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Screening of Potential Biocontrol Agents for *Ligustrum robustum* subsp. *walkeri* in Sri Lanka for Possible Release in the Island of La Réunion

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ABSTRACT. A survey and laboratory studies were conducted to identify phytophagous insects associated with Ligustrum robustum (Roxb.) Bl. subsp. walkeri (Decne.) P.S. Green (Oleaceae) in Sri Lanka, which is an invasive weed in the island of La Réunion in Indian Ocean in an attempt to select biocontrol agents for plant species,. Previous studies yielded 17 insect species from 6 orders as natural enemies of L. robustum. Due to apparent host specificity, 6 species namely, Palpita celsalis (Lepidoptera: Pyralidae), Epiplema albida (Lepidoptera: Epiplemidae), Problepis deliaria (Lepidoptera: Geometridae), Dermorhytis lewisi, Dermorhytis ornatissima and Hyphasis sp. 1 (Coleoptera: Chrysomelidae) were tested further in the field and under quarantine conditions in Sri Lanka and United Kingdom to identify their host plant preference. Although the results revealed that most of the above species are not completely host-specific to L. robustum, P. celsalis and E. albida have considerable promise as biocontrol agents of this weed found in La Réunion.

INTRODUCTION

Ligustrum robustum subsp. *walkeri*, commonly known as tree privet (*Bora* - Sinhala) is an evergreen tree in the Family *Oleaceae*. It was first observed in the Mauritius as a weed in 1968 and later invaded the proximate island of La Réunion. The seeds of this weed were deliberately transported from Sri Lanka to Mauritius *via* seed exchange programmes (Royal Botanic Garden, 1905-1906).

Since its introduction, *Ligustrum* has spread rapidly and now found along most of the natural forests of La Réunion islands. An exotic bulbil facilitates the spread of this invasive species, and an expansion of this spp is inevitable (Lavergne and Shaw, 1999). All infestations in La Réunion are within the forest reservations or in forest interiors, and the use of classical biological control has been identified as a strategy for controlling this weed.

The crucial factor for determining the safety of any weed biological control programme is to ensure that candidate phytophagous organisms (insects, mite, nematodes or pathogens) can be introduced to control a particular weed without endangering desirable non-target plants (Cock, 1982). The traditional host-range testing protocols established

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over many decades that rely mainly on no-choice or starvation studies intend to prove beyond all reasonable doubt, that a given natural enemy will not damage any non-target plants after release in the area where control of the target weed is required (Cock and Holloway, 1982). However, for effective biological control it is essential to know the exact area of origin or centre of diversity of the target plant since this will be the region where co-evolved natural enemies will be found. Studies on DNA mapping have confirmed that Sri Lanka is the Centre of Origin for the species found in La Réunion and Mauritius (http://www.st-andrews.ac.uk).

Host-specific insects are good candidates for weed biological control agents. The CABI-Bioscience UK Centre, in collaboration with Postgraduate Institute of Agriculture, Sri Lanka, began searching for natural enemies of *Ligustrum robustum* in June 1997. This resulted in the discovery of 17 natural enemies six of which are considered potential candidates as biocontrol agents (Sakalasooriya *et al.*, 2000). These six natural enemies include three species of Coleoptera and three species of Lepidoptera that damage, *Ligustrum* seeds, leaves and flowers. This paper reports the results of field and laboratory studies carried out on host plant preference of these natural enemies of *Ligustrum robustum* in Sri Lanka to identify potential biocontrol agents for subsequent release in La Réunion.

MATERIALS AND METHODS

Description of the collection sites

Over twenty sites were surveyed in Sri Lanka between June 1998 and August 2000 for *Ligustrum robustum* and its natural enemies. The sites represented six districts namely Ratnapura, Kandy, Badulla, Nuwara-Eliya and Monaragala (Table 1). These regions fall in to five different agro-ecological zones as defined by the Department of Agriculture (1979). Five sites representing different districts namely Loolecondera (Kandy), Kalupahana (Ratnapura), Boralanda (Badulla), Halpe-Belihuloya (Ratnapura) and Boragas/Boragasketiya (Nuwara-Eliya) were selected for regular monitoring owing to the ease of access, high plant populations and low human disturbance.

