

BIOLOGICAL CONTROL OF CITRUS RUST MITE USING INDIGENOUS AND INTRODUCED GENERALIST ACARINE PREDATORS IN ISRAEL

Yael ARGOV¹, Eric PALEVSKY², and Uri GERSON³

¹Israel Cohen Institute for Biological Control
Plant Production and Marketing Board, Citrus Division
POB 54
Bet Dagan, 50250, Israel
yael@jaffa.co.il

²Institute of Plant Protection, Agricultural Research Organization
The Neve-Ya'ar Research Center
P.O. Box 1021
Ramat Yishay 30095, Israel
palevsky@volcani.agri.gov.il

³Department of Entomology
Faculty of Agricultural, Food and Environmental Quality Sciences
Hebrew University of Jerusalem
POB 12
Rehovot 76100, Israel
Gerson@agri.huji.ac.il

41

Citrus rust mite (CRM), *Phyllocoptruta oleivora* (Ashmead) (Acarina: Eriophyidae), is a major pest of citrus in Israel. Two approaches were evaluated in order to improve CRM biological control.

1. Classical biological control - five species of predatory mites: *Amblyseius herbicolus* Chant, *Euseius victoriensis* (Womersley), *Euseius elinae* (Schicha), *Typhlodromus rickeri* Chant and *Euseius stipulatus* (Athias-Henriot) (all Acarina: Phytoseiidae), were imported. Mite rearing and sampling methods were developed and improved, and the predators were released. Although some recovery was recorded, only *E. victoriensis* became established in the north of Israel. Despite the latter's establishment CRM control was not improved.
2. Conservation of indigenous acarine predators - damage caused by CRM is usually negligible in minimally to unsprayed isolated groves located in the central coastal plain of Israel. Assuming that resident natural enemies were responsible for this situation, we monitored the pest's potential predators in five unsprayed citrus plots, and concurrently determined their feeding habits in the laboratory. In the field *Iphiseius degenerans* (Berlese) and *Amblyseius swirskii* Athias-Henriot (both Acarina: Phytoseiidae) were the main predators found, the former being dominant during the critical winter and spring

months, the period of low pest populations. In the laboratory, when solely CRM was offered, the decline in pest numbers was similar in leaf arenas containing either phytoseiid or the stigmatid *Agistemus cyprius* Gonzalez, but *I. degenerans* killed fewer CRM in the presence of pollen. While the cessation of pesticide applications during two years was insufficient for reducing CRM populations, observations suggest that a three year break from broad spectrum pesticides would be the turning point for the reestablishment of *I. degenerans*, the postulated major winter time predator. Our field and laboratory data indicate that a complex of indigenous, generalist predators could be responsible for the control of CRM in isolated, unsprayed citrus groves on the central coastal plain of Israel.

Session 9: Role of Generalist Predators in Biological Control

STAGE PREFERENCE AND FUNCTIONAL RESPONSE OF *EUSEIUS HIBISCI* TO *TETRANYCHUS URTICAE* (ACARI: PHYTOSEIIDAE: TETRANYCHIDAE)

M. H. BADI, E. HERNÁNDEZ-ORTIZ¹, A. E. FLORES, and J. LANDEROS²

UANL

Ap. 391

San Nicolás, N. L., México

mhbadii@yahoo.com.mx

¹SAGAR, Chis

²UAAAN, Coahuila, México

The aims of this study were: a) determine the prey stage preference of female *Euseius hibisci* (Chant) (Phytoseiidae) at constant densities of different stages of *Tetranychus urticae* Koch (Tetranychidae), b) assess the functional response of the predatory females to the varying densities of eggs, larvae, or protonymphs of *T. urticae*, and c) estimate the functional response of *E. hibisci* when pollen of *Ligustrum ovalifolium* Hassk. (Oleales: Oleaceae) was present as well. We conducted experiments on excised pieces of strawberry leaf arenas (*Fragaria ananassa* Duchesne (Rosales: Rosaceae)) under laboratory conditions of $25\pm 2^\circ\text{C}$, $60\pm 5\%$ RH and 12 h photophase. Our results indicated that the predator consumed significantly more prey eggs than other prey stages. Consumption of prey deutonymphs and adults was so low that they were excluded from the non-choice functional response experiments. The functional response on all food items was of type II. The two parameters of the functional response were estimated for each prey type by means of the adjusted nonlinear regression model. The highest estimated value of a' (instantaneous rate of discovery) and the lowest value of T_h (handling time, including digestion) were found for the predator feeding on prey eggs, and a' was lowest and T_h highest when fed protonymphs. Using the jack-knife method, the values for the

functional response parameters were estimated. The values of a' and T_h produced by the model were similar among all prey types except for the eggs, which were different. Using pollen simultaneously with prey larvae decreased the consumption of the latter over the full range of prey densities. The suitability of this predator for biological control of *T. urticae* on strawberry is discussed.

Session 9: Role of Generalist Predators in Biological Control

POPULATION INTERACTION BETWEEN *PHYTOSEIULUS LONGIPES* AND *TETRANYCHUS PACIFICUS* ON BEAN PLANTS

M. H. BADI, J. A. MCMURTRY¹, and A. E. FLORES

Ap. 391, San Nicolas,
N. L., México,
mhbadii@yahoo.com.mx,

¹University of Oregon,
Corvallis, OR, U.S.A.

