

**NORTH AMERICAN HOST RANGE EXPANSION OF AN INTRODUCED PEST,  
THE LILY LEAF BEETLE, *LILIOCERIS LILII* (SCOPOLI) (COLEOPTERA:  
CHRYSOMELIDAE) AND POTENTIAL TO ATTAIN ENEMY FREE SPACE  
FROM ITS PARASITOID, *TETRASTICHUS SETIFER***

by

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## ABSTRACT

The lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae) is an introduced invasive pest of lilies (*Lilium* spp.) in North America. Classical biological control using a larval parasitoid, *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), was implemented in 2010 in Ottawa. As the beetle expands its geographic range in North America, it is adding new host plant species to its diet. The objectives of this study were to examine preference of the lily beetle for different host plants and larval performance on those plants. In addition, the ability of *T. setifer* to find beetle larvae on various plants was also examined.

Feeding and oviposition experiments suggested that several novel host plants might be suitable for the beetle: *Lilium canadense*, *Lilium michiganense*, *Lilium philadelphicum*, *Lilium superbum*, *Streptopus lanceolatus* and *Polygonatum biflorum*. Field experiments revealed that the beetle is able to attain enemy free space on some of these novel native host plants.

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## CHAPTER 1

### GENERAL INTRODUCTION

Introduced insects, defined as exotic species residing outside of their native range, attract public attention when they become pests (Simberloff, 1986; Keane and Crawley, 2002). According to Sailer (1978), human commerce and travel have resulted in the introduction of numerous species at multiple locations. Each of these introductions may result in a biological invasion, defined as an increase in the abundance and distribution of an exotic species, creating disruptions in natural ecosystems (Keane and Crawley, 2002). Biological invasions are hypothesized to be one of the main causes of biodiversity loss (Vitousek *et al.*, 1997). The enemy release hypothesis (ERH) suggests that introduced species become invasive and spread quickly because their co-evolved natural enemies are no longer with them in the new area (Liu and Stiling, 2006).

Phytophagous insects that have been purposely or accidentally introduced to a new area can result in the evolution of new plant-insect interactions (Strong, 1974; Berenbaum and Zangerl, 1991; Singer *et al.*, 1992; Thompson, 1998; Ernst *et al.*, 2007). Although some novel plants may provide suboptimal conditions as a food source for larval development, host switches occur nonetheless due to potential advantages in survival. In pea aphids, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), fecundity is reduced on *Trifolium pratense* (Fabaceae) and *Medicago sativa* (Fabaceae) relative to the usual food plant, *Vicia faba* (Fabaceae). The benefit of feeding on the novel hosts included the presence of fewer natural enemies, resulting in reduced predation, in comparison to the typical host (Vosteen *et al.*, 2016). This is an example of enemy-free

space in which herbivores that move onto a novel host plant to which they are only partly physiologically adapted might nevertheless enjoy increased survivorship if their natural enemies do not effectively search for them on that plant (Jeffries and Lawton, 1984; Brown *et al.*, 1995). Therefore, herbivore performance depends not only on the suitability of a host plant as a food resource, but also on the mortality caused by natural enemies that search for and kill the herbivore on the host plant(s) it has coevolved with.

Classical biological control, the introduction of a natural enemy from the pest's native range to the invaded area, can help manage these new plant-insect interactions by suppressing populations of the pest (Mason and Brodeur, 2013). One prominent classical biological control strategy involves the introduction of parasitic wasps. However, this strategy also has its limitations because host switches by the herbivore resulting in enemy-free space can decrease the success of the program. For instance, in the case of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), the wild host plants, *Brassica nigra* (L.), *Cardamine integrifolia* (Nuttal) and *Erysimum cheiranthoides* L. (Brassicaceae) were preferred for oviposition and similarly these plant species were the preferred search targets of its primary parasitoid, *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae). However, when the moth switched to cultivated varieties of *Brassica oleracea* L. (Brassicaceae), although larval performance was reduced, parasitism also decreased, thus providing the diamondback moth larvae with a refuge from their enemy (Fox and Eisenbach, 1992). The ability of a pest to escape its natural enemies demonstrates the importance of understanding the role novel hosts play in the biology of pest species.

The lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae), feeds on *Lilium* spp. and *Fritillaria* spp. (Liliaceae), which support complete development (Salisbury, 2008). This beetle is native to the east Palearctic region, spanning mostly European countries to Russia (Salisbury, 2008). However it has recently been suggested that the true native origin of this beetle is only in Asia (Orlova-Bienkowskaja, 2013). The beetle is an invasive pest in the UK and parts of North America and threatens the lily industry in the US, which in 1997 was worth over US\$65 million (Gold, 2003; Salisbury, 2008). Lily leaf beetle also occurs in commercial lily fields in European countries, such as France and Switzerland (part of the native range); however, the beetle rarely causes significant damage there, potentially due to the high levels of parasitism in those areas (Kenis *et al.*, 2001; Casagrande and Gold, 2002).

The lily leaf beetle is hypothesized to have been introduced to North America by two separate introductions (Dieni *et al.*, 2016). The first introduction of the lily leaf beetle occurred in Montreal, Quebec, Canada in 1943 (Brown, 1946; Dieni *et al.*, 2016). For the first 30 years, the beetle seemed to be confined to the island of Montreal; however, it can now be found in nine Canadian provinces (from Alberta to Newfoundland) (Brown, 1946; LeSage, 1983; Gooderham, 1993; Casagrande and Livingston, 1995; LeSage and Elliott, 2003; Majka and LeSage, 2008; Calgary Horticultural Society, 2009; Hicks and Sellars, 2014; Brace, 2016). The second accidental introduction into North America likely occurred in the United States at Cambridge, Massachusetts in 1992 (Dieni *et al.*, 2016). It is hypothesized that the spread of the beetle in North America was due to the movement of lily bulbs or plants as well as their packing material (Fox-Wilson, 1942). Additionally, Ernst (2005) noted that adults

are strong fliers and can travel large distances, potentially further facilitating their quick spread.

### **Lily Leaf Beetle Biology and Life Cycle**

Lily leaf beetle adults are bright red with a black head, antennae, legs and abdomen and are approximately 6-8mm long (Ernst, 2005). Adults burrow into the soil in fall to overwinter, emerging in early spring the following year to feed and mate. Mated females will lay eggs on the underside of host leaves often parallel to leaf veins in rows of 3-12 (Emmel, 1936; Ernst, 2005). The eggs are approximately 1.0 x 0.5 mm in size, being bright orange immediately after oviposition, then transitioning to a dark brown as the eggs mature. Eggs are covered in a sticky coating, which is believed to help them adhere to the underside of leaves (Reinecke, 1910; Salisbury, 2008). Individual females lay between 200-367 eggs (Lataste, 1932; Fox-Wilson, 1942). After 4-10 days the larvae emerge from the eggs with the aid of egg-bursting spines on the first abdominal segment; they are a pale orange/yellow with a dark head and legs and range from 8-10 mm in length once mature (Fox-Wilson, 1942; Cox, 1994). Larvae move their own feces onto their backs from their dorsally situated anus by utilizing abdominal bristles (Balachowsky and Mensil, 1936; Emmel, 1936). It has been hypothesized that this 'fecal shield' prevents desiccation, aids in thermoregulation and provides protection against generalist enemies; however, specialist enemies can exploit this shield (Reinecke, 1910; Nolte, 1939; reviewed in Olmstead, 1994; Salisbury, 2008). When a lily leaf beetle larva is disturbed it will regurgitate a dark brown/green droplet, adding another possible defense mechanism to its repertoire. The larva continuously feeds throughout its four instars, first

consuming the leaves of a plant and eventually feeding on the petals and stem. The instars can be differentiated by head capsule size (Livingston, 1996). Early-instar larvae feed gregariously on the ventral epidermis of the leaf, while single late-instar larvae consume entire leaves (Nolte, 1939; Haye and Kenis, 2004).

Prior to pupation, the larva ceases to feed, sheds its fecal shield and moves down the plant to the soil in which it buries itself. At this point the pale larva becomes a bright orange pre-pupa that begins to construct a well-camouflaged silken cocoon using oral secretions and small soil particles. The beetle remains on average 9 days in the pre-pupal and pupal stages (Haye and Kenis, 2004). Adults emerge in late summer, feed until early fall, and then overwinter in soil until next spring.

### **Management of the Lily Leaf Beetle**

According to Gold and colleagues (2001), North American natural enemies of the lily leaf beetle have not been documented. Management of this species therefore includes handpicking or utilization of broad-spectrum insecticides, with frequent repetition needed (Salisbury, 2007). These methods are often not effective. For example, when the adult beetle is disturbed, its defense mechanism includes dropping off the leaf, making handpicking difficult. On the other hand, insecticides raise consumer concerns, are heavily regulated in Canada and are not recommended for use when the plants are in flower due to the danger to pollinating insects and other non-target organisms (Salisbury, 2007; Health Canada, 2009; Royal Horticultural Society, 2016). A third method, which has been successful, involves classical biological control.

## **Lily Leaf Beetle Management: Classical Biological Control**

Four larval parasitoids are associated with lily leaf beetle in France and Switzerland: *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), *Lemophagus pulcher* Szepligeti, *L. errabundus* Gravenhorst, and *Diaparsis jucunda* (Holmgren) (Hymenoptera: Ichneumonidae) (Gold *et al.*, 2001). In 2003, *T. setifer*, *L. errabundus*, and *D. jucunda* were approved for release in the United States after host-specificity tests demonstrated their safety (Casagrande and Kenis, 2004). Once *T. setifer* was released in trial plots in Massachusetts, 60% parasitism was recorded that year; however, low winter survival occurred, possibly due to the use of bark mulch affecting the overwintering of the parasitoid (Casagrande and Gold, 2002; Tewksbury *et al.*, 2005). A few years after *T. setifer* was released in Wellesley, Massachusetts, it became established (Tewksbury *et al.*, 2005), had spread at a rate of approximately 1.0 km per year (Tewksbury, 2014), produced parasitism levels ranging from 37% - 100% and reduced lily leaf beetle numbers (Tewksbury *et al.*, 2005).

### **Biology and Behavior of *T. setifer***

The larval parasitoid *T. setifer* is the most widely distributed parasitoid of the lily beetle in Europe (Casagrande and Kenis, 2004). It is a gregarious endoparasitoid, producing an average of seven (2-26) parasitoid larvae per host. It is univoltine, overwintering as mature larvae in the host's cocoon. *T. setifer* adults emerge over a span of several weeks in the spring. Female parasitoids oviposit in all four larval stages (Haye and Kenis, 2004).

In contact bioassays, *T. setifer* responded positively to fecal shields and fecal extracts of the lily leaf beetle (Scarborough, 2002). When the fecal shields of lily leaf beetles were switched to those of the related *Lilioceris merdigera* L. (Coleoptera: Chrysomelidae) and *vice versa*, *T. setifer* switched its preference and spent more time on *L. merdigera* (Scarborough, 2002), confirming the importance of the fecal shield in lily leaf beetle larvae detection. This type of information can be crucial and needs further investigations as the fecal shield may be chemically altered as the lily leaf beetle expands its host range to novel plant species.

### **Host Range Expansion of the Lily Leaf Beetle and Thesis Objectives**

Recently, the lily leaf beetle has been reported feeding and laying eggs on several novel host plant species native to North America. In a natural population of *Lilium michiganense* Farwell (Liliaceae) in southwestern Ontario, 40% of the plants were infested with lily leaf beetle (Blackman *et al.*, 2016, Chapter 5). In Québec, 4.6% of Rose twisted stalk, *Streptopus lanceolatus* (Aiton) Reveal (Liliaceae) were infested (Blackman *et al.*, 2016, Chapter 5). Additionally, 40% of natural Canada lily, *Lilium canadense* L. (Liliaceae) populations surveyed in Québec contained the pest as well (Bouchard *et al.*, 2008). However, none of the wood lily, *Lilium philadelphicum* L. (Liliaceae) sites surveyed were infested (Bouchard *et al.* 2008). In laboratory host range experiments, *L. canadense* and *L. philadelphicum* were highly suitable as hosts of lily leaf beetle and in no-choice oviposition tests lily leaf beetle eggs were laid on *S. lanceolatus* [= *S. amplexifoliosus* (L.) DC] (Liliaceae) (Ernst *et al.*, 2007). This raises the question of why lily leaf beetle is not found on *L. philadelphicum* in nature. Perhaps the beetle cannot find

*L. philadelphicum* in nature or the plant is not suitable for oviposition (Bouchard *et al.*, 2008). Also, if the lily leaf beetle is found to colonize *L. philadelphicum* or other native plants in the future, it is of interest to know whether its biological control agents will be able to detect it on these novel hosts. These questions are important because *L. philadelphicum* and other potential native hosts are endangered or threatened species in certain jurisdictions (Ernst *et al.*, 2007).

To explore these questions, the objective of this study was to further elucidate the potential host range of lily leaf beetle and better understand the interaction with its biological control agent *T. setifer*. Specific objectives were the following: (i) test host preference and performance of the lily leaf beetle (Chapter 2), (ii) investigate the possibility that novel host plants may provide a refuge from parasitism by *T. setifer* (Chapter 3), (iii) determine if the parasitoid had successfully spread from its release sites in the Ottawa area (Chapter 4), and (iv) document the host range expansion of the beetle onto novel hosts in Ontario (Chapter 5).

## CHAPTER 2

### **PREFERENCE OF ADULT LILY LEAF BEETLE, *LILIOCERIS LILII* (SCOPOLI) (COLEOPTERA: CHRYSOMELIDAE), AND LARVAL PERFORMANCE ON NOVEL HOSTS**

#### INTRODUCTION

The lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae), is an invasive pest of lilies in the UK and parts of North America (Casagrande and Kenis, 2004; Salisbury, 2008). Both adults and larvae feed on leaves, buds, flowers, seed capsules and epidermis of the stem of host plants, where all that is left over after a devastating attack is a desiccated stem. Study of this pest is of importance as biological invasions are predicted to be one of the main causes of biodiversity loss (Vitousek *et al.*, 1997). Therefore, continuous monitoring of the spread of this pest and possible novel host expansions is required.

There are a few generalist enemies known to attack the lily leaf beetle larvae, such as earwigs, *Forficula auricularia* L. (Dermaptera: Forficulidae) (Schaffner and Kenis, 1999), nymphs of the Brassica shield bug *Eurydema oleracea* L. (Hemiptera: Pentatomidae) (Nolte, 1939), lacewing larvae (Neuroptera) and minute pirate bug nymphs (Hemiptera: Anthocoridae) (Salisbury, unpublished, as cited in Salisbury, 2008). An unidentified stinkbug (Hemiptera: Pentatomidae) has been observed feeding on a larva as well (personal observation). In North America, no native parasitoids are known to attack lily leaf beetle (Gold *et al.*, 2001). There are, however, four larval parasitoids known to attack this pest in Europe: *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), *Lemophagus pulcher* Szepliget, *L. errabundus* Gravenhorst, and *Diaparsis*

*jucunda* (Holmgren) (Hymenoptera: Ichneumonidae) (Gold *et al.*, 2001). One egg parasitoid, *Anaphes* sp. (Hymenoptera: Mymaridae) and two parasitic flies *Meigenia simplex* Tschorsnig and Herting and *Meigenia uncinata* Mesnil (Diptera: Tachinidae) have also been reported attacking larvae (Haye and Kenis, 2004). No parasitoids are known to attack lily leaf beetle adults. To rectify the lack of enemies in North America, classical biological control, the introduction of a natural enemy from the pest's native range to the introduced range, has been implemented using *T. setifer* (Tewksbury *et al.*, 2005). This larval parasitoid has been very successful, as a few years after releases in Rhode Island, Massachusetts, New Hampshire and Maine, USA, it attained parasitism levels of 37-100% (Massachusetts) and populations of lily leaf beetles began to decline (Tewksbury *et al.*, 2005).

The lily leaf beetle is rapidly dispersing throughout North America, underscoring the importance of investigating possible native host plants onto which the beetle can expand. The beetle has been documented on a wide range of hosts; in Europe it was found on over 18 plant species that are also native or naturalized in North America, mainly in the orders Liliales and Asparagales (as reviewed in Ernst, 2005). Additionally, Salisbury (2008) reported at least one life stage of lily leaf beetle on species in 23 plant genera, including 57 hybrid *Lilium*, 30 *Lilium*, one *Cardiocrinum* and five *Fritillaria* spp. However, a distinction must be made between plants on which adults can feed, those upon which they will oviposit and those that support larval development (Salisbury, 2008). To date, complete development has mainly been documented on *Lilium* spp. and *Fritillaria* spp. (Salisbury, 2008). However, Ernst (2005), documented complete development of one lily leaf beetle feeding on a *Streptopus* spp. Determining the host

range of the lily leaf beetle is important because as the beetle expands its North American host range, some plant populations may be susceptible to serious infestations that they may not be able to survive.

Previously, it was believed that in North America lily leaf beetle mainly attacked cultivated *Lilium* spp. and *Fritillaria* spp. Recent discoveries found lily leaf beetle on wild populations of *Streptopus lanceolatus* (Aiton) Reveal (Liliaceae), *Lilium michiganense* (Farwell) (Liliaceae) (Blackman *et al.*, 2016) and *Lilium canadense* L. (Liliaceae) in Ontario and Québec (Bouchard *et al.*, 2008). More than 20 native lily species are present in North America, many of which are protected in one or more US states (USDA, NRCS, 2016). The ranges of several species including wood lily *Lilium philadelphicum* L., Canada lily *L. canadense* L., Turk's-cap lily *L. superbum* L. and Michigan lily *L. michiganense* F., (Liliaceae), overlap with the eastern North American range of the lily leaf beetle. Therefore, it is important to investigate potential lily leaf beetle hosts, specifically native hosts that are protected or endangered. The objective of this study was to examine potential host plant species to determine which host species adult lily leaf beetle prefer either as a food choice or for oviposition, and which plant hosts are suitable for larval development. This information can then help predict which plant species may be at risk in a natural habitat.

## **MATERIALS AND METHODS**

### **A - Adult Host Plant Acceptance**

#### *Experimental Material*

##### *Plant Species*

Several potential hosts were included in this study: Canada lily, *L. canadense* L., Michigan lily, *L. michiganense* Farwell, Turk's cap, *L. superbum* L., Wood lily, *L. philadelphicum* L., Rose twisted stalk, *Streptopus lanceolatus* (Aiton) Reveal and Solomon's seal, *Polygonatum biflorum* Elliott (Walter) (Asparagaceae). Day lily, *Hemerocallis fulva* L. (Asphodelaceae), was included as a non-host and the normal host was Asiatic hybrid lily cultivars (Lily Looks Tiny series; Wetering Potlilium, Den Hoorn, The Netherlands). *L. canadense*, *L. michiganense*, *L. superbum*, *L. philadelphicum* and *S. lanceolatus* were chosen because they are protected in one or more states in the USA and their ranges overlap with that of the lily leaf beetle (USDA, NRCS, 2016).

##### *Insects*

Overwintered wild adult lily leaf beetles were collected in spring (May to mid-June) from Asiatic lily plants in residential gardens in the Ottawa area in 2015 and 2016. Adults were placed in 47.5 x 47.5 x 47.5 cm nylon rearing cages containing potted Asiatic hybrid lilies, with no more than 60 beetles per cage. New generation beetles were obtained from lab rearing and were kept separate from the collected overwintered beetles. Cages were maintained under a 16:8 h light:dark cycle,  $21.80 \pm 0.03$  °C and  $44.40 \pm 0.06$

RH. Adults were not collected after mid-June since there is the potential for new generation beetles to emerge at this time (Salisbury *et al.*, 2012).

### ***Field Experiments***

#### ***Plot Preparation***

In 2015 and 2016, potential host plant species were planted in a small plot at the Agriculture and Agri-food Canada's Central Experimental Farm in Ottawa, ON (45°23'18.5"N 75°42'45.2"W). The 10 m x 8 m plot was tilled and enriched with compost and manure. In 2015, straw mulch was used to inhibit weed growth between rows of host plants; in 2016, landscaping cloth was laid down between the rows before the straw was spread. In November 2015, a 3-foot high and 1-foot deep ¼ inch galvanized hardwire fence was constructed for protection against voles (Witmer, 2000).

In the 2015 season, plants were grown from bulbs in the greenhouse in February in 10 – 15 cm biodegradable pots and planted in the garden in early May. Plants used in the 2016 season were planted in fall 2015 in biodegradable pots; the pots were then sunk into the ground in an outdoor garden adjacent to the common plot, covered with approximately 10 cm of straw and left to overwinter naturally. This assured that growth in the spring would occur on a more natural schedule.

### ***Experimental Plot Design***

Host plants were transplanted into the plot using a stratified random design (Figure 1). The plot contained 10 rows, each 1 m apart, with plants in each row 30 cm apart. In summer 2015, *L. philadelphicum* was planted in the plot; however, due to poor growth it was excluded in summer 2016 and *L. michiganense* was planted in its place.



