

# Selecting arthropod biological control agents against arthropod pests: Can the science be improved to decrease the risk of releasing ineffective agents?

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## Abstract

With greater emphasis being placed on management of the risks attached to natural enemy releases for biocontrol programs and the need to justify research budgets, the efficient selection of effective natural enemies is increasingly important. Historically there has been little agreement regarding how or whether this can be accomplished. Recent studies have demonstrated that there is good correspondence between insect host-finding behavior and attack rates in well-designed laboratory studies and their performance of this behavior in the field. Success in measuring efficacy of candidate agents remains somewhat of an art due to the multitude of factors influencing efficacy, but will be improved by attention to: (1) characterization of natural enemy candidates using morphological taxonomy or genetic markers at the onset of a program, (2) climatic matching candidate agents when possible, and (3) evaluations in semi-field or field cage conditions following quarantine evaluations whenever possible before proceeding with widespread releases. The application of these principles is discussed in regard to US biocontrol programs for *Bemisia tabaci* (Gennadius), *Lygus* spp., and *Aphis glycines* Matsumura. Proper project planning and interdisciplinary cooperation will enhance the chances for a successful project.

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## 1. Introduction

The single most important decision in a biological control program is the determination that a biological control solution is obtainable for the proposed target pest. This decision should be based on a scientific assessment, without which there can be no reasonable guarantee that effort will not be wasted on agents that eventually prove to be ineffective. Some of the factors relevant to feasibility assessments were discussed by

Barbosa and Segarra-Carmona (1993); other issues include consideration of not only the potential for unintended impacts (van Lenteren et al., 2003), but also the potential efficacy of existing indigenous agents (Michaud, 2002) and other ecological effects (Heimpel et al., 2004).

After it has been decided that biological control can be a solution to a pest problem, the goal is to establish agents that are effective at limiting the target pests. There are various reasons why ineffective natural enemies should not be released, including the possibility of undesirable impacts on non-target organisms, but these latter aspects are not the subject of this paper. In our view, releasing inefficient agents should be avoided primarily because it wastes precious resources—researchers' time

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and funding—and it delays or avoids arriving at an eventual solution to the problem.

Ecologists and practitioners of biological control have long argued among themselves about the feasibility of predicting the efficacy of arthropod biological control agents prior to their release into the field. Although the basic characteristics of an effective agent are generally agreed upon (summarized, for example, by van Lenteren, 1980), many biological control researchers believe as did DeBach and Huffaker (1971) that “no amount of planning and preliminary research can replace actual and empirical research for natural enemies in the field” and “there are no reliable criteria for determining, a priori, which of several attributes is ‘best’” (panel consensus, Anonymous, 1978). Reviewing the range of opinion on evaluation methods for arthropod natural enemies several decades ago, van Lenteren (1980) posed the rhetorical question “does art have to become science?” and concluded that trial-and-error introductions still offered the best approach, especially for field releases of predators and parasitoids into complex natural environments (as opposed to the controlled environments of greenhouses).

Even using trial-and-error approaches, however, selectivity may be forced upon researchers by limited budgets and the necessity to demonstrate progress and results. This is less of a problem when only a few candidate agents are available, but in other cases an abundance of natural enemies may be available for evaluation. For example, over 40 geographic populations comprising 17 species of aphelinid parasitoids were maintained in quarantine laboratory cultures during a program to establish new biocontrol agents against *Bemisia tabaci* (Gennadius) in the US (see below).

Nobel laureate Sir Peter Medawar elegantly described research as “the art of the soluble” (Medawar, 1967). This relates to biological control research and implementation programs quite well, for complex interactions of biological, behavioral, and physical factors must be considered by researchers for successful outcomes. Because of this complexity many biological control programs have unique aspects that do not apply to other projects, so methods that work well for evaluating a specific pest–natural enemy pair may be ineffective or misleading for another. Traditional ecological and behavioral methods for studying parasitoids and predators continue to be very useful when properly used in evaluations, but biological control practitioners today also have an array of new tools available to assist them in designing and analyzing appropriate assays. Computer hardware and software for recording observations and data have improved the ease and accuracy of data gathering. Analytical software has also greatly simplified the task of managing and extracting patterns from large amounts of data. Development of a wide range of marking techniques, such as rare elements and monoclonal

antibodies (e.g., Lavandero et al., 2004), are also very powerful new tools for monitoring insects. These are generally more useful for evaluations of agents already present in the new environment than ones still being tested in controlled or confined settings, but they may still be useful to track movement and survival within large cage evaluations. The widespread application of molecular technology has also provided many new analytical tools, however, the availability of new technologies has not rendered the traditional approaches to biological control irrelevant. These approaches are well-grounded in ecology, systematics, and behavior, and if followed, are still likely to improve the chances for successful selection of effective agents. In this paper, we discuss how they have become more powerful by incorporating new tools, such as molecular biology, and we illustrate this with several examples.

