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## CHAPTER 10. EVALUATION OF LILY LEAF BEETLE PARASITOIDS FOR NORTH AMERICAN INTRODUCTION

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### INTRODUCTION

The lily leaf beetle, *Lilioceris lili* (Scopoli) (Coleoptera: Chrysomelidae), has spread into five Canadian provinces and six states in the northeastern USA in the 60 years since its introduction into North America. It is a serious pest of native and ornamental lilies and a candidate for classical biological control given that it is well regulated by a complex of seven parasitoids in Europe. However, unlike many of the key agricultural and forest pests, the arrival of this pest is not regarded as a disaster (except by lily growers). This has afforded us the opportunity to carefully evaluate host specificity using many different approaches without the serious time constraints that affect many programs. We have evaluated the parasitoid complex through field and laboratory tests with congeneric species in Europe; with studies of chemical ecology of the pests, their parasitoids, and their host plants in Europe; and with laboratory host range testing in the USA. These multiple approaches yield generally supportive, but sometimes contradictory results, which provide useful insight into the value and interpretation of these techniques.

### **LILIOCERIS LILII**

The first published record of *L. lili* in North America was by Brown (1946), who found it in Montreal, Canada, in 1945. The beetle, which had been found on Montreal Island as early as 1943 (LeSage, 1992), apparently did not cross the St. Lawrence River until 1978. Within three years, the beetle was found in Ottawa (140 km distant). It was subsequently found in Wellington, Nova Scotia, in 1992 (LeSage, 1992); Boston, Massachusetts, in 1992 (Livingston, 1996); Toronto, Ontario in 1993 (Gooderham, 1993); Portage la Prairie, Manitoba, in 1999 (LeSage, pers. comm); and Fredericton, New Brunswick, in 2002 (LeSage, pers. comm.). Since its dis-

covery in Boston, the beetle has spread throughout New England and into northern New York.

The genus *Lilioceris* contains 142 species, of which 35 are found in the holarctic region, 60 are Oriental, 16 Australian, 20 Ethiopian, three neotropical, and the remaining eight species are of unknown distribution (Berti and Rapilly, 1976). Among the European species, *Lilioceris lili* (Scopoli) 1863 appears to be the most widely distributed, with specimens recorded from as far north as Siberia and south through North Africa (Livingston, 1996). Berti and Rapilly (1976) trace the origin of *L. lili* to the Orient. Lu and Casagrande (1998) and Yu *et al.* (2001) report the insect to occur in China.

This univoltine beetle overwinters as an adult and after initiating feeding in the spring, oviposits on the undersides of lily leaves. Larvae, which carry a fecal shield, pass through four instars before pupating in the soil. In North America, larval feeding often results in severe defoliation of cultivated *Lilium* and *Fritillaria* species as well as native lilies (Livingston, 1996). There are 21 species of lilies native to North America, including three (*Lilium canadense* L., *Lilium philadelphicum* L., and *Lilium superbum* L.) that lie within the eastern North American range of *L. lili* (Woodcock and Stearn, 1943).

In Europe, the beetle is widespread and relatively common but seldom achieves pest status except in the United Kingdom, where it is an exotic species (Salisbury, 2003), and in some ornamental plantings in continental Europe, where natural enemies are likely disturbed by cultural practices such as bulb removal in winter (Kenis *et al.*, 2003).

## NATURAL ENEMIES

No insect natural enemies have been reported on *L. lili* in North America (LeSage, 1992; Livingston, 1996; Gold, 2004). In Europe, Gold *et al.* (2001) surveyed France and Switzerland and identified four larval parasitoids of *L. lili*: *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), *Lemophagus pulcher* Szepligeti (Hymenoptera: Ichneumonidae), *Lemophagus errabundus* Gravenhorst (Hymenoptera: Ichneumonidae), and *Diaparsis jucunda* (Holmgren) (Hym: Ichneumonidae). Haye and Kenis (2004) subsequently reported the occurrence of an egg parasitoid, *Anaphes* sp. (Hymenoptera: Mymaridae), reared from *L. lili*, along with two tachinid flies attacking larvae, *Meigenia simplex* Tschorsnig and Herting, and *Meigenia uncinata* Mesnil (Diptera: Tachinidae). These three species are not being considered as potential biological control agents because the tachinids are known from other, unrelated hosts and *Anaphes* sp. needs to overwinter in an alternate host in order to complete its development (Haye and Kenis, 2004).

*Tetrastichus setifer* is the most widely distributed of the European parasitoids of *L. lili*. In our surveys, it was found from the United Kingdom to Bulgaria and from northern Germany to Italy (Kenis *et al.*, 2002; Haye and Kenis, 2004). It is also known from the Czech Republic, Slovakia, France, the former Yugoslavia, and Sweden (de V. Graham 1991). *Tetrastichus setifer* is a gregarious species, averaging 7 parasitoid larvae per host (range = 2 to 26). It is univoltine, and mature larvae overwinter in the host's cocoon in the soil. Adult emergence is protracted over a period of several weeks in the spring. Females oviposit in all four larval stages of *L. lili* (Haye and Kenis, 2004).

*Diaparsis jucunda* was reported by Horstmann (1971) from Sweden, Finland, Denmark, Germany, and the Czech Republic. Haye and Kenis (2004) found it to be the dominant parasitoid of *L. lili* in central and southern Europe (Switzerland, Austria, Italy) on both cultivated and wild lilies. This species is nearly absent from western and northern Europe. This solitary larval parasitoid attacks all larval stages of *L. lili* and kills the pre-pupa in the host cocoon, where it overwinters as a larva (Haye and Kenis, 2004).