The predator-prey interactions between *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) and *Tetranychus pacificus* McGregor (Acari: Tetranychidae) and also between *Phytoseiulus persimilis* McGregor (Acari: Phytoseiidae), and *T. pacificus* were studied on bean plants. The plants were fertilized with Shultz-instantä, and RA.PID.GROä. Young potted lima bean plants, each having 7-8 leaves, were placed in wooden boxes (60x46x10cm) surrounded by a water barrier. Newly formed leaves on the plants were removed every 2-3 days to prevent excessive growth. The plants were watered every 2-3 days and were fertilized every two weeks. Experiments were conducted under constant conditions. Due to excessive time required to make counts of prey and predators, this study was conducted in three parts:

Part 1. Twenty five mated female *T. pacificus* were introduced to each of 10 plants by placing the mites on a single leaf at the base of the plant. When about half of the female prey had moved to the adjacent leaves (after about three hrs.), one 3-4 day old mated female *P. longipes* was introduced to each of the first 5 plants by placing the predator on a single leaf at the top of the plant. The additional 5 plants containing only prey individuals served as predator-free controls.

Part 2. Five plants were each infested with 25 *T. pacificus* females and one *P. persimilis* female in the same manner as in part one.

Part 3. Five plants were each infested with 25 *T. pacificus* females and two *P. longipes* females in the same manner as in part 1 and 2. The plants were placed in the wooden tray and spaced so that they did not touch each other. All stages of both predator and prey were counted on each plant every four days.

Spatio-temporal relationship between predator and prey populations. The parameter m^*/m (Lloyd, 1967) was used to estimate the patchiness or degree of aggregation between the populations of the prey and the predators, where m is the mean density and the m^* is the mean crowding, i.e., number/individual of other individuals/quadrat. The degree of spatial overlapping in the distribution between the populations of prey and each of the predators was estimated using the Kuno's overlapping index (Kuno, 1968). The value for each of the above two indices was calculated for the interaction between populations of the prey and each of the predators, on the basis of data gathered from the first 4 population censuses after the start of experiment, and the mean values for each index was calculated at each population census. Both predators required an average of 12-16 days to suppress the prey population. The degree of spatial overlapping between the distribution of both predators and their prey declined with time and decreasing prey density. The degree of aggregation of the prey increased while that of both predators decreased with prey decline and time. Regression between mean crowding and mean density revealed a clumped distribution in the population of the prey and the predators.

Session 9: Role of Generalist Predators in Biological Control

GENERALIST PREDATOR COMMUNITIES EXERT TOP-DOWN CONTROL OF A NEW INVASIVE PEST, THE SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA)

Alejandro C. COSTAMAGNA and Douglas A. LANDIS

Department of Entomology
204 Center for Integrated Plant Systems
Michigan State University
E. Lansing MI 48824, U.S.A.

costamag@msu.edu, landisd@msu.edu

The soybean aphid (*Aphis glycines* Matsumura) (Heteroptera: Aphididae), is a major new invasive pest of soybean in North America. In 2003, over 42 million acres of soybean in the North Central US were infested and over 7 million acres were treated with insecticides to control soybean aphid. A complex of generalist predators including both native and previously introduced natural enemies, is abundant in soybean fields and may contribute to soybean aphid regulation. Alternatively, host plant quality, pathogens and abiotic conditions may combine to influence the abundance of this pest. During 2003, we conducted field studies to study the relative impact of generalist predators (top-down regulation) and agronomic practice (bottom-up regulation) on *A. glycines* by contrasting aphid growth under zero, low and conventional high-input production practices with and without access by predators. In addition we investigated the role of the generalist predator community in suppressing aphid

populations at three spatial scales; patches within a field, plants within patches, and within-plant distribution. In both studies, the major natural enemy species observed included *Harmonia axyridis*, *Coccinella setempunctata* (Coleoptera: Coccinellidae) and *Orius insidiosus* (Heteroptera: Anthocoridae).

Experiments reveal a significant top-down effect of generalist predators on aphid abundance with 3 - 10 fold higher aphid numbers in exclusion cages over sham or control treatments. There was also a smaller (1.5 - 2 fold) bottom-up effect when predators were excluded, indicated by a significant interaction between natural enemies and agronomic treatments. In 2004, we conducted a similar experiment in a field under conventional high-input production practices and found a 200 fold reduction in aphid numbers in sham or control treatments over exclusion cages. This season-long control resulted in a significant biomass increase of 2 fold and yield increase on the plants exposed to naturally occurring generalist predators over predator exclusion cages. These results present strong evidence of top-down regulation due to predator assemblages coupled with comparatively weak bottom-up effects. At the field scale, predators rapidly detected high-density patches of aphids and reduced their abundance and net replacement rates. At the plant level, the community of generalist predators reduced aphid populations to similar low levels, despite initial aphid density. Finally, at the within plant level, the proportion of aphids at each node was shifted lower on the plant in the presence of predators indicating that these areas may constitute a refuge from predation allowing aphid persistence in the field. As a whole, these studies document the critical importance of the generalist predator community in regulating soybean aphid abundance and suggest practices that may conserve and enhance their effectiveness.

