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

Charles H. PICKETT[1], Dominique COUTINOUT[2], Kim A. HOELMER[3], and Ulrich KUHLMANN[4]

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

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

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

[4]CABI 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

Lygus 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 *Lygus 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 *Lygus rugulipennis* (F.) to a higher degree (20-32%) than *Lygus 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 attempt at classical biological control on the east coast of the United States against *L. lineolarus* 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 (Catalongnia, Navata), south to the province of Granada. Parasitoids were collected from native *Lygus* (mainly r*ugulipennis*) 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 vermiculate 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 from nymphs swept while monitoring Lygus densities. Four sets of 10 to 50 , 180° 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 nymphal parasitoids were dissected from the1,980 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

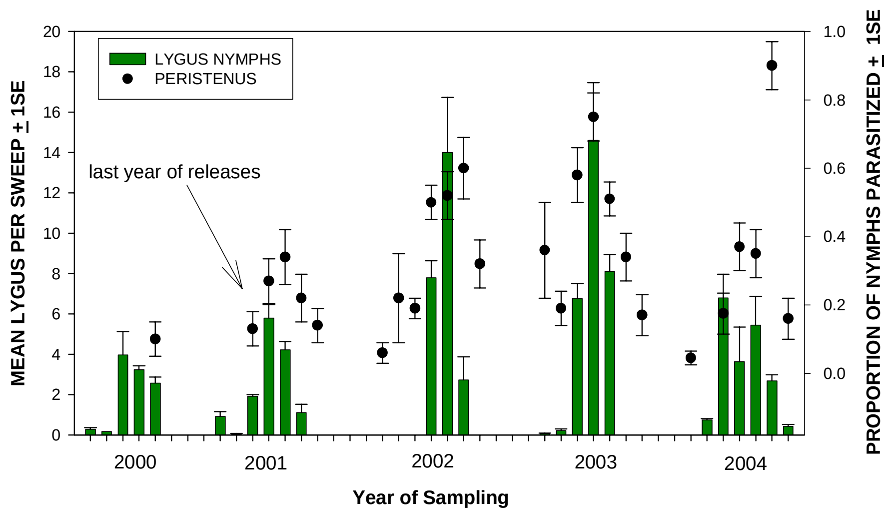

Figure 1. Density of Lygus and proportion in parasitized monthly averages, April-October, North B St., Sacramento.

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

Location	Maximum Parasitism (year)				
	2000	**2001**	**2002**	**2003**	**2004**
Sacramento, N B St.	10.0	34.0	60.0	75.0	90.0
UC Davis	0.0	4.0	2.0	3.5	—
Merced Ranch	—	4.0	2.0	3.5	—
UC Kearney Ag Ctr.	24.0	12.0	10.0	3.3	7.0
Shafter Res. and Ext. Ctr.	—	5.0	0.0	0.0	—
Castroville1	—	—	24.0	7.14	23.0
Castroville2	—	—	—	—	15.0
Watsonville1	—	—	—	7.0	25.0

recoveries. Recoveries at the two more recent coastal sites while still low, have generally increased and have had far less time for increase.

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.

Date Sampled	***P. stygicus* Recovered**		***P. digoneutis* Recovered**	
	#	%	#	%
July 2002	76	95.0	4	5.0
August 2002	86	82.7	18	16.3
October 2002	5	50.0	5	50.0
January 2003A	8	66.6	4	33.4
June 2003	12	85.7	2	14.3
July 2003B	16	84.2	3	15.8
March 2004 A	3	30.0	7	70.0
June 2004	37	80.0	9	20.0
July 2004	3	75.0	1	25.0
September 2004	2	29.0	5	71.0

[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.

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CLASSICAL BIOLOGICAL CONTROL OF CODLING MOTH: THE CALIFORNIA EXPERIENCE

Nick MILLS

Department of Environmental Science, Policy and Management
Mulford Hall, University of California
Berkeley, CA 94720-3114, U.S.A.

nmills@nature.berkeley.edu

ABSTRACT

Codling moth is a notorious fruit-boring pest that has extended its original distribution from the natural apple forests of Central Asia to cover all apple growing regions of the world. Having been discovered in California as early as 1872, codling moth has continued to be the dominant pest of apple, pear and walnut production causing extensive damage in the absence of insecticide treatment. In an effort to reduce reliance on insecticides, a classical biological control program was initiated in 1992. Following an initial survey for parasitoids of codling in Central Asia, three species were selected for importation and release in California; one larval parasitoid, *Bassus rufipes* (Braconidae) and two cocoon parasitoids *Liotryphon caudatus* and *Mastrus ridibundus* (Ichneumonidae). The outcome of releases made from 1993 to 2000 was that insufficient *B. rufipes* were released to gain establishment, *L. caudatus* established at least temporarily, and *M. ridibundus* became well established and continues to impact codling moth populations in the region. Although not a dramatic success in terms of the level of reduction of codling moth population densities, parasitism has played an important role in reducing the frequency of fruit and nut damage in orchards. This project provides an interesting example of what can be expected from parasitoid introductions against a notorious direct pest that belongs to a taxonomic family with a very poor history of success in the biological control record.

INTRODUCTION

California has a long history of classical biological control (henceforth referred to simply as biological control) originating with the successful control of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), as a pest of citrus in southern California in 1889 (Caltagirone and Doutt 1989). Since this first historical success, more than 100 years ago, California has been one of the most active regions of the world with regard to the pursuit of biological control solutions for invasive pests. There have also been numerous subsequent successes in California, including the suppression of invasive armored scales, whiteflies and mealybugs on citrus, as well as other notable cases such as olive scale and walnut aphid (Mills and Daane 2005).

The biological control record provides consistent evidence that homopteran pests have been the most successful targets for biological control and that lepidopteran pests have been more difficult to control through natural enemy importation (Greathead 1995; Mills 2000; 2005a). In addition, both Lloyd (1960a) and Gross (1991) have shown that projects against borers have been less successful than those against pests that have less of a physical refuge from parasitism. Thus, the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a notorious fruit boring pest of pome fruit, walnuts, and some stone fruits, would appear to rank very low in terms of the chances for success as a target for biological control. However, as argued by Mills (2005b), it should not be neglected as a target, due to its economic importance in California and the fact that it is an invasive species in the western U.S., on an exotic crop plant, in a relatively undisturbed environment, and has a lower level of abundance in its region of origin in Central Asia.

Codling moth first appeared in California in 1872 (Simpson 1903), and has since become a devastating pest of apples causing almost complete crop loss in the absence of effective management, and up to 40% loss of early-harvest pear cultivars and early-harvest walnut cultivars (Barnes 1991; Mills unpublished observations). As an invasive species it has extended its original distribution from the natural apple forests of Central Asia to cover all apple growing regions of the world, with the exception of eastern China and Japan (Mills 2005b). Here, I review the biological control project against codling moth in California that ran from 1992-2000, with an emphasis on the selection of parasitoids for introduction and the outcome of the parasitoid releases.

SELECTING EFFECTIVE PARASITOIDS FOR INTRODUCTION

Codling moth in California supports a small assemblage of indigenous parasitoids (Mills 2005b), including an egg parasitoid *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae), a larval-prepupal parasitoid *Macrocentrus ancylivorus* Rowher (Hymenoptera: Braconidae), a cocoon parasitoid *Mastrus carpocapsae* (Cushman) (Hymenoptera: Ichneumonidae), and a pupal parasitoid *Coccygomimus hesperus* Townes (Hymenoptera: Ichneumonidae). It is also attacked by an egg-larval parasitoid *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae) that was introduced into Washington State in the 1920s. In general, using corrugated cardboard bands to intercept codling moth larvae seeking cocooning sites on the trunk of orchard trees, parasitism of codling moth in California at the start of this project was low and typically less than 5% both in coastal and inland regions (Mills unpublished observations). However, egg parasitism by *T. platneri* frequently rose to 30-60% later in the season in unsprayed orchards, and parasitism of overwintering cocoons by *M. carpocapsae* was recorded to be as high at 23% in 1995 in one apple orchard on the Central Coast.

