

## Review

# Perspectives on the development of genetically modified arthropod natural enemies for agricultural pest management programmes

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**Received:** 31 October 2006

**Accepted:** 13 November 2006

doi: 10.1079/PAVSNNR20061058

The electronic version of this article is the definitive one. It is located here: <http://www.cababstractsplus.org/cabreviews>

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## Abstract

The history of genetic improvement of arthropod natural enemies is reviewed briefly and I describe the successful genetic improvement and deployment in California almond orchards of a pesticide-resistant predatory mite. Lessons learned from our efforts to deploy this and other natural enemies in alfalfa, citrus and walnuts from several genetic improvement projects conducted over the past 30 years are described, because they may provide useful lessons for future projects. Gaps in our knowledge are identified, especially in deploying such genetically modified natural enemies, and recommendations are made regarding how to plan such projects. Finally, issues of risk and regulation are discussed based on our experiences with a short-term field trial of a transgenic predatory mite in Florida.

**Keywords:** Genetic improvement, Artificial selection, Heterosis, Transgenesis, Augmentative Biological Control, Risk assessments, Integrated pest management, Regulatory issues

## History of Genetic Improvement Projects

The potential benefits to be achieved in improved biological control through the use of genetic improvement of arthropod natural enemies were recognized by Mally [1] in 1916. The concept languished until Paul DeBach [2] and Reece Sailer [3] once again discussed the potential for genetic improvement of arthropod natural enemies. Like Mally [1], they argued that agricultural ecosystems are artificial and selection or hybridization might be used to improve the effectiveness of arthropod natural enemies. By the 1970s, several laboratory selection projects had been conducted with several natural enemy species to improve climatic tolerance, sex ratio, host-finding ability, host preference and insecticide resistance, but there was widespread scepticism among many entomologists as to whether laboratory selection could produce sufficiently 'fit' insects that they would perform well in the field because none of the modified natural enemies had been deployed in a pest management programme [4].

The diversity of new mutations and the abundance of genetic variability in insects were impressed on me when I worked in Alex Sokoloff's *Tribolium* genetics

laboratory at the University of California at Berkeley. This convinced me that genetic variability in beneficial insects was not being exploited sufficiently. As a result, I decided to combine my interests in genetics and biological control during my Ph.D. programme at Berkeley and was thrilled subsequently to obtain a position advertised by the Connecticut Agricultural Experiment Station that involved genetic improvement of parasitoids of the gypsy moth, *Lymantria dispar* (L.) (Lep.: Lymantriidae). This position possibly represented the first full-time position devoted towards the genetic improvement of a natural enemy.

## Genetic Improvement of a Gypsy Moth Parasitoid

After arriving in Connecticut in 1973, a review of the gypsy moth literature led me to acknowledge that we rarely know why a natural enemy is less than fully effective and that without this knowledge it is difficult to select 'for' anything. The inability to define a selection goal remains a significant deterrent to the genetic improvement of natural enemies, whether by artificial selection or by

recombinant DNA methods. However, our ability to rear a strain of the gypsy moth throughout the year in the laboratory was improved by selecting a 'non-diapausing' strain from a very few field-collected eggs that did not require a chill to terminate their diapause [5]. The selected line could be reared continuously or its eggs could be held in a cold room for several months because, surprisingly, this new gypsy moth strain retained cold tolerance and could overwinter in field cages or be held in cold storage [6–8]. Because the gypsy moth was reputed to have an 'obligatory diapause', the ability to select a non-diapausing strain within about eight generations was surprising to some, but reinforced my previous experience with the enormous amount of genetic variability in *Tribolium* that led me to conclude that selection on rare variants in insect populations could produce results that might be useful to pest managers.

Because I lacked a specific selection goal, I decided to investigate whether hybrid vigour could improve a natural enemy of the gypsy moth and discovered that genetic improvement of a parasitoid of the gypsy moth could be achieved through the use of hybridization. *Apanteles* (= *Cotesia*) *melanoscelus* (Hym.: Braconidae) was being reared for inoculative releases at the leading edge of the gypsy moth's range extension in the north-eastern USA and also was being evaluated by colleagues at the Connecticut Agricultural Experiment Station to resolve whether augmentative releases could prevent incipient outbreaks of the gypsy moth. To investigate the potential value of hybrid vigour as a mechanism to improve mass rearing, I crossed a colony collected from the forest in Connecticut with two populations initially collected from France and Yugoslavia and reared under uncontrolled daylengths in a rearing facility. The hybridized colony was improved in several attributes [9, 10]. Hybridization partially repaired the damage done by inadvertent selection for non-diapause in the French and Yugoslavian populations by producing a higher incidence of diapause in the hybrid colony. Additionally, mass rearing for the augmentation field trial was improved because the hybrid females produced more progeny and a higher proportion of female progeny [9].

