

Host range testing *Leptoypha hospita*: a candidate biological control agent for privet (*Ligustrum* spp.) in New Zealand

Leptoypha hospita

Leptoypha hospita is a sap sucking tingid bug that is native to China and Malaysia where *L. sinense*, *Ligustrum quihoui*, *Ligustrum obtusifolium* were reported as its hosts (Zhang et al. 2011). During surveys for natural enemies *L. hospita* was found in abundance on Chinese privet in China, where its feeding resulted in a bleached appearance of leaves and premature defoliation (Zhang et al. 2011). The host-specificity of *L. hospita* has been investigated to determine their potential for biocontrol of Chinese privet in the USA (Zhang et al. 2012), the results are summarised in *Summary of host-range testing conducted for the USA*, below.

Taxonomy of Ligustrum and its phylogenetic proximity to native New Zealand plants.

Ligustrum belongs to the family Oleaceae, which is within the Order Lamiales (Fig 1.). The Oleaceae is represented in the native New Zealand (NZ) flora by just one genus (*Nestegis*; Fig. 2), of which there are four native species (De Lange & Rolfe 2010). In addition, plants from several exotic genera that belong to the Oleaceae are cultivated and sold in NZ, notably olive (*Olea europaea*; Table 1; <http://findaplant.co.nz/>). Ideally at least one representative from each of these native and exotic genera should be tested.

Fig. 1. Phylogenetic tree of the Order Lamiales (source: <http://www.mobot.org/mobot/research/apweb/orders/lamialesweb.htm>).

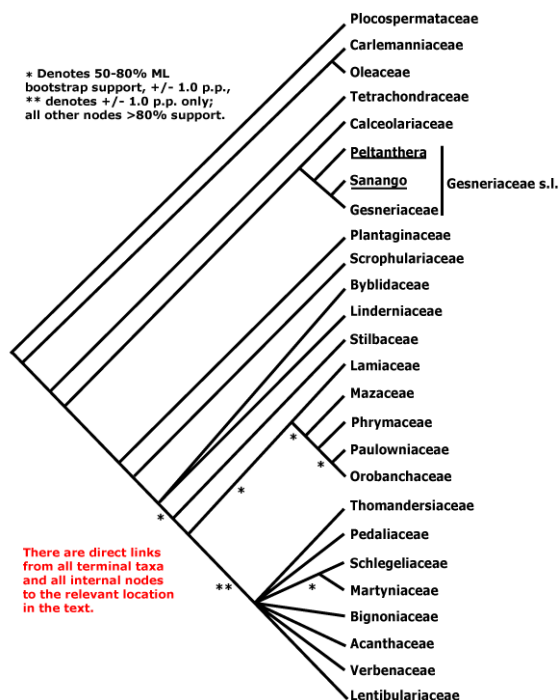
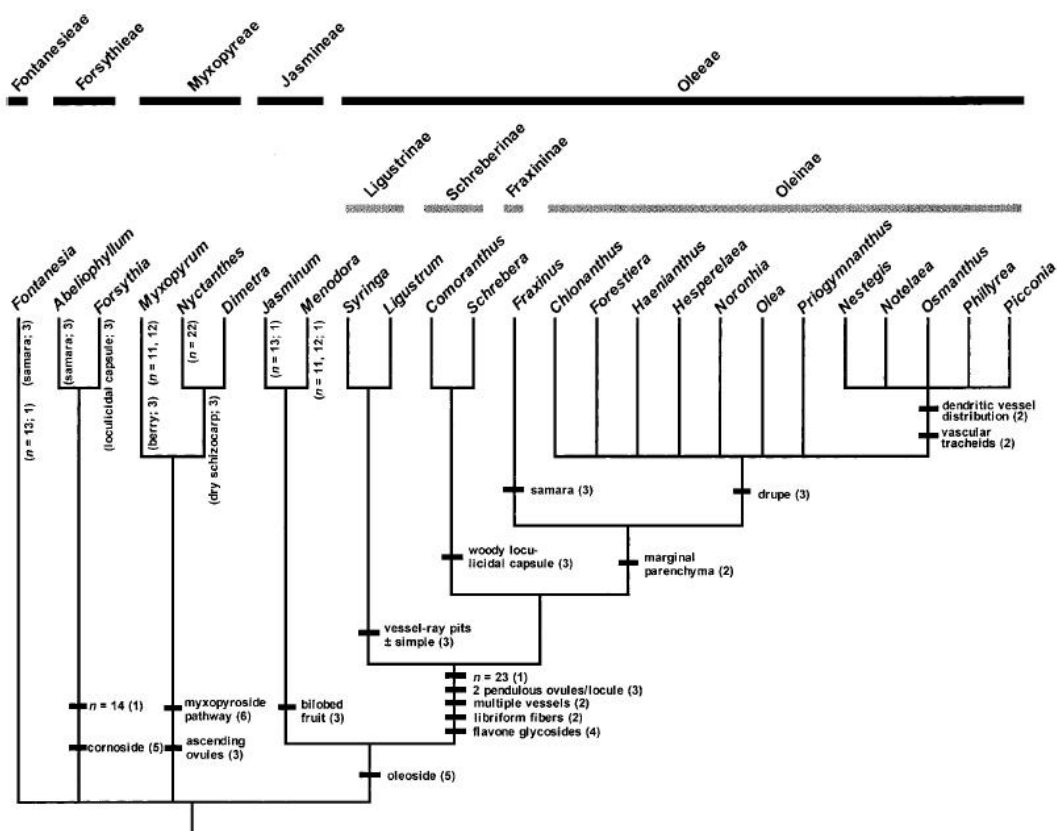