## 2. Characterization of pests and natural enemies

Inadequate taxonomic knowledge of the target pest and candidate natural enemies is frequently cited as a reason for failure of candidate agents to perform in the field as expected after their release (Rosen, 1986). Researchers become aware of taxonomic complexity in some cases only when the failure of a released agent is analyzed. For example, Clarke and Walter (1995) reviewed cases of multiple introductions of different populations of biocontrol agents, and attributed the low success rate to (initially) unrecognized cryptic species. The existence of cryptic species or species complexes, or the misidentification of species, may result in agents not behaving according to expectations after release because (1) the target pest population may not be the same in the release area as the population in the area of origin where the candidate natural enemy was obtained, or (2) an unrecognized mixture of candidate agents comprise the pool of candidate agents evaluated, and this mixture changes following release. In a similar fashion, a lack of awareness of the genetic differences between and within populations of target pests and the candidate agents may cause problems in biological control programs. Inadequate taxonomic knowledge of both host and its principal natural enemies, parasitoids in the aphelinid genus *Aphytis*, delayed significant progress in efforts to establish effective natural enemies of the California red scale for over 50 years (Rosen, 1986). Cryptic (or sibling) species remain problematic in *Aphytis*; cross-mating studies were useful in distinguishing a cryptic species from other populations of *Aphytis lingnanensis* Compère (Fernando and Walter, 1997). The green vegetable bug, *Nezara viridula* (L.), is another recent example. Several populations of what was believed to be the egg parasitoid *Trissolcus basalis* (Wollaston) were introduced from various geographic sources into Australia. Some were given credit

for providing satisfactory control in different parts of the country, and a population from Pakistan was credited with controlling *N. viridula* in the inland regions of Australia (Clarke, 1990). However, voucher specimens from the original Pakistan material were later recognized as *Trissolcus crypticus* Clarke and not *T. basalis*. No specimens of *T. crypticus* were found in any field recovery collections, and it was then realized that the introduction of material from Pakistan had not been successful (Clarke, 1993). Other populations regarded as *T. basalis* were also found to exhibit differences; strains from the US had lower fecundity than strains established in Australia (Powell and Shepard, 1982). The taxonomic status of these and other geographic populations has not yet been resolved (Waterhouse and Sands, 2001). As populations of this parasitoid have been widely distributed to other countries, the task of taxonomists has been further complicated.

These problems can be minimized by placing a high priority on involvement of taxonomists and/or molecular biologists at the start of a project to ensure that all collections of natural enemies received, and their hosts or prey at the collection source and the target release area, are properly identified at the onset. Ideally, well-tested primers should be selected to obtain molecular fingerprints for all populations, even those of the same species that will be reared and released in the field. Even if taxonomists are unable to conclusively identify the insects, the molecular fingerprint may be able to distinguish different populations, and help to ensure the purity of laboratory cultures and track the establishment and movement of released insects.

### 3. Climatic adaptation and matching climates and genotypes

There are clearly underlying biophysical factors that cause climate to influence the establishment and performance of candidate agents. A frequently asked question is: how important is climate matching in determining the efficacy of a biological control agent? The premise that new agents should be obtained from areas having close climatic matches with the intended release area is generally accepted (e.g., Messenger and van den Bosch, 1971; Stiling, 1993) and lack of a proper climatic match is often cited as a presumed reason for lack of establishment of released agents (Clausen, 1978). Climate matching software (e.g., CLIMEX, BioSIM) has been developed to help simplify the task. These programs use data banks of historical weather data from numerous locations worldwide and allow weighting of specific environmental factors to match climates and map distributions, or link meteorological data to information on how temperature affects a given species to make phenological or distribution predictions (Régnière et al., 1996;

Sutherst et al., 1999). In some cases there is sufficient information on physical tolerances to make climatic predictions, as was done for *Thrips palmi* Karny (Dentener et al., 2002). More often, however, precise tolerances are unknown, and climate matching is frequently an exercise in searching for foreign collection sites with climates similar to that of the target release area(s). Despite limitations in the ability to predict distributions, the ability to locate candidate agents in similar climates is still a useful starting point for evaluations, particularly for pest species with wide distributions outside the target range.