Whether hybrid vigour will improve other natural enemies has been little studied. In principle, hybridization of colonies could restore lost variability due to founder's effects, inbreeding, and inadvertent laboratory selection [4]. However, few experiments have been conducted to evaluate this and it is also possible that hybridization could disrupt coadapted gene complexes or cause other damage. For example, reproductive incompatibility could occur if one colony contained *Wolbachia* and others did not. Additional research on the value of hybridization, and the optimal methods by which to achieve it, could resolve whether effectiveness of natural enemies released in both augmentative and inoculative (classical) biological control programmes can be improved.

## Genetic Improvement of a Spider Mite Predator

In 1976 I returned to the University of California at Berkeley to teach acarology and conduct research to improve mite management programmes in agriculture. I maintained my interest in genetic improvement of natural enemies and my goal was to determine whether it was possible to select a strain of the western predatory mite, *Metaseiulus* (= *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae), for resistance to pesticides. Prior work had set the stage to evaluate whether selection could produce a useful change in a natural enemy; this predator had been shown to be an effective natural enemy of spider mites in Washington apple orchards because it had developed resistance to organophosphorus (OP) pesticides used to suppress the codling moth [11]. As a result, OP-resistant *M. occidentalis* populations could persist after sprays were applied to control codling moth, reducing the need for acaricides to suppress spider mites, which thereby reduced production costs. In addition, Croft and Barnes [12] demonstrated it was feasible to establish that the OP-resistant population from Washington in southern California orchards. So, we knew that resistance in this predator could improve pest management programmes and that we could establish a resistant strain in new geographic locations (Figure 1).

Our challenge was to conduct laboratory selection for novel resistance(s) and to demonstrate that the resultant predator colony was sufficiently fit that it could suppress spider mite populations in pesticide-treated crops [4, 13]. Questions to be answered included whether sufficient genetic variability was present in this predator so that resistance could be selected for and whether the level of resistance obtained would allow the predator to survive 'field rates' of pesticides. The general perception at that time was that natural enemies could not develop useful levels of resistance during laboratory selection programmes and that fitness costs were always associated with resistance alleles [13, 14].

To increase the likelihood of obtaining at least one resistant strain of *M. occidentalis*, two selection projects were initiated. To increase the genetic variability in the selected colonies, populations were collected from diverse sites in California, Washington and Oregon from apple, pear and almond orchards as well as from California vineyards. Selection for resistance to carbaryl was considered risky because it was known to be very toxic to phytoseiids [15, 16], but carbaryl was used in the almond integrated pest management (IPM) programme (along with azinphosmethyl) to control the navel orange-worm so a carbaryl-resistant strain was desirable because it would provide a new option for pest managers. In the second project, we selected *M. occidentalis* for resistance to permethrin, which was not yet registered for use [17]. Successful laboratory selection with permethrin would demonstrate that resistant natural



**Figure 1** The phytoseiid mite *M. occidentalis* (Nesbitt) is an obligatory predator that feeds on spider mites, as well as other small arthropods, and is an effective natural enemy of pest mites in deciduous orchards and vineyards in the western USA, where it is native. It has also been introduced into other countries in classical biological control programmes.

enemies could be produced prior to registration of a product, providing a model in which chemical and biological control tactics could be integrated more rapidly.

Selection with carbaryl was successful [16], but diverse colonies of *M. occidentalis* had to be screened with permethrin before a colony supplied by S.C. Hoyt from a Washington apple orchard responded to selection. Adequate sampling is thus very important if selection is to be successful. The resistant strains were evaluated in the laboratory for differences in longevity, sex ratio, developmental rate, diapause, and fecundity under sprayed and unsprayed conditions, and mode of inheritance tests were conducted [16–20]. Significant differences between the carbaryl-resistant strain and the wild populations were not found, suggesting that laboratory selection had not reduced viability or vigour [19]. The permethrin-resistant strain was less fit than the control population in unsprayed conditions, but still survived ‘field rates’ of permethrin, so was evaluated in the field.