*Lemophagus errabundus* was described by Gravenhorst in 1829 from Germany and was reported to attack *Lilioceris merdigera* (L.) in France (Elliott and Morley, 1911). Haye and Kenis (2004) found it to displace *D. jucunda* as the dominant parasitoid in western and northern Europe (United Kingdom, Netherlands, western France, and northern Germany), but it is rare elsewhere. This solitary, univoltine larval parasitoid kills the beetle in the pre-pupal stage and overwinters as a teneral adult in the host cocoon.

*Lemophagus pulcher*, first described from Hungary, was found by Kenis et al. (2002) and Haye and Kenis (2004) to be widespread, occurring in nearly all regions investigated (except the United Kingdom), but dominating only in Bulgaria. It is very similar to *L. errabundus*, but 4-58% of the individuals emerge for a second generation when parasitized larvae are reared in the laboratory, and there are evidences that a partial second generation also occurs in the field. This species is commonly attacked by the hyperparasitoid *Mesochorus lilioceriphilus* Schwenke, and hyperparasitism rates of 30% are common. *M. lilioceriphilus* also occasionally attacks *Lemophagus errabundus*.

Parasitism rates of *L. lili* are generally high throughout Europe, with averages of 25-78% (Haye and Kenis, 2004). Different parasitoids predominate in different regions and at different times of the season (Haye, 2000; Kenis et al., 2002; Kenis and Haye 2004).

## OTHER HOST SPECIES IN EUROPE

Berti and Rapilly (1976) report six species of *Lilioceris* in Europe, but only three are known from western and Central Europe. *Lilioceris merdigera* L. is a widespread species, feeding on *Polygonatum multiflorum* L., *Polygonatum verticillatum* (L.) *Polygonatum odoratum* (Miller), *Convallaria majalis* L., *Allium ursinum* L., and in gardens on chive (*Allium schoenoprasum* L.) (Haye and Kenis, 2004). *Lilioceris tibialis* (Villa) is a rare species found in the Alps that feeds on wild *Lilium martagon* L. and *Lilium bulbiferum* L. (Haye and Kenis 2004). These congeneric species can serve as hosts for the same parasitoids as *L. lili*, and they were used to evaluate host range of the parasitoids found on *L. lili*. There is no record of the dominant parasitoids of *L. lili* (*T. setifer*, *D. jucunda*, *L. errabundus*, *L. pulcher*) attacking other hosts in Europe.

In addition to *L. lili*, three other criocerid beetles have become important pests in North America, and these species have been subjected to extensive biological control research: the cereal leaf beetle, *Oulema melanopus* (L.); the common asparagus beetle, *Crioceris asparagii* (L.); and the spotted asparagus beetle, *Crioceris duodecimpunctata* (L.). A complex of European parasitoids of the cereal leaf beetle has been established in North America, including *Tetrastichus julis* (Walker), *Lemophagus curtus* Townes, *Diaparsis temporalis* Horstmann, and *Anaphes flavipes* (Foerster) (Haynes and Gage, 1981). The introduced asparagus beetles also have European parasitoids, including *Tetrastichus asparagi* Crawford and *Lemophagus crioceritor*

Aubert, both of which were released in North America against *C. asparagi*; and *Tetrastichus crioceridis* Graham and *Diaparsis truncatus* (Gravenhorst), which were released against *C. duodecimpunctata* (Hendrickson *et al.*, 1991). Despite the extensive collection and rearing of the European parasitoids of these species for these biological control programs, none of the parasitoids of *Lilioceris* species were reported from these hosts.

## RELATED BEETLES IN NORTH AMERICA

*Lilioceris lili* is among the 1,720 North American species in the family Chrysomelidae, which are divided among 195 genera (Triplehorn and Johnson, 2005). North American species are grouped in 11 subfamilies, including Criocerinae, which includes the genera *Crioceris*, *Oulema*, *Neolema*, *Lilioceris*, and *Lema* (Triplehorn and Johnson, 2005).

The only insects in North America in the genus *Crioceris* are the introduced asparagus beetles *C. asparagi* and *C. duodecimpunctata* (Arnett, 2000). *Oulema* is represented by at least 10 species, including the European cereal leaf beetle, *O. melanopus*. About 15 species of *Lema* are known to occur in the eastern and southern United States, including *Lema trilineata* White, which feeds on potatoes and other solanaceous plants (Triplehorn and Johnson, 2005). *Neolema* contains at least four North American species, including *Neolema sexpunctata* (Oliver), which feeds on the common dayflower, *Commelina communis* L. The lily leaf beetle, *L. lili*, is the only species of *Lilioceris* that presently is known to exist in North America.

## RESEARCH RATIONALE

We evaluated four parasitoids for possible introduction against *L. lili* in North America: *Tetrastichus setifer*, *Diaparsis jucunda*, *Lemophagus errabundus*, and *Lemophagus pulcher*. All four of these species were found to cause high levels of parasitism in various locations in Europe, and unlike the tachinid species, they appeared to have reasonable host specificity.

Host specificity research in Europe concentrated on the congeneric beetles *L. merdigera*, *L. tibialis*, and the pest itself, *L. lili*. These three species are the only *Lilioceris* species occurring in western and Central Europe. They all have a similar biology and ecology, and we presumed that if parasitoids proposed for introduction distinguished among these congeneric species in Europe, they would also do so among potential U.S. hosts related to *L. lili* at more distant taxonomic and ecological levels. Thus, we believed that a parasitoid of *L. lili* that would not attack *Lilioceris merdigera* and *L. tibialis* would be highly unlikely to attack a species from another genus.