Table 1. Oleaceae genera sold in or native to NZ found by searching <http://findaplant.co.nz/>

Oleaceae genera in NZ	Tribe (subtribe)	Origin
<i>Fontanesia</i>	Fontanseiae	Ornamental
<i>Abeliophyllum</i>	Forsythiae	Ornamental
<i>Forsythia</i>	Forsythiae	Ornamental
<i>Jasminum</i>	Jasminiae	Ornamental
<i>Ligustrum</i>	Oleeae (Ligustrinae)	<i>L. "japonica"</i> sold as ornamental
<i>Syringa</i>	Oleeae (Ligustrinae)	Ornamental
<i>Fraxinus</i>	Oleeae (Fraxininae)	Ornamental
<i>Chionanthus</i>	Oleeae (Oleinae)	Ornamental
<i>Nestegis</i>	Oleeae (Oleinae)	Native
<i>Olea</i>	Oleeae (Oleinae)	Crop/ornamental (olive)
<i>Osmanthus</i>	Oleeae (Oleinae)	Ornamental

The Oleaceae is divided into five tribes (Fig. 2.). *Syringa* and *Ligustrum* form a clade within the tribe Oleaceae. The native NZ genus *Nestegis* also belongs to the tribe Oleaceae, but resides within a different subtribe to *Ligustrum*. The tribe Jasmineae is sister to Oleaceae; the remaining tribes (Fontanesieae, Forsythiae, Myxopyreae) are more distantly-related and basal within the Oleaceae (Wallander & Albert 2000).

Fig. 2. Molecular phylogeny of the Oleaceae (Wallander & Albert 2000). The dark bar at the top delimits the various tribes, the grey bar beneath, the subtribes of the Oleaceae.



Testing additional native NZ plant species is unnecessary because the basal elements of the Oleaceae (tribes Jasmineae, Fontanesieae, Forsythiae, Myxopyreae) were included in host-range testing performed for the USA that has already delineated the limits of the fundamental

host-range of *Leptoypha hospita* (which is confined to the tribe Oleae - see Fig. 3. and *Summary of host-range testing conducted for the USA*, below). After *Nestegis*, the next most closely-related native NZ plant species *Tetrachondra hamiltonii* belongs to the Tetrachondraceae (Fig. 1.) (De Lange & Rolfe 2010), which is estimated to have diverged from a common ancestor during the Paleocene to the Middle Eocene (65-50 Myr B.P.) (Wagstaff et al. 2000) and is much more distantly related to *Ligustrum* than *Nestegis*.

Summary of host-range testing conducted for the USA

A summary of host-range testing that has been conducted against plants in the Oleaceae in the USA (Zhang et al. 2012) is given in Tables 2 - 5. Note that plants from other plant families were also tested, but because the fundamental host-ranges of *L. hospita* was restricted to the tribe Oleae, it is considered unnecessary to report the test results for other plant families here.

Table 2. Summary of host-range testing conducted on *Leptoypha hospita* in the USA with plants in the Oleaceae (greater detail is provided in Table 3).

Oleaceae genus	Tribe (subtribe)	Species tested	Notes
<i>Fontanesia</i>	Fontanseiae	<i>F. fortunei</i>	Not a host
<i>Forsythia</i>	Forsythiae	<i>F. × intermedia</i>	Not a host
<i>Jasminum</i>	Jasminiae	<i>J. nudiflorum</i>	Not a host
<i>Syringa</i>	Oleeae (Ligustrinae)	<i>S. patula</i> , <i>S. meyeri</i> , <i>S. oblata</i> , <i>S. vulgaris</i>	All spp. tested are fundamental hosts but development to adult was consistently lower than that on <i>L. sinense</i> controls. <i>L. hospita</i> did not oviposit on <i>S. meyeri</i> in choice tests.
<i>Ligustrum</i>	Oleeae (Ligustrinae)	<i>L. japonicum</i> , <i>L. vulgare</i>	Both fundamental hosts, but <i>L. japonicum</i> appears to be a poor host
<i>Fraxinus</i>	Oleeae (Fraxininae)	<i>F. pennsylvanica</i> , <i>F. nigra</i> , <i>F. Americana</i> , <i>F. caroliniana</i> , <i>F. profunda</i> , <i>F. quadrangulata</i>	Two of the six species were fundamental hosts, but development (egg to adult) was very low compared to <i>L. sinense</i> controls indicating they are very poor hosts and are considered highly unlikely to be a realised host in field conditions. The remaining four species were not fundamental hosts.
<i>Chionanthus</i>	Oleeae (Oleinae)	<i>C. virginicus</i> , <i>C. pygmaeus</i>	Both species were fundamental hosts, but oviposition and development (egg to adult) was so low compared to <i>L. sinense</i> controls, that plants in this genus are considered highly unlikely to be a realised host in field conditions.
<i>Forestiera</i>	Oleeae (Oleinae)	<i>F. acuminata</i> , <i>F. godfreyi</i> , <i>F. neomexicana</i> , <i>F. segregata</i>	Variable risk. No development occurred on <i>F. godfreyi</i> in no-choice tests, but development was similar to <i>Ligustrum</i> controls on <i>F. neomexicana</i> in no-choice tests (although feeding and oviposition was much lower on <i>F. neomexicana</i> in choice tests). To our knowledge, this genus is absent in NZ, so the potential risk to <i>Forestiera</i> spp. is not of relevance to NZ.
<i>Olea</i>	Oleeae (Oleinae)	<i>O. europaeus</i>	Not a host
<i>Osmanthus</i>	Oleeae (Oleinae)	<i>O. americanus</i>	Not a host