Small-plot trials were conducted in California, Washington and Oregon for two years with both the carbaryl- and permethrin-resistant colonies [18, 21–25]. Replicated single-tree tests indicated that the resistant strains could survive field rates of carbaryl, low rates of permethrin, spread from initial release trees to adjacent trees, overwinter, and reduce spider mite populations. To broaden the potential use of the carbaryl-resistant strain, crosses were made between the carbaryl-OP-resistant strain and a colony with a high level of sulphur (S) resistance and the resultant strain was called the COS strain [25]; this strain was very vigorous and was used in the subsequent almond integrated mite management (IMM) programme.

At this point, both research projects could have been considered complete. We had demonstrated that a predator could be selected for resistance to pesticides in the laboratory and that laboratory-selected strains were effective in field trials. However, if the new strains were not actually used in pest management programmes, the concept of genetic improvement of arthropod natural enemies would continue to be viewed as just another academic exercise. The decision to attempt to deploy the COS strain ultimately was successful, but required at least as much effort as its development and field-testing.

Transferring the results of academic research into an IPM programme was a complex, challenging, time-consuming, often frustrating, and costly process. It required a team effort and very different skills. The IMM project in California almond orchards could be divided into ‘Developmental Research’ (Phase I), ‘Implementation Research’ (Phase II) and ‘Maintenance Research’ (Phase III).

During 1977 and 1978 (Phase I), almond orchards in California were surveyed to determine whether native *M. occidentalis* were present and potentially capable of suppressing spider mite populations. When the project began, it was unclear whether this obligatory predator could be sustained during the entire growing season because alternative prey species were not common in almond orchards. Another concern was that almond trees are more susceptible to spider mite feeding than are apples and the densities of spider mites that can be tolerated in almonds are therefore lower [26, 27]. During the surveys, leaf samples were collected from several almond orchards during two field seasons and individual leaves were counted to determine which spider mite species were present and when, their relative densities, as well as the species of natural enemies and their abundance on a per-leaf basis [28, 29]. The data demonstrated clearly that *M. occidentalis* could control spider mites in some commercial almond orchards. Ultimately, these data were used to develop two sampling methods, a presence/absence and a brush-and-count method, that were integral to the IMM programme [19, 30–32]. Both sampling schemes included consideration of predator (*M. occidentalis*) densities for making spray decisions.



**Figure 2** The carbaryl–OP–sulphur-resistant strain of *M. occidentalis* was introduced into the almond orchard on the left in May, while the orchard on the right received none. Despite the application of carbaryl, which typically causes outbreaks of spider mites, the orchard on the left shows little damage from spider mites in August. The orchard on the right was not sprayed with carbaryl, but spider mite damage caused defoliation. The carbaryl-resistant predatory mites were thus successful in surviving carbaryl and providing control of the spider mites.

After consulting with several key Farm Advisors working in almonds in California, we designed a project to implement the COS strain of *M. occidentalis*, found funds from several sources and hired two pest management graduates to assist with Phase II of the project. Three years were spent developing the information and methods necessary to test the COS strain in larger-scale field trials and to develop other information necessary for an IMM programme for almonds. Among other things, we did not know if the COS strain would work in full-sized commercial almond orchards; additional pest management tools were needed to actually use these predators.

One Phase II tool required was a method for producing large numbers of the genetically modified predators, so we tested two mass-rearing methods [33]. In addition, we learned how to release predators in commercial almond orchards in diverse sites in the Central Valley of California, monitored release sites on a weekly basis during the growing season to document that *M. occidentalis* could control spider mites in commercial almond blocks, developed management guidelines that included the use of lower-than-label rates of acaricides and documented the importance of aerial movements of both

spider mites and predators within and out of almond orchards [19, 25, 27, 34] (Figure 2).

The deployment project (Phase III) was a team effort and University of California Cooperative Extension personnel played key roles in the project. Extension personnel identified key growers who would provide almond orchards, communicated with the growers, coordinated management and spray decisions, provided information on almond pest management and helped monitor the release sites. They also identified rapid, inexpensive and effective sampling of spider mites and predators as a key issue and took a leadership role in developing and testing a presence/absence sampling method using data we had obtained during the first two years of the project [31, 32]. The project was made feasible, as well, by the extensive research that had been conducted by other University of California and Cooperative Extension personnel on how to manage the navel orangeworm, *Amyelois transitella* (Walker) (Lep.: Pyralidae) and the peach twig borer, *Anarsia lineatella* Zeller (Lep.: Gelechiidae), in an integrated manner [35–37].

