ARTIFICIAL DIET FOR REARING *TRICHOGRAMMA* WASPS (HYMENOPTERA: TRICHOGRAMMATIDAE) WITH EMPHASIS ON PROTEIN UTILIZATION

Simon GRENIER[1], Silvia M. GOMES[2], Gérard FEBVAY[1], Patrice BOLLAND[1], and José R.P. PARRA[2]

[1] UMR INRA/INSA de Lyon
BF2I, Bât. L. Pasteur, 20 Ave. Einstein
69621 Villeurbanne Cedex France
Simon.Grenier@jouy.inra.fr

[2] Departemento de Entomologia
Fitopatologia e Zoologia Agricola, ESALQ, USP
13418-900 Piracicaba SP, Brazil

ABSTRACT

Trichogramma wasps are tiny hymenopterous egg parasitoids widely used in biological control programs worldwide. The huge quantities of insects necessary for inundative releases are mainly produced on factitious hosts like *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), or on silkworms. In order to simplify production, increase its flexibility, and potentially reduce cost, studies on artificial media for the development of the parasitoids have been ongoing for many years. Some successes were obtained, mainly with artificial media containing insect extracts such as pupal hemolymph from Lepidoptera. To define new artificial media devoid of insect components or improve the performances of existing ones, a better knowledge of parasitoid nutrition would be useful. Proteins are key components in artificial media, and research was conducted on *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) to better understand the nutritional value of proteins by investigating to what degree they are assimilated by the insect.

A method was developed for studying the assimilation of these nutrients by the preimaginal stages of *T. pretiosum* based on adding a mixture of free 14C-radiolabelled amino acids to the medium to be tested. The basic composition of the medium already included proteins, and proteins to be tested were also added. Amino acid analyses were performed on medium (for free and protein amino acids) and on *T. pretiosum* grown in the medium (for protein amino acids). For each radiolabelled amino acid, comparison of the specific activity in total amino acids in *T. pretiosum* pupae with the specific activity in free and protein amino acids in the medium, allowed us to determine the degree and the means by which the protein was utilized.

We showed that the proteins included in the hemolymph-based medium, as well as casein added at final concentrations of 1.6 or 3.2 %, were completely assimilated. This protein, incorporated into the hemolymph-based medium to increase its protein content, led to im-

proved body composition and some development parameters of *T. pretiosum*. Even media containing hemolymph could be improved by protein addition because of the relatively low content of proteins in the hemolymph. The addition of 3.2% casein increased the protein content of *T. pretiosum* pupae by 25% and normal adult emergence yield by 40%.

INTRODUCTION

Oophagous Hymenoptera of the genus *Trichogramma* are used in many countries in biological control programs to regulate pest populations (mainly lepidopteran species) (Li 1994; Parra and Zucchi 2004). These parasitoids are generally reared in factitious host eggs, the most common belonging to Lepidoptera like *Ephestia kuehniella* Zeller, *Corcyra cephalonica* (Stainton) (Pyralidae), *Sitotroga cerealella* (Olivier) (Gelechiidae) or silkworms, but their multiplication on a large scale remains expensive. This limitation to their use can be overcome by the possibility of artificial rearing systems. Studies have been conducted in different countries on *in vitro* rearing of egg parasitoids for many years. Presently, different kinds of artificial media are available enabling immature development of many species of *Trichogramma*. The best results have been obtained with media mainly composed of insect-derived elements such as hemolymph, body, or egg juices, but media without insect additives have also been tested with some success (Consoli and Parra 1997; Grenier 1994; Grenier *et al.* 1995; Thompson 1999; Thompson and Hagen 1999). In these latter media, one of the main concerns is protein supply, and this is true even with artificial media containing insect hemolymph, which is usually poor in protein content compared to lepidopteran eggs.

This work was conducted in order to define artificial diets for *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) that are more suitable for the development of this oophagous parasitoid, based upon a better knowledge of the nutritional value of proteins and of their utilisation by larvae. The assimilation of the proteins was evaluated by adding a mixture of radiolabelled amino acids to the medium. In addition, a hemolymph-based medium, also supplemented with proteins, was tested for *Trichogramma* development. Assimilation and development tests were performed with the basic medium and with casein supplementations.

MATERIALS AND METHODS

Stock cultures of a thelytokous strain of *T. pretiosum* originating from Uruguay, were maintained on *E. kuehniella* eggs killed by UV irradiation. Adults were fed on a diluted honey solution (30% in water). For experiments, rearing was conducted in 1 litre-glass jars (10 cm diameter, 16 cm high) with the proportion of one female for 10 host eggs glued on cardboard. Climatic conditions were 23 ± 0.5 °C, 75 ± 5 % R.H., and a 16:8 h light-dark regime.

The method of investigation was based on the adding of a mix of 14C-labelled amino acids (aa) to the artificial medium in which the *Trichogramma* larvae were grown. The specific activity of each labelled aa is defined as the radioactive activity of the aa in counts per min / mg (cpm/mg) divided by the concentration of the aa in nmol/ mg. We analyzed free and protein aa in the medium, but only the protein aa in the insect body, considering that i) if the

Trichogramma larvae do not digest and assimilate the proteins in the medium (utilization of free aa only), the specific activity of the aa in the insect body will be the same as the specific activity of the free aa of the medium, ii) if the *Trichogramma* larvae completely digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be the same as the specific activity of the total aa of the medium, iii) if the *Trichogramma* larvae partly digest and assimilate the proteins in the medium, the specific activity of the aa in the insect body will be intermediate between the specific activity of the free and total aa of the medium.

Artificial host eggs made of a polyethylene film (15 µm thick) in the form of hemispherical cupules were filled with artificial medium (about 5 µl) used as the diet for larval development. Each rearing device contained 30 cupules arranged as a 6 x 5 matrix. The experiments were conducted under aseptic conditions as described earlier (Grenier 1994; Grenier and Liu 1990; Grenier *et al.* 2002). Climatic conditions were the same as for the stock culture.

The basic artificial medium contained pupal hemolymph from *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (40%), hen's egg yolk (20%), semi-skimmed cow's milk (20%), Neisenheimer salt solution (10%) and distilled water (10%). Besides this medium, two other media enriched with casein (BDH) at two concentrations (final concentrations in the medium of 1.6 or 3.2%) were used for investigating protein assimilation.

The experimental process consisted of incorporating into the media a radiolabelled aa solution of a 14C-protein hydrolysate containing Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val (Sigma). This labelled medium was distributed in 4 out of 30 cupules of each matrix, the remaining cupules being filled with the same medium without radiolabelled aa. Analyses were performed on the medium (free and total aa). *T. pretiosum* females were allowed to lay eggs for 24 hours. After the larvae had completed development, the pupae were analysed for total aa. Each experimental condition was replicated three times.

For total aa analysis of media and pupae, all samples were hydrolysed under nitrogen in HCl vapour at 120°C for 24 hours using a Pico-Tag work station (Waters, St. Quentin Les Yvelines, France). Along with 2-(beta)-mercaptoethanol (4%) to preserve sulphur-containing aa, 200 µl of 6N HCl were placed in the hydrolysis tank. After hydrolysis, 10 nmol of glucosaminic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a Speedvac apparatus (Savant Instrument Inc., Farmingdale, New York) and taken up with 0.05 M lithium-citrate buffer (pH 2.2). Samples were submitted to ion exchange chromatography in an automatic amino acid analyser (Beckman 6300, Roissy, France). Amino acids were detected by the ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm (440 nm for proline). For each condition, 3 to 5 replicates were analysed. Free aa of media were analysed by the same procedure without hydrolysis, but after precipitation of the proteins by TCA (trichloro acetic acid, final concentration 5%) followed by the elimination of TCA and lipids by chloroform extraction. Again, 3 to 5 replicates were analysed.

Biological (parasitism, adult emergence rate, normal adult rate) and biochemical data (pupal body composition in aa) of *Trichogramma* reared in the different media were compared. The diets were prepared and the experiments were performed as described above, but no radiolabelled aa were added. The degree of parasitism was measured by the number of eggs laid per cupule. The percentage of emergence was evaluated by dividing the number of cupules per box producing adults by the total number of cupules x 100. The percentage of normal adults was calculated by dividing the number of adults with normal wings and abdomen by the total number of adults per box x 100. The compositions in aa were expressed in nmol/mg of fresh pupal weight.

RESULTS AND DISCUSSION

ASSIMILATION

The quantity of labelled aa represented 1% of the quantity of the free amino acids in the medium, and thus was not intended to modify the original balance in aa. The external contamination of the pupae grown in labelled medium, checked by washing them several times, was negligible. The feces, rejected just after the emergence by the adults obtained from *E. kuehniella* eggs, were collected on a glass tube and analysed for aa presence. They contained mainly ammonium and only very small quantities of aa (0.52 nmol / *Trichogramma* vs. 10-30 for body content according to their size and consumed food).

In the three media, the specific activity for all free aa was quite high (up to 15000 cpm/nmol), while the specific activity for total (free and protein) aa in the pupal body was lower (less than 1000 cpm/nmol) (Fig. 1). The specific activities of the aa in the pupal body were quite similar to the specific activity of the total aa of the medium for most of the amino acids, mainly essential ones. The lower amounts of labelled total aa observed in pupal body compared to media, for some aa (Thr, Ser, Glu, Gly, and mainly Pro and Ala) could be explained by the importance of the intermediate metabolism in which these energetic aa are implicated. These differences were greater in control medium and lower in medium with 3.2% of casein, showing a better efficiency of protein utilisation in the latter medium. For essential basic aa (Lys, His, Arg), the proteins did not seem to be completely assimilated, because the values for total aa content of pupae were slightly higher than those for the media. Nevertheless, the differences were very small.

In the control medium as well as in media with casein added, all the proteins present were almost completely digested and assimilated. Subsequently, the effect of adding casein was tested on biological and biochemical parameters.

DEVELOPMENT IN MEDIA

Female wasps readily laid eggs inside artificial host eggs (Fig. 2). The parasitization rate, measured as the mean number of eggs laid per artificial host egg (cupule) was not significantly modified when 1.6% casein was added to the control medium (139.1 vs. 146.9), but was significantly reduced with 3.2% casein (111.6). Free aa are usually known as egg laying stimulants (Xie *et al.* 1991), thus this lower parasitization rate was possibly due to a reduction of the relative concentration in aa resulting from the addition of pure casein. Larvae successfully developed and after excreting a black substance turned into pupae (Fig. 2).

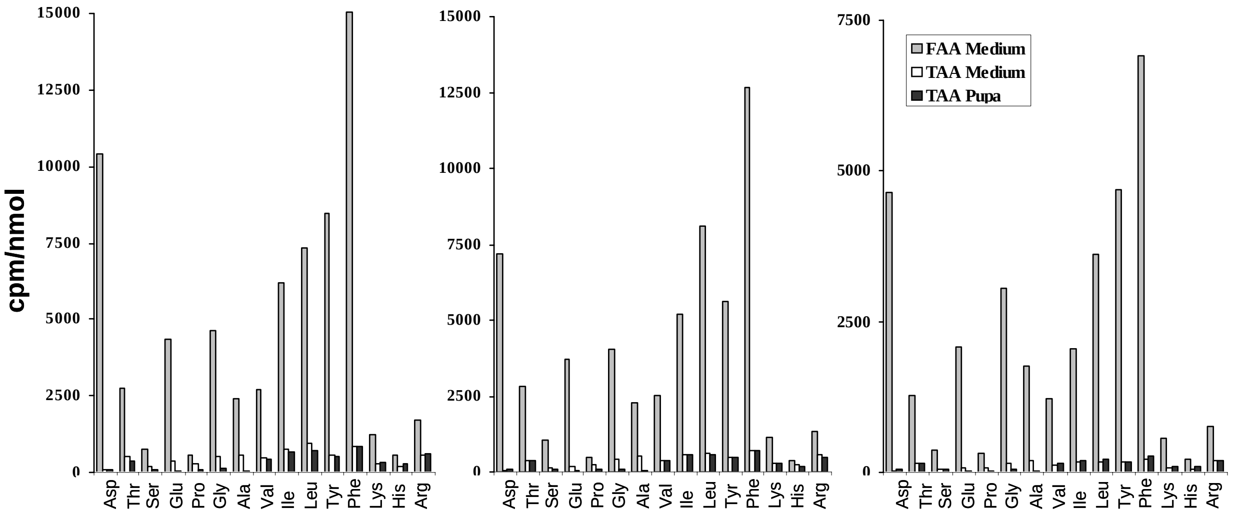

Figure 1. Specific activities (cpm/nmol) of free (FAA Medium) and total amino acids (TAA Medium) in the basic artificial medium as control, in this basic medium supplemented with 1.6 or 3.2% of casein, and of the total amino acids (TAA Pupa) in *Trichogramma pretiosum* pupae grown in these three media.