Table 3. Details of host-range testing conducted on *Leptoypha hospita* in the USA with plants in the Oleaceae. Results for *Forestiera* are included although, to our knowledge, this is absent from NZ. Comparisons are in relation to *L. sinense* controls (e.g. an oviposition of 10.5% indicates that the number of eggs laid on a test plant was 10.5% of the number that were laid on *L. sinense* controls). Fundamental host = a species on which *Leptoypha hospita* could development from egg to adult in no-choice tests, which may or may not be utilised as a host in natural field conditions. Realised host = a plant species which is utilised in the field

Oleaceae genus	Tribe (subtribe)	Species tested	Result no-choice test	Result choice test	Notes
<i>Fontanesia</i>	Fontanseiae	<i>F. fortunei</i>	No adult feeding No oviposition No development (egg to adult)	Not required	Not a host
<i>Forsythia</i>	Forsythiae	<i>F. × intermedia</i>	No adult feeding No oviposition No development (egg to adult)	Not required	Not a host
<i>Jasminum</i>	Jasminiae	<i>J. nudiflorum</i>	Minor adult feeding (13.5%). Minor oviposition (10.5%). No development (egg to adult)	Not required	Not a host
<i>Syringa</i>	Oleeae (Ligustrinae)	<i>S. patula</i>	Similar adult feeding (105%) Lower oviposition (27.2%) Lower development (egg to adult; 29.2%)	Not done	Fundamental host, no-choice tests indicates it is a poor host, but there may be a risk of spill-over attack.
		<i>S. meyeri</i>	Lower adult feeding (22.8%) Lower oviposition (21.5%) Lower development (egg to adult; 29.1 %)	Very low feeding (0.2%) No oviposition	Fundamental host, but low development in no-choice test and lack of oviposition in choice-test indicates it is unlikely to be attractive to <i>L. hospita</i> in field conditions
		<i>S. oblata</i>	Lower adult feeding (18.1%) Similar oviposition (69.7%) Lower development (egg to adult; 22.6 %)	Not done	Fundamental host, no-choice tests indicates it is a poor host, but there may be a risk of spill-over attack.
		<i>S. vulgaris</i>	Low adult feeding (9.4%) Similar oviposition (51.3%) Lower development (egg to adult; 11.9 %)	Not done	Fundamental host, no-choice tests indicates it is a poor host, but there may be a risk of spill-over attack.
<i>Ligustrum</i>	Oleeae (Ligustrinae)	<i>L. japonicum</i>	Lower adult feeding (41.1%) Lower oviposition (19.6%). Very low development (egg to adult; 2.1%)	Lower feeding (10.4%) Low oviposition (7.2%)	Low development in no-choice tests indicates it is unlikely to be a good field host
		<i>L. vulgare</i>	Similar feeding (70.6%) Similar oviposition (78.7%) Similar development (egg to adult;	Lower feeding (56.1%) Lower oviposition (39.4%)	Likely field host, but clear preference for <i>L. sinense</i>