Actually using *M. occidentalis* in an IMM programme in almonds was more complex than in apples because

almonds can tolerate only about 130 spider-mite days of feeding damage before defoliation begins. As a result, the ratio of *M. occidentalis* to spider mites is critical early in the growing season [25, 38]. A brief lag in the buildup of predator populations can result in substantial spider mite feeding damage during July and August [28]. Therefore, rapid and effective monitoring systems for *M. occidentalis* and spider mites were crucial. Another important tool was the use of low rates of selective acaricides to assist the predators should monitoring indicate that the predators were lagging behind the spider mite populations. We learned that the standard label rates of propargite, cyhexatin, or fenbutatin-oxide, acaricides in use at that time, are toxic to *M. occidentalis* and can therefore disrupt predator–spider mite interactions because this obligatory predator will die or disperse from the almond orchard if its prey is temporarily reduced to near zero. We discovered that lower-than-label rates of these products would reduce spider mite densities sufficiently to assist *M. occidentalis* populations, but not cause secondary outbreaks of spider mites due to predator mortality [19, 39]. We also discovered that spot treating the edges of orchards or other ‘hot spots’ could be sufficient to restore adequate predator/prey ratios, particularly if this was done very early in the season [27].

The recommendation to use low rates of acaricides created considerable controversy. Pesticide companies were concerned that the low rates would be used where *M. occidentalis* numbers were inadequate and they would be blamed for control failures. Concerns also were raised about the possibility of selecting spider mites for resistance through the use of low acaricide rates. Yet, the use of low rates of selective acaricides was a key component of the programme because it is difficult for *M. occidentalis* to suppress spider mite populations adequately in all almond orchards every year in this hot, dry irrigated agricultural system [26]. We attempted to educate growers that predators could, in fact, delay the development of resistance in the spider mites, as was demonstrated by Tabashnik [40]. Probably the best inducement for growers to use the lower acaricide rates was that it reduced costs, even though monitoring costs of approximately US\$10.00 per acre (~US\$24.71/ha) were needed [41, 42]. Cultural practices also were important in enhancing predator efficiency. Water-stressed and dusty almond orchards were associated with spider mite problems, so growers were encouraged to improve their irrigation practices and reduce dust [27].

In the end, the IMM programme in almonds did not rely solely on the genetically improved COS strain; native OP-resistant *M. occidentalis* were used, as well. If growers had abundant native OP-resistant *M. occidentalis* in their orchard and they had no need to use carbaryl to control navel orangeworm or peach twig borer, the IMM programme could be implemented without the costs of releasing *M. occidentalis*. If the orchard lacked sufficient *M. occidentalis* and/or the grower wanted to use carbaryl,

then the genetically modified strain could be purchased and released for approximately US\$20.00 per acre (~US\$49.42/ha). Growers, always cost conscious, wondered whether they needed to release the COS strain in their orchard or whether they could wait until it migrated in from surrounding release sites. After studying dispersal of *M. occidentalis* (and spider mites) into and out of almond orchards for two years, we determined that natural dispersal is relatively slow and inefficient. If growers wanted the COS strain, they needed to release it [25, 34].

Releases were successful only if prey was present in the orchard; densities of 0.5–1.0 spider mites per leaf ensured sufficient prey was present for these obligatory predators to survive and reproduce. Releases of approximately 100 *M. occidentalis* into every third tree in every third row (one tree in nine) were successful in obtaining establishment, although releases into every tree improved the level of control achieved during the current season. Because the COS strain successfully overwintered in the release sites, one release could result in effective control for several years if a compatible spray programme was maintained [19, 25, 39].

Adoption of the IMM programme in California almonds was rapid (Phase III). The reasons for this probably involved several factors. First, growers were eager to reduce production costs, and the use of lower-than-label-rates of selective acaricides reduced them by approximately US\$24 or 44 per acre (~US\$59.30 or 108.72 per hectare), depending on whether predator releases were necessary [41, 42]. Secondly, Farm Advisors, a number of key growers and private pest control advisors were already trained in the new technology because they had participated in field trials during Phase II. In addition, the two project assistants became commercial pest control advisors and began deploying the techniques they had helped to develop. Information on the programme was readily available because a long series of progress reports had been made at meetings with growers, Cooperative Extension personnel and pest control advisors.