Table 1. Physical Characteristics of the Collection Sites.

Name of the sites	Lat. N*	Lon. E*	Alt. (m)	Climate type
Loolecondera	07 07 742	080 42 382	1175	Up country-wet zone RF>125@
Boralanda - Rahangala	06 49 475	080 53 267	1325	Up country intermediate RF>85@
Boragasketiya Boragas	06 54 445	080 49 962	1625	Up country wet RF>55@
Kalupahana	06 45 828	080 45 003	950	Mid country intermediate RF>45@
Halpe-Belihuloya	06 39 047	080 40 735	825	Mid country wet RF>50@

@Rainfall in inches (1 inch = 2.54 cm)
Lat. N = Northern latitudes, Lon. E = Eastern longitudes, Alt = altitude

Collection methods

Collections were made throughout the year but primarily between November and March. At each site *Ligustrum* plants were examined for adult and immature insects. The presence of insects and feeding damage were recorded and representative samples were taken from each accessible tree from each site. Each part of the plant was carefully inspected visually before a standardised beating process was undertaken using beating trays (Julien and White, 1997). The insects were then transported in ventilated plastic vials or boxes at ambient temperature.

Laboratory cultures

Laboratory cultures were set up using materials collected at the sites. Larval instars were reared on *Ligustrum* foliage in ventilated plastic food containers (0.5 l to 3 l in size). Moist paper towels or filter papers were placed at the bottom of the rearing containers in order to avoid desiccation. The mated adults collected were allowed to oviposit in the presence of plant material and 20% honey water in cotton-wool feeders in 5 ml vials.

Host specificity tests

Host-specificity studies were conducted in the quarantine insectaries in the Faculty of Agriculture, University of Peradeniya, Sri Lanka and CABI-Bioscience, United Kingdom, which were air-conditioned to 24-27°C, with indirect natural light supplemented by artificial florescent bulbs. The potential host range of the selected insects was determined by comparing larval feeding response to the leaves of selected test plants (Table 2), which were selected using the criteria suggested by Wapshere (1974), Julien and White (1997) and Sakalasooriya *et al.* (2000). In the feeding trials, leaf materials were kept clean and changed every other day. Each test was replicated at least three times. The larval feeding was assessed by the palatability of each plant, which was evaluated by rating the feeding damage on the leaves as given below :

0	-	No feeding
*	-	Small feeding punctures *i.e.*, exploratory feeding
**	-	Small feeding lesions/holes *i.e.*, restrained feeding
***	-	Large feeding holes/lesions *i.e.*, normal feeding

Even after feeding, the final qualification was made by counting the number of faecal pellets produced. The suitability of the test plants as alternative hosts was evaluated by recording the growth and the survival of the larvae. Observations were made at three day intervals.

Table 2. Plants exposed to potential natural enemies in host specificity testing.

