



## Flora surrounding rice fields as a source of alternative prey for coccinellids feeding on the pests of rice

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**Abstract.** Coccinellids are effective predators and a key component of the predator guild in rice ecosystems. In order to enhance their efficacy, a study was undertaken to assess the seasonal movement of coccinellids into rice fields and the role of the surrounding flora on their colonization. The seasonal abundance of coccinellids and their prey was recorded on the rice crop and the surrounding flora at fortnightly intervals from 2012 to 2015. Coccinellid prey range was assessed using PAGE electrophoresis. The herbivorous insects associated with weeds were *Aphis gossypii* Glover, *Aphis craccivora* (Koch), *Cicadulina bipunctata* (Meli-char), *Schizaphis graminum* (Rondani), *Sitobion* sp., *Thaia oryzivora* Ghauri and *Zygina maculifrons* Matsumura. Of the species of coccinellids recorded in rice fields, *Harmonia octomaculata* (Fabricius), *Micraspis discolor* (F.), *Propylea dissecta* (Mulsant), *Coccinella transversalis* Fabricius, *Cheilomenes sexmaculata* (Fabricius), *Scymnus nubilus* Mulsant and *Brumoides suturalis* (Fabricius) were also recorded on weeds. The esterase profiles indicated that the leafhoppers and aphids on the weeds were the prey of the coccinellids before they colonized the rice fields. The coccinellids recorded on the weeds showed bands corresponding to the insects present on the weeds. Beetles collected from rice fields had different bands, some of which corresponded to the green leafhopper (GLH) *Nephotettix virescens* Distant, the brown planthopper (BPH) *Nilaparvata lugens* Stal and white backed planthopper (WBPH) *Sogatella furcifera* Hovarth infesting rice. In addition, some bands corresponded to hoppers and aphids that were present on the surrounding flora. The results indicate the importance of surrounding flora in the conservation and colonization of rice fields by coccinellids.

## INTRODUCTION

Generalist predators play an important role in regulating insect pests in agricultural ecosystems (Joon & Seung, 2001). Although their ecological role in pest management was underestimated in the past, recently, the importance of generalist predators has been reconsidered (Symondson et al., 2002). In particular, the development of molecular techniques for detecting the presence of prey in the guts of the predators has enhanced our understanding of the trophic links in agricultural ecosystems (Pompanon et al., 2012). By manipulating the abundance and assemblage of generalist predators it is possible to significantly affect the abundance of pests (Symondson et al., 2002). For example, a recent meta-analysis of small-scale field studies reveal that an increase in plant diversity, realized by a minimal control of weeds, fosters the activity of generalist predators in suppressing herbivorous pests (Dassou & Tixier, 2016). Generalist predators can thus be a key component of conservation biological control. Simple techniques for conservation of indigenous natural enemy diversity can

enhance natural control and prevent pest outbreaks. Conservation of native natural enemies also reduces pesticide use and their detrimental effects on the environment (Boreau de Roincé et al., 2012). Among generalist predators, some species of coccinellids are widely reported occurring in rice fields, where they mostly feed on aphids (Rekha et al., 2009; Shanker et al., 2013a).

Many weeds are also known to harbour coccinellids (Schmid, 1992; Kranz et al., 2002) on which they oviposit and feed on pollen/nectar and alternate prey. Some plants, in particular those belonging to the aromatic family Apiaceae, are more attractive than others (Schaller & Nentwig, 2000; Lixa et al., 2010). As a conservation biological control measure, these species of weeds can be used to increase the abundance of coccinellids in or within the borders of field crops (Wäckers & van Rijn, 2012). In this respect, it is of primary importance to investigate the prey spectrum of coccinellids and their seasonal movement into and from the rice field. In particular, knowledge of coccinellid trophic relationships would provide a better under-

standing of terrestrial food webs and the alternative food items that enhance the survival of these predators during periods when their main prey is scarce.

The natural food of coccinellids can be ascertained in several ways (Harwood & Obrycki, 2005; Weber & Lundgren, 2009; Hodek & Evans, 2012). Most studies are based on the dissection of their gut contents. Other techniques such as radio-labelling of prey, microscopic examination of faecal samples, electrophoresis, stable isotope analysis and ELISA using antibodies have been used in field studies with varying degrees of success (reviewed by Michaud & Harwood, 2012). Recent advances in molecular biology have resulted in the development of group-specific or species-specific DNA primers, which can be used in standard or multiplex PCR to identify the prey in a predators' gut (Michaud & Harwood, 2012). While this method is the most accurate for detecting a given type of prey it is expensive when screening a large number of predators for several types of prey. In addition, the development of an appropriate primer is rather time-consuming. In fact, primer design based on sequenced individuals, evaluation of prey specificity using a set of non-target organisms, primer sensitivity and laboratory feeding trials to estimate the DNA half-detectability time should be done before undertaking a field study (Harwood & Obrycki, 2005; Hodek & Evans, 2012). Considering the small size and cryptic nature of their prey, an analysis of the prey-specific enzymes in the gut of generalist predators, such as coccinellids, could be used to more rapidly identify their prey and define their feeding patterns. In this study, we used an electrophoretic approach rather than PCR-based methods. Our approach proved to be useful in a first assessment of the predators' food. In particular, when the prey and predator enzyme banding patterns are distinct, electrophoresis can be a cheap and quick tool for the detection of prey items in field-caught predators (Solomon et al., 1996). It has already been shown that electrophoretic techniques can be used to identify the prey of aquatic and terrestrial invertebrate predators (Lister et al., 1987; Dicke & De Jong, 1988; Schulz & Yurista, 1995; Corey et al., 1998). In order to determine the importance of alternative prey in conservation biological control we used an electrophoretic approach to detect the predation by coccinellids on essential and alternative prey occurring in rice fields and surrounding areas.