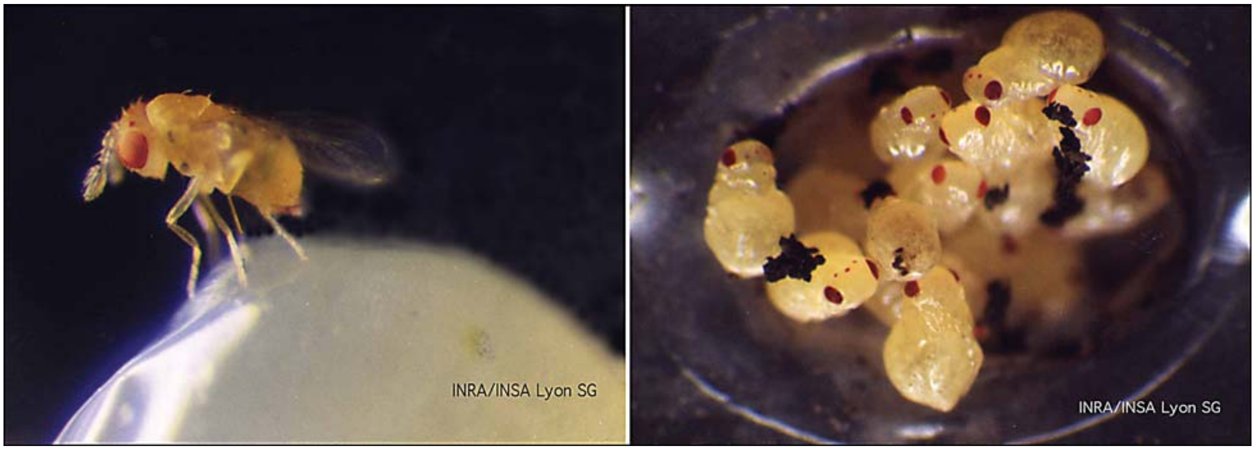

Figure 2. *Trichogramma* female laying eggs inside an artificial host egg (left); *Trichogramma* pupae grown in an artificial host egg (right). Photos: INRA/INSA de Lyon, Simon Grenier. UGA1390018, UGA1390019

Adult production (emergence rate or normal adult rate) was increased when casein was added either at 1.6 or 3.2% (Fig. 3). The lower emergence rate with 3.2% casein compared to 1.6% casein could be explained by the lower parasitization observed in the 3.2% casein medium: if the number of larvae in a cupule is too low, the larvae will become bloated and no further development can occur (Grenier *et al.* 1995). The percentage of normal adults was the highest in medium with 3.2% casein, probably in correlation with a higher amount in aa content of the pupae. The total aa content was 672.3 ± 38.0, 729.3 ± 28.0, and 839.6 ±36.4 nmol/mg for pupae grown in basic medium, and in medium with 1.6% or 3.2% of casein, respectively. The highest value for total aa content of pupae obtained in medium with 3.2% casein was lower than the control values obtained with pupae grown in *E. kuehniella* eggs (88.7 vs. 118.4 expressed in ng/µg), and also than the value (128.1 ng/µg) found for *Trichogramma dendrolimi* Matsumura grown in *E. kuehniella* eggs (Grenier *et al.* 1995).

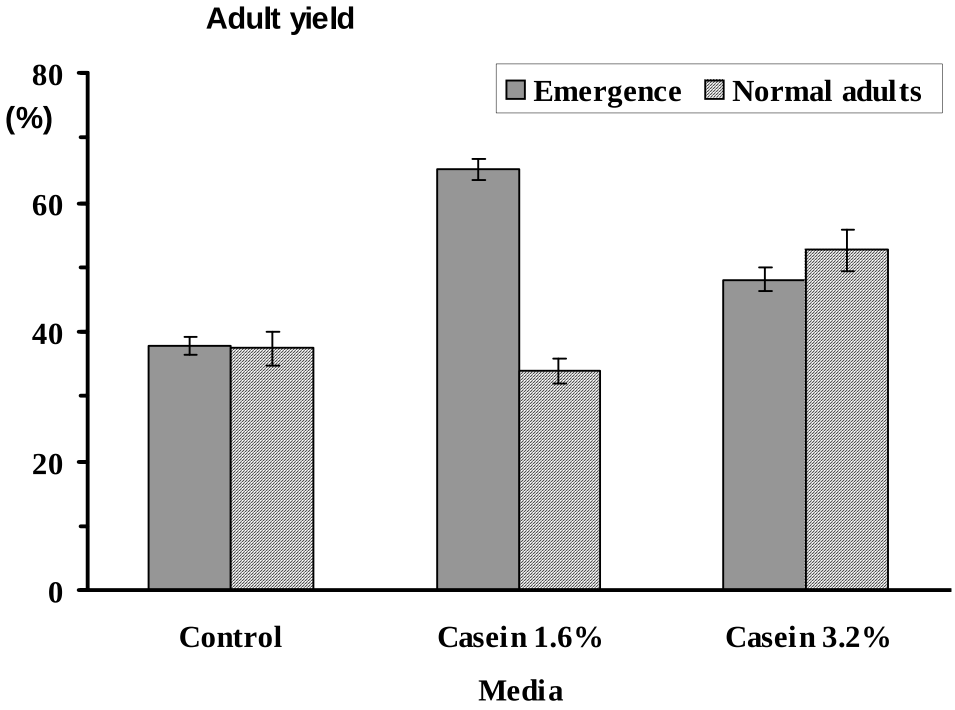

Figure 3. Percentages of emergence and normal adult rates of *Trichogramma pretiosum* on the basic artificial medium, and on the basic artificial medium supplemented with 1.6 or 3.2% casein. Means are given with their SE.

CONCLUSIONS

It was demonstrated that the principle of studying the assimilation rate of proteins can be applied successfully to tiny endoparasitoid insects such as *Trichogramma* species (pupal weight around 30 µg). The results revealed a complete utilisation of the proteins for essential aa, and showed the high level of implication in intermediate metabolism for the other aa.

The methodology, although quite complex and difficult to perform, was shown to be efficient. Through several experiments it appeared that the *Trichogramma* larvae completely assimilate all the proteins present inside the basic medium. Also the casein added into the medium was completely assimilated at the tested concentrations of 1.6 or 3.2%. Thus, casein could be used in artificial media to increase the protein content and improve the performance of the basic medium.

For further experiments, different proteins should be tested at various concentrations to enlarge the spectrum of the components to be used in artificial media. Experiments using this method could also be conducted on *Trichogramma* strains harbouring or not *Wolbachia*, a symbiont inducing thelytokous parthenogenesis in *Trichogramma*, to elucidate the possible role of this symbiotic rickettsia in the digestive physiology of the host. Artificial media could be used not only for production purposes, but also as a powerful tool to study the physiology of immature parasitoids, particularly endoparasitoids, by simplification of their environment (Grenier 2000), as shown again in this study.

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REFERENCES

Consoli, F. L., and Parra, J. R. P. 1997. Development of an oligidic diet for *in vitro* rearing of *Trichogramma galloi* Zucchi and *Trichogramma pretiosum* Riley. *Biological Control* 8, 172-176.

Grenier, S. 1994. Rearing of *Trichogramma* and Other Egg Parasitoids on Artificial Diets. *In* "Biological Control with Egg Parasitoids" (E. Wajnberg and S. A. Hassan, Eds.), pp. 73-92. CAB International, Wallingford, U.K.

Grenier, S. 2000. Rearing in artificial conditions as a tool for physiological or behavioural studies of egg parasitoid insects. Proceedings XXI International Congress of Entomology, Foz de Iguaçu, Brazil, abstract no. 1541, p. 389.

Grenier, S., Gomes, S. M., Pintureau, B., Lassabliere, F., and Bolland, P. 2002. Use of tetracycline in larval diet to study the effect of *Wolbachia* on host fecundity and clarify taxonomic status of *Trichogramma* species in cured bisexual lines. *Journal of Invertebrate Pathology* 80, 13-21.

Grenier, S., and Liu, W. H. 1990. Antifungals: mold control and safe levels in artificial media for *Trichogramma* (Hymenoptera, Trichogrammatidae). *Entomophaga* 35, 283-291.

Grenier, S., Yang, H., Guillaud, J., and Chapelle, L. 1995. Comparative development and biochemical analyses of *Trichogramma* (Hymenoptera: Trichogrammatidae) grown in artificial media with hemolymph or devoid of insect components. *Comparative Biochemistry and Physiology* 111B, 83-90.

Li, L. Y. 1994. Worldwide Use of *Trichogramma* for Biological Control on Different Crops: A Survey. *In* "Biological Control with Egg Parasitoids" (E. Wajnberg, and S. A. Hassan, Eds.), pp. 37-53. CAB International, Wallingford, U.K.

Parra, J. R. P., and Zucchi R. A. 2004. *Trichogramma* in Brazil: Feasibility of use after twenty years of research. *Neotropical Entomology* 33, 271-281.

Thompson, S. N. 1999. Nutrition and culture of entomophagous insects. *Annual Review of Entomology* 44, 561-592.

Thompson, S. N., and Hagen, K. S. 1999. Nutrition of Entomophagous Insects and other Arthropods. *In* "Handbook of Biological Control: Principles and Applications" (T. S. Bellows, and T. W. Fisher, Eds.), pp. 594-652. Academic Press, San Diego, CA.

Xie, Z. N., Xie Y. Q., Li, L. Y., and Li, Y. H. 1991. A study of the oviposition stimulants of *Trichogramma neustadt*. *Acta Entomologica Sinica* 34, 54-59.