Closely related plants		Closely related plants	
Family and Genera	Species	Family and Genera	Species
Acanthaceae		Apocynaceae	
Acanthus	*A. mollis*	*Asclepias*	*A. curassavica*
Thumbergia	*T. alata*	*Cryptostegia*	*C. grandiflora*
Hypoestes	*Hypoestes sp.*	Camptocarpus	*C. mauritiana*
Bignoniaceae		*Hoya*	*H. carnosa*
Colea	*C. colei*	Loganiaceae	
Bignonia	*B.unguis-cati*	*Nuxia*	*Nuxia sp.*
Eccremocarpus	*E.scaber*		*Geniostoma sp.*
Buddlejaceae		Convolvulaceae	
Buddleja	*B. davidii*	*Convolvulus*	*C. arvensis*
Gesneriaceae		*Ipomoea*	*I. purparia*
Streptocarpus	*S. rexii*	Rosaceae	
Lamiaceae		*Chaenomeles*	*Chaenomeles sp.*
Clerodendron	*C. heterophyllum*	*Fragaria*	*Fragaria sp.*
	C. laciniatum	*Prunus*	*P. spinosa*
Myoporaceae			*Rosa sp.*
Myoporum	*M. mauritianum*	Oleaceae	
Nesogenaceae		*Ligustrum*	*L. robustum*
Nesogenes	*N. orerensis*		*L. ovalifolium*
	N. decumbens	*Syringa*	*S. josiflexa*
Pedaliaceae		*Fraxinus*	*F. excelsor*
Sesame	*S. indicum*	*Olea*	*O. polygoma*
Scrophulariaceae			*O. europaea*
Digitalis	*D. ambigua*		*O. Lancea*
Hebe	(lilacolour)	*Chionanthus*	*C. albidiflora*
	(wiry splash)		*C. zeylanica*
	x franciseana		*C. broomeana*
Verbascum	*V. blatteria*	*Osmanthus*	*O. hetephyllus*
Calceolariya	*C. biflora*		*O.delahayii*
	C. herbeohybrida	*Jasminum*	*J. polyanthus*
Antirrhinum	*A. orantium*		*J. angustifolium*
Verbanaceae			*J. flexile*
Premna	*P. obtusifolia*		*J. rotterianum*
Lantana	*L. camara*		
Carissa	*C. grandiflora*		
	C. xylopicron		
Vinca	*V. major*		

Continued ....

Table 2: Cont'd..............

Economically Valued Plants		Economically Valued Plants	
Family and Genera	Species	Family and Genera	Species
Anacardiaceae		Musaceae	
Mangifera	*M. indica*	*Musa*	*M. acuminata*
Annonaceae			*M. coccinea*
Anona	*A. squamosa*		*M. velutina*
Bromeliaceae		Orchidaceae	
Ananas	*A. comosus*	*Vanilla*	*V. planiflora*
Solanaceae		Palmaceae	
Capsicum	*C. frutescens*	*Chamaedorea*	*C. elegans*
Lycopersicon	*L. esculentum*	Papilionoideae	
Nicotiana	*N. tabacum*	*Phaseous*	*Phaseous sp.*
Solanum	*S. tuberosum*	Rosaceae	
Cruciferae		*Prunus*	*P. spinosa*
Brassica	*Brassica sp.*		*Rosa sp.*
Poaceae		Rutaceae	
Saccharum	*S. officinarum*	*Citrus*	*Citrus sp.*
Fabaceae		Rubiaceae	
Acacia	*A. heterophylla*	*Coffea*	*C. arabica*
Delonix	*D. regina*		

RESULTS AND DISCUSSION

Identification and diversity of natural enemies

A wide range of natural enemies were found attacking the aboveground parts of *Ligustrum robustum* plant throughout its native range at elevation above 1000 m in the central highlands of Sri Lanka (Table 3). The majority of natural enemies are Lepidopteran and Coleopteran foliage-feeders. Of these the larvae of the moth *Phyllocnistis citrella* caused substantial leaf mining damage, and two other moth species caused heavy damage to fruits and flowers. Among the insects that caused substantial damage to *Ligustrum* trees in Sri Lanka, a Lepidopteran *Rhopobota naevana* and pentatomid sap-sucker *Antestiopsis picturata* were the most abundant species.

The inter-species feeding preference varied. For example, there was a significant difference between diameters of woody stem attacked by two Scolytid beetles: *Euwallacea fornicatus*, the tea shot hole borer, which showed a preference for stems of pencil thickness whilst *Xylosandrus arquatus* fed on relatively young stems. Furthermore, trees with tender flushes, sustained more damage attributable to leaf-eating caterpillars whilst trees with mature leaves supported more beetles and stem borers.

Table 3. Updated database of natural enemies attacking Ligustrum robustum in Sri Lanka, their feeding pattern and host range [updated after Sakalasooriya *et al.* (2000)].