In contrast, in Central Asia, codling moth supports a more diverse parasitoid assemblage (Fig. 1), including two hyperparasitoids *Perilampus tristis* Mayr (Hymenoptera: Perilampidae) and *Dibrachys cavus* (Walker) (Hymenoptera: Pteromalidae). Levels of parasitism were greater in this region (Mills 2005b), with a maximum of 33.3% recorded for *Bassus rufipes* (Nees) (Hymenoptera: Braconidae) and 43.9% for *Mastrus ridibundus* (Gravenhorst)

(Hymenoptera: Ichneumonidae). A stage-structured model of codling moth population growth also identified that, of the life stages in the codling moth life cycle that are vulnerable to parasitism, the 2nd instar and cocoons stages would be most vulnerable to additional parasitism (Mills 2005b). In selecting parasitoids for introduction, the criteria used were the absence of antagonistic interactions between parasitoid species (Mills 2003), greater than

30% parasitism observed in the region or origin (Hawkins and Cornell 1994), and parasitoids targeting the 2nd instar and cocoon stages (Mills 2005b). Using these combined criteria, the larval endoparasitoid *B. rufipes*, and the two prepupal ectoparasitoids *Liotryphon* spp. and *M. ridibundus* were selected for introduction to California (Fig. 2).

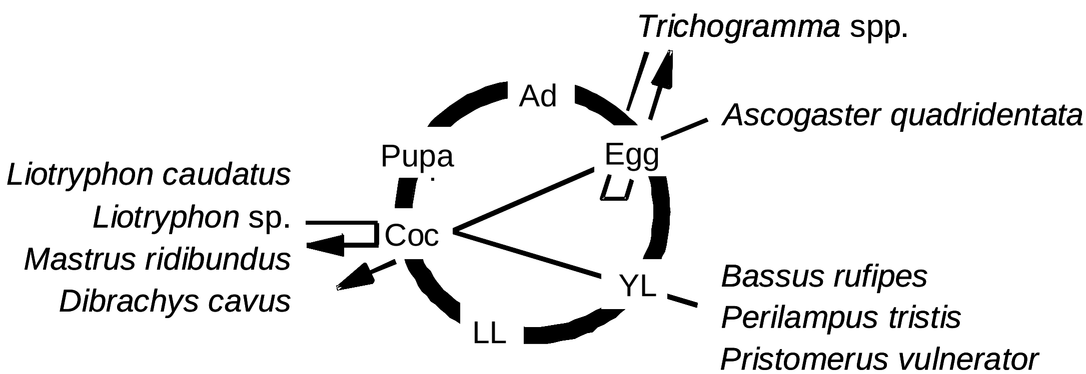

Figure 1. The parasitoid assemblage associated with codling moth in Central Asia. The circle represents the life cycle of the codling moth with YL = young instar larva, LL = late instar larva, and Coc = cocoon. The arrows represent the life stages attacked and killed by the associated parasitoids, those remaining outside of the circle being ectoparasitoids, and those passing through the circle being endoparasitoids.

Figure 2. Parasitoids selected for introduction into California, (a) *Bassus rufipes*, (b) *Liotryphon caudatus*, and (c) *Mastrus ridibundus*.
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CURRENT STATUS OF THE OUTCOME OF THE PROJECT

The introduced parasitoids were released in a total of 130 orchards in California, comprising 37 apple orchards, 21 pear orchards, and 72 walnut orchards. *L. caudatus* was the first parasitoid to be released, from 1992-1997, with a total of 45,981 individuals (males and females) released (Table 1). In the case of *M. ridibundus*, a total of 316,986 individuals were released from 1995-2000. A much larger number of individuals were released for two reasons. Firstly, *M. ridibundus* is a gregarious parasitoid with an average of 4 individuals per host cocoon, and thus greater numbers could be produced in rearing, and secondly, during the course of the release phase of the project it became clear that it was more effective than *L. caudatus* and thus greater effort was devoted to the release of this species. Releases of *B. rufipes* were constrained to 196 individuals from 1995-1997 due to the lack of success in rearing this species effectively in captivity.

Recoveries were monitored in several, but not all, orchards each year by installing corrugated cardboard bands on the trunks of at least 50 trees in the orchard early enough in the season to trap the naturally descending codling moth larvae. Both *L. caudatus* and *M. ridibundus* were recovered from codling moth cocoons in the year of release (Table 2), but there have been no recoveries of *B. rufipes*. Average rates of parasitism varied between parasitoid species ($F = 5.84$, df = 1, 85, $P < 0.02$, arcsine transformed data), but not between commodities ($F = 1.15$, df = 2, 85, $P = 0.32$), although there was a significant interaction ($F = 3.19$, df = 2, 85, $P = 0.05$) as in contrast to the situation in apple and walnut, *L. caudatus* appeared more successful in pears than *M. ridibundus*.

Table 1. The numbers of individuals of each of the three parasitoids that were released against codling moth in California orchards during the course of the project.

	1992	1993	1994	1995	1996	1997	1998	1999	2000
Liotryphon caudatus									
Current year	1,464	7,053	6,452	10,467	11,382	9,000			
Cumulative	1,627	8,680	15,132	25,599	36,981	45,981			
Mastrus ridibundus									
Current year				10,850	29,186	39,150	82,800	115,000	40,000
Cumulative				10,850	40,036	79,186	161,986	276,986	316,986
Bassus rufipes									
Current year				38	127	31	0	0	0
Cumulative				38	165	196	196	196	196

Table 2. The percent parasitism of codling moth cocoons, pooled across orchards and years, for each of the parasitoids and commodities from which they were recovered in the year of release.

	Apple		Pear		Walnut	
	L. caudatus	*M. ridibundus*	*L. caudatus*	*M. ridibundus*	*L. caudatus*	*M. ridibundus*
Mean ± SD	1.58 ± 0.03	16.39 ± 0.19	7.51 ± 0.17	4.58 ± 0.08	1.59 ± 0.05	8.64 ± 0.11
Maximum	14.29	56.29	50.00	21.74	24.57	36.86
N	17	13	8	6	25	22

Although *L. caudatus* has been recovered occasionally from orchards in years after the release it seems likely that this species has not become established in California. In contrast, *M. ridibundus* has continued to be recovered from orchards and is almost certainly established in the region.

CONCLUSIONS

Although considered a target for classical biological control both in the 1920s and again in the early 1960s (Lloyd 1960b), codling moth has been ignored more recently by biological control practitioners due to the fact that it is a direct pest. However, a need to reduce insecticide residues in fruit crops (e.g., Melnico 1999) together with a consideration (Mills 2005b) of orchards as a suitable environment for biological control success, the lower level of abundance of codling moth in its region of origin, and the vulnerability of 2nd instar and cocoon stages of the life cycle to parasitism, raised the profile of codling moth as a target for biological control in California.

Codling moth damage in walnuts in California has declined since the release of *M. ridibundus* in 1995, with parasitism of overwintering cocoons reaching 56% in some unsprayed orchards (Mills unpublished observations). The outcome of the project cannot be considered a dramatic success, as should be expected in the case of a direct pest (Gross 1991; Lloyd 1960a), but as noted by Goldson *et al.* (1994), the value of parasitism and the contribution of partial biological control to the overall management of such notorious and intractable pests as the codling moth should not be underestimated.