LARGE-SCALE AUGMENTATIVE BIOLOGICAL CONTROL OF ASIAN CORN BORER USING *TRICHOGRAMMA* IN CHINA: A SUCCESS STORY

Zhenying WANG [1], Kanglai HE [1], and Su YAN [2]

[1]State Key Lab for the Biology of the Plant Diseases and Insect Pests
Institute of Plant Protection, Chinese Academy of Agricultural Sciences
Beijing 100094, China

[2]National Agro-technology Extension and Service Center
Ministry of Agriculture
Beijing 100026, China

ABSTRACT

Asian corn borer, *Ostrinia furnacalis* (Guenée), is the most destructive pest of corn in China. It causes 6 to 9 million ton yield loss in an average year. Biological control using releases of *Trichogramma* has increased greatly since *T. dendrolimi* Matsumura can be successfully mass reared on eggs of the Chinese oak silkworm, *Antheraea pernyi* Guérin-Méneville. The process and technique for mass production and releasing of *Trichogramma* has been greatly improved in recent years. A series of machines and devices for mass rearing the *Trichogramma* with the eggs of oak silkworm has been developed, which promote *Trichogramma* production and make application for control of the Asian corn borer more practical and efficient. Asian corn borer control by release of *T. dendrolimi* on a large scale has been the key pest management technique in North China. In *T. dendrolimi* release areas parasitism of corn borer eggs ranged from 73.4% to 87.8%, with a 92.5% decrease of stalk-boring. Overall, augmentative releases have been made on 4.1 million ha of corn from 1990 to 2002 in Jilin Province with good pest control effects. *T. dendrolimi* and *T. chilonis* have been successfully produced by means of artificial host eggs and releases of these have had similar effects to the same species reared from factitious host eggs. Field application techniques also have been greatly improved. Large ecological and economic benefits have been achieved in the area where *Trichogramma* have been released continuously for many years. In Miyun County of Beijing, where *Trichogramma* have been released for more than 20 years, populations of natural enemies in cornfields have increased, which allow natural control of other insect pests without application of pesticides. Parasitism due to natural *Trichogramma* increased from 1% and 79.3% in 1980 to 33% and 92% in 1991 for first and third generation of Asian corn borer eggs, respectively. In Gongzhuling City, Jilin Province, the mean borer holes and number of larvae per hundred stalks decreased by 73.66% and 75.93%, respectively, where the *Trichogramma* were released from 1990 to 1996. In recent years *Trichogramma* releases for control of Asian corn borer cover 1 to 1.3 million ha annually, and have become one of the key techniques for IPM of corn pests in China.

INTRODUCTION

Corn (*Zea mays* L.) is playing a very important role in grain production in China. Among the grain crops grown in China, corn ranks second after rice in planting area, total yield and average yield. The average annual planting area is 24 million ha, total yield is 125 million ton, the average yield was 4,839 ton/ha. China is also the second largest corn production country in the world. The Asian corn borer, *Ostrinia furnacalis* (Guenée), is distributed in East and Southeast Asian countries, such as China, Japan, Korea, Thailand, The Philippines, Indonesia, Malaysia, and some islands in the Pacific Ocean (Nafus and Schreiner 1991). It causes serious damage to corn, sorghum, millet and cotton. It remains the most significant economic insect pest of corn in China. Estimated average annual losses in China due to this insect range from 6 to 9 million tons. These losses can be much greater in an outbreak year (Zhou and He 1995).

In China, the Asian corn borer is distributed in most corn growing areas. It goes through one to seven generations a year from the far northern Heilongjiang Province to the southern Hainan Province, according to different latitudes and elevations (All China Corn Borer Research Group, 1988). Among these, one- to three-generation areas are of greater economic importance, owing to the extensive cultivation of corn in these regions. The generations that occur in whorl stage corn cause more serious direct reduction in yield than those that occur in the silking/pollen-shedding stages (Zhou and He 1995). However, the indirect yield loss caused by the generations occurring in later crop stages is much greater than that in whorl stage because the larvae feed on silk and kernels inducing ear and kernel rot which result in contamination of corn grains by mycotoxins produced by fungi, such as *Aspergillus*, *Fusarium*, and *Penicillium*.

Since the early 1950s, a comprehensive study of utilization of the egg parasitoid *Trichogramma* has been conducted for controlling Asian corn borer. Shandong Academy of Agricultural Sciences successfully produced *Trichogramma dendrolimi* Matsumura on the Chinese oak silkworm, *Antheraea pernyi* Guérin-Méneville in the 1960s (Wang 2001). As the eggs of *A. pernyi* were used as host for mass rearing *Trichogramma* in 1970s, research and application of *Trichogramma* have expanded in China and it has been widely used in the successful biological control of many insect pests, especially the Asian corn borer in North China (Gou 1986).

Since 1983, the Chinese government has funded National IPM Technique Research Projects as one of the State Key Research Programs in four successive State Five-year Plans. Biological Control Technique Research is one of those research projects. Since then, there have been improvements in process and technique for mass production and releasing of *Trichogramma*, especially for the Asian corn borer.

TRICHOGRAMMA SPECIES USED FOR ASIAN CORN BORER IN CHINA

There are 12 *Trichogramma* species identified from parasitized Asian corn borer eggs throughout China. Among them, *T. dendrolimi*, *T. chilonis* Ishii, *T. ostriniae* Pang et Chen, and *T.*

evanescens Westwood are distributed throughout the country, and *T. leucaniae* Pang et Chen, *T. poliae* Nagaraja, *T. closterae* Pang et Chen, *T. pintoi* Voegele, *T. ivelae* Pang et Chen, *T. exiguum* Pinto and Platner, *T. forcipiformis* Zhang and Wang, and *T. tielingensis* Zhang and Wang are distributed in some regions. *T. ostriniae* is the dominant species attacking the Asian corn borer in most corn growing regions of China, comprising from 72.2% to 100% of the *Trichogramma*. However, *T. dendrolimi* comprises 97.3%, 28.9% and 45.1% of the total *Trichogramma* in Heilongjiang, Jilin, and Liaoning Provinces in Northeast China, respectively, and *T. chilonis* comprises 88.9% of all *Trichogramm*a in Guizhou Province in Southwest China. *T. ostriniae* accounts for up to 90% of the total parasitized Asian corn borer eggs in Beijing (Zhang *et al.* 1990).

Although *T. ostriniae* is the dominant species attacking Asian corn borer eggs in China and it is more effective for corn borer control than *T. dendrolimi*, the cost for mass rearing *T. ostriniae* is higher and the production efficiency is lower. This species can only be mass reared on small eggs, such as the rice grain moth eggs, *Corcyra cephalonica*, and not use oak silkworm eggs or artificial host eggs. As a result the application of *T. ostriniae* is limited in practice (Feng 1996). *T. dendrolimi* and *T. chilonis* are the two *Trichogramma* species which can be mass reared on the eggs of *A. pernyi* and artificial host eggs. Some field releases showed that *T. chilonis* provided better control for Asian corn borer control in some areas than *T. dendrolimi* (Feng *et al.* 1999; Tan 1999; Wu *et al.* 2001).

In northeastern China, the Chinese oak silkworm is reared on oak tree as sideline occupation in forest regions. The Chinese oak silkworm cocoons are harvested in autumn and transported to biological stations throughout the country, and then stored in cool room for *Trichogramma* mass rearing the following year. The cocoons of oak silkworm can be stored under -5°C for 5 months. In the early summer, the cocoons are hung in the emerging room before mass rearing begins. The eggs squeezed from abdomens of female moths are better for parasitization of *Trichogramma*. These eggs are obtained by squeezing female moth abdomens 1 or 2 days after adult emergence, where upon they are washed and dried. Each female moth can produce 200 eggs and between 50-262 wasps emerge from each egg. A number of 60 per egg is optimal and more than 80% of emerged adult parasitoids are females (Liu *et al.* 2000).

Procedures and equipment for mass production of *T. ostriniae* by using *Sitotroga cereallela* (Olivier) eggs have been developed and *T. ostriniae* will be available for improved Asian corn borer control in the near future (Jia *et al.* 2002; Zheng *et al.* 2003)

IMPROVEMENT OF MASS PRODUCTION OF *TRICHOGRAMM*A USING CHINESE OAK SILKWORM EGGS

Given the large size of an oak silkworm egg, adjustments must be made in the ratio of female wasps to host eggs as well in the exposure time to avoid superparasitism and degeneration. For oak silkworm eggs, the optimum ratio between the number of parent female wasps and host eggs is 2:1. The optimum time of exposure is shorter than 24h. The parasitism of fresh eggs usually reaches 90% (Liu *et al.* 2000).

At least 5 different production components have been developed, which are basically composed of (1) collection of *Trichogramma* from the field that are then cultured on host eggs and reserved as founder population for the following year; (2) selection of host eggs and their storage, and (3) mass propagation (Piao and Yan 1996). The selection of female cocoons which will produce host eggs and treatment of host eggs have been mechanized. A set of machines and devices has been designed, which includes machines for collecting emerged silkworm moths, squeezing of female moths, washing and drying host eggs, preparing egg cards, and parasitizing eggs (Liu *et al.* 1980; Liu *et al.* 1991; Song *et al.* 1994). Equipment for separating immature eggs from mature eggs was also developed. An automatic production line, with the capacity of producing 40 billion *Trichogramma* annually was established in Jilin (Song *et al.* 1994).

The procedure for *Trichogramma* mass production has been simplified. *Trichogramma* spp. are reared simply by the method of releasing wasps in a small empty room with the egg cards on glass windows or on hanging screens (room-rearing). Sometimes the parasitized host eggs (before emergence of *Trichogramma*) are mixed with fresh unparasitized host eggs on wooden trays. When the wasps emerge, they parasitize the host egg directly. Every day in such biological stations 800-1000 million wasps are produced (Liu *et al.* 2000).

To maintain high quality of *Trichogramma* reared on oak silkworm eggs, an instrument for selecting healthy parasitized host eggs was designed. It can distinguish parasitized from unparasitized host eggs, and healthy from infected host eggs based on the elasticity of parasitized host eggs (Wang *et al.* 1999). In addition, the processes of mass production, quality control and field release are standardized in Jilin, Liaoning and Beijing in North China (Piao and Yan 1996).

Technical regulations for *T. dendrolimi* mass production using *A. pernyi* eggs were standardized in 2004 and await approval. This will regulate procedures for *Trichogramma* production and improve the quality of the parasitoid using *A. pernyi* eggs in China.

TRICHOGRAMMA SUCCESSFULLY MASS REARED ON ARTIFICIAL HOST EGGS

Research conducted since 1975 in China has resulted in successful rearing of *Trichogramma* in vitro on artificial host eggs. Breakthroughs have been made on the rearing of *T. dendrolimi* and *T. chilonis* by means of artificial eggs and further research has shown that their efficacies were similar to the same species reared from factitious eggs. Oviposition synergists that improved oviposition by *T. dendrolimi* and *T. chilonis* were selected (Han *et al.* 1994). With the addition of tricosane in a polyvinyl alcohol hydrophilic colloid, parasitism and pupation of *T. dendrolimi* on artificial host eggs reached 100% and 81.25%, respectively (Zhang 1993). The system closest to commercial production is that developed for *T. dendrolimi* on a basis of insect hymolymph. This diet has been packaged in plastic host egg-cards. Mechanized production of *T. dendrolimi* and *T. chilonis* with artificial host eggs has been successful. A model GD-5 automatic machine for mass production of artificial host egg cards was successfully created in 1995, and the technological process of *Trichogramma* produced with artificial host egg cards was developed. A computer controlled machine automatically completes all five processes for egg production including setting-up the synthetic membrane, forming the "egg shells", injecting the artificial media into the shells, sealing the double-layered membrane, and separating into egg cards. Operating rules for mechanized production of artificial host eggs for *Trichogramma* and techniques for propagating parasitoids, quality control, and releasing were formulated (Dai *et al.* 1996; Liu *et al.* 1996). Two artificial host egg production lines for *Trichogramma* were set up in Guangzhou and Beijing in the late 1990s. The parasitoids from in vitro rearing have been used on more than 150,000 ha with parasitism and control effects equal to parasitoids from natural host eggs (Wang 2001). Field experiments showed that the egg parasitism was 65.44% to 68.16%, when using *T. dendrolimi* and *T. chilonis* reared on artificial host eggs to control summer corn borer. In comparison with chemical control, the percentage of tunnels and broken tassels was reduced by 66.67% to 70.37%, and 73.33% to 80.00%, respectively. *T. chilonis* also showed good control of corn earworm, *Helicoverpa armigera,* on corn with 71.1% control, significantly better than that of *T. dendrolimi* (20% control) (Feng *et al.* 1999). China is the first country to make use of in vitro rearing of *Trichogramma* for commercial production and use on a large scale (Wang 2001).

