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HOST PLANTS, BIOLOGY AND CHEMICAL ECOLOGY OF THE INTRODUCED LILY LEAF BEETLE, *LILIOCERIS LILII* (SCOPOLI) (COLEOPTERA: CHRYSOMELIDAE)

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of

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ABSTRACT

The lily leaf beetle, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) is a recently introduced pest of lilies (*Lilium* spp.) (Liliales:Liliaceae). I analyzed preference and fitness measures to determine if the beetle has the potential to use wild, native Liliales (and closely related Asparagales) as hosts. It was found that adult beetles will oviposit occasionally on some native species in a no-choice situation, although they oviposit preferentially on *Lilium* species. First instar larvae feed on all Liliales and Asparagales, but not on species from dicot outgroups. Neonate host plant preference is significantly related to host plant phylogeny and relatedness to the genus Liliales, indicating that phylogeny may be a predictor of host shifting. Larvae can complete their development on *Streptopus amplexifolius* (Liliales), and probably on *Medeola virginiana*. This study indicates that the host range of the lily beetle is larger than previously thought and that it includes several wild, native, non-*Lilium* species.

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INTRODUCTION

Although introduced phytophagous pests are widespread and common, it is difficult to predict if, and to what extent, an exotic species will utilize plants in a novel environment. Thousands of alien species arrive in North America every year, usually as passengers hidden in the cargo of international trade ventures. Most do not persist in their new environment, but occasionally one is able to exploit it and thrive, sometimes with disastrous consequences for agricultural, lumber or horticultural industries. The Canadian Wildlife Federation (2003) has estimated that 56 introduced insect species have become invasive in Canada.

The lily leaf beetle, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae) is an introduced oligophagous pest of cultivated lilies (*Lilium* spp.) and fritillaria. Discovered for the first time in Montreal, Quebec in 1943, this striking red and black beetle is spreading throughout central and eastern Canada as well as in the north-eastern United States. Lily beetles cause extensive damage to their host plants; the larvae and adults rapidly defoliate plants, damaging buds and flowers as well.

Lilioceris lilii has been reported widely feeding on hybrid, Asiatic hybrid and oriental lilies (*Lilium* spp.) in private and public gardens in Ontario. Wild, uncultivated *Lilium* species are uncommon (only three species exist in the province), especially in the Ottawa region, and the plants are usually patchily distributed. However, the forests of Ontario support a rich diversity of abundant related species within the order Liliales that could be potential host plants for the beetle.

Phytophagous insects generally have close associations with certain plant species or groups of species. The plants that are included in their normal mating, oviposition

and feeding behaviours are considered part of the insect's realized host range, which can differ greatly from the fundamental host range. The fundamental host range includes species that may not be used normally, but *can* be used for one, some or all stage(s) of development. Traditionally, novel insect-host associations have been thought to develop very gradually. Recent evidence, however, indicates that novel associations can arise quickly, within a hundred-year time span (Thompson, 1998).

Oligophagous herbivores have been shown to develop relationships with novel host plants in a relatively short period of time after either the insect or the plant is introduced to a new geographic range (Berenbaum and Zangerl, 1991). The mechanisms enabling novel host use vary, but generally they are preceded by exposure to a new combination of plant species that fall within the insect's fundamental host range. Exposure to new plants may be the result of human disturbances. Human-induced ecosystem disturbances leading to loss of plant species or different plant assemblages have been demonstrated to result in rapid, adaptive novel host use (Singer *et al.*, 1992; Singer *et al.*, 1993). Insects introduced to new geographical areas generally encounter entirely new plant assemblages. If the novel plants encountered are similar to the usual hosts, introduced insects may possess sufficient preadaptations to include them in their diet.

Novel host use can occur even when the novel plant is suboptimal in terms of offspring development (Chew,1980; Louda *et al.*, 1997; Zangerl *et al.*, 2002). Insects will feed and oviposit on novel host plants despite low larval survival rates and retarded larval or pupal development. The weak correspondence often demonstrated between adult herbivore host selection and offspring performance could be explained, in part, by

benefits that result in an overall increase in net fitness by means of reduced competition, parasitism or predation. Changes in an insect's behaviour or life history that reduce the impacts of their enemies are highly advantageous (Jeffries and Lawton, 1984). For introduced insect species, novel plants inherently lack associations with the insect's natural enemies resulting in new or different predation pressures for the insect, or even enemy-free space. Enemy-free space associated with the novel hosts may be enough to facilitate a host shift (Zangerl *et al.*, 2002). Utilization of a novel host can lead to reduced mortality by predation, which could offset any physiological fitness costs associated with developing on a novel host plant (Gratton and Welter, 1999).

Relatedness of a novel host to the usual host plant may be a predictor for host shifting. Classical biological control efforts typically involve an evaluation of the introduced control agent's performance on species related to the target plant pest, under the assumption that species closely related to the target could fall within the insect's fundamental host range, and therefore be the most vulnerable to a potential host shift (van Klinken and Edwards, 2002). Others have been able to demonstrate close relationships between host plant phylogeny and host plant use by phytophagous insects. Ehlrich and Raven (1964) demonstrated that many groups of insects have taxonomically restricted host associations. These restrictions may be strong enough to allow us to predict which plants would be susceptible to a host shift by a particular insect (Futuyma and McCafferty, 1990; Mitter *et al.*, 1991).

There appears to be a high number of examples of so-called "rapid evolution" involving novel host associations with introduced species (i.e. for biological control) (Thompson, 1998). These examples of rapid changes in host range can be classified as

changes in the pattern of host use rather than a change in the fundamental host range (vanKlinken and Edwards, 2002). To evaluate the risks posed to native plants by exotic insects we must evaluate and identify plants that fall within the insect's fundamental host range. In doing so, we should be able to predict, at least to some extent, which native species are at risk for colonization.

Novel host-plant use by *Lilioceris lilii* has not been reported in our area; to date, there is only one record of an adult *L. lilii* feeding on a native plant in Ontario: *Medeola virginiana* (LeSage, 1983). There are several possible explanations why the lily beetle has not yet been recorded using native species as host plants. The beetle was introduced to our area (Ottawa, Ontario) only recently (1981) and therefore has had little time to encounter other potential hosts. Unexploited potential hosts are also generally found in the understories of forests, unlike the cultivated garden plants that are the lily beetle's usual hosts. It is also conceivable that novel host use is already occurring but has gone undetected since the beetle has only recently gained notoriety among people other than ornamental lily enthusiasts and horticulturists. There may also be physiological or chemically-related factors inhibiting colonization. Oviposition stimulants may be lacking. If eggs are laid on novel hosts, larvae may encounter physical, chemical, or phenological barriers to feeding and development. An insect's adaptations to its usual host(s) may facilitate colonization to closely related novel species, but development and survival may initially be poor, delaying successful establishment (Berenbaum and Zangerl, 1991).

In this study, I quantify the preference and performance of adult and larval lily beetles reared on plant species closely related to their typical host plants, the *Lilium*

genus, in an attempt to assess the risk this relatively new introduction poses to our native plants. I hypothesized that there would be a correlation between the beetles' feeding propensity on various potential hosts and the relatedness of these plants to *Lilium*. I looked for evidence of chemicals present in *Lilium* species and in *Lilioceris lilii* (sequestered or endogenous) that either deter or harm carnivorous predators; extracts derived from the beetle and their hosts were evaluated for anti-feedant properties.

Background

Basic biology and ethology of the lily beetle

Lilioceris lilii is a bright red beetle with a black head and antennae, black legs and black underparts. Approximately 6-8mm long, the adult insect has prominent eyes, a narrow thorax and broad elytra. Although it has not been confirmed experimentally, the adult lily beetle's striking red and black body is suggestive of a predator-deterring aposematic coloration. Shepard (1997), who observed beetles of the genus *Lilioceris* (the species was never successfully identified) feeding on cycads in Thailand, speculated that they could be sequestering the secondary metabolites of that host plant. The secondary chemicals found in lilies (genus *Lilium*) include alkaloids such as lilaline (Figure 1) and cordatine (Figure 2). It is possible that lily beetles are able to sequester these agents for their own defence as well. An adult lily beetle will also exhibit thanatosis (or feigning death) if disturbed: it folds its appendages against its body and drops off the plant onto the ground, becoming difficult to detect when its dark underparts are facing upwards (Jolivet and Verma, 2002). It remains immobile for a time then quickly retreats to its host plant or to undergrowth and debris beneath the plant. A third defence mechanism of the adult beetle is stridulation, which is audible when a

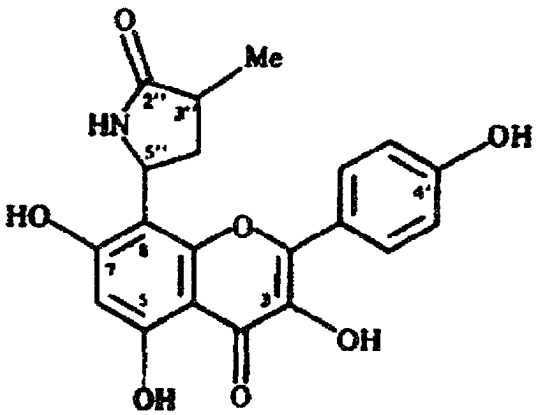

Figure 1. Chemical structure of lilaline, a flavonoid alkaloid from *Lilium candidum* (from Mašterová *et al.*, 1987)

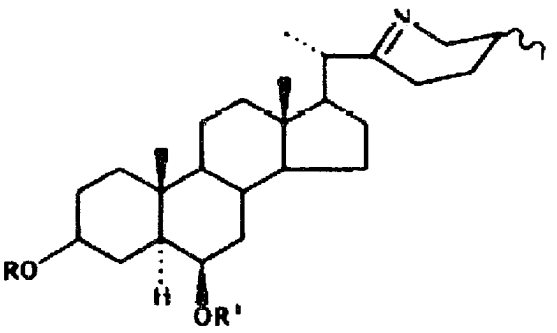

Figure 2. Basic chemical structure of cordatine A and B, two steroidal alkaloid glycosides from *Lilium cordatum* (from Nakano *et al.*, 1987)

beetle is picked up with one's fingers. Stridulation is common in Chrysomelids, and is thought to startle predators into releasing them or may condition predators before they are swallowed. In the lily beetle, stridulation is achieved by rapidly rubbing the striated section near the base of the last abdominal tergite over apices of the elytra (Ruppel and Smith, 1965).

Adults overwinter in the soil beneath the lily plants on which they fed the previous summer. They emerge from dormancy in early spring and feed on young lily leaves. Pairing commences shortly after emergence. After mating, the female oviposits irregular lines of 3-12 reddish-orange eggs along the midrib of the underside of the host plant leaves. The eggs are approximately 1-2 mm long and are protected by a sticky, viscous brown coating. A single female will oviposit between 200 and 300 eggs, although one individual in captivity was recorded as laying over 520 in one season (Fox-Wilson, 1942). Eggs hatch after an average of 8-10 days in the field. First instar larvae were found to possess a pair of oviruptors (or egg-bursters) on the dorso-lateral thorax, which were observed to assist in eclosion. Oviruptors are commonly found in chrysomelids (Jolivet and Verma, 2002). Larvae are stout, pale yellowish-white, with large black heads and a dorsally-situated anus. The larvae are particularly repugnant as they deposit their faeces on their backs. This fecal shield may help prevent desiccation (Jolivet and Verma, 2002). Larval fecal shields are generally thought to provide some protection against predators as well (Eisner *et al.*, 1967) although Schaffner and Muller (2001) have described a specialist hymenopteran parasitoid that may exploit the shield in locating and accepting lily beetle larvae as hosts for their young. When disturbed, a larva will curl its body upwards and regurgitate a dark brown droplet, also considered to be a

defence mechanism (Pasteels *et al.*, 1988). The larvae feed voraciously, initially from the undersides of the leaves, producing large amounts of damage. Larvae will also feed on flower buds and petals. Larvae feed for the duration of their 4 instars.

Just before pupation, the larvae stop feeding, dislodge their fecal shields from their bodies and move down the plant, seeking soil in which they can bury themselves. At this time their integument begins to change colour from yellowish to orange. Also, in the absence of soil (i.e. in a paper-lined petri dish), the larvae were observed to produce a whitish, shiny salivary secretion. Larvae at this stage would secrete this white discharge instead of the brown discharge mentioned earlier when disturbed. These behaviours and changes in appearance were consistently observed throughout the duration of my experiments, and have been reported by others (LaTaste, 1931). As these were reliable indicators of pre-pupation, I have defined this as the pre-pupal stage. Occasionally larvae would pupate when a proper substrate was not provided (i.e. soil), although other researchers have had little success rearing *Lilioceris* larvae to adults under these conditions (e.g. Shepard, 1997).

The pre-pupae will use their oral secretions and small particles of soil to construct a well-camouflaged, ovoid cocoon. Opening the cocoon will reveal a striking fluorescent orange pupa encapsulated within. The duration of pupation has been recorded as 20 to 22 days in the field (Fox-Wilson, 1947). After hatching, the imagos will feed on lilies until early fall. These adults will overwinter and resume the cycle in the spring. Lily beetles are generally described as univoltine, although it there has been one record that up to three generations can be produced in a single breeding season, and that females may survive to reproduce in two successive years (Brown, 1946).

Native range and introduction of the beetle to North America

The lily leaf beetle is thought to have originated in Asia, although its exact point of origin is unknown (Berti and Rapilly, 1976). It is now naturalized throughout Eurasia, parts of Europe, the Middle East and northern Africa. Recent studies indicate that the range of *L. lilii* in Europe is expanding. In 2002 it was reported in northern Ireland and Scotland for the first time, and by September 2003 the beetle was present in all counties of England from Yorkshire southward (Salisbury, 2004).