**Figure 1:** Stratified random design of experimental plot, including eight plant species.

In 2015, due to poor growing conditions the following number of each species was planted: five *L. philadelphicum*, 38 *H. fulva*, 24 *L. canadense*, 21 *L. superbum*, 18 Asiatic (Tiny Dancer) and 17 *P. biflorum*. In the 2016 field season, the following number of each species was planted: 37 *L. michiganense*, 31 *L. canadense*, 34 *L. superbum*, 13 *S. lanceolatus*, 36 *H. fulva*, 34 Asiatic hybrids (Tiny Dancer and Tiny Bee) and 11 *P. biflorum*. At the beginning of each season, when most of the plants were fully developed, the following biomass indices were recorded: number of leaves, length and width of leaves. A border of flowering Alyssum (*Lobularia maritima* (L.) Desvaux

(Brassicaceae)) was planted around the perimeter of the experimental plot to create a more consistent surrounding and to provide an energy source for the parasitoid *T. setifer*. The plot was weeded approximately once every two weeks and watered every couple of days; however, neither were performed during beetle or larvae releases.

### ***Weekly Field Surveys***

In 2015 and 2016, the experimental plot was surveyed once a week from May until plant senescence in early August by counting the number of lily leaf beetle adults and eggs on each plant and estimating percent damage. Lily leaf beetle individuals were removed after each survey. Multiple beetle releases were made throughout the season, therefore any leftover beetles from the experimental releases were likely recovered during weekly surveys.

### ***Adult Beetle Field Releases***

Due to the low population of lily leaf beetles in the experimental plot, multiple releases in 2015 and 2016 were made using beetles that had been collected from Ottawa-area gardens and held in rearing cages with Asiatic lilies for food. Prior to release, beetles were held at 5 °C for four hours in order to slow them during release. In May 2015, 35 overwintered individuals were released randomly (1 – 2 beetles dropped between random plants) into the plot. The plot was then surveyed the next day and adults and eggs were collected to protect the plants from further damage. A second release of 25 beetles was done in the same way in June 2015; however, all Asiatic lilies were covered to assess

preference in the absence of the beetle's normal host. Each Asiatic lily was covered with a breathable plastic bread bag and tied tightly with string at the base of the plant to ensure that the beetles had no access to these plants. In June 2016, the first release was done with 50 beetles and the second release (Asiatics covered) was also done in June with 50 beetles as well following the above procedures. An additional release in July 2016 was made with 106 new generation beetles, obtained from lab rearing.

### ***Estimated Plant Biomass Consumed in Field***

To account for the variety of leaf shape, size and thickness among the host plants in the plot, estimated biomass consumed (EBC) was calculated using the formula:

$$(1) \text{ EBC (g)} = (\text{proportion of leaf area damaged}) \times (\# \text{ leaves}) \times (\text{average biomass (g/cm}^2\text{)}) \\ \times (\text{average leaf area (cm}^2\text{)})$$

This estimate included overall damage from lily leaf beetle adults and larvae. This method was only used in 2016, as plants were too small in 2015 to estimate damage. In order to calculate EBC, at the beginning of the season after most plants had completed growth, the number of leaves was counted for each plant. At the end of the season, or before each plant senesced, the proportion of leaf area eaten was estimated.

To calculate the average biomass per cm<sup>2</sup> for each species, ten 0.5 cm - diameter (total disk area of 1.96 cm<sup>2</sup>) leaf disks were cut from one leaf from each of 15-20 individuals of each species. Leaves of *L. philadelphicum* were small; therefore, five disks (total disk area of 0.98 cm<sup>2</sup>) were sampled per leaf for 30 leaves of 30 individuals. Each

sample of 5-10 disks was then placed in a metal weighing tray in a drying oven set at 60 °C for 48 h. The samples were weighed on a Mettler Toledo AG245 scale ( $\pm 0.0001\text{g}$ ). Dry weight for each sample was divided by applicable total disk area and an average biomass ( $\text{g}/\text{cm}^2$ ) for each species was obtained.

In order to determine average leaf size of each species, 20 random leaves of each species (30 for *L. philadelphicum* due to its more variable size) were photographed with a Canon Power Shot SD 1300 IS camera. Leaf area was obtained by tracing leaves in ImageJ64 software (<http://imagej.net/>). The averages were as follows: *L. philadelphicum*  $2.07 \pm 0.11 \text{ cm}^2$  ( $n = 30$ ), *L. superbum*  $3.27 \pm 0.35 \text{ cm}^2$  ( $n = 20$ ), *L. michiganense*  $5.18 \pm 0.44 \text{ cm}^2$  ( $n = 20$ ), *L. canadense*  $3.40 \pm 0.28 \text{ cm}^2$  ( $n = 20$ ), Asiatic hybrids  $7.14 \pm 0.47 \text{ cm}^2$  ( $n = 20$ ), *S. lanceolatus*  $16.99 \pm 0.92 \text{ cm}^2$  ( $n = 20$ ) and *P. biflorum*  $16.30 \pm 1.85 \text{ cm}^2$  ( $n = 20$ ). Due to the size of *H. fulva* leaves, length was simply multiplied by width of 20 random leaves to obtain the average leaf area ( $68.85 \pm 5.53 \text{ cm}^2$ ). The four values obtained were substituted into formula (1) to obtain estimated biomass consumed.

## **Laboratory Experiments**

### ***No-Choice Oviposition and Adult Feeding***

From 11 – 24 plants per species were utilized to test whether each plant species was suitable as an oviposition host for the lily leaf beetle (Asiatic variety Tiny Shadow was used for this section). These trials were conducted over two successive seasons due to the limited availability of plants. In 2015, plants were cut and kept at 5 °C until used. When used in trials, they were held upright in 12 cm waterpics (Aquapic) filled with

water in a 125 ml flask and placed in a 32.5 x 32.5 x 32.5 cm nylon cage. In 2016, plants were taken from the field when at least 10cm in height. Leaves that had damage were removed and the rest of the plant was washed with distilled water, potted in a small plastic 5 cm diameter pot and placed into a 30 x 30 x 30 cm plastic cage in the laboratory. One overwintered copulating pair was placed into each experimental cage and left undisturbed for 72 hours to mate, feed and lay eggs. After 72 hours, beetles were removed and the number of eggs on the plant, cage and vial were recorded. Cages for these trials were randomly placed on shelves lit with fluorescent bulbs using a 16:8 light:dark cycle. The room conditions for the 2015 season was  $23.5 \pm 1.0$  °C and the 2016 season were  $20.9 \pm 0.2$  °C,  $43.8 \pm 0.1$  RH . Some plants were used multiple times if numbers of that species were few; however, as stated above, damaged leaves were always removed and the plant washed prior to use.

In order to determine which host plant was preferred for adult feeding, estimated biomass consumed was calculated after each replicate from the above no-choice oviposition trials (72 h). Prior to the commencement of the oviposition trials, the number of leaves was recorded for each plant. After the trials were complete, leaves were photographed (Canon Power Shot SD 1300 IS) from approximately a 30cm distance. Leaf area consumed in each trial was calculated by tracing the area of leaves that were consumed by beetle in software ImageJ64. If there were missing leaves (whole leaves eaten) average values from the field estimates of leaf area were used. The total leaf area consumed per trial was calculated by adding all the ImageJ64 consumed leaf area tracings and if applicable, addition of missing leaf values for that specific trial.

Different leaf thicknesses for the species were also accounted for by using the leaf disks as described above in the estimated biomass methods. The estimated biomass consumed was calculated with the following formula:

$$(2) \text{ EBC(g)}_{(\text{no-choice trials})} = \text{Average biomass (g/cm}^2\text{)} \times \text{Total Leaf Area Consumed (cm}^2\text{)}$$

## **B - Larval Performance on Potential Novel Hosts**

### ***Plants and Insects***

The performance of lily leaf beetle larvae on *L. canadense*, *L. michiganense*, *L. philadelphicum*, *L. superbum*, *S. lanceolatus*, *P. biflorum*, *H. fulva* and Asiatic lilies (Tiny Dancer) was tested from June to August 2016. Leaves of tested plant species were obtained from the field plot and stored in plastic bags at 5 °C until needed.

Wild adult lily leaf beetles were collected from May to mid-June 2016 from Asiatic lilies in residential gardens in the Ottawa area and placed in holding cages. Cages were inspected regularly for larvae, which were moved into a separate cage and used when needed.

### ***No-Choice Host Suitability Experiment***

For each trial, one mid- to late-instar larva was placed individually using a soft paintbrush on a test plant leaf in a 9.0 x 2.5 cm petri dish. The petri dishes were placed on a shelf under 16:8 light/dark cycle with room conditions of  $23.10 \pm 0.04$  °C,  $47.7 \pm 0.92$  RH. Due to a shortage of insects, a second trial was run at the same time. The biological

control agent, *T. setifer*, was introduced to each petri dish 24 h after the beetle larvae were placed on the leaf. Therefore, this experiment tested food source acceptability in the presence of an enemy. Twenty-four hours after the introduction of the parasitoid the following variables were recorded: a) larva initiated feeding versus did not; b) larva on leaf versus wandering; c) larva made a fecal shield versus did not; d) larva survived versus did not. The number of replicates ranged from 8 - 42 larvae for each of the eight plant species tested. Due to limited insect material, complete larval development tests on each species were not performed. A 48 h trial time was chosen for this study because according to Ernst (2005) and Kealey (2013), results regarding proportion of larvae surviving on each plant species were relatively clear after this length of time.

### ***Recovery Rate of Larvae on Various Host Plant species in the Field***

In 2015 and 2016, the suitability of host plants in the presence of the biological control agent *T. setifer* was tested in the plot using larvae that had been reared in the lab on Asiatic lilies. One late-instar beetle larva with fecal shield intact was placed on each plant using a soft paintbrush and left for 24 h. At the same time, 100 *T. setifer* adults were released into the plot at random locations, supplementing the natural population present at the site. The number of larvae recovered on each plant was recorded. In June 2015, larvae were placed on 17 Asiatic lilies (Tiny Dancer), 17 *L. philadelphicum*, 13 *L. canadense*, 6 *L. superbum*, 13 *S. lanceolatus* and 14 *P. biflorum*. In 2016, this experiment was repeated three times: twice in June and once in July. Larvae were placed on 70 Asiatic Lilies (Tiny Dancer and Tiny Bee), 66 *L. philadelphicum*, 67 *L. canadense*, 74 *L. superbum*, 76 *L. michiganense*, 75 *S. lanceolatus*, 43 *P. biflorum* and 39 *H. fulva*.

## **C - Adult Host Acceptance Versus Larval Performance**

The eight host plant species were ranked according to their values for each adult preference and larval performance variable, with the highest rank assigned to the most suitable plant species. Ranks for the three adult preference variables (overwintered beetle field release, no-choice oviposition and no-choice food) were summed then averaged to create an overall preference index. Likewise, a performance index was calculated as the average of the ranks of the five larval performance variables from the no-choice host suitability trials (% larvae initiating feeding, % remaining on the plant, % producing a fecal shield, % surviving 48 hours and % recovered from the field trial).

## **DATA ANALYSES**

All statistical comparisons were made with SPSS version 24.0 (IMB Corp., 2015). Field data on host plant colonization by naturally occurring adult beetles were analyzed by comparing median values of lily leaf beetle specimens (eggs and adults) found on each plant species using the non-parametric Kruskal-Wallis H test because the data did not meet the assumptions of parametric tests. When the overall effects were significant, post-hoc pairwise comparisons were performed using Dunn's test (1964) with a Bonferroni-corrected significance level of  $\alpha = 0.0024$ . Colonization of host plants in the field was only analyzed for the 2016 season, as the native plants were far too small in 2015. The same analysis was also done for estimated leaf biomass consumed in the field. The beetle preference following releases of known numbers of adults was analyzed in the same manner; however, data from both field seasons were combined and analyzed as one set. This allowed for three separate analyses: overwintered beetle release, overwintered beetle

release excluding Asiatics and new generation beetle release. The Bonferroni-corrected significance level for the above analyses was  $\alpha = 0.0018$ .

Adult host acceptance laboratory experiments, no-choice plant consumption and oviposition experiments were also analyzed with Kruskal-Wallis H tests. When main effects were significant, post-hoc pairwise comparisons were performed using Dunn's test (1964) using a Bonferroni-corrected significance level of  $\alpha = 0.0018$ . A Chi-squared test was utilized to determine whether the binary outcomes of the laboratory no-choice larval performance trials differed among plant species. When a significant effect of species was obtained, post hoc pairwise comparisons via 2 x 2 Fisher's exact tests were performed using a Bonferroni-corrected significance level of  $\alpha = 0.0018$ .

The effect of host plant species on larval recovery in the field trial (yes/no) was analyzed via a Chi-squared test. Data from both field seasons were combined for this analysis. A difference was noted and pairwise comparisons were also made using 2 x 2 Chi-squared tests, using the Bonferroni-corrected significance level of  $\alpha = 0.0018$ .

Lastly, the relationship between the larval performance index and the adult host acceptance index was analyzed using Pearson's Correlation.

## **RESULTS**

### **A - Adult Host Plant Acceptance**

#### ***Adult Preference of Hosts Plants in Field***

In 2016, Asiatic hybrids and *L. superbum* were more frequently visited by naturally occurring adult beetles in comparison to the other plants tested (except for *L. michiganense* and *L. canadense*) (Figure 2). Less frequent visits were made to *L. canadense* and few to none were made to *S. lanceolatus*, *P. biflorum* and *H. fulva*. The 2015 experiment was considered to be a pilot study because the native plants were very small compared to the large Asiatic lily plants. Therefore, data from 2015 were not analyzed; however, there was trend in such that Asiatic hybrids were more frequently visited than the tiny native plants (Figure 2).

#### ***Adult Beetle Releases: Preference for Host Plants in Field***

When Asiatic lilies were present as a choice in the plot, lily beetles significantly preferred this host (were recovered more often and laid more eggs) to any of the native hosts. This was documented in releases of both overwintered beetles (Figure 3) and new generation beetles (Figure 4). The only exception was in the new generation beetle release, *L. superbum* and *L. michiganense* were also preferred (Figure 4).

When Asiatic hybrids were covered, after 24 h, beetles were recovered from the four native *Lilium* species as well as *S. lanceolatus*; none were found on *P. biflorum* and *H. fulva* (Figure 5).

### ***Adult Preference: Overall Field Plant Damage***

Estimated biomass consumed indicated that adult lily leaf beetles preferred to forage on Asiatic hybrid lilies and *L. michiganense*, followed by *L. superbum* and *S. lanceolatus* (Figure 6). However, the latter species were not significantly different than hosts preferred less such as *L. canadense*. Hosts preferred the least were *P. biflorum*, followed by *H. fulva*. No damage attributed to the lily leaf beetle was documented on *H. fulva*.

### ***Lab No-Choice Oviposition and Feeding Test***

In no-choice 72 h oviposition trials, there was no significant difference between median number of eggs laid on each host plant species (Figure 7). That being said, Asiatic hybrids had the highest median number of eggs (three eggs) in comparison to the other potential hosts. Despite the similarity in oviposition among all plant species, eggs laid on *P. biflorum* were often dried out and shriveled, and laid in a scattered manner, unlike the neat arrangement on *Lilium* species. Scattered and shriveled eggs also occurred on *S. lanceolatus*; however, this did not occur as frequently as on *P. biflorum*.

Adults foraged on all host plant species, but fed more, in terms of estimated biomass consumed, on *L. philadelphicum*. This plant was consumed significantly more than Asiatic hybrid, *L. canadense*, *S. lanceolatus* and *P. biflorum*. Plants fed on intermediately were *L. michiganense* and *L. superbum* (Figure 8).

## **B - Larval Performance on Potential Novel Hosts**

### ***No-Choice Host Suitability Experiment***

Percentage of larvae that initiated feeding on each plant species in no-choice experiments were similar, except for *P. biflorum* and *H. fulva*, which were fed on significantly less (Figure 9). Percentage of larvae that stayed on each plant species yielded mixed results, with a significant difference among various plant species (Figure 10). The percentage of larvae remaining on four of the potential hosts — *L. superbum*, *L. canadense*, *L. michiganense* and *S. lanceolatus* — was not significantly different from that of Asiatic hybrids. Significantly fewer larvae stayed on *L. philadelphicum*, *P. biflorum* and *H. fulva* than Asiatic hybrids.

Percentage of larvae that formed a fecal shield was also different among plant species; however, those placed on *Lilium* spp. attained similar high percentages (Figure 11). Larvae placed on *P. biflorum* and *H. fulva* never formed a fecal shield and were significantly different from larvae placed on all other plant species. Larvae placed on *S. lanceolatus* constructed fecal shields significantly less frequently in comparison to those on Asiatic hybrids, *L. canadense* and *L. superbum*.

The percentage of larvae that survived differed significantly among plant species (Figure 12). Larvae placed on *H. fulva* survived significantly less frequently in comparison to those placed on other plant species except *S. lanceolatus* and *P. biflorum*. There was no significant difference in larval survival between *Lilium* species and *S. lanceolatus*.

### ***Recovery Rate of Larvae on Various Host Plant species in the Field***

There were significant differences in the percentage of larvae recovered among plant species (Figure 13). Recovery rates of larvae on all five *Lilium* species were high and did not differ significantly. The recovery rate of larvae on *S. lanceolatus* was significantly lower than recovery rates on all *Lilium* species, and significantly higher than *P. biflorum* and non-host *H. fulva*. It is important to note the variation between seasons 2015 and 2016 for recovery rates of larvae placed on *S. lanceolatus*, as the average in the first season was 88% (n = 16) and in 2016 was 25% (n = 75).

### **C - Adult Host Acceptance Versus Larval Performance**

Larval performance was strongly correlated with adult plant acceptance (Table 2, Figure 14;  $r = 0.870$ ,  $p = 0.005$ ). In all performance experiments larvae performed best on Asiatic hybrids. However, this was not the case for adult preference experiments, where Asiatic hybrids were ranked as the third most accepted host (Table 2). Adults seemed to accept *L. philadelphicum* and *L. superbum* to Asiatic hybrid lily in the majority of tests. It should be noted that these tests often consisted of no-choice tests. When given a choice, the adult beetles always preferred Asiatic lilies or hybrids as seen in Figures 2-4. *H. fulva* was the least accepted host for both larvae and adults.

## DISCUSSION

All plant species studied, with the exception of non-host *H. fulva*, are potentially suitable hosts for the lily leaf beetle. In the field, adult lily leaf beetle strongly preferred Asiatic hybrid lilies compared to all other plant species (Figures 2-4). However, despite this strong preference, some adult beetles chose the novel hosts or may have used multiple hosts in addition to the Asiatic hybrid lilies, as seen in field release experiments and in the overall damage of plants at the end of the season (Figure 2-4, 6). This suggests that even if Asiatic lilies are present in the same environment, some adult beetles will move onto native hosts, especially native *Lilium* spp. Even in the absence of Asiatic hybrids, 36% of released beetles were recovered on native species (*Lilium* spp. and *Streptopus* spp.). Although this is a much lower recovery rate in comparison to the 62% of beetles that remained when Asiatic lilies were present, it demonstrates the potential for the lily leaf beetle to colonize native plants (Table 1). Generally few to no lily leaf beetle individuals were found on *S. lanceolatus* and *P. biflorum*, suggesting that on the rare occasion the beetle had an option in a natural setting, it would be the least favorable choice.

All potential hosts, *H. fulva* excluded, were consumed to some extent in adult no-choice consumption experiments (Figure 8) and all seven were suitable as hosts in no-choice oviposition tests (Figure 7). Although *S. lanceolatus* and *P. biflorum* were accepted as oviposition hosts in the no-choice trials, eggs on these two plants were often dried and shriveled, making the suitability of these hosts for oviposition questionable.

Larval consumption results provide a more realistic predictor of the capability of lily leaf beetle to survive on novel hosts. The no-choice larval feeding data indicated that

six of the tested plant species were more suitable when comparing response variables (initiated feeding, stayed on leaf, formed fecal shield and survived 48 h) than *P. biflorum* and *H. fulva* (Figure 9 – 12). However, *P. biflorum* may be a suitable host for adults, as many gardeners and I have noted adult lily leaf beetles feeding on this species. It should be noted that the no-choice larval trials were performed in the presence of one female *T. setifer*. This set-up was not ideal; however, the limited number of larvae available necessitated combining trials involving host suitability (this chapter) and ability of *T. setifer* to locate hosts on different plant species (Chapter 3). On the other hand, this setup allowed for a realistic simulation of a natural environment, where enemies are present, potentially allowing for more natural behavior to occur.

Results from the larval field release study supported the 48 h laboratory results. The field study tested the suitability of each host plant species in a more natural setting, exposing larvae to a variety of abiotic factors (wind, temperature, rain) and biotic factors (*T. setifer*, as well as other potential enemies). These results indicated once again that the true lilies—Asiatic hybrids, *L. superbum*, *L. philadelphicum*, *L. canadense* and *L. michiganense*—were the most suitable hosts for lily leaf beetle larvae (Figure 13). *S. lanceolatus* was a somewhat intermediate host with recovery at 36%. The low recovery rates on *P. biflorum* and *H. fulva* indicated that the larvae were either rejecting these hosts or experiencing higher mortality on them in the field plot.

The above results were consistent with those of other studies. Lily leaf beetle larvae were able to survive as well on *L. canadense* and *L. philadelphicum* as on Asiatic hybrid *Lilium* cultivars (Ernst *et al.*, 2007; Bouchard *et al.*, 2008). When reared on *L. canadense* in comparison to Asiatic hybrid *Lilium* cultivars, fewer adults emerged

(Bouchard *et al.*, 2008). This is consistent with the lower adult preference for this species in the field and in no-choice feeding tests (Figures 2-5, 8).