Another reason for rapid adoption probably was due to the educational efforts made possible by a University of California publication that was disseminated widely to growers, pest control advisors and extension agents. Progress reports were published regularly in *California Agriculture* [15, 22, 23, 25, 28, 29, 32, 34, 39, 41]. In fact, we often received more requests for articles in this publication than for comparable papers published in entomology technical journals. Guidelines for the IMM programme also were published in a University of California Integrated Pest Management Program publication [27]. Although publications such as *California Agriculture* usually do not receive much recognition in university promotion systems, they were particularly effective in helping to achieve implementation of the COS strain of *M. occidentalis*. The availability of reliable commercial sources of resistant predators also was vital and

starter cultures of the COS strain, as well as advice and information on mass production methods, were provided to anyone requesting them. Several commercial companies produced and sold this strain.

How many almond growers adopted the IMM programme and used the genetically modified *M. occidentalis* strain? A survey conducted during 1986 indicated that approximately 48% of growers routinely monitored for mites and an additional 20% said they sometimes monitored [37]. Another survey conducted during 1987 indicated that 70.6% of pest control advisors working in almonds had tried the presence/absence sampling programme and 49% used the 'low acaricide rates for mite control based on predator counts' [42]. It is not clear how many growers released the COS predator strain because the appropriate question was not asked. The surveys, and information provided by Farm Advisors, suggest that 60–70% of the almond growers adopted the IMM programme, at least in part. This suggests that benefits to the almond industry through the adoption of the IMM programme were between US\$6 636 000 and 10 452 000 per year, which is a return on the original research investment (Phases I and II) of approximately 34:1 [43]. These benefits accrued each year as long as the programme was maintained and were due primarily to the reduced costs of applying acaricides for mite control; no effort was made to evaluate the potential benefits that these reduced pesticide applications had on worker health and safety or to reduced negative effects on the environment.

The cost of conducting research in Phase I was estimated to be US\$294 536, the cost of Phase II research was estimated to be US\$529 306, while the costs of Phase III were not calculated. Phase I took approximately three years to complete and an equal amount of time was devoted to Phase II research into implementation methodology. However, IMM requires periodic additional inputs to maintain it (Phase III). New problems always arise and new IPM practices, such as new pesticides, can disrupt the established programme. Maintenance research (Phase III) will be needed indefinitely in any IPM programme.

Although selection for pesticide resistance was the focus of the genetic improvement project with *M. occidentalis*, I also selected the COS strain for an inability to diapause because we suspected that *M. occidentalis* could perform better during the winter in greenhouses if it did not enter diapause [44]. This non-diapausing strain was evaluated in greenhouse-grown roses during the winter and showed promise of being effective in augmentative releases for managing spider mites, although low rates of a selective acaricide would be needed during its establishment phase to ensure that no economic injury occurred [45, 46]. The non-diapausing COS strain was not tested in commercial greenhouses, but the laboratory selection and small-scale evaluation indicated this predator strain was promising.

## Other Resistant Natural Enemies for IPM Programmes and Lessons Learned

The success of the almond IMM programme led to additional selection projects on natural enemy species. A number of people questioned whether the project with *M. occidentalis* could be repeated with insect natural enemies; phytoseiid mite predators were perceived to be different somehow in their ability to develop resistance to pesticides.

Selection was conducted on the common green lacewing (*Chrysoperla carnea* (Stephens); Neur.: Chrysopidae) for carbaryl resistance [47], on *Aphytis melinus* DeBach, an aphelinid parasitoid of the California red scale, *Aonidiella aurantii* Maskell (Hom.: Diaspididae), for carbaryl resistance [48–52], and on *Trioxys pallidus* (Haliday) (Hym.: Aphididae), a parasitoid of the walnut aphid, *Chromaphis juglandicola* (Kaltenbach) (Hom.: Aphididae), for resistance to azinphosmethyl [53, 54]. The general selection approach employed with *M. occidentalis* proved successful with all three species: multiple colonies of each were collected from commercial orchards or fields in an effort to increase the probability of selecting for rare resistance alleles and selection in the laboratory involved gradual increases in pesticide concentrations over six to eight generations until no selection response occurred.