IMPROVEMENT OF FIELD APPLICATION TECHNIQUES FOR *TRICHOGRAMMA* AND CONTROL EFFICACY

Field application techniques have been greatly improved since the 1980s. Release sites have decreased from 90 to 45 per ha based on the dispersal distance of *T. dendrolimi* in the field. The frequency of release has decreased from 3 to 2 and the number of *Trichogramma* released has increased from 135,000 wasps to 150,000-300,000 wasps/ha. Release timing is determined by monitoring of Asian corn borer pupation rate. When the pupation rate of the overwintering generation is 10%, the first release is made 10 days later. The second release is usually done seven days after the first release. Parasitoid releases have shown consistent levels of 60-85% parasitism, with reductions in damage of 65-92% (Piao and Yan 1996). Meanwhile, long-period egg cards were exploited for corn borer control by mixing different stages of *Trichogramma* development on a card, thereby staggering emergence, so that only one or a few releases need to be made. This approach ensures that there are always some females actively searching throughout host oviposition (Zhang *et al.* 1993).

The mean parasitism of Asian corn borer egg masses was 76% compared with 12% in the uncontrolled area on a 72,400 ha scale trial in 1988 in Yushu City, Jilin Province. The parasitism of corn borer eggs by *T. dendrolimi* ranged from 73.4% to 87.8%, with a 92.5% decrease of the stalk-bore rate (Liu *et al.* 2000). Overall, releases were made in 4.1 million ha of corn from 1990 to 2002 in Jilin Province with good control efficacy. In two-generation areas, additional *Trichogramma* release was needed when the egg masses of the second generation were observed, leading to an average reduction of 46.3-73.6% for the overwintering population of Asian corn borer. The strategy for controlling the Asian corn borer in two-generation areas consists of inundative release for the first generation, and inoculative release for the second generation. This strategy has been exploited on a large-scale in Liaoning Province where it has resulted in sustainable management of Asian corn borer (Cong *et al.* 2000). Where large pest outbreaks occurred chemical insecticide granules and *Bacillus thuringiensis* were applied in the late whorl stage.

Large ecological and economic benefits have been achieved in areas where *Trichogramma* have been released continuously for many years. In Miyun County of Beijing, where *Trichogramma* have been released for more than 20 years, the populations of natural enemies in corn fields have increased. This helps keep other insect pests under control without application of pesticides. Parasitism due to natural *Trichogramma* increased from 1% and 79.3% in 1980 to 33% and 92% in 1991 for first and third generation eggs of the Asian corn borer, respectively (Shi 1996). The number of overwintering larvae was reduced to 5.6 larvae per hundred stalks with a yield of 7500 kg/ha in Xifeng County, Liaoning Province, where *Trichogramma* was released continuously on a large scale for over 30 years, compared with 193.6 larvae per hundred stalks and 5250 kg/ha in other surrounding counties when the Asian corn borer outbreak occurred in 1997 (Cao and Sun 2002). In Gongzhuling City, Jilin Province, mean bores and number of larvae per hundred stalks decreased by 73.66% and 75.93%, respectively, where the *Trichogramma* were released from 1990 to 1996.

Trichogramma release for control of the Asian corn borer has become one of the key techniques of IPM of corn pests in China (Wang *et al.* 2003). It has been commonly adopted by the farmers in the northeastern provinces in China because of its easy use and good control efficacy. *Trichogramma* releases to control Asian corn borer comprise 1 to 1.3 million ha each year. With the Chinese government paying attention to grain production and environmental protection, the technique has been expanded to the Huang-Huai-Hai summer corn region and the Northwestern corn region in recent years. The Jilin, Liaoning and Heilongjiang provincial governments have provided some subsidies for controlling the Asian corn borer with *Trichogramma* in recent years. This has expanded the *Trichogramma* release area to 2 million ha in 2004.

REFERENCES

All China Corn Borer Research Group. 1988. Studies on the identification of the dominant corn borer species in China. *Acta Phytophylacica Sinica* 15, 145-152.

Cao, J. L, and Sun, H. J. 2002. Control effect analysis for Asian corn borer by releasing *Trichogramma dendrolimi*. *Rain Fed Crops* 22, 116.

Cong, B., Yang, C. C., Yang, S. W., Li, Y. Q., Wen, H., Liu, W., Yi, Y. Q., Fu, B., and Chen, G. 2000. Establishment, development and perspective of IPM of maize diseases and pest insects in Liaoning Province. *Journal of Shenyang Agricultural University* 31, 413-417.

Dai, K. J., Ma, Z. J., Pan, D. S., Xu, K. J., and Cao, A. H. 1996. Standardization on Mechanized Production of Artificial Host Eggs of *Trichogramma* and Techniques for Propagating Wasps. *In* "Proceedings of the National Symposium on IPM in China" (Z. L. Zhang, Y. F. Piao, and J. W. Wu, Eds.), pp. 1138-1139. China Agricultural Scientech Press, Beijing.

Feng, J. 1996. The effect and influence factors on the use of *Trichogramma dendrolimi* to control *Ostrinia furnacalis*. *Entomological Journal of East China* 5, 45-50.

Feng, J. G., Tao, X., Zhang, A. S., Yu, Y., Zhang, C. W., and Cui, Y. Y. 1999. Studies on using *Trichogramma* spp. reared on artificial host egg to control corn pests. *Chinese Journal of Biological Control* 15, 97-99.

Gou, X. 1986. Research and application *Trichogramma* in China. *Natural Enemies of Insects* 8,113-120.

Han, S. C., Chen, Q. X., and Li, L. Y. 1994. A study on the oviposition synergists for in vitro rearing *Trichogramma* spp. *Entomologia Sinica* 1, 333-338.

Jia, N. X., Huang, Y. G., Li, Q. Z., and Li, Y. 2002. Studies on reproduction *Trichogramma* by using *Sitotroga cerealella* (Olivier) eggs. *Journal of Jilin Agricultural University* 24, 58-60.

Liu, Z. C., Wu, J. Q., Xie, W. D., and Wu, Y. N. 1980. Preliminary study on the mechanized production of *Trichogramma. Acta Phytophylacica Sinica* 7, 233-237.

Liu, Z. C., Yang, H. W., Wang, C. X., Liu, J. F., Liang, Y. F., and Zeng, J. C. 1991. A machine for obtaining clean infertile eggs of oak silkworm used in *Trichogramma* production. *Chinese Journal of Biological Control* 7, 38-40.

Liu, Z. C, Liu, J. F., Yang, W. H., Li, D. S., and Wang, C. X. 1996. Research on technological process of *Trichogramma* produced with artificial host egg and quality standard. *Natural Enemies of Insects* 18, 23-25.

Liu, Z. C., Liu, J. F., Zhang, F., Li, D. S., and Feng, X. X. 2000. “Production and Field Application Techniques of *Trichogramma.*” Golden Shield Press, Beijing.

Nafus, D. M., and Schreiner, I. 1991. Review of the biology and control of the Asian corn borer, *Ostrinia furnacalis* (Lep: Pyralidae). *Tropical Pest Management* 37, 41-56.

Piao Y. F., and Yan, S. 1996. Progress of Mass Production and Field Application of *Trichogramma dendrolimi. In* “Proceedings of the National Symposium on IPM in China” (Zhang, Z. L., Piao, Y. F., and Wu, J. W. Eds.), pp. 1135-1136. China Agricultural Scientech Press, Beijing.

Shi, G. R. 1996. Ecological structure becomes better after releasing *Trichogramma* year by year. *In* “Proceedings of the National Symposium on IPM in China”(Zhang, Z. L., Piao, Y. F., and Wu, J. W. eds.) pp 488. China Agricultural Scientech Press, Beijing.

Song, R. C., Chi, Y. M., Wang, H. P., Lu, G. Y., Wang, Q. X., and Zhang, Q. B. 1994. A study on setting equipment of manufactured reproduction of *Trichogramma. Transactions of the CSAE* 10, 48-52.

Tan, Y. Q. 1999. Present status and prospect in *Trichogramma* application in Heilongjiang Province. *Chinese Agricultural Science Bulletin* 15, 50-51.

Wang, C. L., Mao, G., Gao, Y., Li, L. J., and Cui, D. J. 1999. An instrument for selecting healthy parasitized host eggs. *Chinese Journal of Biological Control* 15,139-140.

Wang, S. 2001. Research progress in *Trichogramma* mass rearing by using artificial host eggs. *Plant Protection Technology and Extension* 21, 40-41.

Wang, Z. Y., He, K. L., Zhao, J. Z., and Zhou, D. R. 2003. Implementation of Integrated Pest Management In China. *In* "Integrated Pest Management in the Global Arena" (K. M. Maredia, D. Dakouo, and D. Mota-Sanchez, Eds.), pp.197-207. CABI Publishing, Oxon.

Wu, L., He, N. P., and Hou, D. W. 2001. Studies on controlling effect of *Trichogramma chilonis* to corn borer. *Journal of Jilin Agricultural Sciences* 26, 35-37.

Zhang, L. W. 1993. Attractive material for the oviposition of *Trichogramma dendrolimi* on artificial "eggs". *Natural Enemies of Insects* 15, 101-105.

Zhang, F., Li, Y. H., Sun, T., Sun, G. Z., Cui, J. A., Ning, S. M., and Wang S. H. 1993. Control effect on Asian corn borer by using long-period effective egg-cards of *Trichogramma*. *Journal of Jilin Agricultural University* 15, 11 -15.

Zhang, J., Wang, J. L., Cong, B., and Yang, C.C. 1990. A faunal study of *Trichogramma* species on *Ostrinia furnacalis* in China. *Chinese Journal of Biological Control* 6, 49-53.

Zheng, L., Song, K., and Zheng, S. H. 2003. Mass production of *Trichogramma brassicae* on eggs of *Sitotroga cerealella* (Olivier). *Journal of Hebei Agricultural Sciences* 7(suppl.), 29-32.

Zhou, D. R., and He, K. L. 1995. "Asian Corn Borer and Its Integrated Management", Golden Shield Press, Beijing.

EFFECTIVE AUGMENTATIVE BIOLOGICAL CONTROL – IMPORTANCE OF NATURAL ENEMY DISPERSAL, HOST LOCATION, AND POST-RELEASE ASSESSMENT

Mark G. WRIGHT[1], Thomas P. KUHAR[2], Joselito M. DIEZ[1], and Michael P. HOFFMANN[3]

[1]Department of Plant and Environmental Protection Sciences
University of Hawai'i at Mnoa
3050 Maile Way
Honolulu, HI 96822, U.S.A.
markwrig@hawaii.edu

[2]Department of Entomology, ESAREC
Virginia Polytechnic Institution
Painter, VA, U.S.A.

[3]Department of Entomology
Cornell University
Ithaca, NY, U.S.A.

ABSTRACT

Augmentative biological control in outdoor cropping systems is often considered to be ineffective. High release rates are often needed for effective control and may be so frequently required that they become prohibitively expensive, especially when natural enemies are purchased from commercial suppliers. Natural enemies released argumentatively may provide control levels that are considered too low to be economically viable. Other germane issues are the selection of appropriate natural enemy species or strains for specific crops, and protocols related to timing and density of releases relative to crop phenology and other pest management strategies.

