

RESEARCH-POTENTIAL VERSUS FIELD-APPLIED SUCCESS AND USE OF AUGMENTED NATURAL ENEMIES IN NORTH AMERICAN FIELD CROPS

Kent M. DAANE¹, Rodrigo KRUGNER², and Vaughn M. WALTON¹

¹Division of Insect Biology, University of California
Berkeley, CA 94720, U.S.A.
daane@ucl.ac.uk, vaughn@ucl.ac.uk

²Department of Entomology, University of California
Riverside, CA 92521, U.S.A.
rkrug001@student.ucr.edu

ABSTRACT

The effectiveness of augmentation programs varies depending on natural enemy species released, targeted pest, and release environment. For example, open-fields, row crops, and orchards present a more difficult environment for successful natural enemy release than protected environments, such as glasshouses. Released natural enemies may disperse from the target site, perform poorly at ambient temperatures, or fall prey to resident predators. Successful programs consider characteristics of the released natural enemy, the target pest, and the release environment before developing commercial release programs. Too often, matching the natural enemy to the target pest and environment is overlooked. To illustrate the impact of natural enemy biology on the success (or failure) of an augmentation program, we present results from research on augmentation programs for the vine mealybug, *Planococcus ficus* (Signoret), obliquebanded leafroller, *Choristoneura rosaceana* Harris, and variegated leafhopper, *Erythroneura variabilis* Beamer.

INTRODUCTION

Three broad categories describe how natural enemies are used in biological control: classical biological control, augmentation and conservation. Augmentative biological control is used when resident natural enemies occur too late in time or too low in number to provide adequate pest control, and includes inoculation - “seeding” natural enemies in the release area, and inundation - mass-releasing natural enemies to overwhelm the pest population (Daane *et al.* 2004). The effectiveness of augmentation programs varies depending on natural enemy species released, targeted pest, and release environment. How are natural enemy species selected for augmentation programs? The requirements for species selection and their successful use may include an ability (a) to rear or collect predictable quantities of natural enemies of high quality, (b) to store, transport, and release natural enemies effectively, and (c) to understand the compatibility of released natural enemies with the target pest(s) and other manage-

ment practices (Daane *et al.* 2002; Tauber *et al.* 2000). Nevertheless, the importance of the natural enemy's biological attributes is often undervalued as compared with advantageous insectary-rearing and shipment attributes.

Information regarding aspects of reproductive development, brood sizes, and dispersion along with culturability, sex ratio, food requirements, and host preference has greatly aided in the interpretations of the dynamics in biological control successes and provide a basis to evaluate natural enemy performance in different areas (Ehler 1990; Legner and Bellows 1999). To illustrate the impact of natural enemy biology on the success (or failure) of an augmentation program, we highlight research results from augmentation programs for *Macrocentrus iridescens* French (Hymenoptera: Braconidae) attacking obliquebanded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) (Fig. 1), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) attacking variegated leafhopper, *Erythroneura variabilis* Beamer (Hemiptera: Cicadellidae) (Fig. 2), and *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) attacking vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Fig. 3).

Figure 1. *M. iridescens* pupae near dead OBLR.
Photo: Kent Daane. UGA1390002



Figure 2. *C. carnea* feeding on variegated leafhopper.
Photo: Kent Daane. UGA1390003

Figure 3. *A. pseudococci* adult on honeydew.
Photo: Kent Daane. UGA1390004



OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

The obliquebanded leafroller (OBLR) is a polyphagous feeder that can cause economic damage to several different crops over a wide geographic range in North America. In California pistachios, recent high OBLR population densities and resultant crop losses have led farm managers to apply insecticides, most commonly tebufenozide and carbaryl. Additional control tools for OBLR are needed to reduce the dependence on insecticide applications, prevent yield losses, and maximize profits.