There were also mixed results in regards to larval host suitability on *S. lanceolatus*, a host on which the beetle has been reported feeding and ovipositing in the understory of the Gatineau Park in Québec, Canada (Kealey *et al.*, 2013; Blackman *et al.*, 2016). Although small larvae have been seen at this location, no late-instar larvae have been recorded (Blackman *et al.*, 2016), indicating either that *S. lanceolatus* is of low suitability as a food plant or that mortality caused by other factors, such as natural enemies is high. A laboratory study by Ernst *et al.* (2007), found that larvae fed *S. lanceolatus* [= *S. amplexifolius* (L.) DC] were unable to develop to the adult stage. In contrast, in a second laboratory study, Kealey (2013) found that 42% of larvae were able to complete development to the adult stage on *S. lanceolatus* [= *S. amplexifolius* (L.) DC]. Mixed results were also seen in this study as in season 2015, 24-hour field recovery rates of larvae were 88% (n=16); however, in season 2016 recovery rates were low at 25% (n=75). Larvae used in all these experiments came from different sources suggesting that natural genetic variation among individuals and populations of the lily leaf beetle can affect the ability of this species to incorporate novel food plants into its diet. Genetic variation among populations has been shown to influence host range expansion in the apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae) (Prokopy *et al.*, 1988), tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae) (Schneider and Roush, 1986), and lice species in the genera *Physconelloides* and *Columbicola* (Johnson *et al.*, 2002).

Futuyma *et al.* (1995) demonstrated that the host range expansion of an herbivore could be predicted by considering the hosts of its closest relatives. Although the beetles did not perform well on *P. biflorum* in the present study, it should be noted again that various gardeners have observed the beetle on this plant species in gardens. Laboratory feeding trials by Ernst *et al.* (2007), documented that this host was relatively suitable for larval development; however it was not comparable to *Lilium* spp. A close relative of the lily leaf beetle, *Lilioceris merdigera* L., feeds on plants within the genus *Polygonatum* (Haye and Kenis, 2004). Common ancestry might explain the tendency of lily leaf beetle to recognize *Polygonatum* as a potential host. This possibly explains the occasional reports of the beetle on *P. biflorum* in gardens.

On a different note, some novel host plants that were extremely suitable for the lily leaf beetle to expand onto—*L. superbum* and *L. philadelphicum*—have not been reported as hosts in natural environments. Larvae performed extremely well on the hosts in comparison to the normal Asiatic hybrid host (Figure 9-13). In previous studies, adult emergence, proportion of larvae surviving and efficiency of conversion of ingested food were higher on *L. philadelphicum* in comparison to Asiatic hybrids (Ernst, 2005; Bouchard *et al.*, 2008). Although larval development time on *L. philadelphicum* was longer than on Asiatic hybrids, weights of pre-pupae and adults were similar (Ernst, 2005; Bouchard *et al.*, 2008). Bouchard *et al.* (2008), hypothesized that the beetle may not have expanded onto this host as it potentially was not suitable for oviposition. However, the present study demonstrated in no-choice experiments that *L. philadelphicum* is suitable for oviposition and the beetle will lay relatively the same number of eggs as on the Asiatic hybrids (Figure 7). Therefore, potentially the beetles simply have not

expanded into locations containing these hosts and will eventually include them in their host range.

Females of certain insect species order host plants for oviposition in terms of how well their larvae can develop on those hosts; however, these correlations are often weak (Jaenike, 1990). In the present study, it appears that adult lily beetles ranked hosts in accordance to which host plants were most suitable for larvae to develop on (Figure 14). There were minor deviations, for instance the Asiatic host was always the host the larvae performed best on; however, in the oviposition tests, some native lilies received more eggs instead. This is not surprising as larvae still performed extremely well on most of the native lily hosts (Figure 9-12).

Although the lily leaf beetle was not as successful on all native plant species offered in these trials as it was on its normal hybrid *Lilium* host, there are many insects that develop in suboptimal conditions on novel hosts. For instance butterflies *Pieris napi macdunnoughii* Remington and *P. occidentalis* Reakirt (Lepidoptera: Pieridae) have been found to oviposit on certain crucifers, despite the negative consequences of slower larval growth and even mortality (Chew, 1977). Additionally the ovipositional preferences of the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) did not correlate with larvae performance levels (growth) on several of its host plants (Fox and Eisenbach, 1992). One possible explanation for these host switches is the ability to attain enemy-free space by switching host plants. Enemy-free space is defined as ways an insect lives that reduces or even eliminates its vulnerability to natural enemies (Jeffries and Lawton, 1984).

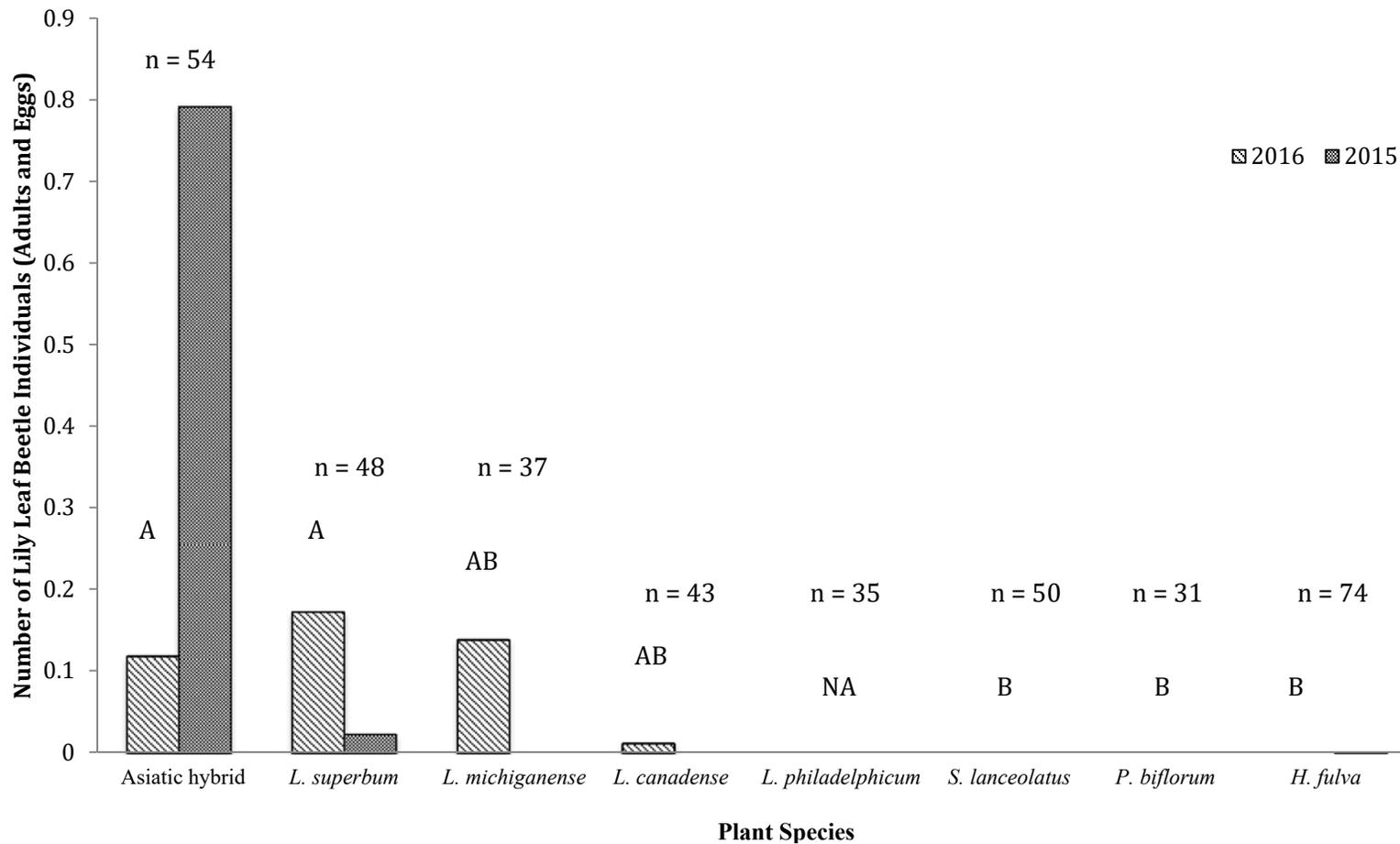
In conclusion the results of this study demonstrate that the lily leaf beetle has the capability to further expand its host range, particularly to native North American host plant species. This is of utmost importance as many of the hosts studied are protected species and may not be able to maintain populations if a serious lily leaf beetle infestation occurs. Investigations on the ability of *T. setifer* are therefore extremely important to determine if the parasitoid can find its host if the beetle moves onto some of these vulnerable plant populations.

**Table 1:** Recovery of beetles from different host plants 24 h after release in field plot. Table shows percent beetle recovery using combined data over field seasons 2015-2016. Numbers in parentheses are total number of beetles recovered on each plant species from each release.

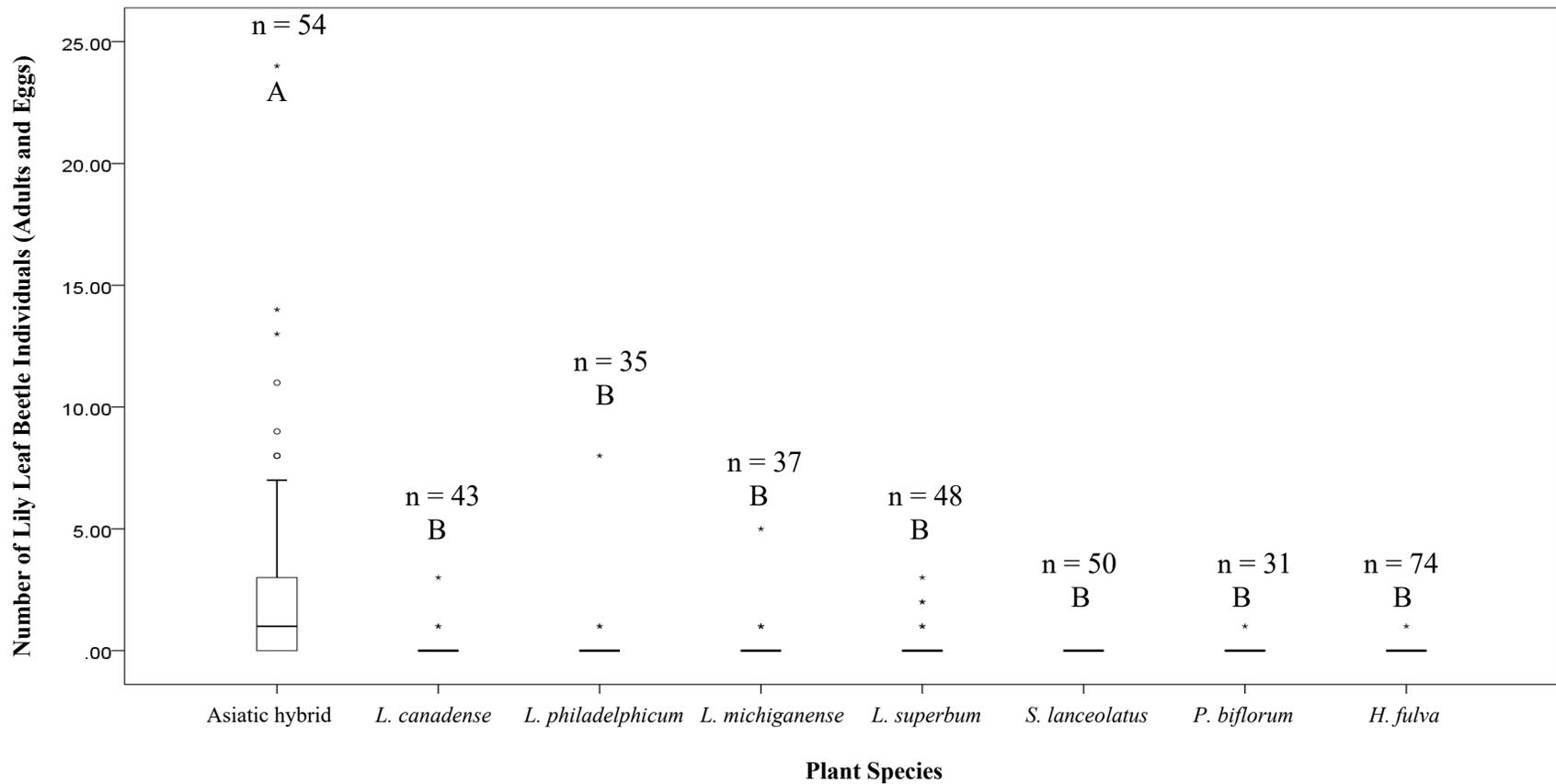
Beetle Release Type (# beetles released)	Percent Beetles Recovered (total beetles recovered)							
	Asiatic	<i>L. superbum</i>	<i>L. michiganense</i> **	<i>L. canadense</i>	<i>L. philadelphicum</i>	<i>S. lanceolatus</i>	<i>P. biflorum</i>	<i>H. fulva</i>
All plants (n = 100)	75.4 (49)	10.8 (7)	4.6 (3)	4.6 (3)	3.1 (2)	0.0	0.0	1.5 (1)
No Asiatics (n = 85)	NA	29.0 (9)	16.1 (5)	12.9 (4)	38.7 (12)	3.2 (1)	0.0	0.0
*New generation beetles (n = 106)	52.0 (13)	16.0 (4)	32.0 (8)	0.0	0.0	0.0	0.0	0.0

\* New generation beetle release only for season 2016.

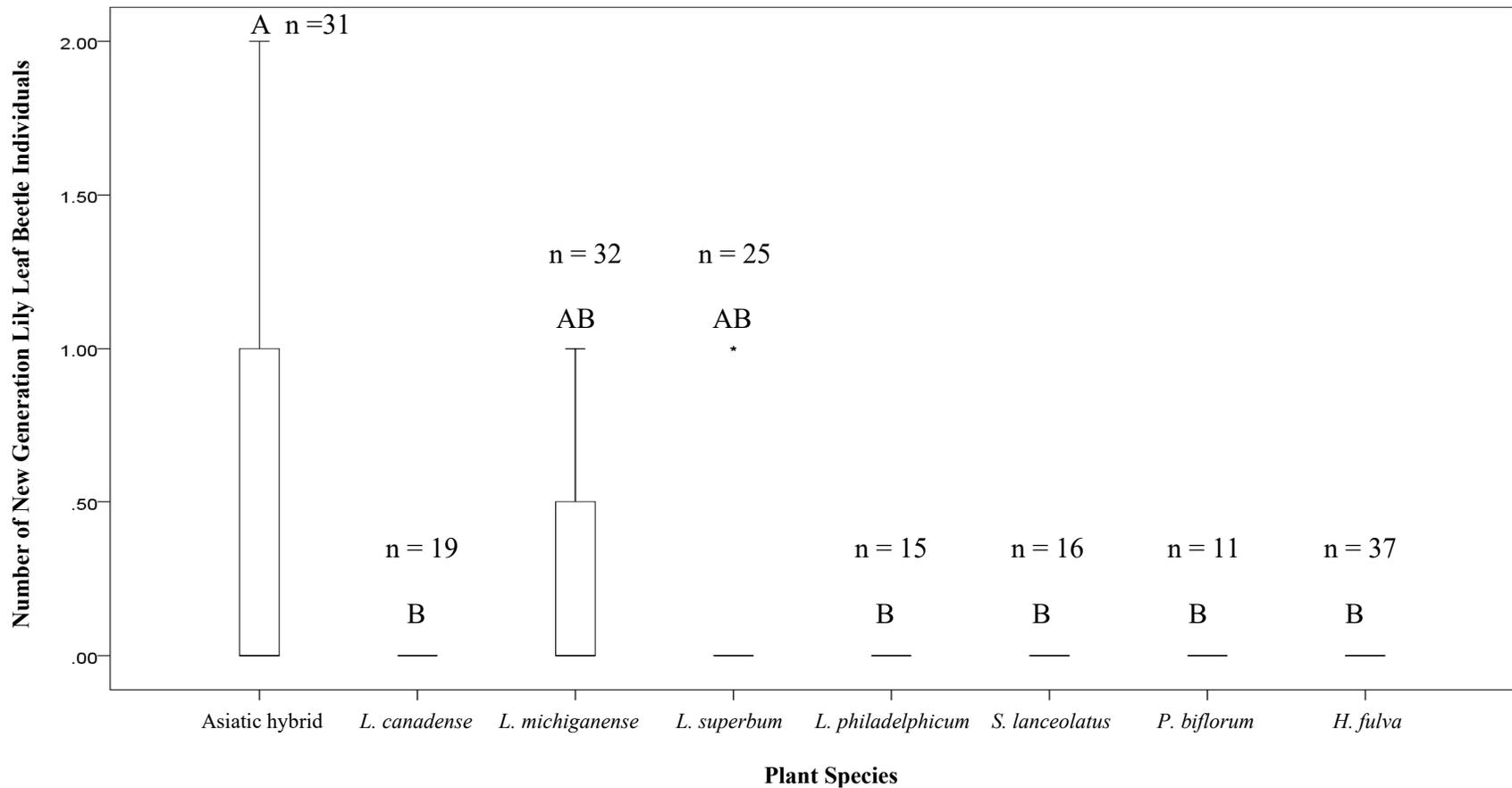
\*\* Only 2016 data.



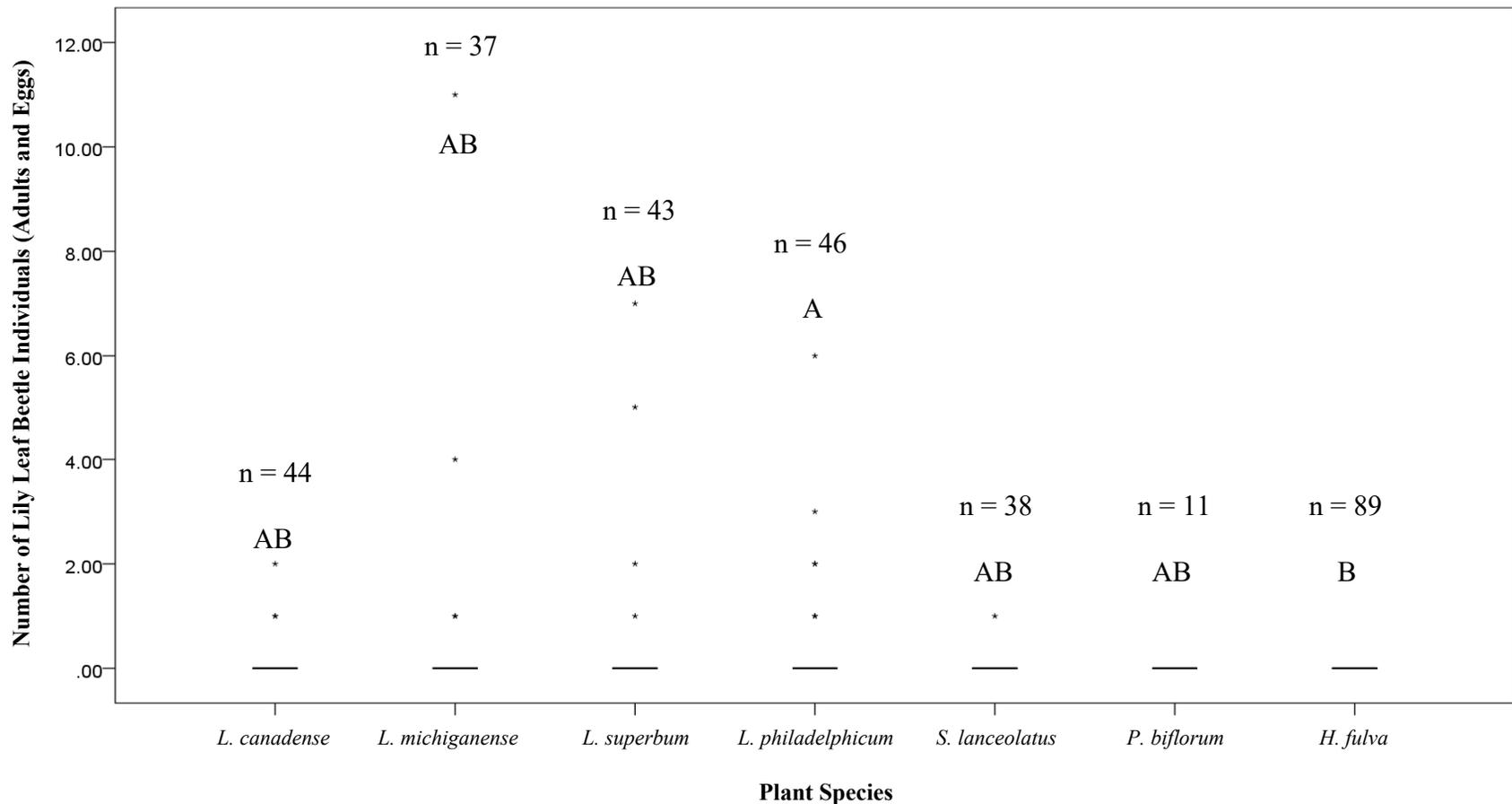
**Figure 2:** Number of lily leaf beetle individuals (adults and eggs) on various potential host plant species in weekly surveys during the 2015 and 2016 field seasons. There was a significant difference between numbers of lily leaf individuals on different plant species, analysis only for season 2016 (Kruskal-Wallis test;  $H = 32.114$ ,  $df = 6$ ,  $p < 0.001$ ). Species with different letters denote significantly different medians according to Dunn's test (1964).