There are indeed cases where effective augmentative programs have been implemented in outdoor crops. This paper addresses grounds for the effectiveness of these programs, with special reference to the use of *Trichogramma ostriniae* in sweet corn and field corn, where low-density inoculative releases can be highly effective. The importance of understanding dispersal capacity and host location behavior of the biological control agents is examined. Host-seeking behavior of parasitoids in different crop habitats is considered and expanded upon as an aspect of central importance in ensuring effectiveness of augmentative biological control.

This is compared to less successful efforts at developing augmentative biological control in other crops with other parasitoid species (Trichogrammatidae and Scelionidae), in an attempt to identify key characteristics of a potential augmentative agent that are likely to result in success or failure.

Appropriate post-release assessment procedures are also considered. Measurement of the impact that augmentative releases have on integrated pest management systems are explored, to determine whether current approaches to measuring success of augmentative releases are reasonable and adequate. Measuring success of augmentative biological control releases as a component of a holistic IPM program, rather than in isolation, is considered with emphasis on reduced dependence on insecticides.

INTRODUCTION

Augmentative biological control of insect pests in outdoor cropping systems is an attractive option for IPM programs. Augmentative releases of biological control agents have promise as environmentally safe applications of biological control, and as an approach that should be compatible with the application of appropriate pest monitoring and economic injury levels. However, the effectiveness and economic value of augmentative biological control options is questionable in many cases – 64% of augmentative control projects are failures, and in many cases the costs associated with these programs are as high or higher than insecticides (Collier and van Steenwyk 2004). The generally low success rate is attributable to unfavorable environmental conditions, compensatory mortality, enemy dispersal, host refuges from released natural enemies, and predation of released agents (Collier and van Steenwyk 2004). Situations in which augmentative control may be particularly valuable include IPM systems that include pesticides that disrupt natural enemies periodically and crops with moderate to high economic injury levels. Both inundative and inoculative release approaches have the potential to be effective.

Natural enemy dispersal and host location are among the most important components identified by Collier and van Steenwyk (2004). These characteristics of biological control agents have long been recognized as essential components of classical biological control (e.g., Caltigirone 1981).

In spite of the recognized importance of these aspects of the ecology of augmentative biological control agents, they have receive scant attention. In this paper, we discuss some case studies illustrating the importance of understanding dispersal and host location, and the need for post-release assessment. We emphasize the importance of understanding searching behavior and dispersal in specific habitats, and the implications for effective augmentative biological control. Dispersal is defined here as the organism "moving from a point of release, to the place where they reproduce" (*sensu* Caughley 1980). This is an essential aspect of the effectiveness of parasitoids as biological control agents – although they might move throughout a habitat quickly, they must be able to locate and parasitize the target host to be effective.

SOME CASE STUDIES

A SUCCESSFUL AUGMENTATIVE BIOLOGICAL CONTROL PROJECT

While there are many cases of augmentative biological control that are considered ineffective, there also are success stories. Here we examine a system with which we are intimately familiar, and then compare this with another effort at augmentative biological control that has been less successful.

Trichogramma ostriniae Pang et. Chen (Hymenoptera: Trichogrammatidae), released augmentatively against European corn borer (*Ostrinia nubilalis* (Hübner), Lepidoptera: Crambidae) in sweet corn (*Zea mays* L.) is an example of an augmentative biological control agent with great potential. After initial efforts to use this wasp in a classical biological control program failed, an interest was developed in augmentative releases, particularly inoculative releases. This was based on field observations by M.P. Hoffmann and colleagues, which indicated that *T. ostriniae* seemed to have pronounced dispersal characteristics and appeared to establish effectively for a season following low-density release early in the season. Subsequent work on *T. ostriniae* demonstrated that this insect is indeed an excellent candidate for inoculative augmentative biological control. Hoffmann *et al.* (2002) showed that *T. ostriniae* does establish effectively in sweet corn fields in the northeast USA, and can survive insecticide applications at certain times. The wasp demonstrated a Type-I functional response under field conditions, and was thus able to maintain a consistent rate of parasitism across the range of *O. nubilalis* egg mass densities typically encountered in the northeastern U.S. (Hoffmann *et al.* 2002). Further work demonstrated that following low density (70,000 females per hectare), early season release, *T. ostriniae* contributes substantial and significant indispensable mortality to *O. nubilalis* populations, increasing pest mortality from ~60% to more than 95% (Kuhar *et al.* 2002). This mortality level was adequate to consistently reduce damage to ears of corn by ~50%, and the costs of conducting these releases were minimal, based on rearing costs for mass production of the wasps (Wright *et al.* 2002). *Trichogramma ostriniae* has indeed since been made commercially available. The effectiveness of *T. ostriniae* in augmentative biological control releases is attributed largely to its remarkable dispersal and host-location abilities, and the considerable indispensable mortality it is able to contribute as a result. Wright *et al.* (2001) showed that *T. ostriniae* could disperse throughout a large area (~10 ha) within less than seven days, and were able to effectively locate *O. nubilalis* egg masses during their dispersal. Laboratory work in Y-olfactometers showed that *T. ostriniae* females are attracted to the scales of female *O. nubilalis*, presumably to kairomones emitted from these, and field-deployed sentinel egg masses were indeed more attractive to the wasps when lightly sprinkled with fresh wing scales from moths (M. Wright and S. Pitcher, unpublished data).

Further investigation into the ecology of *T. ostriniae* showed that the wasps were substantially more effective at locating and parasitizing hosts in corn fields than in other habitats. When released in broad-leaf vegetable crops, they were relatively ineffective unless released at high density (Kuhar *et al.* 2004). When released in forest habitat, they were less than 10% as effective as in adjacent corn fields, with equal release densities (Wright *et al.* 2005). It was also evident from work done to measure dispersal of *T. ostriniae* out of corn fields and into adjacent habitat, that the wasps prefer to remain within cornfields unless the plants are shorter than about 50 cm (Wright *et al.* 2005). When plants are shorter than this the wasps appeared to readily disperse from the release field (M. Wright, unpublished data).

In summary, factors that make *T. ostriniae* an effective augmentative biological control agent are: effective dispersal; effective host location in the target crop; habitat fidelity; and persistence within the release field.

In addition to the above considerations, it is clear that the selection of an appropriate species of natural enemy is of cardinal importance. For example, attempting to use *T. ostriniae* for the control of an orchard pest is unlikely to be effective, considering the searching behavior demonstrated.

A LESS THAN SUCCESSFUL AUGMENTATIVE BIOLOGICAL CONTROL PROJECT

Nezara viridula (Hemiptera: Pentatomidae) is a perennial pest of macadamia nuts in Hawaii (and many other crops) (Jones 2002). A number of natural enemies have been introduced to control *N. viridula* in Hawaii, including adult parasitoids (*Trichopoda* spp., Diptera: Tachinidae) and an egg parasitoid *Trissolcus basalis* (Hymenoptera, Scelionidae). While *T. basalis* is considered to be a landmark success story in classical biological control in many areas (Jones 1995), it shows variable effectiveness in Hawaii. Parasitism levels may exceed 95% of *N. viridula* eggs on some islands (e.g., Oahu), yet be less than 5% in other areas (southern regions of the Big Island, Hawaii). This variability prompted an investigation into the possibility that augmentative biological control using *T. basalis* may be useful in areas where it has limited effectiveness as a classical agent (Wright *et al.* 2003). The dispersal capacity and host location abilities of *T. basalis* were investigated within macadamia orchards and in adjacent weedy habitats, to determine effective augmentative biological control release sites (Wright *et al.* 2004). The results from numerous releases of 5,000 female *T. basalis* within orchard areas of 5 ha have been uniformly disappointing – low parasitism rates were recorded, and dispersal was sporadic (Wright *et al.* 2004). Other work has shown that *T. basalis* probably contribute negligible indispensable mortality to *N. viridula* in Hawaii (Johnson *et al.* 2005; Jones 1995), at least in tree-habitats. Jones (1995) showed that parasitism by *T. basalis* was minimal within trees in orchards (up to 2.5%), but considerably higher in weed-infested orchard boundaries (up to 13.8%).

The effectiveness of *T. basalis* as an augmentative parasitoid of *N. viridula* eggs appears to be limited by ineffective host location and choice of release site within macadamia orchards and weedy areas. Local climatic conditions may also play an important role, with minimal parasitism resulting even after augmentative releases in dry areas, but high parasitism in areas with predictably high humidity levels.

POST RELEASE ASSESSMENT

Assessment of effectiveness in augmentative biological control programs is probably as important as releasing the natural enemies. Comprehensive life table studies show the extent of indispensable mortality attributable to a specific natural enemy, and can be used to measure the expected impact on the target pest. An understanding of expected yield gains attributable to natural enemies is also a useful measure that may be used in deciding whether to employ augmentative biological control. This approach will also allow the development of a meaningful measure of effectiveness, viz. to what extent a natural enemy reduces dependence on chemical or other pest management options.

CONCLUSIONS

The many failed attempts at augmentative biological control are primarily attributable to a poor understanding of the natural enemy's ability to locate hosts in specific crops after release. This is also identified as an important constraint by Collier and van Steenwyk (2004) in their comprehensive review of success and failures in augmentative biological control. A lack of knowledge of the expected dispersal behavior of a natural enemy influences the decision on release rates and the crop system targeted for augmentative biological control. Work on *T. ostriniae* has shown that low density, early-season releases are effective in corn (Wright *et al.* 2002), yet in solanaceous crops, release rates have to be orders of magnitude higher to achieve even moderate parasitism levels (Kuhar *et al.* 2004). This will clearly impact the benefit-to-cost ratio of using the same species in different crop systems.

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REFERENCES

Caltigirone, L. E. 1981. Landmark examples in classical biological control. *Annual*

Review of Entomology 26, 213-32.

Caughley, G. 1980. "Analysis of Vertebrate Populations," John Wiley and Sons Ltd., New York.

Collier, T., and van Steenwyk, R. 2004. A critical evaluation of augmentative

biological control. *Biological Control* 31, 245-256.

Hoffmann, M. P., Wright, M. G., Pitcher, S. A., and Gardner, J. 2002. Inoculative

releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) for suppression of *Ostrinia nubilalis* in sweet corn: field biology and population dynamics. *Biological Control* 25, 249-258.

Kuhar, T. P., Wright, M. G., Hoffmann, M. P., and Chenus, S. A. 2002. Lifetable

studies of European corn borer (Lepidoptera: Crambidae) with and without inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). *Environmental Entomology* 31, 482-489.

Kuhar, T. P., Hofmann, M. P., Fleischer, S. J., Groden, E., Gardner, J., Wright, M. G., Pitcher, S. A., Speese, J., and Westgate, P. 2004. Potential of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) as a biological control agent of European corn borer (Lepidoptera: Crambidae) in solanaceous crops. *Journal of Economic Entomology* 97, 1209-1216.

Johnson, M. T., Follett, P. A., Taylor, A. D., and Jones, V. P. 2005. Impacts of biological control and invasive species on a non-target native Hawaiian insect. *Oecologia* 142, 529-540.

Jones, V. P. 1995. Reassessment of the role of predators and *Trissolcus basalis* in biological control of southern green stink bug (Hemiptera: Pentatomidae) in Hawaii. *Biological Control* 5, 566-572.

Jones, V. P. 2002. Macadamia Integrated Pest Management: IPM of Insects and Mites Attacking Macadamia Nuts in Hawaii. College of Tropical Agriculture and Human Resources, University of Hawaii at Mnoa.

Wright, M. G., Hoffmann, M. P., Chenus, S. A., and Gardner, J. 2001. Dispersal behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: implications for augmentative releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Biological Control* 22, 29-37.

Wright, M. G., Kuhar, T. K., Hoffmann, M. P., and Chenus, S. A. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. *Biological Control* 23, 149-155.