IMMATURE PREDATORS: AN UNDERAPPRECIATED RESOURCE FOR BIOLOGICAL CONTROL

Matthew GREENSTONE¹, John RUBERSON², and Jorge TORRES³

¹USDA-ARS Insect Biocontrol Laboratory
Beltsville, MD 20705 U.S.A.
greenstm@ba.ars.usda.gov

²Department of Entomology
University of Georgia
Tifton, GA 31794 U.S.A.
ruberson@uga.edu

³Departamento de Agronomia, Entomologia
Universidade Federal Rural de Pernambuco
Recife, PE 52171-900, Brasil
jtorres@ufrpe.br

46

The vast majority of arthropod predators that are considered important natural enemies in agriculture and forestry are predaceous not only as adults but also as larvae. However, for all but a few groups, such as coccinellids, chrysopids, and syrphids, we know almost nothing about the significance of the immatures in biological control. This ignorance arises from difficulties in identification and sampling. Identification of adult predators is challenging enough when closely related species are found at a given locality, but identification of the immature stages is especially problematic, because distinguishing morphological features are lacking or difficult to use. Using data from carabids and spiders, we show that immatures can be correctly identified by using polymerase chain reaction (PCR) primers to amplify cytochrome oxidase I sequences of immatures and matching them to those of correctly identified adults. The sampling problem is less tractable: many sampling technologies, such as sweeping and pitfall trapping, do not yield absolute density estimates, but rather some index of activity and density that is affected by stage- and species-specific responses to weather and catchability. Also, adults and immatures may tend to be found in different strata, e.g., carabid beetles, whose adults are mainly epigeal or foliar while the larvae tend to be found in litter and soil, sometimes to depths of several centimeters.

Here we present previously unpublished absolute density data from cotton and potato, and review the quantitative literature from other cropping systems. We show that immature predator densities can be considerable, often rivaling or exceeding those of adults. Absolute density data, coupled with PCR-based species identifications and molecular gut analysis, will enable an appreciation of the biocontrol potential of immature arthropod predators.

CARABID BEETLE ASSEMBLAGES IN INSECTICIDE-SPRAYED AND UNSPRAYED POTATO FIELDS

Kiyomitsu ITO

National Agricultural Research Center for Hokkaido Region
1 Hitsujigaoka
Sapporo, Hokkaido 062-8555, Japan

kymt@affrc.go.jp

Aphids, which cause direct sucking damage and transmit virus diseases, are the most important pest insects in northern Japan, and the farmers usually spray insecticides for this pest 3 – 4 times on average during a cropping season. However, a recent study (Ito et al., in press) showed that no aphid outbreaks occurred in the field when no insecticides sprayed due to the activity of indigenous predators (i.e. *Harmonia axyridis* (Pallas), *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *Orius* spp. (Heteroptera: Anthocoridae)), nor other leaf-eating pests such as *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) were conspicuous. It is considered that generalist predators are also contributing to leaf-eating pests control in the unsprayed potato field. Therefore, ground beetles assemblages were surveyed in the insecticide-free (=N) and conventionally sprayed (=S) plots. From early July to mid-August, total of 13 and 11 carabid species were collected in N and S plots, respectively. The most abundant species was *Bembidion morawitzi* Csiki (Coleoptera: Carabidae) in both plots (84.8% of the total catches in N plot and 77.1% in S plot) (see Table). Several dominant carabids (i.e. *B. morawitzi*, *Pterostichus planicollis* Motschulsky, *P. haptoderoides japonensis* Tschitscherine, *Amara chalcites* DeJean, *Chlaenius pallipes* Gebler and *Campalita chinense* (Kirby)) were experimentally confirmed that they fed lepidopteran larvae. Diversity indices calculated showed that S plot was more diverse than N plot. This might be due to the smaller catches of the most abundant species, *B. morawitzi* in S plot, though the species richness did not differ much. Since *B. morawitzi* is smaller in size, this species is likely to be more susceptible to insecticides than other species, but details are unknown. These results indicate that carabid beetle assemblage and diversity do not remarkably change even if conventional aphid control was carried out, unlike the case of aphid predators.

Total number of carabid beetles collected by pitfall traps in the potato field (July 3 – August 13, 2004).

Species	Applx. Size	N 0(%)	S (%)
<i>Bembidion morawitzi</i>	4 mm	870 (84.8)	356 (77.1)
<i>Pterostichus planicollis</i>	11 mm	54 (5.2)	28 (6.1)
<i>Amara chalcites</i>	9 mm	30 (2.9)	35 (7.6)
<i>Chlaenius pallipes</i>	14 mm	13 (1.3)	8 (1.7)
<i>Anisodactylus signatus</i>	12 mm	12 (1.2)	18 (3.9)
<i>Bembidion paediscum</i>	3 mm	12 (1.2)	3 (0.6)
<i>Clivina fossor sachalinica</i>	6 mm	10 (0.9)	6 (1.3)
<i>Campalita chinense</i>	30 mm	7 (0.7)	4 (0.9)
<i>Dolichus halensis</i>	19 mm	7 (0.7)	0 (0)
<i>Pterostichus haptoderoides japonensis</i>	9 mm	5 (0.5)	2 (0.4)
<i>Amara plebeja</i>	6 mm	3 (0.3)	1 (0.2)
<i>Bembidion semilunium</i>	6 mm	2 (0.2)	1 (0.2)
<i>Chlaenius micans</i>	16 mm	1 (0.1)	0 (0)
Total		1026 (100)	462 (100)

STUDY ON POPULATION DYNAMICS OF EUROPEAN ELM SCALE (HOMOPTERA: ERIOCOCCIDAE) AND ITS PREDATOR, EXOCHOMUS UNDULATUS (COLEOPTERA: COCCINELLIDAE) IN ISFAHAN, IRAN

Alireza JALAIZAND¹, Bijan HATAMI², and Jahangir KHAJEHALI³

¹College of Agriculture, Islamic Azad Univ., Ardestan Branch
Ardestan, Iran
jalalizand@yahoo.com