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THE IMPACT OF PARASITOIDS ON *PLUTELLA XYLOSTELLA* POPULATIONS IN SOUTH AFRICA AND THE SUCCESSFUL BIOLOGICAL CONTROL OF THE PEST ON THE ISLAND OF ST. HELENA

Rami KFIR

Plant Protection Research Institute, Private Bag X134
Queenswood 0121, Pretoria, South Africa

KfirR@ARC.Agric.ZA

ABSTRACT

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most injurious insect pest of brassica crops throughout the world. In many countries it has developed resistance to almost every synthetic insecticide used against it including *Bt* formulations. In addition to resistance, the destruction of its natural enemies through indiscriminate use of broad-spectrum insecticides is considered responsible for its high pest status. Population studies of *P. xylostella* and its parasitoids in the Eastern Cape, Gauteng and North-West Provinces of South Africa revealed that the pest is naturally controlled if insecticides are not used. A total of 3 egg-larval parasitoids, 8 larval parasitoids, 4 larval-pupal parasitoids, 6 pupal parasitoids and 12 hyperparasitoids have been identified as being associated with *P. xylostella* in South Africa.

An insecticide check method was used to assess the impact of parasitoids on levels of infestations by *P. xylostella*. In the sprayed plots parasitism of *P. xylostella* larvae and pupae fluctuated between 5-10% whereas in the untreated plots parasitism peaked above 90%. As a result population levels of *P. xylostella* on the sprayed plants were about five times higher than on the control plants, which is an indication that parasitoids played an important role in controlling the pest populations.

Plutella xylostella was a severe pest on the Island of St Helena, South Atlantic Ocean. Farmers were heavily depended on chemical control, often spraying cocktails of several insecticides when the required control failed. A survey in brassica crops on St Helena revealed that natural enemies were not an important factor in controlling *P. xylostella* and that the only parasitoid on the Island was the larval-pupal parasitoid *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae). Following an agreement between NRInternational and the Plant Protection Research institute (PPRI) of South Africa two consignments of the larval parasitoid, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), and the pupal parasitoid, *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae), were shipped in 1999 from South Africa to St Helena. The parasitoids were mass reared on the Island and released on 10 different farms. An early survey of 19 farms (release and non-release sites) in 2000 indicated that both introduced parasitoids became established. *Cotesia plutellae* was found in 15 farms

with up to 80% parasitism and *D. collaris* on 5 farms with up to 55% parasitism. Further surveys during 2002 - 2004 indicated very low levels of *P. xylostella* populations. However, *C. plutellae* cocoons were present throughout the Island which is an indication that parasitoids had been the cause for the decline in the pest populations. Farmers in St Helena reported that *P. xylostella* infestations remain low and that no chemical control has been necessary since 2001. This is a strong indication for the success of the biological control of *P. xylostella* on St Helena.

INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is cosmopolitan in its geographical distribution, occurring in all major zoogeographical regions of the world wherever crucifer crops are cultivated (Talekar and Shelton 1993). It is the most universally distributed of all Lepidoptera and has the ability to migrate and disperse over very long distances.

The host range of *P. xylostella* is limited to plants of the family Brassicaceae that contain mustard oils and their glucosides. Cultivated crops on which the diamondback moth feeds include cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), radish (*Raphanus sativus*), turnip (*B. rapa pekinesis*), brussels sprouts (*B. oleracea* var. *gemmifera*), kohlrabi (*B. oleracea* var. *gongylodes*) and more (Fig. 1). In addition, *P. xylostella* feeds on numerous crucifer weeds (Talekar and Shelton 1993). Lohr (2001) observed for the first time the ability of *P. xylostella* to switch hosts; severe outbreaks of *P. xylostella* on commercial peas (*Pisum sativum*) in the Rift Valley of Kenya.

Figure 1. A young *Plutella xylostella* larva feeds on a cabbage leaf.
UGA1390043

In many countries, *P. xylostella* has developed resistance to almost every synthetic insecticide used against it in the field including *Bacillus thuringiensis* Berliner (*Bt*) formulations (Liu *et al*. 1995; Tabashnik *et al*. 1990). *Plutella xylostella* was the first crop pest to develop resistance to DDT and the first insect to develop resistance to *Bt* in the field. The increasing usage of *Bt* products resulted in an increasing number of reports of field resistance by *P. xylostella* populations (Tabashnik 1994). New insecticides are continuously being developed as existing insecticides become useless, but *P. xylostella* has developed resistance very quickly to many of these (Nisin *et al*. 2000; Shelton *et al*. 2000). The pest has also developed cross-resistance and multiple-resistance to different chemical pesticides (Shelton *et al*. 2000).

Plutella xylostella is the most injurious insect pest of cabbage and other crucifer crops throughout the world (Fig. 2). Lack of effective natural enemies is considered to be the major reason for its high pest status in most parts of the world (Lim 1986). In many countries, in addition to the development of resistance, the destruction of natural enemies by the widespread use of broad-spectrum insecticides is also considered responsible for this imbalance (Talekar and Shelton 1993). The annual cost of managing *P. xylostella* worldwide is estimated to be about US$1 billion (Talekar 1992).

Figure 2. Severe Damage to cabbage caused by *Plutella xylostella* in St Helena Island before the introduction of parasitoids. UGA1390044

Mediterranean area of origin has been repeatedly suggested for *P. xylostella* (Harcourt 1954; Hardi 1938) but no evidence for such assumption has ever been provided. This was based on the idea that the pest evolved there on crucifer plants and has been accidentally distributed from Europe around the world with the cultivated brassicas. More recently, Kfir (1998) challenged this hypothesis by suggesting a South African origin for *P. xylostella*. This was based on the diversity of wild crucifer plants and the numerous *P. xylostella* parasitoids recorded in South Africa. However, there is not yet conclusive evidence for the exact origin of the pest.

Numerous parasitoids and predators attack all developmental stages of *P. xylostella*. In addition, general predators such as birds and spiders often consume adult moths. Over 90 species of parasitoids have been recorded worldwide (Goodwin 1979) attacking all developmental stages of *P. xylostella*. Of these, the most predominant and effective larval parasitoids belong to three major genera, *Apanteles*, *Cotesia* and *Diadegma* and pupal parasitoids belonging to the genus *Diadromus*. For biological control of *P. xylostella* some parasitoid species have been introduced to Southeast Asia, the Pacific Islands, North and Central America, Africa, the Caribbean, Australia and New Zealand with various degrees of success (Lim 1986).

In South Africa Ullyett (1947) studied *P. xylostella* and its natural enemies and recorded parasitoids, predators, bacteria and an entomopathogenic fungus associated with it. He concluded at the time that in South Africa *P. xylostella* was well controlled by its natural enemies. Later Dennill and Pretorius (1995) demonstrated that high infestation levels by *P. xylostella* are a result of excessive insecticide applications. At one study site where insecticides were applied only once every three weeks, parasitism of *P. xylostella* reached 90% and the pest did not cause economic losses. In contrast, at a second study site with regular and excessive chemical applications, parasitism levels were negligible and serious outbreaks of *P. xylostella* caused total crop loss. Other studies in the Eastern Cape, Gauteng and North-West Provinces of South Africa revealed very high parasitism levels of *P. xylostella* in unsprayed cabbage crops (Kfir 1997a,b; Smith and Villet 2002; Waladde *et al.* 2001) whereas at the same regions economic damages were recorded by farmers who regularly sprayed their cabbage fields. This indicated that insecticides interfered with the natural control of *P. xylostella* in South Africa. During these studies a total of 3 egg-larval parasitoids, 8 larval parasitoids, 4 larval-pupal parasitoids, 6 pupal parasitoids and 12 hyperparasitoids have been identified as being associated with *P. xylostella* in South Africa (Kfir 2003; Lohr and Kfir 2004).

This paper reviews the impact of parasitism by indigenous parasitoids on populations of *P. xylostella* in South Africa and a successful biological control of *P. xylostella* on the Island of St Helena with parasitoids introduced from South Africa.

IMPACT OF PARASITOIDS ON *P. XYLOSTELLA* POPULATIONS

An insecticide check method was used to assess the effect of parasitoids on levels of infestation by *P. xylostella* in cabbage (Kfir 2004). The field trials were conducted at Gauteng and North-West Provinces in South Africa. Previous studies at these regions indicated that the number of *P. xylostella* moth caught in pheromone traps, and *P. xylostella* larval infestations on the crops normally peaked during the spring months of September-October (Kfir 1997b). The planting dates in this study were chosen to coincide with high populations of DBM in the field to ensure maximum natural infestations.

