MAKING BIOLOGICAL CONTROL INTRODUCTIONS MORE EFFECTIVE

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ABSTRACT

Although biological control introductions have many documented successes and the strategy as a whole is cost effective, three-fifths to three-quarters of introductions against insects pests have failed to establish and the rate of successful control is even lower. A number of hypotheses have been suggested for failures in establishment and control, but few experiments have been done to test these hypotheses. However, the opportunities for doing experiments during biological control introductions beg for such tests. I illustrate the possibilities by describing research my colleagues and I have been doing during introductions against *Diuraphis noxia* and other pests introduced into North America. This includes experiments on impact of natural enemies in the area of pest origin, genetic variation in natural enemies within and among geographical regions, genetic variation in host specificity, characteristics of effective natural enemies, impact of laboratory rearing, demographic barriers to introductions, and genetic adaptation after introduction.

INTRODUCTION

Biological control of insect pests and weeds by introduction of natural enemies from elsewhere in the world has had many well-documented successes (DeBach and Rosen 1991) and is in general cost effective (Tisdell 1990, Cullen & Whitten 1995). However, somewhere between two-thirds and three-quarters of arthropods introduced against insect pests have failed to establish (Hall and Ehler 1979, Stiling 1990, Greathead 1995). Furthermore, only 16% of attempted projects have been completely successful at controlling the target pest (Hall et al. 1980). Insects introduced to control weeds have established with greater frequency, with only about one-third failing (Crawley 1986, Greathead 1995), and 34% of attempted projects against weeds have been successful (Julien et al. 1984). Introductions against weeds may have been more successful because the greater screening required for herbivorous insects may lead to better selection of candidates and greater understanding of how to use them. Nevertheless, improvements seem possible here as well.

Various hypotheses have been proposed to explain the failure of introduced natural enemies to establish: for example, lack of pre-adaptation to the novel climate, insufficient genetic variation, adverse conditions at the time of introduction, and inadequate number of organisms introduced. Additional hypotheses have been proposed to explain lack of control of the target pest by natural enemies that have established: for example, poor phenological synchrony with the pest, temperature ranges for development and activity of the natural enemy more restricted than those of the pest, poor searching ability of the natural enemy, insufficient natural enemy aggregation in areas of high pest density, low host suitability, and a highly ephemeral habitat with greater colonizing ability for the pest than for the natural enemy. Reviews of the criteria for selecting candidate species for introduction (Waage 1990) and managing the genetics of introductions

(Hopper et al. 1993) point out the lack of experiments or crucial observations to reject or accept these and other hypotheses. Little hard evidence is available to support current practice or to provide a basis for improvements. Fortunately, this lack can be remedied by using the research opportunities inherent in biological control introductions. Here, I will discuss how this can be done using examples from research my colleagues, Z. Basky, K. Chen, D. Coutinot, R. Dabire, O. R. Edwards, A. Farias, X. Fauvergue, P. G. Mason, D. J. Kazmer, N. Ramualde, and R. T. Roush, and I have been doing over the last six years in conjunction with introductions to control *Diuraphis noxia* (Homoptera: Aphididae) and other pests in the United States. By restricting the examples to our research, I do not mean to imply it is superior to that of others; nly that I know it more intimately and can with more impunity point out weaknesses as well as strengths.

The guiding question behind our research has been: How can choices made at the various steps during biological control introductions be improved so that introduced natural enemies establish more frequently and have greater impact on the target pest? The process of biological control by introduction consists of choosing a target pest, finding and collecting natural enemies, transporting and perhaps rearing the natural enemies collected, and releasing the natural enemies against the target. At various points in this process, the natural enemies are evaluated in a more or less thorough fashion for their potential efficacy at controlling the target and for potential impacts on non-target species. In this process, we must choose the target pest, the sites and dates of collection, the taxa and numbers collected, the methods of shipment and rearing, the methods of pre-release evaluation, the sites, dates, and methods of release, and the numbers to release. Each hypothesis concerning why natural enemies fail to establish or to control the target pest may impinge on more than one of these choices, and an exhaustive discussion of all the hypotheses and all the possible combinations of choices is beyond the scope of this paper. Therefore, I will concentrate on several hypotheses and choices that we have studied in some detail to illustrate how we have tackled what are fundamental, difficult questions.

TARGET PEST

Because pest taxa and habitats have widely different success rates (Hall & Ehler 1979, Hall et al. 1980, Stiling 1990, Greathead 1995), choosing target pests based on amenability to control by natural enemies seems indisputably sound idea. Although systems for ranking pests have been developed (e.g., Peschken & McClay 1995), the weighting of various criteria for ranking has been somewhat arbitrary, and no experiments have been done to test the validity of such rankings. Many biological control researchers working with insect pests have implicitly or explicitly assumed that all organisms must be regulated by density-dependent processes and that many, if not most, herbivores are regulated in this way by natural enemies (Huffaker et al. 1976). Although the importance of density-dependence in host-parasitoid interactions has been challenged (Murdoch et al. 1984, Stiling 1987), the viewpoint persists that most introduced herbivores are good candidates for biological control because they are very likely limited by natural enemies in their country of origin (Debach & Rosen 1992). However, the choice of targets is seldom based solely, or even mostly, on whether pests are likely to be controlled by natural enemies, and is more often driven by the politics of funding agencies and the economics of pest problems. Our lack of deep understanding concerning why some taxa and habitats have better track records than others leaves us open to such political pressure.