We suggest that climate matching should be used to identify suitable areas for initial exploration for natural enemies on the basis that organisms from regions with homologous climates are likely to be adapted to those conditions in areas targeted for eventual release. Such areas may not always coincide with the presumed center of origin of the target pest. It is a widely held tenet of biological control that natural enemies associated with a pest at its geographic evolutionary center of origin are more likely to be well adapted to the pest as a result of long-periods of co-evolution and therefore capable of preventing pest population outbreaks. If the center of origin is known, it is logical to consider exploration in this region first, especially if the climate is also similar. However, the center of origin is often not known with any degree of certainty. Genetic matching of a pest from both its new exotic distribution and its known areas of natural distribution will help this and close genetic matches may be more relevant whether or not the center of origin is known. Genetic analysis of sufficient populations may show a match between the exotic pest and part of its native range. If so, exploration for natural enemies should then be focused on the native distribution area of the best genetically matched exotic pest population.

### 4. Laboratory assessment of efficacy paired with field assessments

Evaluations of candidate agents while they are still in quarantine cannot be convincingly validated with field studies until after permits are obtained for their release. Limitations of space in quarantine usually result in evaluations being confined to small cages in greenhouses or controlled-environment chambers. Researchers must therefore design the most realistic tests possible under the constraints of quarantine, given their knowledge of the candidate's biology. Once release permits are obtained, the next step in efficacy evaluation—especially if many agents are being evaluated—should be to conduct enclosure studies under field or near-field conditions. Such cages provide much larger environments for candidate insects than is feasible in quarantine culture, and expose agents to natural climatic conditions. Researchers can also take advantage of any new

biological information obtained during quarantine culture and assays to design such studies.

Native range studies of candidate agents are often proposed as part of efficacy evaluation processes. These may reduce the need for expensive quarantine work and can give a better indication of the efficacy of a natural enemy in the field. However, native range studies cannot usually be carried out during initial overseas exploration trips, and to avoid lengthy and expensive overseas visits by researchers, it is usually necessary to rely on local co-operators to carry out the research. Conducting studies of candidate agents in environments where their hosts or prey may be relatively rare may also be more difficult than evaluations under conditions of abundant host or prey. Studies of gypsy moth parasitoids that limit their host in non-outbreak years provide an example: surveys for natural enemies that attacked gypsy moth at low densities required placing host larvae as ‘sentinels’ in the field at numerous locations for exposure to parasitism, which were then retrieved for rearing in the laboratory (Mills and Nealis, 1992). The tachinid fly *Aphantorhaphopsis samarensis* (Villeneuve) was identified as an important natural enemy under these conditions. Evaluation of its host specificity required extensive field collections and rearing of potential alternate hosts, paired with laboratory studies of host suitability. This research was a joint effort that involved a number of European and North American laboratories (Fuester et al., 2001). The importance of native range studies (in this case, field collections of potential alternate hosts and their parasitoids) requiring collaboration between researchers in the native range and the prospective release area cannot be overstated.

Concurrent with laboratory evaluations, complementary studies should be conducted in the potential release area to document gaps in indigenous natural enemy activity or other local ecological factors that contribute to high pest populations. This requires a committed cooperating biological control researcher to be present in the intended release area.

Several recent field studies have examined the relationship between laboratory observations of behavior and how natural enemies performed these behaviors in the field. Although such comparisons are not yet very numerous, it is encouraging to find examples of good correspondence. For example, by using detailed field observations, Casas et al. (2004) found that previously published laboratory studies of the host-finding behavior of the scale parasitoid *Aphytis melinus* DeBach closely described the actual performance of these events by the parasitoid in the field, although the field rate of host rejection was higher, apparently due to high encounter levels with dead hosts. A second example is that of evaluations of introduced parasitoids of *B. tabaci* (Gennadius) in the US. Numerous accessions of *Eretmocerus* and *Encarsia* species collected from Africa,

Europe, and Asia were first screened in the quarantine (Goolsby et al., 1996, 1998). After permission for release was obtained, the top performing species in quarantine were then evaluated in large cages in semi-field tests in the south western US (Hoelmer, 1998; Hoelmer and Roltsch, in press). The most effective species in these trials were then released on a wider scale for establishment. This example will be discussed in more detail below.