To suppress natural enemies a selective insecticide, dimethoate, an organophosphate compound with both systemic and contact action was applied twice weekly to cabbage plots. Similar untreated plots were used as control. Dimethoate was shown to suppress natural enemies in California cotton fields, which in turn caused an increase in abundance of *Spodoptera exigua* Hübner (Eveleens *et al*. 1973), and *Trichoplusia ni* Hübner (Ehler *et al*. 1973). This indicates that dimethoate can be detrimental to natural enemies of Lepidoptera but causes no harm to the pests.

At weekly intervals ten plants were randomly selected from each plot and thoroughly scouted for *P. xylostella* larvae, pupae and parasitoid cocoons. To determine parasitism all collected material was kept individually in glass vials in the laboratory until either parasitoids or moths emerged. All emergent parasitoids were identified and their incidence calculated.

At North-West Province, populations increased very rapidly from the second half of September and peaked during the second half of October at 47.0 larvae/plant in the sprayed plots and 12.4 larvae/plant in the control plots (Fig. 3a). At Gauteng Province populations peaked at 27.7 larvae/plant in the sprayed plots and at 4.7 larvae/plant in the control plots (Fig. 3b). At the two sites population levels of *P. xylostella* on the sprayed plants were significantly higher than on the control plants (t-test between two independent samples (Snedecor and Cochran 1967). At North-West Province, a total of 8205 DBM larvae and pupae were collected from the sprayed plants and 1607 from the control plants ($t = -16.59$, 4 df, $P < 0.001$). At Gauteng Province 3648 DBM were collected from the sprayed plants as compared with 734 DBM from the control plants ($t = -16.28$, 4 df, $P < 0.001$) (Kfir 2004).

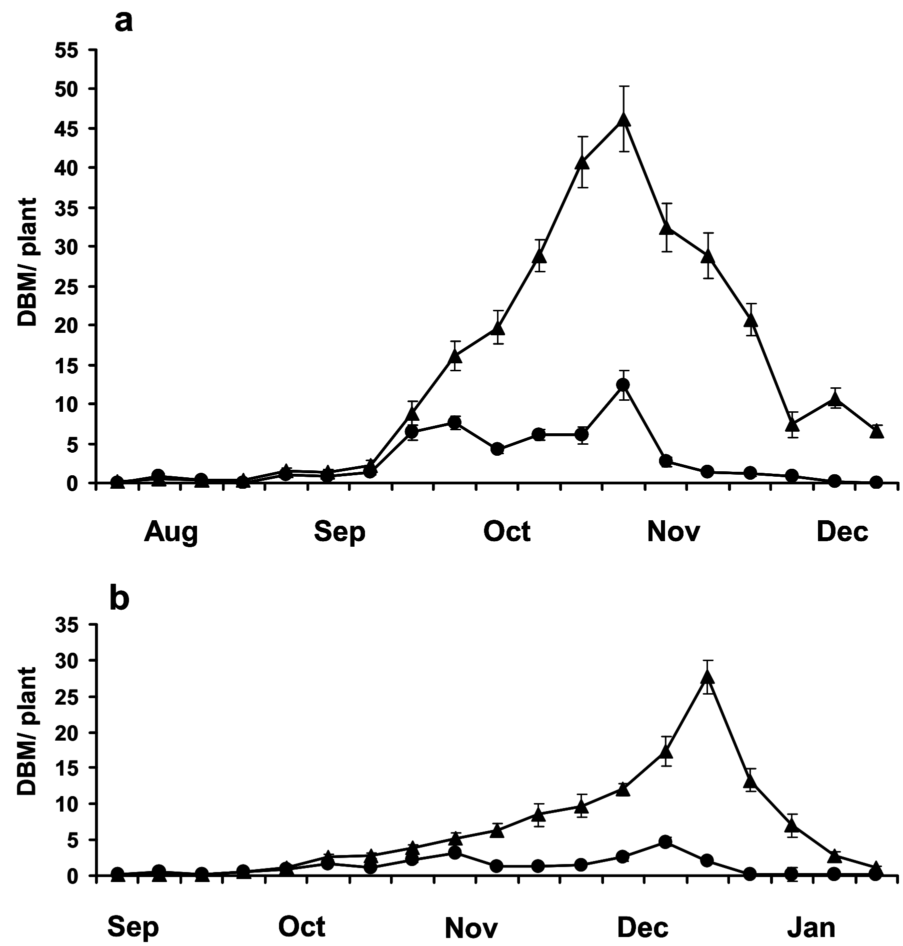

Figure 3. Abundance of diamondback moth, *Plutella xylostella*, larvae and pupae on sprayed (triangles) and control (circles) cabbage. Bars represent standard errors (SE) when larger than symbol size. (a) North-West Province, (b) Gauteng Province, South Africa (from Kfir 2004).

Percent parasitism of *P. xylostella* at both sites throughout the season was higher on the unsprayed plots (Fig. 4). At North-West Province, in the sprayed plots percent parasitism fluctuated around 5% (seasonal mean of 4.9%) whereas in the control plots parasitism increased rapidly to above 90% towards the end of the season (seasonal mean of 65.9%) (Fig. 4a). At Gauteng Province, parasitism in the sprayed plots fluctuated around 10% with a peak of 17.9% in middle of December (seasonal mean of 12.8%) and in the control plots parasitism was high (70-95%) from the middle of November to the middle of January (seasonal mean of 64.9%) (Fig. 4b).

The most abundant parasitoids were the larval parasitoids *Cotesia plutellae* (Kurdjumov) and *Apanteles halordi* Ullyett (Hymenoptera: Braconidae) (Fig. 5), the larval-pupal parasitoid *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), which is the only known gregarious primary parasitoid *of P. xylostella,* the pupal parasitoid *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) and the hyperparasitoids *Mesochorus* sp. (Hymenoptera: Ichneumonidae) and *Pteromalus* sp. (Hymenoptera: Pteromalidae). Both emerged from cocoons of their primary parasitoid hosts (Kfir 2004).