Since its appearance in Texas in 1986 (Stoetzel 1987), *D. noxia* has become a major pest of wheat in the United States, causing over $850 million in direct and indirect losses from 1987 to 1992 (Brooks et al. 1994). It is also an important pest in South Africa (Walters et al. 1980) and

Ethiopia (Haile & Megenasa 1987). Because it attacks crops with relatively low value per unit area, chemical control can be prohibitively expensive. Thus, there has been great interest in using other management tactics. Among these, biological control has been prominent, even though biological control introductions have not been outstandingly successful in annual field crops. Fortunately, there are several reasons to expect that introducing Eurasian natural enemies would reduce *D. noxia* abundance. Introduced natural enemies have substantially controlled several other introduced aphid pests, e.g., *Therioaphis trifolii*, spotted alfalfa aphid, and *Chromaphis juglandicola*, walnut aphid (Laing & Hamai 1976). Although these successes were in orchards or perennial crops, introduced natural enemies have also substantially reduced the abundance of some introduced cereal pests, e.g., cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae) (Haynes et al. 1974), in the U.S. and greenbug, *Schizaphis graminum* (Homoptera: Aphididae), in Chile (Zuniga 1990). Furthermore, a wide variety of predators and parasitoids attack cereal aphids, and many of these have been reported in association with *D. noxia* (Pike et al. 1991). In Eurasia, where it is thought to have originated, *D. noxia* is seldom a pest, although occasional, short-lived outbreaks are reported (e.g., Grossheim 1914, Tuatay & Remaudière 1964, Fernández et al. 1992). However, climates, aphid biotypes, alternative host plants, wheat varieties, cultural practices, and natural enemies all differ to greater or lesser degree between Eurasia and North America and may all contribute to the difference in pest status of *D. noxia*. Nonetheless, during 1989-1994, staff at the European Biological Control Laboratory collected at least 29 species of *D. noxia* predators and parasitoids in 7 families, as well as several species of fungal pathogens, from throughout the endemic range of *D. noxia*, and shipped over 85,000 natural enemies to the U.S. for rearing and release (Hopper et al. in press). These collections, together with those by other groups, provided the basis for a substantial program of natural enemy introductions to control *D. noxia* in the U.S.

Although the politics of funding and the economics of pest outbreaks may constrain choice of targets for biological control, it would seem that one should at least determine whether natural enemies suppress the target in its area of origin, given that a fundamental assumption of biological control by introductions is that exotic pests are problems because they have been introduced without their natural enemies (Debach & Rosen 1992). However, some have argued against such studies because of their complexity and thus cost and because natural enemies and prey/hosts may behave differently in the complex
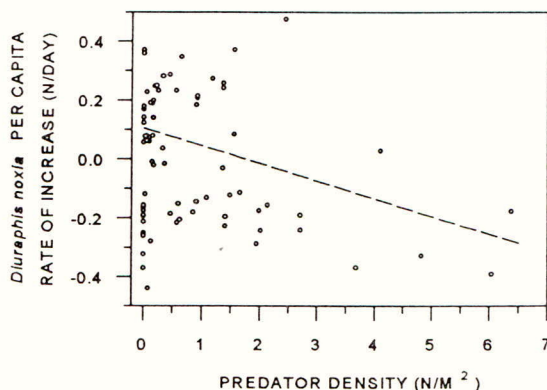

Fig. 1. Decline in *Diuraphis noxia* per capita rate of increase with increase in predator density. Each point represents on field and sample period.

trophic web of their native environment than they will in the new, and perhaps simplified, environment of introduction (Zwolfer et al. 1976). Nonetheless, we tested whether natural enemies were important in regulation of *D. noxia* in southern France and other areas of Eurasia. In a two year field survey near Montpellier, France, we found that *D. noxia* per-capita population growth rate declined with increasing predator density but did not vary significantly with plant maturity, rainfall, temperature, and the densities of *D. noxia* and parasitoids (Fig. 1; Chen & Hopper 1997). Sampling with potted plants, artificially infested with *D. noxia*, over a 2-year period revealed peak parasitism of 24-72% by six parasitoid species in two families (Aphelinidae and Aphidiidae) (Farias 1995). During 1990-2 in field exclosure experiments in wheat near Montpellier, aphid densities reached peaks 10- to 18-fold higher on plants where natural enemies were excluded than on plants where natural enemies had access (Fig. 2; Hopper et al. 1995). Differences in microclimate and emigration were eliminated as explanations for these increases. On the other hand, natural enemies could easily account for the observed differences in aphid densities. Furthermore, in field enclosure experiments, larvae of *Leucopis ninae* (Diptera: Chamaemyiidae) and *Sphaerophoria scripta* (Diptera: Syrphidae) significantly reduced the density of *D. noxia* compared to cages without fly larvae (Dabire 1995, unpublished data). All of this evidence suggests that natural enemies do limit *D. noxia* population growth in the area of origin. On the other hand, although field exclosure experiments have also been done in Hungary (Basky and Hopper, in review), we do not have comparable experiments for a variety of other areas in Eurasia. It it noteworthy that this work was done in parallel with, rather than prior to, the extensive effort to collect and introduce *D. noxia* natural enemies into the U.S. Many biological control projects dispense entirely with such studies in the area of origin, and it is not certain that negative evidence from our research would have stopped the effort to introduce natural enemies of *D. noxia* or that our evidence concerning the importance of predators would have shifted the collection effort more towards them. The problem is that we cannot predict impact of natural enemies in one setting from their impact in another. If more research on the impact in the area of origin were available to compare to impact in the area of introduction, we would at least have material for generating hypotheses to test.
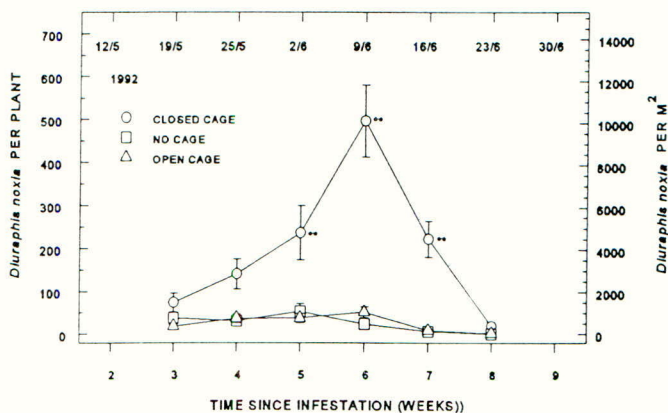

Fig. 2. Temporal dynamics of *Diuraphis noxia* in closed-caged, open-caged, and uncaged populations, 1992. Vertical bars are standard errors of the mean; two asterisks indicate closed-caged mean differed from open-caged and uncaged means. (From Hopper et al. 1995).

