Host range tests of *Hydrellia lagarosiphon* a candidate agent for *Lagarosiphon major* in New Zealand: assessing the risk to non-target species in New Zealand

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Materials and Methods

Selection of test plants

A test plant list for New Zealand (henceforth NZ) (Table 1) was compiled using the centrifugal phylogenetic method (Wapshere, 1974) with amendments, as suggested by Briese and Walker (2002) and Briese (2003). Recent development in phylogenetics for the Hydrocharitaceae and its relation to taxonomically similar plant families were obtained by consulting the Angiosperm Phylogeny Website (Stevens, 2001 onwards; Fig. 1).

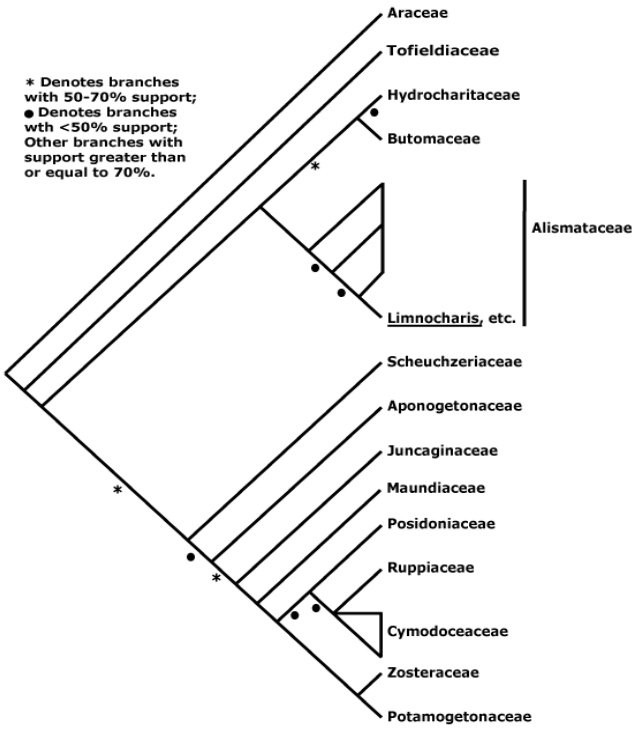

Figure 1 Phylogeny of the Alismatales obtained from the Angiosperm Phylogeny Website: **(Stevens, 2001 onwards)**

The most recent checklist of native NZ plants (De Lange and Rolfe, 2010) was examined to identify the NZ plant species that are most closely-related to lagarosiphon in order to compile a list of native plants for inclusion in host-range testing. Given that the aquatic lifestyle is a highly specialised one, relying totally on taxonomic position without due consideration of habitat has the potential to result in unsuitable test plants being included in a test list. For example, arthropod herbivores that feed on lagarosiphon are adapted to being submerged in freshwater and we can be sure that the marine eelgrass *Zostera muelleri* (Zosteriaceae) cannot be a suitable host for an insect herbivore because no insects have followed seagrasses into the ocean (Ollerton and McCollin, 1998). *Zostera muelleri* was, therefore, excluded from host-range testing. *Wolffia australiana* was also excluded from host-range testing as the tiny (0.3-1 mm long) platelets are far too small to be at risk of supporting the development of a leaf-mining fly. *Lemna disperma* is also considered to be too small to allow complete development of *H. lagarosiphon*, but it was retained in the list, so that the potential for spill-over attack could be determined.

The family Hydrocharitaceae (to which *Lagaroisiphon major* belongs) is absent from the New Zealand native flora (De Lange and Rolfe, 2010). Therefore, exotic species that belong to this family that are present in in New Zealand were included in host-range tests. Species selection widened to include six other families in the order Alismatales, which included key New Zealand natives (Table 1).

Table 1 Test plant list for host-range testing *Hydrellia lagarosiphon* for New Zealand.

Family	Test plant species/genus	Status in NZ
Araceae	*Lemna disperma* Hegelm.	Native
Hydrocharitaceae	*Lagarosiphon major* (Ridley) Moss ex Wager	Target weed
	Egeria densa Planch.	Exotic weed
	Elodea canadensis Michx.	Exotic weed
	Hydrilla verticillata (L.f.) Royle	Exotic weed
	Ottelia ovalifolia (R.Br.) Rich.	Exotic weed
	Vallisneria gigantea (Graeb.)	Exotic weed
Alismataceae	*Alisma lanceolatum* With.	Exotic weed
	Sagittaria sagittifolia L.	Exotic weed
	Hydrocleys nymphoides (Humb. & Bonpl. ex Willd.) Buchenau	Exotic weed