## 5. Project examples

Until comparatively recently, many classical biocontrol programs were constrained by (1) the limitations of morphological taxonomy, which could not distinguish biotypes or cryptic species, (2) the loss of natural enemies during the long transport times from the area of origin to the release area, (3) dependence on field observations of impact in the area where the natural enemies were collected rather than experimentation, and (4) the lack of reliable climatic data from large areas of the world. The earliest biocontrol programs, e.g., against cottony-cushion scale (Koebele, 1890), were remarkably successful given such constraints. Koebele made massive collections of a dipteran parasite, *Cryptochetum iceryae* (Will), coincidentally also obtaining the beetle *Rodolia cardinalis* Muls. Both these insects were individually effective in controlling the pest, but together they brought about permanent control (Wilson, 1960). The approach of prospecting for natural enemies in their area of origin and the methods that were used (massive collections in species diversity and numbers of individuals) by early practitioners laid the foundation for many later programs. To assess what has changed and how the selection of efficient agents has moved from more of an art to a science by adopting new methods, we present three recent or ongoing examples of biological control programs that illustrate the incorporation of modern methods with traditional approaches.

### 5.1. *Bemisia tabaci* biotype B in the US

A new biotype of the sweetpotato whitefly, *B. tabaci*, invaded North America in the late 1980s and caused massive losses for vegetable and ornamental growers in several areas of the country. Because crop production in markedly different climatic regions of the US was affected, foreign exploration for natural enemies was focused for areas matching three distinct climates of the US southeast, south central, and southwestern desert regions, using CLIMEX and existing literature reports of *B. tabaci* in Africa and Eurasia (Lacey et al., 1993). The best matches proved to be in the area of Multan, Pakistan, climatically similar to the lower Rio Grande Valley TX; the desert region of Ethiopia and the United Arab Emirates, climatically similar to the Imperial Val-

ley desert climate in California; and the hot, dry region of Murcia in southeast Spain, climatically similar to the San Joaquin Valley, CA, and these areas were made priorities for exploration. Collections were also made in Thailand, Malaysia, Indonesia, India, and Nepal, each with areas determined to be climatically similar to Florida where *B. tabaci* was also a problem (Goolsby et al., 1998). Genetic typing and matching of *B. tabaci* populations using genetic markers was initiated at the start of the program (Brown et al., 2000); this was matched with co-occurring natural enemies (Kirk et al., 2000) at several of the foreign collection sites. Thus, foreign explorers and taxonomists worked in collaboration from the outset.

Twenty-eight countries were visited at least once during the explorations, some several times. *B. tabaci* has a very large host range, which facilitated collection of natural enemies on weedy hosts, ornamentals, and crops. Parasitism rates varied widely among host plants, but the extensive and widespread collections made it possible to sample a wide range of diversity of natural enemies from more than 50 host species of *B. tabaci*, including 17 crops, 13 ornamentals, and 20 weeds. Many crops were intensively treated with pesticides, so the opportunity was taken to collect both on treated crops when natural enemies were observed as well as untreated crops. Laboratory evaluations for tolerance to pesticides showed that parasitoids obtained from some of these treated crops exhibited higher levels of tolerance to several insecticides in common use against *B. tabaci* (Jones et al., 1996).

Approximately 130 shipments of natural enemies were made by express mail and airfreight over a 5-year period to the USDA APHIS Mission Biological Control Center quarantine laboratory in Mission, Texas. Express shipment meant that most parcels arrived after only 2–4 days. The foreign collections resulted in the establishment of 41 quarantine cultures, of which 17 proved to be distinct species of *Eretmocerus* and *Encarsia*. Preliminary taxonomic identification was made using morphological characters of adult parasitoid specimens and whitefly nymphal case remains. Concurrently, molecular markers using RAPD-PCR were developed by APHIS as an alternative for lacking morphological identifications, and to ensure the purity of quarantine cultures. These markers proved to be an essential means in the project for identifying parasitoid population diversity and making preliminary separations of geographic populations or strains, and potential new species. Later in the program it also allowed researchers to document the presence and establishment of exotic parasitoids in the field (Goolsby et al., 2000; Zhang et al., 2002). Physical voucher specimens matched with their RAPD fingerprints allowed taxonomists to clarify the identity of important indigenous parasitoids, and to identify the introduced parasitoids and describe several new species

(Heraty and Polaszek, 2000; Rose and Zolnerowich, 1997; Zolnerowich and Rose, 1998). Morphological and/or molecular characterizations are needed from the inception of the project. In this instance, traditional names based on morphological species definitions were often not available until evaluation of candidates was well advanced and some field releases had already been made.