COLLECTION TACTICS

Genetic diversity

Many researchers have argued that maximizing genetic diversity in the natural enemies collected and released is important for the establishment and efficacy of natural enemies introduced for biological control (for review, see Roush 1990). High genetic diversity is supposed to increase the likelihood of adaptation to the new environment. However, few data are available concerning the magnitude and spatial distribution of genetic variation in fitness components in natural enemies (Hopper et al. 1993). This makes design of collection programs difficult: should one collect a few insects from many countries and environments or collect more insects from fewer locations? Although sampling over a wide geographic range has frequently been suggested (Bartlett and van den Bosch 1964; Doutt and DeBach 1964; Messenger, Wilson and Whitten 1976; Coppel and Mertins 1977; Roush 1990), this suggestion has been based on deductions from theory, not from field evidence, and recommendations have often been qualitative rather than quantitative.

To address these issues, we measured variation in fitness components within and among cultures from collections of the aphid parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae) throughout the endemic range of *D. noxia* (Hopper et al., in preparation). We measured egg load, adult longevity, and walking speed. These are traits
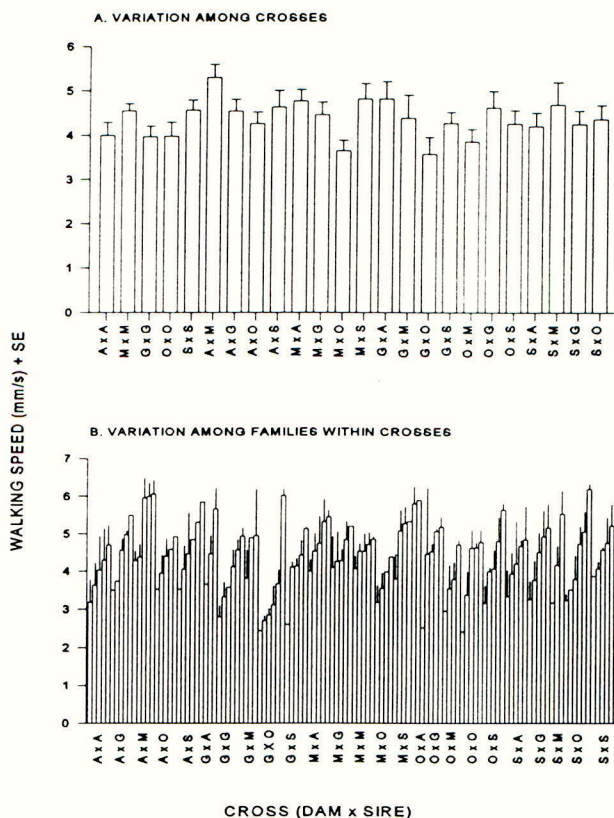

Fig. 3. Variation in mean walking speed among cultures and crosses (A) and families within cultures and crosses (B) from throughout Eurasia. A = Antibes, France; G = Xino Nero, Greece; M = Montpellier, France; 0 = Annoceur & Settat, Morocco; S = Lleida, Spain.

likely to affect the success of this parasitoid at finding and parasitizing large numbers of its host and thus are traits likely to be important for biological control. Egg load (i.e., the number of eggs carried by adult females) and adult longevity are particularly important for aphid parasitoids whose hosts are extremely patchily distributed. To be effective in biological control, aphid parasitoids must carry enough eggs to parasitize large numbers of hosts when they encounter an aphid colony, but they must also be able to survive long periods without encountering hosts. *A. asychis* searches for hosts and mates primarily on foot, thus high walking speed is also likely to be important for finding hosts and thus for its impact as a biological control agent. None of the fitness components varied significantly among cultures of *A. asychis* from collections in China, Kazhakstan, France, Greece, Morocco, and Spain (e.g., Fig. 3a). This was true both for pure cultures and for crosses among cultures. However, all of these components varied significantly among families within cultures (e.g., Fig. 3b). Thus, for egg load, longevity, and walking speed, collection at one geographical location would have provided as much genetic variation as collections from throughout Eurasia. This was so despite our discovery that cultures from China and Kazhakstan were reproductively isolated from one another and from those from the Mediterranean basin (Kazmer et al., 1996).

Climate matching

A widely accepted principle in biological control is that natural enemies should be collected from climates that match as closely as possible the environment into which they will be introduced (Messenger & van den Bosch 1971, Stiling 1993). In a review of the factors correlated with establishment of introduced parasitoids, Stiling (1990) found that tropical parasitoids introduced into temperate areas established with lower frequency (24%) than when introduced into tropical areas (35%). However, he found that temperate parasitoids established with about equal frequency in tropical and temperate areas (35% versus 38%, respectively). Furthermore, although these are interesting results, tropic versus temperate zones is more crude a match than would be useful during
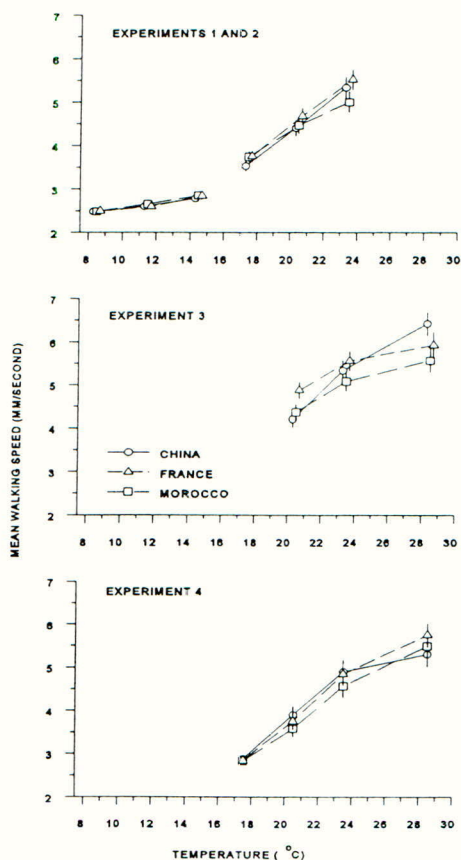

Fig. 4. Mean walking speed (mm/second) of *Aphelinus asychis* females from China, France and Morocco at various temperatures. Points are means of 12 (Experiment 4) or 20 individuals (Experiments 1-3); vertical lines are standard errors of the means.

most biological control programs. For many case histories, failure of a natural enemy to establish or control the target pest has been attributed to lack of climatic match (Clausen 1978, Stiling 1993). However, for some frequently cited cases, factors other than climate of origin differed among releases. For example, although *Trioxys pallidus* collected in France for release against *Chromaphis juglandicola* (walnut aphid) failed to establish in California's central valley, *T. pallidus* collected in Iran established and provided control (van den Bosch et al. 1962, 1979). However, parasitoids from the two areas where released in different numbers at different sites in different years and thus it is unclear how much these other factors affected establishment. Furthermore, apart from such case histories, few data are available concerning genetic variation in responses to temperature or other climatic variables among populations from different geographical areas. Thus, it is hard to determine how often climate matching has been warranted.