A rich complex of more than 45 parasitoid species has been reported attacking OBLR; however, the level of parasitism and the parasitoid species present varies greatly among crops and regions surveyed. *Macrocentrus iridescens* is a polyembryonic parasitoid with a wide host and geographic range in North America. While *M. iridescens* is relatively ubiquitous, being reared from larvae in the Tortricidae, Lasiocampidae, Gelechiidae, Plutellidae, and Geometridae families in surveys from Ontario to California, it has rarely been reported as the dominant parasitoid or a key biological control agent (references in Krugner *et al.*, 2005). A recent exception was a survey of California pistachio orchards, where *M. iridescens* was the dominant parasitoid species and it was considered a promising biological control agent for OBLR in this crop and region. We developed a laboratory colony of *M. iridescens* and conducted inoculative field release studies. Because little is known about *M. iridescens* biology or ecology, we conducted a series of laboratory assays of *M. iridescens* biology to determine its potential for mass culture, as well as its impact in an augmentation program.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

In San Joaquin Valley (California) vineyards, the variegated leafhopper became the dominant insect pest in the 1980s (Daane and Costello 2000). At high densities, leafhoppers cause chlorotic spotting and defoliation, and their excretion acts as a substrate for sooty molds, resulting in cosmetic damage to fruit. Before the successful development and use of nicotenoïd (imidacloprid) insecticides in the mid-1990s, farm managers sought alternative to insecticide applications, and some used inundative releases of green lacewings. Numerous experimental releases of *Chrysoperla* spp. have been tested against a variety of arthropod pests (for reviews, see Daane and Hagen 2000; Tauber *et al.*, 2000); however, large-scale *Chrysoperla* spp. release programs for leafhoppers required better guidelines than were currently available. We evaluated green lacewing release impact and release methodologies in vineyards. Here, we present pertinent results from four years of field and laboratory experiments.

VINE MEALYBUG AND *ANAGYRUS PSEUDOCOCCI*

Vine mealybug has become a primary insect pest of vineyards in South Africa, Mexico, and California (Daane *et al.* 2005). When left uncontrolled, vine mealybug infestations result in spoiled, infested fruit. Thick layers of excreted honeydew covering the vine also promote sooty mold growth, which can result in defoliation and reduced yield, and a further reduction in crop quality from sunburn. In California, suggested mealybug insecticide treatments include multiple insecticide applications, often with organophosphates. However, because the vine mealybug can feed on all vine sections, there is often poor insecticide coverage and mealybug control in the more protected areas of the vine, such as under the bark, where mealybugs often reside is difficult (Geiger and Daane 2001). Moreover, repeated insecticide use also has adverse impacts on mealybug natural enemies (Walton and Pringle 1999). For these reasons, the development of effective, species-specific, and environmentally safe control programs is needed to work in combination with or as an alternative to insecticides.

Natural enemies attacking vine mealybug in California vineyards include the encyrtid parasitoids *A. pseudococci*, *Allotropa* nr sp. *mecrida* Walker, and *Leptomastidea abnormis* (Girault); several species of green (*Chrysoperla* and *Chrysopa* spp.) and brown (*Hemerobius* spp.) lacewings, and coccinellid beetles. Of these, *A. pseudococci* is currently the most effec-

tive natural enemy, with percentage parasitism as high as 90% of the exposed mealybugs collected near-harvest-time (Daane *et al.* 2004). *Anagyrus pseudococci* is well-known as a parasitoid of the citrus mealybug, *Planococcus citri* (Risso) (Noyes 1994). A polyphagous parasitoid, it also attacks distantly-related species such as *Pseudococcus comstocki* (Kuwana), *Phenacoccus herreni* Cox and Williams, *Dysmicoccus brevipes* (Cockerell), and *Maconellicoccus hirsutus* Green (Noyes 1994). While *A. pseudococci* has been well-studied as a parasitoid of the citrus mealybug (Islam and Copland 2000; Rosen and Rössler 1966; Tingle and Copland 1989), there are no comparable studies with the vine mealybug. Therefore, along with augmentation trials, we conducted a series of studies with *A. pseudococci* reared on vine mealybug to improve effectiveness of biological control in California vineyards.