Implementation of these new natural enemy strains required different approaches, however, and led us to recognize that each natural enemy demanded its own crop- and site-specific deployment plan. In addition, we learned how critical it was to have a thorough knowledge of the biology, ecology and behaviour of the natural enemy, ideally before initiating the project.

For example, the resistant strain of *Chrysoperla*, a generalist predator, was not evaluated in field trials because we could not devise a method of containing the carbaryl-resistant strain in a particular alfalfa plot that would yield realistic data on efficacy. Our initial concept for implementing this strain involved inoculative releases into alfalfa in the San Joaquin Valley of California. In planning the selection project, we had not focused on the fact that newly emerged adults of *C. carnea* have an obligatory dispersal behaviour and can fly for miles prior to oviposition. The prospect of monitoring the entire San Joaquin Valley for the resistant *C. carnea* was daunting and we chose not to evaluate this strain in the field, in part because other projects appeared more promising. However, this project demonstrated that resistance alleles were present in different field populations of *C. carnea*, that resistance levels in these populations were correlated with past treatment histories, and that laboratory selection could yield a carbaryl-resistant population [13, 55].

The successful development of a carbaryl-resistant strain of the red scale parasitoid *A. melinus* demonstrated that it was possible to select a parasitoid for a useful level of resistance and, again, that different field populations varied in their resistance levels, which correlated with

past treatment histories [48]. This variability in responses to pesticides by a parasitoid was, at the time, considered unusual. The implementation of the genetically modified strain might, depending on the geographic location, have been achieved either with augmentative releases or by inoculative releases. In California citrus groves, *A. melinus* is mass reared for release into citrus by commercial and cooperative insectaries and the resistant strain was provided to two insectaries for rearing and release [52]. This strain also was inoculatively released in Israel in a classical biological control programme [56], but improvements in pest suppression were not evaluated.

Deployment of the azinphosmethyl-resistant strain of *T. pallidus* was expected to involve permanent establishment of the new strain in California walnut groves after inoculative releases [57]. Several issues affected our ability to deploy this genetically modified strain in an IPM programme: this strain could, potentially, interbreed with the enormous numbers of susceptible wild *T. pallidus* and we also knew that *T. pallidus* has a rapid dispersal rate. The question was how to get growers to modify their pest management practices to encourage the establishment and spread of the resistant strain, which could only be released in relatively small numbers because *T. pallidus* is very difficult to rear. Rearing this host-specific parasitoid required a tri-trophic system that required rearing potted walnut trees that produced new growth, introducing the walnut-specific aphid on to new growth and introducing *T. pallidus* into cages containing the aphid-infested trees. Unfortunately, no artificial diets for aphids or *T. pallidus* were available. Thus, one limitation to large-scale releases of genetically modified natural enemies may be cost-effective mass-rearing methods.

Our initial conceptual model for implementing the azinphosmethyl-resistant strain of *T. pallidus* involved inoculative releases after treatment of individual walnut groves with azinphosmethyl to eliminate the susceptible *T. pallidus*. We expected that, after one or two sprays (selections) with azinphosmethyl, the resistant strain would replace the susceptible population and begin to disperse into adjacent walnut groves that were also treated with azinphosmethyl. We envisioned that walnut groves in the entire San Joaquin Valley could be colonized by the resistant strain through a series of inoculative releases, subsequent selections with azinphosmethyl, and coordinated azinphosmethyl sprays on walnut orchards adjacent to the release sites that would allow the resistant strain to disperse and establish. These larger-scale releases would take place over two or three years, perhaps beginning in the southern portion of the Valley and moving north. Unfortunately, this implementation model could not be tested before I moved to Florida in 1992.

When the resistant strain was tested in several orchards in California, some of the flaws in our conceptual model were revealed. A simulation model revealed that San Joaquin Valley-wide establishment would involve a complex interplay between dispersal rate, relative fitness

of the two parasitoid strains, the mode of inheritance of the azinphosmethyl resistance and selection pressures exerted by pesticide applications in walnut orchards [57–60]. One surprising fact that emerged during the field trials was that selection intensity was much lower than expected because the very tall walnut trees rarely were sprayed completely when growers failed to properly adjust their spray rigs. This left susceptible *T. pallidus* in the tops of the trees in the sprayed groves to interbreed with the resistant population. The outcome of the field releases also was difficult to evaluate because the resistant and susceptible *T. pallidus* looked the same and could only be discriminated with time-consuming bioassays or by a combination of molecular markers [61]. True area-wide implementation of *T. pallidus* would have required a longer time frame than initially predicted because selection in the field probably would be less effective than expected, economical mass-rearing methods could not produce sufficient resistant *T. pallidus*, and coordinated spray programmes would have required considerable education and recruitment of growers.