For this reason, we did experiments on variation in the relationship between locomotion (mean and maximum walking speed and proportion of time walking) and temperature among *A. asychis* cultures collected from various geographical areas with differing climates. The relationship between locomotion and temperature will affect the daily and seasonal rhythms in parasitoid foraging and thus the likelihood that a parasitoid will be pre-adapted to a particular climate. Although mean and maximum walking speed increased with temperature from 8.5 to 28.5°C and maximum walking speed varied among geographical sources, the relationship between locomotion and temperature did not differ among *A. asychis* cultures from China, France, and Morocco (Fig. 4; Mason & Hopper, in review). These results suggest that although locomotion depended on temperature, this dependence has not led to adaptation to different temperature regimes and thus climate matching would not be important for this trait in this species.
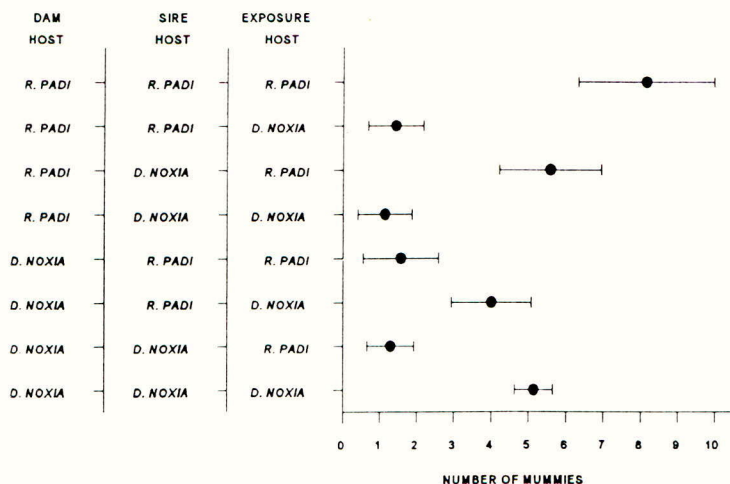

Fig. 5. Number of mummified aphids produced versus host from which dam and sire emerged and host to which adult females were exposed. Points are means and horizontal bars are standard errors.

Host specificity

Because exotic pests are often rare in their area of origin, collecting sufficient numbers of natural enemies is often difficult. When natural enemies attack other hosts or prey, one solution to this problem is to collect from more abundant hosts or prey. However, this may be a mistake if the parasitoids or predators collected from other hosts or prey are substantially less able to attack the target pest than those collected directly from the target.

D. noxia is very rare in Eurasia, but its parasitoids attack other, more abundant hosts. To test the effects of the host species from which a parasitoid was collected, we exposed *Aphelinus varipes* collected from *Rhopalosiphum padi* (Homoptera: Aphididae) and from *D. noxia* to *R. padi* and *D. noxia*. *A. varipes* from each host species oviposited equally in the other host species, but survival was reduced in the novel host. Reciprocal crosses of *A. varipes* from the two host species showed that this effect depended only on the original host of the female parent (Fig. 5; Chen & Hopper, in preparation). Such a maternal effect could arise transient conditioning to the host, but it could also arise from cytoplasmically inherited factors (e.g., mitochondria or *Wollbachia*-like parasites). Selection experiments would reveal whether adaptation of *A. varipes* from *R. padi* to *D. noxia* could be improved.

Traits important for natural enemy efficacy

Despite 100 years of biological control introductions, the traits of effective natural enemies have not been agreed upon (van Lenteren 1980, Waage 1990). Some biological control practitioners argue that it is very difficult, and perhaps inherently impossible, to predict which natural enemies will establish and regulate a pest. The problem is that few experiments have actually been done to address this issue, so that it is not surprising that we cannot make such predictions. Although a few excellent studies have been done (e.g., Bigler et al. 1988, Kazmer & Luck 1995), the relationship between laboratory or field-cage measures on one hand and field performance on the other is unknown for most traits and species. Studies done after introduction when the outcome is known, like those with *Apoanagyrus (Epidinocarsis) lopezi* and other natural enemies introduced to control *Phenacoccus manihoti* (cassava mealybug) in Africa (Gutierrez et al. 1993, Neuenschwander & Ajuonu 1995, Pijls et al. 1995), help in developing a list of traits associated with successful biological control. However, we need measurements and predictions before introduction and experimental introductions to test whether the predictions are valid.

We have begun research on this question with parasitoids of *D. noxia*. Field surveys near Montpellier, France, showed that mummies of *D. noxia* parasitized by *Aphelinus asychis* were 3-fold more abundant than those parasitized by *Aphidius matricariae* (Hymenoptera: Aphidiidae) (Farias 1995). Furthermore, during collections throughout Eurasia, aphelinids were in general more abundant on *D. noxia* than aphidiids (Hopper et al., in press). To test whether this difference might arise from differences in responses of the parasitoids to host odors or from differences in the ability of *D. noxia* to defend itself from attack by the two parasitoids, we did olfactometer and defense-behavior experiments (Farias & Hopper, in review). In a four-way olfactometer, field-collected and laboratory reared *Aphelinus asychis* females did not move preferentially towards odors from *D. noxia* or barley plus *D. noxia* compared to filtered air. On the other hand, *Aphelinus asychis* females spent more time in the plant-host odor field after they were allowed to oviposit in hosts on plants. *Aphidius matricariae* females moved preferentially towards odors from barley plus *D. noxia*, and females allowed to oviposit in hosts also moved towards odors from *D. noxia* alone. Furthermore, *Aphidius matricariae* females spent more time

in the plant-host odor field than in the other odor fields, whether or not they were allowed to oviposit in hosts prior to being tested (Fig. 6). Although *D. noxia* nymphs and adults exposed to *Aphelinus asychis* and *Aphidius matricariae* defended themselves, this defense did not reduce parasitism by either wasp species. Thus greater attraction to the host or ability to overcome host defenses cannot explain higher parasitism by *Aphelinus asychis*, and more experiments are needed to test other hypotheses.