Insect	Foliage	Stem	Flower	Fruits	Roots
Heteroptera					
Pentatomidae					
Antestiopsis picturata	+,1		+	+	+
Coleoptera					
Chrysomelidae					
Dermorhytis lewisi[P]	+,1				+
D. ornatissima[P]	+,1				+
Hyphasis sp. 1[P]	+,1				
Hyphasis sp. 2	+,1				
Curculionidae					
Lepropus immunis	+,1				
Scolytidae					
Euwallacea fornicatus		+,4			
Xylosandrus arquatus		+,4			
Lepidoptera					
Epiplemidae					
Epiplema albida[P]	+,1				
Geometridae					
Problepis deliaria[P]	+,3				
Gracillariidae					
Phyllocnistis citrella	+,4				
Tortricidae					
Rhopobota naevana	+,4		+	+	
Noctuidae					
Pangrapta grisangula	+,3				
Pyralidae					
Palpita celsalis[P]	+,1		+	+	
Phycita eulepidella			+,2	+,2	
Sphingidae					
Psilogramma menephron	+,3				
Cossidae					
Zeuzera pyrina			+,4		

\+ Indicates the feeding habit of the insects.
1 = feed on *Ligustrum* + *Jasminum* only 2 = feed on Oleaceae family only
3 = feed on hosts other than Oleaceae 4 = feed on economically important crops
[P] Sakalasooriya *et al.*, (2000) identified these species as high priority natural enemies that require further study.

Insects feeding on reproductive parts

Ligustrum robustum inflorescence has a large number of minute flowers. Although the species host various insects seeking pollen or nectar, this section only deals with those which actually damage the reproductive structures. Various *Lepidoptera* and *Hemiptera* feed on the inflorescence and developing berries including *Palpita celsalis* (Pyralidae). One of the major flower and fruit damaging insects recorded in Sri Lanka is the Pentatomid, *Antestiopsis picturata* (family: Heteroptera), formerly known under the genus *Antesia*. It has long been known that this genus includes a serious pest group that damages coffee plantations around the world including Sri Lanka and India. Pests from the genus *Antestiopsis* have been recorded from coffee plantations in Sri Lanka, but no reference was made to the species involved. In Athulgama (Kandy District), *Ligustrum* trees were found growing close to a coffee plantation, which enabled a realistic field preference study. Even under heavy population density of this pentatomid, no visual impact was seen on nearby Coffee trees.

Ongoing studies also revealed that there is no visual damage by this insect to coffee flowers where all the insects died after about three to four days. However, the introduction of this genus to Mascarine islands (*i.e.* La Réunion, Mauritius) has been strictly prohibited by plant protection agreement for the Asia and Pacific region with respect to Article I(a), in November 1983 (http://www.fao.org/Legal/TREATIES/Treaty-e.htm).

Leaf feeders

Approximately 80% of the natural enemies of *Ligustrum robustum* are foliage feeders (Table 3). In the high priority group of insects, the most widespread and abundant leaf feeder on the privet in Sri Lanka is *Palpita celsalis* (Sakalasooriya *et al.*, 2000). The genus *Palpita* is distributed in Asia, United Kingdom on *Oleaceae* plants including Jasmine and *Ligustrum* and this genus is commonly known as Jasmine moth (http://www-ache.socs.uts.edu.au/pyralidae.html). *Palpita celsalis* larvae cause severe defoliation of *Ligustrum* trees in Sri Lanka. Their attack begins in the flower buds and later spread to young shoots. This moth has shown the potential as a biocontrol agent due to its apparent restriction to the *Oleaceae* (Table 4 and 5). They are mobile enough to disperse onto surrounding trees and branches at the larval stage. This species has not been reported or found on other non-*Oleaceae* tree species in Sri Lanka or elsewhere. The results of the field research showed that *Palpita celsalis* does not appear on other tree species. However, a major drawback to continued work on this natural enemy is the difficulty encountered with rearing it in the laboratory as eggs tend not to be laid on the plants in culture, and are subject to desiccation or fungal attack depending on the humidity.

The no-choice tests (Table 6) indicate that *Palpita* are capable of feeding and developing on a few *Oleaceae* but will not develop on *J. polyanthus* and *Olea europaea* var. *africana*. However, this test was confounded by high larval mortality. It appears that this species will not feed as well on non-*Ligustrum* hosts. Previous no-choice tests carried out in Sri Lanka by feeding native *Oleaceae* species, showed that *Palpita* was unable to develop and feed on these plants (Sakalasooriya *et al.*, 2000).

