ESTABLISHMENT OF PERISTENUS SPP. IN NORTHERN CALIFORNIA FOR THE CONTROL OF LYGUS SPP.

Charles H. PICKETT1, Dominique COUTINOUT2, Kim A. HOELMER3, and Ulrich KUHLMANN4

1Biological Control Program, California Department of Food & Agriculture
3288 Meadowview Rd, Sacramento, California, U.S.A
cpickett@cdfa.ca.gov

2European Biological Control Laboratory, USDA ARS
Montferrier, France
dcoutinot@ars-ebcl.org

3USDA ARS BIIR
Newark, Delaware, 19713, U.S.A.
 khoelmer@ars-ebcl.org

4CABI Bioscience
Delemont, Switzerland
u.kuhlmann@cabi.org

ABSTRACT

_Lygus hesperus_ is native to western United States and is a pest to numerous field and seed crops. In California, it is a key pest of cotton and strawberries, both highly valued crops. Extensive surveys for natural enemies in western United States have found one egg and two nymphal parasitoids attacking _Lygus_ species, primarily _L. hesperus_. However in central California surveys in alfalfa by ourselves and others have failed to find any nymphal parasitoids. Beginning in the early 1970’s the USDA ARS initiated importation of parasitoids associated with _Lygus rugulipennis_ infesting alfalfa in central Europe. Van Steenwyk and Stern attempted but failed to establish _Peristenus stygicus_ during the mid 70’s in the southern region of the San Joaquin Valley in central California. Importation of nymphal parasitoids into eastern United States during the 1980’s, however, successfully reduced _Lygus lineolaris_ infesting alfalfa, a close relative of _L. hesperus_.

Several populations of _Peristenus stygicus_ and _Peristenus digoneutis_ were released in Sacramento, California in alfalfa managed by CDFA. Parasitoids were collected from southern France, central Italy and Spain by CABI Bioscience and the European Biological Control Laboratory, USDA ARS. Beginning in 1999, parasitoids have been released at several sites in central California, both inland and on the coast. Parasitism has increased each year at our original release site of alfalfa in Sacramento. Three years following our last releases there, we continue to find abundant numbers of both _P. stygicus_ and _P. digoneutis_. Maximum summer parasitism has increased each year since releases were made, reaching 90% summer 2004.
Parasitized nymphs of *L. hesperus* and *Closterotomus norvegicus* have been collected from nearby vacant lots infested with black mustard and wild radish. Identification of adults is pending. Results indicate that these parasitoids are permanently established in the Sacramento region. Over the same period of time, maximum *Lygus* counts has varied from 3 to 14 per sweep, and appears to be declining.

In contrast to results at the first release site in Sacramento, parasitism at our other central California release sites, including one at UC Davis has yet to increase, despite additional releases in 2002 and 2003. However at one of our new central coast sites we recovered parasitoids, as larvae, at a control site 300 m from where they were first released 6 weeks earlier. Only the introduced parasitoids *Peristenus stygicus* and *P. digoneutis* were recovered, i.e. no native braconids. Native parasitoids, *Peristenus nr. howardi*, have been recovered from *Closterotomus norvegicus* at the same locations.

**INTRODUCTION**

*L. hesperus* Knight (Heteroptera: Miridae) is native to western United States and a pest to several field and seed crops in California (University of California Cooperative Extension 2000; Zalom *et al.* 1990) and across North America (Broadbent *et al.* 2002; Coulson 1987; Strong 1970). Currently *Lygus* spp. in North America are managed on most crops through applications of broad spectrum insecticides. Cultural and biological alternatives are not considered useful. Importation of nymphal parasitoids in eastern United States during the 1980’s, however, successfully reduced *L. lineolaris* Palisot de Beauvois infesting alfalfa, *Medicago sativa* L., a close relative of *L. hesperus* (Day 1996; Day *et al.* 1990).