The chemical ecology research in Europe attempted to elucidate the stimuli for attraction and oviposition of the four key parasitoids of *L. lili* using the three *Lilioceris* species and their host plants, as well as extracts from *L. trilineata* from North America. The rationale for this research in the context of host specificity is that we expected to find stimuli that were specific to the lily/*Lilioceris* system whose absence would preclude the use of other species as hosts.

Further host range testing was conducted in quarantine in North America to expand upon the work with congeneric species done in Europe and determine if parasitoids were specific at the genus level. *Lilioceris lili* is the only North American insect in its genus, and it arrived

relatively recently. Thus parasitoids with genus-level specificity could be safely released without fear of affecting other insect populations. In selecting potential hosts, we focused upon the most closely related species, attempting to get at least one species from each of the North American genera within the Criocerinae. Specifically, we selected *Oulema melanopus*, *Crioceris asparagi*, and *Lema trilineata* for our tests. We would have evaluated *Neolema sexpunctata*, but we were unable to collect this species in numbers adequate for experimentation. We broadened the taxonomic scope of our tests by including three additional chrysomelids: the imported willow leaf beetle (*Plagioderma versicolora* Laicharting), the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), and two *Galerucella* species introduced for biological control of purple loosestrife (*Lythrum salicaria* L.). Additionally, we tested one coccinellid: the Mexican bean beetle (*Epilachna varivestis* Mulsant). These non-criocerid test species were selected based upon availability and relative ease of rearing. The laboratory experiments required growing (or collecting) the host plants for the test species and inducing these beetles to oviposit so that non-parasitized larvae were available at the time that *L. lili* and its parasitoids were available for testing. Our laboratory host preference tests were expanded to include parasitoid oviposition response to previously parasitized *L. lili* larvae to test for cleptoparasitism and to help evaluate some of the field results observed in Europe.

## STUDIES ON CONGENERIC BEETLE SPECIES

### METHODS

Two different investigations were conducted in Europe: (1) evaluation of sympatric populations in the field and (2) laboratory host specificity screening.

**Sympatric populations** This research was conducted at CABI Bioscience in Switzerland by Claire Scarborough, working under the direction of Marc Kenis. She collected third and fourth instar larvae of various *Lilioceris* species between May and July, 2002, from four natural sites in the Jura region of Switzerland (Scarborough, 2002). All four sites had sympatric populations (separated by less than 500 m) of the beetle *L. lili* feeding on *Lilium martagon* and the beetle *L. merdigera* feeding on *P. multiflorum* and *P. verticillatum*. At a fifth site, situated in the Alps, sympatric populations of the beetles *L. lili* and *L. tibialis* were found feeding on the lily *L. martagon*.

Larvae from all sites were reared on excised host plants in 1.3 liter plastic containers with a bottom layer of wet fine vermiculite and allowed to pupate. After emergence of adult beetles and some non-diapausing parasitoids, the containers were sifted and the parasitoids that had emerged from beetles were identified based on cocoon features and adult emergence

**Host specificity screening** These tests were carried out by Haye (2000), Kenis et al. (2001, 2002), and Scarborough (2002). Laboratory rearing of the three species was set up in cages using adults or eggs collected from field populations in Switzerland. Larvae were fed with cultivated lily (for *L. lili* and *L. tibialis*) and cultivated onion (for *L. merdigera*)

Parasitoids used in these experiments (*L. pulcher*, *L. errabundus*, *D. jucunda*, and *T. setifer*) were reared from cocoons collected in previous years and held over winter at 2°C. The cocoons, held in Petri dishes in polystyrene boxes lined with damp cellulose paper, were moved

to room temperature (20–24°C) and monitored daily for adult emergence. For the ichneumonid species, males of a single species were held together in 1.3 liter containers in groups of four or five and provided with moist cotton wool dipped in honey. Females were placed in cages with males for approximately 24 hours for mating and then held separately for another 24 hours before use in experiments. Between tests, parasitoids were kept in incubators at 11–17°C, 16:8 L:D photoperiod and ambient humidity, with access to moist cotton wool and honey.

In choice tests, three larvae of *L. lili* and three larvae of either *L. merdigera* or *L. tibialis* were placed in a 9.4 cm diameter Petri dish and one parasitoid was introduced for ten minutes, during which time ovipositions on individual larvae were directly observed. Because the eulophid *T. setifer* oviposits for up to 30 minutes compared to a few seconds for the three ichneumonids, experiments with *T. setifer* were run for 3 hours. Following each test, larvae were reared on their proper host plants and held over wet fine vermiculite in 0.15 liter containers until they were dissected to determine parasitism.

In no-choice tests, a single female was introduced into a dish of its dominant host (typically *L. lili*) and observed for 10 minutes to count ovipositions. She was removed and allowed 10 minutes before a second exposure to three larvae of the alternate host. Again, ovipositions were recorded during this second exposure, after which the female was provided a second 10-minute rest. A third 10-minute exposure to the initial test species was conducted to confirm her ability (or willingness) to oviposit. Exposed beetle larvae were reared over wet, fine vermiculite before dissection to determine parasitism.

## STUDIES WITH CONGENERIC BEETLES: RESULTS AND DISCUSSION

*Sympatric populations* Scarborough (2002) found high parasitism among the natural sympatric populations of *L. lili* and *L. merdigera* (86.1% and 79.2%, respectively, among the total 957 *L. lili* and 524 *L. merdigera* larvae that she collected). *Diaparsis jucunda* was the principal parasitoid of *L. lili*, accounting for 142 of 198 (71.1%) parasitoids recovered from that host, but it accounted for only 10% of the total parasitism of *L. merdigera* (8 of 80 recovered). Conversely, the *Lemophagus* species (principally *L. pulcher*) were more prevalent in *L. merdigera* than in *L. lili*, accounting for 71 of 80 (87.6%) parasitized larvae vs. 56 of 198 (28.3%), respectively. *Tetrastichus setifer* was found only in one *L. merdigera* (1.2% of total).

