles were “dumping” eggs randomly in the absence of their preferred host plant. When a second analysis was performed counting only eggs laid on plants, the test result was even more clear-cut (Fig 2c; Kruskal-Wallis chi-squared = 49.89, df = 7, $P < 0.001$)

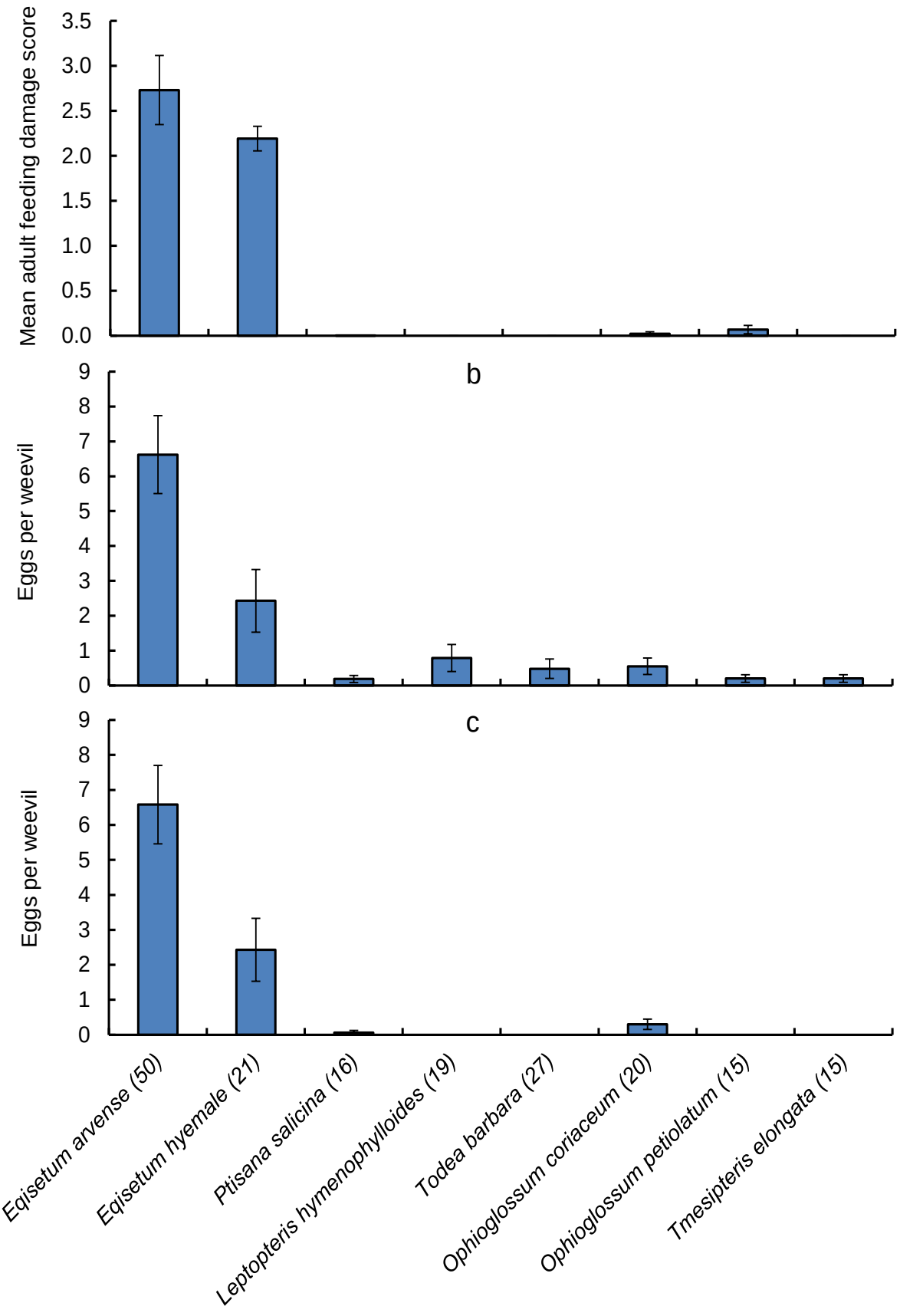

Fig 2. (a) Results of the preliminary tests on adult *Grypus equiseti* conducted in 2015 (see text for details). (a) Mean feeding damage score ± SEM; (b) mean ± SEM number of eggs laid in Petri dishes containing *Equisetum arvense* and a range of test plants and (c) mean ± SEM number of eggs laid in Petri dishes containing *Equisetum arvense* and a range of test plants

excluding eggs not laid on plant material The numbers in brackets above each plant species indicate the number of replicates performed.

LARVAL DEVELOPMENT

Minor larval feeding occurred on some native NZ species but larvae generally died within a few days. No living larvae were found beyond 9 days on any NZ native test plant and development to pupation only occurred on *Equisetum arvense* and *E. hyemale*. The treatment (plant species) effect on larval survival was highly significant at 15 days (Fig. 3; $\chi^2 = 15{\cdot}82$, df = 7, $P < 0{\cdot}001$) and at 236 days, when all larvae had either died or pupated ($\chi^2 = 5.06$, df = 7, $P < 0{\cdot}001$).