Brown (1946) first identified the beetle in North America (Montreal, Quebec) in 1945. However, more recent examinations of entomological collections place the earliest appearance of the beetle closer to 1943 (LeSage, 1983). It is likely that the insect was introduced in one of three locations in Montreal through which many novel plant species pass: the Port of Montreal, the Montreal Botanical Garden, or MacDonald College of Megill University (LeSage, 1983). The lily beetle's spread to North America was probably facilitated by the import of lily plants or bulbs and their associated packing material, as this has been noted to occur between regions in their native range (Fox-Wilson, 1942). *Lilioceris lilii* adults are very strong fliers and can travel sizeable distances; this has likely played a role in their spread since their initial introduction. The lily beetle remained exclusively in Montreal until 1978, when it was encountered north of the Saint-Lawrence River; it arrived in Ottawa, Ontario in 1981 (LeSage, 1983). Since this time it has been recovered in all of the New England states (U.S.) (Gold *et al.*, 2001) and in the Canadian provinces of Quebec, Ontario, Nova Scotia, and as far west as Manitoba (Dolores Nelson, NALS, *pers. comm.*). It has been surmised that the beetle will continue to expand its range, given its successful Europe/Eurasian expansion. I

found the beetle to be well established in gardens in several neighbourhoods throughout the Ottawa area.

***Predators and parasitoids of* L. lilii**

There are no known parasitoids of *L. lilii* in North America. Seven hymenopteran parasitoids attack the beetle in Europe. *Tetrastichus setifer* (Eulophidae), *Lemophagus errabundus*, *Lemophagus pulcher* and *Diaparsis jucunda* (all Ichneumonidae) will parasitize *L. lilii* larvae (Gold *et al.*, 2001). *Anaphes* sp. (Mymaridae) parasitizes the eggs. Dipteran *Meigenia* sp. (Tachinidae) have been shown to occasionally attack larvae as well. A biological control program using introduced *T. setifer* was initiated in Massachusetts (US) in 2001 (Kenis *et al.*, 2002)

Known host plant range

Lilioceris lilii adults and larvae are well known to attack both native and cultivated lily species as well as fritillaria (Fox-Wilson, 1946; Haye and Kenis, 2004). An extensive list of 81 lilies and hybrid lilies and four *Fritillaria* species from which *L. lilii* has been recorded in larval or adult form is described by Salisbury (2003). Observations in Europe have shown *L. lilii* to be found occasionally on plants that are also native or naturalised in North America, mostly as adult beetles (Table 1). In most cases, adult feeding was unconfirmed, or else the beetle was observed only to "taste" the plant. Only two non-*Lilium* species (*Maianthemum canadense*, *Convallaria majalis*) have been described as being viable food plants for larvae and none are recorded as being suitable for oviposition (Table 1). In Canada, the beetle seems to prefer cultivated lilies (Cox, 2001). *Lilioceris lilii* adults have been found on other garden (cultivated) species, including *Nomocharis* spp., *Iris graminea*, *Campanula* sp., *Nicotiana* sp., *Alstroemeria* sp. and

Table 1. Records of plants on which *Lilioceris lilii* has been found (from Cox, 2001). Note that only native/naturalised species of North America have been included in this list. Plant family classification follows that of Vinnersten and Bremer (2001).

Key: N = not stated; +- will feed sparingly; + will feed readily; - will not feed; ?+ adult(s) present, but feeding not confirmed; P = eggs present

Plant name	Status in North America	Adult(s)	Larva(e)	Egg(s)	References
Liliales					
(Smilacaceae)					
Smilax sp.	Native	+	N	N	Fabre, 1900
		+-	N	N	Casagrande, pers. obs.
(Liliaceae)					
Lilium bulbiferum	Introduced	+	N	N	Palmqvist, 1945
Lilium candidum	Introduced	+	N	N	Barton, 1941; Fox-Wilson, 1943; Palmqvist, 1945; Coghill, 1946; Halstead, 1990
		+	+	N	Donisthorpe, 1943; Southgate, 1959; Cox, 2001
		+	-	N	Berti & Rapilly, 1976
Lilium formosanum	Introduced	+	N	N	Fox-Wilson, 1943
		?+	+	N	Salisbury, 2001
Lilium lancifolium (=*trigrinum*)	Introduced	+	N	N	Fox-Wilson, 1942, 1943; Barton, 1940; Halstead, 1990
		+	+	N	Southgate, 1959
		+	-	N	LeSage, 1983; Cox, 2001

Lilium longiflorum	Introduced	+	-	N	Cox, 2001
		?+	+	N	Salisbury, 2001
Lilium pardalinum	Native	+	N	N	Halstead, 1990
		+	+	N	Cox, 2001
		?+	+	N	Salisbury, 2001
Lilium regale	Introduced	+	-	N	Barton, 1940
		+	N	N	Fox-Wilson, 1942, 1943; Palmqvist, 1945; Cox, 2001
		+	+	N	Coghill, 1946; Southgate, 1959; LeSage, 1983; Cox, 2001; Salisbury, 2000
Lilium superbum	Native	?+	N	P	Salisbury, 2001
Asparagales					
(Hemerocallidaceae)					
Hemerocallis sp.	Introduced	?+	N	N	Cox, 2001
(Ruscaceae)					
Maianthemum canadeNe	Native	+	+	N	Lesage, 1983
Polygonatum sp.	Unknown	+	-	N	Casagrande & Livingston, 1995
(Convallariaceae)					
Convallaria majalis	Introduced	+	N	N	Fabre, 1900; Hesse, 1932; Reinecke, 1910; Halstead, 1989
		+	+	N	Harlow, 1991
		+-	-	N	Salisbury, 2000
		+	N	N	Casagrande & Livingston, 1995
Convallaria sp.	Unknown				
(Asparagaceae)		+	N	N	Fabre, 1900
Asparagus sp.	Unknown				
Solanales					
(Solanaceae)		+	-	N	Halstead, 1990
		+-	-	N	Salisbury, 2000

Solanum dulcamara	Introduced	+	-	N	Cox, 2001
		+	N	N	Fox-Wilson, 1942
		+-	N	N	Casagrande, pers. obs.
Solanum tuberosum	Introduced	+-	N	N	Casagrande, pers. obs.
Nicotiana sp.	Unknown				

Cardiocrinum giganteum (see Cox, 2001). It has been surmised that larvae can probably only develop on *Lilium* spp, *Fritillaria* spp, and *Cardiocrinum* (Salisbury, 2003).

MATERIALS AND METHODS

Initial neonate preference and host plant phylogeny tests

Wild adult *Lilioceris lilii* were collected from Asiatic hybrid *Lilium spp.* plants in residential gardens in the Ottawa area in May, 2003. These adults were placed with several potted lilies (Asiatic *Lilium* hybrid "Orange Pixie", henceforth referred to as "lilies") in a breeding cage in a greenhouse, where they were permitted to mate randomly. Lily leaves were examined daily for eggs, which were harvested. Using a soft bristle brush, eggs were placed singly and randomly on a novel "food" leaf. Each food leaf with a *L. lilii* egg was placed in its own petri dish lined with a moistened filter paper. Fifteen species were used as food plants; 10 from within the Liliales: *Clintonia borealis*, *Erythronium americanum,* Asiatic hybrid lilies *Lilium spp.*, *Medeola virginiana*, *Streptopus amplexifolius*, *Smilax herbacea*, *Tulippa spp.*, *Trilium erectum*, *Trilium grandiflorum*, *Uvularia perfoliata*; three from the Asparagales (a closely related monocot outgroup): *Maianthemum canadense*, *Smilacina racemosa*, *Polygonatum biflorum*, and two from dicot outgroups: *Vincetoxicum rossicum* (Asclepiadacaeae) and *Solidago altissima* (Asteracaeae) (Table 2). Between 70 and 89 eggs were randomly placed on individual leaves of each species of food plant.

The petri dishes were kept in a laboratory under a 16:8 light/dark cycle. Each developing beetle was examined every 24 hours until the time that it died. If the food leaf upon which an egg was placed became desiccated before eclosion it was replaced with a new leaf so that the first-instar larva would have a suitable food leaf. Larvae were permitted to feed for a 24-hour period after hatching. After this time, the food leaf was removed and I noted: a) whether or not the larva fed on its food leaf; b) whether or not

Table 2. List of plant species used in larval feeding and adult oviposition trials. Taxonomy of the Liliaceae taken from Vinnersten and Bremer (2001).

Species name	Common Name	Family
Asiatic *Lilium* hybrid "Orange Pixie"	Asiatic Lily	Liliaceae
Lilium philadelphicum L.	Wood Lily	Liliaceae
Tulipa spp.	Tulip	Liliaceae
Erythronium americanum Ker-Gawl	Dogtooth Violet	Liliaceae
Medeola virginiana L.	Indian Cucumber	Liliaceae
Clintonia borealis (Ait.) Raf.	Bluebead Lily	Liliaceae
Streptopus amplexifolius (L.) DC.	Claspleaf Twistedstalk	Liliaceae
Smilax herbacea L.	Smooth Carrionflower	Liliaceae
Trillium erectum L.	Red Trillium	Liliaceae
Trillium grandiflorum (Michx.) Salisb.	Snow Trillium	Liliaceae
Uvularia perfoliata L.	Perfoliate Bellwort	Liliaceae
Maianthemum canadense Desf.	Canada Mayflower	Asparagaceae
Smilacina racemosa (L.) Desf.	Feathery False Lily of the Valley	Asparagaceae
Polygonatum biflorum (Walt.) Ell.	Smooth Solomon's Seal	Asparagaceae
Solidago altissima (L.)	Tall Goldenrod	Asteraceae
Vincetoxicum rossicum (Kleopov) Barbarich	Pale Swallow-wort	Asclepiadaceae

the larva remained on its food leaf or abandoned it; c) the area of the leaf consumed by the larva. To calculate the area of feeding damage, the food leaves were scanned and measured using imaging software (Scion Image Beta 4.02, Scion Corporation, Frederick, Maryland, available online at http://www.scioncorp.com). Larvae were regularly supplied with fresh food leaves afterwards, until they either died or pupated.

I used stages of growth that were readily identifiable to track the beetles' development: egg, larva, pre-pupa, pupa, and adult. An individual was defined as a pre-pupa if it met all of the following conditions: feeding ceased; abandonment of food leaf; fecal shield dropped from body; and integument colour change from pale yellowish-beige to deep orange-yellow. At this point, the larva would typically attempt to construct a cocoon in which to pupate, evident by a white oral secretion deposited on the filter paper. Pupation would occasionally occur regardless of the absence of soil in the petri dishes.

For each plant species, I obtained the estimated time of the divergence of the clade containing that species from the clade containing the genus *Lilium*. Divergence times of species within the Liliales were taken from Vinnersten and Bremmer's recent revision of their phylogeny (2001). The estimate of the divergence of the genera in the Asparagales outgroup (*Maianthemum*, *Polygonatum* and *Smilacina*) from the Liliales was obtained from a review of angiosperm group ages compiled by Bremmer (2000). The estimate of age of the common ancestor of the monocotyledonous Liliales and the dicot outgroup genera (*Vincetoxicum* and *Solidago*) were taken from Soltis and others (2000). The phylogenies are based on branch lengths from variation in the chloroplast *rcbL* gene sequences, and are dated using the mean branch-length method described by Bremer and Gustafsson (1997). Time of divergence is stated in terms of millions of years.

I evaluated three neonate larval feeding parameters for each food plant species: a) percentage of larvae that abandoned their food leaf; b) percentage of larvae willing to at least "taste" their food leaf; and c) mean area of damage caused by feeding.

Subsequent tests

In order to more closely examine the relationships between the beetle and host plants, I reduced the number of plant species for further studies. My selection of plants was based on the results of the initial study; those upon which larvae fed most readily were considered to be the best candidates for further study. The plants selected for adult oviposition studies were Asiatic *Lilium* hybrid "Orange Pixie", *Clintonia borealis*, *Polygonatum biflorum*, *Medeola virginiana,* and *Streptopus amplexifolius*. I included one other species in the larval performance tests that was not fed upon as readily, for comparative purposes: *Trillium grandiflorum.*

Adult oviposition

Wild adult *Lilioceris lilii* feeding on Asiatic hybrid *Lilium spp.* were collected from approximately 25 private and public gardens within a 10km radius of Carleton University in Ottawa, Ontario. Two to ten individual beetles were collected from each location, beginning in early June 2004. Collection sites were frequented throughout June and July to maintain a breeding population of adults at the University.

Potted lilies were obtained from the greenhouses at Ritchies Feed & Seed (1390 Windmill Lane, Gloucester, Ontario). The other four plant species were transplanted from wild populations. *Medeola virginiana* plants were obtained from the Mer Bleue sector of the National Capitol Greenbelt (Ottawa, Ontatio). *Clintonia borealis* and *Polygonatum biflorum* were obtained from Gatineau Park in Gatineau, Quebec, as well as

from the Mer Bleue sector. *Streptopus amplexifolius* were obtained exclusively from Gatineau Park. All plants were placed singly in ProMix soil (Premier Horticulture, Ltd., Dorval, QC) in 6" plastic pots, stored in a greenhouse module, and watered regularly.

Adult oviposition tests took place between June and August 2004. I constructed 19 holding cages and two breeding cages measuring 24"x24"x26" out of 1" square pine boards and fiberglass insect screening. Cage interiors could be accessed using removable flaps that were secured using velcro strips. The two breeding cages, each containing three potted lily plants, were placed in a small greenhouse module. All of the wild adult beetles were placed in the two breeding cages, where they were permitted to mate randomly. Lily plants in the breeding cages were regularly replaced when feeding damage became severe.

In a large greenhouse module, the holding cages were distributed evenly on three benches. Cages were designated either for "no-choice" oviposition tests, or for "choice" oviposition tests. There were three no-choice cages for each of the five host plant species under investigation (total no-choice cages=15). Each no-choice cage contained five uniformly arranged potted plants of the same species. I also prepared four choice cages that contained one potted plant of each of the five species, arranged randomly within. These 19 cages were assigned random positions on the three benches.

Single pairs of adult beetles, obtained by inspecting the breeding cages daily for mating beetles, were placed in each of the 19 oviposition test cages and were left undisturbed for 72 hours. After this time, the beetles were removed and all plants within the cage were thoroughly inspected for eggs, which were counted if present. Oviposition

by twenty mated females was tested for each plant species under no-choice conditions, and twenty pairs were also evaluated under choice conditions.