Extensive surveys for natural enemies in western United States have found one egg and two nymphal parasitoids commonly attacking *Lygus* spp. (primarily *L. hesperus* and some *L. elisus* Van Duzee; [Clancy and Pierce 1966; Clancy 1968; Craig and Loan 1987; Graham *et al.* 1986]). In California, *Lygus* eggs are commonly attacked by *Anaphes iole* Girault (=ovijentatus) (Hymenoptera: Mymaridae) (Graham *et al.* 1986), and in Idaho *Peristenus howardi* Shaw (Hymenoptera: Braconidae) has been reported attacking nymphs on alfalfa (Day *et al.* 1999; Mayer *et al.* 1998). Although *Euphoriana uniformis* (Gahan) (Braconidae) has been reported in southern California, only rarely has it been found attacking *Lygus* in alfalfa. In Europe, nymphal parasitoids were reported attacking *L. rugulipennis* (F.) to a higher degree (20-32%) than *L. lineolaris* found in eastern United States (8-13%) prompting their importation (Day *et al.* 1990).

Alfalfa is a major crop in central California (over 623,000 ha of hay alfalfa in 2004, California Agricultural Statistics Service, www.nass.usda.gov) and considered a major source for *Lygus* infesting other crops (Goodell *et al.* 2000; Stern *et al.* 1969). Surveys by Clancy and Pierce (1966) and others (S. Rao and S. Mueller pers. comm.) have found *Lygus* nymphs infesting alfalfa in central California free of any parasitoids. An attempt at classical biological control of *Lygus spp.* in Canada and parts of western U.S.A. over the last 30 years using *Peristenus* spp. imported from Europe has met with failure (Broadbent *et al.* 2002; Coulson 1987). Attempts to colonize *P. stygicus* in central California in the 1970’s resulted in limited recoveries, but no permanent establishment (Van Steenwyk and Stern 1977). A similar at-
tempt at classical biological control on the east coast of the United States against *L. lineolaris* in the 1980’s has met with much better success (Day 1996; Day et al. 1990). *Peristenus digoneutis* Loan collected off *Lygus rugulipennis* was imported from central Europe, where alfalfa is native. A recent survey showed that *P. digoneutis* is established over a wide area and has reduced *L. lineolaris* to much lower levels in alfalfa than prior to importation of this natural enemy. Parasitism of nymphs increased from 15% by native parasitoids to 50% two years later following establishment of *P. digoneutis*. *Lygus* numbers in alfalfa decreased by 75%. Correlative data suggests *P. digoneutis* is responsible for a reduction of damage to apples by *L. lineolaris* on the east coast (Day et al. 2003).

Since attempts to colonize parasitoids collected on closely related *Lygus* in Europe were successful in reducing populations of *Lygus lineolaris* in alfalfa on the east coast of the United States (Day 1996), we felt another, more enduring effort was warranted in California. Furthermore, *L. hesperus* attacks a broad range of crops, including strawberries and cotton in California, both of high economic value (Schuster 1987; Zalom et al. 1990). Laboratory and field studies show that these parasitoids have a high degree of host specificity (Condit and Cate 1982; Day 1999; Haye 2004; Kuhlmann et al. 1999; Lachance et al. 2001), supporting the notion that imported parasitoids would have a minimal, if any negative side affect on the environment. We report on an on-going effort to permanently establish these parasitoids in several regions of California where *Lygus* is a serious pest to high value crops. Central California was again surveyed for the presence of nymphal parasitoids and two species of *Peristenus* were imported and released at several locations.

**MATERIALS AND METHODS**

In 1997 and 1998 we surveyed alfalfa in Kern, Fresno, Sacramento and Yolo counties for the presence of nymphal parasitoids in *Lygus* spp. Three alfalfa fields in each county were sampled each year during July and August using a standard 37 cm diameter sweep net. Nymphs were dissected by teasing apart the abdomen and examining their contents for the presence of immature parasitoids with the aid of a dissecting microscope.