<i>Fraxinus</i>	Oleeae (Fraxininae)	<i>F. pennsylvanica</i>	142%) Minor adult feeding (15.2%) No oviposition.	Very low adult feeding (4.8%) Very low oviposition (0.4%).	Fundamental host, but low oviposition in both tests indicated this is unlikely to be a realised host.
		<i>F. nigra</i>	Very low development (egg to adult; 1.1%) Lower adult feeding (48.3%)	Lower low adult feeding (15.3%) Low oviposition (5.1%)	Fundamental host, but low oviposition and development (egg to adult) indicates that it is a sub-optimal host.
		<i>F. americana</i>	Low oviposition (6.6%). Lower development (egg to adult; 10.3%) Lower adult feeding (11.4%) No oviposition.	Low adult feeding (9.8%) No oviposition.	Not a host
		<i>F. caroliniana</i>	No development (egg to adult). Lower adult feeding (45%) No oviposition.	Low adult feeding (6.3%) Very low oviposition (2.5%).	Not a host
		<i>F. profunda</i>	No development (egg to adult). Very low adult feeding (4.6%) Very low oviposition (1.9%).		Not a host
		<i>F. quadrangulata</i>	No development (egg to adult). Very low adult feeding (0.4%) Lower oviposition (14.4%).		Not a host
		<i>Chionanthus</i>	Oleeae (Oleinae)	<i>C. virginicus</i>	No development (egg to adult). Lower adult feeding (29.4%).
<i>C. pygmaeus</i>	Lower oviposition (29%). Lower development (egg to adult; 17.6%) Lower adult feeding (37.2%). Lower oviposition (16.4%). Very low development (egg to adult; 2%)			-	Fundamental host. No-choice test indicates that it is a very poor host.
<i>Forestiera</i>	Oleeae (Oleinae)	<i>F. acuminata</i>	Greater adult feeding (207%)	Low adult feeding (10.4%)	Fundamental host. Low number of newly emerged adults indicates it is a relatively poor host and choice tests indicate a preference for <i>Ligustrum</i> , but spill over non-target attack is possible

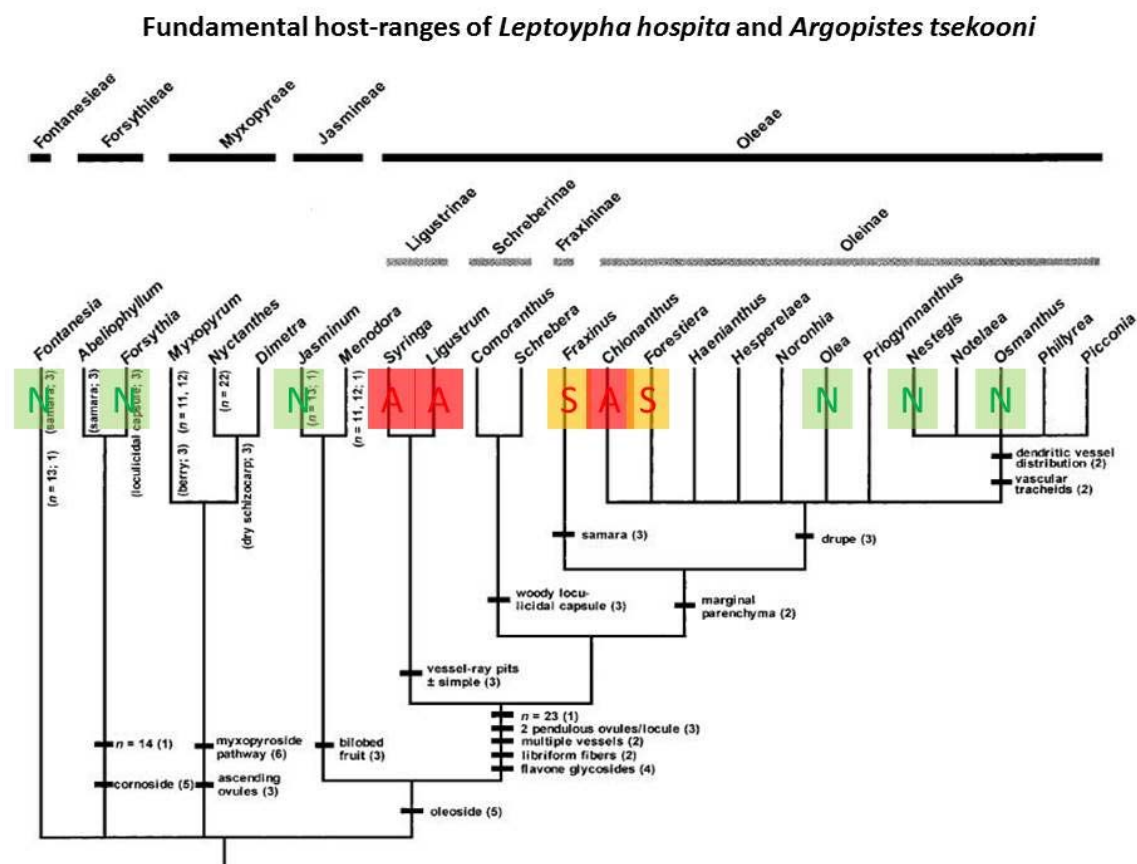
		<i>F. godfreyi</i>	Similar oviposition (110%) Fewer newly emerged adults (28%) No adult feeding Few eggs (11%) No newly emerged adults	Low oviposition (16.3%)	Not a host
		<i>F. neomexicana</i>	Similar adult feeding	Very low adult feeding (4.6%)	Fundamental host. Reduced attack in choice tests indicates it is not as attractive to <i>L. hospita</i> as <i>Ligustrum</i> , but spill-over non-target attack is possible
		<i>F. segregata</i>	Similar oviposition Similar newly emerged adults Lower adult feeding (26.7%)	Low oviposition (23.1%) Low adult feeding (8%)	Fundamental host. Low number of newly emerged adults in the no-choice test indicates it is a poor host and low adult feeding and oviposition in choice tests indicates it is not as attractive to <i>L. hospita</i> as <i>Ligustrum</i> , but spill over non-target attack is possible
<i>Olea</i>	Oleeae (Oleinae)	<i>O. europaeus</i>	Fewer eggs (49.5%) Few newly emerged adults (8.9%) Very low adult feeding (3.8%) No oviposition. No development (egg to adult)	Low oviposition (17.1%) Very low feeding (0.3%) Very low oviposition (0.4%)	Not a host
<i>Osmanthus</i>	Oleeae (Oleinae)	<i>O. americanus</i>	No adult feeding No oviposition. No development (egg to adult)	Not required	Not a host