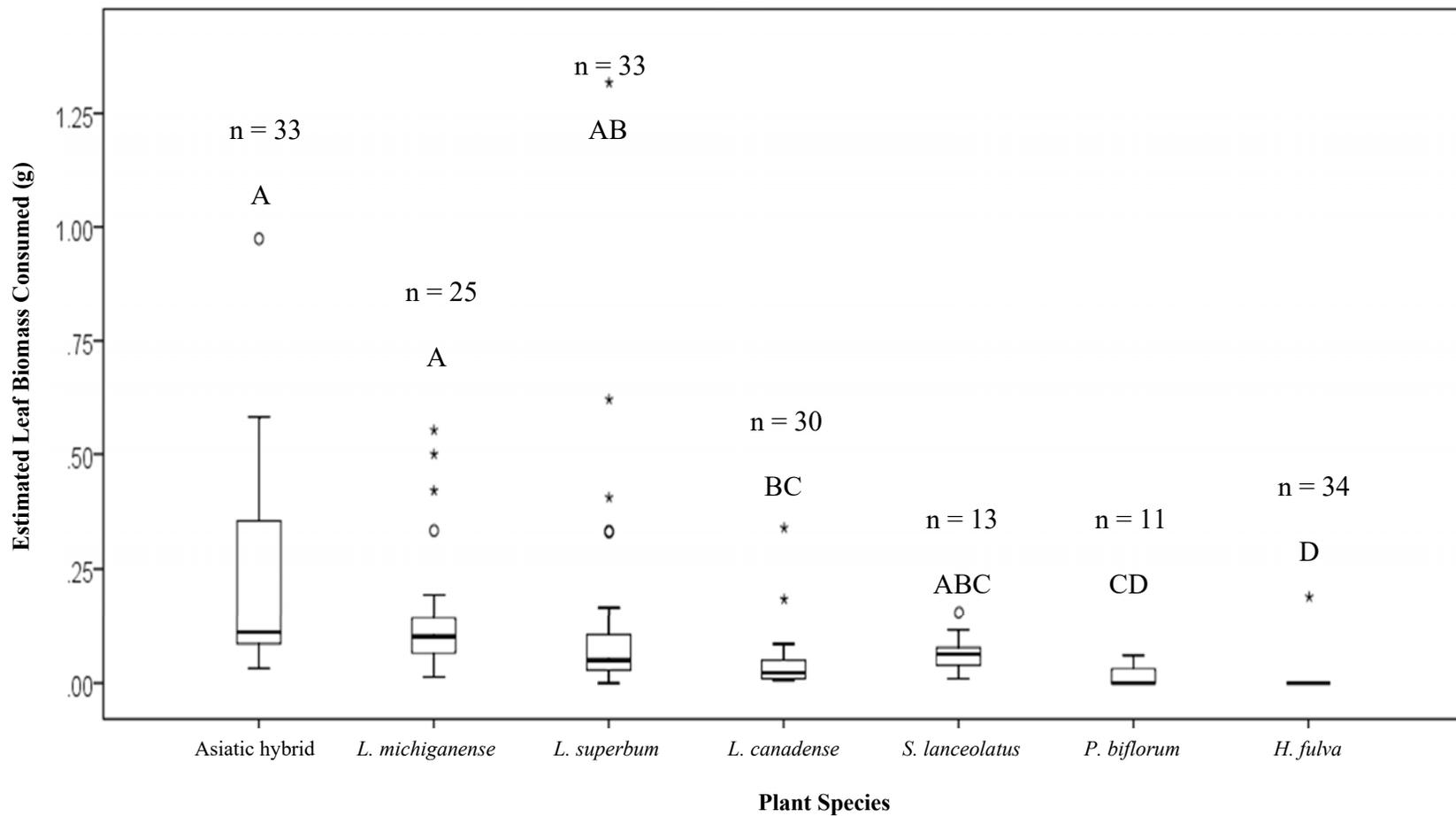
**Figure 3:** Number of lily leaf beetle individuals (adults and eggs) on various potential host plant species in combined 2015 and 2016 choice 24 h beetle field releases of 100 beetles total. There was a significant difference between numbers of lily leaf beetle individuals on different plant species (Kruskal-Wallis test;  $H = 111.940$ ,  $df = 6$ ,  $p < 0.001$ ). Species with different letters denote significantly different medians according to Dunn's test (1964). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Outliers denoted by dots and extreme outliers denoted by asterisk.



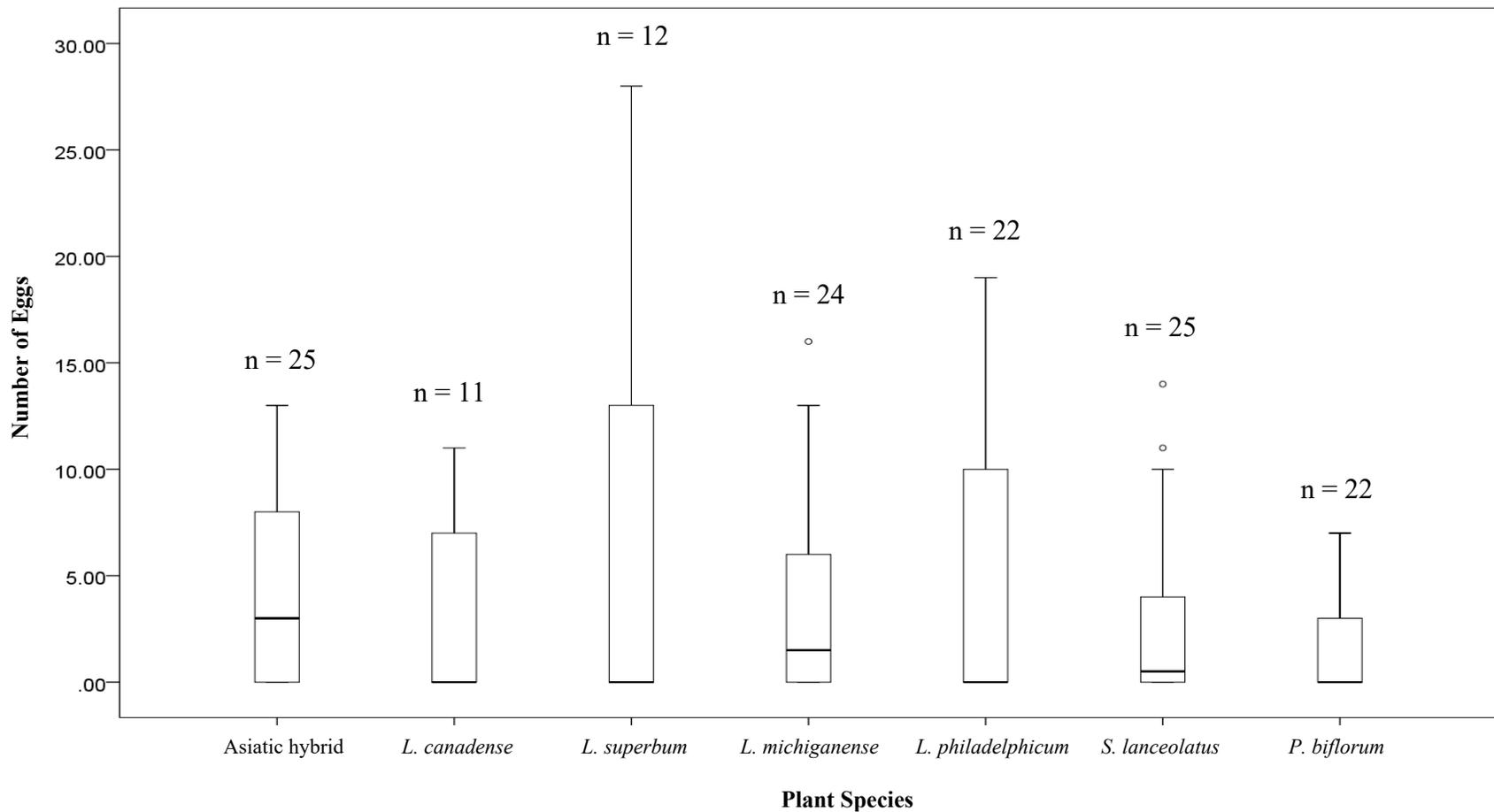
**Figure 4:** Number of lily leaf beetle individuals (adults and eggs) on various potential host plant species in 2016 choice 24 h new generation beetle field release of 106 beetles total. There was a significant difference between numbers of lily leaf beetle individuals on different plant species (Kruskal-Wallis test;  $H = 37.233$ ,  $df = 6$ ,  $p < 0.001$ ). Species with different letters denote significantly different medians according to Dunn's test (1964). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Extreme outliers denoted by asterisk.



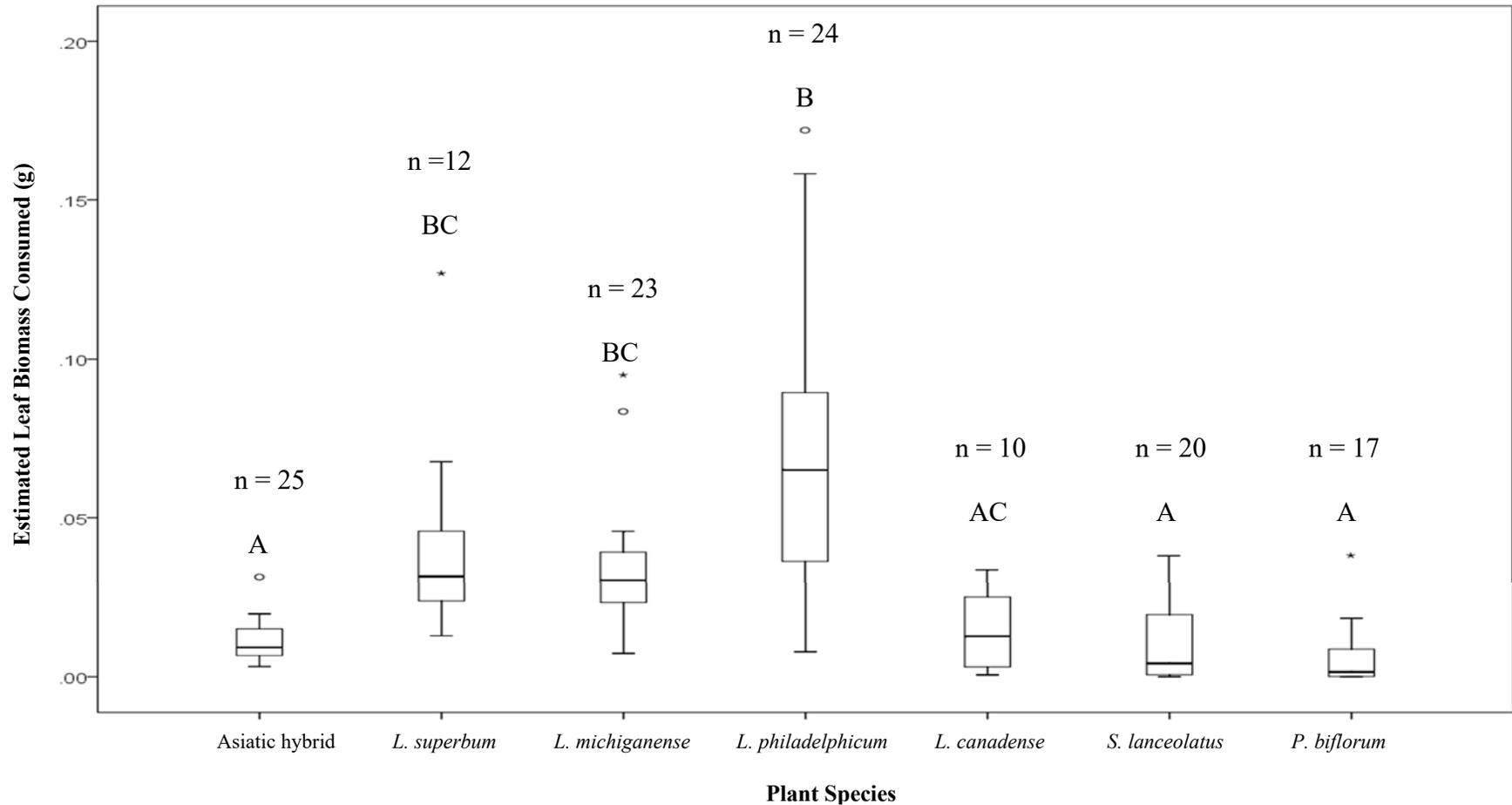
**Figure 5:** Number of lily leaf beetles individuals (adults and eggs) on various potential host plant species, when normal Asiatic host excluded, in combined 2015 and 2016 choice 24 h beetle field release of 85 beetles total. There was a significant difference between numbers of lily leaf beetle individuals on different plant species (Kruskal-Wallis test;  $H = 14.024$ ,  $df = 6$ ,  $p < 0.05$ ). Species with different letters denote significantly different medians according to Dunn's test (1964). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Extreme outliers denoted by asterisk.



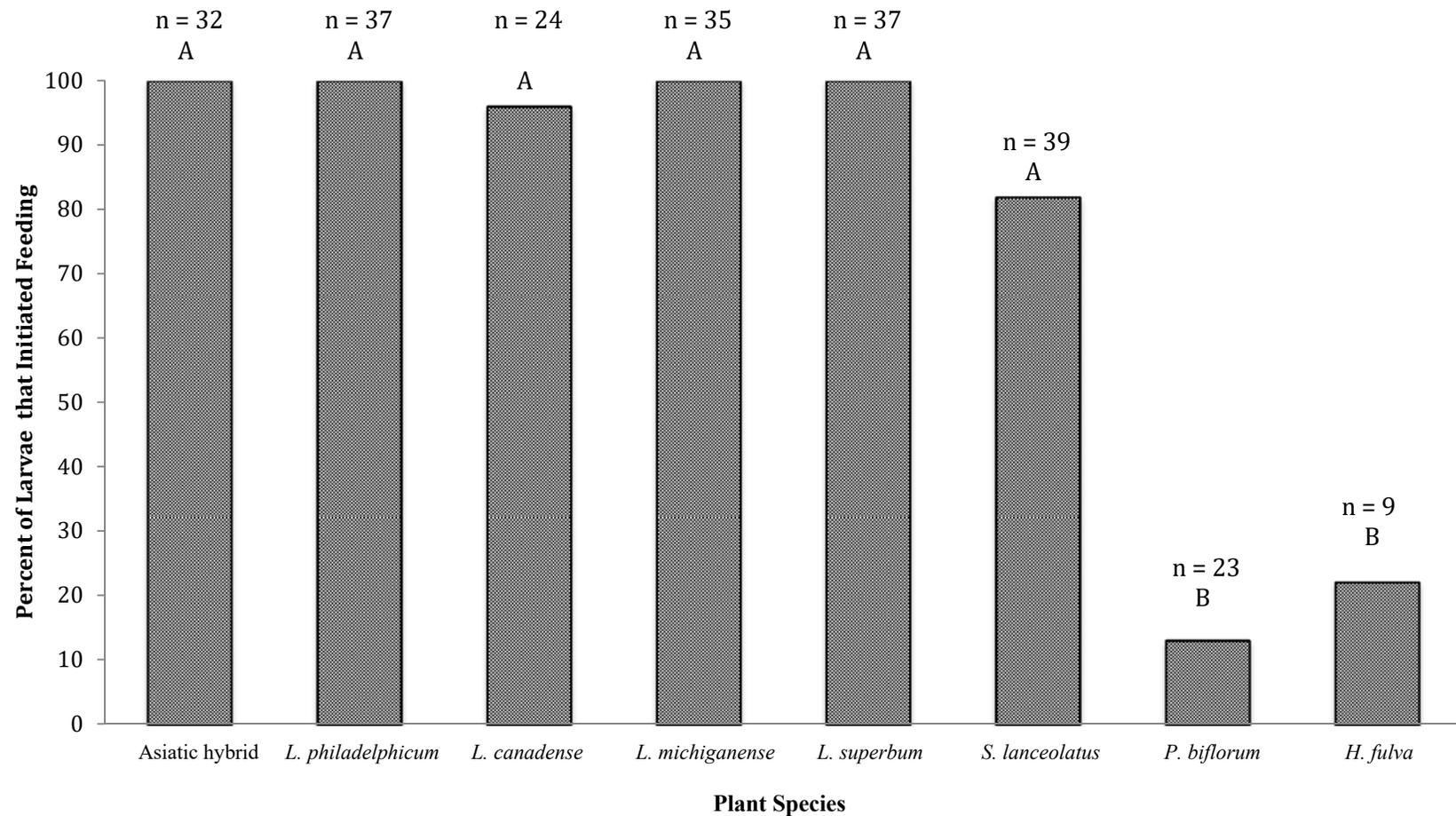
**Figure 6:** Estimated overall leaf biomass consumed (g) per plant species in field at the end of season 2016. There was a significant effect of plant species on plant biomass consumed (Kruskal-Wallis test;  $H = 109.967$ ,  $df = 6$ ,  $p < 0.001$ ). Species with different letters denote significantly different medians according to Dunn's test (1964). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Outliers denoted by dots. Extreme outliers denoted by asterisk.



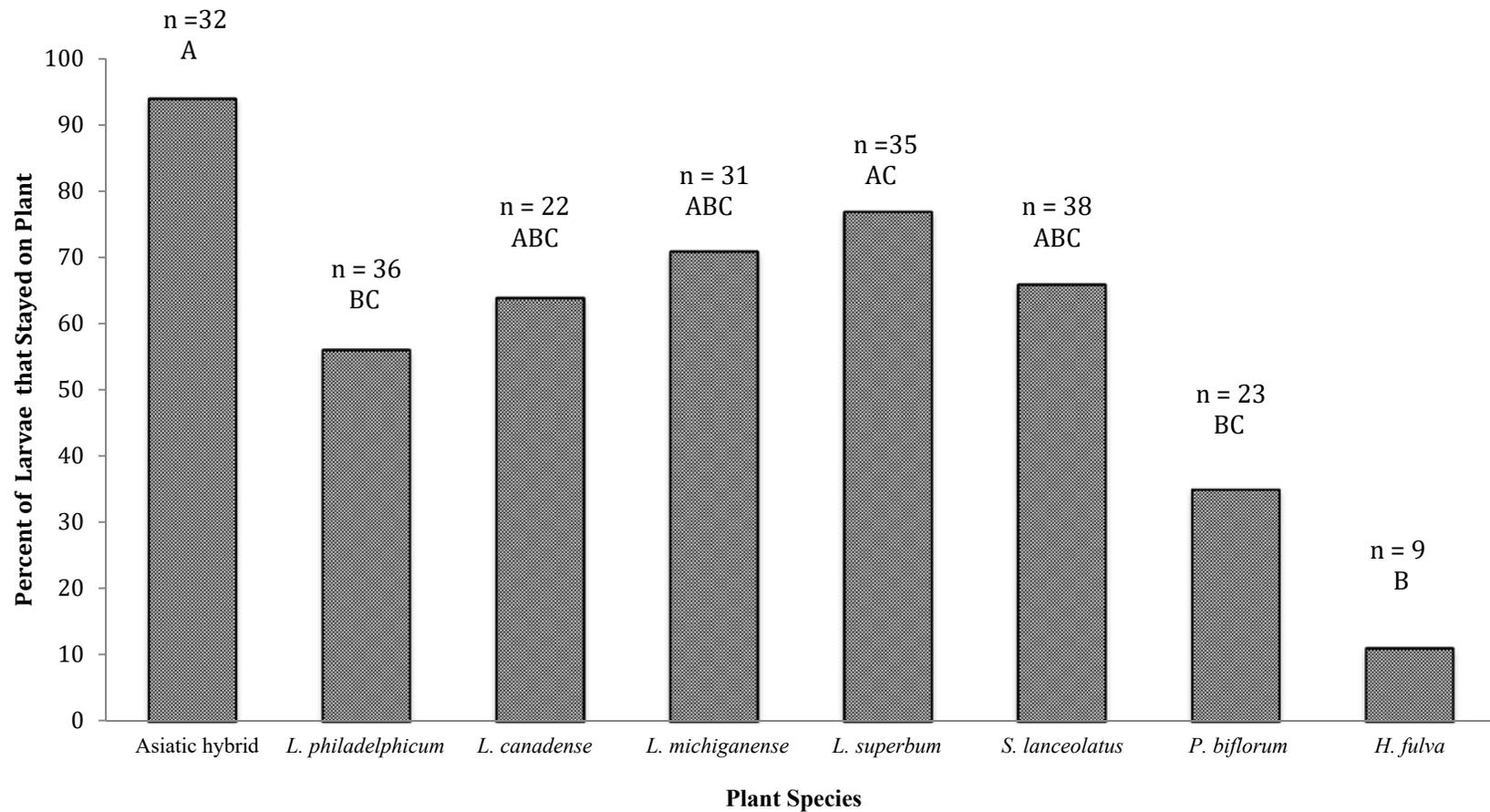
**Figure 7:** Number of eggs laid on various potential host plant species in no-choice 72 h oviposition trials. There was no significant difference between numbers of eggs laid on different plant species (Kruskal-Wallis test;  $H = 3.805$ ,  $df = 6$ ,  $p = 0.703$ ). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Outliers denoted by dots.