After oviposition tests were complete, all the leaves of the plants in the holding cages were harvested, dried to a constant mass at 50° C, and weighed. The combined dry masses of each species in the no-choice and choice tests were calculated.

Another novel host plant

Halfway into my study, a population of wild Wood Lilies, *Lilium philadelphicum,* was discovered growing in the Burnt Lands Alvar in Almonte, Ontario. I was unable to obtain permission to remove entire plants from this protected ecosystem, and thus was unable to include this species in my oviposition tests as described above (only a few leaves from each plant were permitted for collection). However, I did place two mated pairs in a small cage with a single stalk of *L. philadelphicum* to ascertain if the insects would oviposit on this species. Also, I randomly examined 400 *L. philadelphicum* plants growing in the Burnt Lands Alvar for signs of *L. lilii* eggs, adults, larvae or feeding damage. I decided, given the protected status this species in our region, to include it in the long-term larval performance studies.

Larval Performance

Food plants and insects

I tested the performance of *L. lilii* larvae reared on the five plant species previously described in the ovipostition tests, as well as on *L. philadelphicum* (a wild novel *Lilium* species) and on *T. grandiflorum* (a species on which they fed very seldom in the initial larval tests) , between the months of June to August, 2004. Leaves of the native species were collected from the wild populations described earlier, stored in plastic bags

under refrigeration (4°C) until used in feeding trials. Fresh foliage was obtained from the field whenever refrigerated leaves showed signs of wilting.

Larvae were obtained by regularly inspecting the two breeding cages for eggs, which were harvested by removing entire leaves on which eggs had been oviposited. These leaves were kept in batches in 10" petri dishes containing moistened filter papers, to prevent dessication of either the leaves or the eggs. Eggs were inspected twice daily for eclosion. Neonate larvae were removed from the lily leaves using a soft-bristle brush and placed singly and randomly on a new "food" leaf, in a petri dish with moistened filter paper. In each dish I also placed a "control" leaf of the same species, of roughly equal size and shape, which was protected from larval feeding with a breathable nylon bag. Forty-five larvae were reared on leaves of each species of food plant.

Survivorship

Larvae were inspected daily. I recorded if they had died or if they had reached the pre-pupal stage of development. I calculated the percentage of larvae that survived between successive 48-hour periods. The leaves in the dishes of larvae reaching the pre-pupal stage were removed (as feeding had ceased) and the petri dish was filled with moistened ProMix soil. The pre-pupal larvae were placed on top of the soil and were permitted to bury themselves for pupation. The dishes containing pupae were inspected daily for emergence of adult beetles. If emergence did not occur after five weeks, I considered it an unsuccessful pupation.

Larval growth

Larvae were removed from their food leaves every 72 hours and their weights were recorded. Before being weighed, all frass (including the fecal shield) was gently

removed from the body with a soft bristle brush. Weights were recorded only every three days to minimize disturbance to the larvae and desiccation caused by removal of the fecal shield. Pre-pupal larvae were also weighed prior to being placed in soil. Adult beetles were weighed upon emergence from the pupae. Pupae were not weighed, as the larvae construct a cocoon around themselves using soil. This cocoon is almost indiscernible from the soil, and is therefore difficult to detect; it is also difficult to open these cases without causing harm to the pupae.

Food leaf consumption

Leaf masses (both food leaves and control leaves) were measured at the same time larvae were weighed. Control leaves were used to correct for weight loss in the food leaves not accounted for by larval feeding (i.e. due to moisture loss), using the method described by Candy and Baker (2002). The mass of the portion of each food leaf consumed by the larvae between each weighing period (72 hours) was calculated based on the corrected leaf weights. The total weight of food leaves consumed by each larva was calculated. The efficiency of conversion of ingested food (ECI) (Scriber and Slansky, 1981) is a measure of gross dietary utilization (ECI=weight gained(g)/amount of food ingested(g)). The ECI was calculated for larvae at day 6 (144 hours) using the corrected leaf weights

Chemical Ecology

Plant and insect material

I collected lily leaves from potted plants kept in the greenhouse, and *L. philadelphicum* leaves from the wild population described earlier. Fifty adult lily beetles that had been feeding exclusively on potted lilies (from the breeding cages for the

oviposition study) were collected. Plant material and insects were stored whole in 80% ethyl alcohol. All materials were collected and stored in August, 2004.

Preparation of extracts

I prepared the extracts during the months of November and December, 2004. The stored *Lilium philadelphicum* leaves were blended into fine pieces; leaf material and 500mL of 80% ethyl alcohol were added to a flask. The flask was placed in a sonicating bath for 15 minutes, then shaken overnight. The mixture was filtered using suction and the ethanol filtrate was collected and labelled E1. The remaining leaf material was placed in a flask with 500mL of 80% ethyl alcohol, then placed in a sonicating bath for 15 minutes, then shaken overnight. A second ethanol filtrate (E2) was obtained by filtering with suction. The remaining leaf material was dried for three days at 40° C and then weighed. The entire procedure was repeated for the Asiatic hybrid lily leaves to obtain two ethanol filtrates.

The beetles were crushed by hand using a mortar and pestle. The same extraction procedure as outlined above was performed. Only one filtrate was prepared from the insect material.

Each plant and beetle filtrate was evaporated until only the water/extract solution was remaining. These were placed in small plastic vials, put in a freezer overnight, and then in a freeze-drier for 48 hours until the water had also been removed. The resultant extracts were then weighed and stored under refrigeration until feeding trials commenced.

Model predators

The convergent lady beetle, *Hippodamia convergens* (Coleoptera, Coccinellidae) was selected as a model carnivorous insect, as they are generalist predators, readily

attainable and easy to keep under laboratory conditions. Live adult lady beetles were purchased from Plant Products Co., Ltd. (Brampton, Ontario). As I was unsuccessful in maintaining a breeding colony of beetles, feeding trials were performed using the adult beetles.

Artificial diet preparation

The beetles were fed an artificial diet suspended in agar. The artificial diet was composed of organic dandelion honey, bee pollen, brown rice protein, agar, and the preservatives methyl paraben and sorbic acid. The procedures for making this diet are described by Neil Cunningham of the Minnosota Department of Agriculture at http://www.mda.state.mn.us/biocon/plantscape/artificaldiet.htm (Appendix A).

I melted six separate 100g batches of plain agar diet using a hot plate; I added treatments to five of these and then all treated agar diets were poured into separate petri dishes and refrigerated. A total of six diet treatments were made: one control (C) (plain, untreated agar diet), one ethanol control (EC) (with 1.0 mL of %50 ethanol) and four with plant or insect extracts. Each extract (0.01g) was dissolved in 1.0 mL of %50 ethanol. Each suspension was added to 100g of melted agar diet: Asiatic hybrid lily extract 1 (LE1) and 2 (LE2); *Lilium philadelphicum* extract 1 (LP); and *Lilioceris lilii* (LL). I did not obtain a full 0.01g of the second *L. philadelphicum* ethanol extract (E2), so I was unable to include it in the feeding trial.

Feeding trials: acceptance/avoidance of treated agar diet

Feeding trials took place in March, 2005. I placed twenty adult *Hippodamia convergens* into separate petri dishes containing a small piece of agar diet. This was repeated for each of the six diet preparations for a total of 120 beetles. Each beetle was

checked at five minute intervals, for 60 minutes, for active feeding (beetles simply resting on or near the piece of agar diet were not counted as feeding). The proportion of five-minute intervals spent feeding was calculated for each lady beetle, and the average proportion was calculated for each of the six treatments.

Analyses

I used JMP-IN version 5.1 (SAS Institute, Cary, NC) to perform all statistical analyses.

Initial neonate preference and host plant phylogeny tests

Since the response variables "percent of neonates that abandoned food leaf" and "percent of neonates that fed on food leaf to some extent" are ratios, they were arcsine/squareroot transformed prior to analysis. Food leaf abandonment could not be normalized with this transformation, so I used a non-parametric Spearman's Rho analysis. For percent feeding, I used a regression analysis to compare the effect of the age of the divergence of the clade containing the food plant from that containing *Lilium* on these two variables. The response variable "area of leaf damaged by neonates" was not transformed, and was not normally distributed. As such, I performed a nonparametric Wilcoxon/Kruskal-Wallis to determine the effect of host/food plant age of divergence on feeding damage.

Adult oviposition

Insufficient data were obtained to perform statistical analyses; general trends of female oviposition behaviours are therefore presented instead.

Larval performance: larval growth and leaf consumption

I analyzed the effects food plant on larval growth parameters and leaf consumption. The effects of food plant on the median weight of larvae, the median weight of food leaf consumed by larvae, and the median ECI of larvae at the sixth day of development were analyzed using non-parametric Wilcoxon/Kruskal-Wallis tests. When I found a significant effect, I used Dunn's post-hoc method of multiple comparisons using rank sums to perform pairwise comparisons (1964).

The effects of food plant on median pre-pupal weight, the median duration of larval development to the prepupal stage, and the median duration of the pupal stage (measured from pre-pupa to adult emergence) were also analyzed using non-parametric Wilcoxon/Kruskal-Wallis tests. Post-hoc pairwise comparisons were performed if a significant effect was detected using Dunn's test (1964). The effects of food plant on adult beetle weights were evaluated using ANOVA. The effects of food plant on survival of pre-pupae to adults were evaluated using a likelihood ratio ChiSquare test

Predator feeding trials

I analysed the effects of treatment (i.e. extract added to agar diet) on the mean proportion of five-minute intervals spent feeding. Data were arcsine/squareroot transformed prior to performing ANOVA. A significant effect was detected, so a post-hoc Tukey-Kramer test was used to separate treatment means via all pairwise comparisons.

RESULTS

Initial neonate preference and host plant phylogeny tests

The percentage of eggs placed on food leaves that survived to eclosion varied by species. Only 22.7% of eggs placed on *Smilax herbacea* and only 24.0% of those placed on *Medeola virginiana* survived (Table 3). Otherwise, survival rates ranged between 50-82.9%.

The average area of feeding damage caused by 24 hours of larval feeding was negatively correlated with the estimated divergence of the host plant genus from the genus *Lilium* (Spearman Rho=-0.6745, P=0.0058) (Figure 3). Genera with a greater divergence from *Lilium* suffered less feeding damage than those genera which diverged more recently. *Streptopus amplexifolius,* lilies, and *M. virginiana* sustained the greatest average area of feeding damage (57.5, 42.0, and 28.2, respectively) (Table 3).

The average area of feeding damage is significantly correlated with the percentage of larvae that fed on their host plant (F=13.1610, df=14, P=0.0031). The percentage of larvae that fed to some degree also showed a significant negative correlation with the divergence of the host plant genus from *Lilium* (F=4.9487, df=14, P=0.0444) (Figure 4). The Liliales upon which the greatest percent of larvae fed were *Smilax herbacea* (100%), lilies (97.8%), and *Streptopus amplexifolius* (86.8%). The dicot species *Solidago altissima* and *Vincetoxicum rossicum* were seldom fed upon (0% and 13.3% respectively). *Trillium grandiflorum* also sustained infrequent damage (5.8%), but *Trillium erectum* was fed upon often (41.5%). Interestingly, two species from the Asparagales outgroup were also fed upon frequently: *Polygonatum biflorum* (66.7%) and *Smilacina racemosa* (46.0%) (Table 3).

Table 3. Summary of initial *L. lilii* larval preference and host plant phylogeny tests.

Food Plant	Estimated age of divergence (mya)	# eggs placed	% eggs surviving to eclosion	% larvae that abandoned food leaf	% larvae that fed on food leaf	Mean area of leaf consumed by larvae
Asiatic *Lilium* hybrid	0	80	64.0	0	97.8	42.0
Tulippa spp.	28	76	64.0	89.2	37.0	8.4
Erythronium americanum	28	89	57.5	78.8	42.1	4.2
Clintonia borealis	30	75	77.5	56.0	51.2	4.0
Medeola virginiana	30	75	24.0	0	77.8	28.2
Streptopus amplexifolius	37	75	64.0	29.0	86.8	57.5
Smilax herbacea	58	75	68.4	64.7	100.0	6.0
Trillium grandiflorum	63	73	22.7	91.7	41.5	1.3
Trillium erectum	63	82	82.9	84.6	41.5	2.1
Uvularia perfoliata	82	77	50.7	88.2	25.0	2.8
Maianthemum canadense	134	80	50.0	78.1	29.0	2.1
Smilacina racemosa	134	76	71.2	94.3	46.2	1.7
Polygonatum biflorum	134	75	60.5	75.0	66.7	20.2
Solidago altissima	135+	70	62.3	93.1	0	0
Vincetoxicum rossicum	135+	70	64.3	91.1	13.3	2.0