	Baldellia ranunculoides (L.) Parl.	Exotic ornamental
Aponogetonaceae	*Aponogeton distachyos* L.f.	Exotic weed
Juncaginaceae	*Triglochin striata* Ruiz et Pav.	Native
Ruppiaceae	*Ruppia polycarpa* R. Mason	Native
Potamogetonaceae	*Lepilaena bilocularis* Kirk	Native
	Stuckenia pectinata (L.) Börner	Native
	Potamogeton cheesemanii A. Benn.	Native
	Potamogeton ochreatus Raoul	Native
	Zannichellia palustris L.	Native

Origin of H. lagarosiphon and location of host-range testing

All host-range testing was done at a quarantine facility at University College Dublin in Ireland. The laboratory culture of *H. lagarosiphon* originates from a field collection made in July 2009 from the Eastern Cape Provence, South Africa.

Larvae for experiments

Host specificity trials were conducted for the duration of larval and pupal development (1st instar larva through until adult emergence). Neonate larvae were obtained from laboratory cultures by exposing approximately 20 newly emerged adult pairs for a series of 24 hr periods to obtain eggs deposited in the first week. Eggs deposited on *L. major* shoots were monitored for larval emergence twice daily. Neonate 'naïve' 1st instar larvae were removed from plant material and transferred to undamaged shoots of test plants in experimental containers.

No-choice larval development trials

No-choice trials were conducted in 1.5 L transparent plastic containers and covered with a nylon mesh. Five larvae were placed in each container with sufficient excised shoots of the plant species and topped up with 6- to 7 cm of water. The containers were checked after 25 days for mining damage typical of the larvae. The survivorship to the pupal and adult stage was recorded for each trial.

Paired-choice larval development trials

Choice tests were only conducted for a subset of test plants that belong to the Hydrocharitaceae, *Aponogeon distachyos* (Aponogetonaceae) and most of the NZ native plant species that belong to the Araceae, Juncaginaceae and Potamogetonaceae. Excised shoots of *L. major* and 1 non-target plant species were placed together in 1.5 L transparent plastic containers and 5 fly larvae were added. Seven *L. major* shoot tips were provided and also a similar amount of plant material from the non-target plant ensuring that a sufficient number of shoot tips with young leaves was provided. Trials were conducted as in the no-choice tests.

Results

No-choice larval development and survival

The successful development of the larval stages of *H. lagarosiphon* was restricted to *L. major* (Table 2). All other plant species tested, including the non-native Hydrocharitaceae and the remaining 6 families in the order Alismatales, proved to be unsuitable host plants. The larval survival rates on *L. major* were in excess of 70%, with a mean of 4.35 larvae (±0.15) surviving out of five placed in each replicate (n=29). In addition to the test list, a few additional plant species were included in the no-choice tests (*Vallisneria nada, Vallisneria spiralis, Alisma plantago-aquatica, Lemna minor*; Table 2). However, four plant species were not obtained for testing: *Ottelia ovalifolia* (exotic), *Hydrocleys nymphoides* (exotic), *Lepilaena bilocularis* (native) and *Ruppia polycarpa* (native). Surrogate species (*R. cirrhosa* and *R. maritima*, which were present in Ireland) that belong to the same genus as *Ruppia polycarpa* were obtained for inclusion in host-range tests, so that only three genera in the original test plant list were not tested, and only one of these was a native to NZ.

Table 2 List of plant species exposed to the 1st instar larvae of *Hydrellia lagarosiphon* showing a rating of feeding damage and larval survival under no-choice conditions.

Family	Test plant	*n*	Feeding	Survival
Araceae	*Lemna disperma*	60	None	-
	L. minor	60	None	-
Hydrocharitaceae	*Lagarosiphon major*	145	High	++++
	Egeria densa	75	None	-
	Elodea canadensis	80	None	-

	Ottelia ovalifolia	0	Not tested	Not tested
	Hydrilla verticillata (L.f.)	85	None	-
	Vallisneria gigantea	60	None	-
	Vallisneria nada	60	None	-
	Vallisneria spiralis	60	None	-
Alismataceae	*Alisma lanceolatum* With.	65	None	-
	Alisma plantago-aquatica L.	75	None	-
	Baldellia ranunculoides (L.)	65	None	-
	Hydrocleys nymphoides	0	Not tested	Not tested
	Sagittaria sagittifolia L.	65	None	-
Aponogetonaceae	*Aponogeon distachyos*	60	None	-
Juncaginaceae	*Triglochin striata* L.	65	None	-
Ruppiaceae	*Ruppia cirrhosa* (Petagna)	65	None	-
	R. maritima L.	65	None	-
	R. polycarpa	0	Not tested	Not tested
Potamogetonaceae	*Lepilaena bilocularis*	0	Not tested	Not tested
	Potamogeton cheesemanii	60	None	-
	Potamogeton ochreatus	60	None	-
	Stuckenia pectinata	60	None	-
	Zannichellia palustris L.	60	None	-

n, Number of replicates. Nymphal survival rating (to adult stage): +, ≤ 20%; ++, 21–40%; +++, 41–70%; ++++, 71–100%; -, no nymphal survival and/or development.