## MATERIALS AND METHODS

### Spatial and temporal distribution of coccinellids

Field and laboratory studies were carried out on the experimental farm of the ICAR-Indian Institute of Rice Research, Hyderabad, India for three years (2012–2015). In this region rice is grown during the wet season (July to October) and dry season (January to April). This study, therefore, continued throughout each year in order to include the two cropping seasons and the non-crop period in between.

The coccinellids were sampled every fortnight by sweep netting and visual counts in rice fields and on field bunds at fortnightly intervals at five different locations. Sampling was done in the morning up to 10:00. Ten sweeps were made in each rice field in plots of 100 m × 25 m and 40 m of field bund. Total coccinellid

abundance was averaged for the five locations in order to reveal the overall seasonal trend.

### Electrophoretic assessment of the prey of the coccinellids

A polyacrylamide gel electrophoresis (native PAGE) method, modified from Murray & Solomon (1978) and Giller (1982, 1984 and 1986) was used to characterize banding patterns for different species of prey and predator. Esterases were studied because they are ubiquitous enzymes in organisms and as they have species specific patterns they can be used to discriminate between the different species of prey (Murray & Solomon, 1978).

Two coccinellid species, *Harmonia octomaculata* (Fabricius) and *Micraspis discolor* (F.) and three hopper species, the green leaf hopper (GLH) *Nephotettix virescens* Distant, the brown planthopper (BPH) *Nilaparvata lugens* Stal and the white backed planthopper (WBPH) *Sogatella furcifera* Hovarth, were used to confirm the unique banding patterns for coccinellids and their prey. Adults of each species were released individually into 100 × 41 mm polystyrene Petri dishes with a 4-cm diameter ventilation hole in the lid covered with a 0.05 mm fabric mesh (SPL Life Sciences Inc., Korea). Five third-instar nymphs of each of the three species of planthopper from glasshouse cultures were placed in the Petri dishes. There were five replicates of each combination of predator and prey, totalling 15 adults per taxa. Beetles were removed for analysis after they were observed to feed on one hopper. In order to assess the feeding behaviour of coccinellids collected from field, the insects were analyzed in the following categories:

Adult beetles of *H. octomaculata*, *M. discolor*, *Propylea dissecta* (Mulsant), *Coccinella transversalis* Fabricius, *Cheilomenes sexmaculata* (Fabricius), *Scymnus nubilus* Mulsant and *Bruroides suturalis* (Fabricius) were collected in the field between 8:00 and 9:00, placed in a refrigerator below 4°C within 1 h of collecting and then quickly analyzed in order to preserve their prey content.

All possible homopteran prey viz., *Aphis gossypii* Glover, *Aphis craccivora* (Koch), *Schizaphis graminum* (Rondani), *Cicadulina bipunctata* (Melichar), *Thaia oryzivora* Ghauri and *Zyginia maculifrons* (Matsumura) were collected from weeds and preserved in a refrigerator below 4°C until analyzed.

The ricehopper pests viz., green leafhopper *N. virescens*, brown planthopper *N. lugens* and white backed planthopper, *S. furcifera* were collected from glasshouse cultures.

Adult coccinellids of all the species listed above were previously starved for 24 h so that their guts did not contain any remnants of prey. This allowed the identification of bands specific for each predator.

The signature banding pattern for esterase activity was identified using the Bio-Rad Mini-PROTEAN-3 apparatus following the procedure of Murray & Solomon (1978). Individual predators and prey were macerated in small glass wells using a glass rod. The sample buffer consisted of 6–10 µl aliquots of a 0.1 M Tris/HCl (pH 7.0) or a 0.15 M phosphate pH 8.2 buffer, with 0.2% Triton X-100 and 10% sucrose. The samples were later centrifuged for 10 min at 6500 rpm and the supernatant transferred using a micropipette to the sample holders at the top of the gel. 5–10 µl of supernatant from each sample was loaded onto the gels, and a constant current of 90 V was applied to the gels for 2.5–3 h, or until the molecular weight standard reached the bottom of the gel. After electrophoresis, the gel was stained briefly in sodium phosphate buffer pH 7.4 containing 0.1% fast blue RR and 0.05% α-naphthyl acetate for 30 min, and then photographed. The bands recorded for the field-collected coccinellids were screened against the bands of each individual prey, in order to detect the presence of any prey remnants in the predator.