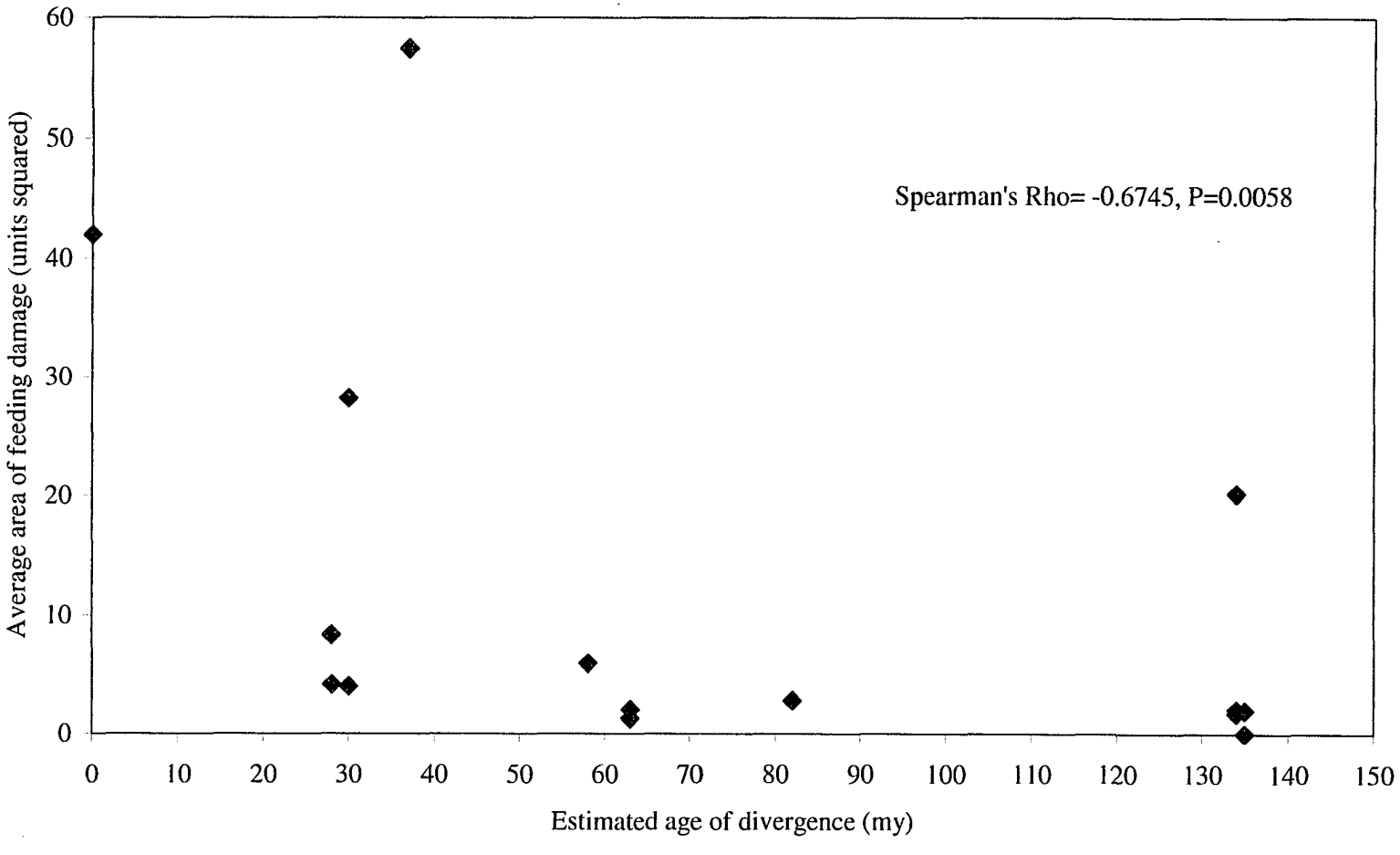

Figure 3. Average area of damage by neonate *L. lilii* larva feeding expressed as a function of the estimated age of the food plants' divergence from the clade containing *Lilium.*

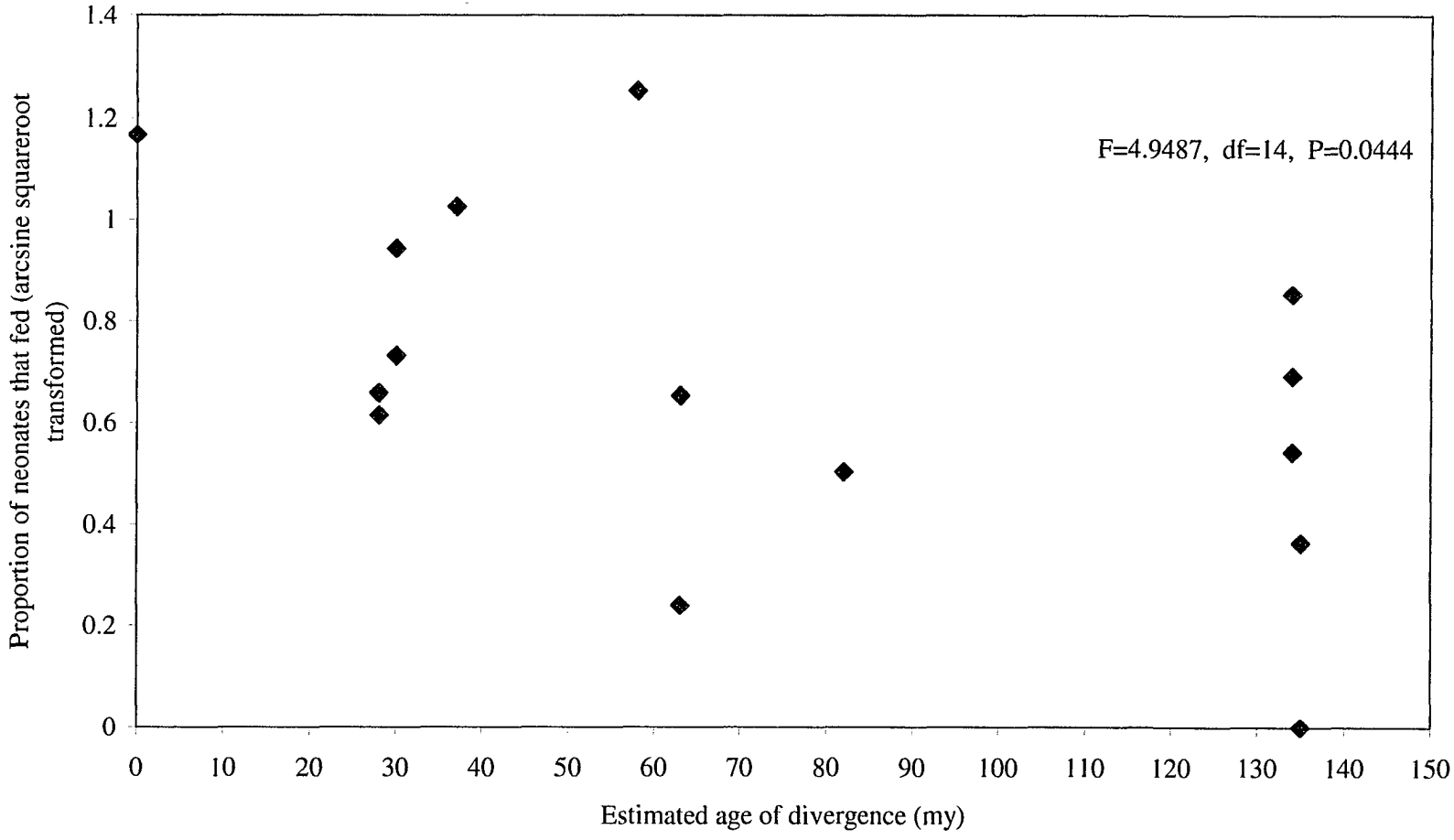

Figure 4. Proportion of neonate *L. lilii* larvae that fed to some extent expressed as a function of the estimated age of the food plants' divergence from the clade containing *Lilium*.

The percentage of larvae that abandoned their food leaf by the end of the 24 hour period showed a significant positive correlation with the divergence of the food leaf genus from *Lilium* (F=7.4350, df=14, P=0.0173) (Figure 5) and a significant negative correlation with the area of feeding damage (F=25.3658, df=14, P=0.0002). On average, larvae never abandoned their food leaf if it was either lily or *M. virginiana*, and only seldom when it was *S. amplexifolius* (29.0%) (Table 3). At least half of all larvae abandoned their food leaf when feeding on any other species.

Adult oviposition

No-choice tests

In a no-choice situation, the twenty pairs of lily beetles oviposited the most eggs on lilies, with an average of 5.33 eggs per g drymass (300 eggs in total) (Table 4). One egg was laid on *S. amplexifolius* (0.13 eggs/g), four eggs on *C. borealis* (0.35 eggs/g), one egg on *P. biflorum* (0.09eggs/g) and six eggs on *M. virginiana* (1.03 eggs/g). The highest number of eggs laid by a single pair in a 72-hour period was 26 on lilies. Two eggs were also found oviposited on a petri dish left inside a cage containing *C. borealis*, and three were found on the frame of the cage holding *P. biflorum*. The eggs laid on the non-*Lilium* species tended to look dark and somewhat sunken.

I observed adults feeding on leaves of all host plant species in the no-choice cages except *Clintonia borealis*.

Choice tests

No eggs were oviposited on any host plant species other than lilies in a choice situation. The total number of eggs oviposited by 20 pairs of beetles was 239. The highest number of eggs laid by a single pair in a 72-hour period was 46.

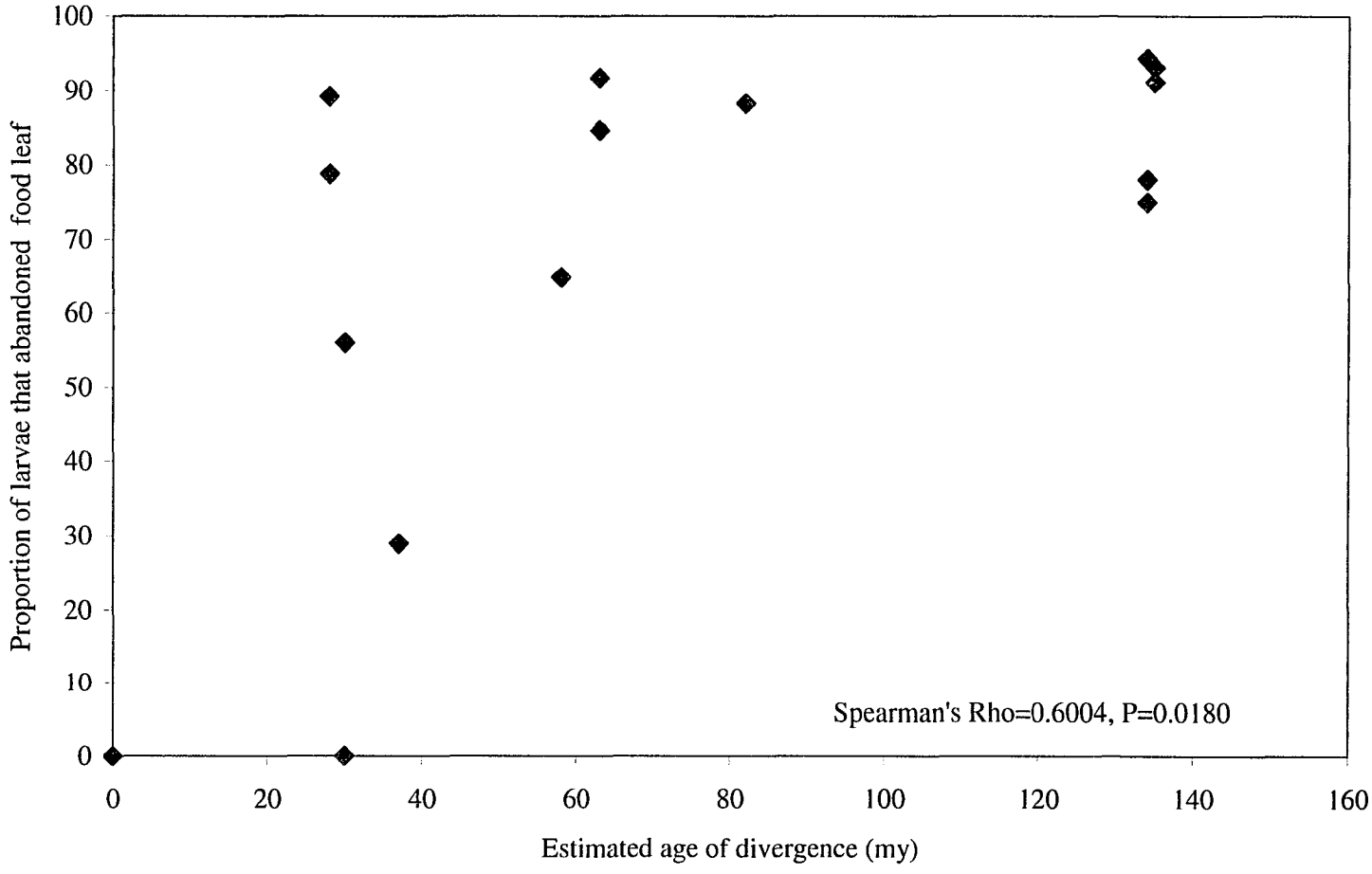

Figure 5. Proportion of neonate *L. lilii* larvae that abandoned their natal food leaf expressed as a function of the estimated age of the food plants' divergence from the clade containing *Lilium.*

Table 4. Oviposition by adult *Lilioceris lilii* on five host plant species in no-choice trials

Host plant species	Total leaf drymass (g)	Total # eggs oviposited	# eggs/g drymass
Asiatic *Lilium* hybrid.	58.26	300	5.33
Medeola virginiana	5.83	6	1.03
Clintonia borealis	11.42	4	0.35
Polygonatum biflorum	11.33	1	0.09
Streptopus amplexifolius	7.96	1	0.13

Lilium philadelphicum

Two females from mated pairs of beetles oviposited 16 eggs within three hours of being placed on the single stalk of *L. philadelphicum*. This stalk was not monitored for a full 72 hours because it began to wilt shortly after being placed in the greenhouse. No eggs were observed on any of the 400 wild *L. philadelphicum* examined in the Burnt Lands Alvar.