MATERIALS AND METHODS

OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

Field augmentation. Parasitoids and OBLR were cultured as described by Krugner *et al.* (2005). *Macrocentrus iridescens* females, derived from a laboratory colony, were released in late April to early May in two commercial pistachio fields, near Hanford, California (Kings Co.). Each commercial field (8–20 ha blocks), was split into release and control plots (10 rows x 10 trees) that were separated by »50 buffer rows. Release adults were 1–2 days old, and fed honey and water prior to release. There were from 1,210–2,279 adult *M. iridescens* released in each 100-tree plot, with releases timed to attack the overwintered OBLR larvae (8 April through 9 May). To determine the impact of released parasitoids on OBLR density, we recorded the number of OBLR strikes (infested pistachio leaves) during timed counts (20 trees per plot per sampling date) and made collections of live OBLR (100 per plot per sampling day) to determine percentage parasitism.

***Macrocentrus iridescens* biology.** We report here on two studies that were particularly pertinent to the impact of *M. iridescens* in the augmentation program (described in Krugner *et al.* 2005). First, the ideal temperature range for *M. iridescens* was determined by comparing development and mortality at eight constant temperatures (between 12.6–36.8°C). The upper and lower temperature thresholds, and development rates were estimated by graphing inverse development rates against temperature and fitting a nonlinear curve. Second, the OBLR host stage preferred by *M. iridescens* and the possible range of OBLR host stages that *M. iridescens* can attack were determined in both choice and non-choice tests. In the choice test, all five OBLR instars were placed in an oviposition cage and adult parasitoids added for a 24 hour exposure period. In the non-choice test, each oviposition cage had only one OBLR development stage present. In both experiments the exposed larvae were individually isolated in diet cups and reared to adult parasitoids or OBLR. The experiment was a randomized complete block design with six replicates.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

Field augmentation. The effectiveness of commercial *C. carnea* release programs was evaluated in three vineyards located near Madera, CA (Madera Co.) from (1990 to 1993) (described in Daane *et al.* 1996). *Chrysoperla carnea* were released at rates varying from a total of 37,065

eggs per ha over five periods. Leafhopper densities were estimated 7 days before and 14 and 21 days after lacewing releases with counts of leafhopper nymphs on 20 leaves per plot, following sampling guidelines described by Daane and Costello (2000).

***Chrysoperla carnea* prey-consumption.** Results from these field studies brought into question the effectiveness of release methods, such as egg vs. larval release. For this reason, we studied release methodology, and describe here results from one experiment on the impact of varying release rates, which helps highlight the impact of target prey selection for the “generalist” predator, *C. carnea* (described in Daane and Yokota 1997). To test different release rates, we used a vineyard block at the Kearney Agricultural Center, located near Parlier, CA (Fresno, Co.). Individual vines were isolated by pruning canes on either side. Treatments consisted of a no-release control and 10, 50, 100, 250, 500, and 1000 *C. rufilabris* eggs per vine. These rates correspond to 12,350, 61,750, 123,500, 308,750, 617,500, and 1,235,000 eggs per ha, respectively, with the higher release rates clearly uneconomical. To determine impact leafhopper nymphs were counted on 15 leaves just before and 14 days after treatment application, as described previously. Treatments were set in a randomized complete block design with nine replicates.

VINE MEALYBUG AND ANAGYRUS PSEUDOCOCCI

Field augmentation. Field studies were conducted in five commercial raisin vineyards located near Del Rey, California (Fresno Co.). Treatments were *A. pseudococci*-release and a no-release control, with 0.6 ha treatment plots set in a randomized split plot design, and with each vineyard serving as a replicate. Treatment plots were separated by a buffer zone to minimize dispersion of released *A. pseudococci* into control plots. We released 8,090 *A. pseudococci* per ha on 12 June, 3 July, and 30 July; the release dates were selected based on mealybug movement to exposed locations on the vine. To measure the impact of *A. pseudococci* release, vine mealybug density was determined by a 5-minute search per vine on each of 10 randomly selected vines per plot, as described in Geiger and Daane (2001). Additionally, parasitoid activity was evaluated by collecting 100 mealybugs from each treatment plot (all mealybug stages were sampled). The collected mealybugs were stored in gelatin capsules and held for parasitoid emergence. Crop damage was evaluated at harvest-time by ranking damage of 50 randomly selected vines per treatment plot (five clusters per vine).