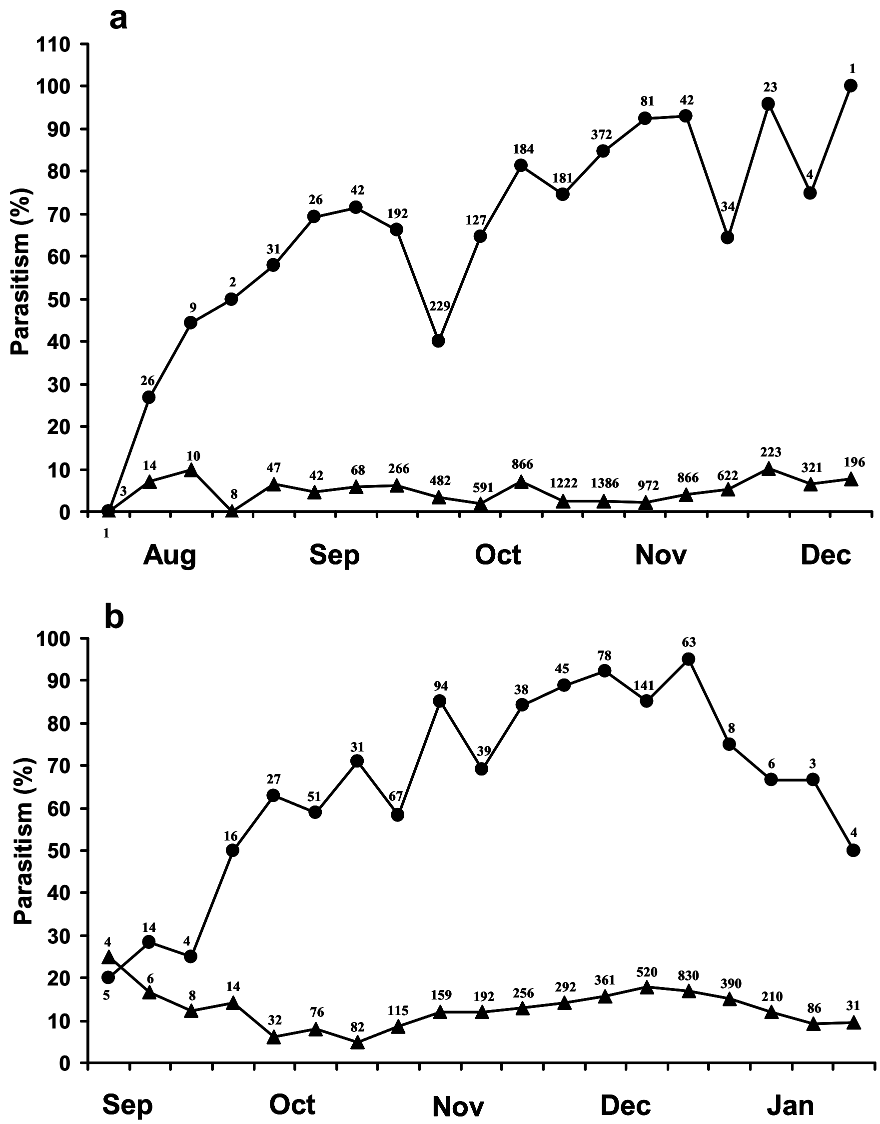

Figure 4. Percentage parasitism of diamondback moth, *Plutella xylostella,* larvae and pupae on sprayed (triangles) and control (circles) cabbage. Numbers represent sample size. (a) North-West Province, (b) Gauteng Province, South Africa (from Kfir 2004).

Figure 5. Adults of *Apanteles halfordi* (right) and *Cotesia plutellae* feeding on honey.
UGA1390045

The findings from this study demonstrated that the higher infestation level of cabbage by *P. xylostella* in the insecticide-treated plots was caused by partial elimination of parasitoids and that parasitoids play an important role in the natural control of *P. xylostella* populations in South Africa.

BIOLOGICAL CONTROL OF *P. XYLOSTELLA* ON ST. HELENA

Until recently *Plutella xylostella* was a serious pest of crucifer crops on the island of St Helena, a small British volcanic Island (122 sq km) in the South Atlantic Ocean (15 57'S, 5 42'W), 1,850 km from the west coast of Africa. Farmers on St. Helena were heavily dependent on insecticides to control *P. xylostella*, often overdosing and mixing cocktails, containing several pesticides, when the recommended dose failed to control the pest. Surveys in crucifer fields revealed that the only parasitoid of *P. xylostella* present on St Helena was *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), which also occurs on the African mainland and some Indian Ocean Islands (Azidah *et al*. 2000).

Since most supplies, including fresh produce, are shipped from Cape Town in South Africa to St Helena, it was assumed that *P. xylostella* together with this single parasitoid had been introduced into the Island on imported cabbages from South Africa. However, because *D. mollipla* on its own was unable to reduce *P. xylostella* to below economic damage levels a biological control project, funded by DFID, UK, was hence initiated. The Plant Protection Research Institute (PPRI) of South Africa was contracted by the IPM Project on St Helena and NRInternational to supply additional parasitoids of *P. xylostella* to St Helena and to train the IPM Project personnel in mass rearing and handling procedures for *P. xylostella* and its parasitoids, release techniques for parasitoids and methods to follow-up parasitoid dispersal and establishment in the field (Kfir and Thomas 2001).

In order to reduce likelihood of competition between the introduced parasitoids and the resident larval-pupal *D. mollipla* parasitoid, it was decided to introduce into St Helena the larval parasitoid, *C. plutellae*, and the pupal parasitoid, *D. collaris.*

During 1999 two consignments of *C. plutellae* and, *D. collaris* were sent to St Helena by ship since there is no airport on the Island. The consignments contained all developmental stages of these two parasitoids, i.e. adult wasps, parasitoid cocoons and parasitised *P. xylostella* larvae and pupae (Fig. 6). During the 6-day voyage adult wasps were fed daily with honey and water, while the parasitised, but active, *P. xylostella* larvae were provided with fresh cabbage leaves, until parasitoid cocoons formed or until the hosts pupated.

Figure 6. *Diadromus collaris* parasitising a pupa of *Plutella xylostella*. UGA1390046

The parasitoids were mass reared in a rearing facility established for the project by the IPM Project of the Department of Agriculture and Natural Resources on the Island. Before releases were undertaken, extension officers visited the intended release sites, and spoke to all farmers using the local radio station advising them to stop using insecticides and to switch to more selective Bt sprays, so as to give the introduced parasitoids the best possible chance of survival. A total of 17,500 *C. plutellae* and 23,500 *D. collaris* were then released on ten different farms across the Island, continuously from May 1999 to September 2000 (Kfir and Thomas 2001).

A follow-up survey of 19 farms, conducted during 2000, at the release sites and on another 9 non-release farms, found that both parasitoids were well established: *C. plutellae* was present on 15 out of 16 farms sampled, 8 of which were farms where no parasitoids had been released. The percentage parasitism of *P. xylostella* larvae by *C. plutellae* was relatively high. For example, on Briars farm it was 32.7% (n = 104 larvae), on Mulberry Gut farm it was 27.7% (n = 70) and on Pouncey's farm (a non-release site) it was 80% parasitism (n = 30). *Plutella xylostella* pupae were likewise collected on 14 farms and *D*. collaris parasitoids were found on 5 of these, one of which was a non-release site. Percentage parasitism of pupae by *D. collaris* ranged from zero up to 55% on Nr Half Way farm (n = 20). This was an indication that both parasitoids had survived their initial release and had found and successfully parasitized the respective host stages. They were also actively dispersing into adjacent farms. However, at this stage of the project, *D. mollipla,* the resident species, still proved the most abundant and widely distributed parasitoid present. It emerged in *P. xylostella* samples from 17 out of 19 farms surveyed (Kfir and Thomas 2001).

Further surveys during 2001 – 2004, even in spring (September-October), which is normally a time when *P. xylostella* outbreaks occurred on St Helena, indicated low levels of *P. xylostella* populations. Moreover, cocoons of *C. plutellae* were reportedly found to be present throughout the Island, which is an indication that parasitoids had been the cause for the decline in the pest populations. Farmers in St Helena reported that *P. xylostella* infestations remain low and that no insecticides or *Bt* applications have been necessary since 2001. This is a strong indication for the success of the biological control of *P. xylostella* on St Helena.

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ESTABLISHMENT IN NORTH AMERICA OF *TETRASTICHUS SETIFER* THOMSON (HYMENOPTERA: EULOPHIDAE), A PARASITOID OF *LILIOCERIS LILII* (COLEOPETERA: CHRYSOMELIDAE)

Lisa TEWKSBURY[1], Marion S. GOLD[1], Richard A. CASAGRANDE[1], and Marc KENIS[2]

[1]Department of Plant Sciences, University of Rhode Island, Kingston, Rhode Island U.S.A.
lisat@uri.edu, mgold@uri.edu, casa@uri.edu

[2]CABI Bioscience Switzerland Centre
Delémont, Switzerland
m.kenis@cabi.org

The lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae), first reported in North America in 1945, was found in Boston in 1992 and has since spread throughout the New England States, New York, and five Canadian provinces. This pest of ornamental and native lilies in North America is generally under good biological control in Europe from a complex of larval parasitoids, including three ichneumonid species and *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae). Based upon surveys in Europe and host range testing in the U.S.A. and Europe, we determined that *T. setifer* was the best candidate to control *L. lilii* throughout its current range and it was the first species released against this pest. *Tetrastichus setifer* is a univoltine, gregarious parasitoid which overwinters in a host cocoon in the soil. Adults emerge in the spring and females oviposit in all four larval instars, spending in excess of 15 minutes laying an average of nine eggs per host. They are host specific to the genus *Lilioceris*, with a preference for *L. lilii*.