After receiving foreign material for culture, and developing a process for categorizing and characterizing the collections, quarantine screenings were the next step and the first stage of efficacy evaluations. Evidence suggested that indigenous natural enemies exhibited different levels of parasitism of *B. tabaci* whitefly on different plant hosts (e.g., Gruenhagen and Perring, 2001; Headrick et al., 1996), resulting in significant gaps in parasitoid activity during the winter and spring. Because it was important to ensure that newly introduced species would be effective on several different crops that were key to whitefly population increase during these times, Goolsby et al. (1998, 1996) evaluated attack rates of 19 imported and two indigenous parasitoid species on the host whitefly on three key crop hosts and one common weed host in assays conducted in the quarantine. These tests measured the number of hosts attacked per female in small cages as an indicator of efficacy, a number that ranged from 0.2 to 63.0 over the lifetime of a female. The results showed that the Palearctic *Eretmocerus* species had significantly higher attack rates than any *Encarsia* species or the indigenous Nearctic *Eretmocerus*.

The best performers in these quarantine screenings were selected for further testing in semi-field evaluations in the desert south western US after release permits were obtained. Field cages were used to confine known numbers of each species or population of parasitoid tested with whitefly-infested alfalfa, broccoli, cantaloupe, and cotton, key crops at different times of the year (Hoelmer, 1998), and efficacy was compared by measuring the number of progeny in the F<sub>1</sub> generation per female. The top performing species from quarantine tests were usually, but not always, the same species that did best in the field tests. The top performing species or populations from each set of crop tests were then re-tested in a final comparison on cantaloupe. Overall, the best-performing species included populations of *Eretmocerus emiratus* Zolnerowich and Rose from the United Arab Emirates (66.1 F<sub>1</sub> progeny per female) and Ethiopia (66.6 F<sub>1</sub> progeny), several geographic populations of *E. mundus* Mercet from Spain (50.7 F<sub>1</sub> progeny), India (45.1 F<sub>1</sub> progeny), and Israel (54.8 F<sub>1</sub> progeny), and *E. hayati* Zolnerowich and Rose from Multan, Pakistan (26.7 F<sub>1</sub> progeny). The most effective species identified in the field cage tests were then mass-reared and widely distributed in release programs.

Curiously, the thelytokous species tested performed well in quarantine evaluations but did poorly in the field

cage tests. For example, in whitefly hosts on broccoli, results ranged from 0.42 to 3.48  $F_1$  progeny per female *Encarsia* (sp. nr. *hispid*a and sp. nr. *pergandiella* from Brazil, and sp. *parvella* group from Dominican Republic) in the field cage studies vs. 10.5–18.2  $F_1$  progeny per female in the quarantine evaluations. This may be indicative of a more limited genetic basis of the uniparental populations resulting in less adaptability to field conditions. The quarantine tests were conducted in growth chambers maintained continuously at warm temperatures and moderate levels of humidity, while the field cages experienced wide daily fluctuations in temperature and humidity. These differences highlight the necessity to pair laboratory evaluations with tests under actual field conditions.

Goolsby et al. (2005) retrospectively analyzed the establishment of whitefly parasitoids introduced in the US and showed that those species having the best climate match between source and release locations tended to be the most efficient in the evaluations. They were also the ones that became established in release areas with closely similar climates to the collection localities. Given this retrospective history, CLIMEX was then used to determine the closest climatic match between the whitefly-impacted areas in the US and north eastern Queensland, Australia, where *B. tabaci* had become established. The Lower Rio Grande Valley of Texas was the best match, leading to the recommendation that *Eretmocerus hayati* Zolnerowich and Rose should be the first candidate considered for establishment in Australia.