Wright, M. G., Diez, J., and Follett, P. A. 2003. Green Stinkbug Damage and Biological Control. pp. 21-26. Proceedings of the 43rd Hawaii Macadamia Nut Association Conference,

Wright, M. G., Diez, J. M., and Follett, P. A. 2004. Dispersal and Effectiveness of Mass-Released Green Stinkbug Egg Parasitoids. Proceedings of the 44th Hawaii Macadamia Nut Association Conference. In press.

Wright, M. G., Hoffmann, M. P., Kuhar, T. P., Gardner, J., and Pitcher, S. A. 2005. Evaluating risks of biological control introductions: a probabilistic risk-assessment approach. *Biological Control*, in press.

REMOVAL OF A PREDATORY BUG FROM A BIOLOGICAL CONTROL PACKAGE FACILITATED AN AUGMENTATIVE PROGRAM IN ISRAELI STRAWBERRY

Moshe COLL[1], Inbar SHOUSTER[1], and Shimon STEINBERG[2]

[1]Department of Entomology, the Hebrew University of Jerusalem
Rehovot 76100, Israel
coll@agri.huji.ac.il, inbars@bio-bee.com

[2]Bio-Bee Biological Systems
Kibbutz Sde Eliyahu
Beit Shean Valley 10810, Israel
S_stein@bio-bee.com

ABSTRACT

The demands of export and domestic markets have led growers to adopt a biological control-based integrated pest management program in low-tunnel strawberry fields in Israel. The program consists of the mass release of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) against red spider mites and of the parasitic wasp *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) against the cotton aphid. A study was launched to assess the potential use of *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) to control the western flower thrips (WFT), Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae), in strawberry. After first developing economic thresholds for WFT in strawberry, we investigated (i) the ability of *O. laevigatus* to reproduce on vegetative and reproductive plant parts, (ii) the potential damage to fruits caused by *O. laevigatus* feeding and oviposition, and (iii) the species composition of the naturally-occurring WFT predator complex in strawberry fields.

ORIUS REPRODUCTION

Laboratory experiments show that *O. laevigatus* females prefer to deposit most of their eggs in reproductive parts of strawberry plants, including flowers, green, white and ripened fruits, and their petioles. Inspection of strawberry plants collected from commercial fields revealed a similar distribution pattern of *Orius* eggs. A similar egg deposition pattern was found on field-collected strawberry plants. The egg deposition pattern corresponded with egg hatch: hatching rate was significantly higher for eggs deposited in flowers than in those deposited in leaf tissues.

ORIUS-INFLICTED DAMAGE

To test whether *Orius* feeding and oviposition cause damage to strawberry fruits, we confined 10 female *O. laevigatus* on intact flowers, green fruits and white fruits for 72 hrs. After removing females, we allowed the fruits to develop and recorded their quality at harvest. Inspection of the flowers and fruits revealed an extremely high density of *Orius* eggs imbedded in plant tissues. Nonetheless, no *Orius*-inflicted damage was visible on the harvested fruits as compared to controls. *Orius* feeding and oviposition thus do not inflict appreciable damage to strawberry fruits even at extremely high and un-realistic densities.

PREDATOR POPULATIONS IN STRAWBERRY FIELDS

The predominant WFT predators found in strawberry flowers were *O. albidipennis*, *O. niger* and predaceous thrips of the genus *Aeolothrips.*

CONCLUSIONS

In light of the established thresholds, the natural abundance of *Orius* predators in strawberry fields in Israel, their spatial and temporal co-occurrence with WFT, and their ability to reproduce successfully in this crop, *O. laevigatus* could be excluded from the commercial biological control package. This step made the package much more economically attractive to growers and accelerated its implementation, so that more than 80% of the strawberry acreage in Israel is now under a biologically-based integrated management program.

INTRODUCTION

The IPM/biocontrol program in Israeli strawberries was initiated as a direct result of the Western European export market's demand for significantly lower chemical input in plant protection. During the season of 1998/99, 15 ha. of commercial strawberries were designated as a pilot/demonstration field. Since then, the area encompassed by the program has increased steadily, reaching 300 ha. in the 2004/05 season (Fig. 1), which is ca. 80% of the total strawberry acreage grown in Israel. The majority of the crop is produced under low tunnels on Israel's coastal plain between the months of September and May. About 120 growers currently participate in the program.

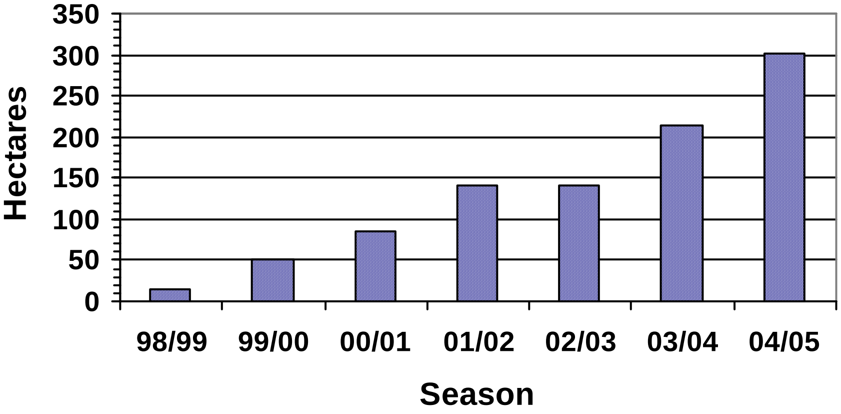

Figure 1. Area of the Israeli strawberry crop under IPM/biocontrol program.

From the onset, the IPM/biocontrol program for Israeli strawberries has been financially supported by the export marketing companies, growers' association and the Ministry of Agriculture. The technical implementation of the program is conducted by Bio-Bee Sde Eliyahu Ltd, the sole commercial producer of natural enemies for biological pest control in Israel. Professional scouts, supervised by Bio-Bee's technical advisory service, monitor the IPM/biocontrol plots on a weekly basis. They provide the grower with detailed reports on the status of pests and natural enemies, as well as recommendations for biological or chemical control action.

We report herein on the major biological components of the biologically-based IPM program for Israeli strawberries. Special emphasis is placed on the predatory bug *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) and the rationale behind its exclusion from the commercial biocontrol package.

DEVELOPMENT OF THE BIOLOGICAL CONTROL COMPONENT OF THE PROGRAM

USE OF THE PREDATORY MITE *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT (ACARINA: PHYTOSEIIDAE) AGAINST THE RED SPIDER MITE

In most plots, *P. persimilis* is introduced in early November, when plastic mulch is in place. The red spider mite is present in the majority of the fields at that time. During the last two seasons, the release rate of *P. persimilis* has stabilized at 20-24 predatory mites per m^2, a dramatic decrease from the 1999/2000 season when an average of 86 predatory mites were released per m^2 (Fig. 2). The continuous reduction in predatory mite release rate can be attributed to experience gained by the growers and scouts during the course of the project regarding both timing and mode of introduction of the predatory mites, and to economic considerations: during the last two seasons, growers have paid for *P. persimilis* on the basis of product used, rather than a lump sum paid in the past for a "biocontrol package" including an almost unlimited supply of natural enemies. In addition, since the 2003/04 season, the new acaricide 'bifenazate' has been applied with *P. persimilis*. 'Bifenazate' is harmless to the predatory mites or to any other natural enemies in the system. Hence, it is an ideal chemical for use against the red spider mite in this system, where needed. 'bifenazate' is mainly effective against the adult spider mites, allowing *P. persimilis* to sustain itself on the immature stages (eggs, larvae and nymphs). In this case the biological and the chemical agents act synergistically. During the 2003/04 season, 35% of the 67 participating IPM/biocontrol plots did not correct with 'bifenazate' at all, 23% used one application, and 42% corrected selectively in hot spots.

USE OF THE PARASITOID *APHIDIUS COLEMANI* VIERECK (HYMENOPTERA: APHIDIIDAE) AGAINST THE COTTON APHID

A. colemani is released following a single application of 'imidacloprid' or 'thiamethoxam' at the beginning of fruit-set. During the last two seasons, the average release rate of *A. colemani* has ranged from 0.7-1.0 parasitoids per m^2. As with *P. persimilis*, this rate also reflects a sharp decrease in the number of parasitoids released per m^2, from 13 per m^2 in the 1999/2000 season (Fig. 3). The reasons for this trend are the same as discussed regarding *P. persimilis*, i.e., experience, economics and availability of compatible aphicides.

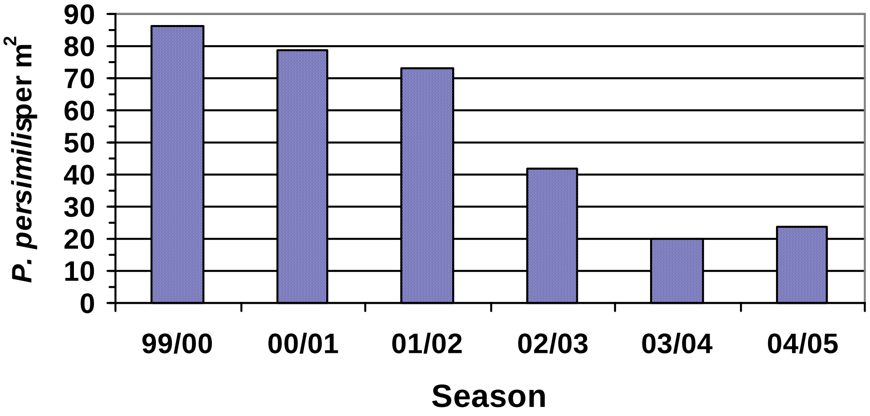

Figure 2. Average release rate of *P. persimilis* (number of predatory mites per m^2) in IPM/biocontrol strawberry fields in Israel during six growing seasons.

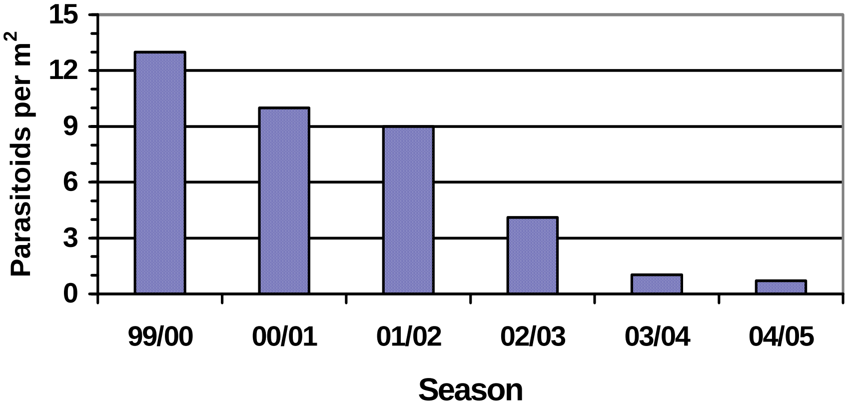

Figure 3. Average release rate of *A. colemani* (number of parasitoids per m^2) in IPM/biocontrol strawberry fields in Israel during six growing seasons.

USE OF THE PREDATORY BUG *ORIUS LAEVIGATUS* (FIEBER) (HETEROPTERA: ANTHOCORIDAE) AGAINST WESTERN FLOWER THRIPS

During the 1999/2000 winter growing season, an average of 3.5 predatory *O. laevigatus* bugs were introduced per m^2 of strawberry. There was no significant recovery of this species from the release fields. During the spring of the 2000/01 growing season, an average of 0.8 predatory bugs was released per m^2. Again, no recovery was recorded of the released bugs. As a result of an intensive research effort (see below), no commercial applications of *O. laevigatus* bugs were made on the subsequent growing seasons in strawberry fields.