Conclusions

The results of the testing for the USA should be interpreted bearing in mind that no-choice tests (where a candidate agent is presented with a test plant and can either feed on it or starve to death) are good at determining the fundamental host-range (i.e. all the plant species that can support development from egg to adult). However, because agents are confined to test plants, no-choice tests commonly produce ‘false positives’ where some plant species are fed on that would not be utilised as a host under natural field conditions because the insect would normally disperse in search of its preferred host plant rather than feed. The *realised host range* comprises plant species that are actually utilised in the field. Plants that are demonstrated not to be ‘fundamental hosts’ by no-choice host-range tests are not at risk of non-target attack. Additional testing (e.g. oviposition tests) or analysis of quantitative no-choice data is used to determine the risk to plants which are shown to be fundamental hosts.

The no-choice tests indicated that the fundamental host-range of *L. hospita* is confined to the tribe Oleae (Fig. 3) and plants outside this tribe (*Fontanesia*, *Forsythia*, *Jasminum*) are not at risk. Within the tribe Oleae, the genera *Olea* and *Osmanthus* are also not fundamental hosts of *L. hospita* and can be discounted as potential field hosts.

Fig. 3. Fundamental host-ranges of *L. hospita* in relation to the molecular phylogeny of the Oleaceae: **N** = none of the tested species are fundamental hosts (development to adult did not occur); **S** = some, but not all tested species are fundamental hosts; **A** all tested species are fundamental hosts (development to adult can occur).



Development to adult occurred in three genera of the Oleaceae that are of relevance to the NZ nursery industry, namely *Fraxinus*; *Chionanthus* and *Syringa*. The risk of non-target attack on these species is considered below:

1. *Fraxinus*: Most *Fraxinus* spp. (4/6) tested did not support the development of *L. hospita*. Although the potential for minor spill-over attack on the two species which did support development (*F. pennsylvanica*; *F. nigra*; Table 3) cannot be totally ruled out, it is considered highly unlikely because compared to the *L. sinense* controls, oviposition and developmental success was very low. Moreover, none of the 22 *Fraxinus* spp. that occur in China has been reported to be a host of *L. hospita*. No further testing is deemed necessary.
2. *Chionanthus*: Although *L. hospita* was able to complete development on both *C. virginica* and *C. pygmaea*, low developmental success on these species indicates that *Chionanthus* is also unlikely to become a permanent host of either agent in the field. Again the possibility that minor spill over attack may occur on plants growing in close proximity to *Ligustrum* cannot be ruled out, but is also considered to be unlikely. None of the 7 *Chionanthus* spp. that occur in China has been reported to be a host of *L. hospita*. No further testing is deemed necessary.
3. *Syringa*: This is the most closely-related genus to *Ligustrum* (Fig 2.) and was therefore identified as the genus that is at the greatest risk of non-target attack. Developmental success (egg to adult) was significantly lower on all four *Syringa* spp. tested in no-choice tests (but numbers reared were c. 30% of those on *L. sinense* controls in two of the species tested). In a choice-test, feeding was trivial and no eggs were laid on *S. meyeri* but choice tests were not performed on the other species. The testing done for the USA, coupled with the lack of field records of *L. hospita* on *Syringa* spp. in China, indicates that the risk of non-target attack on *Syringa* is low. However, not all of the cultivars that are sold in New Zealand were tested for the USA or present in China. For this reason additional testing was performed on *Syringa* cultivars that are commonly sold in NZ (see *Additional host-range testing for New Zealand*, below).

Two Oleaceae genera (*Nestegis* and *Abeliophyllum*) that are present in NZ were not tested in the USA. *Nestegis* is the sole NZ native genus in the Oleaceae. The genus *Nestegis* is placed in the tribe Oleaceae and the potential for it to be a fundamental host of *L. hospita* could not be ruled out. It was therefore decided to conduct additional host range tests on this genus (see *Additional host-range testing for New Zealand*, below).

Abeliophyllum belongs to the tribe Forsythieae and is represented in NZ by an exotic ornamental (*A. distichum*). The results of the testing already conducted indicate that plants in the tribe Forsythieae are highly unlikely to be suitable hosts because the fundamental host-ranges of *L. hospita* was shown to be confined to the tribe Oleaceae. Testing of *Abeliophyllum* was therefore, considered to be unnecessary.