Foreign exploration for *Lygus* spp. was conducted by CABI Bioscience and the USDA ARS European Biological Control Laboratory beginning summer 1998. The first release of *P. digoneutis* and *P. stygicus* was in September 1998. Parasitoids were collected in regions of southern France (Herault, Lattes), northern (San Dona’de Piave) and central (Umbria) Italy, and northeastern Spain (Catalongia, Navata), south to the province of Granada. Parasitoids were collected from native *Lygus* (mainly *rugulipennis*) infesting alfalfa, shipped as cocoons, and sent to either the USDA ARS quarantine facility in Newark, Delaware, or the Agriculture Agri-Food Canada quarantine in London, Ontario. Both agencies stored cocoons through the winter, then shipped adult parasitoids to CDFA in Sacramento, California. Each year collections were made in increasingly more southern sites starting with southern France then moving to southern Spain, which closely matches the climate of central California (Climex® climate matching software).

A quarter ha plot of alfalfa was planted at CDFA’s field insectary in Sacramento fall 1997 for the sole purpose of colonizing imported parasitoids of *Lygus*. In 1999 and 2000,
three other plots of alfalfa were planted in central California also for establishing Lygus parasitoids: the University of California, Davis (Student Experiment Farm), ca. 0.5 ha, University of California Kearney Agricultural Center near Fresno (ca. 0.5 ha), and the Shafter Research and Extension Center, near Shafter (ca.1 ha). In 2002 (Santa Cruz County), and in 2003 (Monterey County), parasitoids were released within 10 km of the coast into non-crop vegetation near strawberry (Fragaria L.) farms.

Parasitoids received from these two quarantine facilities were either released directly into study plots of alfalfa or reared for future release. About 100 to 500 parasitoids were shipped to us each summer. Additional parasitoids released into fields were either produced ourselves or collected from our field insectary, the initial release site in Sacramento. Each year from 1998 to 2003 1,100 to 20,000 were released among these 6 locations. Beginning in 2001, we reduced our cultures of Peristenus for release to two populations of P. stygicus (Umbria, Italy and Granada, Spain) and one culture of P. digoneutis (Catalonia, Spain). All populations and species were released at all locations. After four years (summer 2001), releases of Peristenus spp. ceased at the first release site (North B St., Sacramento). Releases were discontinued at the Shafter site in 2003 due to poor recoveries.

Lygus were reared on a mix of green beans and artificial diet following methods developed by the USDA ARS and others (Cohen 2000a,b; Patana and Debolt 1985). Parasitoids were reared on L. hesperus nymphs, both placed in 1 liter clear plastic containers, fitted with a screened false bottom. The bottom of the container was layered with autoclaved vermiculite for diapausing parasitoids.

Parasitoids and Lygus were monitored at release sites beginning one to two years following initial releases. The proportion of nymphs parasitized by Peristenus spp. was measured by subsampling of nymphs swept while monitoring Lygus densities. Four sets of 10 to 50, 180º sweeps were made across the tops of alfalfa plants. Numbers of Lygus were recorded when aspirating nymphs dumped onto a beat sheet. A subset of the same nymphs of all instars were returned to the laboratory and used for dissections or identification of parasitoids. The abdomens of 15 to 60 nymphs were teased apart and examined using a dissecting scope. Nymphs in which we found eggs or larvae of Peristenus were scored as parasitized. Samples of 100 or more nymphs from the same sampling event were placed in rearing cages (above) allowing for adult development and identification. Sampling was initiated each spring just prior to making the first releases of additional parasitoids.

Beginning in 2004, three vacant fields within 5 km of our original release site in Sacramento were surveyed for the presence of Peristenus spp. Herbacious annuals known to harbor Lygus were swept in mid spring, March – June. Nymphs were returned to the laboratory and dissected as above for the presence of parasitoids. If enough nymphs could be collected (>50), some were reared to adults.