Paired-choice larval development and survival

When placed in paired choice conditions the 1st instar larvae only located and fed on the target weed (*L. major*) (Table 3). No feeding, exploratory or other was recorded on the test species in each of the paired-choice trial. Development on *L. major* was similar to those recorded in choice trials, indicating that the non-target plants, in close proximity, did not affect the host finding behaviour and subsequent survival of the larvae to the adult stage. Over 80% survival occurred in each of the paired-choice trial (Table 3), similar to those recorded during the no-choice tests. The relative survival recorded on *L. major* was 1.00, in each case which indicates that even under conservative conditions where spillover could occur, the larvae still showed a preference to feed and develop on the target species *L. major*.

Table 3 Relative survival of the 1st instar *H. lagarosiphon* larvae exposed to paired choice conditions with equal access to both *L. major* and the test species (5 larvae per replicate).

Family/		Relative survival		Mean (±SE) survival (out of 5)	
Test plant species	***n***	***L.***	**Test**	***L. major***	**Test species**
Araceae					
Lemna disperma	12	1.00	0.00	4.16 ± 0.24	0.00 ± 0.00
Lemna minor	12	1.00	0.00	4.58 ± 0.15	0.00 ± 0.00
Hydrocharitaceae					
Vallisneria gigantean	12	1.00	0.00	4.16 ± 0.24	0.00 ± 0.00
Vallisneria nada	12	1.00	0.00	4.08 ± 0.29	0.00 ± 0.00
Vallisneria spiralis	12	1.00	0.00	4.08 ± 0.23	0.00 ± 0.00
Aponogetonaceae					
Aponogeon distachyos	12	1.00	0.00	4.33 ± 0.28	0.00 ± 0.00
Juncaginaceae					
Triglochin striata	12	1.00	0.00	4.50 ± 0.15	0.00 ± 0.00
Potamogetonaceae					
Potamogeton cheesemanii	12	1.00	0.00	4.00 ± 0.25	0.00 ± 0.00
Potamogeton ochreatus	12	1.00	0.00	4.17 ± 0.27	0.00 ± 0.00

Conclusions

The tests conducted clearly show that *Hydrellia lagarosiphon* is specific to *Lagarosiphon* and poses no threat to either exotic or native related plants that belong to the order Alismatales that grow in NZ. The risk to more distantly related plant families that have aquatic native New Zealand representatives (e.g. Halagoraceae, which belongs to the order Saxifragales) is considered to be trivial: 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 (Briese, 1996; Briese and Walker, 2002). This pattern of strong phylogenetic conservatism in diet indicates the non-target plants at 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 (Briese and Walker, 2002; Louda et al., 2003; Paynter et al., 2004; Pemberton, 2000) and fungal (Barton, 2004) weed biological control agents.

Not all plant species/genera on the proposed test plant list were obtained and successfully shipped to Ireland for host-range testing (species that were not tested were: *Ottelia ovalifolia*, *Hydrocleys nymphoides*, *Lepilaena bilocularis* and *Ruppia polycarpa*), although the use of 'surrogate' species to replace *Ruppia polycarpa* ensured that this genus was tested. The genus *Ottelia* belongs to the same subfamily of the Hydrocharitaceae as *Elodea* and *Egeria* (Anacharidoideae), which were not hosts of *H. lagarosiphon*, so we conclude that the likelihood of *O. ovalifolia* being a host of *H. lagarosiphon* is very low. Furthermore, *O. ovalifolia* is not considered to be an essential test plant as it is not native to NZ and has no economic importance as a crop or an ornamental - indeed it is listed as an environmental weed in NZ (Howell, 2008).

The only NZ native genus on the test list not to be tested was *Lepilaena*, which could not be tested because the *L. bilocularis* plants did not survive shipment to Ireland. *Lepilaena bilocularis* belongs to the family Potamogetonaceae. Given the clear-cut host-range test results, which showed that *H. lagarosiphon* failed to develop any other test plant species, including several species within the Hydrocharitaceae which are much more closely-related to *L. major*, it is considered unnecessary to test *L. bilocularis*, which is only distantly related to *L. major*.

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