Additional host-range testing for New Zealand

Based on the review of the host-range testing done for the USA it was decided that additional testing should be conducted in New Zealand to investigate the potential for *L. hospita* to attack native New Zealand *Nestegis* spp. and to further investigate the risk to ornamental *Syringa* cultivars grown in New Zealand.

Methods

Host-range tests using similar techniques to those reported by (Zhang et al. 2012) were set up as follows:

Insect Colony

Leptoypha hospita adults were provided by Dr Jim Hanula (USDA Forest Service) from a culture that was originally field collected from *L. sinense* growing in Huangshan city, Anhui province, China in March 2009; the same population that was tested for the United States. Upon arrival, lace bugs were transferred to potted privet plants in the Beever Containment Facility, Auckland. Plants were covered with white polyester sleeves to keep lace bugs from escaping and the colony was maintained transferring bugs to new plants as old plants deteriorated.

Experimental Design

No-Choice Tests

Plants were tested in a series of 3 separate trials from May 2013 to February 2014. Each trial included two to four test species plus *L. sinense* control plants. Lace bugs were sexed based on the shape of the terminal sternite (Zhang et al. 2012) and pairs of adults (1 male and 1 female) were caged separately within polyester sleeves (25 cm × 15 cm) placed over randomly selected individual branches of test plants. Typically, four sleeves of paired lace bugs were tied to each plant. Approximately one week later (range 1 week-11 days), half the sleeves (henceforth 'adult feeding and oviposition sleeves') were randomly selected and cut from each test species. Feeding damage was assessed by counting chlorotic spots or points on leaves caused by *L. hospita* and eggs deposited in leaves were counted using a dissecting microscope. Lace bugs in the remaining sleeves (henceforth 'development sleeves') were collected after 9-14 days, depending on trial, using an aspirator and cages were placed back on the branches to allow nymphs to develop. These remaining sleeved branches were checked and newly emerged adults were collected and counted weekly.

Trial 1 comprised *L. sinense* controls (4 plants) and two plants each of the four native NZ *Nestegis* spp. (*N. apetala*; *N. cunninghamii*; *N. lanceolata*; *N. montana*).

Trial 2 comprised *L. sinense* controls; *Syringa x josiflexa* 'Bellicent' and *Syringa x laciniata* (5 plants of each species). In this trial some adult *L. hospita* reared through on the *Syringa* test plants, so testing was continued over a second generation (Trial 2b) to determine whether *L. hospita* is likely to be capable of maintaining populations on *Syringa* plants as follows:

additional ‘development sleeves’ were set up using the adults reared in Trial 2: 4 control sleeves were set up on *L. sinense* plants using *L. hospita* reared from *L. sinense*; 10 sleeves (2 per plant) were set up on *Syringa x josiflexa* 'Bellicent' plants using *L. hospita* adults reared from *Syringa x josiflexa* 'Bellicent' plants and 6 sleeves (2 per plant) were set up on *Syringa x laciniata* plants using *L. hospita* adults reared from *Syringa x laciniata*.

Trial 3 comprised *L. sinense* controls; *Syringa hyacinthiflora* (3 plants; one each of the following cultivars: Alice Eastwood; Clarks Giant and Esther Staley) and *Syringa vulgaris* (6 plants, one each of the following cultivars: Belle de Nancy; General Pershing; Sensation; Madame Lemoine; Princess Clementine; Rhum Von Horstenstein). As in Trial 2 some adult *L. hospita* were reared from *Syringa* test plants, so testing was continued over a second generation (Trial 3b) as follows: additional ‘development sleeves’ were set up using the adults reared in Trial 2: 4 control sleeves were set up on *L. sinense* plants (one per plant) using *L. hospita* reared from *L. sinense*; 3 sleeves (1 per plant; one each of the following cultivars: Alice Eastwood; Clarks Giant and Esther Staley) were set up on *Syringa hyacinthiflora* using *L. hospita* adults reared from *Syringa hyacinthiflora* and 5 sleeves (one each on the following cultivars: Belle de Nancy; General Pershing; Madame Lemoine; Princess Clementine; Rhum Von Horstenstein) were set up on *Syringa vulgaris* plants using *L. hospita* adults reared from *Syringa vulgaris*.

Statistical Analyses

Analyses were done using the R statistical program (R Development Core Team 2008). Kruskal Wallis tests were performed to investigate the effect of treatment (i.e. plant species) on adult feeding, the number of eggs laid and the numbers of adults reared in each trial. Where an analysis indicated a significant treatment effect, a post-hoc test was performed (Mann-Whitney test with Bonferroni correction) to compare means between plant species.

Results and Discussion

Trial 1. Although some feeding damage was recorded on the *Nestegis* test plants in the adult feeding and oviposition sleeves, the number of feeding holes was much lower than on *Ligustrum sinense* controls for all species tested and oviposition was only reported on the *L. sinense* controls (Table 4). Moreover, an average of 43.13 adults was reared from *L. sinense* in the ‘development sleeves’ and none were reared from any of the *Nestegis* species.