At the site with *L. tibialis* and *L. lili* in the western Alps, Scarborough found 98.3% parasitism of 88 *L. lili* larvae, but only 29.3% parasitism of 527 larvae of *L. tibialis*. As with the other sites, *D. jucunda* was most common in *L. lili*, accounting for 38 of the 45 parasitized larvae from that host (84.4%) vs. 2.7% (3 of 111) parasitized larvae of *L. tibialis*. *Lemophagus* species were more common in *L. tibialis* than *L. lili*, comprising 77 (69.4%) of the 111 parasitized larvae of *L. tibialis* vs. 2 (4.4%) of the 45 parasitized larvae of *L. lili*. At this site, *T. setifer* was relatively common, accounting for 27.9% (31/111) of total parasitism of *L. tibialis* and 11.1% (5/45) of the parasitized *L. lili* larvae.

These results are consistent with those observed by Haye and Kenis (2004) in non-sympatric populations at natural sites on wild plants in Switzerland. Although all of the four major parasitoids occasionally attack all three congeneric hosts in natural settings, strong host preferences are shown. *L. lili* is mainly attacked by *D. jucunda*, which is found in very low numbers on the two other hosts and only in the vicinity of *L. lili* populations. *L. pulcher* is by far the

main parasitoid of *L. merdigera*. *T. setifer* has been observed in high numbers attacking populations of *L. tibialis*, and *L. errabundus* is found occasionally on all the three hosts. These strong host preferences shown in natural habitats in Switzerland do not necessarily reflect their potential as biological control agents, given that all four parasitoids have been found as dominant parasitoids of *L. lili* in gardens in different regions of Europe (Haye and Kenis, 2004).

**Host specificity screening** All four parasitoids were successfully reared on the three host species. In choice tests with pairs of species, *L. pulcher* attacked *L. tibialis* and *L. merdigera* as readily as *L. lili*, and this response was not affected by the parasitoid's rearing host (Scarborough, 2002). Eggs and larvae were found in all three hosts, supporting field observations that all three congeners are adequate hosts for this species.

*Lemphagus errabundus* also readily attacked both *L. lili* and *L. tibialis* in choice tests with species pairs, but it demonstrated a borderline significance ( $P = 0.07$ ) in preference for *L. lili* over *L. merdigera*, a preference that was supported by no-choice tests in which significantly fewer ( $P = 0.004$ ) larvae of *L. merdigera* were selected for oviposition compared to *L. lili*.

Contradictory results were obtained with *Diaparsis jucunda*. Haye (2000) and Kenis et al. (2001) observed that *D. jucunda* showed a strong preference for *L. lili* in choice-tests ( $P < 0.001$ ). In no-choice tests, it oviposited in *L. lili* very frequently, whereas ovipositions in *L. merdigera* occurred at much lower frequency. In contrast, two years later, Scarborough (2002) observed *D. jucunda* attacking *L. tibialis* and *L. merdigera* as readily as *L. lili*. The parasitoid's rearing host did not affect the parasitoid's oviposition preferences.

*Tetrastichus setifer* reared from *L. lili* spent significantly more time on *L. lili* than on *L. tibialis* in paired choice tests ( $P = 0.009$ ) and showed a significant preference for *L. lili* over *L. merdigera* ( $P = 0.0002$ ). However *T. setifer* reared from *L. tibialis* showed no preference between that host and *L. lili*.

Collectively, these screening studies indicate that all three beetle species are attacked by all four parasitoid species. The preference for particular hosts observed in sympatric populations in the field did not clearly appear in the laboratory tests. The contradictory results obtained with *D. jucunda*, perhaps due to the use of different parasitoid populations, emphasize the need for large sample sizes and replicates with different strains in such screening tests.

## STUDIES ON CHEMICAL SCREENING

### METHODS

The research on the chemical ecology of the parasitoids of *L. lili* has been led by Dr. Urs Schaffner at the CABI Bioscience Switzerland Centre. Both olfactometer bioassays and contact bioassays were used in this research. The olfactometer tests used a round, static-air, four-chamber olfactometer (Steidel and Schöller, 1997) in which the test substance was placed at random in one or two of the chambers while the other two or three chambers remained empty. A single parasitoid was released onto a fine mesh screen over these four chambers and the time spent over the test chamber and the three controls was recorded during a five-minute assay

period. Contact bioassays were conducted in 9 cm glass Petri dishes in which two or three substrates placed equidistant from one another were offered simultaneously to a single wasp placed in the center of the arena. Contact frequency, contact duration, and frequency of ovipositor probing were recorded for five-minute periods.

Tested substrates included, among others, *L. lili* larvae with or without their fecal shield; fecal shield of *L. lili* alone; shield extracts of *L. lili*, *L. merdigera* and the North American non-host *Lema trilineata* on paper dummies; lily leaves damaged by *L. lili*, by other defoliators or artificially damaged; *Polygonatum verticillatum* leaves damaged by *L. merdigera*; and larvae and fecal shield of the cereal leaf beetle, *Oulema melanopus*.

All four larval parasitoids were tested. However, *L. errabundus* did not respond consistently to the contact bioassays, and both *L. errabundus* and *T. setifer* were unresponsive to the olfactometer bioassays. Therefore, most tests were carried out with the more cooperative *L. pulcher* and *D. jucunda*. When possible, naïve and experienced females were compared in their response to signal sources. The experimental approaches and results are described in detail in various publications and unpublished reports (Schaffner and Kenis, 1999; Kenis *et al.*, 2001; Schaffner and Müller, 2001; Scarborough, 2002; Schaffner, 2002). Only a summary of the most relevant results is presented herein.