The ranking procedure suggested by Hobson (1974) and adapted for studying the gut content of fish (Eya et al., 2011) was used based on the presence or absence of the prey items in the predators' gut as follows:

$$RI = (A/B) * (C)$$

where RI = Ranking Index of each prey item, A = number of coccinellids per taxa containing each prey item, B = total number of coccinellids per taxa with stomach contents, C = percentage of each prey item, which can be computed as:

$$C = \frac{\text{No. of each prey detected}}{\text{Total of all prey items combined}}$$

## RESULTS

### Spatial and temporal distribution of coccinellids

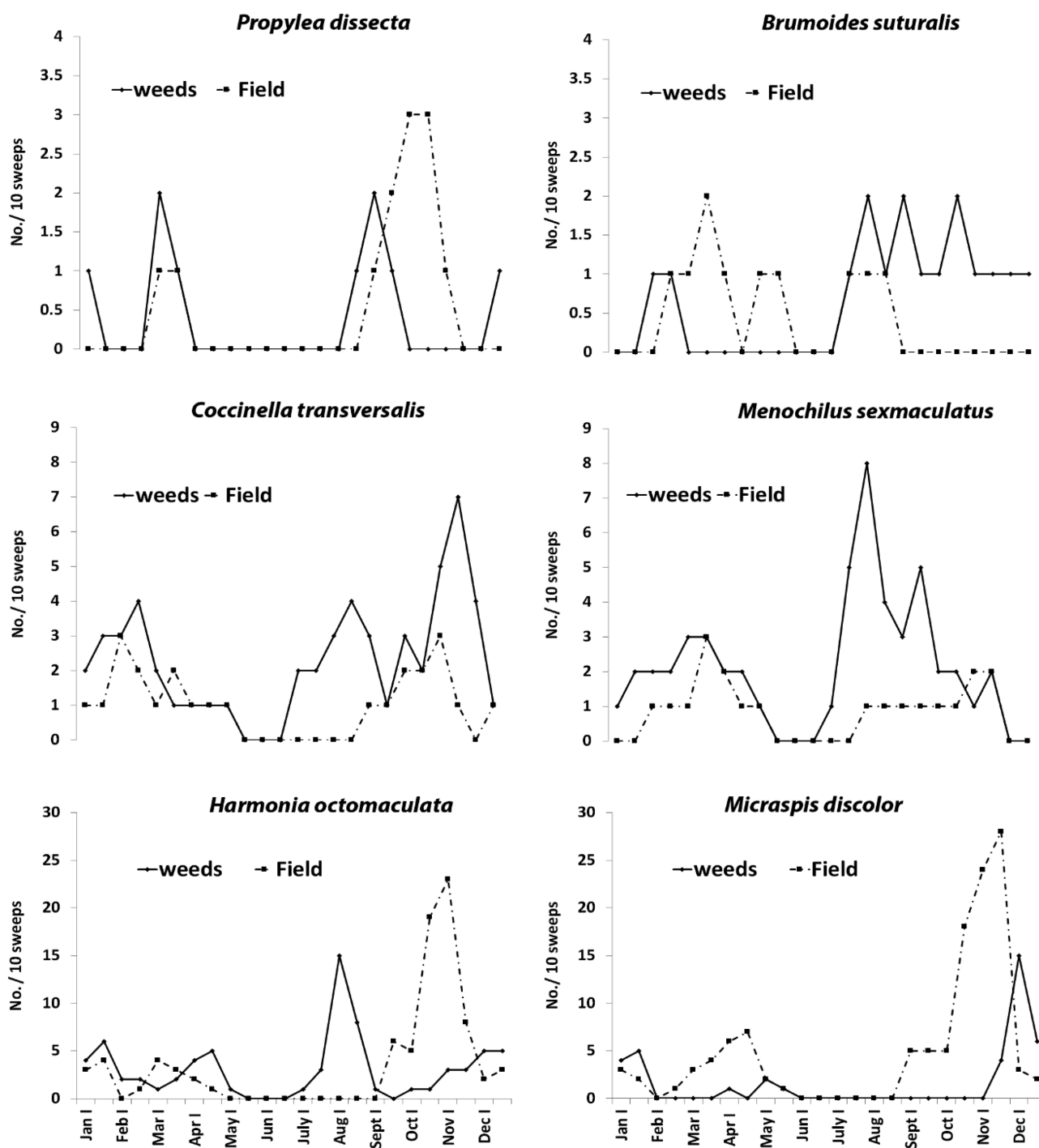
Around 30 weeds, including grasses sedges and broad leaved plants, were surveyed on field bunds and a total of 1620 coccinellids were collected from twelve species of plants over a period of three years. In total, nine species of coccinellids were recorded on weeds and on rice: *B. suturalis*, *C. sexmaculata*, *C. transversalis*, *H. octomaculata*, *Illeis cincta* (Fabricius), *M. discolor*, *P. dissecta*, *Scymnus latemaculatus* Motschulsky and *S. nubilus* Mulsant. All of them were recorded on weeds, while only seven were also recorded on rice. *Illeis cincta* was recorded on *Acmella uliginosa* where it was feeding on fungal spores. Direct observations confirmed feeding on insects or pollen on twelve species of weeds (Table 1). Feeding activity was also confirmed by the feeding studies conducted in the laboratory. In all the three years, *H. octomaculata*,