### 5.2. Tarnished plant bug in the US

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), and western tarnished plant bug, *Lygus hesperus* Knight, are economically damaging insects in North America with wide host ranges that include many food, fiber, and seed crops. Because surveys showed that there were no effective indigenous natural enemies in the northeastern US (Day, 1987) or California (Clancy, 1968), and most crops damaged by *Lygus* had been introduced to North America, biological control has been a desired goal even though these species are considered to be native. In Europe, parasitism of congeneric *Lygus* species in alfalfa is notably higher than in North America (Day et al., 1990). Collections of the braconid nymphal parasitoids *Peristenus digoneutis* Loan and *P. stygicus* Loan were made in various countries and shipped to North America, with material from northern Europe generally designated for releases in the northeastern US and in Canada, and that from southern Europe and Turkey generally designated for releases in the south western US (Coulson, 1987). European studies and field collections with subsequent releases in North America continued sporadically for 25 years without apparent success. Finally, *P. digoneutis* Loan, a braconid

parasitoid of *Lygus*, collected in northern Europe was successfully established in the northeastern US and adjoining provinces of Canada (Day et al., 1990, 2000) where it has reduced *L. lineolaris* numbers in alfalfa to low levels (Day, 1996) and is believed also to be reducing damage in other crops. Several years after the initial establishment of *P. digoneutis*, it continues to spread to the northeast and northwest, but it has become apparent that it does not survive the warm summers common south of latitude 40.5°. An analysis of climatic factors suggested that high summer temperatures and their duration were probably responsible (Day et al., 2000).

More recently, releases of *P. stygicus* and *P. digoneutis*, both from populations obtained in hot-summer climate regions of southern Europe, have become established in California at several study sites. In this case, based upon the successful climate match of *P. digoneutis* in the northeastern US with its source in northern Europe, special attention was given to focusing foreign collections in areas with very similar climates to central and coastal California (Pickett et al., 2002).

It is noteworthy that establishment in the northeastern US and in California was reported at only some of the release sites although all release locations had been selected to ensure continuity of alfalfa, the host crop, and lack of pesticide use. The microclimate of the sites varied somewhat, however, due to differences in elevation and latitude. This highlights the utility of making releases at a number of sites with differing ecological characteristics to increase chances of establishment.

Efforts are being continued to obtain populations of *P. digoneutis* and *P. stygicus* from warmer regions of southern Europe that will establish in the eastern US south of the range limits of *P. digoneutis*. Likewise, there is continued interest in additional releases of suitable geographic populations of these species in western Canada because releases several decades ago were not successful (Broadbent et al., 2002). Various studies have reported on aspects of the biology of these and other *Peristenus* species, but the most comprehensive study of fecundity as a predictor of efficacy against *L. lineolaris* was conducted only recently (Haye et al., 2005). The authors concluded that previous workers underestimated the reproductive potential of both *P. digoneutis* and *P. stygicus* for various experimental and technical reasons. Their study examined egg load, age-specific and potential lifetime fecundity, and oviposition period and longevity, and they concluded that *P. stygicus* should be the more effective agent of the two due to its greater fecundity.

Several *Peristenus* species and hyperparasitoids often occur together in *Lygus* populations in Europe and in North America. *Peristenus* species overwinter in nature as pupae within cocoons in the soil or leaf litter, but when field-collected material is held in the laboratory for rearing there are significant losses due to unexplained

rearing mortality. For these reasons it has been necessary to devote considerable time and effort to obtain quantities of desired species of parasitoids for research in their native range and for shipment to cooperators in the US. Field parasitism has often been assessed by rearing, but this method significantly understates actual levels of parasitism (Day, 1994), and rearing results are often not obtained until the following spring. Conversely, dissections accurately indicate parasitism levels but do not allow for the identification of parasitoids. Molecular methods that are capable of both accurately estimating parasitism and distinguishing among *Lygus* parasitoids have now been developed for a number of *Peristenus* species and a common hyperparasitoid (Ashfaq et al., 2004, 2005; Bon et al., 2003; Erlandson et al., 2003; Tilmon et al., 2000). These will greatly assist studies aimed at understanding field patterns of parasitism and the interaction of native and exotic *Peristenus* species with overlapping geographic and host ranges. The role of taxonomy in characterizing the species diversity of *Peristenus* was appreciated early in the project, and the important species were distinguished and described. As the project progressed, and the surveys including non-target mirids became increasingly important, it became evident that the taxonomic diversity of *Peristenus* had been incompletely understood. The continued involvement of taxonomists has been essential in recognizing the diversity of species attacking *Lygus* and other mirids. Further development of molecular methods is also needed to determine whether different geographic populations of *Peristenus* species can be differentiated with molecular markers.