Table 4. Results of the choice test on five insects after feeding leaves of selected Oleaceae trees.

	Damage after 5 days		Damage after 5 days	
	Ligustrum	Test plant	*Ligustrum*	Test plant
Oleaceae				
L. robustum (La Réunion)	❶❷❸❹❺	❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺
L. ovalifolium	❶❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺
J. flexile	❶❷❸❹❺	❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺
J. angutifolium	❶❷❹❺	❶❷❺	❶❷❹❺	❶❷❹❺
J. polyanathus	❶❺	❺	❶❺	❶❺
Chionanthus zeylanica	❶❷❺		❶❷❺	❺
Chionanthus broomeana	❶❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺	❶❷❸❹❺
Olea europaea	❶❸❹❺		❶❸❹❺	
Olea lancea	❶❹❺		❶❹❺	❶❺
Osmanthis heterophyllus	❶❷❸❹❺	❺	❶❸❹❺	❷❸❺
Osmanthus delahi	❶❺		❶❺	❺
	❶❺		❶❺	❺

Trace damage by insect: ❶ = *Dermorhytis lewisi*, ❷ = *Dermorhytis ornatissima*, ❸ = *Hyphasis* sp.1, ❹ = *Palpita celsalis*, ❺ = *Problepis deliaria*
Ligustrum = *Ligustrum robustum* (Sri Lanka)

Table 5. Results of the no-choice test on three insects using leaves of different plant species as feeding material (Number of replicates = 3 or more).

Species	Feeding*	Dead after 5 days	Alive after 5 days
Oleaceae			
Ligustrum robustum	❷❸		❷❸
L. ovalifolium	❷❸		❷❸
J. flexile	❶❸	❶❷	❸
J. anguistifolium	❸	❷	❸
J. polyanthus	❸	❷	❸
C. broomeana	❸	❷	❸
C. zeylanica		❶❷❸	
Olea polygama		❶❸	
Olea europaea	❸	❷	❸
Olea lancea	❷❸		❷❸
Non-Oleaceae			
Carisa xylopicron		❶❷	
Ochrosia borbonica		❶❷	
Antirrhium sp.		❶❷	
Hebe species	❸		❸

* feeding considered when more than trace level
❶ = *Dermorhytis lewisi* ❷ = *Palpita celsalis* ❸ = *Problepis deliaria*

Table 6. Results of no-choice test on *Palpita celsalis* carried out under quarantine conditions in UK.

Plant species	Rep.	Day 5		Day 7		Pupation Days	Adult Day*
		No. alive	Damage	No. alive	Damage		
L. robustum	1	2	3	2	4	16,16	21
L. robustum	2	1	2	1	3	16	N/A
L. robustum	3	3	3	3	3	N/A	N/A
O. europaea	1	0	0	0	0	N/A	N/A
O. europaea	2	0	0	0	0	N/A	N/A
O. europaea	3	3	2	3	3	N/A	N/A
L. ovalifolium	1	3	2	3	4	12/14/14	N/A
L. ovalifolium	2	3	2	3	3	14,19	24,26
C. broomeana		1	1	0	1	N/A	N/A
O. lancea		2	1	2	2	19	24
J. polyanthus	1	0	0	0	0	N/A	N/A
J. polyanthus	2	0	0	0	0	N/A	N/A

Damage level is indicated according to Wapshere (1974): t = <1% damage, 1 = 1-5%, 2 = 5-10%, 3 = 10-20%, 4 = 20-50%, 5 = >50%; N/A = not assessed due to dead of larvae
No. alive - number of insects alive; * days to emergence of adult insect from L1 larvae

Another Lepidopteran, *Problepis deliaria* (Family: *Geometridae*) seemed promising initially due to its remarkable camouflage against discovery on *Ligustrum* including lenticels on the skin of its larvae. This moth was subject to extensive host range testing in UK quarantine, and it was found that its physiological feeding range was restricted to *Oleaceae* in no choice tests, except one outlier, which completed development on a *Hebe* sp. (Table 5). Over 90 species of plants were tested with this insect under quarantine conditions (Shaw, 2000) and only those in the *Oleaceae* sustained anything other than trace feeding damage. Choice tests (Table 5) revealed a much more restricted host preference but still revealed feeding on at least one Réunion native non-target plant so it has since been rejected as a potential biocontrol agent, despite the insect not being recorded from any of the tree species in Sri Lanka India or Vietnam (Shaw, 2000).