#### CHEMICAL SCREENING RESULTS AND DISCUSSION

In both olfactometer and contact bioassays, *L. pulcher* was found to be attracted to *L. lili* larvae with and without their fecal shields, to the fecal shields alone, to shield extracts on dummies, and to lily leaves that had been damaged by *L. lili*. Females were induced to oviposit on dummies by shield extracts. Larvae and the fecal shield of *O. melanopus* and fecal shield extracts of *L. trilineata* were found to be significantly attractive to *L. pulcher*.

*Diaparsis jucunda* responded rather similarly, being attracted to fecal shields with and without larvae and lily foliage that had been damaged by *L. lili* larvae, and being stimulated to oviposit by extracts from the fecal shields of *L. lili* larvae. *Diaparsis jucunda* showed ovipositor probing on dummies with shield extracts of *L. trilineata* but only a nonsignificant preference for such dummies over untreated controls. Interestingly, *D. jucunda* was not attracted to *P. verticillatum* leaves damaged by *L. merdigera*. In contrast, it was attracted to dummies treated with fecal extracts from *L. merdigera*, displaying ovipositor probing (*L. pulcher* was not tested with *L. merdigera* extracts).

Scarborough (2002) also investigated in these chemical screening experiments the effect of prior experience on host selection. She found that, while *L. pulcher* host selection behavior is largely innate, it may change with experience. Naïve *L. pulcher* females did not probe a dummy treated with an extract of *L. lili* fecal shield, but after experience with *L. lili* larvae, the females showed increased frequency of probing and increased duration of contact with the dummy. In contrast, *D. jucunda* host specificity appears fixed regardless of experience: both naïve and experienced females respond to fecal extracts of *L. lili* with frequent ovipositor probing and prolonged contact with the larva. These observations may indicate greater host plasticity in *L. pulcher* given that its behavior may change with experience. In contrast, the innate host-selection behavior of *D. jucunda* suggests a narrower host range.

*Tetrastichus setifer* females also responded positively to fecal shields and fecal shield extracts of *L. lili*. When presented with *L. lili* and *L. merdigera* larvae, they were found to spend less time on the *L. merdigera* larvae in contact bioassays. When the fecal shields of these hosts were switched (putting *L. merdigera* feces on *L. lili* and vice-versa), the parasitoids switched their preference, spending significantly more time on *L. merdigera* (Scarborough, 2002).

Overall, these experiments, while incomplete, were useful in assessing host preference. *Lemophagus pulcher*, while attracted to *L. lili*, its fecal material, and its damaged host plants, also was attracted to, and oviposited in, dummies treated with extracts from *L. trilineata*, a North American insect in a different genus from the normal host. *Lemophagus pulcher* also demonstrated a greater plasticity in host response based upon prior experience than *D. jucunda*. These results, combined with the field results of sympatric populations and laboratory host screening tests with congeneric species, indicate that special attention might be given this parasitoid in further host specificity studies. *Diaparsis jucunda* showed generally similar responses to those of *L. pulcher*, including a non-significant positive response to *L. trilineata*. *Tetrastichus setifer* showed selectiveness in fecal shield attractiveness – responding more strongly to fecal material from *L. lili* than to that from *L. merdigera*. This is consistent with the laboratory screening with intact larvae.

Like the congeneric studies, the chemical screening tests indicate possible host preferences in some species, but they do not identify any of the four parasitoids as host specific to a particular species. Furthermore, one parasitoid (*T. setifer*) responded in only the contact bioassay and another (*L. errabundus*) did not respond in either test. It is likely that, with additional experimentation, it would be possible to establish test conditions that allowed these species to respond, but this problem brings into question the general utility of this approach to host specificity screening.

## TESTS IN QUARANTINE

### SOURCE AND REARING OF PARASITIDS

Parasitoids used in these experiments were reared from *L. lili* larvae collected in Europe. In 1998, these were collected in northwestern France (Gold et al. 2001), and in subsequent years, they were collected throughout Europe (Haye and Kenis, 2000; Gold, 2004). Field-collected larvae were held in 1.4 l plastic containers under laboratory conditions (ca 25°C) and fed lily leaves until cocoon formation. Resultant cocoons were then held under similar conditions until all adult *L. lili* emerged. Parasitized cocoons were then held at 4°C in a growth chamber for a minimum of two months before shipment in chilled containers to the URI Biological Control Laboratory. In our quarantine laboratory, parasitoids were held at 4°C until needed for experiments and then warmed to 25°C for adult emergence. From 1999-2003, 12,978 parasitized *L. lili* cocoons were shipped to URI, including 4,352 *T. setifer*, 4,895 *D. jucunda* and 3,731 *Lemophagus* spp. Parasitoids that emerged were used in research. The remaining cocoons were dissected and information on species was provided to Marc Kenis at CABI in Switzerland for

parasitoid distribution surveys. Only field-collected parasitoids were used in our host specificity studies.

We maintained the pest beetle *L. lili* in quarantine at the URI Biological Laboratory in a colony that was started (and periodically refreshed) with adults collected near Boston, Massachusetts. Beetles were reared on potted Asiatic and Oriental lilies grown from organically produced bulbs in a greenhouse under ambient temperature conditions and a minimum of 16h daylight, supplemented by 400 watt sodium vapor or 1000 watt mercury vapor lights on timers. In the laboratory, beetles were reared in screen cages (45 cm on a side) under fluorescent lights with a 16:8 (L:D) photoperiod. Newly emerged adult beetles were fed for a minimum of one week and then stored in plastic freezer cartons with paper towels in a refrigerator at 7°C for three months, after which they were removed and used in rearing (Gold, 2004).