One of the problems with comparing species of natural enemies is that they differ in many traits. As part of our research on genetic variation in fitness components within and among natural enemy populations, we have searched for and found molecular markers to distinguish isofemale
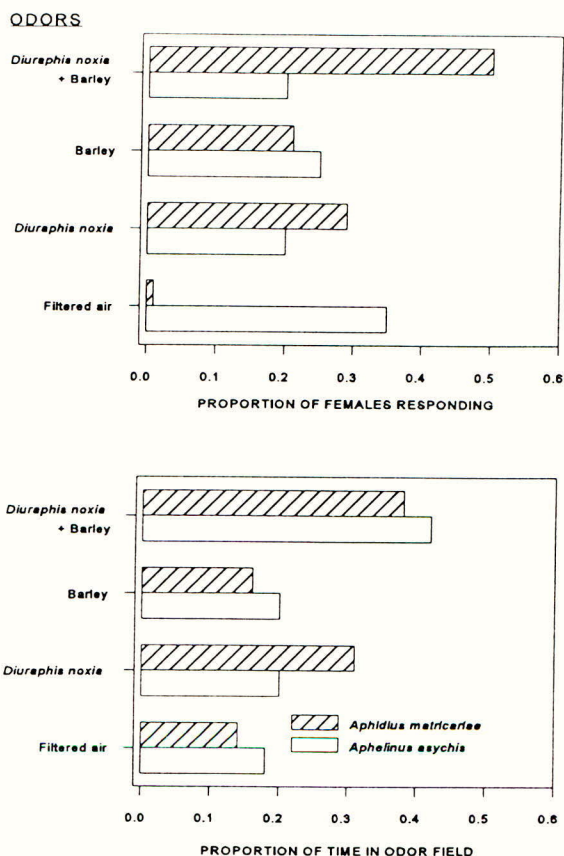
ODORS

Fig. 6. Responses of *Aphelinus asychis* and *Aphidius matricariae* females in four-way olfactometer: proportion of females choosing various odor sources and proportion of time spent in each odor field.

lines of *A. asychis* within populations and to distinguish *A. asychis* from different geographical areas. We plan to use these markers to test whether lines or populations differ in their field performance (e.g., parasitism, overwintering mortality). We will then compare various laboratory-measurable traits among these strains to find correlates that could explain differences in field performance.

REARING AND RELEASE

Laboratory rearing

During biological control introductions, one is frequently forced to rear natural enemies in the laboratory to meet quarantine requirements, to produce sufficient numbers for release, or to

synchronize them with target pest populations. Such rearing may select for laboratory-adapted genotypes that are maladapted to the field (Mackauer 1976, Roush 1990). However, evidence for or against such maladaptation is extremely rare (Hopper et al. 1993).

We did experiments to measure changes in fitness components during long-term laboratory rearing of *A. asychis* (Kazmer et al., in preparation). A key feature of our experimental design was the use of replicate cultures founded from a common gene pool, which allowed us to distinguish between random changes in fitness components, which may arise from drift, and unidirectional changes, expected under selection. In one experiment, we compared replicate cultures started in 1992 (F_{47}) and 1993 (F_{29}) to insects collected in 1995. We found no unidirectional changes in fitness components after 29 and 47 generations of laboratory rearing. However, we did find significant variation among replicate cages of the same age in 5 of 7 cases. We also examined random amplified polymorphic DNA (RAPD) variation within and among the replicate cultures. Analysis of 64 polymorphic RAPD loci showed significant differences among cultures of the same age and the amount of difference was greater after 47 generations than after 29 generations, suggesting that drift had occurred. In another experiment, heritabilities of the four fitness components estimated using a half-sib analysis were low (6.3-11.1%). With such low heritabilities, high selection intensities would be necessary to overcome drift. Thus, drift may sometimes be more important than selection in genetic changes under laboratory rearing. This is encouraging because drift can be avoided with large populations and in any case will not necessarily lead to maladaptation to the field. One way to preserve genetic variation under laboratory rearing is to take advantage of drift by maintaining isofemale lines (Roush & Hopper 1995). The idea is that drift within lines started from single females will fix different alleles in different lines; thus as long as all the lines are maintained no losses should occur from inadvertent selection. For arrhenotokous species only 25 such lines would be needed to preserve common alleles, which are those that seem most likely to be important in field performance.

Demographic barriers

Several researchers have suggested that insufficient numbers released may cause introductions to fail (Beirne 1975, 1985; Hopper & Roush 1993). This may arise from an Allee effect: Allee (1931) proposed that per-capita population growth rate may decrease as density decreases when organisms are rare. Introduced insects dispersing into a new environment may become so rare that males and females often fail to encounter one another. In this case, a high proportion of females remain virgin and so either do not reproduce at all, or in arrhenotokous species, produce all male progeny. If the proportion of virgin females is high enough, natality will fall below mortality and the introduced population will decline to extinction. Analysis of past introductions shows that establishment probability decreases as numbers released decreases (Fig. 7; Hopper & Roush 1993). Sensitivity analysis of a reaction-diffusion model shows that, with reasonable values for net reproductive rate, mate detection distance, and dispersal, an Allee effect may prevent populations from establishing (Fig. 8; Hopper & Roush 1993). The problem is that little is known about net reproductive rates or dispersal rates of parasitoids in the field, and almost nothing is known about how males search for females or about the relationship between male density and probability that females mate.