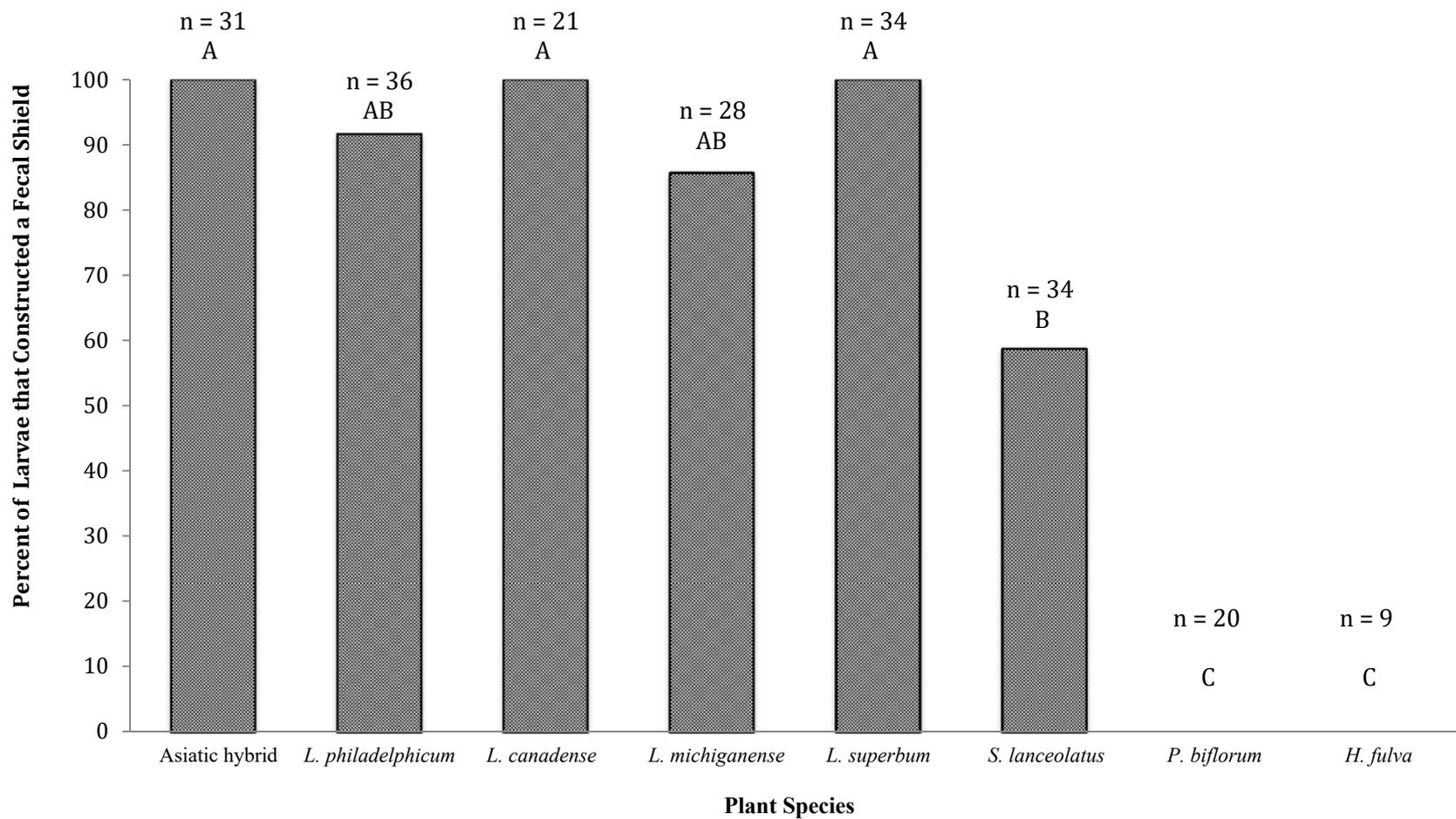
**Figure 8:** Estimated overall leaf biomass consumed (g) per plant species in a no-choice 72 h feeding trial. There was a significant effect of plant species on plant biomass consumed (Kruskal-Wallis test;  $H = 81.623$ ,  $df = 6$ ,  $p < 0.001$ ). Species with different letters denote significantly different medians according to Dunn's test (1964). (Horizontal line denotes median value. Vertical line denotes 10<sup>th</sup> and 90<sup>th</sup> percentiles. Box denotes 25<sup>th</sup> and 75<sup>th</sup> percentiles). Outliers denoted by dots. Extreme outliers denoted by asterisk.



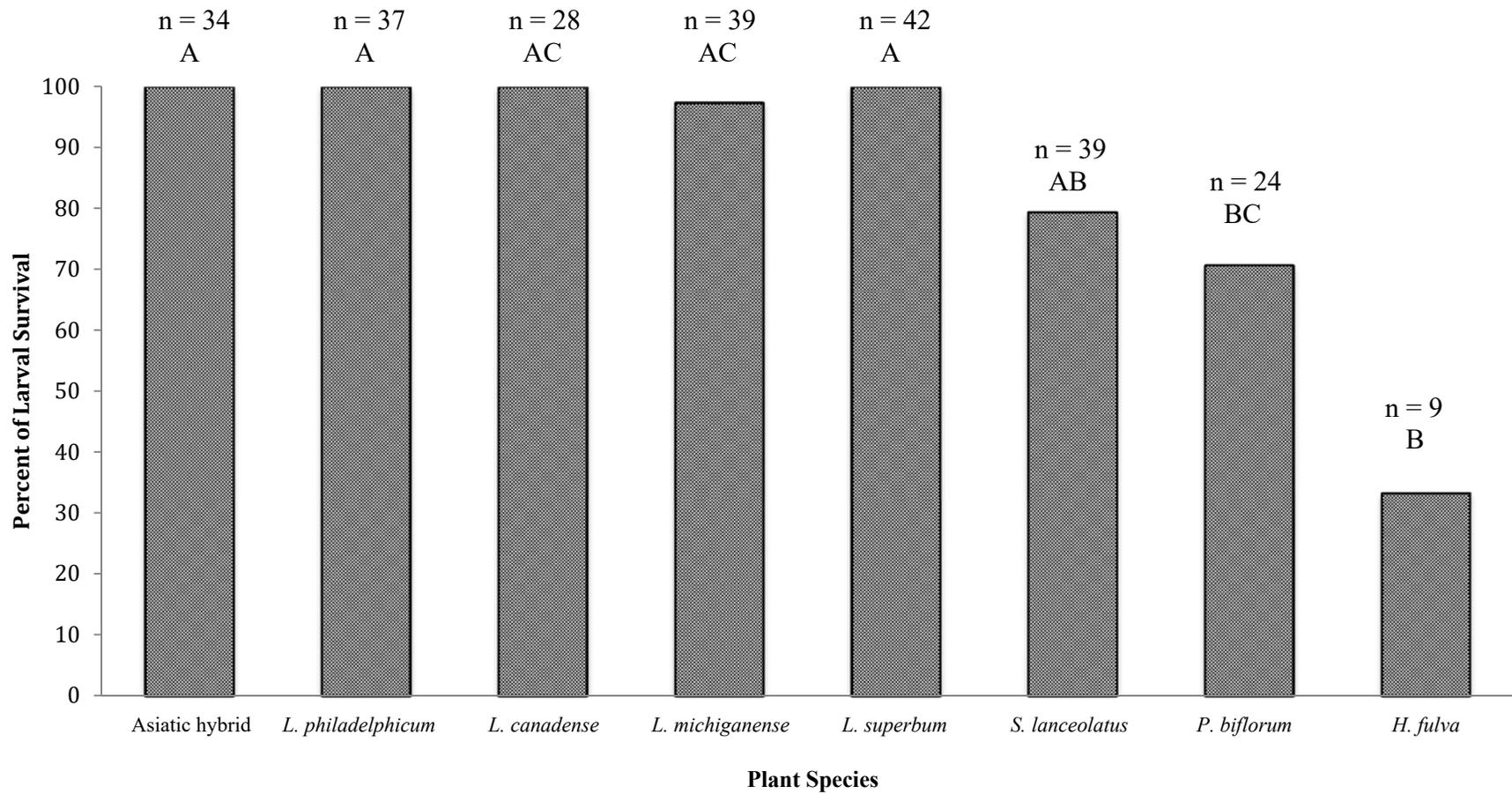
**Figure 9:** Percentage of lily leaf beetle larvae that initiated feeding on plant species in no-choice 48 h trial. There was a significant difference between plant treatments for percentage of lily leaf beetle larvae that initiated feeding on host plant species ( $X^2 = 150.658$ ,  $df = 7$ ,  $n = 237$ ,  $p < 0.001$ ). Different letters denote differences in significance (Chi-squared test).



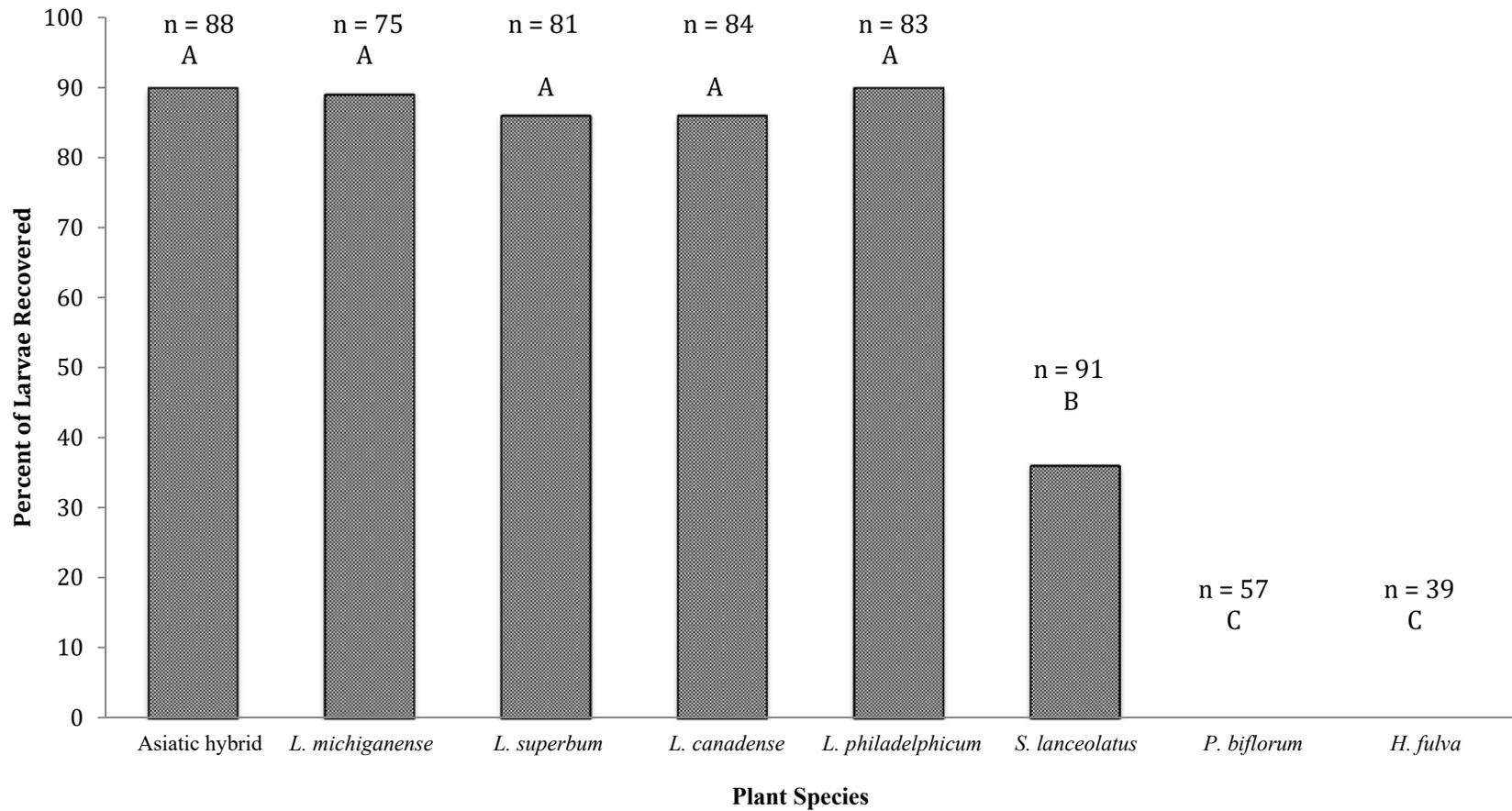
**Figure 10:** Percentage of lily leaf beetle larvae that stayed on plant species in no-choice 48 h experiment. There was a significant difference between plant treatments for percentage of lily leaf beetle larvae that stayed on host plant species ( $X^2 = 36.561$ ,  $df = 7$ ,  $n = 226$ ,  $p < 0.001$ ). Different letters denote differences in significance (Chi-squared test).



**Figure 11:** Percentage of lily leaf beetle larvae that constructed a fecal shield while on plant species in no-choice 48 h experiment. There was a significant difference between plant treatments for percentage of lily leaf beetle larvae that constructed a fecal field while on host plant species ( $X^2 = 132.762$ ,  $df = 7$ ,  $n = 213$ ,  $p < 0.001$ ). Different letters denote differences in significance (Chi-squared test).



**Figure 12:** Percentage of lily leaf beetle larvae that survived post 48 h on plant species in no-choice experiment. There was a significant difference between plant treatments for percentage of lily leaf beetle larvae that survived on host plant species ( $X^2 = 72.637$ ,  $df = 7$ ,  $n = 252$ ,  $p < 0.001$ ). Different letters denote differences in significance (Chi-squared test).



**Figure 13:** Percentage of lily leaf beetle larvae recovered from each plant species in combined 2015 and 2016 24 – 48 h field plot experiment. Percentage of larvae recovered differed significantly between different plant species ( $X^2 = 312.66$ ,  $df = 7$ ,  $n = 598$ ,  $p < 0.001$ ). Plant species with different letters denote significant differences between percentages of larvae recovered (Chi-squared).

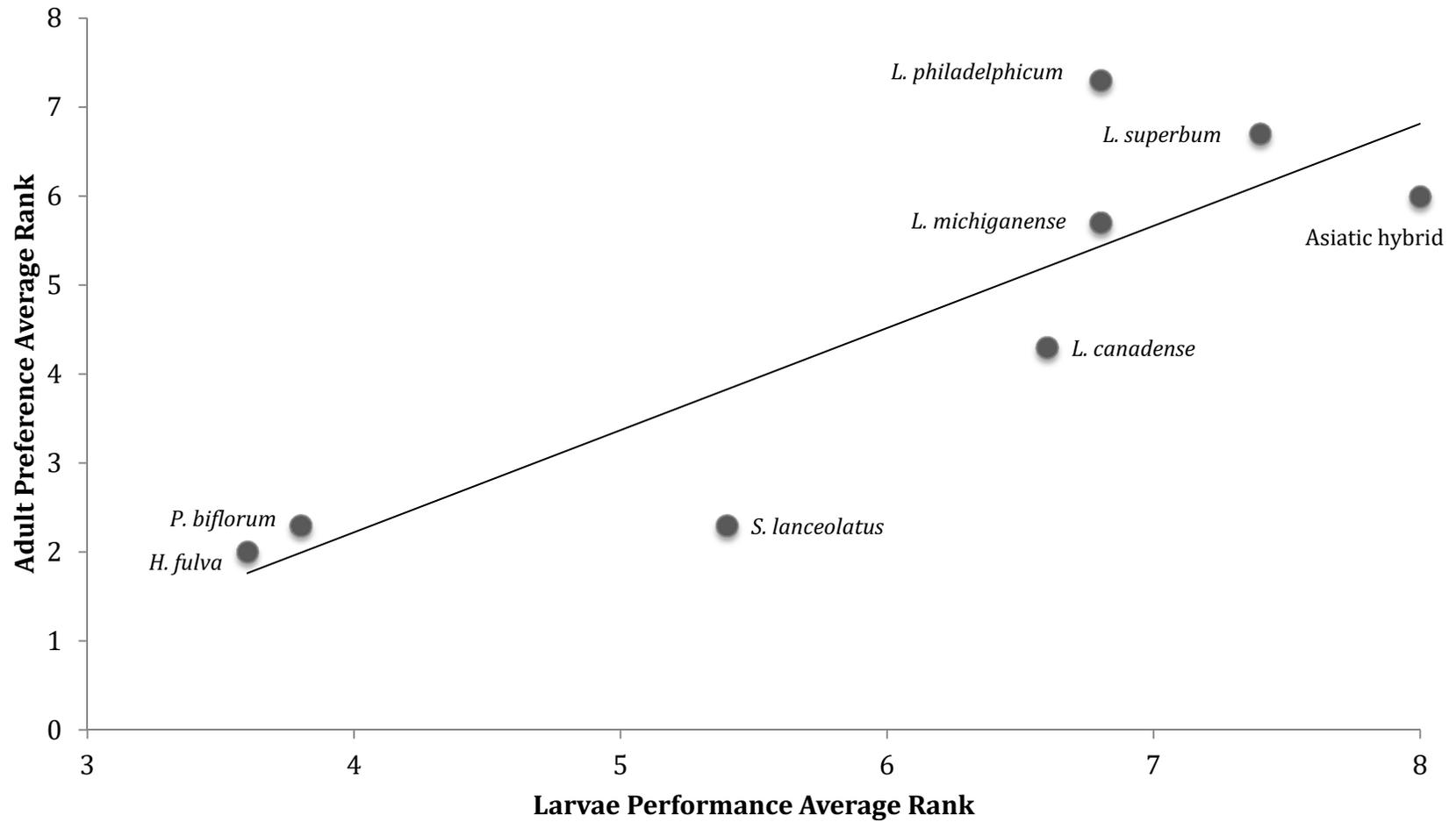
**Table 2:** Table 2A (larval performance) and 2B (adult preference) showing ranks assigned for each experiment and average rank.

<b>A</b>	<b>Larvae Suitability Test (ranks)</b>					
<b>Plant Species</b>	% Initiated Feeding	% Stayed on	% Fecal Shield	% Dead	% Recovered	Average Rank
Asiatic hybrid lily	8	8	8	8	8	8.0
<i>L. superbum</i>	8	7	8	8	6	7.4
<i>L. michiganense</i>	8	6	6	7	7	6.8
<i>L. philadelphicum</i>	8	3	7	8	8	6.8
<i>L. canadense</i>	7	4	8	8	6	6.6
<i>S. lanceolatus</i>	6	5	5	6	5	5.4
<i>P. biflorum</i>	4	2	4	5	4	3.8
<i>H. fulva</i>	5	1	4	4	4	3.6

<b>B</b>	<b>Adult Beetle Preference (ranks)</b>				
<b>Plant Species</b>	Beetle Field Release*	End of Season Plant Damage**	No-Choice Oviposition	No-Choice Food	Average Rank
Asiatic hybrid lily	8	8	6	4	6.0
<i>L. superbum</i>	5	6	8	7	6.7
<i>L. michiganense</i>	6	7	5	6	5.7
<i>L. philadelphicum</i>	7	NA	7	8	7.3
<i>L. canadense</i>	4	4	4	5	4.3
<i>S. lanceolatus</i>	1	5	3	3	2.3
<i>P. biflorum</i>	3	3	2	2	2.3
<i>H. fulva</i>	2	2	NA	NA	2

\* Overwintered beetle release, including all plant species

\*\* Not included in average rank, as *L. philadelphicum* was not included in experiment.



**Figure 14:** Correlation between adult preference index and larval performance index on different plant species using the average rank values (higher numbers = performed or preferred more) from Table 2. (Pearson's Correlation;  $r^2 = 0.870$ ,  $n = 8$ ,  $p = 0.005$ ).

## **CHAPTER 3**

### **NATIVE PLANTS PROVIDE ENEMY FREE SPACE FOR LILY LEAF BEETLE, *LILIOCERIS LILII* (SCOPOLI) (COLEOPTERA: CHRYSOMELIDAE) FROM THE LARVAL PARASITOID *TETRASTICHUS SETIFER* (THOMSON)**

#### **INTRODUCTION**

Over 2,000 species of insects have been either intentionally or unintentionally introduced into North America (Kim and McPheron, 1993). One unintentional introduction was the lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae). Introduction of this beetle was hypothesized to have occurred via importation of Asiatic lilies (Fox-Wilson, 1942). The first introduction of the lily leaf beetle occurred in Montreal, Québec, Canada in 1943 (Dieni *et al.*, 2016; Brown, 1946); the beetle can now be found in nine Canadian provinces (from Alberta to Newfoundland) (Brown, 1946; LeSage, 1983; Gooderham, 1993; Casagrande and Livingston, 1995; LeSage and Elliott, 2003; Majka and LeSage, 2008; Calgary Horticultural Society, 2009; Hicks and Sellars, 2014; Brace, 2016). The second accidental introduction into North America is thought to have occurred in the United States at Cambridge, Massachusetts in 1992 (Dieni *et al.*, 2016) and the lily leaf beetle has now expanded into several northeastern states (Livingston, 1996; Gold, 2003). No native natural enemies of the lily leaf beetle have been documented in North America (Gold *et al.*, 2001). Therefore, management methods have included handpicking or utilization of broad-spectrum insecticides, which are heavily regulated in Canada (Health Canada, 2009), with frequent

application needed (Salisbury, 2007); however, these methods are often not effective. This is partially due to the defense behavior of the beetle to drop when disturbed, as well as pesticides not being effective enough. A third management method, which has been successful, is classical biological control, the introduction of a pest's natural enemy from its native range into its introduced range, to suppress the pest's population (Mason and Brodeur, 2013).