### HOST RANGE TESTS

**Methods for host range tests** Newly emerged adult parasitoids were held in 1.8 liter plastic jars in growth chambers under fluorescent lights with a 16:8 (L:D) photoperiod and a day:night temperature cycle of 20:15°C. The jars were removed from the growth chambers for 4h during host specificity tests at ambient room temperature (25°C). These tests were conducted on a table next to a window with supplemental fluorescent lighting. Putative hosts evaluated in these experiments included the *Criocerinae* species *O. melanopus*, *C. asparagi*, and *L. trilineata*. We also tested three non-*Criocerinae* chrysomelids: *P. versicolora*, *L. decemilineata*, and *Galerucella* sp. and the coccinellid *E. varivestis*.

Test larvae were placed on stems of their host plant for a minimum of 2h before exposing them to parasitoids in all experiments because Schaffner and Müller (2001) showed that some species of *L. lili* parasitoids are attracted to plants damaged by *L. lili* larvae. For these feeding periods and subsequent parasitoid exposures, 10-12 second or third instar larvae were placed on an excised stem of a host plant, and that stem was placed in a water pic filled with tap water. In the tests with ichneumonid species, one to five female wasps (generally three, rarely one) and one male wasp were placed in a jar with the test larvae for 2 hours. In the tests with eulophid species, ten females and at least one male *T. setifer* were placed in a jar for 2 hours. Wasps were provided water and honey with either a damp wick in a water pic and a streak of honey or honey water on a wick. Immediately after exposure to the test larvae, the same parasitoid adults were given a second exposure to 10-12 second or third instar *L. lili* larvae on a lily stem using the same protocol as above. When parasitism was found in a test larva, as well as in the subsequent test with lily leaf beetle larvae, the results were analyzed using a Chi-square test (Johnson and Bhattacharyya, 1987).

After parasitoid exposure, larvae were reared in 240 ml plastic containers with a bottom layer of 50 cc of damp vermiculite and fed leaves of the host plant for approximately ten days before they were dissected to determine parasitism. In all experiments, the first exposure of a female parasitoid was to a nontarget test species (other than *L. lili*), and these exposure data were used only if parasitoids successfully attacked *L. lili* larvae after that first exposure. Depending upon the parasitoid species, between 35% and 71% of the tests were rejected because of lack of attack on *L. lili*, involving well over 1,500 test larvae and an equivalent number of *L. lili*. Among the possible 32 tests (8 test larvae x 4 parasitoid species) we obtained useful results

(with positive results in controls) in 27 combinations with an average of 35.6 test larvae per test. The *L. lili* controls in these tests averaged 27.3% parasitism.

**Results from Host Range Tests** Among the ichneumonids, Gold (2004) found that neither *D. jucunda* nor *L. errabundus* oviposited in any of the eight nontarget hosts tested. *Lemophagus pulcher* oviposited in two nontarget insects, *L. trilineata* and *C. asparagi*. We found 6 of 76 (7.8%) *C. asparagi* larvae were parasitized by *L. pulcher* in a test where the controls showed 30 out of 102 (29.4%) parasitized. A significant difference in these ratios (Chi-square test,  $P = 0.001$ ) indicates a preference for *L. lili* over *C. asparagi*. *Lemophagus pulcher* parasitized 11 of 33 *L. trilineata* (33%) vs. 9 of 35 *L. lili* (25.7%). This non-significant difference (Chi-square test,  $P = 0.30$ ) indicates that *L. trilineata* is as acceptable as *L. lili* to this parasitoid.

None of the putative hosts exposed to *T. setifer* were attacked except a single larva of *L. trilineata*, which was found to contain *T. setifer* larvae. The parasitoid ratio (1/73) was significantly different (Chi-square test,  $P = 0.001$ ) from the parasitism of the *L. lili* control in this test (15/63), indicating a distinct preference of *L. lili* as a host by this species. Gold (2004) also conducted preliminary tests in which *T. setifer* was exposed to *L. trilineata* using a slightly different protocol, and in those tests 0 of 79 larvae were parasitized. We consider the parasitism of a single *L. trilineata* larva out of 150 tested to be an anomaly, perhaps due to confinement in too small a container.

#### HOST PREFERENCE TESTS (PARASITIZED VS. NON-PARASITIZED)

**Methods for host preference tests (after Gold, 2004)** We assessed the behavior of parasitoids exposed to previously parasitized hosts in a series of choice tests conducted in 8.5 cm diameter Petri dishes. To obtain larvae stung by the ichneumonid wasps, we placed three second or third instar *L. lili* larvae on lily leaf fragments in a covered Petri dish. Individual female wasps were placed in the Petri dish, and the larvae were removed once they were stung. For *L. errabundus*, *L. pulcher*, and *D. jucunda*, a sting entailed insertion of the ovipositor for a minimum of two, two, and three seconds, respectively (Haye and Kenis, 2000). Because of the long oviposition time of *T. setifer*, we used a different protocol to obtain stung larvae. We placed 20 female wasps in a Petri dish with ten second or third instar lily leaf beetle larvae. Larvae were removed from the dish once they had been stung, which in this case was defined as insertion of the ovipositor for at least 15 minutes, exceeding the 13 minute minimum oviposition requirement reported by Haye and Kenis (2000).