Larvae of *L. lilii* were collected throughout Europe and fed lily leaves until forming a cocoon in vermiculite. Host cocoons containing overwintering parasitoid larvae were held at 2°C for a minimum of five months before shipment to Rhode Island. In Rhode Island they were stored at 4°C before warming to 25°C for adult emergence and field release into 6m x 6m plots of approximately 800 lilies. We released *T. setifer* in Wellesley, Massachusetts in 1999 and 2000. Following release, we recorded in-season parasitism, but no winter survival. In 2001 we removed the shredded bark mulch from our plot and released 810 parasitoids. Again, we recorded high levels of in-season parasitism, but parasitoids also successfully overwintered in the plot. With no further parasitoid releases, we recorded 37% parasitism of fourth instars at peak density in 2002, followed by 100% and 57% parasitism of fourth instars in 2003 and 2004, respectively. Peak *L. lilii* larval density in the plot declined from seven per stem in 2000 to one per stem in 2004. We had similar results with a release plot in Cumberland, Rhode Island, where a release of 584 parasitoids in 2001 resulted in high in-season parasitism, but no winter survival in mulched plots. Following mulch removal, 984 parasitoids were released in 2002 and *T. setifer* successfully overwintered, causing 95% parasitism of peak fourth instar larvae in 2003 and 75% in 2004. Peak *L. lilii* larval density in the plot declined from six larvae per stem in 2001 to two per stem in 2004.

In 2003 we began distributing *T. setifer to* cooperators in other New England States who established lily plots, monitored beetles, and released parasitoids at four sites. We recovered successfully overwintered *T. setifer* in Bridgton, Maine in 2004 with 6% parasitism of fourth instar larvae. Based upon high parasitism rates following releases in 2004, we expect to find overwintered *T. setifer* in New Hampshire in 2005.

Tetrastichus setifer is established in at least three New England States and is substantially impacting populations of *L. lilii* in release plots in Massachusetts and Rhode Island. We are presently evaluating the spatial distribution of this parasitoid around release sites and evaluating release protocols.

RETROSPECTIVE EVALUATION OF THE BIOLOGICAL CONTROL PROGRAM FOR *BEMISIA TABACI* BIOTYPE "B" IN THE U.S.A.

John GOOLSBY[1], Paul DE BARRO[2], Kim HOELMER[3], and Alan KIRK[4]

[1]USDA-ARS, Beneficial Insects Research Unit, 2413 E. Hwy. 83
Weslaco, Texas, U.S.A. 78596
jgoolsby@weslaco.ars.usda.gov

[2]CSIRO Entomology
Indooroopilly, QLD, Australia

[3]USDA-ARS, Beneficial Insects Introduction Research Unit
Newark, Delaware, U.S.A.

[4]USDA-ARS, European Biological Control Laboratory
Montferrier sur Lez, France

ABSTRACT

A retrospective evaluation of the biological control program for *Bemisia tabaci* biotype B in the U.S.A. was conducted. The use of climate matching to direct foreign exploration led to discovery of *B. tabaci* parasitoids from diverse climates, which proved useful in selecting species which would establish in the varied climates of the impacted agricultural areas of the U.S.A. The parasitoids which established on the B biotype in the U.S.A. came from several Old World biotypes. Field and laboratory evaluation demonstrated significant differences in their attack rates when searching for *B. tabaci* on cotton, broccoli, or melons. These tritrophic interactions could also have influenced their competitiveness and is also evidence of how plant hosts influence host range of parasitoids. It is also suspected that hybridization of the *Eretmocerus* spp. may have occurred, and molecular methods for testing this hypothesis are discussed. This retrospective evaluation of the program in the U.S.A. was used to develop predictive tools for selection of agents for biological control of *B. tabaci* in Australia and China.

INTRODUCTION

Bemisia tabaci biotype 'B' Gennadius (Homoptera: Aleyrodidae) (= *Bemisia argentifolia* Bellows and Perring) became a major pest of cotton, cucurbits, winter vegetables and ornamental plants in the southern U.S.A. during the 1990's. Foreign exploration for natural enemies of *B. tabaci* was implemented, and as a result, over 56 populations of parasitoids were established in quarantine culture from collections made between 1992 and 1998 (Kirk and Lacey 1995 Kirk *et al*. 1993; Kirk *et al.* 2000; Legaspi *et al.* 1996). Imported natural enemies were evaluated in laboratory and field cage tests and then released in AZ, CA, and TX (Goolsby *et al.*

1996; Goolsby *et al*. 1998; Goolsby *et al.* 2000; Gould *et al*. 1998; Hoelmer *et al*. 1998; Simmons *et al*. 1998; Pickett *et al*. 1999). Several species of parasitic Hymenoptera are now established in Texas, Arizona and California (Goolsby *et al.* 2005). A retrospective analysis of the program was conducted, and a set of predictive tools was developed to assess the parasitoids of *B. tabaci* being considered for importation by other countries (Goolsby *et al.* 2004). These tools were put into practice to predict which parasitoid species would be the best candidate for introduction into areas of Queensland, Australia, and more recently southern China, which are currently affected by *B. tabaci*.

DISCUSSION

FOREIGN EXPLORATION

Due to the wide distribution of *B. tabaci* and the unknown origin of the B biotype population, a worldwide search for natural enemies was conducted. By the early 1990's, *B. tabaci* biotype B was distributed across the southern tier of North America, including Florida, Texas, Arizona, and California. The climates and agroecosystems of these areas were climatically similar in that they supported a mix of year-round crops, including winter vegetables and summer row crops. However, their climates differed in terms of their seasonal minimum and maximum temperatures, relative humidities, and rainfall patterns and amounts. Climate-matching using CLIMEX (Sutherst *et al.* 1999) showed strong affinities between: Southeast Asia and south Florida; Mediterranean Europe with the San Joaquin Valley of California; the Arabian Peninsula with the Imperial Valley of California and South Asia and the Lower Rio Grande Valley of Texas as shown in Fig. 1.

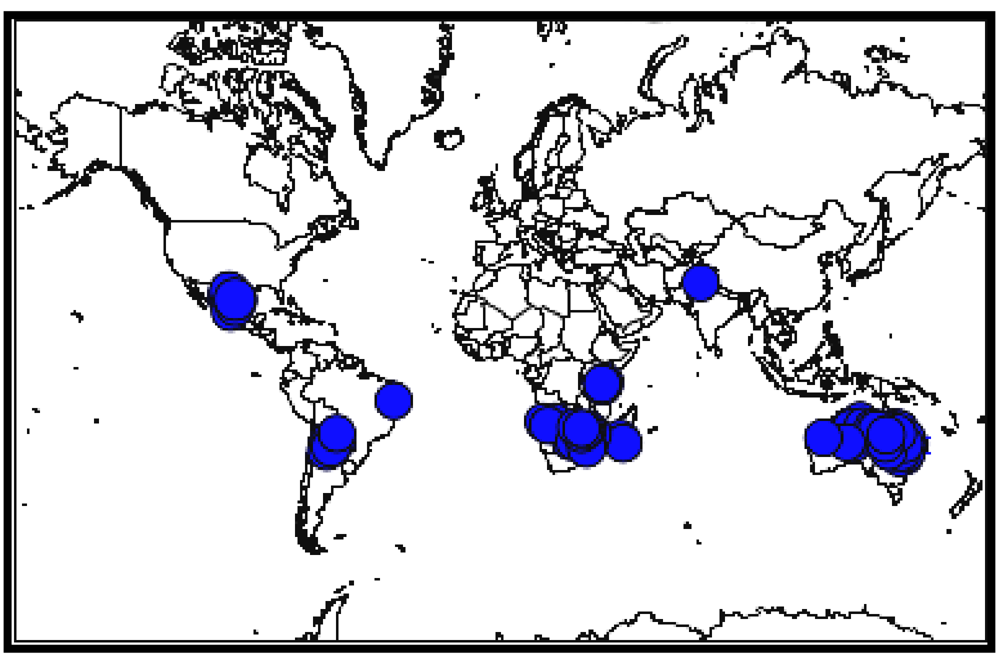

Figure 1. Worldwide locations with climatic similarity to the Lower Rio Grande Valley of Texas, U.S.A. Blue dots indicate a CLIMEX match of 75% or better.