RESULTS
Roughly equal numbers of nymphs were collected from each of four counties while surveying for alfalfa. No nympha! parasitoids were dissected from the 1,980 Lygus nymphs collected in 1997 and 1998. Of 400 adult Lygus collected at the same time and then later identified, 98%
were *Lygus hesperus* and 2% *L. elisus* Van Duzee (det. M. Schwartz, Agriculture and Agri-Food Canada, Ottawa)

*Peristenus* spp. were first recovered May 2000 at our first release site located in Sacramento, two years after the first releases of parasitoids and when we first began to dissect nymphs. Each subsequent year annual maximum parasitism levels have climbed at this location reaching a high of 90% in 2004 (Fig. 1). However, recoveries and levels of parasitism from our other locations in the central valley have remained low and highly variable (Table 1). Releases at the Shafter Research and Extension Center were discontinued due to poor recoveries. Recoveries at the two more recent coastal sites while still low, have generally increased and have had far less time for increase.

![Figure 1. Density of Lygus and proportion in parasitized monthly averages, April-October, North B St., Sacramento.](image)

**Table 1.** Maximum parasitism levels of releases *Peristenus* spp. at release sites.

<table>
<thead>
<tr>
<th>Location</th>
<th>Maximum Parasitism (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000</td>
</tr>
<tr>
<td>Sacramento, N B St.</td>
<td>10.0</td>
</tr>
<tr>
<td>UC Davis</td>
<td>0.0</td>
</tr>
<tr>
<td>Merced Ranch</td>
<td>—</td>
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<tr>
<td>UC Kearney Ag Ctr.</td>
<td>24.0</td>
</tr>
<tr>
<td>Shafter Res. and Ext. Ctr.</td>
<td>—</td>
</tr>
<tr>
<td>Castroville1</td>
<td>—</td>
</tr>
<tr>
<td>Castroville2</td>
<td>—</td>
</tr>
<tr>
<td>Watsonville1</td>
<td>—</td>
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</tbody>
</table>
Parasitism at the Sacramento site steadily increased the three years following last releases of parasitoids. Increases in parasitism have paralleled increases in the Lygus population in alfalfa and were positively correlated (Fig. 1; \( r = 0.55, p = 0.01, n = 20 \) sample months), suggesting a density dependent relationship between these two insect populations. In 2004 parasitism reached a maximum of 90\% in August. Lygus densities dropped dramatically from a seasonal average of 7.4 nymphs per sweep in 2003 to 3.42 in 2004. Densities of Lygus increased during the first 6 years of the alfalfa plot most likely because it was the first field of its kind in the area in many years, an industrial region of the city.

Both species of Peristenus have persisted at the Sacramento release site. Peristenus stygicus has in general been the dominant species, varying each year from 29 to 95\% of the species identified (Table 2). However, P. digoneutis towards the end of summer’s 2002 and 2004, increased in relative proportion and was equal or dominant in numbers by the end of the summer.

Parasitized L. hesperus were found near the original release site in Sacramento. Weedy annuals swept at these lots included wild radish, (Raphanus sativus L.) black mustard, (Brassica nigra (L.) and vetch (Vicia sp.). In 2003 and 2004 parasitized Lygus were found in vacant lots 0.16, 0.50, and 2.0 km from the original release site. On one occasion, a single adult P. digoneutis was reared from a collection of Lygus made at the vacant lot 0.16 km from the release site.

Table 2. Species composition of Peristenus sp. at Sacramento release site.

<table>
<thead>
<tr>
<th>Date Sampled</th>
<th>P. stygicus Recovered</th>
<th>P. digoneutis Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td>July 2002</td>
<td>76</td>
<td>95.0</td>
</tr>
<tr>
<td>August 2002</td>
<td>86</td>
<td>82.7</td>
</tr>
<tr>
<td>October 2002</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>January 2003A</td>
<td>8</td>
<td>66.6</td>
</tr>
<tr>
<td>June 2003</td>
<td>12</td>
<td>85.7</td>
</tr>
<tr>
<td>July 2003B</td>
<td>16</td>
<td>84.2</td>
</tr>
<tr>
<td>March 2004 A</td>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>June 2004</td>
<td>37</td>
<td>80.0</td>
</tr>
<tr>
<td>July 2004</td>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td>September 2004</td>
<td>2</td>
<td>29.0</td>
</tr>
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\( ^{A} \) All recoveries made from soil samples
\( ^{B} \) 2 P. stygicus and 3 P. digoneutis recovered from soil samples
DISCUSSION