One very successful biological control agent of the lily leaf beetle is the larval parasitoid *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae). This parasitoid is the most widely distributed of the lily leaf beetle parasitoids in Europe, ranging from the United Kingdom to Bulgaria and Northern Germany to Italy (Kenis *et al.*, 2002; Casagrande and Kenis, 2004). Female *T. setifer* parasitoids oviposit in all four larval stages of the lily leaf beetle (Haye and Kenis, 2004). In the spring, the emergence of adults occurs over a span of several weeks and female parasitoids have been found to oviposit at as young as two days old (Haye and Kenis, 2004). The parasitoid larvae are gregarious, with parasitized lily leaf beetle cocoons containing an average of seven (2-26) larvae. It is univoltine, overwintering as mature larvae in the host's cocoon (Haye and Kenis, 2004). *T. setifer* was released in Massachusetts, USA from 1999 to 2001 (Tewksbury *et al.*, 2005) and Ontario, Canada in 2010 (Cappuccino *et al.*, 2013). Near the release site in Wellesley, Massachusetts, parasitism of fourth-instar lily leaf beetle larvae a few years after release ranged from 37–100% (Tewksbury *et al.*, 2005).

The fecal shield of the lily leaf beetle is an important cue *T. setifer* uses when searching for a host. This dorsally situated structure is made out of the larval feces. The lily leaf beetle larvae may use their fecal shield as a defense mechanism; however, it is

hypothesized that the shield mainly protects against only generalist enemies, such as the earwig *Forficula auricularia* L. (Dermaptera: Forficulidae) (Schaffner and Kenis, 1999). Specialist enemies, such as *T. setifer*, respond positively to fecal shields and fecal shield extracts of lily leaf beetle (Scarborough, 2002). Furthermore, when *T. setifer* was offered larvae of potential hosts—lily leaf beetle and the closely related *Lilioceris merdigera* L. (Coleoptera: Chrysomelidae)—they spent less time on *L. merdigera* (Scarborough, 2002). However, when the fecal shields of these two species were switched, the parasitoid switched its preference and spent more time on *L. merdigera* (Scarborough, 2002). This demonstrates how crucial the fecal shield is in *T. setifer* host detection. Other potential cues for *T. setifer* include the lily leaf beetle larvae itself and damaged plants (Scarborough, 2002).

Some novel hosts the beetle has recently exploited in North America include *Lilium michiganense* Farwell, *Lilium canadense* L. and *Streptopus lanceolatus* (Aiton) Reveal (Liliales: Liliaceae) (Bouchard *et al.*, 2008; Blackman *et al.*, 2016). With this continuous host expansion one must consider the enemy-free space hypothesis. This is defined as a herbivore escaping its specialized parasitoids by expanding its host range to include one or more novel hosts (Jeffries and Lawton, 1984; Brown *et al.*, 1995). An example of this involves the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae). The wild host plants, *Brassica nigra* (L.), *Cardamine integrifolia* (Nuttal) and *Erysimum cheiranthoides* L. (Brassicaceae) were preferred for oviposition and similarly these plant species were the preferred search targets of its primary parasitoid, *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae). However, when the moth switched to cultivated varieties of *Brassica oleracea* L. (Brassicaceae), although larval

performance was reduced, parasitism also decreased, thus providing the diamondback moth with a refuge from its enemy (Fox and Eisenbach, 1992).

The enemy-free space phenomenon has the potential to occur in the lily leaf beetle - *T. setifer* dynamic. The objective of this study was to investigate factors that affect the ability of *T. setifer* to detect and successfully parasitize lily leaf beetle. Specific objectives were to determine if *T. setifer* can detect lily leaf beetle larvae present on novel host plants and to determine if sunny open field versus shaded deciduous forest habitats affect the ability of *T. setifer* to successfully parasitize host larvae. These factors are of extreme importance to help determine if native host species are at risk of serious lily beetle infestations.

## **MATERIALS AND METHODS**

### **Insects**

Approximately 200 lily leaf beetle cocoons parasitized by *T. setifer* were imported from Delémont, Switzerland during 2015 and 2016. These were maintained at the Agriculture and Agri-Food Canada National Arthropod Containment Facility in Ottawa, Ontario. Cocoons were placed in 9.0 x 2.5 cm petri dishes, with a 16:8 h light:dark cycle at approximately 23 °C, until emergence of adult parasitoids. Once emerged, parasitoids were transferred to a  $17.73 \pm 0.012$  °C,  $47.81 \pm 0.42$  RH room and placed in a nylon 32.5 x 32.5 x 32.5 cm cage, with 10% sucrose solution in vials with cotton dental wicks protruding through a hole in the cap. Additional *T. setifer* were also reared from overwintered cocoons of parasitized lily leaf beetles from an Ottawa, ON population.

### **Field Experiments**

#### ***Parasitoid Host Detection on Novel Plants***

A small plot design as described in Chapter 2 was utilized (Methods: Plant Species and Experimental Plot Design) to evaluate the ability of *T. setifer* to detect hosts on novel plants in a natural setting. One late-instar lily leaf beetle larva with fecal shield, reared on an Asiatic hybrid lily host, was placed on each test plant using a soft bristled paintbrush. Fifty male and 50 female *T. setifer* were collected from the rearing cages, placed in vials and chilled in a cooler for one hour prior to release. Parasitoids were released by placing the vials at five points in the plot, just inside each of the four corners

and in the middle of the plot. After 24 h (July 2016 replicate was left for 48 h, due to poor weather conditions), larvae were collected, were sorted into groups by plant species and were reared on Asiatic hybrid lily hosts. Three weeks after pupation, lily leaf beetle cocoons were dissected to determine parasitism. A total of four replicate trials were conducted. One replicate was run in June 2015 and three replicates in 2016, two in June and one in July. Data were pooled for 2015 and 2016. The following host plants were in the plot: Asiatic hybrid lily (Tiny series), Wood lily, *Lilium philadelphicum* L., Canada lily, *Lilium canadense* L., Turks cap lily, *Lilium superbum* L., Michigan lily, *Lilium michiganense* Farwell and Rose twisted stalk, *Streptopus lanceolatus* Aiton (Reveal) (Liliaceae).

#### ***Parasitoid Host Detection in Different Habitats (Light/Shade)***

At Moffat Farm Veterans Park, Ottawa, ON, (45°21'37.4"N 75°41'41.3"W) five Asiatic hybrid lilies were placed in pots 3 m from the forest boundary in shade and five were placed 3m from the forest boundary in full sun. The plants in each treatment group were placed in a line approximately 1m apart. Prior to setting the plants in the field, 10 late-instar lily leaf beetle larvae were placed on each plant and allowed to forage for 12 hours. Once the plants were in place, 100 *T. setifer* were released at the forest boundary to forage and oviposit. After 48h, the lily leaf beetle larvae were collected and reared, with larvae from each replicate plant in a separate container. Three weeks after pupation, the cocoons were dissected to determine parasitism.

## Laboratory Experiment

### *Parasitoid Close-Range Host Detection on Novel Plants*

Lily leaf beetle larvae were obtained from cages as described in Chapter 2. Fecal shields were removed from each larva using a moist paintbrush. One larva was placed on a whole leaf of one of eight host plants in a 9.0 x 2.5 cm petri dish under a 16:8 light/dark cycle at  $23.10 \pm 0.04$  °C,  $47.7 \pm 0.92$  RH. Larvae were allowed to feed for 24 h and develop a new fecal shield if possible. After 24 h, the leaves were replaced with a new leaf of the same species. One female *T. setifer* was then placed in the petri dish and left undisturbed for 24 h. The parasitoid was then removed and the lily leaf beetle larvae were reared to pupation on Asiatic hybrid lilies and later dissected to determine presence of parasitism. The following plant species were tested: *L. canadense*, *L. michiganense*, *L. superbum*, *L. philadelphicum* and *S. lanceolatus*, and the preferred Asiatic hybrid lily (Tiny series). Depending on plant availability, 28 - 42 replicates were done for each plant species. However, once cocoons were formed sample size decreased to 10 – 35.

### Data Analyses

All statistical comparisons were made using SPSS version 24 (IMB Corp., 2015). Field parasitism levels of larvae on different host plants were analyzed via a Chi-squared test. Data from 2015 and 2016 were pooled and analyzed as one data set. If a difference was noted, pairwise comparisons were made using a 2 x 2 Fisher's Exact test using the Bonferroni correction where  $\alpha = 0.003$ . After the above post hoc tests were done, only one pair was observed to have a significance difference. This was thought to have

resulted from the relatively small sample size. Therefore, the data were rearranged to compare the preferred host (Asiatic hybrid) versus all novel hosts combined (*L. canadense*, *L. michiganense*, *L. superbum*, *L. philadelphicum*, *S. lanceolatus* and *P. biflorum*) and analyzed via a 2 x 2 Chi-Squared analysis. Similarly, a Chi-Squared analysis was used to compare whether or not host plant species affected parasitism levels of larvae in the close range laboratory system with the parasitoid.

Parasitism in sun and shade treatments was analyzed using the average of each replicate (total of 5 replicate plants for each treatment). Since these data were not normally distributed, a Mann-Whitney U test was used to compare the medians of these two treatments.

## **RESULTS**

### ***Parasitoid Host Detection on Novel Plants***

Data were pooled for 2015 and 2016 to provide the following total numbers of successfully reared individuals for each plant species: Asiatic hybrid lily (n = 43), *L. philadelphicum* (n = 35), *L. canadense* (n = 36), *L. superbum* (n = 29), *L. michiganense* Farwell (n = 37) and *S. lanceolatus* (n = 23). Upon combining all the novel native hosts, this resulted in 160 cocoons.

In the field plot experiment, significantly more larvae were parasitized when placed on Asiatic hybrid lilies in comparison to novel native hosts (Figure 1). Larval parasitism was lower on all native plant species compared to the preferred Asiatic hybrid host, although it was significantly different only for *L. michiganense* (Figure 2).

### ***Parasitoid Host Detection in Differing Environments (Sun/Shade)***

No lily beetle larvae were parasitized in the shade; however, because of the small sample size, parasitism level was not significantly different in the two treatments (Figure 3). Mean parasitism level in the sun was  $18 \pm 22\%$  and in the shade  $0 \pm 0\%$ . Originally 50 larvae were used for each treatment. However, recovery of the larvae after 48 h was low; 29 larvae were recovered in the sun and 43 in the shade. Of those, only 18 larvae from the sun treatment and 29 from the shade formed cocoons. It is therefore suggested that this experiment be replicated with 200 larvae for each treatment.

### ***Parasitoid Close-Range Host Detection on Novel Plants***

In the close-range host detection laboratory experiments, the host plant did not have a significant effect on the proportion of individuals parasitized by *T. setifer*, number of adult lily leaf beetles or lily leaf beetles dying from unknown causes in the cocoon (Figure 4).

## **DISCUSSION**

The native North American Liliaceae plants *L. michiganense*, *L. superbum*, *L. philadelphicum*, *L. canadense* and *S. lanceolatus* are all potentially suitable to varying degrees as novel hosts for the lily leaf beetle (Chapter 2). Although there may be some negative effects on lily leaf beetles that utilize novel plants as hosts, such as the reduced adult preference and larval performance (Chapter 2), there may also be benefits. One of these potential benefits is enemy-free space. This was evident in the 24 – 48 h field experiment, which showed that parasitism was 31% lower when larvae were placed on native plant species compared to larvae on the preferred Asiatic hybrid host (Figure 1). More specifically, the lily leaf beetle attained the greatest refuge from parasitism on *L. michiganense* (Figure 2). Although the other native host plants individually were not significantly different than the Asiatic hybrid lily, convincing trends were observed in that parasitism was about half (17-29%) that observed on the preferred host (55%). Further research would clarify whether these low parasitism levels persist, providing partial enemy-free space, or if the parasitoids would eventually adapt to find lily leaf beetle on these novel hosts.

Various factors may affect the ability of *T. setifer* to detect lily leaf beetle on novel native plants. Plants emit general volatiles and often emit more specific volatiles when damaged by herbivores (Paré and Tumlinson, 1999). Many parasitoids use these volatile cues for habitat selection and host plant-finding behavior (Tumlinson *et al.*, 1992). *T. setifer* is a specialized natural enemy. Therefore, according to De Moraes *et al.* (1998) and Powell *et al.* (1998), they should locate their hosts using infochemicals that are unique to the plant-herbivore complex. For instance, in y-tube olfactometer studies,

host detection by the specialist *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) was guided by signals associated with *Brassica oleracea* var. *capitata*, the preferred food plant of its host, the diamondback moth. This association reduced the ability of the parasitoid to detect larvae on their novel host plant, snow pea *Pisum sativum* var. Oregon sugar pod (Rossach *et al.*, 2005). These laboratory results were confirmed in the field when *D. semiclausum* was introduced into Kenya. Parasitism of diamondback moth larvae feeding on the preferred kale, *Brassica oleracea* var. *sabellica*, increased from 26 -75%; however, parasitism levels only increased from 2 - 4% for larvae feeding on the novel host, snow pea, *Pisum sativum* (Fabaceae) (Rossbach *et al.*, 2006).

The fecal shield of the lily leaf beetle is essential for allowing the parasitoid to detect its host. When the similar host species, *L. merdigera*, was placed in a petri dish, it was not as attractive to *T. setifer* as the lily leaf beetle. However, it was more attractive than lily leaf beetle after the fecal shields were switched (Scarborough, 2002). Fecal shields are believed to play a part in the short-range host-selection behavior of *T. setifer*, as they do in that of another lily leaf beetle parasitoid, *Lemophagus pulcher* (Szepligeti) (Hymenoptera: Ichneumonidae) (Schaffner and Müller, 2001). It is hypothesized that when the lily leaf beetle forages on novel hosts, the chemistry of the fecal shield is slightly altered, potentially reducing the familiarity of the olfactory cue to its parasitoids. This in turn interrupts the ability of the parasitoid to detect the host, therefore affecting parasitism levels when the lily leaf beetle switches host plants. This is supported by the work by Vencl and Schaffner (unpublished report, as cited in Schaffner and Müller, 2001) who found that a number of chemical components from the fecal shield most likely originated from the host plant. Therefore, if the lily leaf beetle switches host plants, the

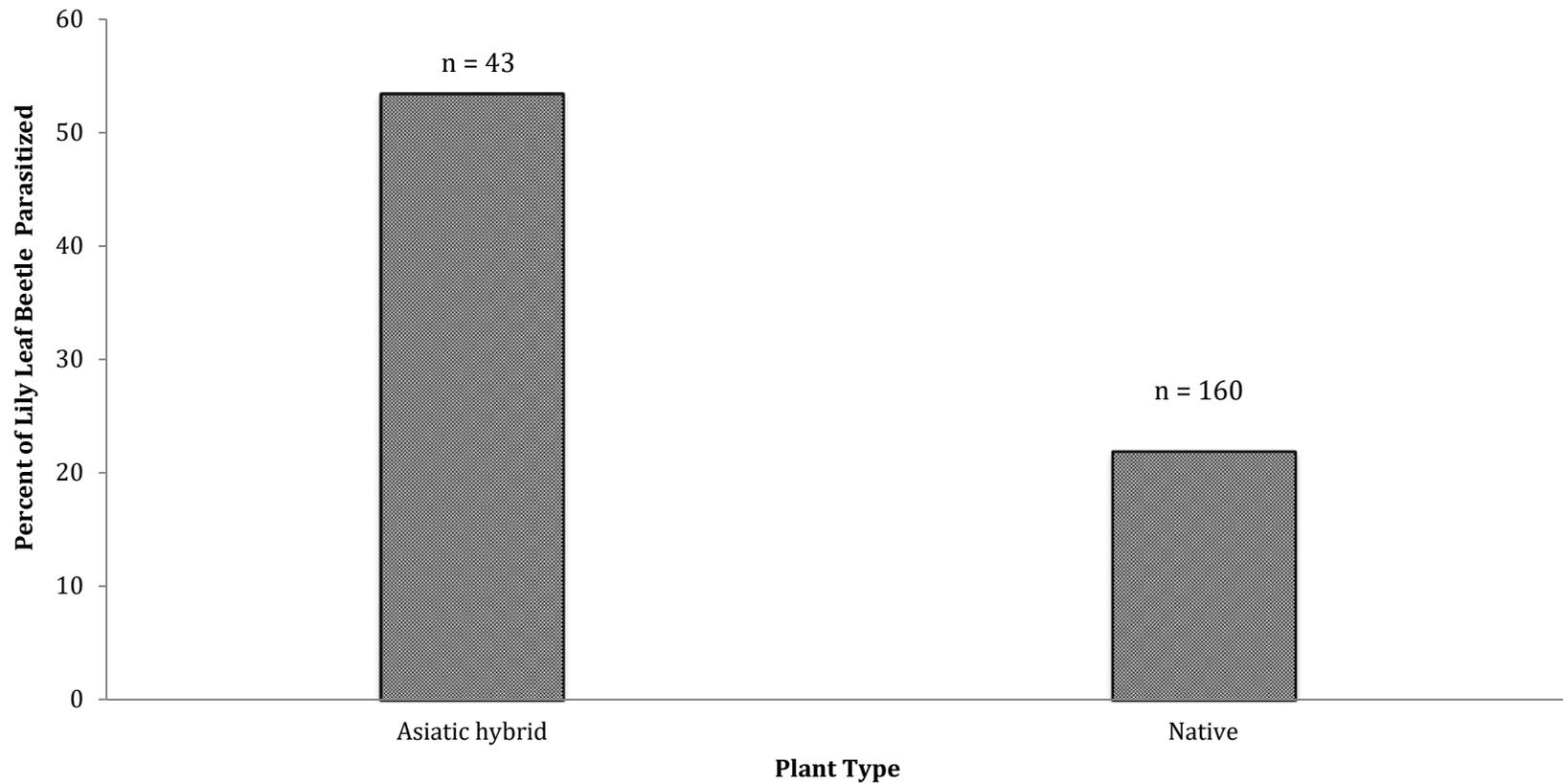
olfactory cues may be slightly different not only from the plant volatiles emitted, but also from volatiles emitted from the fecal shield, resulting in greater difficulty in host detection for *T. setifer*.

Despite the differences in parasitism of larvae placed on Asiatic hybrid lily versus native plant species in the field, laboratory no-choice experiments in petri dishes demonstrated that the parasitoid was able to find lily leaf beetle larvae on all six novel host plants and parasitize them at similar levels (Figure 4). Since the larvae were able to construct a new fecal shield from the novel host plants, this suggests that the fecal shields created from novel host plants did not change host-searching cues sufficiently for *T. setifer* to have difficulty detecting larval hosts at short range. Although one must keep in mind, leftover feces from prior feeding on Asiatic hybrids may have been excreted after removal of the initial fecal shield, possibly affecting the results. Therefore, fecal shields may not be the main hindrance to *T. setifer* detecting lily leaf beetle hosts on novel plants. The reason parasitism of lily leaf beetle larvae on novel host plant species was lower in a natural field environment may be due to a complex system involving multiple cues.