ASSESSMENT OF THE PEST STATUS OF THE WESTERN FLOWER THRIPS AND JUSTIFICATION FOR *ORIUS LAEVIGATUS* RELEASES

BACKGROUND

The first report of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (WFT), in Israel dates back to 1987 (Argaman *et al.* 1989). This species is reported to be the dominant thrips species on strawberries in Israel (Shouster 2003) and is thought to be a key pest of this crop elsewhere (Allen and Gaede 1963; Tommasini and Maini, 1995). It has been credited for causing serious damage, mainly through flower drop and fruit distortion. Yet the pest status of WFT in strawberries and the nature of the damage it inflicts are the subject for much debate in many parts of the world.

The few published studies provide contradictory reports regarding WFT damage to strawberry. Damage to the flowers is typically caused by feeding punctures (Tommasini and Maini 1995) that lead to browning and premature withering of the stigmas and anthers, occurring after fertilization (Ribes 1990; Zalom *et al.* 2001). This damage can result in malformation of fruits, sometimes called cat-facing or monkey-facing (Allen and Gaede 1963; Buxton and Easterbrook 1988), which is unacceptable to consumers (Houlding *et al.* 1995). It has been suggested that thrips inject toxic saliva into the plant tissues, which also results in fruit deformation (Buxton and Easterbrook 1988). However, Allen and Gaede (1963), Easterbrook (2000) and Schaefers (1966) reported that various thrips species did not cause fruit malformation through their feeding but instead sometimes caused fruit discoloration. Damage to styles and stigmas may also lead to irregular fertilization and consequent failure of some achenes to develop. WFT may therefore be responsible for uneven ripening and yield loss (Parker 2004). Feeding by thrips on fruit surface and underlying cells often results in discoloration, sometimes accompanied by a silvery sheen caused by air filling the emptied cells (Lyth 1985). Hancock (1999) suggested that thrips feeding on developing seeds and the tissues between seeds results in damaged, small fruit with a seedy, dull or bronze-colored surface, and unevenly developed berries. Views on WFT-inflicted damage in strawberry thus remain ambiguous. Determining the extent and nature of the damage inflicted by WFT to strawberry flowers and fruits was therefore our first step toward the development of a thrips management program in this crop. Specifically, we (i) characterized damage symptoms, (ii) established WFT thresholds, and (iii) monitored pest population densities and compared them to the established threshold levels.

The second stage of this research involved assessing the possible use of *Orius* predators (Heteroptera: Anthocoridae) for the biological control of WFT in strawberry. Predatory bugs of this genus, such as *Orius laevigatus* (Fieber), are known to be effective natural enemies of WFT and are currently used for its control in a number of agricultural systems (Riudavets 1995). Towards this end, we (iv) investigated the ability of *O. laevigatus* to establish itself and reproduce on strawberries, and (v) determined the natural occurrence of *Orius* predators and other natural enemies of WFT in strawberry fields.

WESTERN FLOWER THRIPS AS A STRAWBERRY PEST IN ISRAEL

To characterize WFT damage and determine the vulnerable stage of fruit development, we confined 20 WFT adults for three days on flowers and on white, green and pink fruits. The fruits were then allowed to develop to maturity. At harvest, we compared the weight, size, shape, and coloration of fruits from the different treatments (i.e., time of exposure to WFT) to those of uninfested control fruits. In an additional experiment, we varied the number of adult WFT confined for four days on pink fruits (0 to 25 adults per fruit) and assessed the WFT density-fruit damage relationship.

A significant reduction in fruit fresh weight was recorded only when WFT infestation occurred at the green and pink fruit stages. Fruits in these treatments weighed approximately 40% less than controls. Bronzing was the only type of fruit damage attributable to WFT infestation, and this symptom appeared only when thrips fed on pink fruits. Thrips feeding resulted in punctures around the achenes and the appearance of silvery spots. At low WFT densities, light spotting and slight browning of the calyx were visible. At higher densities, fruit damage was characterized by bronzing, surface russeting and feeding punctures on the fruit surface. WFT-inflicted damage was clearly visible on the fruit surface beneath the calyx; these brown spots due to WFT feeding were particularly apparent at high densities (25 thrips per fruit). No fruit deformation was recorded in any of the treatments and no fruit damage was visible when WFT infestation occurred at the flowering stage. Field experiments, in which thrips populations were kept low in half of the plots but allowed to attain high densities in the others, showed similar results. The field experiments also suggest that WFT may play an important role in flower drop: a tendency toward higher flower drop was recorded in the high-WFT plots in the field. WFT feeding on strawberry blossoms was characterized by brown and withered stigmas and anthers. Necrotic spots were detected on the calyx of the flowers at high thrips densities and flower receptacles were significantly smaller at thrips densities greater than 10 per flower, compared to uninfested control.

These results were used for the establishment of economic thresholds for WFT in strawberry. Two thresholds were established, one for fruits grown for winter export between December and February, and the other for fruits for the local market (March-May). Thresholds for WFT sampling in strawberry flowers were set according to density-damage relations on the fruits, and the recorded ratio of 1:3 of WFT found on fruits and in flowers, respectively. Our calculations indicate that the economic threshold for WFT for exported fruits is 10 adults and second instars per flower. The threshold for the local market was set at 25 adults and second instars per flower.

Weekly sampling of strawberry flowers showed that WFT appears in strawberry fields during the winter, but populations become well established only in early spring. WFT numbers per flower rarely exceed the above thresholds. Typically, an average of 2-7 adult and second instar thrips were found per flower at peak population densities, with high variability among fields and years. WFT density on strawberry flowers began to decrease in April, and the population level remained low until the end of the season (an average of < 2 individuals per flower). Based on our field monitoring, it therefore appears that WFT populations rarely exceed the economic thresholds and, usually, no control measures are warranted against thrips in strawberry.

POSSIBLE USE OF *ORIUS LAEVIGATUS* TO CONTROL WFT IN STRAWBERRY

Laboratory experiments demonstrated that the predatory bug *Orius laevigatus* is able to reproduce on strawberries. Most oviposition takes place on plants that are in the reproductive stages of growth, and oviposition was higher on flowers than on leaves. Flowers and both unripe and ripe fruits were the preferred oviposition sites, and significantly fewer eggs were deposited between flowering cycles, when flowers and fruits were not available. *Orius* oviposition did not cause any visible damage to strawberry fruits even under excessive deposition of eggs in fruits and flowers (approx. 70 eggs per plant part). These results indicate that while inoculative releases of *O. laevigatus* could be considered for the control of thrips in strawberry, the bugs should not be released before flowers and fruits appear in the field, or between flowering cycles. The establishment of the bug in the field could be confirmed by examining egg deposition in flowers and fruits.

Our field monitoring indicated that the dominant natural enemies of thrips in strawberry flowers were *O. niger* (Wolff) and *O. albidipennis* (Reuter). Contrary to expectations, *O. laevigatus* was rare in our fields. *Orius* spp. became established in the crop in April and appeared to reduce WFT populations at that time. Other thrips natural enemies that spontaneously occurred in strawberry fields included predatory thrips of the genus *Aeolothrips* and the hymenopteran parasitoid *Ceranisus menes* (Walker).

RECOMMENDATIONS

Taken together, our results indicate that the western flower thrips is not a key pest of strawberries in Israel, and that under most circumstances no steps are needed for its control. WFT is present on the crop mainly during the second half of the growing season (spring), when the market value of the yield is relatively low and the fruit is destined for the local market, which tolerates a moderate level of cosmetic damage. Also, thrips density in flowers is generally kept in check by naturally occurring natural enemies that are abundant in un-sprayed, biological control-IPM fields. The predatory bug *Orius laevigatus* has the potential to serve as an effective biological control agent of WFT in strawberries; it reproduces on the crop, its presence is compatible with standard agrotechnical practices, and it causes no damage to flowers or fruits. In the Israeli strawberry system, however, the release of *O. laevigatus* is not economically justified; other *Orius* species appear spontaneously in high numbers in insecticide-free fields and the cost of *Orius* production is prohibitive.

CONCLUDING REMARKS

Several important lessons could be derived from the biological control-IPM program in Israeli strawberry. First, it is important to address all major pests in the system so that all used control measures are compatible with the employed biological control agents. Second, it is crucial to secure, early on, the financial and strategic support of private and government sectors, to allow the development of a viable and sustainable program. Third, to maximize profits, biological control producers and suppliers must not seek to maximize sales of a particular biological control agent. Rather, they should aim at developing a system-wide program, even at the cost of excluding a particular biological control agent from the package. Finally, stakeholders often include growers, extension people, natural enemy producers, marketing companies, and retailers that spread across several countries. An international coordinated effort is therefore warranted to match the interest of all parties.

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REFERENCES

Allen, W. W., and Gaede, S. E. 1963. The relationship of *Lygus* bugs and thrips to fruit deformity in strawberries. *Journal of Economic Entomology* 56, 823-825.

Argaman, Q., Klein, Z., Ben Dov, Y., and Mendel, Z. 1989. *Frankliniella occidentalis* (Thysanoptera: Thripidae), an injurious intruder. *Hassadeh* 69, 1268-1269.

Buxton, J. H., and Easterbrook, M. A. 1988. Thrips as a probable cause of severe fruit distortion in late-season strawberries. *Plant Pathology* 37, 278-280.

Easterbrook, M. A. 2000. Relationships between the occurrence of misshapen fruit on late-season strawberry in the United Kingdom and infestation by insects, particularly the European tarnished plant bug, *Lygus rugulipennis*. *Entomologia Experimentalis et Applicata* 96, 59-67.

Hancock, J. F. 1999. "Strawberries," CABI, New York, 237 pp.

Houlding, B., and Woods, B. 1995. Mite and insect pests of strawberries. Department of Agriculture, Western Australia. http://agspsrv34.agric.wa.gov.au/agency/pubns/farmnote/1995/F07195.htm (last accessed Apr. 15 2005)

Lyth, M. 1985. The evidence that thrips are a growing menace. *Grower* 26, 52-53.

Parker, D. 2004. Western flower thrips in strawberries. http://www.clemson.edu/scg/ipm/reports/03parkerb.html (last accessed Apr. 15 2005)

Ribes, A. 1990. Problematica del trips *Frankliniella occidentalis* en el cultivo del freson. *Cuadermos Phytoma España* 4, 17-24.

Riudavets, J. 1995. Predators of *Frankliniella occidentalis* (Perg.) and *Thrips tabaci* Lind.: a review. *In* "Biological Control of Thrips Pests" (A. J. M. Loomans, J. C., Van Lenteren, M. G., Tommasini, S., Maini, and J., Riudavets, Eds.), pp. 43-87. Vol. 1. Veenman Drukkers, Wageningen.

Schaefers, G. A. 1966. The reduction of insect-caused apical seediness in strawberries. *Journal of Economic Entomology* 59, 698-706.

Shouster, I. 2003. Ecological and agricultural implications of tritrophic level interactions between strawberry plants, the western flower thrips *Frankliniella occidentalis*, and the predatory bug *Orius laevigatus*. MS Thesis, The Hebrew University of Jerusalem, Rehovot.

Tommasini, M. G., and Maini, S. 1995. *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe. *In* "Biological Control of Thrips Pests" (A. J. M. Loomans, Van Lenteren, J. C., Tommasini, M. G., Maini, S., and Riudavets, J., Eds), pp. 1-31. Vol. 1. Veenman Drukkers, Wageningen.

Zalom, F. G., Phillips, P. A., Toscano, N. C., and Bolda M. 2004. Strawberry, Western flower thrips. *UC IPM Pest Management Guidelines: Strawberry* UC ANR Publication 3468, Insects and Mites http://www.ipm.ucdavis.edu/PMG/r734301211.html (last accessed Apr. 15 2005).