No *Peristenus* spp. were recovered from *Lygus* nymphs collected from alfalfa in central California in 1997 and 1998, prior to releases of exotic parasitoids reported herein. The same was found more recently by S. Mueller (unpubl. data). These results show that *Lygus* spp., infesting alfalfa in central California have remained free of nymphal parasitoids since earlier surveys by Clancy and Pierce (1966) and that releases of *P. stygicus* by Van Steenwyk and Stern (1977) in the southern part of the valley have never established. The vast majority of the Lygus were *L. hesperus* (98%), the remainder being *L. elisus*.

Populations of *P. stygicus* and *P. digoneutis* have persisted and increased in numbers at our original release site since last releases in 2001. Correlative data suggests together they have caused the local population of Lygus in alfalfa at this site to drop from a high of 7.4 to 3.4 per sweep. Parasitized Lygus nymphs have been collected up to 2 km from this release site suggesting that their populations are spreading. Both species of *Peristenus* have coexisted since the last releases in 2001, with *P. stygicus* dominating in numbers recovered. Recent work on its biology shows that *P. stygicus* has twice the lifetime fecundity as *P. digoneutis* (Haye et al. 2005). However, seasonal trends at this same site also suggest that the proportion of each species approaches 50:50 towards the end of the summer, similar to findings by Haye (2004) who surveyed Europe in the native range of these parasitoids. Although *P. stygicus* has a higher reproductive output, *P. digoneutis* may outcompete this species over the summer. Laboratory studies show that *P. digoneutis* is a superior intrinsic competitor (LaChance et al. 2001). Furthermore, host range studies show that *P. digoneutis* has a higher degree of host specificity (Haye 2004), an attribute often associated with greater searching ability and survivorship at low host densities (Varley et al. 1973).

There may be several reasons for the lack of parasitoid establishment at sites other than Sacramento. The two most likely reasons are poor climatic match and lack of *Lygus* nymphs at key times of the year. The Shafter Research and Extension Center is about 580 km south of Sacramento (38.5° N). The University of California Kearney Agricultural Center is in between. Many of the released *Peristenus stygicus* have come from the Granada region of southern Spain which has a climatic match of 77 (out of 100) with Sacramento, and climatic match of 55 with Bakersfield about 20 km southeast of Shafter (Climex software, Sutherst et al. 1999). Therefore, Sacramento may be at the southern range, in terms of climatic limits, for these parasitoids. Day et al. (2000) found that *P. digoneutis* collected from central Europe has been limited in its dispersal southward on the eastern seaboard of the United States. He found a good agreement between summer high temperatures of 30° C for 14 to 30 days and this parasitoids southern-most establishment. However, the UC Davis release site is in the exact same climatic region as the Sacramento site, being only 20 km away in a flat valley. The most likely cause for poor establishment there is the low numbers of *Lygus*. While densities of nymphs at the Sacramento site have varied from an annual average of 1.32 to 7.4 per sweep each year, densities at UC Davis have varied from 0.4 to 1.2 per sweep.

Another key factor in establishment of *Peristenus* spp. at Sacramento is how the alfalfa has been grown. At Sacramento, unlike the other sites, the cuttings were never baled, allowing for buildup of thatch on the ground. This may have provided additional protection from desiccation for the parasitoids pupating in the soil. The ground at other locations was far
more barren. We also used overhead sprinkler systems rather than surface irrigation. Although these latter two practices were adopted at the Shafter research center in 2002 and 2003, parasitoids still failed to colonize.

REFERENCES