Larval performance

Survivorship

All larvae fed *Clintonia borealis*, *Polygonatum biflorum* or *Trillium grandiflorum* died within 48 hours of feeding. The other hosts displayed a noticeable drop in larval survivorship between day 0 and day 2 although this drop was greatly pronounced for larvae feeding on *Medeola virginiana* (26.6% survived) (Figure 6). Also, on the fourth day, survivorship of larvae feeding on *Streptopus amplexifolius* declined greatly (from 66.6% to 22.2%). The survivorship of *L. lilii* larvae, prepupae and adults was similar for those reared on hybrid lilies and on *L. philadelphicum* (Figure 6). *Lilium philadelphicum* supported 82.2% of larvae to the prepupal stage while hybrid lilies only supported 66.7%, but this difference was not significant (ChiSquare=2.897, P=0.0887). The survivorship of larvae reared on all four species tended to level off by day 4 to 6, showing another marked decline in survivorship only between the pre-pupal and adult stages. All four plants were able to support larval development up to the pre-pupal stage. Over 13% of larvae reared on *S. amplexifolius* survived to the prepupal stage, and I successfully reared almost 9% of larvae on *M. virginiana*. Survivorship of pre-pupae showed a similar trend in terms of the suitability of their host plants: 53.3%, 42.2%, 2.2% and 0%

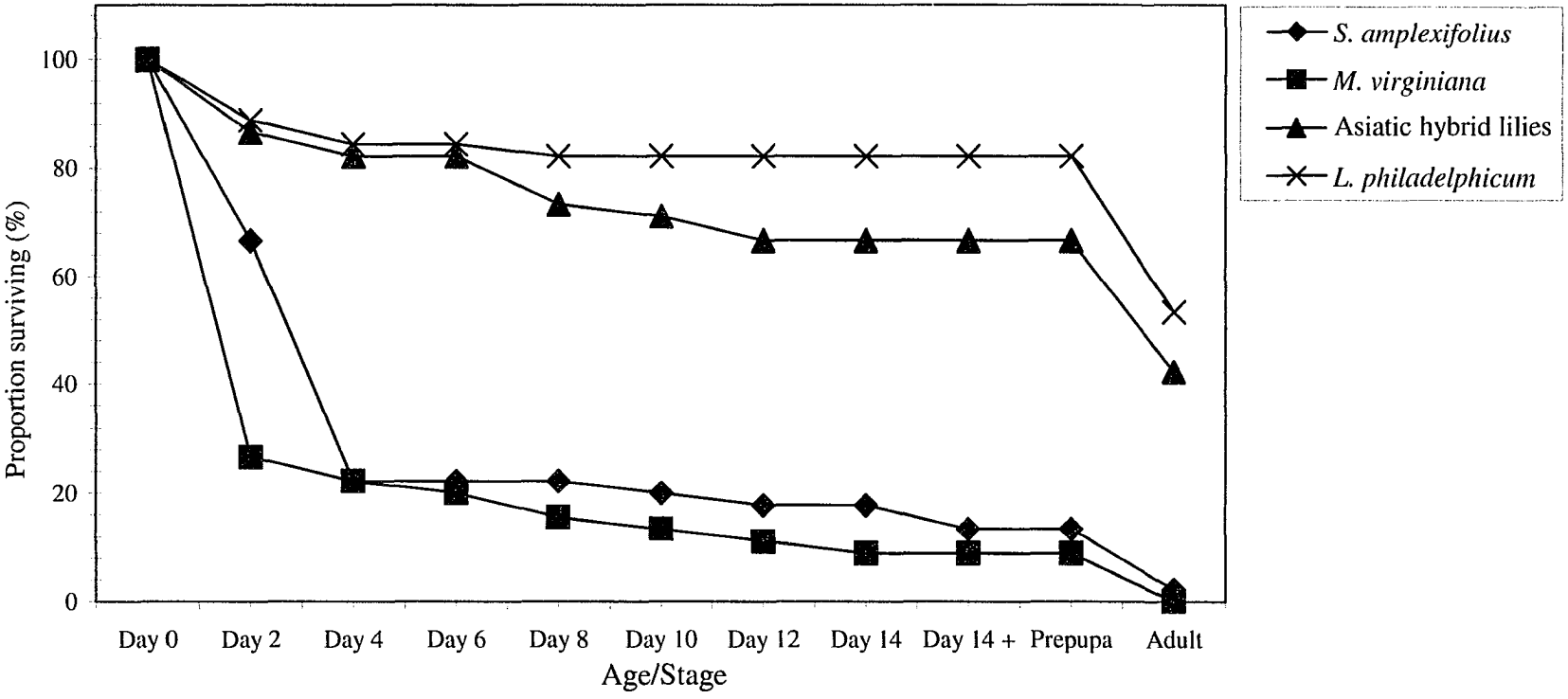

Figure 6. Proportion of surviving *L. lilii* reared on various species of Liliales (N=45 for each species), for larvae (at 2-day intervals), prepupae and adults

Larval growth

The average weights of larvae feeding on the two *Lilium* species were very similar throughout the development of the larvae (Figure 7). Overall, larvae feeding on *Lilium* grew quickly and to large sizes (up to about 0.06g) compared to those feeding on *S. amplexifolius* or *M. virginiana*, which seldom grew larger than 0.03g. For example, by the sixth day of development, the median weight of larvae reared on lilies and on *L. philadelphicum* was significantly higher than those reared on *Medeola* or *Streptopus* (Figure 8). The efficiency of conversion of ingested food (ECI) at this point in larval development was significantly higher for larvae feeding on *L. philadelphicum* compared to either hybrid lilies, *S. amplexifolius* or *M. virginiana* (Figure 9). There was no difference in the ECI of larvae eating hybrid lilies and *Streptopus* but the ECI was higher for larvae eating lilies compared to *Medeola,*

Development time and pupal weight

The median number of days it took hybrid lily-feeding larvae to reach the pre-pupal stage was significantly lower than those feeding on any other species (Figure 10). Larvae feeding on *L. philadelphicum* became pre-pupae significantly sooner than larvae reared on either *M. virginiana* or *S. amplexifolius* (Figure 10). There was no difference in overall development time for larvae feeding on *Medeola* or *Streptopus.*

The weights of the pre-pupae showed similar trends compared to the larval weights. Pre-pupae that developed from larvae reared on hybrid lilies and *L. philadelphicum* were significantly larger than those reared on either *Medeola* or *Streptopus*, with no significant difference between the two *Lilium* species (Figure 11).

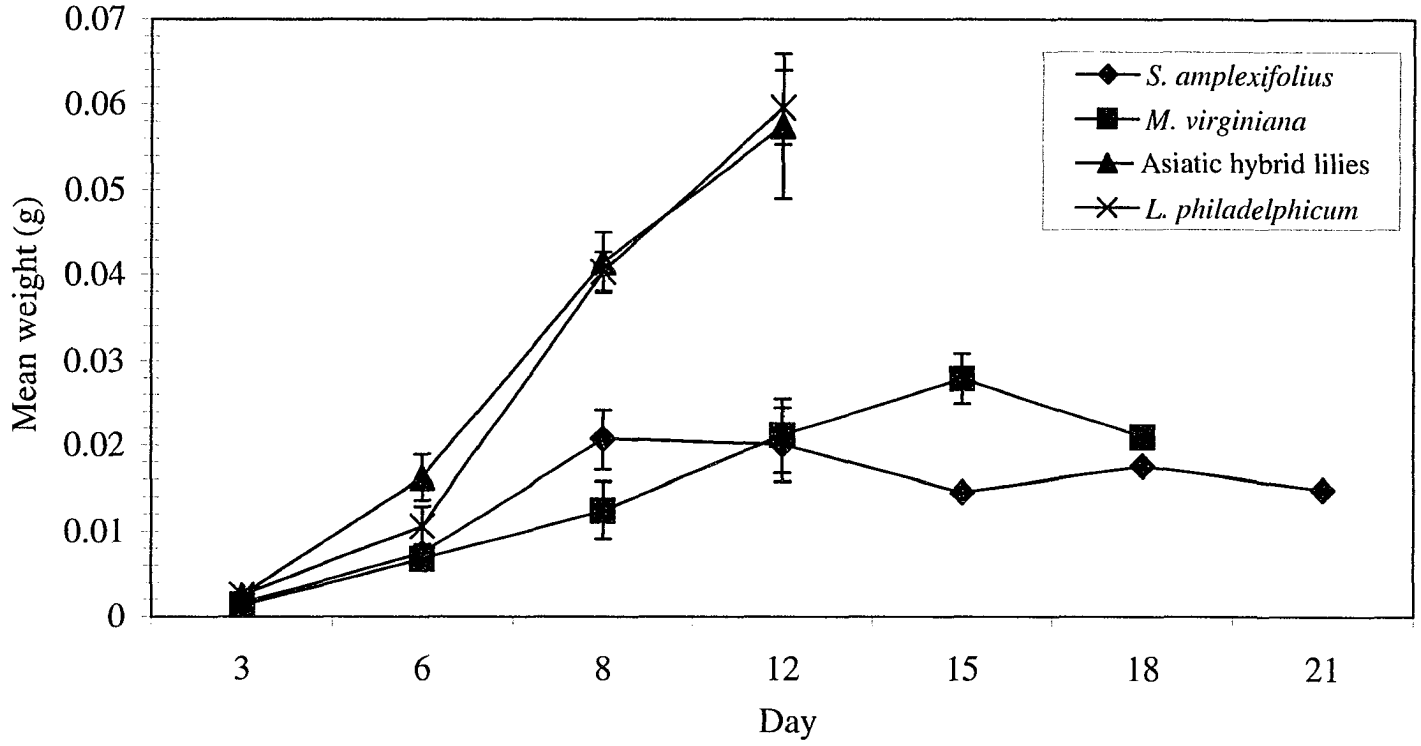

Figure 7. Mean weight (g ± SE) of *L. lilii* larvae reared on various species of Liliales, at 3 day intervals

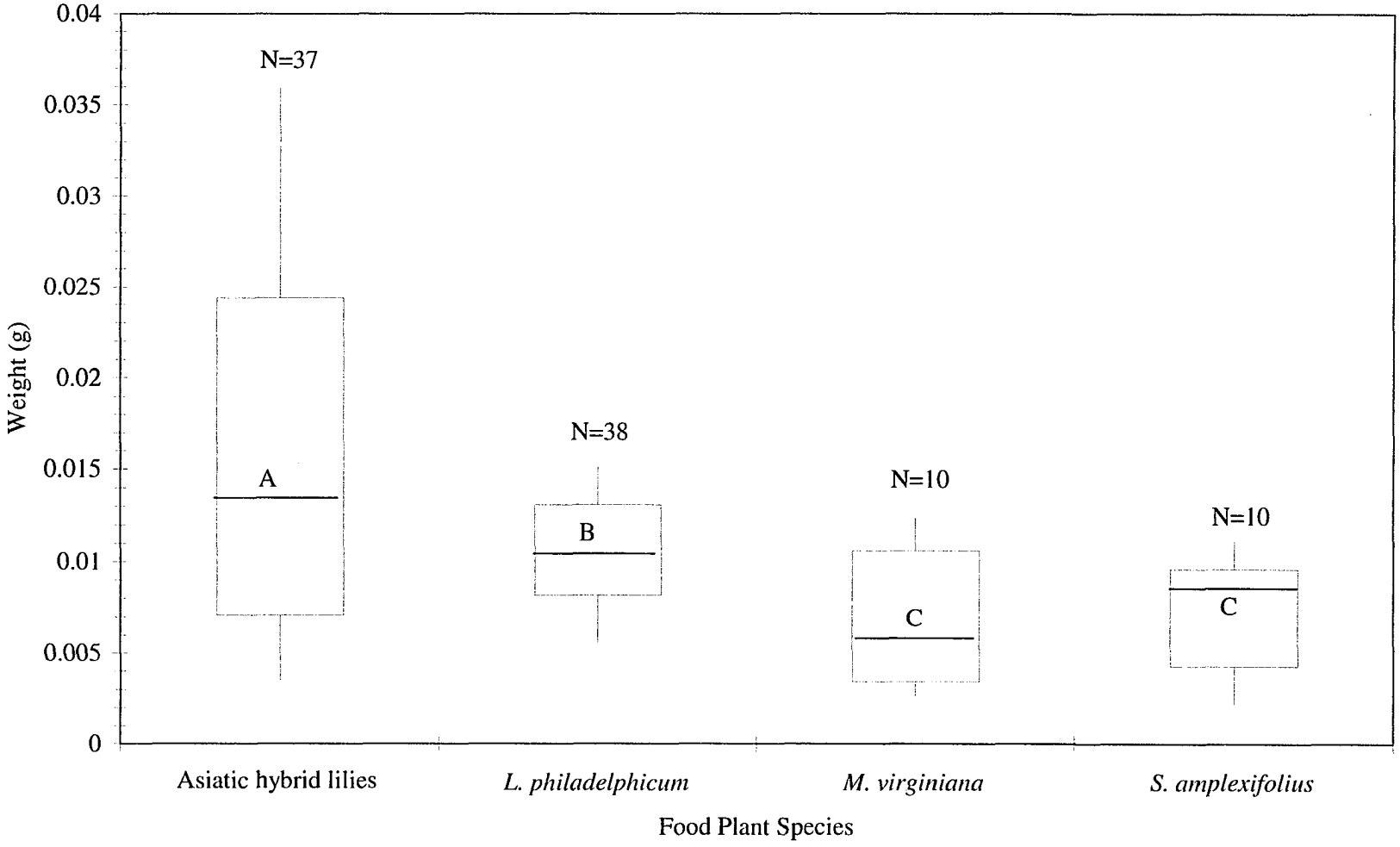

Figure 8. Weights of *L lilii* larvae (day 6) reared on various species of Liliales. Species with different letters denote significantly different medians according to Dunn's test (1964) (ChiSquare=14.6841, P=0.0021) . (Horizontal line denotes median value. Vertical line denotes 10th and 90th percentiles. Box denotes 25th and 75th percentiles.)