*P. dissecta*, *C. transversalis*, *C. sexmaculata*, *S. latemaculatus* and *B. suturalis* were recorded frequently on weeds during the early part of the season and on rice in both the wet and dry seasons (Fig. 1). Conversely, *M. discolor* was not recorded initially on weeds in the wet season, which is the main season for the rice crop. It was detected on rice during its tillering stage and then moved to weeds when the rice crop reached the harvesting stage (Fig. 1). In the dry season, however, a few were recorded on weeds that had moved from the rice crop to weeds. The phytophagous insects associated with weeds included *A. gossypii*, *A. craccivora*, *Schizaphis graminum* and *Sitobion* sp. (all Hemiptera: Aphididae); *C. bipunctata*, *T. oryzivora*, *Z. maculifrons* (Hemiptera: Cicadellidae) and all stages of *Altica cyanea* (Weber) (Coleoptera: Chrysomelidae). All species of coccinellids were recorded on weeds when the rice crop neared the harvesting stage both in December–January following the wet season and in April–May following the dry season. The numbers decreased over time, but coccinellids remained on the weeds until the start of the next rice crop. The presence of coccinellid eggs on the surrounding flora increased greatly as the rice approached the harvesting stage. Many adults of *M. discolor* and *H. octomaculata* were recorded on *Acmella uliginosa* (Sw.) Cass., *Ageratum conyzoides*, *Caesularia axillaris* Roxb., L. and *Eclipta alba* (L.) Hassk. (Family: Asteraceae) and *Echinocloa colona* (L.), (Family: Poaceae), when the rice crop was near the harvesting stage and the hopper population had also started diminishing in the field. The adults were observed mostly feeding on pollen from flowers and

**Table 1.** Visual observations of feeding by coccinellids on insects associated with weeds growing on rice bunds.

Weed	Food / Prey	Classification	Associated coccinellid	Stages observed
Family: Asteraceae				
<i>Tridax procumbens</i> L.	<i>Aphis gossypii</i>	Hemiptera: Aphididae	<i>Coccinella transversalis</i> Fabricius <i>Cheilomenes sexmaculata</i> (Fabricius) <i>Propylea dissecta</i> Mulsant	E, L, P, A A A
<i>Ageratum conyzoides</i> L.	<i>Myzus persicae</i> , pollen	Hemiptera: Aphididae	<i>Harmonia octomaculata</i> (Fabricius) <i>Micraspis discolor</i> (Fabricius) <i>Scymnus nubilus</i> Mulsant <i>C. sexmaculata</i>	A A A A
<i>Parthenium hysterphorus</i> L.	<i>Uroleucon</i> sp.	Hemiptera: Aphididae	<i>C. transversalis</i> <i>Scymnus latemaculatus</i> Motschulsky <i>H. octomaculata</i>	A, L, E A A
<i>Acmella uliginosa</i> (Sw.) Cass	<i>Aphis gossypii</i> , pollen, powdery mildew	Hemiptera: Aphididae	<i>M. discolor</i> <i>Illeis cincta</i> (F.)	A L, A
<i>Caesularia axillaris</i> Roxb	Pollen		<i>M. discolor</i>	A
<i>Eclipta alba</i> (L.) Hassk	Pollen		<i>M. discolor</i>	L, A
Family: Fabaceae				
<i>Macroptilium atropurpureum</i> (DC.) Urb.	<i>Aphis craccivora</i>	Hemiptera: Aphididae	<i>C. sexmaculata</i>	E, L, P, A
<i>Vigna trilobata</i> (L.) Verdc.	<i>Aphis craccivora</i>	Hemiptera: Aphididae	<i>C. sexmaculata</i>	E, L, P, A
<i>Chloris barbata</i> Sw.	<i>Sitobion</i> sp.	Hemiptera: Aphididae	<i>C. transversalis</i>	A, L
Family: Lythraceae				
<i>Ammania baccifera</i> L.	Eggs of <i>Haltica cyanea</i>	Coleoptera: Chrysomelidae	<i>H. octomaculata</i>	A, E, L, P
Family: Poaceae				
<i>Echinocloa colona</i> (L.)	<i>Schizaphis graminum</i> Pollen Pollen	Hemiptera: Aphididae	<i>C. transversalis</i> <i>M. discolor</i> <i>Brumoides suturalis</i> (Fabricius)	L, A A A
Family: Amaranthaceae				
<i>Alternanthera</i> sp.	Pollen		<i>M. discolor</i>	L, A

E – egg; L – larva; P – pupa; A – adult.