We are studying the potential for an Allee effect during introductions of *A. asychis* with experiments designed to address the following questions: (1) To what degree does mating occur locally within populations? Is this on a sufficiently large spatial scale that mate finding is a

problem? (2) Do females manipulate progeny sex ratio to compensate for low probability of mating? (3) What are the relationships between the proportion of mated females on one hand and the number of insects released and male density in subsequent generations on the other hand, given the net reproductive rates and dispersal rates of introduced parasitoids? Using allozymes as genetic markers, we examined the genetic structure of
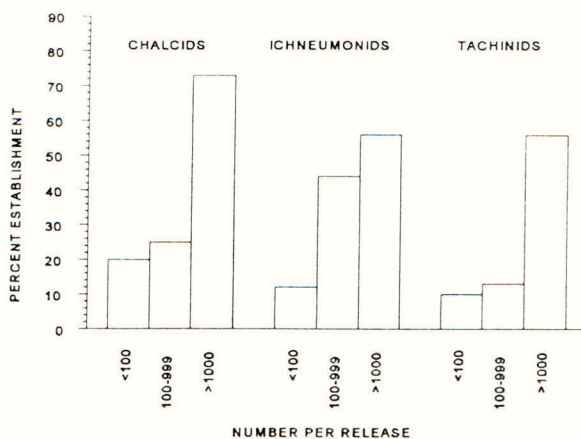

Fig. 7. Percent of attempted introductions that led to establishment versus number per release of chalcids, ichneumonids, and tachinids introduced to control Lepidoptera. (From Hopper & Roush 1993.)

A. asychis populations near Montpellier, France. These data, used in models of Kazmer (1992), indicated that three female *A. asychis* visited each patch on average. Because each patch contained only about 8 parasitoid progeny, each foundress may contribute only 2-3 progeny to a patch. Field surveys also show low frequencies of parasitized aphids per colony (Chen & Hopper 1997). These results suggest female *A. asychis* do not mate in the aphid colony in which they emerge, and thus must disperse to mate. On the other hand, in laboratory experiments, the frequency of males among progeny of mated females decreased with increased time to mating, which could compensate for low probability of mating (Fauvergue et al., in preparation). We are now testing the effect of numbers released and subsequent population density on parasitoid sex ratio by replicated introductions of small (500/replicate) and large (5000/replicate) numbers of parasitoids in southwestern Colorado. We sampled parasitoid density and sex ratio for three parasitoid generations by collecting plants infested with *D. noxia* at various distances from the release points. We also collected and dissected adult females to determine whether they were mated. These data will allow us to estimate dispersal and net reproductive rate and test the effect of these and numbers released on mating success.

GENETIC ADAPTATION AFTER INTRODUCTION

Arguably the most important research concerning the genetics of biological control introductions is to determine whether introduced populations adapt genetically so that establishment or control of target pests improves (DeBach 1965, Wilson 1960, Roush 1990, Hopper et al. 1993). If genetic adaptation were not important for introductions, one need not worry about genetic variation in collections or releases. This could greatly reduce costs for biological control exploration and introduction. On the other hand, if genetic adaptation turns out to be important, the nature of that adaptation will influence how one should collect and release natural enemies.

However, there are few experiments on whether genetic adaptation has been important for establishment of natural enemies or for control of target pests.

Inferences based on observations can be misleading. For example, introduced species sometimes remain at essentially undetectable densities for long periods before suddenly increasing rapidly in density over large areas. Some researchers have argued that, during such eclipse periods, introduced species are adapting to the local environment. However, dispersal and exponential population growth can produce just such an eclipse period without local adaptation (Hopper & Roush 1993).

We are now doing a series of laboratory and field cage experiments to test whether genetic adaptation has occurred since *Macrocentrus grandii* was introduced into North America from

Fig. 8. Predictions of model indicating critical number of females for establishment versus net reproductive rate km^2/generation), mean-square displacement, and mate detection distance. Below the curves, populations go extinct, above the curve, they establish. (From Hopper & Roush 1993.)

Korea and northern France against *Ostrinia nubilalis*. Baker et al. (1949) concluded that the Korean introductions are the ones likely to have established because (1) post-release collections retrieved *M. grandii* only in areas where Korean material was released, and (2) the established populations, like those in Korea, attack bivoltine hosts, whereas the French populations attack univoltine hosts. If this conclusion is true, introduced *M. grandii* would have had to switch from
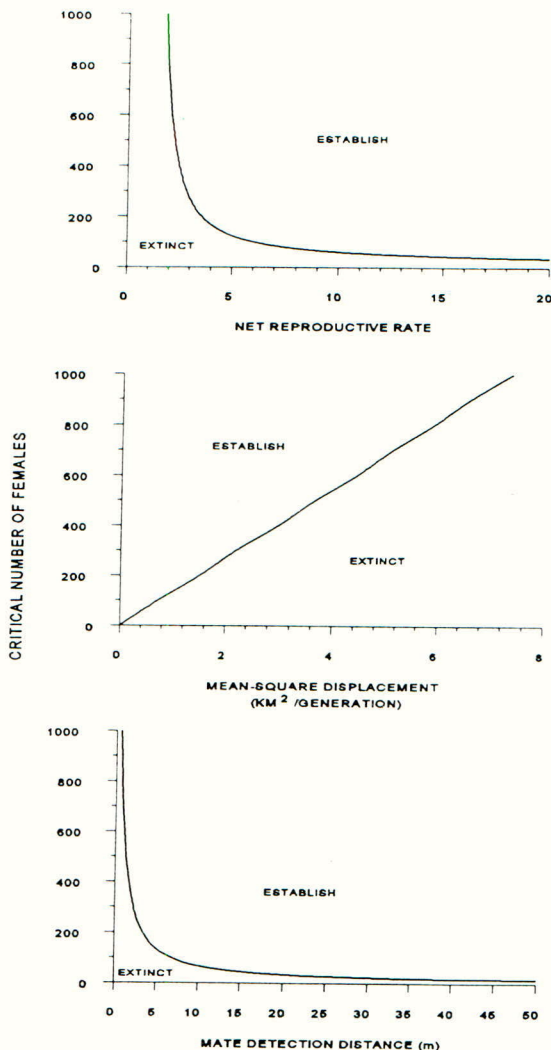
O. furnacalis to *O. nubilalis.* Alternatively, if the French introductions established in North America, introduced *M. grandii* would have had to switch from univoltine to bivoltine hosts. These shifts in host species or phenology may have required genetic adaptation. Climates also differ between the areas of collection and introduction. The Korean source area has greater temperature extremes and higher mean minimum and maximum temperatures during the growing season than most of the area in North America where *M. grandii* has established. The source area in northern France is the opposite, with lower temperature extremes and lower mean minimum and maximum temperatures than most of the area in North America where *M. grandii* has established. With introductions from either source region, genetic adaptation may have been necessary for *M. grandii* to persist. We are addressing the following questions: (1) Do North American populations and the population(s) from which they were derived differ in traits (host suitability, diapause, temperature tolerance, temperature threshold for activity) likely to affect persistence or impact on the target pest? (2) Do any phenotypic differences have a genetic basis?