²Dept. of Plant Protection, College of Agriculture
Isfahan Univ. of Technology, Iran
bhatami@cc.iut.ac.ir

³Dept. of Plant Protection, College of Agriculture
Isfahan Univ. of Technology, Iran
khajeali@cc.iut.ac.ir

The European elm scale *Gossyparia spuria* (Modeer) (Hemiptera: Eriococcidae) is one of the most serious pests of the elms in Iran, Isfahan. An investigation of the population dynamics of European elm scale and its natural enemies revealed that the two coccinellid species, *Eriococcidae undulatus* Weise and *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae), were important predators of this pest. Of these two predators, *E. undulatus* had the highest density. This study was conducted by weekly sampling during 2000 and 2001. Twenty elm trees were randomly selected and the coccinellids on 10 infested branches of each elm tree were collected and counted. In order to investigate the population changes of different life stages of European elm scale, four branches (approximately 30 cm length and 2 cm diameter) were taken weekly from the four cardinal directions of selected trees and the number of each stage was recorded. Adults of *E. undulatus* were active in mid March and began to feed on overwintered *G. spuria* nymphs. The coccinellid population increased rapidly with the increase of temperature and peaked in early June. The population changes of *E. undulatus* were synchronized with the population changes of different stages of *G. spuria* including adults, first nymphal instar and eggs. The coccinellid population decreased rapidly in summer and fall, whereas high populations of *G. spuria* second nymphal instars were observed. In essence, this predator could be regarded as a potential biological agent to control *G. spuria* in the long term and it will be necessary to continue these studies to observe the efficiency of this predator.

Session 9: Role of Generalist Predators in Biological Control

WHICH ARE THE KEY INVERTEBRATE PREDATORS OF EGGS AND LARVAE OF SCARABS?

Anita JUEN and Michael TRAUGOTT

Centre for Mountain Agriculture & Institute of Zoology and Limnology
University of Innsbruck
Technikerstraße 13
6020 Innsbruck Austria

anita.juen@uibk.ac.at
michael.traugott@uibk.ac.at

Larvae of the Scarabaeidae (Coleoptera) are amongst the most abundant and most widespread soil-living pests. They feed on roots and thereby cause considerable damage in grassland, arable land and forestry. Augmentative natural control by bacterial pathogens, fungi and entomopathogenic nematodes are of restricted use due to their sensitivity to environmental conditions and the high production costs compared with the crop value. Conservation natural control by soil-living invertebrate predators may be an inexpensive alternative strategy to regulate white grub populations in the long term. However, up to now, the key invertebrate predators of the most abundant European scarab pests, *Melolontha*, *Amphimallon* and *Phyllopertha*, are unknown.

We developed a PCR-based approach to detect scarab-DNA in the gut of soil-living predators. The new method has been tested in laboratory feeding experiments with one of the most abundant soil living generalist predators in grasslands, *Poecilus versicolor* (Sturm) (Coleoptera: Carabidae). The detection times differed among prey species and depended on the length of the amplified nucleic acid sequence. The half-lives of detectability ranged from 20 to 32 hours.

In contrast to earlier studies on trophic interactions with DNA-based methods, copurified inhibitors caused considerable problems in PCR. Widely used extraction and purification methods failed to eliminate the inhibitors or resulted in an unacceptable high loss of nucleic acids. The inhibition could be reversed by adding BSA in high concentrations to the amplification cocktail. To exclude false negative results in field caught predators a multiplex PCR was developed: the combination of predator-specific primers and prey-specific primers allowed testing for inhibition and detection of prey DNA simultaneously.

For the correct interpretation of field derived data it is highly important to distinguish between scavengers and active predators, as the former group has no or only an indirect influence on the prey population. Feeding experiments showed that carrion prey is equally well detected from the gut of predators as fresh prey. This means additional approaches have to be included to estimate the magnitude of scavenging. First results on field caught soil-living predators including larvae of Carabidae and Staphylinidae as well as Geophilidae will be presented.

Session 9: Role of Generalist Predators in Biological Control

IMPACT OF GENERALIST PREDATORS ON SURVIVAL OF EGGS OF *CHRYSOPERLA CARNEA*

Zahra RAFIEI KERAHROODI¹ and Bijan HATAMI²

¹Department of Plant protection, College of Agriculture
Azad University of Arak
Arak, Iran
z-rafiie@iau-arak.ac.ir

²Department of Plant Protection, College of Agriculture
Isfahan University of Technology
Isfahan, Iran

51

One of the natural enemies of aphids is *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysoptera), the green lacewing, which, in biological programs, is usually released in the egg stage. Predators of the egg have direct effect on the survival of *Chrysoperla carnea* eggs. To compare the effect of an ant as a generalist predator on the survival of lacewing eggs, three methods of egg release were conducted 1) chrysocard, 2) chrysobag and 3) eggs mixed with sawdust, all in 5 replications. Lacewing eggs were released on cabbage plants where ants were present. After 24 hours the remaining healthy eggs were counted. In chrysocard all eggs were healthy, but in chrysobag 35.6 and 0% and with the sawdust method 59.6 and 7.6%, respectively. The results showed that the chrysobag method protects eggs better than other methods.