***Anagyrus pseudococci* biology.** Our research suggests that *A. pseudococci* overwintering biology and host searching efficiency impacts its success in biological control programs. First, we studied *A. pseudococci* overwintering and spring emergence patterns (for details, see Daane et al., 2004). Briefly, mealybugs were exposed to *A. pseudococci* and then placed at either ambient temperatures (outside) or at room temperatures. The inoculation periods were repeated each month with inoculation dates in October, November, December, January, February, and March. We then recorded the period of adult emergence. Second, we studied the impact of mealybug location on *A. pseudococci* effectiveness (for details, see Daane et al. 2005). In commercial vineyards, we collected >100 mealybugs per month per vineyard. Each mealybug was categorized by development stage and location, as “protected” for mealybugs collected under ground, under the bark of the trunk or older canes, or in cavities formed by wood-boring moths, or as “exposed” for mealybugs found on new canes, leaves and clusters. The collected mealybugs were then held for parasitoid emergence.

RESULTS

OBLIQUEBANDED LEAFROLLER AND *MACROCENTRUS IRIDESCENS*

Field augmentation. There were significantly more “old” shoot strikes (plant damage – but no live OBLR) in the control than release plots in the mid-July and late-August surveys ($t = 2.54$, $P = 0.014$ and $t = 2.59$, $P = 0.016$, respectively) (Fig. 4a). Similarly, there was a significantly higher percentage parasitism in the release treatment in the mid-June period, and derived from the targeted overwintered OBLR larvae (Fig. 4a). Still, there were no significant differences between treatments near harvest-time (Fig. 4a,b) and there were no differences in the number of “new” shoot strike (damaged leaves with OBLR larvae). In summary, we had a significant increase in parasitism in release plots in late-May, just after the parasitoids were released. Unfortunately, this success was short-lived and did not carry over to the next collection periods. Particularly significant is the mid-July reduction in percentage parasitism in the release treatment, suggesting no carry-over between OBLR generations in parasitoid activity.

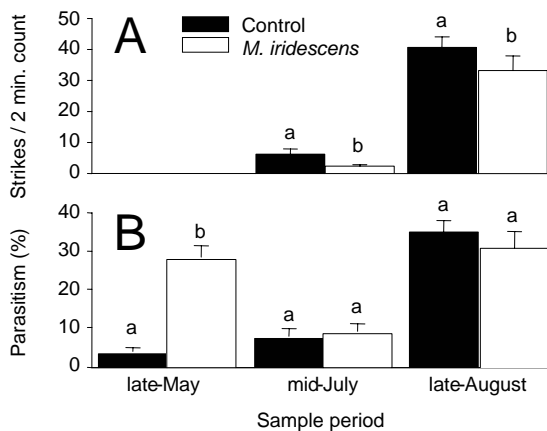


Figure 4. a) Number of OBLR damage leaves (shoot strikes) and b) percentage parasitism by *M. iridescens* in release and no release treatment plots.

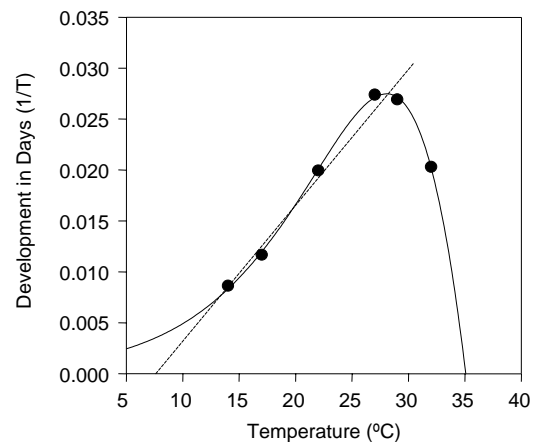


Figure 5. Relationship of temperature and *M. iridescens* development rate at eight constant temperatures.