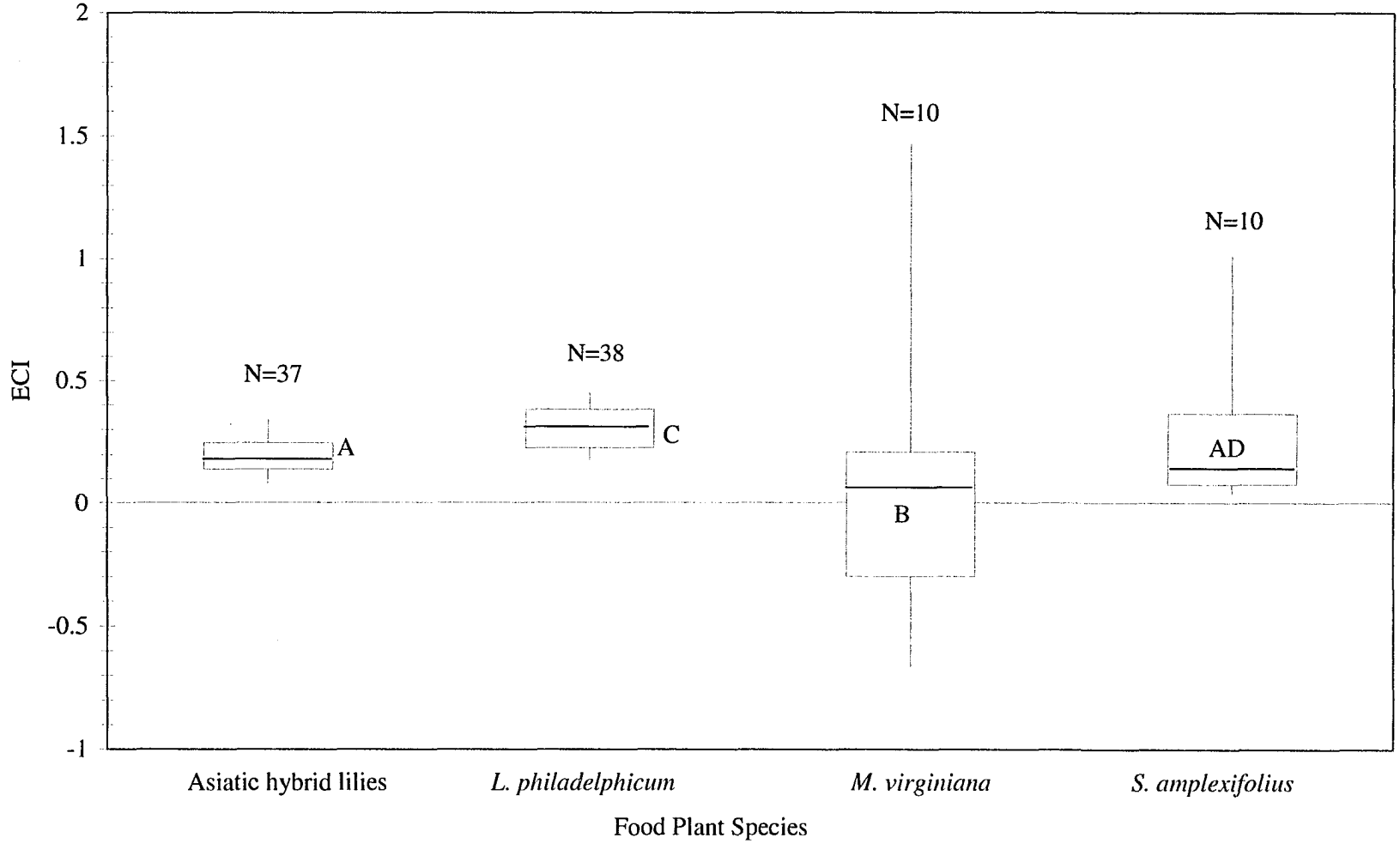

Figure 9. Efficiency of conversion of ingested food (ECI) of *L lilii* larvae feeding on various Liliales. Species with different letters denote significantly different medians according to Dunn's test (1964) (ChiSquare=23.1813, P<0.0001) . (Horizontal line denotes median value. Vertical line denotes 10th and 90th percentiles. Box denotes 25th and 75th percentiles.)

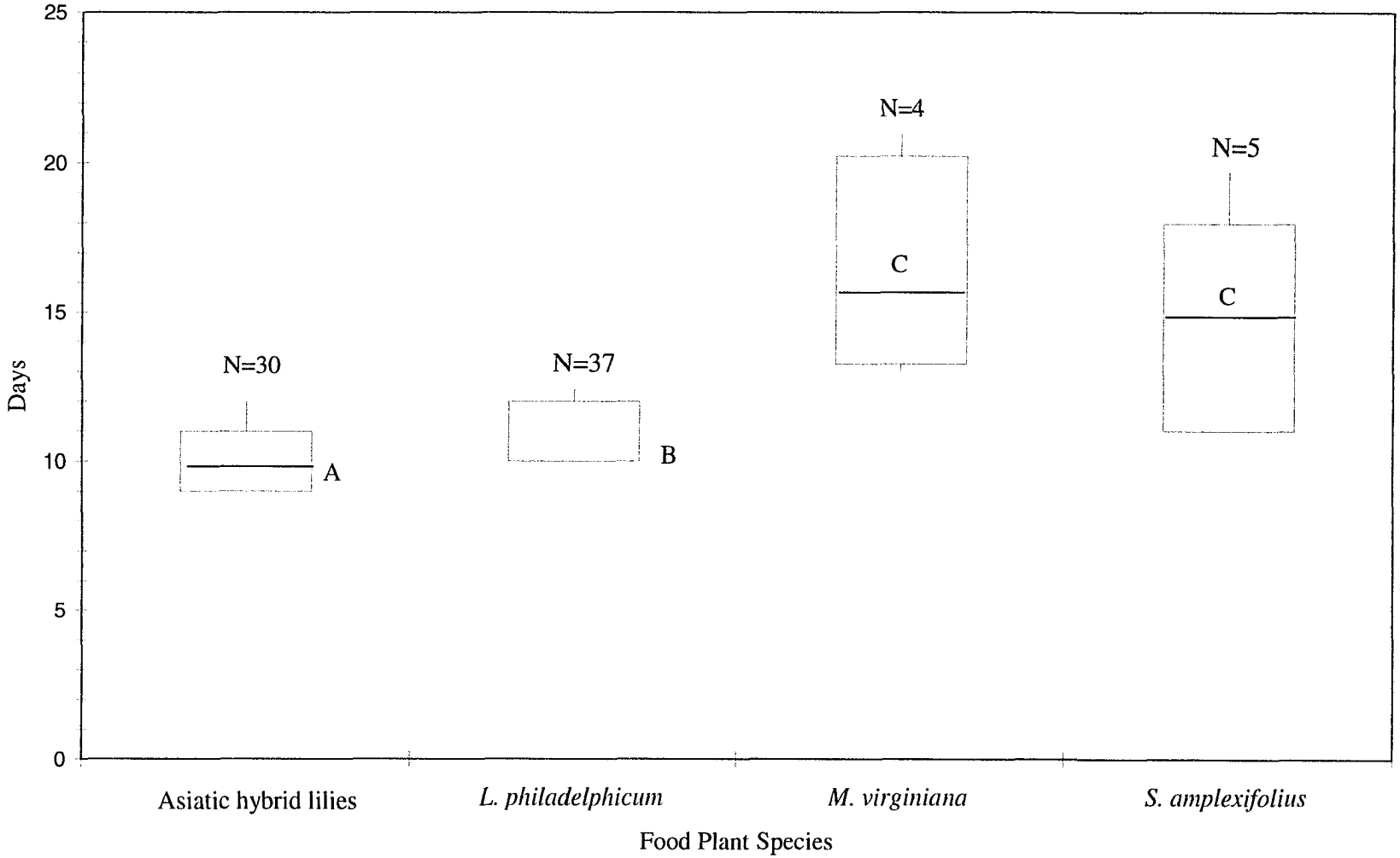

Figure 10. Days required to develop from first instar *L lilii* larvae to pre-pupae. Species with different letters denote significantly different medians according to Dunn's test (1964) (ChiSquare=25.5199, P<0.0001) . (Horizontal line denotes median value. Vertical line denotes 10th and 90th percentiles. Box denotes 25th and 75th percentiles.)

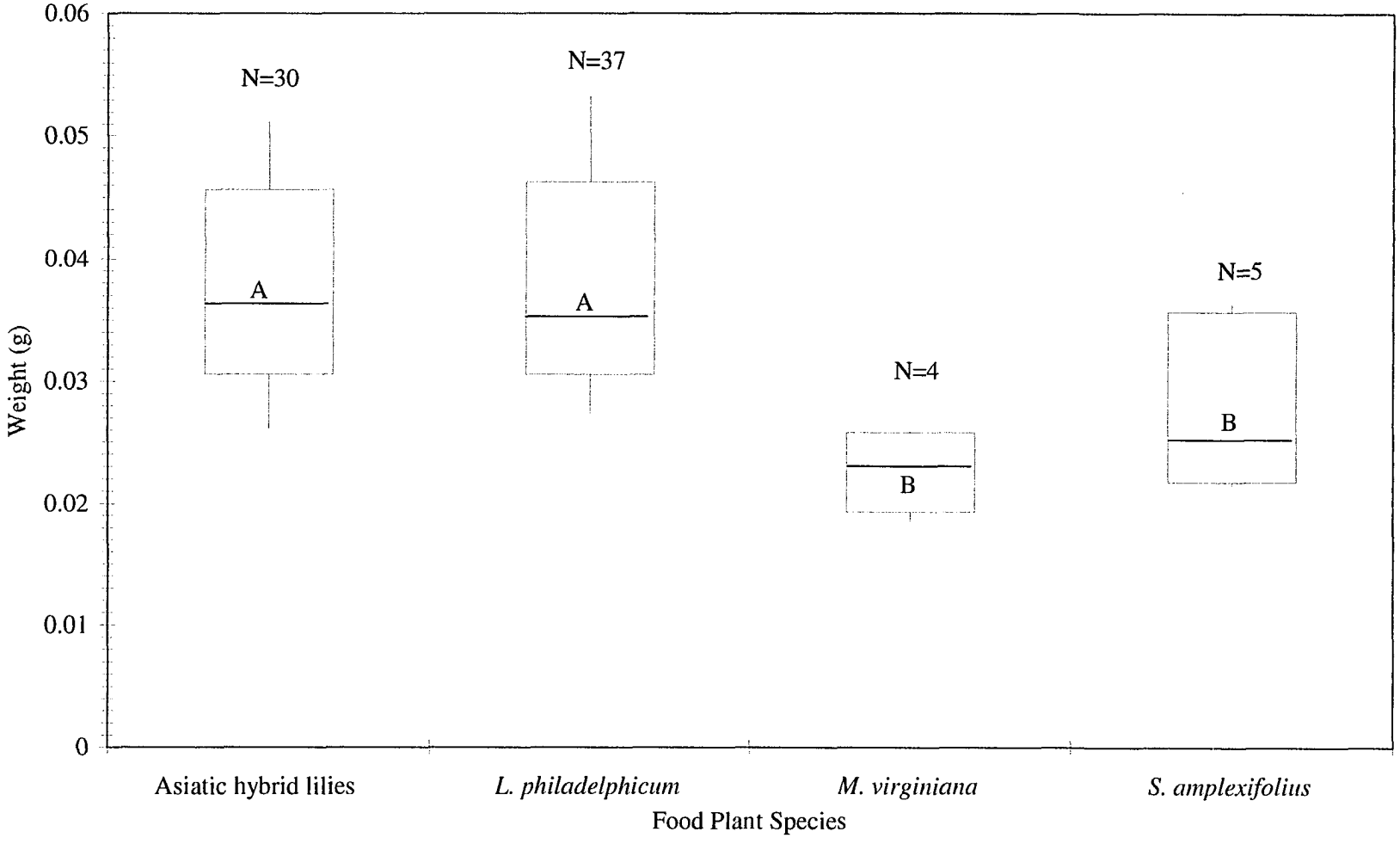

Figure 11. Mean weights of pre-pupae developed from *L. lilii* larvae reared on various species of Liliales. Species with different letters denote significantly different medians according to Dunn's test (1964) (ChiSquare=14.4156, P=0.0024) . (Horizontal line denotes median value. Vertical line denotes 10th and 90th percentiles. Box denotes 25th and 75th percentiles.)

Adults

Sixty-three percent of pre-pupae reared on lily survived to adulthood, while *L. philadelphicum*-reared pre-pupae survived 64.9% of the time (not a significant difference: ChiSquare= 0.017, P=0.897). Only one of the five *Streptopus*-reared pre-pupae survived (20%). None of the four pre-pupae reared on *Medeola* successfully developed into adult beetles.

The median number of days it took pre-pupae to develop into adults differed significantly between the insects reared on the two *Lilium* species (Figure 12). There were no significant differences in the weights of the adult beetles reared on the two *Lilium* species (Figure 13).

Predator feeding trials

There was no effect on convergent lady beetle *(Hippodamia convergens)* feeding behaviour associated with the addition of 10mL %50 ethanol to the agar diet compared to the control (Figure 14). The proportion of five-minute time intervals that lady beetles spent actively feeding on agar diet was significantly lower than the control (C), ethanol (E) and *Lilium philadelphicum* extract treatments (LP) when they were given agar diet treated with extract derived from the lily leaf beetle, *Lilioceris lilii* (LL) (Figure 14). There was no significant difference in feeding between those given agar diet with *L. lilii* extract (LL), Asiatic hybid *Lilium* extract 1 (LE1), or Asiatic hybrid *Lilium* extract 2 (LE2) (Figure 14). There was no significant difference in lady beetle feeding when they were given the C, E, LE1, LE2 or LP treated diets.