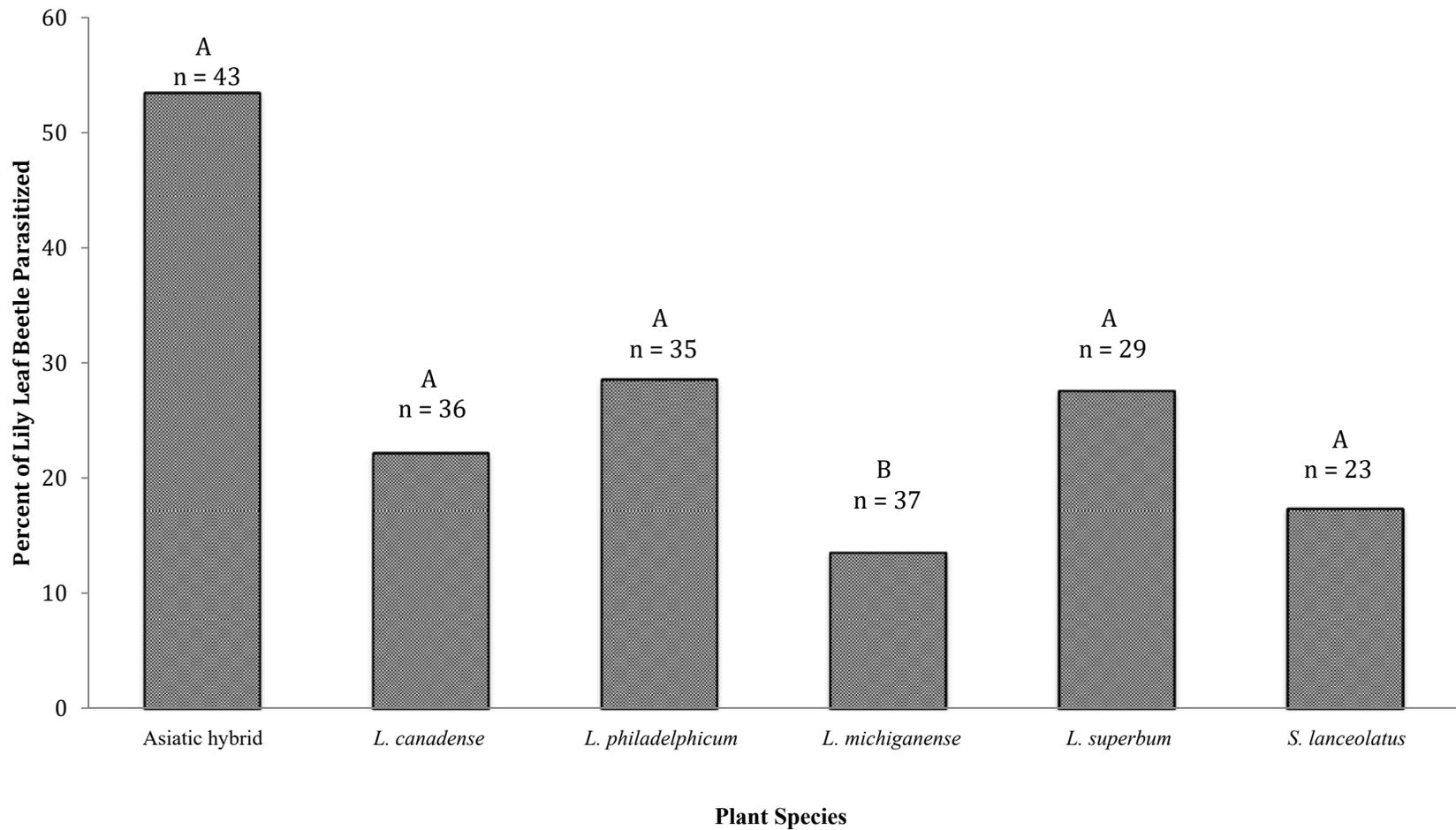
Another factor that may impact the ability of the parasitoid to detect host larvae is the habitat in which the novel host resides. There are many trade-offs between insects living in a shady versus a sunny habitat. Some studies have noted that parasitism in an open habitat is greater in comparison to understory habitats; however, the trade-off included reduced leaf quality in the shade (Zilahi-Balogh *et al.*, 2006; Stoepler and Lill, 2013). Another study found that *Pseudacteon* sp., a phorid fly parasite of ants (Formicidae), had a greater impact on the ant species, *Azteca instabilis* F. Smith (Hymenoptera: Formicidae), in low-shade compared to full shade (Pardee and Philpott,

2011). Comparison of parasitism of larvae on plants in an open field (full sun for most of the day) versus an adjacent forest (shade for whole day) suggested that *T. setifer* might be less likely to forage for hosts in the shade (Figure 3). However, because small sample sizes were used in this experiment, further investigations are needed to support or refute this finding. Species in the Liliaceae, such as *L. canadense*, *L. michiganense* and *S. lanceolatus*, are native to a wide range of habitats, ranging from open fields to woodlands. *Lilium canadense* and *L. michiganense* can reside in moist forests putting them at further risk if the biological control agent does not search for the lily leaf beetle in shaded areas. Furthermore, despite the reduced performance of the larvae on *S. lanceolatus*, if *T. setifer* cannot detect the hosts in the shade, the lily leaf beetle could gain considerable advantage by exploiting this novel understory host. Therefore if habitat were another factor influencing *T. setifer*'s ability to detect its host successfully, it would help determine which native Liliaceae species are at risk to more serious infestations.

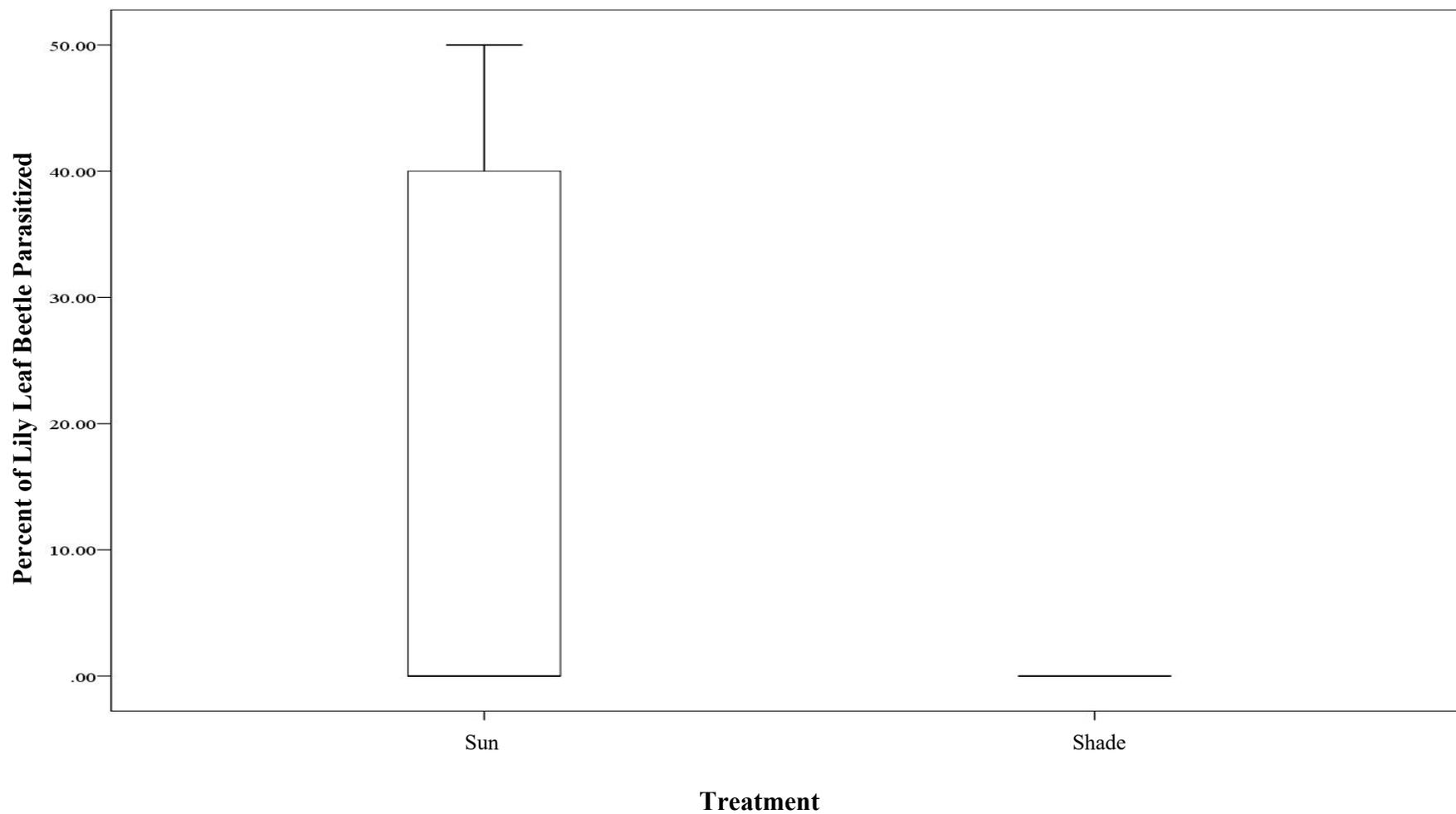
In summary, understanding factors that affect the ovipositional ability of the biological control agent *T. setifer* will help to improve management of lily leaf beetle and to predict which native North American plant species may be prone to serious lily leaf beetle infestations. Several of the species studied here are protected (USDA, NRCS 2016); therefore, serious lily leaf beetle infestations on these plant species could be devastating.



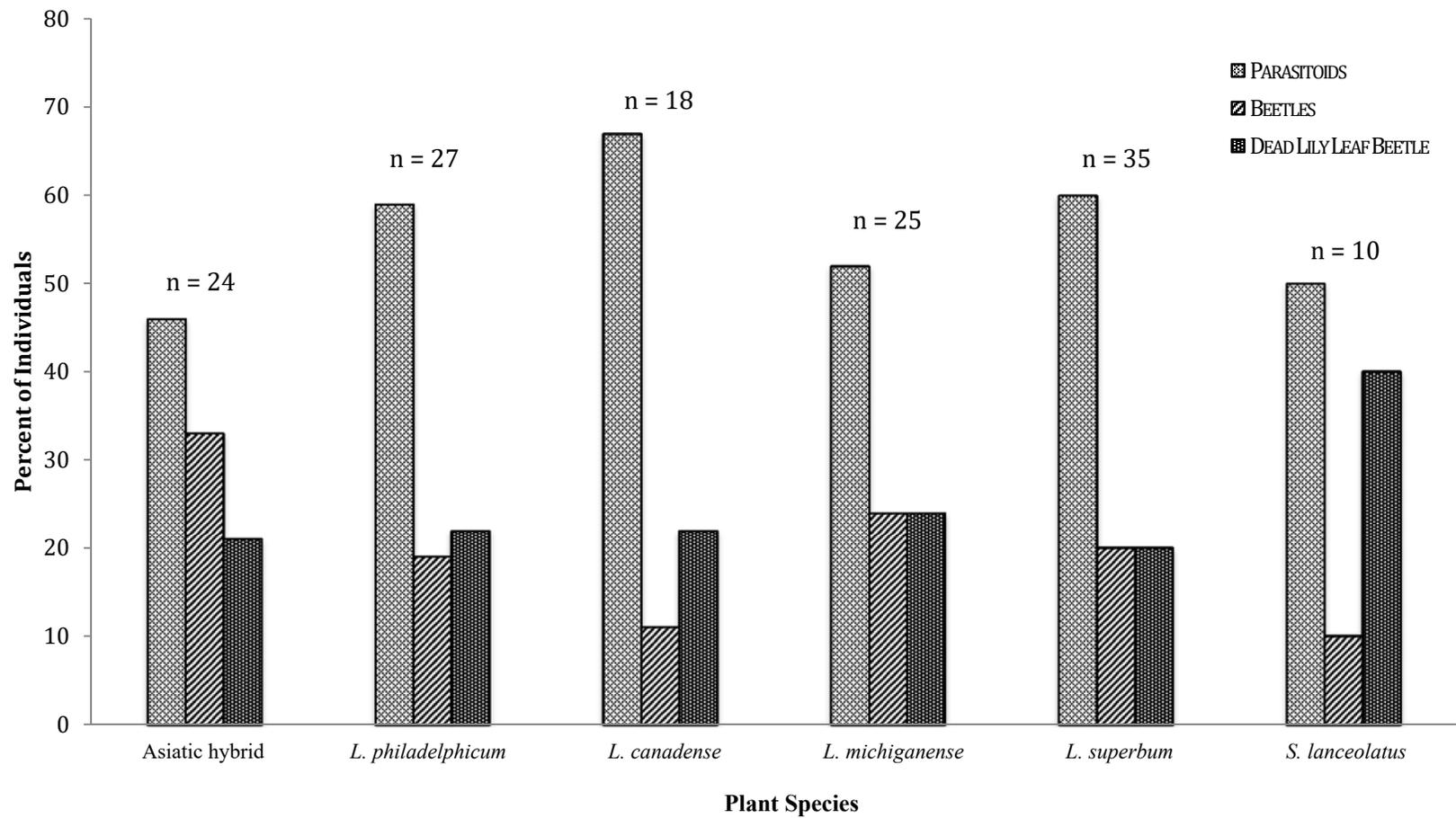
**Figure 1:** Percent of lily leaf beetle larvae parasitized by *T. setifer* in choice 24-48 h field trials in 2015 and 2016 on Asiatic hybrid lilies and native plants (pooled data). (Chi-Squared;  $X^2 = 16.597$ ,  $df = 1$ ,  $n = 203$ ,  $p < 0.001$ ).



**Figure 2:** Percent of lily leaf beetle larvae parasitized by *T. setifer* in choice field trials (24-48 h) in 2015 and 2016 on various host plants. (Chi-Squared;  $X^2 = 19.326$ ,  $df = 5$ ,  $n = 203$ ,  $p = 0.002$ ). Different letters denote significant differences (Chi-Squared).



**Figure 3:** Percent of lily leaf beetle larvae parasitized by *T. setifer* in sun and shade treatments. (Mann-Whitney U test;  $U = 7.50$ ,  $p = 0.136$ ).



**Figure 4:** Percent of lily leaf beetle cocoons that contained parasitoids, lily leaf beetles or dead lily leaf beetle in parasitoid no-choice 24-h laboratory trials on various host plants. Parasitoid emergence ( $X^2 = 2.491$ ,  $df = 5$ ,  $n = 139$ ,  $p = 0.778$ ), beetle emergence ( $X^2 = 4.267$ ,  $df = 5$ ,  $n = 139$ ,  $p = 0.512$ ) and dead lily beetle pupae ( $X^2 = 1.902$ ,  $df = 5$ ,  $n = 139$ ,  $p = 0.863$ ).

## **CHAPTER 4**

### **SCIENTIFIC NOTE**

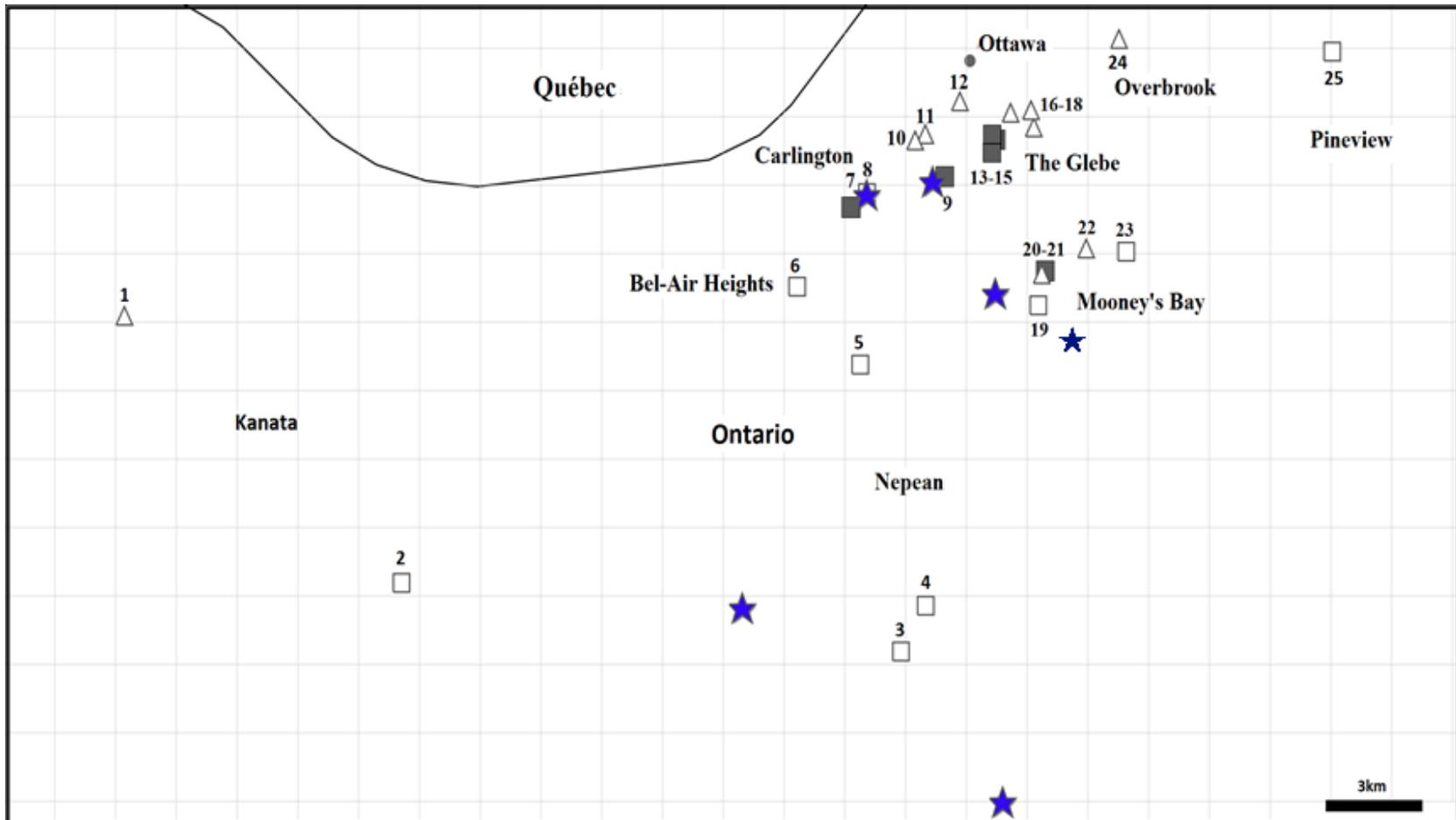
#### **BATTLE OF LILY LEAF BEETLE AND ITS PARASITOID IN THE OTTAWA, ONTARIO AREA**

The lily leaf beetle, *Lilioceris lili* (Coleoptera: Chrysomelidae), is an invasive alien species introduced to North America in 1943 (Brown, 1946), and which was first reported in Ottawa, ON in 1981 (LeSage, 1983). This pest is capable of fully developing on hosts in the genera *Lilium* and *Fritillaria* (Liliaceae) (Livingston, 1996) and has the ability to destroy hosts by feeding on leaves, flowers and buds, leaving only a desiccated stem. The beetle is continuing to spread throughout Canada, currently occurring from Alberta to Newfoundland (Brown, 1946; LeSage, 1983; Gooderham, 1993; Casagrande and Livingston, 1995; LeSage and Elliott, 2003; Majka and LeSage, 2008; Calgary Horticultural Society, 2009; Hicks and Sellars, 2014; Brace, 2016). Additionally it has begun to expand its plant host range to include native North American species, some of which are protected. These hosts include *Lilium canadense* L. (Bouchard *et al.*, 2008), *Lilium michiganense* L. Farwell and *Streptopus lanceolatus* (Aiton) Reveal (Liliaceae) (Blackman *et al.*, 2016). Management has been mostly unsuccessful. Insecticides, which are heavily regulated in Canada (Health Canada, 2009), are often not effective to control this pest. Furthermore, they also pose environmental concerns, such as danger to pollinating insects, specifically when plants are in flower. Handpicking is not effective and is time consuming, as the beetle drops off leaves when disturbed (Salisbury, 2007; Royal Horticultural Society, 2016). On the other hand, classical biological control, which

is the introduction of one or more natural enemies from pest's native range into the newly invaded area, has resulted in tremendous success.

The larval parasitoid, *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), which is native to Europe, has been highly successful in reducing lily leaf beetle populations. This biological control agent is of utmost importance because no native natural enemy of the lily leaf beetle has been recorded in North America (Gold *et al.*, 2001). Once released in the United States in 2001, in Wellesley Massachusetts, 37-100% parasitism of fourth instars from 2002-2004 was achieved and lily leaf beetle population numbers have declined (Tewksbury *et al.*, 2005). *T. setifer* was also released in Ottawa, ON, Canada in 2010 (Cappuccino *et al.*, 2013). To date there have not been any follow-up studies in the Ottawa area.

From May – August 2016, surveys of lily leaf beetle and *T. setifer* populations were made in the Ottawa region. Flyers requesting specimens were circulated to the public, and local gardeners contacted the research team when lily leaf beetle infestations were suspected. The garden sites were visited and the following were recorded: number of lily leaf beetle eggs, larvae, adults and number of lily plants. Larvae were collected from sites, reared to pupation and then dissected to determine which sites had *T. setifer* present. Some larvae collected were in an early instar and may not have had a chance to be located by *T. setifer*, potentially affecting the results. Locations of field collections were then documented on a map (Figure 1). The estimated number of lily leaf beetle specimens per plant and percent parasitism by *T. setifer* in each survey area was recorded (Table 1).



**Figure 1:** Lily leaf beetle and *T. setifer* survey locations in 2016 in Ottawa, ON, Canada. Triangles and squares denote lily leaf beetle locations; triangles denote only surveys taken and no larval specimens collected, squares denote locations in which lily leaf beetle larvae were collected, stars denote *T. setifer* release sites from 2010-2016 and grey squares indicate sites in which *T. setifer* was recovered.

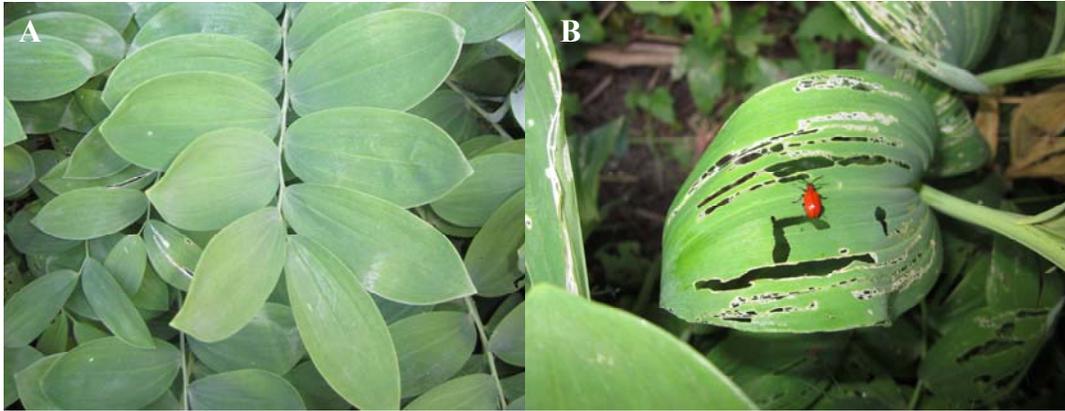
**Table 1:** Survey locations of lily leaf beetle and *T. setifer* in the Ottawa, ON area. Number of lily leaf beetle specimens (adults, eggs and larvae) per lily at each location and percent parasitism by *T. setifer*. Map points correspond to locations in Figure 1.