***Macrocentrus iridescens* biology.** Why was there no season-long impact of the parasitoid release? We believe the answers can be found in the biological data collected in the laboratory. A nonlinear model (Wang *et al.* 1982) gave an excellent fit to the data set ($R^2 = 0.998$) and suggests optimal and upper development temperatures (Fig. 2). The fastest development time, estimated from the upper asymptote, is 36.36 days at 28°C (Fig. 5, dotted line); the upper temperature threshold is 35°C (Fig. 5, solid line) and a lower temperature threshold was determined to be 7.6°C. Using these data, we found that the development time for *M. iridescens* (in degree days) was longer than that reported for OBLR (Gangavalli and AliNiazee 1985). Therefore, there is only one *M. iridescens* generation to each OBLR generation. This by itself can reduce the effective build-up of the natural enemy population.

We also found the mean number of adults emerging from each OBLR significantly decreased at temperatures above 28.2–31.0°C ($F = 12.605$, $\delta f = 5$, $P \leq 0.001$). Since host larvae were parasitized under the same conditions and randomly exposed to different temperatures,

the only variable assumed to affect the size of the emerging progeny was temperature. Therefore, it is possible to conclude that constant temperatures above 28.2°C reduces the number *M. iridescens* individuals emerging from each OBLR larvae. This suggests that during the hot summer temperatures in the Central Valley there will be a reduction in the number of parasitoids produced per OBLR larva. The sex ratio also became more male biased (data not presented, see Krugner et al., 2005). Furthermore, the parasitoid has clear host preference for second and third stage OBLR larvae and if these are not available its reproductive potential will drop. Such circumstances are more likely to occur early in the season because there is clear overlap of OBLR development stages in late July and August when there is also a naturally high level of parasitism.

VARIEGATED LEAFHOPPER AND *CHRYSOPERLA CARNEA*

Field augmentation. In 9 of 20 trials, leafhopper densities were significantly lower in *C. carnea*-release than no-release plots. Data from all trials were combined to determine possible explanations for the variation in the effectiveness of *C. carnea* releases. Possibilities include differences in release trials, rates, and methods, as well as prey density. The average reduction of leafhoppers in *C. carnea*-release plots, as compared with no-release plots, was only 9.6% in commercial vineyards. A significant, although only weakly positive, correlation was found between release rate and effectiveness. There was also a greater reduction of leafhopper nymphs when lacewings were released as larvae, as compared with eggs. Combining data from all studies, the number and percentage reduction of leafhopper nymphs was related to leafhopper density (Fig. 6). Most importantly, when leafhopper densities were above the suggested economic injury level (15-20 nymphs per leaf), the reduction in leafhopper number was frequently not sufficient to lower the leafhopper density below the economic injury threshold.

***Chrysoperla carnea* prey-consumption.** We tested a wide range of release rates (12,350 to 1,235,000 eggs/ha/generation) with the expectation of generating a dose response. However, no correlation between release rate and leafhopper density was found (Fig. 7). One explanation is that higher release rates resulted in increased cannibalism, which reduced the overall impact of added lacewings. Although lacewing larvae are more likely to cannibalize the egg stage, hungry larvae will attack most soft bodied prey, including conspecifics. Satiated larvae are rarely cannibalistic. However, while there was abundant leafhopper prey in these trials, lacewing prey selection is based, in part, on its ability to capture prey (Daane 2000) and small conspecifics may be easier to capture than large leafhoppers. Moreover, because the lacewing are actively moving in search of prey, while the leafhoppers are relatively sessile while feeding, there may be more chance encounters of lacewing to lacewing than lacewing to leafhoppers.