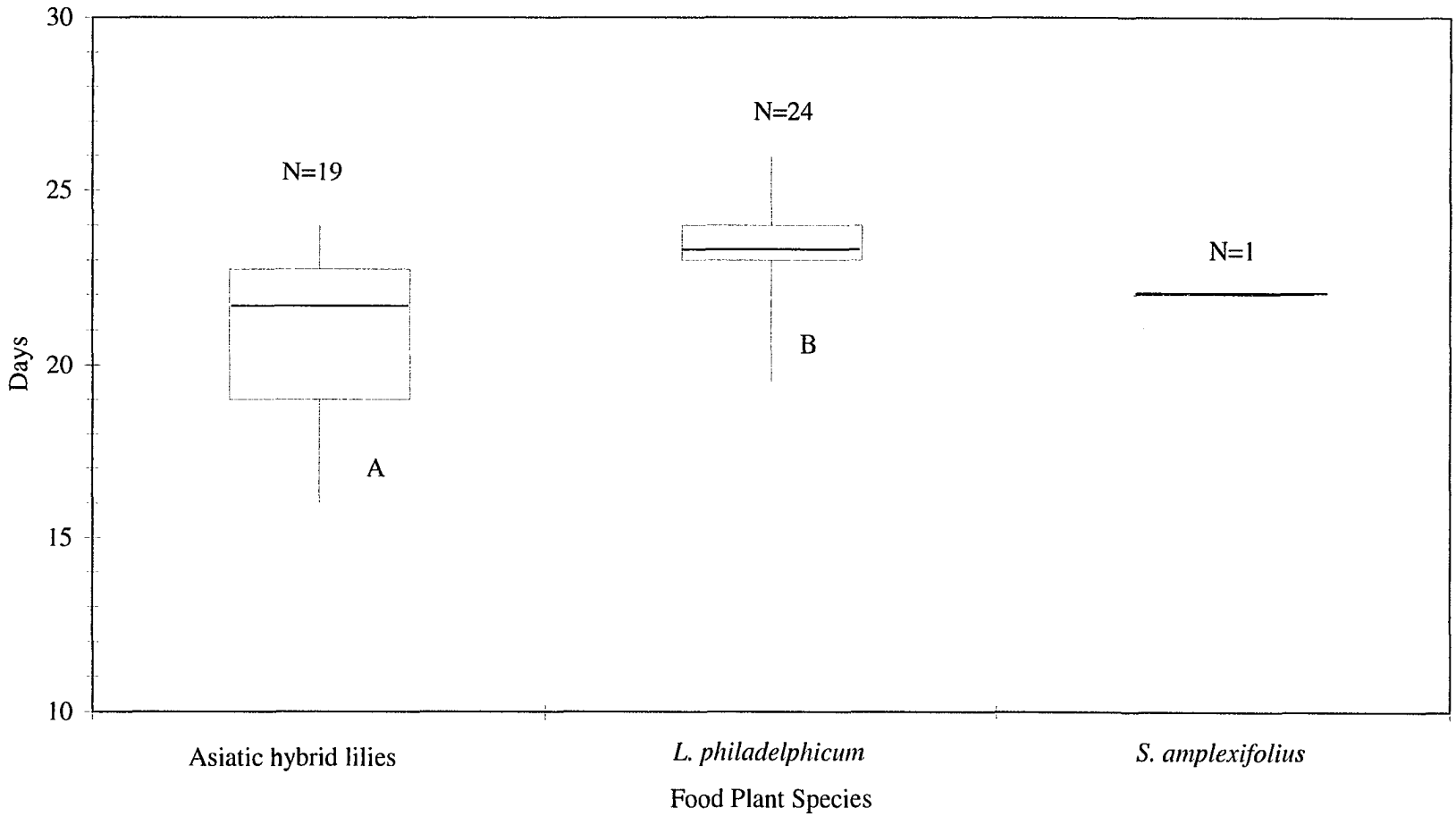

Figure 12. Number of days to development of adult *L. lilii* from pre-pupa reared on several species of Liliales. Species with different letters denote significantly different medians according to Dunn's test (1964) (ChiSquare=9.0558, P=0.0026 with *S. amplexifolius* omitted from analysis). (Horizontal line denotes median value. Vertical line denotes 10th and 90th percentiles. Box denotes 25th and 75th percentiles.)

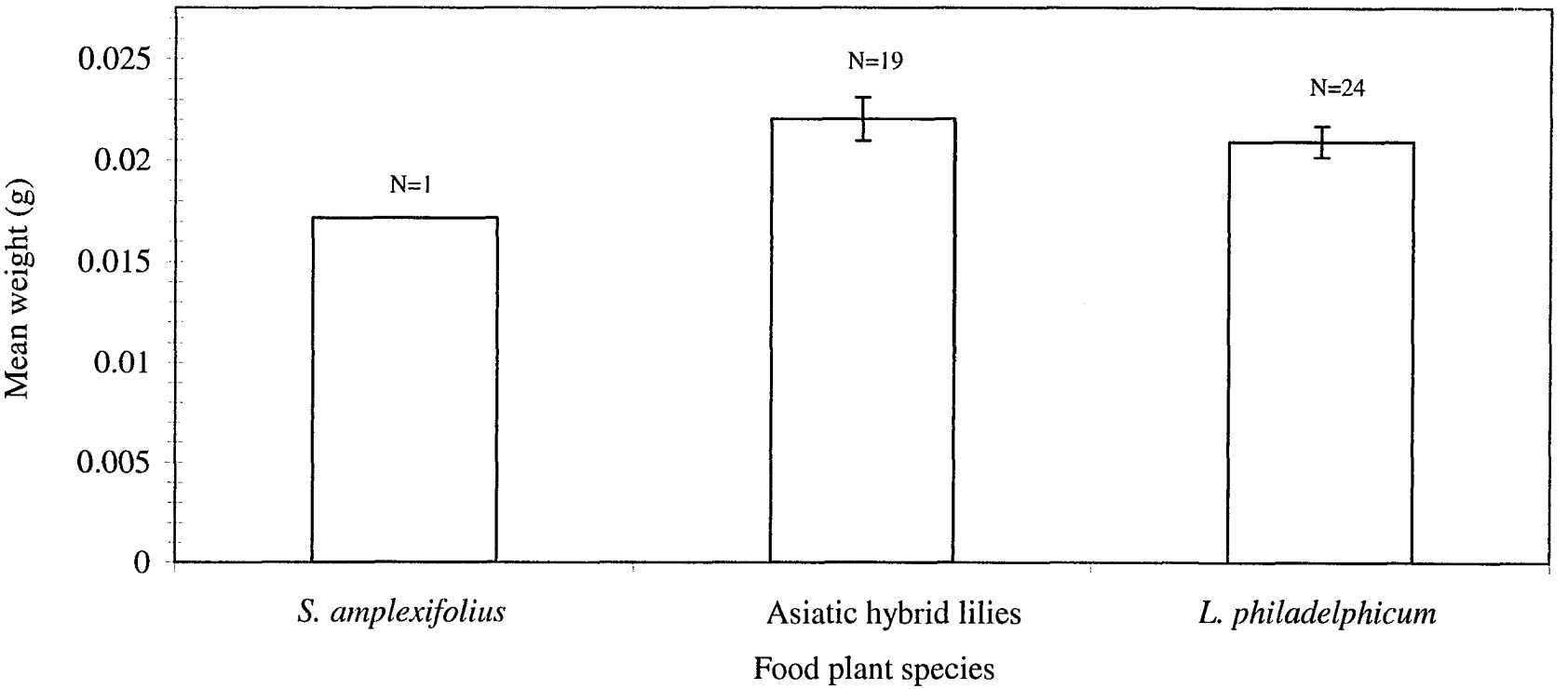

Figure 13. Adult weight (mean ± SE) of *L. lilii* (df=43, F=0.9183, P=0.3434 with *S. amplexifolius* omitted from analysis). Adults developed from larvae reared on diets of different Liliales.

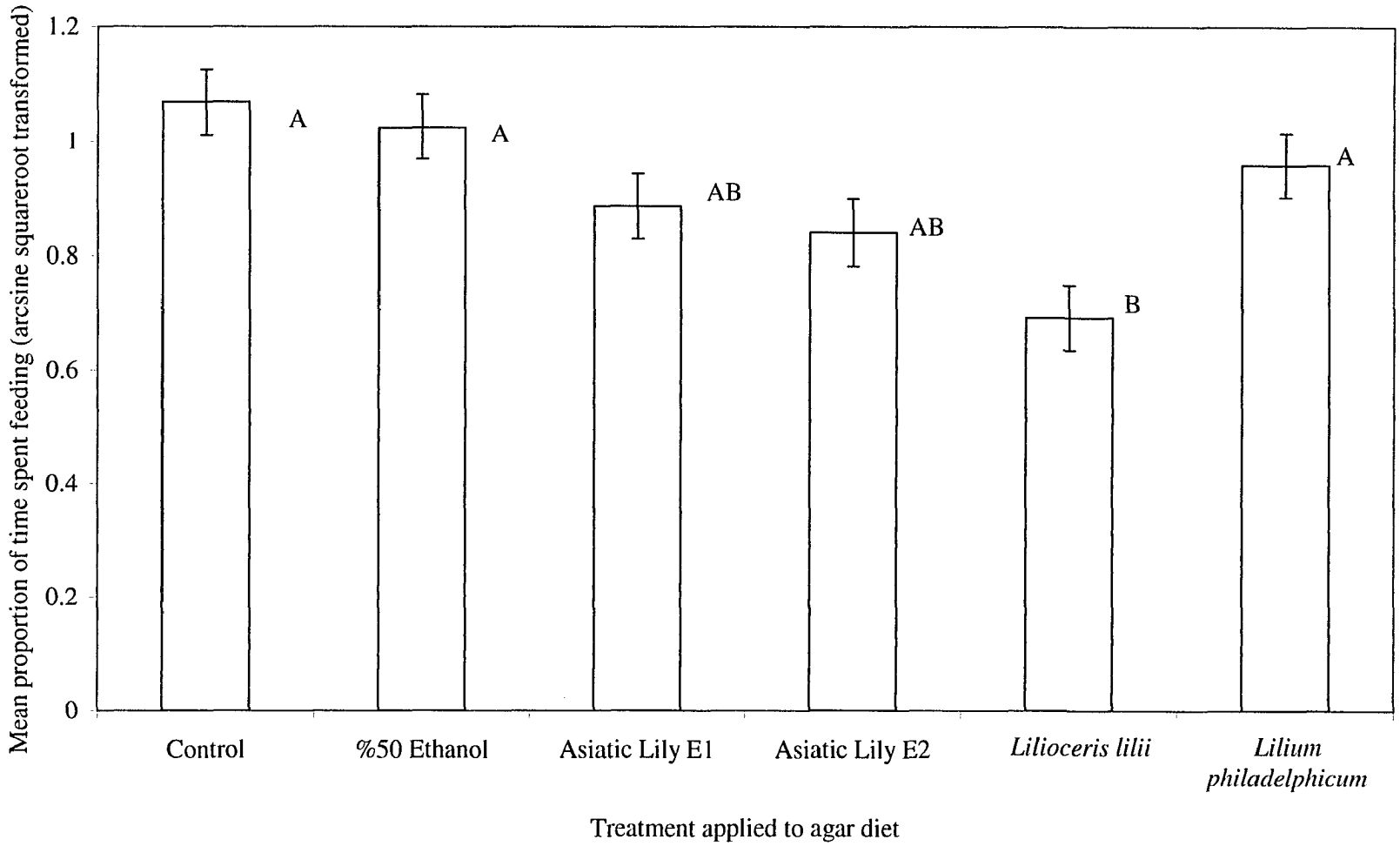

Figure 14. Proportion of time intervals (mean ± SE) (out of 12 five-minute intervals) that *H. convergens* spent actively feeding on supplemental agar diet (F=5.8175, df=118, P<0.0001). Treatments with different letters denote significant differences between treatments (Tukey HSD).

DISCUSSION

Adult oviposition

Mated females no-choice situations oviposited occasionally on host plants other than Asiatic hybrid *Lilium* plants. Some females laid a few eggs on either the petri dish in their cage or on the wood framing of the cage itself, indicating that there may be few specific oviposition stimuli required. While egg viability was not directly measured, the eggs laid on non-*Lilium* species tended to look dark and rather sunken, suggesting questionable viability. However, due to the relatively small sample of these eggs, and the low success rate of eggs surviving to eclosion generally found in this study, it is inconclusive if any eggs laid on species other than *Lilium* would be able to survive. Since specific stimuli may not be required for oviposition, it is possible that the novel host plants contain oviposition deterrents.

Females of some insect species will rank hosts for oviposition roughly in the same order as their suitability for offspring development. However, few of these correlations are strong (see review by Jaenike, 1990). Some insects may not oviposit on novel species that are suitable for larval development, while others may oviposit on species that are not suitable. Our results demonstrate the conflicting nature of preference-performance correlations. Although I was not able to quantify *Lilioceris lilii*'s willingness to oviposit on the wild Wood Lily, *Lilium philadelphicum*, our observations indicate that the beetle recognizes and will use this species as a host. Correspondingly, larval performance was extremely high: survivorship of larvae reared on *L. philadelphicum* was higher than that of larvae reared on their usual host (hybrid lilies), the ECI for larvae feeding on this plant was significantly higher than that of any other species. Conversely, the performance of

L. lilii larvae reared on *Streptopus amplexifolius* and *Medeola virginiana* does not seem to be correlated with female oviposition behaviour. Adult females laid far fewer eggs on these novel hosts than on lilies, but larvae reared on them were still able to develop. This study showed that the lily beetle is able to complete its development to adulthood on *S. amplexifolius*, albeit with longer larval development times and somewhat lower larval weights. It is interesting to note that lower larval weights did not result in significantly different adult weights. Since larvae were able to develop to the pre-pupal stage, I also speculate that, given a larger sample size, I would be able to demonstrate that *Medeola virginiana* is also a suitable host for the complete development of the lily beetle.

Adult feeding on a host plant is often a prerequisite for ovipostion; if the adults recognize and use the plant as a food source, they are more likely to lay eggs on it (Becerra and Venable, 1999). There are several records of adult lily beetles feeding on species other than *Lilium* spp. (Table 1), demonstrating that the beetle may be ultimately capable of exploiting a wider breadth of host plants. Adults in our oviposition experiments left signs of feeding damage on the leaves of all species used except *Clintonia borealis*, indicating that, with this one exception, the beetles may recognize the plants as potential hosts. Compared to all other plant species used throughout this study, *Clintonia borealis* has much larger, thicker, tougher leaves. Leaf toughness can be a major deterrent for feeding. In leaf beetles, mandibular wear, reduced feeding and reduced fecundity can be the result of feeding on tough leaves (Raupp, 1985). Interestingly, *C. borealis* was one of the least likely species to be abandoned by neonate larvae during the first 24 hours of feeding. This may indicate that it is palatable, but

perhaps difficult to feed on or damaging to the mouthparts of newly hatched *L. lilii* larvae.

It should be noted that, due to time constraints and the difficulty in maintaining a multi-generational colony of lily beetles, I was not able to evaluate the oviposition behaviour of naïve insects. Fundamental host-plant range (using no-choice testing) is usually best determined with naïve insects (van Klinken, 2000) and for this reason, oviposition behaviour, and the ability of naïve insects to identify novel plants as oviposition sites, should be more fully evaluated.