Choice tests were conducted 24 hours after the larvae were stung. In the choice tests with the ichneumonid parasitoids, three *T. setifer*-stung larvae and three unstung larvae were placed alternatively in a circle on fragments of lily leaf in an 8.5 cm Petri dish. An individual female wasp was placed in the Petri dish for 15 minutes. Every ovipositor insertion was recorded. When the tests were conducted with *T. setifer*, ten female wasps were placed in a Petri dish with three ichneumonid-stung and three unstung lily leaf beetle larvae for 15 minutes. Total ovipositor insertions of all ten females were recorded. Trials were replicated six to ten times, depending upon the availability of wasps and host insects. New females and a clean Petri dish were used for each replicate. All results were analyzed with the Wilcoxon matched-pairs signed-ranks test (Johnson and Bhattacharyya, 1987). Larvae were dissected after approximately ten days to

determine parasitism, including which parasitoid survived in cases of multiple-species ovipositions.

In a second series of choice tests, a similar protocol was followed except that the choice exposure was conducted within three hours after the first exposure instead of 24 hours later. Results were again analyzed with the Wilcoxon matched-pairs signed rank test, and larvae were dissected after approximately ten days.

**Results for host preference tests** Gold (2004) found that *L. errabundus* does not distinguish between *L. lili* larvae that were and were not previously stung by *T. setifer* or *L. errabundus* in tests conducted 3 and 24 hours after initial parasitism. In three of four trials, the same applied in the reverse direction: *Tetrastichus setifer* did not distinguish between larvae that were and were not previously stung by *L. errabundus*. However, in one test, *T. setifer* stung significantly more *L. lili* larvae (3.7 vs. 2.0) that were previously stung by *L. errabundus*. In one of two tests, *T. setifer* showed a significant preference for unstung larvae vs. those that were previously stung by *D. jucunda* (2.3 vs. 1.0). *Diaparsis jucunda* preferred unstung larvae over those previously parasitized by *T. setifer* in one of three trials, and *L. pulcher* did not distinguish between larvae that were and were not previously by *T. setifer* in a single trial.

Although Gold (2004) did not test all possible combinations of parasitoids, she did test all four species under evaluation and found no indication of cleptoparasitic tendencies in any of them. There is also little or no indication that the parasitoids distinguish between previously parasitized and unparasitized larvae – even among those parasitized by their same species (Gold, 2004). Tests conducted 3 hours after initial parasitoid exposure gave results similar to the exposures conducted 24 hours later. It is possible that this test protocol could have masked behavior that occurs in the field. Following oviposition, female ichneumonids, particularly *L. errabundus*, are frequently observed dragging their abdomens across the leaf on which the parasitized larva resides, possibly marking these leaves as containing parasitized larvae. Our protocol involved using new leaves and clean Petri dishes for each exposure, thereby removing any signals that were not directly associated with the larva.

In dissecting the parasitized *L. lili* larvae that resulted from the behavior experiments, Gold (2004) found that when *T. setifer* oviposits first, it is more likely to survive and develop in lily leaf beetle larvae than are *D. jucunda*, *L. errabundus*, or *L. pulcher*. However, if *L. errabundus* stings the lily leaf beetle first, either *T. setifer* or *L. errabundus* may survive; and when *D. jucunda* stings first, it is more likely to survive and develop than *T. setifer*.

## SUMMARY

The three types of investigations conducted (field studies of congeneric species under sympatry, chemical ecology, and laboratory screening) all provided useful results, which together present a clear picture of host specificity in parasitoids of *L. lili*.

Studies of sympatric populations of *L. lili* and its congeners showed *D. jucunda* to be the most discriminating of the four primary parasitoids of *L. lili*, demonstrating a strong preference for *L. lili* over *L. merdigera* or *L. tibialis*. Laboratory chemical screening results strengthened this observation. *Diaparsis jucunda* is attracted to lily foliage that has been damaged by *L.*

*lilii* larvae, and it is stimulated to oviposit by extracts from the fecal shields of *L. lilii* larvae (Scarborough, 2002). Further, this species is not attracted to *P. verticillatum* leaves damaged by *L. merdigera*. Studies in quarantine showed that *D. jucunda* does not attack any of the eight nontarget test species presented in tests in which *D. jucunda* consistently parasitized *L. lilii*. Furthermore, *D. jucunda* is not a cleptoparasitoid. In competition with *T. setifer* within a *L. lilii* larva, we found that whichever species attacked was most likely to survive. The only negative results with *D. jucunda* are reported by Scarborough (2002), who determined that it attacked *L. tibialis* and *L. merdigera* as readily as *L. lilii* in choice tests based on pairs of species presented in Petri dishes. This may have resulted from the choice of experimental chambers. Livingston (1996) obtained anomalous results when she confined the cereal leaf beetle parasitoid *Anaphes flavipes* (Foerster) with lily leaf beetle eggs in a Petri dish without host plants. Although *Anaphes* readily attacked *L. lilii* under these conditions (and Livingston reared them for several generations in this manner), the parasitoid failed to attack these eggs in larger screened cages (45 cm on a side) in the laboratory or under field conditions.

*Lemophagus pulcher* was shown to be less host specific with all three approaches. In natural sympatric populations with *L. lilii* congeners, *L. pulcher* was more common in *L. merdigera* and *L. tibialis* than in *L. lilii*. Laboratory chemical screening tests with these congeners and their host plants showed that *Lemophagus pulcher* is attracted to *L. lilii*, its fecal material, and its damaged host plants. It also shows attraction and oviposition responses to extracts from *Lema trilineata*, and it demonstrated a greater plasticity in host response based upon prior experience than *D. jucunda*. In choice tests with pairs of hosts, *L. pulcher* attacked both *L. tibialis* and *L. merdigera* as readily as *L. lilii* in Petri dishes, and eggs and larvae were found in all three hosts. Laboratory tests in quarantine showed that *L. pulcher* attacked *L. trilineata* as readily as *L. lilii*, and it also oviposited in *C. asparagi*. This species is clearly the least host specific of the four species under consideration, and despite the potential advantage of having a partial non-dia-pausing population, it is presently not under consideration for release in North America. It is not clear, however, that *L. pulcher* would attack *L. trilineata* if this wasp were released in North America because it also attacked *C. asparagi* in our laboratory tests, and from all indications, it does not attack this host in the field in Europe. It is common for laboratory tests to indicate a wider host range than actually occurs in the field (Federici and Maddox, 1996; Strand and Obrycki, 1996).