VINE MEALYBUG AND *ANAGYRUS PSEUDOCOCCI*

Field augmentation. Mealybug season-long density was significantly lower in the *A. pseudococci* release than control treatment (Fig. 8). Cluster damage rating was a significant 57% lower in the *A. pseudococci* release (0.22 ± 0.03) than control (0.51 ± 0.05) treatment ($t = 5.522$, $df = 1, 444$, $P < 0.001$). However, we are unable to conclude that the released *A. pseudococci* were solely responsible for this reduction. First, while there was no treatment difference in

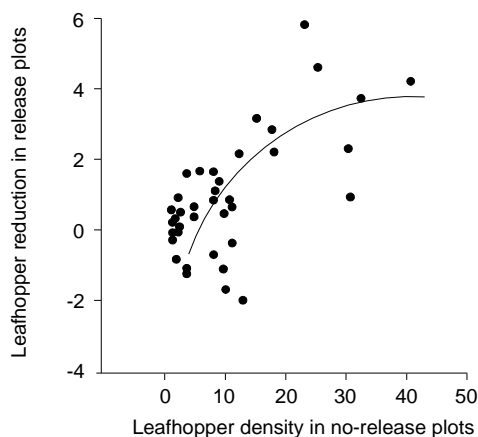


Figure 6. Percentage reduction of leafhopper nymphs in *C. carnea*-release plots plotted against mean number of leafhopper nymphs in associated no-release plots.

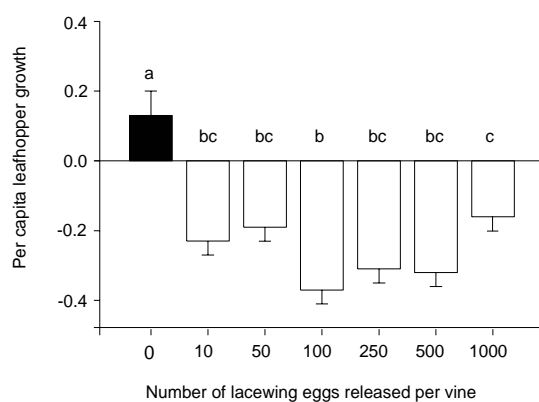


Figure 7. Per capita change (\pm SEM) in leafhopper density after release of 10 to 1000 lacewing eggs per vine. Different letters above each bar indicate a significant difference (Tukey's $P < 0.05$)

mealybug density on 27 March (t -test = 1.659, $P = 0.101$), when treatment plots were randomly assigned, there were significantly fewer mealybugs on 5 June (t -test = 3.701, $P < 0.001$), just before the *A. pseudococci* release. Second, there was no season-long difference in percentage parasitism (Repeated Measures ANOVA: $F = 2.114$, $df = 1, 521$, $P = 0.147$), although percentage parasitism is often an unreliable tool to measure natural enemy impact.

Nevertheless, the data provide encouraging information for the commercial use of *A. pseudococci*. From 7,458 mealybugs collected and held in gelatin capsules, 1,978 were parasitized (26.5%) and 1,235 parasitoid were reared to the adult stage. Parasitoids reared were *A. pseudococci*, *L. abnormis*, *Allotropa* sp. and a hyperparasitoid, *Chartocerus* sp. Of the adult parasitoids, *A. pseudococci* was dominant, comprising >93% of all reared parasitoids. Third instar mealybugs were the most commonly attacked, reflecting the host preference of *A. pseudococci*. Most important, there was a significant reduction in crop damage near harvest-time (data not shown, see Daane *et al.* 2005).

***Anagrus pseudococci* biology.** Earlier studies showed that *A. pseudococci* in California vineyards has an initial period of activity in late May, a result of temperature-dependent development during the overwintering period (Daane *et al.* 2004). For this reason, we believe that early-season inoculation/inundation could dramatically improve parasitism rates. While augmentation with *A. pseudococci* did increase parasitism (Fig. 8) there remained a significant population of the pest in the vineyard. We attribute this resident population to the parasitoids' ineffective host searching attributes for mealybugs located in the more protected locations.