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

Kent M. DAANE[1], Rodrigo KRUGNER[2], and Vaughn M. WALTON[1]

[1]Division of Insect Biology, University of California
Berkeley, CA 94720, U.S.A.
daane@uckac.edu, vaughn@uckac.edu

[2]Department 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 management 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), *Chyrsoperla 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 nicotenoid (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 effective 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.

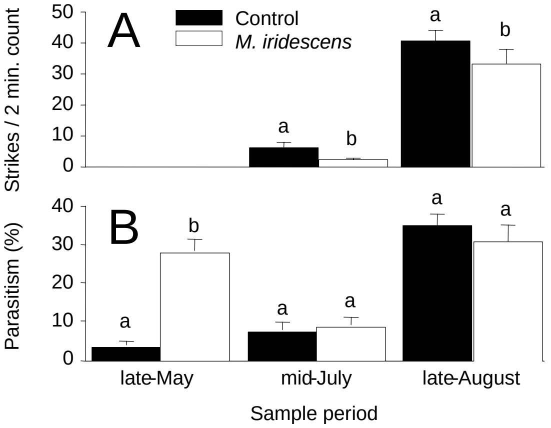

Figure 4. a) Number of OBLR damage leaves (shoot strikes) and b) percentage parasitism by *M. iridescens* in release and no release treatment plots.

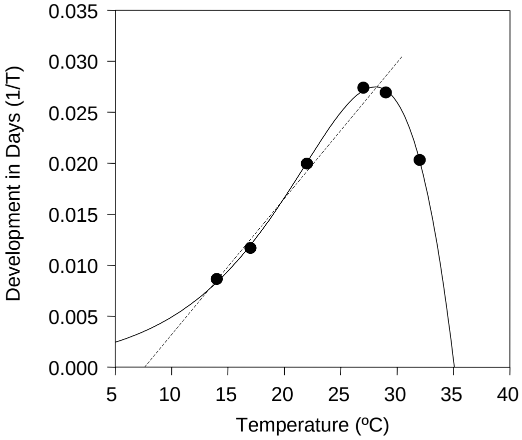

Figure 5. Relationship of temperature and *M. iridescens* development rate at eight constant temperatures.

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, δf = 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

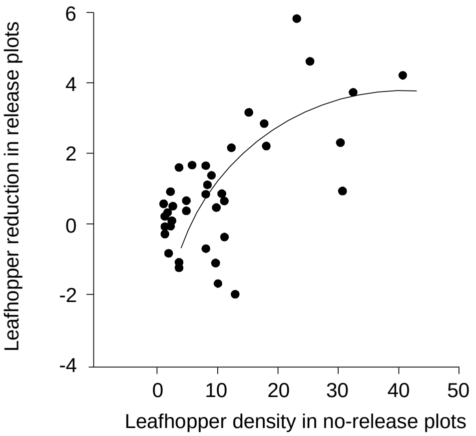

Figure 6. Percentage reduction of leafhopper nymphs in *C. carnea*-release plots plotted against mean number of leafhopper nymphs in associated no-release plots.

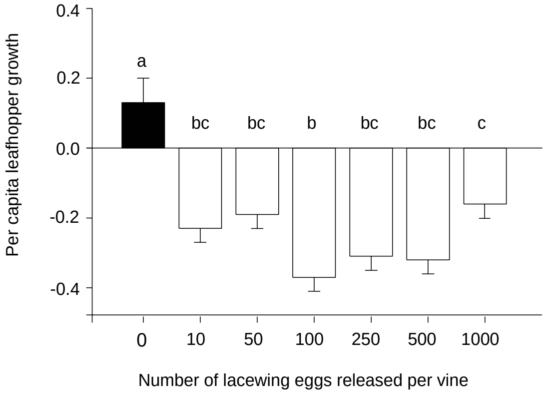

Figure 7. Per capita change (± SEM) in leafhopper density after release of 10 to 1000 lacewing eggs per vine. Different letters above each bar indicate a significant difference (Tukey's P < 0.05)

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).

Anagyrus 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 ± 0.8%, respectively, while from third instar and adult mealybug we reared 95.4 ± 1.1 and 92.9 ± 2.2% females, respectively. More important for parasitoid impact was the great difference in parasitoid effectiveness with respect to mealybug location

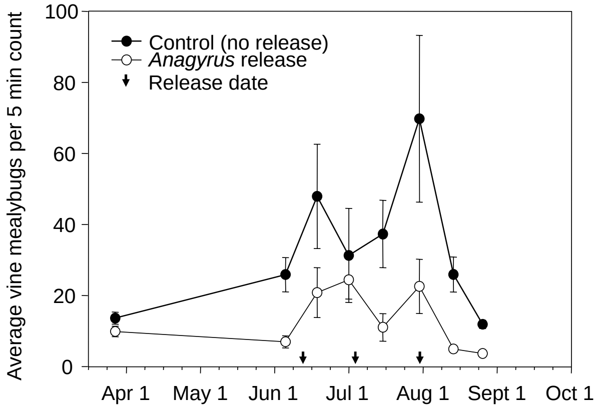

Figure 8. Season-long density (± SEM) of settled vine mealybugs was significantly lower in treatments with *A. pseudococci* release, as compared with no-insecticide control plots (Repeated Measures ANOVA: F=13.27, df=1, 76, $P < 0.001$).

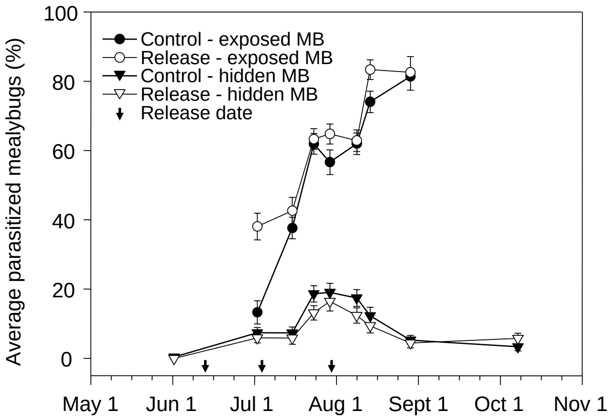

Figure 9. Season-long percentage parasitism (±SEM) of settled vine mealybugs, separated by treatment and location, shows significantly higher in parasitism exposed than hidden locations for both control (F = 247.3, df = 1, 273, $P < 0.001$) and release (F = 501.5, df = 1, 249, P < 0.001) treatments.

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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REFERENCES

Daane, K. M. 2000. Ecological Studies of Released Lacewings in Crops. *In* “Lacewings in the Crop Environment” (P. K. M^{c}Ewen, T. R. New, and A. Whittington, Eds.), pp. 338-350. Cambridge University Press, London.

Daane, K. M., and Hagen, K. S. 2000. An Evaluation of Lacewing Releases in North America. *In* “Lacewings in the Crop Environment” (P. K. M^{c}Ewen, T. R. New, and A. Whittington, Eds.), pp. 398-407. Cambridge University Press, London.

Daane, K. M., and Costello, M. J. 2000. Variegated and Western Grape Leafhoppers. *In* “Raisin Production Manual” (P. Christensen Ed.), pp. 173-181. University of California, DANR Publication 3393. Berkeley, California.

Daane K. M., and Yokota, G. Y. 1997. Release methods affect egg survival and distribution of augmentated green lacewings (Chrysopidae: Neuroptera). *Environmental Entomology* 26, 455-464.

Daane, K. M., Yokota, G. Y., Zheng, Y., and Hagen, K. S. 1996. Inundative release of the common green lacewing to control *Erythroneura variabilis* and *E. elegantula* (Homoptera: Cicadellidae) in grape vineyards. *Environmental Entomology* 25, 1224-1234.

Daane, K. M., Mills, N. J., and Tauber, M. J. 2002. Biological Pest Controls: Augmentative Controls *In* “Encyclopedia of Pest Management” (D. Pimentel Ed.), pp. 36-38. Marcel-Dekker, Inc., New York, NY.

Daane, K. M., Malakar-Kuenen, R., and Walton, V. M. 2004. Temperature development of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae). *Biological Control* 31, 123-132.

Daane, K. M., Bentley, W. J., Walton, V., Malakar-Kuenen, R., Yokota, G. Y., Millar, J. G., Ingels, C. A., Weber, E. A., and Gispert, C. 2005. Sustainable controls sought for the invasive vine mealybug. *California Agriculture* (in press).

Ehler, L. E. 1990. Introduction Strategies in Biological Control of Insects. *In* “Critical Issues in Biological Control” (M. MacKauer, L. E. Ehler and J. Roland, Eds.), pp. 111-134. VCH Publishers, New York.

Gangavalli, R. R., and AliNiazee, M. T. 1985. Temperature requirements for the development of the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae). *Environmental Entomology* 14, 17-19.

Geiger, C. A., and Daane, K. M. 2001. Seasonal movement and sampling of the grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Homoptera: Pseudococcidae), in San Joaquin Valley vineyards. *Journal of Economic Entomology* 94, 291-301.

Islam, K. S., and Copland, M. J. W. 1997. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Science & Technology* 7, 449-456.

Legner, E. F., and Bellows, T. S. 1999. Exploration for Natural Enemies. *In* “Handbook of Biological Control” (T. S. Bellows, and T. W. Fisher Eds.), pp. 87-101. Academic Press, San Diego.

Krugner, R., Daane, K. M., Lawson, A. B., and Yokota, G. Y. 2005. Biology of *Macrocentrus iridescens* (Hymenoptera: Braconidae): A parasitoid of the obliquebanded leafroller (Lepidoptera: Tortricidae). *Environmental Entomology* 34, 336-343.

Nechols, J. R., and Kikuchi, R. S. 1985. Host selection of the spherical mealybug (Homoptera: Pseudococcidae) by *Anagyrus indicus* (Hymenoptera: Encyrtidae): Influence of host stage on parasitoid oviposition, development, sex ratio, and survival. *Environmental Entomology* 14, 32-37.

Noyes, J. S., and Hayat, M. 1994. “Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae).” CAB International - Natural History Museum, London, University Press, Cambridge.

Parrella, M. P., Heinz, K. M. and Nunney, L. 1992. Biological control through augmentative releases of natural enemies: a strategy whose time has come. *American Entomologist* 38, 172-179.

Rosen, D., and Rössler, Y. 1966. Studies on an Israel strain of *Anagyrus pseudococci* (Girault) (Hymenoptera, Encyrtidae). I. Morphology of the adults and developmental stages. *Entomophaga* 11, 269-277.

Sagarra, L. A., and Vincent, C. 1999. Influence of host stage on oviposition, development, sex ratio, and survival of *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), a parasitoid of the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). *Biological Control* 15, 51-56.

Tauber, M. J., Tauber, C. A., Daane, K. M., and Hagen, K. S. 2000. New tricks for old predators: implementing biological control with *Chrysoperla*. *American Entomologist* 46, 26-38.

Tingle C. C. D., and Copland, M. J. W. 1989. Progeny production and adult longevity of the mealybug parasitoids *Anagyrus pseudococci*, *Leptomastix dactylopii* and *Leptomastidea abnormis* (Hym. Encyrtidae) in relation to temperature. *Entomophaga* 34, 111-120.

Walton, V. M., and Pringle, K. L., 1999. Effects of pesticides used on table grapes on the mealybug parasitoid *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae). South African Journal of Enology and Viticulture 20, 31-34.

Wang, R. S., Lan, Z. X., and Ting, Y. C., 1982. Studies on the mathematical models of the relationships between insect development and temperature. *Acta Ecologica Sinica* 2, 47-57.