### 5.3. Soybean aphid in the US

The Chinese soybean aphid, *Aphis glycines* Matsumura, recently became the first major insect pest of soybeans to be introduced into North America. Most of the soybean production in North America occurs within the predicted climatic range of the aphid, and its rapid spread in the US disrupted what had been a satisfactory IPM situation in soybeans.

Venette and Ragsdale (2004) used biome maps and CLIMEX to determine locations in eastern Asia having similar climates to areas of soybean production in North America. A large number of natural enemies are reported from the literature to attack *A. glycines* in Asia (Wu et al., 2004a). Classical biological control was considered to be an attractive option pending the outcome of host specificity testing of Asian parasitoids (generalist predators were considered too risky for introduction). Foreign exploration for candidate agents against soybean aphid was therefore initiated in Asia in areas identified as suitable climatic matches, and quarantine evaluations for efficacy and host specificity of agents collected to date are ongoing (Heimpel et al., 2004; Wu

et al., 2004b). These evaluations are using methods similar to those used by Goolsby et al. (1998) in which female parasitoids are placed with hosts in small cages and the comparative attack rate is an indicator of host acceptability and efficacy. Concurrent native range field studies, such as were conducted in China by Liu et al. (2004) using exclusion cages, are intended to complement the quarantine laboratory evaluations that are in progress in the US and may help to identify effective species. This study reported on the results of a survey conducted in an experiment station soybean field and a companion exclusion cage experiment in the same field. Designated sample sites were inspected every other day throughout the duration of the season to record all natural enemies seen and collect aphid mummies for rearing. The exclusion study used screened cages with several mesh sizes, on the basis that relatively large predators would be excluded from the small mesh cages, and a no-cage control. Although only the results of one season were reported, the study provided useful information on local population dynamics and seasonal activity of natural enemies that has been helpful for designing longer-term studies at additional sites in Asia and in North America. One notable finding was that a large number of parasitized aphids were found at the start of the colonization period on soybean, suggesting that many of the colonizing alate females had been parasitized before their dispersal, and that more extensive surveys on the overwintering host were needed to fully document the suite of natural enemies.

Populations of *Aphelinus* species (Hym.: Aphelinidae) have been frequently collected during foreign explorations for *A. glycines* and many are included in the ongoing evaluations. Cryptic species and species hybridization are known to occur among species of *Aphelinus*. Genetic characterizations and reproductive crossing studies of two of these populations have been completed (Wu et al., 2004b), revealing them to be compatible but genetically divergent. However, outbreeding depression was noted in the crossbreeding tests, and the authors noted that both populations should therefore not be released together to avoid the possibility of reduced levels of biological control. Genetic fingerprints of these populations and of other natural enemy taxa will be useful in monitoring the eventual establishment and spread of any populations that are released in North America.

## 6. Conclusions

The aim of classical biocontrol programs is to obtain effective permanent control of a pest. From that point, the science and the art (for we believe the two are interconnected in the practice of biological control) of evaluating candidate agents and identifying those that are effective

can be greatly improved by retrospective reflections on previous programs, increased communication between practitioners and the availability of new research tools and techniques. The application of new technologies will complement the traditional approaches and increase their analytical and predictive power. It is of great importance that the chain of collaborators in a project should remain intact; i.e., specialists involved in foreign exploration, rearing, testing, selecting, releasing, monitoring spread and establishment post-release and non-target impacts should remain in collaboration with one another. Without such linkages a program is less likely to reach fruition.

We make the following recommendations for improving the selection of arthropod biological control agents against arthropod pests:

1. Climatic matching software should be used in coordination with literature records to identify an initial collection area for foreign exploration.
2. Genetic fingerprinting should compare populations of the pest in its target, introduced range with populations in its natural range; and for all populations of natural enemies obtained before evaluations.
3. Foreign exploration should focus on the natural range of the matched population of pest arthropod if it can be identified.
4. Collaborative studies should focus on the efficacy of natural enemies in their native range.
5. Assuming that key biological characteristics have not been overlooked, laboratory comparisons of the effective kill rate of potential natural enemies can be an effective method to screen for efficacy.
6. Field cage studies should follow laboratory evaluations, prioritizing the most efficacious natural enemies from the laboratory studies.
7. Post-release field collections should use genetic fingerprints to assess distribution and establishment of the natural enemy in the field.

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