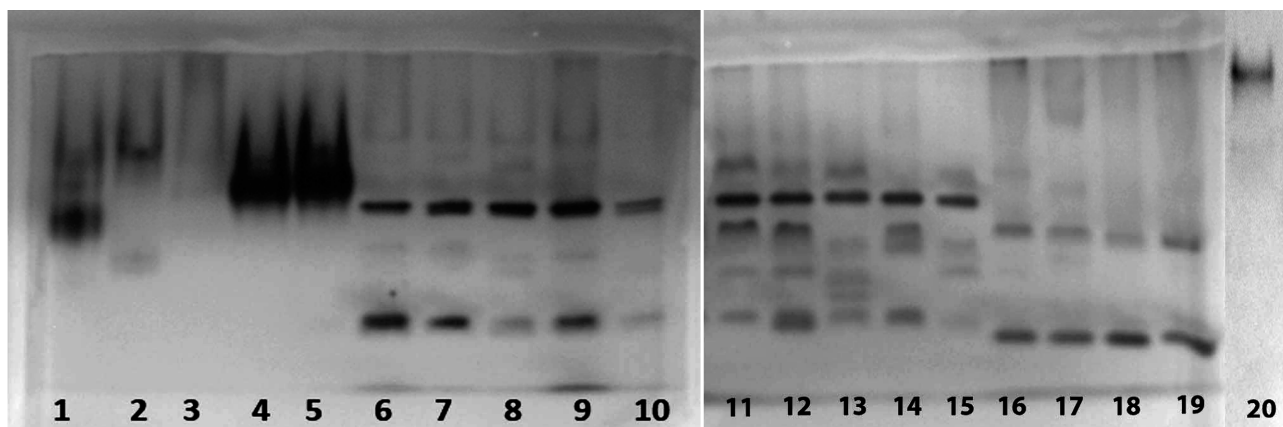


**Fig. 1.** Seasonal abundance of the species of coccinellids recorded in rice fields and on the surrounding flora. Abundances were recorded at fortnightly intervals with each point representing the mean of five replicates collected over a period of three years.

in some cases ovipositing on weeds. Notably, larvae of *H. octomaculata* were observed cannibalizing eggs. *C. transversalis* was recorded on *Chloris barbata* Sw. (Family: Poaceae) and *Parthenium hysterophorus* (Family: Asteraceae), and *C. sexmaculata* mainly on leguminous weeds such as *Macroptilium atropurpureum* (DC.) Urb and *Vigna trilobata* (L.) Verdc (Family: Fabaceae). After the rice harvest, coccinellid adults were recorded on all the surrounding green vegetation.

### Electrophoretic assessment of the prey of the coccinellids

Specific unique bands in the esterase banding patterns for the different predators and their prey were used to identify the different predators and prey (Figs 2 and 3). The comparison of the esterase banding pattern of *H. octomaculata* collected from rice fields (Fig. 2: Lanes 11–15) and weeds (Fig. 2: Lanes 6–10) with that of prey and starved coccinellids revealed that all the adults had additional bands that were not present in starved adults. These bands



**Fig. 2.** Esterase banding recorded for *H. octomaculata*, *M. discolor* and its prey collected from rice fields and weeds: Lane 1 – *Zygina maculifrons*; Lane 2 – *Thaia oryzivora*; Lane 3 – *Cicadulina bipunctata*; Lane 4 – *Sogatella furcifera*; Lane 5 – *Nilaparvata lugens*; Lanes 6–10 – *H. octomaculata* collected from weeds; Lanes 11–15 – *H. octomaculata* collected from rice field; Lanes 16–19 – *M. discolor* collected from field; Lane 20 – *Nephotettix virescens*.

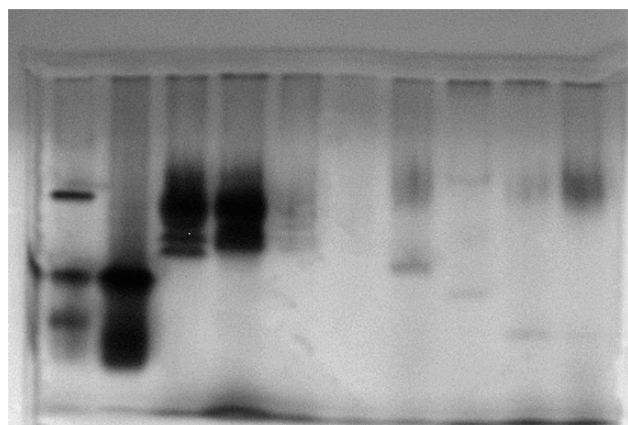
corresponded to *C. bipunctata* and *A. craccivora* from the leguminous creeper, *M. atropurpureum*, *S. graminum* collected from grass, *C. barbata* Sw and the three ricehoppers, mainly GLH and a few BPH and WBPH, which reveals their possible role as prey of *H. octomaculata*. Similarly, a few field collected *M. discolor* individuals showed bands that corresponded with the three species of ricehoppers. In addition, we also recorded other bands that did not correspond with the prey studied indicating that this coccinellid fed on other insects or possibly plant tissues. *Coccinella transversalis* exhibited bands that correspond to *A. gossypii* collected from *Tridax procumbens* L., *A. craccivora* from leguminous weeds and *S. graminum* from grasses, indicating that this predator mainly feeds on aphids (Fig. 2). *C. sexmaculata* had bands corresponding to only *A. craccivora* found on leguminous weeds where all stages of this predator were recorded. The results clearly indicate that leafhoppers and aphids infesting weeds were the main or alternative prey on which the coccinellids fed before moving to rice fields.