Foreign exploration focused entirely on natural enemies of the *B. tabaci* complex, which included several known biotypes (Brown *et al.* 1995; Frohlich *et al.* 1999). It was not known at the time if the parasitoids from non-B biotypes would find the B biotype suitable, but now in retrospect, we know that the Old World *Eretmocerus* parasitoids readily accepted it. With the introduction of the Q Biotype into North America it is likely that the established parasitoids will find this biotype equally suitable. This is the case in southeast Spain, where the Q biotype is established and is readily accepted as a host by *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). The other important outcome of the foreign exploration involved the *a priori* decision to collect natural enemies only from the *B. tabaci* complex. This decision was manifested in the host range and host specificity of the biological control agents. A biological risk assessment was conducted in 1994 prior to release, and it was determined that only natural enemies reared from *B. tabaci* would be permitted for release, and further only primary and autoparasitoids in the genera *Eretmocerus* and *Encarsia* (USDA 1995a,b). The requirement set forth in this assessment precluded the 'new association' strategy, but appears to have resulted in natural enemies with narrow host specificity. A decade later, this decision seems appropriate, given the trend towards natural enemies with narrow host ranges and the call for host-range testing of arthropod biological control agents in the U.S.A. (Van Driesche and Reardon 2004).

QUARANTINE

Several *Eretmocerus* and *Encarsia* species were established in culture between 1993-1998 (Goolsby *et al.* 1998). These candidate biological control agents came from varied climates and from *B. tabaci* infesting a wide variety of host plants. Early experiences with the indigenous North American natural enemies showed that parasitism by a key native species, *Eretmocerus eremicus* Rose and Zolnerowich, was low on *B. tabaci* infesting fall/winter cole crops. This resulted in very low numbers of overwintering *E. eremicus* in the spring and outbreaks of *B. tabaci* on melons (Hoelmer 1995). The same drop in parasitism by the native *Encarsia* was not noted on winter cole crops. This was evidence of a tritrophic interaction between *E. eremicus, B. tabaci* and its cole crop host. To evaluate potential host plant effects (tritrophic interactions) of the imported parasitoids their attack rate was evaluated on several key crop plants, including cotton, broccoli, and cantaloupe melons. Significant differences were noted between plant types (Goolsby *et al.* 1998). For example, *Eretmocerus hayati* Zolnerowich and Rose, performed best on cotton, but was lower ranked on melons and broccoli. In general, the Old World *Eretmocerus* spp. performed the best and were prioritized in the biological control program.

As the science of biological control moves toward more extensive host range testing for arthropod agents, we should use this evidence of the tritrophic effects on the *B. tabaci* parasitoids when we predict the realized host range of agents. Realized host range is a term that is gaining acceptance in the biological control of weeds community. It is defined as the innate host specificity of an organism, including its fundamental or physiological host range, relative acceptability and suitability of hosts, ability to learn, and influence of time-dependent effects in the post-release environment (van Klinken 2000). In the case of the Old World *Eretmocerus* spp., their realized host ranges were influenced by the host plants where they were released. Preference for *B. tabaci* on selected host plant showed evidence of habitat specialization. For these parasitoid species, plant cues, rather than the host insect, may be more important in their host-finding mechanisms. This is similar to the results of Kuhlmann *et al.* (2000) who found distinctive habitat preferences and host plant associations for mirid plant bug parasitoids in Europe. If non-target whitefly species were at risk in future biological control programs involving *Eretmocerus* spp., then the influence of the plant host should be considered in host-range testing. Non-target attack may be ameliorated by the effect of the plant host on their searching behavior. It appears from the research on *Eretmocerus* spp. in the *B. tabaci* program that even studies done in the confinements of quarantine laboratories can produce meaningful results and add to predictions of the realized host range of a biological control agent.

ESTABLISHMENT OF PARASITOIDS IN THE U.S.A.

Five species of Aphelinidae became established in the U.S.A.: *E. mundus*, *E. hayati*, *Eretmocerus emiratus* Zolnerowich and Rose, *Eretmocerus* sp. (ex. Ethiopia), and *Encarsia sophia* Viggiani. A sixth species *Eretmocerus melanoscutus* Zolnerowich and Rose, may be locally established in greenhouses in South Texas (T. X. Liu, pers. comm.). Climatic effects and the influence of tritrophic interactions appears to have had a strong influence on their establishment (Goolsby *et al.* 1998; Goolsby *et al.* 2005). However, several questions remain regarding establishment patterns. *Eretmocerus hayati* only established in Texas despite what appears to be ample heat tolerance for the irrigated desert agriculture of Yuma and the Imperial Valley. Did the crop mix of the Imperial Valley, i.e. large plantings of alfalfa, put it at a competitive disadvantage versus *E. emiratus* and *E.* nr. *emiratus* (ex. Ethiopia)? One of the obvious differences in the crop mix between the two areas is the large plantings of alfalfa in the Imperial Valley. Although year-round densities of *B. tabaci* in alfalfa are low, it is an important bridging host in the fall between cotton and winter vegetables. Field cage evaluations of the parasitoids conducted in the Imperial Valley showed that levels of parasitism on alfalfa were much lower than with cantaloupe melons, cotton or broccoli (Hoelmer 1998; Hoelmer and Roltsch in press). These tests did not include *E.* nr. *emiratus* (ex. Ethiopia), which became available later in the program. This parasitoid species may have been more effective at searching for *B. tabaci* in alfalfa, a trait that would have favored its establishment. In the Lower Rio Grande Valley the situation is reversed, where *E. hayati* has become established, and to date, no recoveries of *E.* nr. *emiratus* (ex. Ethiopia) have been made. Could the lack of alfalfa have influenced the establishment patterns in this agroecoystem? Field studies are planned for Arizona, California and Texas to evaluate the impact of the introduced parasitoids on *B. tabaci* using the methods developed by Naranjo and Ellsworth (In press). The proposed studies will be conducted on series of crops which may also explain the tritrophic interactions of the plant hosts with the introduced parasitoids.

In Yuma and the Imperial Valley, two species, *E. emiratus* and *E.* nr. *emiratus,* have become established. The population of *E.* nr. *emiratus* in Yuma is morphologically identical to the original voucher specimens (Mike Rose, pers. comm.). However, in the Imperial Valley the species appear to grade together, which suggests that the two populations are reproductively compatible and may be hybridizing. Could this be evidence that a more fit hybrid form of the two species exists in the field? Molecular tools may be able to validate this hybridization event. More importantly though, could molecular markers be used in the laboratory to identify hybrids and follow their success or failure in the field? If so, these techniques may open the possibility for selective breeding in quarantine of reproductively compatible species and/or populations followed by field studies to evaluate their efficacy. Biological control programs often seek populations of a natural enemy species from different climates with the belief that they will be more suited to the area of introduction. However, climatically adapted species may lack important biological attributes found in other populations. Multiple populations may have the opportunity to mate in the introduced range which leads to selection of the most fit individuals. However, the opportunity for this to occur may be lacking due to the stochastic effects of the environment and small initial release populations of the biological control agents. The opportunity for hybridization of species may be best done in the laboratory after which molecular markers can be employed to track the failure or success of these hybrids in the field.