Map Point #	Neighbourhood	# Lily Leaf Beetle per lily	% Parasitism
1	Kanata	1.50	NA
2	Kanata	12.86	0.0 (n = 5)
3	Nepean	5.30	0.0 (n = 4)
4	Nepean	3.50	0.0 (n = 2)
5	Nepean	2.20	0.0 (n = 8)
6	Bel-Air Heights	5.32	0.0 (n = 68)
7	Carlington	2.19	1.6 (n = 62)
8	Carlington	NA	0.0 (n = 11)
9	Ornamental Gardens	3.00	23.7 (n = 38)
10	Centretown West	0.00	NA
11	Centretown West	1.00	NA
12	China Town	0.04	NA
13	Glebe	1.33	100.0 (n = 6)
14	Glebe	1.10	38.5 (n = 13)
15	Glebe	9.85	42.3 (n = 26)
16	Glebe	4.00	NA
17	Glebe	2.00	NA
18	Glebe	0.95	NA
19	Hunt Club	0.45	0.0 (n = 12)
20	Mooney's Bay	0.73	NA
21	Mooney's Bay	8.75	7.1 (n = 14)
22	Ridgemont	2.83	NA
23	Ridgemont	8.80	0.00 (n = 18)
24	Overbrook	4.28	NA
25	Pineview	NA	0.00 (n = 4)

NA: Not applicable

Although the results cannot be compared to other years, the information communicated by Ottawa gardeners suggests that lily leaf beetle populations in the Ottawa area have been decreasing. An overall average of 3.6 lily leaf beetle specimens (beetles, eggs and larvae) per lily plant were recovered, with surveys ranging from the Kanata to Pineview area (Figure 1 and Table 1). The largest infestation appeared in a Kanata location with 12.86 lily leaf beetle per lily, while the lowest at 0% was in Centretown West (Table 1). Although a general statement cannot be made that the population is decreasing in this area, personal observations suggest that in the previous year 2015, the beetle population was higher.

Multiple factors may affect the beetle population size in Ottawa. Potentially environmental factors were at play this year, as the season had an early warm spell, allowing a lot of overwintering beetles to emerge. This was followed by a cold spell a couple weeks later, possibly lowering the adult numbers. Additionally, some gardeners were more hesitant to plant lilies, therefore reducing the number of hosts available for the beetle. However if this is the case, beetles may search elsewhere for hosts and discover native plant species to forage on. Native hosts should continue to be monitored, specifically *Lilium superbum* L., *Lilium michiganense* Farwell, *Lilium philadelphicum* L., *Lilium canadense* L., *Streptopus lanceolatus* Aiton (Reveal) (Liliaceae) and even distantly related host, Solomon's seal *Polygonatum biflorum* (Walter) Elliott (Asparagaceae). Multiple gardeners have also noted lily leaf beetles on hosts more distant to *Lilium* such as various Solomon's seal varieties (Figure 2) and *Hosta* spp. (Asparagaceae), thereby supporting the potential host expansion of this beetle.



**Figure 2:** A) Solomon's seal patch where small lily leaf beetle population was discovered  
B) Adult feeding on Solomon's seal in Ottawa, ON garden.

Lastly, the beetle population may be lower as the parasitoid, *T. setifer*, now established in the Ottawa area may have spread widely. From the 25 garden sites surveyed, larvae were collected from 15 sites (ranging from 2 - 88 per site). Six sites (40%) had the parasitoid (Table 1). The lowest parasitism was 2% at one site and it ranged up to 100% at another site. With all sites combined ( $n = 15$ ), average parasitism was 14%. The majority of sites with the parasitoid present, were in the Glebe (3 sites), where parasitism ranged from 38-100%. These three sites were the furthest from a release site, with an average distance of 2.03 km. One site in the Carlington area, the closest to a release site, only yielded parasitism of 2%. While another location 1.25 km from a release site yielded 7% parasitism (Figure 1; Table 1). Considering the initial release site of *T. setifer* in Ottawa in 2010, parasitoids spread a distance of 3.54 km. Research by Tewksbury (2014), determined that *T. setifer* dispersed at approximately 1km per year from release sites in multiple New England States, USA. *T. setifer* was released in the Ottawa area 6 years ago, but may be dispersing at a much slower rate. Data from more survey sites are needed to confirm this. In summary, these results are promising for the

continued decline of lily leaf beetle numbers, but continued monitoring is needed to confirm this finding. Furthermore, more novel native plant species in the Ottawa area should be surveyed, particularly native lily species, to monitor their vulnerability to lily leaf beetle infestations.

## **CHAPTER 5**

### **Published Scientific Note 2016**

Blackman, C.K., Cappuccino, N and P. Mason. First record of *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae) on *Lilium michiganense* Farwell and confirmation of its association with *Streptopus lanceolatus* (Aiton) Reveal (Liliaceae: Liliales). *The Coleopterist Bulletin*. 70(3): 482-484.

### **SCIENTIFIC NOTE**

#### **FIRST RECORD OF *LILIOCERIS LILII* (SCOPOLI) (COLEOPTERA: CHRYSOMELIDAE) ON *LILIUM MICHIGANENSE* FARWELL AND CONFIRMATION OF ITS ASSOCIATION WITH *STREPTOPUS LANCEOLATUS* (AITON) REVEAL (LILIACEAE: LILIALES)**

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The lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae), is an invasive alien pest in North America. It threatens the commercial lily industry and native lily species in North America (Gold 2003; Salisbury 2003; Cappuccino *et al.* 2013). Management of lily leaf beetle requires handpicking or repeated applications of broad-spectrum insecticides (Salisbury 2007), but these methods are often not effective. For example, when the adult beetles are disturbed, they drop off the leaf, making handpicking difficult. Use of insecticides raises environmental concerns, particularly when the plants

are in flower, due to danger for pollinating insects and other non-target organisms (Salisbury 2007; Royal Horticultural Society 2016).

In North America, feeding by the lily leaf beetle causes severe defoliation of cultivated *Lilium* spp. and *Fritillaria* spp. (Liliaceae) and possibly native lily species that are within the beetle's invaded range (Livingston 1996). There are more than 20 native lily species in North America (USDA, NRCS 2016). Some, including wood lily, *Lilium philadelphicum* L., Canada lily, *L. canadense* L., Turk's-cap lily, *L. superbum* L., and Michigan lily, *L. michiganense* Farwell, are protected in one or more states in the US (USDA, NRCS 2016). Bouchard *et al.* (2008) surveyed 20 *L. canadense* populations in Québec and found eight sites that were infested with lily leaf beetle. Although *L. philadelphicum* is an excellent host for lily leaf beetle larval development in the laboratory (Ernst *et al.* 2007), there are no records of lily leaf beetle feeding on this species in natural habitats (Bouchard *et al.* 2008).

In July 2015, we received a report of a lily leaf beetle infestation on *L. michiganense* in Burlington, Ontario (43°25'41.3"N 79°51'07.3"W) (J. Kepkiewicz, personal communication). A survey of 235 *L. michiganense* plants was conducted on 3 August 2015 to document this infestation (Fig. 1a-b). Plants were surveyed to estimate damage (percentage of leaf area damaged per plant) and presence of lily leaf beetle (eggs, larvae and adults). The percentage of plants infested was 40.0% ( $n = 94$ ); 36.2% ( $n = 85$ ) of plants had one or more adults (among these were 12 copulating pairs), 8.9% had one or more larvae, and 0.9% had one or more eggs. The mean feeding damage among plants was 36%. The *L. michiganense* population at this location will be monitored to detect changes in infestation levels of the lily leaf beetle. Voucher specimens of *L.*

*michiganense* were deposited in the National Herbarium (DAO 01-01000615542, Agriculture and Agri-Food Canada, Ottawa, ON.)

Although reports of lily leaf beetle adults feeding on species outside the genus *Lilium* or even outside the family Liliaceae are common, reports of plants on which oviposition or larval feeding has occurred are few (Salisbury 2008). Kealey *et al.* (2013) reported three lily leaf beetle eggs on claspleaf twisted stalk, *Streptopus amplexifolius* (L.) DC (Liliaceae), on King Mountain in Gatineau Park, Québec, Canada (45°29'27.6"N 75°51'46.8"W). In June 2015, the host plant was correctly identified as rose twisted stalk, *Streptopus lanceolatus* (Aiton) Reveal (Liliaceae) (voucher specimen DAO 01-01000678463, Agriculture and Agri-Food Canada, Ottawa).



**Fig. 1.** Lily leaf beetle, *Lilioceris lili*. a) Late instar, b) Adult beetle on *Lilium michiganense* with feeding damage near Zimmerman Park, Burlington, ON, c) Two young larvae, d) Adult beetle on *Streptopus lanceolatus* showing minimal feeding damage on King Mountain, Gatineau, QC.

On 17 June 2015, we surveyed the entire Gatineau Park population ( $n = 282$ ) of *S. lanceolatus*. The percentage of plants infested with at least one life stage — adult, unhatched/hatched eggs or larvae — was 4.6% (Fig. 1c-d). Only one adult was found. Eggs were observed on 11 plants (3.9% of all plants), and two plants (0.7% of all plants) had early instars. Minimal feeding damage was noted on a few leaves where eggs had hatched (larvae present or deceased); however, no late-instar larvae were found.

Kealey *et al.* (2013) hypothesized that lily leaf beetle on *S. lanceolatus* in the Gatineau Park might have been a random, isolated event of an adult beetle dispersing from an urban garden to a natural area. However, the re-occurrence of the lily beetle on this same *S. lanceolatus* population suggests that the beetle may be establishing a population at this location. For this to be the case, *S. lanceolatus* must support lily leaf beetle larval development to pupation. Although Ernst *et al.* (2007) found that lily leaf beetle larval performance was poor on *S. lanceolatus*, Kealey (2013) found that approximately 42% of larvae were able to develop to adulthood on this species. The discrepancy might be caused by natural variation in the lily leaf beetle population for utilizing this novel host. Although we found no late instars larvae on *S. lanceolatus*, it is possible that a few individuals nevertheless manage to complete their development. The population will continue to be monitored, and further studies are planned on the suitability of *S. lanceolatus* as a larval host plant.

No native natural enemy species have been associated with the lily leaf beetle in North America (Gold *et al.* 2001), and classical biological control of this pest has been implemented. *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), a larval parasitoid from Europe, was released in Massachusetts, USA from 1999 to 2001

(Tewksbury *et al.* 2005) and Ontario, Canada in 2010 (Cappuccino *et al.* 2013). At a release site in Wellesley, Massachusetts, parasitism rates of fourth-instar lily leaf beetles ranged 37–100% in the first years after the releases (Tewksbury *et al.* 2005); thus parasitism pressure may be high. The enemy-free space hypothesis suggests that an herbivore can escape its specialized parasitoids by expanding its host range to include one or more novel hosts (Brown *et al.* 1995). This was demonstrated when the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), usually associated with cruciferous hosts such as kale, *Brassica oleracea acephala* L. (Brassicaceae), experienced very low parasitism when found on snow pea, *Pisum sativum* L. (Fabaceae), a novel host, suggesting that diamondback moth had attained enemy-free space (Rossbach *et al.* 2006). Preliminary results of an experiment in which several novel lily leaf beetle hosts were randomly planted in a 10-m<sup>2</sup> experimental plot suggest that *T. setifer* might have difficulty finding lily leaf beetle larvae on *S. lanceolatus*, possibly allowing the lily leaf beetle to attain enemy-free space (C.K. Blackman *et al.*, unpublished data). Although the number of lily leaf beetle observed on *S. lanceolatus* in the Gatineau Park was small, this native plant may be at risk if the beetle establishes a population, particularly if *T. setifer* cannot find it. Further studies are important to investigate the ability of *T. setifer* to detect lily leaf beetle hosts on other novel host plants, such as *L. michiganense*, to establish which plant species may be most vulnerable to lily leaf beetle infestations.

## **Acknowledgments**

John Kepkiewicz and the Ontario Regional Lily Society provided map coordinates for the *L. michiganense* population. The National Capital Commission, Ottawa, Ontario granted permission to conduct a survey and collect specimens of *S. lanceolatus* in Gatineau Park. The Ottawa Field-Naturalists' Club provided financial support. Christopher T. Bonner assisted in a field survey and Stephen Darbyshire (Agriculture and Agri-food Canada) confirmed the plant identifications.

## **Additional Data**

### ***Lilium michiganense***

In 2016, a follow-up survey was conducted. There was a continued strong presence of lily leaf beetle at Zimmerman Park, Burlington, ON, as data from a survey conducted on 18 June 2016 of 388 *L. michiganense* plants document. Of the 51.5% ( $n = 200$ ) of plants infested 10.8% ( $n = 42$ ) had one or more adults (among these were 12 copulating pairs), 36.6% had one or more larvae, and 14.9% had one or more eggs. Feeding damage (percent of leaf consumed) among plants averaged 15.1%. Compared to 2015 (36% average), feeding damage in 2016 was much lower. However, the 2015 survey was conducted two months later in the season (August), therefore documenting feeding damage over a longer period of time. A sample of 45 larvae from the site revealed no parasitism by *T. setifer*. In an attempt to protect this native lily population, 200 *T. setifer* were released in June 2016. Presence of the parasitoid will continue to be monitored as well as beetle infestation levels.

### ***Streptopus lanceolatus***

In Gatineau Park, the *S. lanceolatus* population on King Mountain was surveyed on 15 June 2016 ( $n = 234$ ) and 3 July 2016 ( $n = 162$ ). No lily leaf beetles were present. Although all plants surveyed had some general feeding damage, there was no concrete evidence of the presence of lily leaf beetle (no dead larvae, leftover fecal shields, or egg shells). Potentially, environmental conditions or predation resulted in extermination of this small population. Alternatively, *S. lanceolatus*, being a marginally acceptable host,

was of insufficient quality as a food plant to sustain the population of lily beetles. Despite these findings, this *S. lanceolatus* population should continue to be monitored, because the plant can occasionally support larval development to pupation (Chapter 2; Kealey, 2013). Moreover, the possible reluctance of *T. setifer* to forage in the shade (Chapter 3) might provide an advantage to the beetle on this less-than-optimal food plant.

### ***Lilium philadelphicum***

The Burnt Lands Alvar, Almonte, ON was surveyed 15 June 2016 to determine whether the lily leaf beetle had found the population of *L. philadelphicum*, which has been confirmed to be a suitable host plant (Ernst *et al.*, 2007). Although this site was surveyed in 2015, few lilies were found that year. In 2016, a total of 555 plants were examined and no lily leaf beetles were found. However, 9.2% ( $n = 51$ ) of plants showed some general leaf damage, most often less than 5%. It was noted that ants and tephritid flies were abundant, and occasional spiders were on these plants. The mystery of the absence of lily leaf beetle on this suitable host plant continues.

## **CHAPTER 6**

### **CONCLUSION**

Every year many exotic species are introduced into Canada. Not all become a threat, as many do not become established in the new geographic location. These types of introductions occur more frequently resulting from globalization and increased transport of goods internationally. This thereby increases the chances of an exotic species being imported. A new issue to consider in regards to exotic species is climate change. As northern climates become warmer the environment/habitat may be suitable for the colonization of more exotic species (Gillespie *et al.*, 2013). Further research is needed to deduce the type of impact this may cause as numerous factors are involved.

In a new location there are many constraints affecting the success of an exotic species. Some of these constraints are physiological, environmental and habitat. On the rare occasion the new location is suitable for the exotic species and it is able to reproduce, spread and become abundant and established, it then transitions to an invasive species. Furthermore, often in new locations the invasive species experiences a decrease in enemies, as natural enemies may not be present in the newly colonized area. This is known as the enemy release hypothesis. These invasive introductions are not only negatively affecting agricultural systems (financial loss), but also potentially native habitats (biodiversity loss).

As demonstrated in Chapter 2 and 5, once an exotic species becomes established in a new geographic location, it possesses the ability to expand its host range to novel hosts. As seen in the case of the lily leaf beetle, this not only affects cultivated

ornamental plants, which are precious to many gardeners, but also native plant species. Ornamental plants grown in gardens are often much more protected, as insects can be hand picked off the plants and insecticides may be used if permissible by the law. Native plants do not have this luxury. Without natural enemies of the exotic or invasive species in the new location this issue becomes particularly important, as many native plants are under protected status. Therefore, if infested by an invasive species, eventually whole populations could be lost, potentially resulting in biodiversity loss in the future. This is where classical biological control comes into play, as the introduction of a suitable natural enemy agent can help prevent devastating events as such.

There have been many successful cases of classical biological control, as many pest populations have been suppressed. As in the case for exotic species, the environment, habitat and food sources must also be suitable for the biological control agent to survive and even thrive in the new location. In some cases, such as agricultural systems and gardens, the habitats need to be slightly tweaked to aid in the establishment of the agents. These include the removal of mulches for insect overwintering and planting certain flowers for energy sources. Additionally, upon introduction of an agent, insecticides should not be sprayed as this often results in the agent's mortality. However, certain factors out of control also affect how well the agent is able to suppress pest populations or spread as the pest population expands to new locations. For instance, approximately 35% of biological control agents failed as a result of climate factors. These include temperature, rainfall, humidity and photoperiod (Stiling, 1993). Other potential factors affecting establishment or spread of the agents include attack by generalist predators, lack of genetic diversity, low release numbers and impoverished habitat

(Klinken *et al.*, 2003; Hoddle *et al.*, 2015). If the agent is able to establish in the area, in order to diminish pest populations it is important to ensure it can detect its hosts despite a variety of factors.

One factor that can influence the effectiveness of biological control is enemy-free space, which occurs when a herbivore switches host plants and attains refuge from its natural enemies. Research in Chapter 3 further supports the enemy-free space hypothesis, as lower parasitism levels were documented when lily leaf beetle larvae were placed on novel native plants. This becomes an issue as an exotic species begins to expand its host range to novel plants in the area. In agricultural systems and gardens, multiple management control methods can be implemented to reduce the negative outcomes of the pest's enemy refuge. However, this problem is especially an issue in native plant populations where outbreaks of pests can be undetected and supplemental control methods cannot be established. This leaves many vulnerable plant populations at risk.

Various potential host plants of the lily leaf beetle tested in this study should be considered vulnerable plant populations if infested in the future, especially due to their protected status. These plants include *L. philadelphicum*, *L. superbum*, *L. canadense*, *L. michiganense* and *S. lanceolatus*. Of particular concern is *L. michiganense*, where parasitism levels of lily leaf beetle larvae by *T. setifer* in the field were extremely low (13%) (Chapter 3: Figure 2). Sterile insect technique, a method of insect biological control which would involve the overwhelming release of sterile adult lily leaf beetles into the wild, could be part of the solution in the future if the beetle attains prolonged enemy free space from *T. setifer* (Benedict and Robinson, 2003). This program would involve the sensitive plant populations being exposed to additional damage from the

released adult male beetles; however, it is important to consider that larvae and not adults are what results in the devastating plant damage. The main obstacle to overcome with this potential solution is the correct procedure for sterilization of the lily leaf beetle is required.

In summary this research highlights the importance of native plant monitoring and understanding factors affecting biological control agents. Continued research should be done in regards to host expansions of pests, especially surrounding native plant populations. Currently, classical biological control is one of the only defenses for these native plants if no natural enemies are present. Therefore, the lack of native plant protective strategies should be addressed. Pest management in relation to agricultural systems should also not be undermined, as crop loss is a continuous issue. Many questions are still left unanswered, especially in regards to enemy-free space. Some of these include, how likely are the biological control agents to overcome this barrier? If so, approximately how long would this take? Is this barrier in relation to the experience of the insect; is it genetically based or both? Would rearing these agents on multiple hosts on which the pest have expanded onto aid to decrease this host detection barrier (inundative biological control)? The investigation of these questions should further aid in overcoming some of the obstacles involved in biological control, reducing the impacts numerous invasive species have on the economy and habitat.

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## **Appendix**

## Plant Species Purchase Locations

Plant species were bought from the following locations: Asiatic hybrid (hardware stores in Ottawa, ON), *H. fulva* (Ottawa gardener), *L. canadense* (Thimble Farm Vancouver Islands, BC and Grand Moraine Growers Burlington, ON), *L. philadelphicum* (Thimble Farm), *L. superbum* (Fuller Native and Rare Plants Belleville, ON and Thimble Farm), *L. michiganense* (Fuller Native and Rare Plants and Grand Moraine Growers), *S. lanceolatus* (Sunshine Farm and Gardens West Virginia, US) and some *P. biflorum* (hardware store).