The ranking index of the coccinellids recorded in rice fields with regard to the prey recorded on weeds and in the

rice fields was 64% for *H. octomaculata*, which showed bands corresponding to all the ricehoppers, *N. lugens*, *S. furcifera* and *N. virescens*, and *T. oryzivora*, *C. bipunctata* and *Z. maculifrons* on weeds, and the aphids *A. craccivora*, *S. graminum* and *A. gossypii* on weeds. The green leafhopper *N. virescens* was the preferred prey of this coccinellid with a ranking index of 31.25 (Table 2A, B), followed by the brown planthopper *N. lugens* and *T. oryzivora* (Ranking index of 12.50). The higher preference for the rice pests, GLH and BPH, and all the prey on rice and weeds indicate that *H. octomaculata* might be a suitable candidate for conservation biological control programmes. There was prey in only the guts of 22% of the *M. discolor* adults, the most abundant coccinellid in the rice fields. A greater ranking index (27.27) was recorded for *A. gossypii* than for all three ricehoppers. Sixty to eighty % of the guts of *C. transversalis* and *C. sexmaculata* contained some prey. *C. transversalis* showed higher preference for *A. craccivora*, *A. gossypii* and *S. graminum* developing on weeds, while *C. sexmaculata* only exhibited bands corresponding to *A. craccivora*. *Brumoides suturalis* also had only consumed *A. craccivora*. On the other hand, *P. dissecta* showed equal preference for the *T. oryzivora*, *Z. maculifrons* and *S. graminum* infesting weeds. *Scymnus* spp. were aphidophagous as only the two aphids *A. craccivora* and *S. graminum* were detected in their guts.

## DISCUSSION

Surrounding vegetation and patches of plants seem to have a direct effect on the density of coccinellids by influencing their immigration and emigration patterns (Greze & Prado, 2000). The uncultivated patches of vegetation give stability to the agro-ecosystem by providing refuges, alternative prey, nectar and pollen for natural enemies, thus indirectly contributing to crop pest management (Landis et al., 2000; Sutherland et al., 2001). In order to utilize these reservoirs for conservation biological control it is necessary to understand the population dynamics of natural enemies such as coccinellids and their movement into crops from a particular resource or habitat (Gurr et al., 2000). In



**Fig. 3.** Esterase banding of some coccinellids and their prey. Lane 1 – *H. octomaculata*; Lane 2 – *Brumoides suturalis*; Lane 3–4 – *C. transversalis*; Lane 5–7 – *C. sexmaculata*; Lane 8 – *A. craccivora*; Lane 9 – *S. graminum*; Lane 10 – *A. gossypii*.

**Table 2A.** Prey ranking of coccinellids recorded in rice fields.

Prey	<i>Harmonia octomaculata</i>			<i>Micraspis discolor</i>			<i>Cheilomenes sexmaculata</i>			<i>Coccinella transversalis</i>		
	(A/B) * 100	C	RI	A/B	C	RI	A/B	C	RI	A/B	C	RI
<i>N. lugens</i>	8.00	12.50	1.00	4.00	18.18	0.73	0.00	0.00	0.00	0.00	0.00	0.00
<i>S. furcifera</i>	4.00	6.25	0.25	4.00	18.18	0.73	0.00	0.00	0.00	0.00	0.00	0.00
<i>N. virescens</i>	20.00	31.25	6.25	4.00	18.18	0.73	0.00	0.00	0.00	0.00	0.00	0.00
<i>T. oryzivora</i>	8.00	12.50	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>C. bipunctata</i>	4.00	6.25	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Z. maculifrons</i>	6.00	9.38	0.56	4.00	18.18	0.73	0.00	0.00	0.00	0.00	0.00	0.00
<i>A. craccivora</i>	6.00	9.38	0.56	0.00	0.00	0.00	80.00	100.00	80.00	20.00	33.33	6.67
<i>S. graminum</i>	4.00	6.25	0.25	0.00	0.00	0.00	0.00	0.00	0.00	20.00	33.33	6.67
<i>A. gossypii</i>	4.00	6.25	0.25	6.00	27.27	1.64	0.00	0.00	0.00	20.00	33.33	6.67

(A/B) \* 100 = % beetles with prey content; C = % of prey item in the total prey; RI = Ranking index = (A/B) \* C.

**Table 2B.** Prey ranking of coccinellids recorded in rice fields.

Prey	<i>Brumoides suturalis</i>			<i>Propylea dissecta</i>			<i>Scymnus nubilus</i>		
	A/B	C	RI	A/B	C	RI	A/B	C	RI
<i>N. lugens</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>S. furcifera</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>N. virescens</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>T. oryzivora</i>	0.00	0.00	0.00	20.00	33.33	6.67	0.00	0.00	0.00
<i>C. bipunctata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Z. maculifrons</i>	0.00	0.00	0.00	20.00	33.33	6.67	0.00	0.00	0.00
<i>A. craccivora</i>	20.00	100.00	20.00	0.00	0.00	0.00	20.00	50.00	10.00
<i>S. graminum</i>	0.00	0.00	0.00	20.00	33.33	6.67	20.00	50.00	10.00
<i>A. gossypii</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(A/B) \* 100 = % beetles with prey content; C = % of prey item in the total prey; RI = Ranking index = (A/B) \* C.

this study the movement of coccinellids from rice to weeds was especially marked just before the rice harvest, which also corresponds with a decline in the hopper populations both in the wet and dry seasons (Win et al., 2011). Villegas et al. (2013) report similar movements of coccinellids from thistles in field margins into alfalfa fields, which is synchronised with the changes in the availability of prey on the crop and weeds. The coccinellid population moving from weeds caused a reduction in the aphid population on alfalfa during November. Among the coccinellids included in this study, *H. octomaculata* has the widest prey spectrum on both the rice crop and weeds. In this respect, it is reasonable to use this species in conservation biological control programmes of planthopper pests of rice.