From field collected vine mealybug, we found host size impacted both parasitism and parasitoid gender, as found in earlier studies (Nechols and Kikuchi 1985; Sagarra and Vincent 1999). The percentage of female *A. pseudococci* reared from first and second instar mealybugs was only 2.9 ± 2.9 and $3.6 \pm 0.8\%$, respectively, while from third instar and adult mealybug we reared 95.4 ± 1.1 and $92.9 \pm 2.2\%$ females, respectively. More important for parasitoid impact was the great difference in parasitoid effectiveness with respect to mealybug location

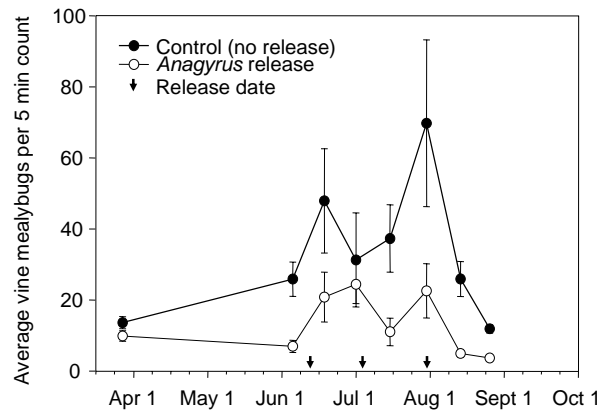


Figure 8. Season-long density (\pm SEM) of settled vine mealybugs was significantly lower in treatments with *A. pseudococci* release, as compared with no-insecticide control plots (Repeated Measures ANOVA: $F=13.27$, $df=1, 76$, $P < 0.001$).

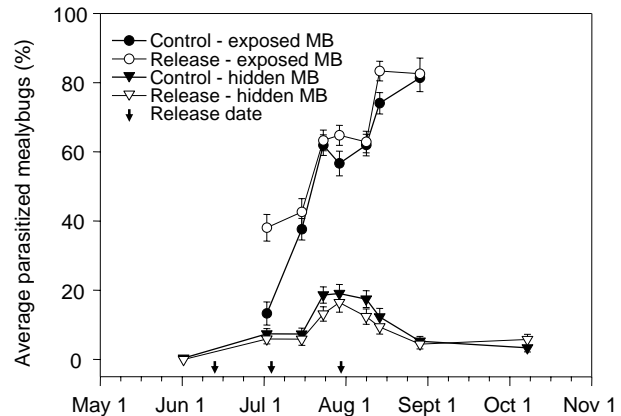


Figure 9. Season-long percentage parasitism (\pm SEM) of settled vine mealybugs, separated by treatment and location, shows significantly higher in parasitism exposed than hidden locations for both control ($F = 247.3$, $df = 1, 273$, $P < 0.001$) and release ($F = 501.5$, $df = 1, 249$, $P < 0.001$) treatments.

on the vine. Season-long percentage parasitism, with data separated by date and location of collected mealybugs, show the importance of timing augmentative release after mealybugs have moved from protected locations (Fig. 9). While there was a low season-long percentage parasitism of mealybug collected from hidden locations (e.g., under the bark) never exceeding 20%, there was a consistent season-long rise in parasitism of mealybugs collected from exposed locations (e.g., on the leaf). No mealybugs could be found in exposed locations on the 1 June sampling date, prior to *A. pseudococci* release. After releases began, there was significantly greater percentage parasitism of exposed mealybugs in release than control plots on the initial sample date (Fig. 9). Parasitism rose steadily in both release and control plots, reaching >80% by late August, after which we could find no live mealybugs in exposed locations.

DISCUSSION

The market for biologically based pest controls is potentially great, driven largely by consumers' desire for pesticide-free produce and loss of current pesticides (Parrella *et al.* 1992). Nevertheless, much of the pest control market is directed towards "soft" insecticides rather than commercially reared and released natural enemies. To meet these needs, researchers and the insectary industry are working to develop more efficient programs. In the insectary, the efficiency of mass culture of beneficial insects is highly dependant on improvement of methods to facilitate and accelerate the insectary process. For this, insectary managers must consider the biology of the host and the parasite in order to produce large numbers while maintaining quality of the mass-reared natural enemy. Here, we describe how natural enemy biology also has considerable impact on its field effectiveness, which is often overlooked.