The number of adults reared from *L. sinense* in the development sleeves greatly exceeded the number of eggs counted on *L. sinense* foliage in the adult feeding and oviposition sleeves (Table 4). Adults were left in the development sleeves for longer (and so had more time to lay eggs), compared to the adult feeding and oviposition sleeves; but this only partially explains the difference: dividing the number of eggs laid or adults reared by the duration of exposure to ovipositing adults in the respective tests indicated that the number of adults reared per day of exposure to ovipositing females was 1.77 times higher than the recorded number of eggs laid per day in the oviposition treatment. This implies that a significant proportion of eggs, which are very small and inserted into the plant tissue, were not detected in the adult feeding and oviposition test. The adult feeding and oviposition sleeve tests are therefore considered to

be an unreliable measure of the quantity of eggs laid (although they are considered to be a good measure of adult feeding preference). By contrast, the development tests (which are a combined measure of both oviposition preference and nymphal survival) are a reliable quantitative measure of the relative acceptability of the host and test plant species.

Table 4. Results of Trial 1. Estimates not followed by the same letter are significantly different (Mann-Whitney test with Bonferroni correction).

Test plant	Mean no. feeding marks (\pm SE)	Mean no. eggs laid (\pm SE)	Mean no. adults reared (\pm SE)
<i>Ligustrum sinense</i>	92.63 (9.23)	14 (3.72)	43.13 (6.18)a
<i>Nestegis apetala</i>	45.25 (2.75)	0 (0.00)	0 (0.00)b
<i>Nestegis cunninghamii</i>	11.50 (3.62)	0 (0.00)	0 (0.00)b
<i>Nestegis lanceolata</i>	10.00 (2.86)	0 (0.00)	0 (0.00)b
<i>Nestegis montana</i>	7.50 (3.52)	0 (0.00)	0 (0.00)b
	Kruskal-Wallis Chi-squared =19.41; df = 4; p-value = 0.0006515	Kruskal-Wallis Chi-squared =18.22; df = 4; p-value = 0.001114	Kruskal-Wallis Chi-squared =21.81; df = 4; p-value = 0.0002189

Trial 2. Feeding damage was consistently higher on *L. sinense* controls, compared to on *Syringa x josiflexa* 'Bellicent' and *Syringa x laciniata*. Oviposition was recorded on both *Syringa* species and the number of eggs counted in the adult feeding and oviposition sleeves did not vary greatly between plant species (Table 5; but note the comments regarding the reliability of the eggs counts in the Trial 1 section of the results, above). The number of adults reared in the 'development sleeves' was an order of magnitude higher on the *L. sinense* controls compared to both *Syringa* spp. (Table 5). Furthermore, when the experiment was repeated over a second generation, large numbers of adults were again reared from *L. sinense*, but none were reared from *Syringa x josiflexa* 'Bellicent' and only two adults were reared from *Syringa x laciniata* (a rearing success of just ~1% compared to the *L. sinense* controls).

Table 5. Results of Trial 2 and 2b. Estimates not followed by the same letter are significantly different (Mann-Whitney test with Bonferroni correction).