*Lemophagus errabundus* and *T. setifer* were somewhat intermediate in their responses in this battery of tests. Neither species was very common in the sympatric populations studied by Scarborough (2002), but both species have shown to be more common on cultivated *L. lilii* in other areas, such as western and northern Europe. In natural environments in Switzerland, *Tetrastichus setifer* was more common in *L. tibialis* than in *L. lilii*. Neither *L. errabundus* nor *T. setifer* responded to the olfactory bioassay test and only *T. setifer* responded in the contact bioassay where it was more attracted to fecal material from *L. lilii* than from *L. merdigera*. In laboratory screenings, *L. errabundus* showed a preference for the beetle *L. lilii* over *L. merdigera*. *Tetrastichus setifer* reared from *L. lilii* was more attracted to *L. lilii* than to *L. tibialis* or *L. merdigera*. However, *T. setifer* reared from *L. tibialis* showed no preference between that host and *L. lilii* (Scarborough, 2002). Finally, in quarantine studies with eight nontarget test species, *L. errabundus* attacked nothing but *L. lilii*, and the same was true for *T. setifer* (except for the single anomalous parasitized *L. trilineata* of 150 exposed). Thus, it appears that both *L.*

*errabundus* and *T. setifer* have host preferences within the genus *Lilioceris*, and they likely would not attack insects outside of that genus.

## RECOMMENDATIONS

When species in the same genus as the target pests are found with sympatric populations, field sampling may be used to determine parasitoid preferences. Our studies suggested host preferences among the species, but fell short of showing any parasitoid to be host specific at the species level. On the other hand, if a parasitoid did attack only a single species among sympatric congeners (particularly on the same host plant), we could be quite certain that it would not attack more distantly related hosts. Given that our survey was relatively inexpensive, it was probably well worthwhile. The same is true for laboratory screening of parasitoids on congeneric species – although such work is more time-consuming.

Chemical ecology studies provide useful insight into parasitoid behavior, but in this case, they were not specifically designed to evaluate host specificity. Theoretically, it would be easier to get fecal extracts from various cicerid hosts and evaluate them in olfactometers than to simultaneously rear various test species and the *L. lili* controls and also have the right size host plants available when the parasitoids are in prime condition for oviposition. However, we probably need a larger body of evidence supporting this approach before we can substitute tests of this nature for the type of host range testing we did in quarantine. These studies do, however, contribute greatly to the growing body of knowledge about parasitoid behavior, and results will influence the design and interpretation of other laboratory experiments. For instance, it was quite clear from the work of Schaffner that the *L. lili* parasitoids are generally attracted to lilies that are damaged by *L. lili*. Thus, in all of our host range tests in quarantine, test species were confined on their host plants prior to the experiment and then kept on the same damaged plant during exposure to parasitoids. Since this is a very common phenomenon among parasitoids, it should be a standard practice for studies of host specificity. The chemical screening tests also showed parasitoid host preferences to be influenced by a parasitoid's prior host exposures. To be conservative, in our choice tests, parasitoids were first tested against the nontarget species (non-*L. lili*) host and then tested on *L. lili* to confirm parasitoid activity.

The choice of testing arena remains one of the key issues in laboratory testing. Our initial testing of *A. flavipes* on *L. lili* eggs in Petri dishes gave completely spurious results. Based upon a high attack rate and successful rearing of this cereal leaf beetle parasitoid on *L. lili* eggs, we attempted several unsuccessful field releases before determining that this parasitoid behaved differently in a large (45 cm on a side) laboratory cage with eggs on their proper host plant (Livingston, 1996). Scarborough (2002) also found *L. lili* parasitoids to be relatively non-discriminating among potential hosts when confined in Petri dishes. For example under such conditions, *D. jucunda* attacked *L. tibialis* and *L. meridigera* as readily as it did *L. lili*, even though all other evidence pointed to a marked preference for *L. lili* over the other two. Based upon these considerations, we used clear plastic jars (1.8 liter) with relatively large (12 cm) screw tops covered by screen. These 16 cm tall containers were large enough to house a host plant stem in a water pic and allow for parasitoid flight, but were small enough that we could readily follow the activity of the parasitoids. Exposures were conducted in front of large win-

dows (out of direct sun), and parasitoids appeared to behave normally during these tests. Test results showed considerable selectivity toward the nontarget test species and a reasonably high level of parasitism in the *L. lili* controls. Oviposition of the ichneumonid *L. pulcher* in the beetle *L. trilineata* was consistent with olfactometer tests and other tests indicating that this parasitoid is relatively non-specific. We do not have field tests or tests in larger cages to validate this experiment. We also observed that *L. pulcher* would occasionally attack *C. asparagi* in our 1.8 liter containers when this test species was confined on asparagus stems. In this case, it is likely that our test is not indicative of field results in Europe, where *C. asparagi* is attacked by a different parasitoid, *Lemophagus crioceritor* Aubert (Hendrickson *et al.*, 1991). It would be interesting to evaluate *L. pulcher* against *C. asparagi* in the field in Europe to determine whether our laboratory tests are predictive of field results.

We are confident that our host specificity tests adequately demonstrate that *T. setifer*, *L. errabundus*, and *D. jucunda* would be restricted to *L. lili* if released in North America. Based upon these tests, we have obtained federal and state permission for field release of all three species.

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