Host plant phylogeny as a predictor of larval acceptance and performance

The relationships between insects and their host plants often demonstrate phylogenetic conservatism (Ehlrich and Raven, 1964; Mitter *et al.*, 1991), meaning that the fundamental host ranges of insects tend to be restricted to related groups of plants. Novel host utilization may be facilitated by physical, chemical or phenological properties shared by related groups of plants and therefore, host use may be predicted from phylogenetic and genetic information (Futuyma and McCafferty, 1990).

I found that the estimated age of divergence of a host plant from the genus *Lilium* was a significant correlate of several neonate larval feeding behaviours. This implies that I may be able to use phylogenetic relatedness to *Lilium* to predict which species are more likely to be accepted by larvae as food plants, and thus which novel species might be at greater risk of colonization.

Some of the species I tested sustained much more feeding damage than I expected (in terms of leaf area consumed), given that they were more distantly related (*Polygonatum biflorum*, for example), while other closely related species (such as

Tulippa spp. and *Clintonia borealis*) sustained much smaller areas of damage. I suspect that some of the discrepancies in leaf area damage are due to differences in the thickness of the food leaves. Of all the species tested, leaf thickness was fairly consistent, with the exception of *Tulippa* and *C. borealis*. These have much thicker leaves, so the damaged area measured may be an underestimation of the actual amount of leaf consumed in comparison to the other species. Dobler and Rowell-Rahier (1994) found that they were unable to definitively quantify larval feeding preference of the leaf beetle *Oreina elongata* for this reason. When they tried to compensate for differences in leaf thickness by factoring in leaf weight, it also underestimated consumed material because of differences in leaf venation. Their two estimation methods yielded different results and they were unable to decide which food plant was preferred. In our tests, the more accurate indicator of larval preference may simply be average willingness to at least "taste" the food plant. There is a significant correlation between this behaviour and the age of host plant divergence from *Lilium*. As well, both willingness to feed and abandonment of the food leaf are significantly related to the leaf area consumed.

Larvae tended to emigrate from (or abandon) their food leaves, with few exceptions (*Lilium spp.* and *Medeola virginiana*), although their tendency to do so increased significantly as the age of divergence of the food plant from *Lilium* increased. Leaf beetle larvae will emigrate from non-natal leaves regardless of their suitability as food. The water-lily leaf beetle *Galerucella nymphaeae* will emigrate from non-natal food leaves even if they have been placed on young leaves (those which adults will tend to favour for oviposition) (Kouki, 1993). *Lilioceris lilii* larvae actively feeding on their usual host plants (*Lilium*) will usually move from their natal leaves only after they have

destroyed them. The results of our tests may have been different had I been able to entice females to lay eggs directly onto the different species of food leaves.

Neonate preferences may not be reliable indicators of long-term larval performance. This was demonstrated by the poor long-term performance of *L. lilii* larvae reared on *Clintonia borealis* and *Polygonatum biflorum.* All larvae feeding on C. borealis died within a 72-hour period of feeding even though most neonates were willing to feed on the plant. Similarly, neonates caused a larger than average amount of leaf damage within 72 hours of feeding on *P. biflorum* but long-term larval development on this plant was extremely poor. It seems that only very high initial acceptance (ie. greater than 75%) of a novel food plant is a consistent indicator of good long-term larval performance. For example, neonates offered *Lilium* spp. and *Medeola virginiana* as a food plant never abandoned their leaves, most of them fed on the leaves to some extent, and feeding damage on these species were higher than average; long-term larval development on both food plant species was correspondingly much better.

Lilioceris lilii is able to complete its development from egg to adult when feeding on *Streptopus amplexifolius* and probably when feeding on *Medeola virginiana.* This was surprising, as neither of these species are as closely related to *Lilium* as some of the other species I evaluated. It is possible that chemical or structural elements of the foliage were more conducive to larval development. Both of these species should be considered a component of the lily beetle's fundamental host range. Lily beetles also performed very well and are able to complete their development on *Lilium philadelphicum*, as expected. These results reiterate that *Lilium* species (whether cultivated or native) should be considered *Lilioceris lilii*'s primary host.

Unfortunately, time constraints and the volume of work required to perform thorough larval feeding trials prevented me from fully evaluating long-term larval performance on all of the plants examined in the initial neonate preference study. The unexpected results described above (as well as the unprecedented success of larvae reared on *Streptopus amplexifolius*, a distant relative of *Lilium*) illustrate the necessity of a more thorough investigation. More work is needed to fully assess suitability of other native plants as hosts for the lily beetle.

Chemical ecology

The colour of many Criocerinae has been shown to elicit an aposematic response in predators (Balsbaugh, 1988). Lily beetles and their relatives are brightly coloured, leading many to assume that they are warning predators of chemical defences. Others have surmised that the beetles sequester secondary metabolites from their host plants for chemical defence (e.g. Shepard,1997). There are no reports in the literature that either of these hypotheses have been tested experimentally with the lily leaf beetle. Using convergent lady beetles, *Hippodamia convergens*, as a model predator, I was able to demonstrate that *Lilioceris lilii* does in fact possess a repellent quality not associated with any type of anti-predation behaviour or physical characters. The lady beetles were significantly less willing to feed on an artificial diet containing a raw extract derived from whole lily beetles than any other diet I presented them.

My results also indicate that the lily beetles may be sequestering chemicals from their hosts. Predator feeding behaviour associated with the Asiatic lily extracts (the host plants on which the lily beetles were feeding prior to being collected for extract preparation) was statistically the same as that associated with the *L. lilii* extract. As well,

although the anti-feedant effects of the Asiatic lily extracts were not significantly different than the controls, there was still evidence of a fairly strong repellent quality. There is also a possibility that the anti-feedant chemicals are being produced *de novo*, or that the beetles use a combination of sequestered and endogenous toxins. Both types of chemical defence have been shown in the leaf beetle genus *Oreina* (Pasteels *et al.*, 1992), and many leaf beetles are generally known to use diverse chemical defences (Pasteels, 1993).

Lilium philadelphicum extracts did not reduce predator feeding. It is possible that this species does not possess anti-feedants, and may explain why some of the lily beetle larval performance measures scored higher when they were feeding on this plant, as opposed to the Asiatic lilies. Dobler and Rowell-Rahier (1994) found that larvae of the leaf beetle *Oreina elongata* performed better on a host plant without sequesterable metabolites than when feeding on a host containing pyrrolizidine alkaloids which they usually sequester for defence. The larvae's preference seemed to be for the alkaloid-containing host plant, however.

It would be of interest to determine the anti-feedant component present in *L. lilii*, and to determine if it is, in fact, identical to the anti-feedant present in Asiatic lilies. If the secondary metabolites present in Asiatic lilies are required components of the lily beetle's defence mechanisms (and if host plants are being selected for this reason), the beetles may reject potential hosts that do not possess the necessary allelochemicals. I did not evaluate larval lily beetle's preference for *Lilium philadelphicum* compared to Asiatic hybrid lilies, so I cannot state if there is a correlation between the chemical composition of the host plants and larval preference. However, there is enough evidence here to

suggest that we may be able to evaluate the suitability of related plant species as hosts in terms of their chemical components. Chemical similarity between host plants and related potential hosts is usually an important factor for host shifting or novel host use (Ehrlich and Raven, 1964; Becerra, 1997).

Lilies at risk of being used as hosts

Based on the lily beetle's acceptance of *Lilium philadelphicum* and the vast reports of *Lilium* species being used as host plants (e.g. Salisbury, 2003), I believe that all native *Lilium* in Canada and the U.S. are at risk of being used as hosts. In the province of Ontario susceptible species are *L. canadense* (Canada Lily) *L. michiganense* (Michigan Lily), and *L. superbum* (Turk's-cap Lily). Throughout the U.S. and other regions of Canada are many other wild *Lilium* species. All should be considered at risk of colonization.

Overall, *Lilioceris lilii* larvae developed best on the two *Lilium* species (one cultivated and one native), and adults demonstrated a willingness to feed and oviposit on both. This is of concern for two reasons. First, the beetle is already well-established on small patches of cultivated lilies (Asiatics, hybrids, and orientals). The many regional lily societies throughout Canada and the U.S. are proof of the popularity of lilies. Many of these groups, as well as horticulturalists and commercial greenhouses, are involved in the sale and/or exchange of lilies. The popularity of cultivated *Lilium* species will likely continue to facilitate the spread of the lily beetle into unaffected regions; it is suspected that the initial introduction of the pest into North America was made possible by such an exchange (Livingston, 1996). Already *L. lilii* has spread throughout eastern Canada and into the north-eastern United States. As the beetle is introduced to new regions, it stands

to reason that they will be more likely to encounter the scattered populations of wild *Lilium*, such as *L. philadelphicum.*

The second reason for concern is that some wild lily populations are at risk from other factors. Rare unspotted orange or yellow morphs of *L. philadelphicum* have been described in New England, U.S. The most recent discovery of a yellow morph has lead to calls for protection of the open heath lands on Nantucket Island (Massachusetts, US), were it was found (Hold and Tiffney, 2001). Small, unique populations such as this (one morph among only 72 individuals in 130km^2) would surely be at risk if the beetle were to come within range. Although *Lilioceris lilii* is established throughout New England (including Massachusetts) the geographical isolation of this particular island population may be enough to spare it from attack.

In our region (Ottawa, Ontario) wild lily populations are extremely rare; the *L. philadelphicum* leaves used in this study came from an alvar ecosystem approximately 52 kilometers west of Ottawa. The Burnt Lands Alvar (in Almonte, Ontario) is currently under government protection due to its assemblage of unusual and rare species, including the Wood Lily (Nature Conservancy of Canada, 2001). The land surrounding this unique ecosystem is in high demand as prime real estate for homeowners and developments are beginning to encroach on the area (Nature Conservancy of Canada, 2001); cultivated lilies planted in gardens could lead to a lily beetle introduction.

Lilium philadelphicum is also an important species in western regions of Canada; indeed, the wild Wood Lily is the official plant symbol of the province of Saskatchewan. Threatened by drought and habitat disturbance, the plant's presence in Saskatchewan has declined since the 1950s. A large-scale production operation was initiated in 2002 with

the intention of restoring natural populations through cultivation and planting (Horne, 2002). Weakened populations of the Wood Lily in this province could be threatened further once the lily beetle crosses over from its neighbouring province of Manitoba.

Risk to other species

I have demonstrated that lily beetles are able to complete their development on *Streptopus amplexifolius* and likely can complete their development on *Medeola virginiana*. Therefore, these species should be considered part of the beetle's fundamental host range, and therefore potentially at risk. These species are extremely common and abundant throughout the forests of Ontario, as well as in other parts of Canada and in much of the U.S. Many other *Streptopus* and *Medeola* species exist in these regions and would likely be able to support lily beetles. It is possible that if the lily beetle were to colonize even one of these native species, its spread could be even more significant and difficult to contain.

CONCLUSION

This study demonstrates the need for broad-spectrum assessment of risk when it comes to identifying native species that could be negatively affected by an exotic invasive species such as the lily leaf beetle, *Lilioceris lilii*. Relatedness of host plants may be a useful indicator of risk, although I have shown that the lily beetle's development is not always predictably associated with this factor.

The chemical ecology of the lily beetle and its host plants deserves further study. I have provided evidence that lily beetles possess chemical defences that may be sequestered from their host plants and possibly modified. Host plant allelochemicals are likely a factor in host selection by the beetle, and may be more helpful in describing the lily beetle's fundamental host range.

I have identified, for the first time, at least two related genera of native plants which are capable of supporting lily beetle development, and have shown that adult females may oviposit on species other than *Lilium*.

All wild *Lilium* species (some of which are endangered or threatened) are at risk of being colonized by the beetle, including *Lilium philadelphicum* in our area. The rapid expansion of this pest in eastern Canada and the north-eastern United States (as well as in Europe) demonstrates that there is ample opportunity for the lily beetle to distribute throughout North America. Control efforts are warranted and further work is needed to identify other plant species that are at risk.

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APPENDICES

Appendix A. Instructions for preparing artificial diet for *Hippodamia convergens* (Cunningham, 2003).

Items Needed:

- Hot plate
- Pot for boiling water and lid
- Bowl for mixing ingredients
- Wire wisk
- 100ml graduated cylinder
- Gram scale
- Three-inch Petri dishes (for storing diet)
- Paper towels
- Mortar and pestle (for grinding bee pollen)

Ingredients needed and purpose

- Distilled water
- Honey - carbohydrates
- Rice protein concentrate – protein and nutrients (can be obtained from health food stores)
- Methyl paraben - preservative (can be obtained from scientific suppliers)
- Sorbic acid - preservative (can be obtained from scientific suppliers)
- Bee pollen - protein (can be obtained from health food stores)
- Agar - suspends the solution in gel form; prevents drying (can be obtained from scientific suppliers)

Instructions

1. Add 500 mLs of distilled water in boiling pot. Cover with lid.

2. Place the lid on the pot and set on hot plate. Set hot plate to High.

3. Pour 60 mLs of honey and pour into mixing bowl.

4. Combine 30 grams of rice protein concentrate with 180 mls water, stir, and pour solution into mixing bowl.

5. Add 0.6 grams of methyl paraben to mixing bowl.

6. Add 0.2 grams of sorbic acid to mixing bowl.

7. Add 2.0 grams of bee pollen (ground into a fine powder) to honey and diet mix.

8. Mix vigorously the honey, diet, methyl paraben, sorbic acid, rice protein concentrate, and bee pollen together using the wire wisk. Dissolve all materials.

9. Measure 7.0 grams of agar and keep separate from all other ingredients.

10. Bring water on the hot plate to a rolling boil and then shut off the hotplate.

11. Carefully remove the lid and stand away from the steam.

12. Slowly add agar to the hot water. Use the wire wisk to stir in the agar as it's added.

13. Mix agar and water until all the agar has dissolved

14. Carefully add agar solution to the already mixed honey/diet solution and mix all ingredients

15. Pour final solution into petri dishes

16. Cover petri dishes with paper towel and allow diet to cool for at least one hour.

17. Cooled diet supplement can then be covered and placed into cold storage for use. Mark the production date on each petri dish to monitor freshness. (Properly refrigerated supplement can remain usable for up to three months after making it.)

(Makes about 14 three-inch petri dishes of diet supplement.)