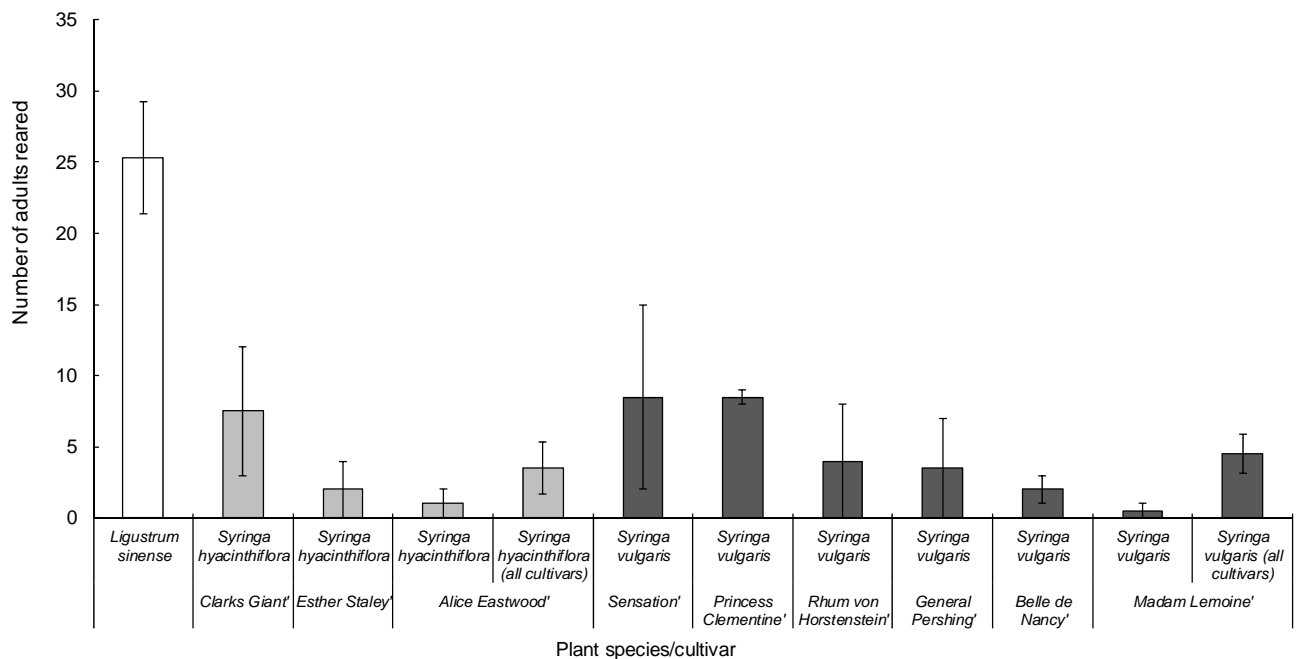
Test plant	Mean no. feeding marks (\pm SE)	Mean no. eggs counted (\pm SE)	Mean no. adults reared (\pm SE)	Mean no. adults reared (\pm SE) in second generation
<i>Ligustrum sinense</i>	149.78 (15.84)a	14.11 (2.80)	52.50 (6.4)a	28.25 (6.25)a
<i>Syringa x josiflexa</i> 'Bellicent'	38.10 (8.39)b	11.30 (2.30)	6.2 (2.80)b	0.00 (0.00)b
<i>Syringa x laciniata</i>	46.00 (7.40)b	11.10 (2.10)	3.60 (0.90)b	0.33 (0.19)b
	Kruskal-Wallis Chi-squared =17.02; df = 2; p-value = 0.0002015	Kruskal-Wallis Chi-squared =0.36; df = 2; p-value = 0.8363	Kruskal-Wallis Chi-squared =18.33; df = 2; p-value = 0.0001048	Kruskal-Wallis Chi-squared =15.07; df = 2; p-value = 0.0005334

Trial 3. Feeding damage was consistently higher on *L. sinense* controls than on *Syringa hyacinthiflora* and *Syringa vulgaris* with little variation between the different cultivars of each species (Fig. 4). The number of eggs counted was also lower on the two *Syringa* species, compared to the *L. sinense* controls (Table 8), but note the comments regarding the reliability of the eggs counts in the Trial 1 section of the results, above. As with trial 2, some adults were reared on both *Syringa* species, but the number of adults reared in the ‘development sleeves’ was almost an order of magnitude higher on the *Ligustrum sinense* controls compared to the *Syringa* test plant spp. (Table 6). When the experiment was repeated over a second generation, large numbers of adults continued to be reared from *L. sinense*, but none was reared from *Syringa hyacinthiflora* and only two adults were reared from the five *Syringa vulgaris* cages (a rearing success of ~2% compared to the *L. sinense* controls).

Table 6. Results of Trial 3 and 3b. Estimates not followed by the same letter are significantly different (Mann-Whitney test with Bonferroni correction).

Test plant	Mean no. feeding marks (\pm SE)	Mean no. eggs counted (\pm SE)	Mean no. adults reared (\pm SE)	Mean no. adults reared (\pm SE) for a second generation
<i>Ligustrum sinense</i>	150.20 (15.18)a	21.90 (3.41)a	25.30 (3.93)a	19.25 (4.52)a
<i>Syringa hyacinthiflora</i>	20.00 (4.03)b	3.00 (1.37)b	3.50 (1.82)b	0.00 (0.00)b
<i>Syringa vulgaris</i>	32.33 (5.90)b	13.83 (1.92)b	4.50 (1.39)b	0.40 (0.40)b
	Kruskal-Wallace	Kruskal-Wallace	Kruskal-Wallace	Kruskal-Wallace
	Chi-squared =19.18;	Chi-squared	Chi-squared =16.40;	Chi-squared =9.29;
	df = 2; p-value =	=13.80; df = 2; p-	df = 2; p-value =	df = 2; p-value =
	6.845e-05	value = 0.001006	0.0002752	0.009574

Fig. 4. Number of adults reared per sleeve in Trial 3, indicating differences between different cultivars of *Syringa hyacinthiflora* and *Syringa vulgaris*, compared to *Ligustrum sinense* controls.



Conclusions

The results of the additional trials in NZ indicate that *Nestegis* spp. are not fundamental hosts of *L. hospita* and that there is no risk of native NZ *Nestegis* spp. becoming field hosts.

The results of the testing on *Syringa* spp. indicate that *L. hospita* is most unlikely to be able to persist on *Syringa* spp. in the absence of nearby *Ligustrum* plants. The potential for minor spill over attack to occur on ornamental *Syringa* spp. cannot be completely ruled out, but the low level of rearing success in the no-choice tests indicates any spill over attack is likely to be minor.

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