The apparent intergradation of the two *Eretmocerus* species in the Imperial Valley could provide an opportunity to test these hypotheses and propose new research that would integrate the use of molecular techniques and crossing studies at the outset of biological control program. To test this hypothesis, material from the sources populations in Ethiopia and the United Arab Emirates would need to be recollected and reared in quarantine for the genetic studies. Other keys species in the program, including *E. hayati* from Pakistan, *E. mundus* from Spain, and *E. melanoscutus* from Thailand, should also be recollected and analyzed in the study. By comparing the genetics of the source populations with the established field populations, we may discover that hybridization occurred between these closely related species. Understanding which parental populations contributed to the hybrid forms may help us determine which biological traits (i.e., climatic tolerance, host range) were contributed from each source population. In the future, we may be able to determine which genes are responsible for the desired traits and select for individuals with the highest potential for success.

USING THE PREDICTIVE TOOLS TO SELECT AGENTS FOR AUSTRALIA AND CHINA

Predictive tools for prioritizing agents were used throughout the biological control program to prioritize agents for mass rearing and release (Goolsby *et al.* 1996; Goolsby *et al.* 1999; Hoelmer and Goolsby 2003). A retrospective study of the establishment of parasitoids in the U.S.A. showed the predictive value of the climate matching and quarantine attack rate studies (Goolsby *et al.* 2005). Based on this retrospective evaluation, a set of guidelines or tools were developed to help select the first agent for evaluation and release in Australia. Regulations in Australia require host range testing of arthropods, which is a considerable commitment in terms of time and resources. Therefore, selecting the best first candidate for testing was imperative. *Eretmocerus* spp. were prioritized because they had generally done well in quarantine attack rate studies. Climate matching showed that McAllen, TX in the Lower Rio Grande Valley was the most similar part of N. America to Queensland, Australia where silverleaf whitefly has become a pest (Fig. 2). In addition, the crop mix in this region of Australia is similar to the Lower Rio Grande Valley. Since *E. hayati* has established in Texas and is the dominant parasitoid in field collections, this species was shipped to Australia for host range testing and evaluation as a biological control agent. In the quarantine studies, *E. hayati* was shown to only attack *B. tabaci* and one other closely related whitefly. Australian regulatory authorities granted a release permit for *E. hayati* and it was released in late 2004. Early indications from the program in Australia are that *E. hayati* is successfully reproducing in the field and dispersing. While it is too early to tell if this species will become permanently established, it appears that the predictive tools worked well in selecting a candidate. It is possible that other parasitoid species might have shown similar results, but given the regulatory framework in Australia, this hypothesis cannot be fully tested unless *E. hayati* is shown to be ineffective and release of a second species is warranted.

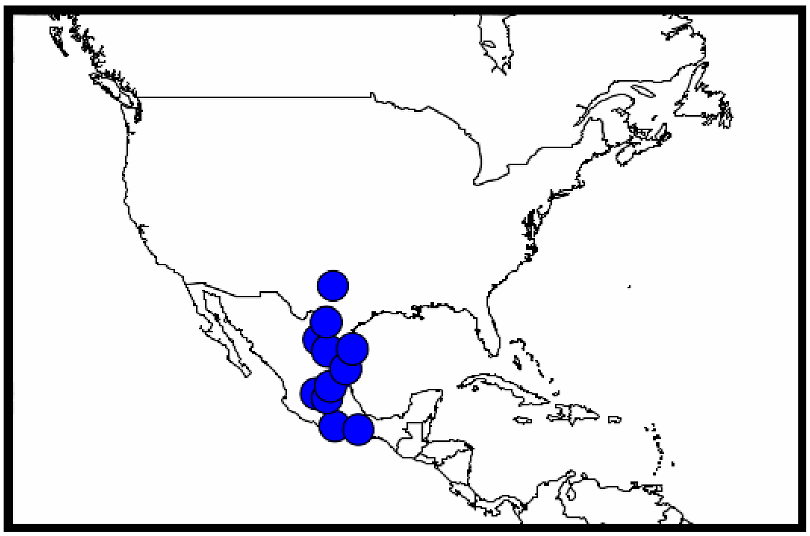

Figure 2. North American locations with climatic similarity to Emerald, Australia. Blue dots indicate a CLIMEX match of 75% or better.

Bemisia tabaci biotype B has also recently become a pest in China (Luo *et al.* 2002). Like Australia, other biotypes of *B. tabaci* are endemic to China along with a suite of indigenous parasitoids (Huang and Polaszek 1998). It is not known if the introduced species will provide additional biological control in the midst of the endemic parasitoids, but the experience in Australia has been that the endemic parasitoids were ineffective (DeBarro 2000). Using our experience in Australia, the predictive tools were used again to select candidates for release in China. The areas of China that are impacted by *B. tabaci* include the subtropical areas of southern China north to the warm temperate areas of Shanghai. In the Shanghai area, *B. tabaci* overwinters in greenhouses and infests field plantings each spring. Climate-matching using Guangzhou, Guandong as the home location showed that the best matches for N. America occurred from Florida westward to Texas (Fig. 3). Both *E. hayati* and *E. melanoscutus* have been recommended for release in China. This provides an opportunity to test the release of two species simultaneously and evaluate their tritrophic interactions in the agroecosystem. In China, banker plant, first developed for augmentation of *Eretmocerus* spp. in melon crops in TX and CA, may be a useful method for passive dispersal of the parasitoids from the greenhouses to field crops (Goolsby and Ciomperlik 1999; Pickett *et al.* 2004).

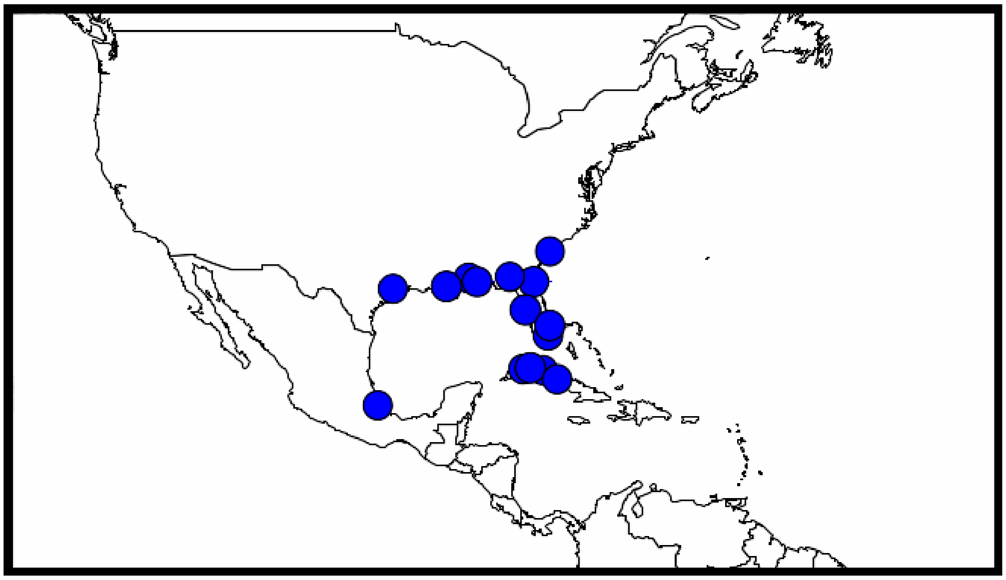

Figure 3. North American locations with climatic similarity to Guangzhou, China. Blue dots indicate a CLIMEX match of 65% or better.

The biological control program for *B. tabaci* provided novel opportunities to use predictive tools to direct foreign exploration and evaluate a suite of natural enemies in quarantine prior to release. These experiences have been used to develop a set of predictive tools for biological control of *B. tabaci*, which have used in the selection of agents for release in Australia and now China. The influences of climate and tritrophic effects appear to have been important factors in the establishment of the *Eretmocerus* spp. for *B. tabaci*. Further studies on the introduced parasitoids, including their impact on *B. tabaci*, the influence of the host plants in the agroecosystem, and their genetics, are warranted, and may provide useful insights and new scientific directions for biological control of arthropod pests.

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