Feeding behaviour of predators can be assessed to a certain extent by direct observations of predation in the field, but is unlikely to identify all the possible interactions between predators and their possible prey spectrum (Harwood & Obrycki, 2005). Predation remains one of the most difficult ecological processes to study. A commonly used approach is the analysis of extracts of homogenized predators (or just their guts) using PAGE gels to record enzyme activity, especially that of esterases. A similar approach was used to assess predation by spiders in the field (Amalin et al., 2000). However, Amalin et al. (2000) only include in their analysis the band patterns recorded for spiders fed with particular prey items in a laboratory. In another study, the predation of *Coccus viridis* in coffee plantations by the foliage dwelling spiders, *Oxyopes* sp.,

*Dieta vires* (Thorell), *Olios milleti* (Pocock, 1901) and *Telomonia dimidiata* (Simon, 1899), was detected using esterase banding patterns (Senthil Kumar & Regupathy, 2009). Similarly, Traugott (2003) use an electrophoretic approach to analyse the prey spectrum of larvae and adults of *Cantharis* spp. collected in an open field system. The combination of our laboratory and field results clearly revealed that coccinellids feed on prey present on the rice crop and surrounding flora. The changes in their seasonal abundance showed that the populations on the crop and weeds were associated with seasonal changes in the spatial availability of prey, with weeds acting as a good reservoir for coccinellids during the lean season when prey is less abundant on rice. Selective conservation of the surrounding flora can enhance the fitness of natural enemies and lead to more effective pest management (Gurr et al., 2010; Shanker et al., 2013b; Horgan et al., 2016). Simple techniques for conserving non-crop flora will enhance the presence of native natural enemies and help create healthy and sustainable ecosystems and a possible reduction in the use of insecticides.

## REFERENCES

- AMALIN M.D., PENA J.E. & MCSORLEY R. 2000: Gut content analysis of three species of sac spiders by electrophoresis. — *Fla Entomol.* **83**: 489–492.
- BOREAU DE ROINCE C.B., LAVIGNE C., RICARD J.M., FRANCK P., BOUVIER J.C., GARCIN A. & SYMONDSON W.O.C. 2012: Predation by generalist predators on the codling moth versus a close-