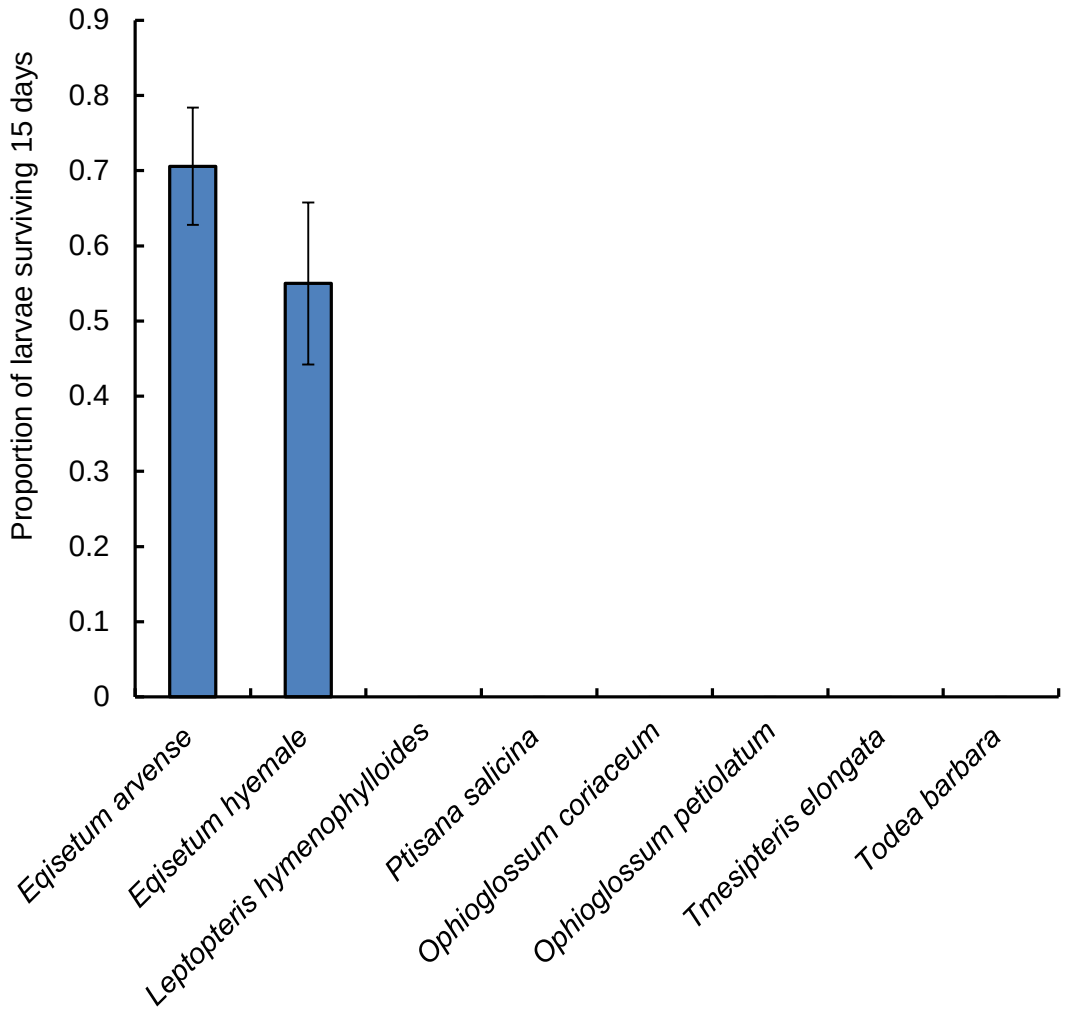

Fig. 3. Proportion of *Grypus equiseti* larvae surviving for 15 days on *Equisetum arvense*, *E. hyemale* and a range of native NZ test plants.

Discussion

Oviposition was higher in 2014, compared to 2015, but this probably reflects differences in the sex ratio of field collected and laboratory-reared beetles: beetle sex could not be reliably determined from external features. However, all beetles imported directly from England laid eggs on *E. arvense* controls, implying the sex ratio of field collections was totally biased towards female beetles. By contrast, 48% of beetles reared in NZ laid eggs on *E. arvense* controls, implying a c. 50:50 sex ratio.

Adult feeding was very low on all native NZ test plant species, indicating that these species are not attractive to *G. equiseti*. The oviposition test results are therefore likely to be conservative and oviposition occurred in Petri dishes containing NZ native test plants because of the no-choice nature of the tests. Moreover, these results of both the 2014 and 2015 tests indicate that oviposition was much higher in dishes containing *E. arvense* and the test results were even more clear-cut when only eggs laid on plant material were counted.

Larval development tests confirmed that NZ test plant species are not hosts of *G. equiseti.* We conclude that *G. equiseti* is unable to form populations on native NZ ferns and that even minor spill over attack on NZ native ferns is highly unlikely.

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