Most of the Coleopteran defoliators are *Chrysomelidae*. Both *Dermorhytis lewisi* and *D. ornatissima* showed little preference between different species of *Ligustrum* (Table 4 and 5). Screening results further revealed that, in the choice test, they damage some non-target *Oleaceae* species including *Chionanthus broomeana*. The preliminary results from studies carried out in Sri Lanka using seven native Oleaceae species have shown that they would not feed on all species except *Jasminum anguistifolium* (Sakalasooriya *et al.*, 2000).

All adult *D. lewisi* introduced to endangered endemic plant species from La Réunion under no-choice conditions died after 5 days without any feeding or causing trace level of damage (Table 5). During the comprehensive field studies sustained for about two years in Sri Lanka, these two species were reported and found only from *Ligustrum* and were restricted in their distribution to some native *Ligustrum* areas, and thus, recognized as specific to the *Oleaceae* family. At present the larva of these two species feed on the roots. Studies are continuing to determine feeding preferences of the below-ground stage of the beetle.

Two new species of beetles (Family: *Chrysomelidae*) from the genus *Hyphasis* have been identified from *Ligustrum* in Sri Lanka. These two are referred to as *Hyphasis* sp. 1 and 2. Sporadic host specificity tests carried out in the field reported that *Hyphasis* sp. 2 feeds and make feeding scars on a wide range of non-related tree species, even non-*Oleaceae* trees. Therefore, further laboratory and field studies will be needed to clarify the detailed host range of these selected Chrysomelid candidates. However, the intensive culture of these insects is still a challenge due to the inherent difficulty of rearing root feeders. Although attempts were made to inoculate minute grubs to live potted *Ligustrum* plants in the laboratory, the grubs disappeared within few days in Sri Lanka, however, the same procedure used to rear larvae in UK found *D. lewisi* feeding on root materials.

One Lepidopteran *Epiplema albida* (*Epiplemidae*) found from several *Ligustrum* sites in Sri Lanka especially in Boralanda and Boragas area is presently being subjected to thorough laboratory investigations in UK where a culture has been established. Field host range tests carried out in Sri Lanka also showed that this insect is specific to *Ligustrum* trees, a conclusion supported by the early host range laboratory studies (R. Shaw - unpublished data).

The present study revealed that *P. deliaria* has a narrow host range within the *Oleaceae* family. However, the damage inflicted by this insect on native plant species of the same family in La Réunion (Table 5) warrants its' rejection as a potential biocontrol agent (based on screening over 90 test species). The screening tests on *Palpita celsalis* indicated that Palpita could damage some of the native *Oleaceae* species in La Réunion. However, high levels of larval mortality suggest that these insects will not feed well on those non-target plant species. The Coleopterans had wider host ranges and *Dermorhytis* species fed several *Oleaceae* family trees without distinction. However, the numbers of replicates were inadequate as laboratory culture of those beetles was difficult.

CONCLUSIONS

The comparative study on natural enemies of the Family *Oleaceae* in Sri Lanka showed that the insects have evolved to feed on different preferences of food plants thus suggesting that most are not completely host specific to *Ligustrum robustum*. The present study indicated that the *Palpita celsalis* (family: Pyralidae) could be a potential biocontrol agent of *Ligustrum robustum*. The moth *Epiplema albida* has shown considerable promise in on-going studies in the UK and may well prove to be host specific enough to be considered for release. However, those agents with inconclusive test results will require more detailed study in order to minimize risks in their release as biocontrol agents.

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