The ultimate objective in developing pesticide-resistant parasitoids and predators was to reduce secondary pest outbreaks caused by the decimation of natural enemies by sprays applied to intractable primary pests. This would reduce pesticide use by allowing natural enemies to survive sprays applied to control other pests, and the savings from the IMM programme in almonds was, in fact, due almost entirely to reduced pesticide use. Despite this oft-stated objective, some critics were concerned that pesticide-resistant natural enemies would encourage the use of pesticides [62]. This concern over genetic manipulation projects was a forerunner of the concern that would be elicited over the use of recombinant DNA methods for developing transgenic arthropods.

### Transgenic Arthropod Natural Enemies

In 1982, *Drosophila melanogaster* Meigen (Dipt.: Drosophilidae) was genetically modified by recombinant DNA methods [63, 64], raising the question as to whether transgenic natural enemies could be produced that could improve biological control programmes [65]. A potential advantage to recombinant DNA methods is that once a useful gene had been cloned it could, in principle, be inserted into several natural enemy species, perhaps allowing scientists to develop a suite of pesticide-resistant natural enemies for specific cropping systems. If this were possible, recombinant DNA methods could speed up the process of developing resistant natural enemies (or natural enemies with other desirable traits).

Genetic improvement by recombinant DNA techniques involves identifying one or more specific traits that, if altered, potentially would achieve the goals of the project. This is a critically important first step, as it is with traditional selection methods. Next, suitable genes must be



**Figure 3** The release of the transgenic strain of *M. occidentalis* at a University of Florida field site was the first release of a transgenic arthropod. State, federal and university regulatory authorities were present for this event.

identified and cloned. Appropriate regulatory sequences must be identified so that the inserted gene will be expressed at appropriate levels in the correct tissues and at the relevant time. Once the construct has been incorporated into the genome and demonstrated to be stable, expressed appropriately, and stably transmitted to progeny, the relative fitness of the transgenic strain should be evaluated. These steps can take place in the laboratory and have become relatively routine [66].

If laboratory tests indicate that the transgenic strain is relatively fit and the trait is stable and appropriately expressed, the transgenic strain(s) should be evaluated in small field plots to confirm their efficacy, stability and fitness [67–69]. Before any field tests can occur, however, regulatory issues relating to the safety of releasing transgenic beneficial arthropods must be resolved and this has turned out to be a greater bottleneck than anticipated. Protocols for evaluating the risks associated with permanent releases of parasitoids and predators that have been manipulated with recombinant DNA techniques do not currently exist and this is a significant barrier. We anticipate that risk analyses will likely include, as a minimum, answers to the questions or principles outlined in [13, 67, 68], which were adapted from principles outlined by Tiedje *et al.* [70].

To initiate regulatory oversight relevant to the release of transgenic natural enemies into the environment, we conducted a release in 1996 of a transgenic strain of *M. occidentalis* that contained a *lacZ* marker gene derived from a micro-organism [67, 71, 72]. This predator is an

obligatory predator, has a low dispersal rate and is unlikely to become a pest [67]. The transgenic strain of *M. occidentalis* was released into a plot on the campus of the University of Florida, where it was unlikely to become permanently established because this species (from the western USA) cannot survive the summer rainy season in Florida [73]. The results of the field trial indicated that we could contain the transgenic strain in the plot and that we could retrieve the released predators at the end of the experiment. Surprisingly, we discovered that there was a fitness cost to the transgene that was not evident from the laboratory tests on stability and fitness. The transgene was unstable under field conditions, for unknown reasons, which is why such short-term field evaluations are necessary before transgenic arthropods can be deployed in pest management programmes [74]. Genetic instability is one type of risk that would reduce the efficacy of a transgenic natural enemy. Risk assessment of transgenic arthropods, as it has with transgenic crops and micro-organisms, adds a significant cost in both time and resources to the project. Thus, it will be important to conduct benefit/cost analyses when transgenic beneficial arthropods are used in future pest management programmes (Figure 3).