To answer these questions, we are doing experiments on mating compatibility, measurements of molecular marker differences, laboratory and field-cage experiments on differences in fitness components, and crosses to determine the genetic contribution to these differences. For these experiments we are using *M. grandii* cultures from Europe and Asia, collected from as near as possible to the original collection sites, and we are comparing these with *M. grandii* in North America.

CONCLUSION

Much of this research is very much work-in-progress so answers are not yet in. The point of presenting our research has been to illustrate that experiments on what limits establishment and efficacy of introduced natural enemies can be done while doing introductions. By doing such experiments, we can test various hypotheses about what is important for the success of introductions, hopefully rejecting some hypotheses and tentatively accepting others. To an experimentalist, this approach seems patently obvious, but it must not be, otherwise we would not have reached the centennial of the first biological control introductions with so few experiments on which to base our understanding of why some introductions succeed and others fail. If we do more experiments during introductions, perhaps we will increase our understanding, and thus our success rate, by the bicentennial.

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REFERENCES

Allee, W C (1931) *Animal Aggregations.* Chicago: University of Chicago Press.

Baker, W A; Bradley W G; Clark C A (1949) Biological control of the European corn borer in the United States. USDA Tech. Bull. No. 983, 185 pp.

Bigler, F; Bieri M; Fritschy; A.; Seidel K (1988) Variation in locomotion between laboratory strains of *Trichogramma maidis* and its impact on parasitism of eggs of *Ostrinia nubilalis* in the field. Entomol. Exp. Appl. 49, 283-290.

Brooks, L; Hein G; Johnson G; Legg D; Massey B; Morrison P; Weiss M; Peairs F (1994) Economic impact of the Russian wheat aphid in the western United States: 1991-1992.

Great Plains Agricultural Council Publication. 147, pp. 250-268.

Chen, K; Hopper K R (1997) *Diuraphis noxia* (Homoptera: Aphididae) population dynamics and impact of natural enemies in the Montpellier region of southern France. Environ. Entomol. In press.

Clausen, C P (ed) (1978) *Introduced parasites and predators of arthropod pests and weeds: a world review.* Washington, DC: Agricultural Research Service, United States Department of Agriculture, Agricultural Handbook No. 480.

Crawley, M J (1986) The population biology of invaders. Philosophical Transactions Royal Society of London, B. 314, 711-731.

Cullen, J M; Whitten M J (1995) Economics of classical biological control. In: *Biological Control: Benefits and Risks*, H M T Hokkanen; & J M Lynch, (eds), Cambridge, Great Britain: University Press, pp. 270-276.

Dabire, R (1995) Impact of *Leucopis ninae* Tanasijtschuk (Diptera: Chamaemyiidae) on *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae). Ph.D. diss., Ecole Nationale Superieure Agronomique de Montpellier, Montpellier, France.

DeBach, P (1965) Some biological and ecological phenomena associate with colonizing entomophagous insects. In: *Genetics of Colonizing Species*, H G Baker; & G L Stebbins, (Eds.), New York: Academic, pp. 287-306.

DeBach, P; Rosen D (1991) *Biological control by natural enemies.* Cambridge University Press.

Farias, A (1995) Parasitoids and hyperparasitoids associated with *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) in the Monpellier region. Ph.D. diss., Ecole Nationale Superieure Agronomique de Montpellier.

Fernández, M V S; Pérez E N; Santero E D; Nafria J M N (1992) Situation of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hom. Aphididae), in the north of Spain. Geórgica. 1, 9-24.

Greathead, D J (1995) Benefits and risks of classical biological control. In: *Biological Control: Benefits and Risks*, H M T Hokkanen; & J M Lynch, (eds.), Cambridge, Great Britain: University Press, pp. 53-63.

Grossheim, N A (1914) The barley aphid, *Brachycolus noxius* Mordwilko. Mem. Nat. Hist. Mus. Zemstwo Province Tavria. 3, 35- 78.

Gutierrez, AP; Neuenschwander P; Van Alphen J J M (1993) Factors affecting biological control of cassava mealybug by exotic parasitoids: A ratio-dependent supply-demand driven model. J. Appl. Ecol. 30, 706-721.

Haile, A; Megenasa T (1987) Survey of aphids on barley in parts of Shewa, Welo and Tigrai, Ethiopia. Ethiop. J. Agric. Sci. 9, 39-53.

Hall, R W; Ehler L E (1979) Rate of establishment of natural enemies in classical biological control. Bull. Entomol. Soc. Amer. 25, 280-282.

Hall, R W; Ehler L E; Bisabri-Ershadi B (1980) Rate of success in classical biological control of arhropods. Bull. Entomol. Soc. Amer. 26, 111-114.

Haynes, D L; Gage S H; Fulton W (1974) Management of the cereal leaf beetle pest ecosystem. Quaest. Entomol. 10, 165-176.

Hopper, K R; Coutinot D; Chen K; Halbert S E; Kazmer D J; Mercadier G; Miller R H; Pike K S; Tanigoshi L K (In press) Exploration for natural enemies to control *Diuraphis noxia* in the United States. Thomas Say Publications, Entomol. Soc. Amer.