SEASONAL ABUNDANCE OF PREDATORS OF THE ELM APHID, *TINOCALLIS NEVSKYI*, IN SHAHREKORD, IRAN

Jahangir KHAJEHALI¹ and Bijan HATAMI²

¹Dept. of Plant Protection, College of Agriculture
Isfahan Univ. of Technology
Isfahan, Iran
khajeali@cc.iut.ac.ir

²Dept. of Plant Protection, College of Agriculture
Isfahan Univ. of Technology
Isfahan, Iran
bhatami@cc.iut.ac.ir

The seasonal abundance of predators of the elm aphid, *Tinocallis nevskyi* Rem., Que. and Heie (Homoptera: Aphididae) was studied in Shahrekord. The predators were sampled with a standard sweep net. The samples of 100 sweeps were taken each week in spring and summer 2003. The elm aphid was sampled on 20 randomly selected trees once per week. Eight shoots were removed from the two heights at ground level and four cardinal directions of selected trees. The number of elm aphids per five randomly selected leaves per shoot was recorded. Seasonal fluctuations of the aphid and predators consisted of Coccinellidae (four species), Miridae, Chrysopidae and spiders were compared. Peaks of populations of Chrysopidae and spiders as well as total population of predators were coincided with the peaks of aphid. Peaks of an active coccinellid, *Oenopia conglobata* L. were observed after the peaks of elm aphid. This lag was due to the developmental time of the coccinellid from larval to adult stages. In spite of decrease of aphid population in summer, some predator populations were increased. This was probably due to increase of other preys such as scale insects on elm trees. The findings can be used in assessing predator potentials in biological control of the elm aphid and choosing suitable selective insecticides and timing of applications.

RAPID SCREENING OF THE GUTS OF INVERTEBRATE PREDATORS TO DETECT DNA FROM MULTIPLE PREY

**R. Andrew KING, Georgina L. HARPER, Samuel K. SHEPPARD,
Daniel S. M. READ, James D. HARWOOD, David M. GLEN,
Michael W. BRUFORD, and William O. C. SYMONDSON**

Cardiff School of Biosciences, Cardiff University
P.O. Box 915
Cardiff, CF10 3TL, UK

kinga2@cardiff.ac.uk
glharper@glam.ac.uk
sheppardsk1@cardiff.ac.uk
readdsm@cardiff.ac.uk
james.harwood@uky.edu
davidmglem@btopenworld.com
brufordmw@cardiff.ac.uk
Symondson@cardiff.ac.uk

It is now possible, for the first time, to rapidly screen large numbers of invertebrate predators to identify the whole range of prey that may be present in their guts, simultaneously. In the past molecular approaches (protein electrophoresis, monoclonal antibodies, PCR) have concentrated upon screening potential natural enemies to confirm trophic links with one or a few major crop pests. Little attention was given to the role of non-pest prey as alternative food for the predators and their effects on the dynamics of the predator-pest interactions. Alternative prey both help sustain predator numbers and, potentially, divert predators away from feeding on pests. Here we describe two approaches that allowed us to study the complex food webs involved.

We developed a multiplex PCR to analyse the gut contents of carabid beetles for 10+ prey targets simultaneously. The prey included various species of aphids, weevils, earthworms and molluscs. During the PCR DNA from many different prey were amplified in the same PCR reaction. Use of fluorescent-labelled primers allowed the PCR products to be screened using a fragment size analyser (an ABI377 or ABI3100 sequencer). As each amplicon for each prey species was a different size or labelled with a different marker, software could assign an identity to each prey DNA fragment. Prey choice could be analysed by comparing the ratios of different species present in the field and in the guts of the predators.

Our multiplex PCR depended upon pre-designing prey-specific primers for each target species. Any prey in the guts for which we did not have primers would go undetected. However, in research concerned with predator responses to prey diversity, 'diversity' becomes the parameter of primary interest. We developed a temperature gradient gel electrophoresis

(TGGE) approach using general invertebrate primers, or conserved primers that amplified groups of prey, such as all aphids or all earthworm species. Small difference between sequences of as little as a single base pair could be clearly separated by TGGE. Although we could run prey standards to confirm species identity, all species within groups would be detected, even those for which we had no sequence information. Prey diversity within the guts of predators can be compared with diversity in the field, revealing predator responses to diversity amongst prey resources.

SESSION 9: ROLE OF GENERALIST PREDATORS IN BIOLOGICAL CONTROL

A TALE OF TWO ENEMIES: WILL THE INTRODUCTION OF A GENERALIST PREDATOR IMPROVE OR DISRUPT BIOLOGICAL CONTROL?

**Sarah MANSFIELD¹, Alison HOPKINS², John BAIN¹, Diane JONES¹,
and Dean SATCHELL³**

¹Forest Research

PB 3020

Rotorua, New Zealand

sarah.mansfield@forestresearch.co.nz

²Department of Science and Technology

University of Waikato

Hillcrest Road

Hamilton, New Zealand

³33(b) Skudders Beach Road

Kerikeri, New Zealand

Generalist predators may act as either an additional source of pest mortality and therefore increase pest suppression or they may suppress more specialised control agents, thus releasing the pest from control. In New Zealand, the recent decision to mass rear and release a predatory ladybird against the eucalyptus tortoise beetle, *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae), has provided an opportunity to examine the effect of a generalist predator on a pest and its specialist parasitoid.