Whenever feasible, early-season, inoculative release is preferred because it requires fewer natural enemies and provides control over a longer period. In the first study reported, we evaluated the inoculative release of *M. iridescens* for OBLR control in pistachios. *Macrocentrus*

iridescens was earlier found to be the most common parasitoid reared from OBLR in California pistachios, and we were able to develop laboratory colonies to conduct release trials. However, well-timed inoculative release against the overwintered OBLR generation did not impact OBLR density near harvest-time. The problem rested in the parasitoids' biological attributes. Parasitoids often exhibit optimum temperatures different from those of their host, and may become ineffective at higher or lower temperatures. For *M. iridescens*, high temperatures reduced its overall reproductive potential and its developmental rate was slightly longer than its host, indicating that there will be a single parasitoid generation for each OBLR generation. Combined with a relatively narrow host stage preference, *M. iridescens* was unable to respond numerically to the increasing host density until late July and August, when the OBLR population age structure presented acceptable hosts throughout the adult's life time.

In the second study reported, we evaluated the commercial use of inundative releases of green lacewing eggs. *Trichogramma*, predaceous mites and green lacewings are some of the most commonly used natural enemies in inundative augmentation programs (Daane *et al.* 2002). Our work on inundative releases with green lacewings illustrates that this generalist predator may not be the best natural generalist predator for all targeted pest species. Released lacewings are subject to predator-predator interactions at the release site (Daane 2000) and information on other predator species may help release decisions. In our studies, the most significant intraguild predation may have derived from lacewing cannibalism.

In the third study reported, we tested what amounted to both inoculative and inundative releases of *A. pseudococci* for mealybug control in vineyards. While we are enthusiastic about the commercial potential of *Anagyrus* to lower economic damage in the grape clusters, we found that augmentation against vine mealybug may be incomplete because mealybugs have protected locations on the vines. In fact, 100% of the live mealybugs found in September and October samples were located in protected locations of the vine and this, we believe, greatly reduces the ability of foraging adult *Anagyrus* to locate and parasitize vine mealybugs that will constitute the overwintering parasitoid population. Furthermore, we reared primarily male *Anagyrus* from first and second instar mealybugs. These results show that *Anagyrus* release should be timed to coincide not only with the presence of mealybugs in exposed locations, but also with the presence of third instar mealybugs. A final problem with the commercialization of this program is the mass-culture of *A. pseudococci*. Currently, vine mealybug is a pest in vineyards only, reducing the demand for this specialized parasitoid and the potential market for insectary production of *A. pseudococci*.

Augmentation in North American field crops has a long history that includes some of the initial research and successful examples (Daane *et al.* 2002; Parrella *et al.* 1992). One of the most successful augmentative release programs has been against California red scale, *Aonidiella aurantii*. Beginning in 1956, mass-production and inoculative releases of *Aphytis melinus* by the Fillmore Citrus Protection District has suppressed red scale populations. One of the first commercially successful uses of augmentation was against spider mites (*Tetranychus* spp.) on strawberries and cotton. Much of this early work helped develop guidelines for the commercial programmes that emerged in the 1980s. Nevertheless, research on the proper use and efficacy of augmentation programmes in field studies often lagged behind concurrent improvements in mass-production methods for parasitoids and increases in their commercial use, especially in glasshouse systems in Europe.

During this past decade, research has once again focused on field-ecology in augmentation programs and, as a result, there have been substantial advances in our understanding of the potential and problems of both inundative and inoculative programs. Future research will include (a) systematic revisions of natural enemy species that make correct identification and evolutionarily-based biological comparisons a reality, (b) improvements in the methodology for mass-production, (c) applying information from chemical ecology and seasonality to conserve and manipulate natural populations, and (d) rigorous experimental evaluation of release methodology (as described for lacewings in Tauber *et al.* 2000).

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