Hopper, K R; Aidara S; Agret S; Cabal J; Coutinot D; Dabire R; Lesieux C; Kirk G; Reichert S; Tronchetti F (1995) Natural enemy impact on the abundance of *Diuraphis noxia* (Homoptera: Aphididae) in wheat in southern France. Environ. Entomol. 24, 402-408.

Hopper, K R; Roush R T; Powell W (1993) Management of genetics of biological control introductions. Annu. Rev. Entomol. 38, 27-51.

Hopper, K R; Roush R T (1993) Mate finding, dispersal, number released, and the success of biological control introductions. Ecological Entomology. 18, 321-331.

Huffaker, C B; Simmonds F J; Laing J E (1976) The theoretical and empirical basis of biological control. In: *Theory and Practice of Biological Control*, C B Huffaker; & P S Messenger, (eds), New York: Academic, pp. 41-78.

Julien, M H; Kerr J D; Chan R R (1984) Biological control of weeds: an evaluation. Protection Ecology. 7, 3-25.

Kazmer, D J (1992) The evolution of clutch size and sex ratio in *Trichogramma* and its relationship to biological control. Ph.D. diss., University of California, Riverside.

Kazmer, D J; Luck R F (1993) Field tests of the size-fitness hypothesis in the egg parasitoid Trichogramma pretiosum. Ecology. 76, 412-425.

Kazmer, D J; Maiden K; Ramualde N; Coutinot D; Hopper K R (1996) Reproductive compatibility, mating behavior, and random amplified polymorphic DNA variability in some *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) derived from the old world. Ann. Entomol. Soc. Am. 89, 212-220.

Laing, J E; Hamai J (1976) Biological control of insect pests and weeds by imported parasites, predators, and pathogens. In: *Theory and Practice of Biological Control*, C B Huffaker; & P S Messenger, (eds.), New York: Academic, pp. 685-743.

Mackauer, M (1976) Genetic problems in the production of biological control agents. Ann. Rev. Entomol. 369-85.

Messenger, P S; van den Bosch R (1971) The adaptability of introduced biological control agents. In: *Biological Control*, C. B. Huffaker, (ed.), New York/London: Plenum, pp. 68-92.

Murdoch W W; Reeve J D, Huffaker C B; Kennet C E (1984) Biological control of olive scale and its relevance to ecological theory. Am. Nat. 123, 371-392.

Neuenschwander, P; Ajuonu O (1995) Measuring host finding capacity and arrestment of natural enemies of the cassava mealybug, *Phenacoccus manihoti*, in the field. Entomol. Exp. Appl. 77, 47-55

Peschken D P; McClay A S (1995) Picking the target: a revision of McClay's scoring system to determine the suitability of a weed for classical biological control. In: Proc. Eighth Internat. Symp. Biol. Control Weeds, E S Delfosse & R R Scott (eds), Melbourne, Australia: DSIR/CSIRO, pp. 137-43.

Pijls, J W A M; Hofker K D; Van Staalduinen M J; Van Alphen J J M (1995) Interspecific host discrimination and competition in *Apoanagyrus (Epidinocarsis) lopezi* and A. (E.) diversicornis, parasitoids of the cassava mealybug *Phenacoccus manihoti*. Ecol. Entomol. 20, 326-332

Pike, K S; Allison D; Tanigoshi L K; Harwood R F; Clement S L; Halbert S E; Smith C M; Johnson J B; Reed G L; Zwer P K (1991) Russian wheat aphid - biology, damage, and management. Pacific Northwest Ext. Publ. PNW371, 24 pp.

Roush, R T (1990) Genetic variation in natural enemies: critical issues for colonization in biological control. In: *Critical Issues in Biological Control*, L Ehler; & M Mackauer, (eds.), Andover, England: Intercept.

Roush, R T; Hopper K R (1995) Use of single family lines to preserve genetic variation in laboratory colonies. Ann. Ent. Soc. Amer. 88, 713-717.

Stiling, P (1987) The frequency of density dependence in insect host-parasitoid systems. Ecology 68, 844-856.

Stiling, P (1990) Calculating the establishment rates of parasitoids in classical biological control. American Entomologist. 36, 225-230.

Stiling, P (1993) Why do natural enemies fail in classical biological control programs? American

Entomolologist. 39, 31-37.

Stoetzel, B M (1987) Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. J. Econ. Entomol. 80, 696-704.

Tisdell, C A (1990) Economic impact of biological control of weeds and insects. In: *Critical Issues in Biological Control*, M Mackauer; L E Ehler; & J Roland, (eds), Andover, Hants, UK: Intercept, pp. 301-316.

Tuatay, N; Remaudiere G (1964) Premiere contribution au catalogue des Aphididae (Hom.) de la Turquie. Rev. Path. Veg. Ent. Agr. Fr. 43, 243-78.

van den Bosch R; Schlinger E I; Hagen K S (1962) Initial field observations in California on *Trioxys pallidus* (Haliday) a recently introduced parasite of the walnut aphid. J. Econ. Entomol. 55, 857-862.

van den Bosch R; Hom R; Matteson P; Frazer B D; Messenger P S; Davis C S (1979) Biological control of the walnut aphid in California: impact of the parasite, *Trioxys pallidus*. Hilgardia 47, 1-13.

van Lenteren, J C (1980) Evaluation of control capabilities of natural enemies: does art have to become science? Netherlands J. Zool. 30, 369-381.

Waage, J (1990) Ecological theory and the selection of biological control agents. In: *Critical Issues in Biological Control*, M Mackauer; L E Ehler; & J Roland, (eds), Andover, Hants, UK: Intercept, pp. 135-158.

Walters, M C; Penn F; Du Toit F; Botha T C; Aalbersberg K; Hewitt P H; Broodryk S W (1980) The Russian wheat aphid. Farming in South Africa, Leafl. Ser. Wheat G.3, 1-6.

Wilson, F (1965) Biological control and the genetics of colonizing species. In: *Genetics of Colonizing Species*, H G Baker; & G L Stebbins, (eds), New York: Academic, pp. 307-329.

Zuniga, S E (1990) Biological control of cereal aphids in the southern zone of South America. In: *World Perspectives on Barley Yellow Dwarf*, Mexico, DF: CIMMYT, pp. 362-367.