P. charybdis was accidentally introduced into New Zealand from Australia in the early 1900s. Adults and larvae feed on eucalypt foliage and this species is a serious pest of eucalyptus plantations in New Zealand. A coccinellid predator, *Cleobora mellyi* Mulsant (Coleoptera: Coccinellidae), was introduced from Tasmania on several occasions from 1979 to 1987. *C. mellyi* became established at only one release site in the South Island (Maori Bay, Marlborough Sounds) and the species persists there today. While *C. mellyi* feeds readily on *P. charybdis* eggs it is not specific to this pest, requiring additional prey, particularly psyllids, to mate and

reproduce successfully. Only one psyllid species was present in New Zealand at the time of introduction so there may have been insufficient prey for *C. mellyi* to establish more widely. Several new eucalyptus psyllid species have established in New Zealand since the original release of *C. mellyi*. A more specific control agent, *Enoggera nassau* (Girault) (Hymenoptera: Pteromalidae), which is a solitary egg parasitoid of *P. charybdis*, was first introduced to New Zealand from Western Australia in 1987 and 1988. An additional release of a cool-adapted strain from Tasmania took place in 2000. This parasitoid is well established in New Zealand and attacks a high proportion of *P. charybdis* eggs.

The possibility that *C. mellyi* will establish more readily now that prey sources on eucalypts have increased will be tested following additional releases. Adults and larvae were collected from the Maori Bay population and mass reared over summer for field releases in the North Island in 2005. We will use this opportunity to examine intraguild interactions between the two agents. Parasitised eggs of *P. charybdis* are exposed to predation longer than unparasitised eggs, because *E. nassau* takes longer to hatch than *P. charybdis*. Feeding preferences of *C. mellyi* for parasitised and unparasitised eggs of *P. charybdis* were measured in comparison with psyllid prey, under laboratory and semi-field conditions. The possible repercussions of widespread establishment of this predator on the biological control of *P. charybdis* is discussed.

Session 9: Role of Generalist Predators in Biological Control

GENERALIST PREDATORS – SPECIES COMPOSITION, MIGRATION, AND MOLECULAR DETECTION OF APHID PREDATION IN SWEDISH SPRING SOWN CEREALS

Sandra ÖBERG and Anna-Karin KUUSK

Department of Entomology, Swedish University of Agricultural Sciences
Uppsala, Sweden

Sandra.Oberg@entom.slu.se
Anna-Karin.Kuusk@entom.slu.se

Spiders and carabid beetles are generalist predators and consume a large number of different arthropods including major agricultural pests. An important pest in cereals in Sweden is the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Homoptera: Aphididae). If generalist predators are to prevent the population of *R. padi* from growing beyond economic thresholds, the predators have to find and consume aphids as soon as the pest starts colonizing the field. Thus, it is important that the predators occur in the fields early in the season. Knowledge about the abundance and distribution of generalist predators at the time of aphid establishment together with quantification of insect predation by these predators will contribute to our understanding of the role of generalist predators for pest control.

The timing of spider and carabid dispersal, their abundance, and distribution were investigated for 3 weeks directly after sowing in cereal fields around Uppsala, central Sweden, in 2003 and 2004. Information was also generated about the species richness of generalist predators on the different farms. Data from 2003 has not yet been completely processed. Results from 2004 showed that both spiders and carabids were present in the field immediately after sowing. No migration movement from surrounding habitats was detected. Different species showed different distributions in the fields. Many spider- and carabid species were bound to the field edge. However, some species were found uniformly spread in the field or even more abundant in the field than at the edge. This group of species included *Pardosa agrestis* (Westring) (Araneae: Lycosidae), *Oedothorax apicatus* (Blackwall) and *Meioneta rurestris* (Araneae: Linyphiidae), and *Bembidion lampros*, *Pterostichus cupreus* and *P. melanarius* (Coleoptera: Carabidae). Twelve Lycosidae, 36 Linyphiidae, and 41 carabid species were found in the fields. Also, an additional 10 spider families were found.

We are also investigating the biological control efficacy of the dominant predators. A method for quantification of insect predation is detection of prey DNA in predators using the Polymerase Chain Reaction (PCR). In order to draw reliable conclusions about predation rates in the field when using this method, it is critical to ascertain how long after a meal it is possible to detect prey DNA remains. As an initial step the half-life (the time after which only half of the meals eaten can be detected) of DNA from *R. padi* was determined for *P. cupreus*, *Bembidion* spp. and *P. agrestis*. Field collected predators were starved and then fed one *R. padi*. After consuming the prey, predators digested their meal for different time periods. Total DNA was extracted followed by PCR reactions with *R. padi* and aphid specific primers.

The entire analysis is not yet complete but preliminary results show that it is possible to detect DNA from one single *R. padi* in all three predatory species. However, the number of positive individuals per time period seems to decline rapidly. In future experiments the effect of temperature on DNA half-life will be examined. This information is essential for design of field collection methods.

Session 9: Role of Generalist Predators in Biological Control

PREDATION RATE AND FUNCTIONAL RESPONSE OF THE PREDATOR *MACROLOPHUS PYGMAEUS* ON THE APHID *MYZUS PERSICAE*

**A. FANTINO¹, D. MASSELOU¹, P. LABROPOULOS¹, D. PERDIKIS²,
and D. LYKOURESSIS²**

¹Laboratory of Ecology and Environmental Sciences
Agricultural University of Athens
Iera Odos 75
188 55 Athens, Greece

²Laboratory of Agricultural Zoology and Entomology
Agricultural University of Athens
Iera Odos 75
188 55 Athens, Greece
dperdikis@aua.gr

The predation rate of the predatory bug *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) was studied for all instars of the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae). A leaf of eggplant was placed in a petri dish with 4, 8, 12, 16, 20, or 24 aphids of each instar. One, fifth instar nymph of the predator was introduced into the dish after a 24hr exposure to an eggplant leaf without prey. Numbers of attacked aphids were recorded 24hr after the introduction of the predator into the dish. The experiments were conducted at 20°C and 25°C, a L16:D8 photoperiod, and 65 ± 5% R.H. For each aphid density, 10 replicates (i.e., 10 predators) were tested. The predation rate was found to be higher at 25°C than 20°C, whereas among the aphid instars, predation was highest on the first instar and decreased proportionally with increasing aphid instar. Apart from attacked aphids, aphids dying from causes other than predation were also recorded, and this number was positively related to aphid instar. Results were used to estimate the functional response curves for *M. pygmaeus*.