### Some Conclusions and Caveats

Although the COS strain of *M. occidentalis* was used successfully in an almond IMM programme, the other

laboratory-selected strains of natural enemies were not implemented in a pest management programme. There were several reasons for the failure to implement these strains, including a lack of knowledge about the biology and behaviour of *C. carnea* at the start of the project (when we were more concerned as to whether selection could be successful), lack of time to complete an implementation programme (*T. pallidus* in walnuts and *A. melinus* in California citrus, the non-diapausing strain of *M. occidentalis* in greenhouse roses), and a failure to recognize in advance how important planning for the implementation phase would be.

Results of these projects, and of projects conducted by others, indicate that manipulation of natural enemies can be achieved through artificial selection, hybridization, or recombinant DNA methods. Selection for pesticide resistance, modified climate tolerances, modified host selection behaviour and diapause capacity can be successful. However, the planning phase of such projects should include more careful consideration of the actual deployment plans for any genetically modified strain. In addition, planners need to recognize that deployment will require the skills of a variety of people in a team, as well as considerable financial and time resources. Thus, during the planning phase, the relative costs and potential benefits need to be considered carefully and adequate recognition given of the importance of, and costs of, the implementation phase of the project.

- If naturally occurring biotypes or other species of natural enemies are available that might solve a particular pest problem, these should be evaluated before beginning a project to genetically modify an ineffective natural enemy because such projects are likely to be more expensive and time consuming.
- Likewise, tactics other than biological control or genetic manipulation of natural enemies should also be considered, including the potential for solving the pest problem through host plant resistance, cultural practices or biorational control tactics.
- In other words, genetic manipulation should not be considered a panacea, although it may be a useful tactic.

A limitation to the broader use of genetic manipulation is our lack of knowledge of the population genetics and dynamics of genetically modified natural enemies. For example, the conceptual basis for releasing the pesticide-resistant red scale parasitoid *A. melinus* into an environment where susceptible *A. melinus* do not occur (equivalent to inoculative releases in classical biological control) is clearly different from releasing resistant *T. pallidus* into walnut orchards where a susceptible population is present. In the *T. pallidus* case, two potential models could be considered: (i) elimination of the susceptible *T. pallidus* population by sprays with subsequent replacement by the resistant population versus (ii) the messier concept of expecting the resistant and susceptible

populations to interbreed but selection for resistance in the hybrid population will yield a resistant and field-adapted population (hybridization followed by selection). The replacement model was operationally difficult to achieve with *T. pallidus* and the hybridization followed by selection model may require substantially more time to achieve, as well as a thorough understanding of the population genetics and dynamics of the species. For example, barriers to interbreeding (partial reproductive isolation) or high rates of immigration of susceptible individuals could dramatically influence the replacement model. In some cases, partial answers may be achieved by species-specific simulation models, while in other cases more basic field research may need to be conducted.

The need for effective implementation plans and population genetics models is not limited to natural enemies that have been genetically modified using traditional genetic methods; projects involving the release of transgenic pest arthropods (especially those in which the transgenic population should become established permanently in the environment) are facing similar challenges. Likewise, transgenic pest and beneficial insects face similar regulatory issues in demonstrating efficacy, safety and cost-effectiveness [75]. It should be noted that risk issues surrounding natural enemies being introduced for classical biological control programmes (non-target effects, efficacy) are relevant to the release of transgenic natural enemies, but concerns about stability and horizontal gene transfer must be added to the assessments.

It may be that the tactic of genetically modifying natural enemies will be most appropriate for augmentative releases into contained environments (interiorscapes or greenhouses) because aesthetic and economic value of plants in such highly managed environments may make genetic improvement more cost-effective.

## Acknowledgements

It is rare that scientists are allowed to discuss what they did 'right' and 'wrong' and it is important that we evaluate our successes and failures if progress is to be made. I have tried to provide an overview of an area of research that has engaged me for the past 30 years. Clearly, more research remains to be done before genetic manipulation, either by traditional genetic selection, hybridization or by recombinant DNA methods, becomes a 'mainstream' method in biological control. I thank the graduate students, postdoctoral scientists, technical support staff and colleagues in Connecticut, California and Florida who have contributed in so many ways to this research; it could not have been done without you. This work was supported in part by the Davies, Fischer and Eckes Endowment in Biological Control and is a contribution of the University of Florida Institute of Food and Agricultural Sciences.

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