57

ENCOUNTERS BETWEEN PREDATORS AND PREY: MOLECULAR DETERMINATION OF WHEN PREDATION OCCURS

**Samuel K. SHEPPARD¹, James R. BELL², John FENLON², David SKIRVIN²,
Keith D. SUNDERLAND², and William O. C. SYMONDSON¹**

¹Cardiff School of Biosciences, Cardiff University
P.O. Box 915
Cardiff CF10 3TL, UK
SheppardSK1@cardiff.ac.uk

²Warwick-HRI, University of Warwick
Wellesbourne, Warwick, CV35 9EF, UK

58 PCR-based gut contents analysis is increasingly used to track predator-prey interactions within invertebrate foodwebs. The gut contents of predators can be analysed using species-specific primers to amplify prey DNA and confirm trophic links. The retention time for DNA within the gut of a predator during digestion is potentially affected by factors including temperature, quantity ingested and size of the target DNA molecule. There is increasing evidence that large DNA fragments are digested relatively quickly within insect guts and even with advanced PCR methodologies small DNA fragments (< 300 bp) are usually targeted to ensure successful amplification of prey DNA. We tested the hypothesis that prey DNA retention/detection time is inversely proportional to fragment length and that this effect can be calibrated to determine, though *post-mortem* analysis, when prey are consumed.

A limiting factor in PCR gut contents analysis is that each predator needs to be analysed with primers that target many different prey amplicons, even though the mean number of targets (usually different prey species) in each gut sample may be low. This requires a large number of separate PCRs and is potentially time consuming. The problem has largely been solved by using a multiplex-PCR approach. Here we found a similar multiplex approach to be effective for amplifying many different sized DNA fragments (71-350 bp) from the same target prey species (the aphid *Sitobion avenae* F. (Homoptera: Aphididae)). This approach successfully amplified degraded DNA from the guts of predators and could amplify and detect many mitochondrial DNA fragments simultaneously.

Primers were designed and selected that could, in combination, detect 13 different sized amplicons using multiplex PCR. Predators (carabids and spiders) were fed on aphids and then killed after a range of time periods. From this it was possible to calculate the time at which 50 % of beetles tested positive, the median detection period (T_{50}), for each DNA frag-

ment. This analysis confirmed that larger DNA fragments decayed more quickly in the predator gut and the relationship between T_{50} and fragment size modelled using regression analysis. The time at which a predator consumed its last aphid meal could, therefore, be determined from the T_{50} of the largest detectable aphid DNA fragment still present in its gut.

The multiplex-fragment analysis approach was then applied to beetles caught fortnightly (May-July) using pitfall traps in a winter wheat field to measure the effects abiotic (time of year, temperature, day length) and biotic factors (aphid numbers) on the time of day when most aphids were encountered and consumed.

Session 9: Role of Generalist Predators in Biological Control

IMPACT OF NATURALLY OCCURRING GENERALIST PREDATORS ON *APHIS PUNICAE* IN A POMEGRANATE ECOSYSTEM

Abraham VERGHESE¹, K. SREEDEVI¹, K. SUDHADEVI¹, and V. VASUDEEV²

¹Indian Institute of Horticultural Research
Hessaraghatta Lake – P.O.
Bangalore – 560 089, India
avergis@ihr.res.in

²Department of Applied Zoology
Kuvempu University, B. R. Project
Shimoga – 577 115, India

The pomegranate (*Punica granatum* L.) (Myrtales: Punicaceae) is a commercial fruit tree in parts of Asia, tropical Africa and Europe. The aphid, *Aphis punicae* Passerini (Homoptera: Aphididae) a serious pest infesting pomegranate, attracts a host of general predators. The study was carried out in an unsprayed 12-year old orchard, for three years (2000 – 2002) at Bangalore (12°58'2" N, 77°35'2" E), S. India. For sampling the predators, each week 1600 shoots were randomly examined (80 shoots/tree x 20 trees). Concurrently, the aphids, weather parameters and crop phenological stages were also quantified. The data were subjected to suitable statistical analyses.

Species diversity. The diversity of predators consisted of three coleopterans two dipterans and a neuropteran. These were *Cheilomenes sexmaculata* (Fab.), *Scymnus* sp., *Pseudaspidemerus circumflexa* (Motsch.) (all Coleoptera: Coccinellidae); *Paragus serratus* (Fab.), *Ischiodon scutellaris* (Fab.) (both Diptera: Syrphidae) and *Chrysopa* sp. (Neuroptera: Chrysopidae).

Species abundance. The significantly most abundant predator was *C. sexmaculata* in all the three years, while others predators showed no difference statistically at $p = 0.05$. *C. sexmaculata* showed a high significant positive correlation with total predator counts ($r = 0.87, 0.94$ and 0.99 in 2000, 2001 and 2002, respectively). On an abundance scale, *C. sexmaculata* was followed by *Chrysopa* sp., *Scymnus* sp., *P. serratus*, *P. circumflexa* and *I. scutellaris*.

Numerical response. This was measured using correlation co-efficient 'r' ($p = 0.05$) and it was found that the predator *C. sexmaculata* had positive numerical response with the prey, *A. punicae* in 2000 and 2002 while *Chrysopa* sp. showed positive correlation in 2002. So, only these could be potential predators of *A. punicae* and the advantage is that both are amenable to mass rearing and release.

Control. Positive numerical response of predator numbers (x) brought about excellent reduction in prey population (y); ($y = 0.0027x + 0.0067, R^2 = 0.81$).

Weather and crop phenology effects. All the predators showed no correlation with major weather factors like temperature, relative humidity, wind speed and rainfall, implying that their occurrence was influenced more by the prey. However, the full mature leaf stage showed significant negative correlation with predator numbers; this again may be linked indirectly to fall in prey number, as at this stage, *A. punicae* density also showed a negative correlation with full mature leaf stage. Interestingly, at senescence stage of the tree, the predator numbers showed a surge with significant positive 'r' value opening new vistas of exploration; do yellow leaves act as cues or are predators responding to other homopterans?

The results of this long-term field study will be discussed in terms of sustainable biological control of *A. punicae* and conservation of the predators.

BIOLOGICAL CONTROL OF THE ROSY APPLE APHID – AN UTOPIAN DREAM?

Eric WYSS¹, Patrik KEHRLI², and Katharina BÜRCEL¹

¹Research Institute of Organic Agriculture
Ackerstrasse
CH-5070 Frick, Switzerland
eric.wyss@fibl.ch
katharina.buergel@fibl.ch

²Soil, Plant and Ecological Sciences Division
PO Box 84, Lincoln University
Canterbury, New Zealand
kehrli@lincoln.ac.nz

The rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), is one of the worlds most detrimental insect pests in apple production. Already single individuals of *D. plantaginea* are causing irreversible damage to leaves, branches and fruits which leads to severe yield losses. The common strategy to control the rosy apple aphid is to spray broad-spectrum insecticides in early spring. However, intense spraying resulted in the appearance of resistance and moreover suppressed the natural enemy complex of the aphids. Thus, the Research Institute of Organic Agriculture (FiBL, Switzerland) was involved in the development of alternative control strategies against the rosy apple aphid. Research was focused on the augmentation of the natural enemy complex of *D. plantaginea*. The aims of our studies were (1) to identify the most suitable control agent, (2) to investigate cost-effective mass-release strategies (eggs versus larvae and adults), (3) to define the optimal predator-prey ratio, (4) to determine the ideal date of release, (5) to understand the behaviour of the predators released and overall (6) to assess the effectiveness of augmentative releases.

Laboratory, semi-field and field screenings showed that the indigenous ladybeetle *Adalia bipunctata* L. (Coleoptera: Coccinellidae) was the most promising aphidophagous antagonist. Mass-releases of *A. bipunctata* pointed out that eggs are not suitable for the release in early spring. However, the release of larvae significantly reduced aphid density, but failed to suppress pest population below the economic threshold level, even at very high predator-prey ratios. Thus, research was focused to target the autumn generation of *D. plantaginea*. Once again the cold weather conditions prevented the hatching of *A. bipunctata* eggs. But the release of larvae decreased the deposition of over-wintering aphid eggs and consequently fewer aphids were observed the following season. Moreover, the release of adults indicated that *A. bipunctata* tended to stay on trees where they were released and significant reductions of aphids could be achieved. Overall, all attempts to reduce *D. plantaginea* below the economic threshold failed. However, the very low threshold value of one rosy apple aphid per fifty buds is a major challenge for any control strategy.

We conclude that the augmentation of natural enemies by mass-releases of ladybird beetles should be considered as a component of an integrated control strategy for *D. plantaginea* in the future. Augmentative release in combination with conservation biological control measures and cultivation of resistant apple varieties could lead to a more sustainable control strategy against *D. plantaginea*, in particular in regions (e.g. Belgium) where insecticide-resistance has evolved.

Session 10: Augmentative Biological Control in Outdoor Annual Crops

CHILO SACCHARIPHAGUS BOJER (LEPIDOPTERA: CRAMBIDAE) IN MOZAMBICAN SUGARCANE-A CASE FOR AUGMENTATION OR CLASSICAL BIOCONTROL, OR BOTH?

D.E. CONLONG¹ and F.R. GOEBEL²

¹ South African Sugarcane Research Institute
Private Bag X02, Mount Edgecombe
KwaZulu-Natal, 4300, South Africa
and

University of KwaZulu-Natal
School of Botany and Zoology
Private Bag X01, Scottsville
Pietermaritzburg, 3209, KwaZulu-Natal, South Africa
Des.conlong@sugar.org.za

² Centre de Coopération Internationale en Recherche Agronomique pour le
Développement
Annual Crops Department, Sugarcane Unit Research
Montpellier, France
Goebel@cirad.fr

In 1999, the spotted stalk borer *Chilo sacchariphagus* Bojer (Lepidoptera: Crambidae) was identified from bored sugarcane at two estates in Sofala Province, Mozambique. This borer, originating from Southeast Asia, is a major sugarcane pest in the Indian Ocean Islands of Mauritius, Madagascar and Reunion. It is the first time it has been recorded as a pest on mainland Africa, and poses a threat to the sugar industries of Mozambique and surrounding countries.