- ly-related emerging pest the oriental fruit moth: a molecular analysis. — *Agr. For. Entomol.* **14**: 260–269.
- COREY D., KAMBHAMPAT S. & WILDE G. 1998: Electrophoretic analysis of *Orius insidiosus* (Hemiptera: Anthrenidae) feeding habits in field corn. — *J. Kans. Entomol. Soc.* **71**: 11–17.
- DASSOU A.G. & TIXIER P. 2016: Response of pest control by generalist predators to local-scale plant diversity: a meta-analysis. — *Ecol. Evol.* **6**: 1143–1153.
- DICKE M. & DE JONG M. 1988: Prey preference of the phytoseiid mite *Typhlodromus pyri*: 2. Electrophoretic diet analysis. — *Exp. Appl. Acarol.* **4**: 15–25.
- EYA A.A., LACUNA D.G. & ESPRA A.S. 2011: Gut content analysis of selected commercially important species of coral reef fish in the southwest part of Iligan Bay, Northern Mindanao, Philippines. — *Publ. Seto Marine Biol. Lab.* **41**: 35–49.
- GILLER P.S. 1982: The natural diets of water bugs (Hemiptera: Heteroptera): electrophoresis as a potential method of analysis. — *Ecol. Entomol.* **7**: 233–237.
- GILLER P.S. 1984: Predators gut state and prey detectability using electrophoretic analysis of gut contents. — *Ecol. Entomol.* **9**: 157–162.
- GILLER P.S. 1986: The natural diet of the Notonectidae: field trials using electrophoresis. — *Ecol. Entomol.* **11**: 163–172.
- GREZ A.A. & PRADO E. 2000: Effect of plant patch shape and surrounding vegetation on the dynamics of predatory coccinellids and their prey *Brevicoryne brassicae* (Hemiptera: Aphididae). — *Environ. Entomol.* **29**: 1244–1250.
- GURR G.M., WRATTEN S.D. & BARBOSA P. 2000: Success in conservation biological control of arthropods. In Gurr G.M. & Wratten S.D. (eds): *Biological Control: Measures of Success*. Kluwer, Dordrecht, pp. 105–132.
- GURR G.M., LIU J., READ D.M.Y., CATINDIG J.L.A., CHENG J.A., LAN L.P. & HEONG K.L. 2010: Parasitoids of Asian rice planthopper (Hemiptera: Delphacidae) pests and prospects for enhancing biological control by ecological engineering. — *Ann. Appl. Biol.* **158**[2011]: 149–176.
- HARWOOD J.D. & OBYRCKI J.J. 2005: Quantifying aphid predation rates of generalist predators in the field. — *Eur. J. Entomol.* **102**: 335–350.
- HOBSON E.S. 1974: Feeding relationships of teleostan fishes on coral reefs in Kona. — *Hawaii. Fish. Bull.* **72**: 915–1031.
- HODEK I. & EVANS E.W. 2012: Food relationships. In Hodek I., Van Emden H.F. & Honek A. (eds): *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. John Wiley & Sons, Chichester, pp. 141–274.
- HORGAN F.G., RAMALA A.F., BERNALA C.C., VILLEGASA J.M., STUART A.M. & ALMAZANA M.L.P. 2016: Applying ecological engineering for sustainable and resilient rice production systems. — *Procedia Food Sci.* **6**: 7–15.
- JOON H.L. & SEUNG T.K. 2001: Use of spiders as natural enemies to control rice pests in Korea. In: *Extension Bulletin – Food and Fertilizer Technology Center for the Asian and Pacific Region (Taiwan), No. 501*. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, 13 pp.
- KRANZ J., TORT N. & SENGONCA C. 2002: Effects of three weeds on the populations of predatory coccinellids and aphids in a kohlrabi field. — *Gesunde Pfl.* **54**: 98–104.
- LANDIS D.A., WRATTEN S.D. & GURR G.M. 2000: Habitat management to conserve natural enemies of arthropod pests in agriculture. — *Annu. Rev. Entomol.* **45**: 175–201.
- LISTER A., USHER M.B. & BLOCK W. 1987: Description and quantification of field attack rates by predatory mites: an example using an electrophoretic method with a species of Antarctic mite. — *Oecologia* **72**: 185–191.
- LIXA A.T., CAMPOS J.M., RESENDE A.L.S., SILVA J.C., ALMEIDA M.M.T.B. & AGUIAR-MENEZES E.L. 2010: Diversity of Coccinellidae (Coleoptera) using aromatic plants (Apiaceae) as survival and reproduction sites in agroecological system. — *Neotrop. Entomol.* **39**: 364–359.
- MICHAUD J.P. & HARWOOD J.D. 2012: Quantifying the impact of coccinellids on their prey. In Hodek I., Van Emden H.F. & Honek A. (eds): *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. John Wiley & Sons, Chichester, pp. 465–487.
- MURRAY R.A. & SOLOMON M.G. 1978: A rapid technique for analysing diets of invertebrate predators by electrophoresis. — *Ann. Appl. Biol.* **90**: 7–10.
- POMPANON F., DEAGLE B.E., SYMONDSON W.O.C., BROWN D.S., JARMAN S.N. & TABERLET P. 2012: Who is eating what: diet assessment using next generation sequencing. — *Mol. Ecol.* **21**: 1931–1950.
- REKHA B.S., RAMKUMAR J., KANDIBANE M., RAGURAMAN S. & SWAMIAPPAN M. 2009: Diversity of coccinellids in cereals, pulses, vegetables and in weeded and partially weeded rice-cowpea ecosystems in Madurai District of Tamil Nadu. — *Madras Agr. J.* **96**: 251–264.
- SCHALLER M. & NENTWIG W. 2000: Olfactory orientation of the seven-spot ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae): Attraction of adults to plants and conspecific females. — *Eur. J. Entomol.* **97**: 155–159.
- SCHMID A. 1992: Investigations on the attractiveness of agricultural weeds to aphidophagous ladybirds (Coleoptera, Coccinellidae). — *Agrarökologie* **5**: 122 pp.
- SCHULZ K.L. & YURISTA P.M. 1995: Diet composition from allozyme analysis in the predatory cladoceran *Bythotrephes cederstroemi*. — *Limnol. Oceanogr.* **40**: 821–826.
- SENTHIL KUMAR C.M. & REGUPATHY A. 2009: Gut content analysis of spiders in coffee ecosystem. — *Curr. Sci.* **96**: 892–893.
- SHANKER C., MOHAN M., SAMPATHKUMAR M., LYDIA CH., KATTI G. 2013a: Functional significance of *Micraspis discolor* (Coccinellidae: Coleoptera) in rice ecosystem. — *J. Appl. Entomol.* **137**: 601–609.
- SHANKER C., MOHAN M., SAMPATHKUMAR M., LYDIA CH. & KATTI G. 2013b: Selection of flowering forbs for conserving natural enemies in rice fields. — *Biocontr. Sci. Technol.* **23**: 480–484.
- SOLOMON M.G., FITZGERALD J.D. & MURRAY R.A. 1996: Electrophoretic approaches to predator-prey interactions. In Symondson W.O.C. & Liddell J.E. (eds): *The Ecology of Agricultural Pests: Biochemical Approaches*. Chapman & Hall, London, pp. 457–468.
- SUTHERLAND J.P., SULLIVAN M.S. & POPPY G.M. 2001: Distribution and abundance of aphidophagous hoverflies (Diptera: Syrphidae) in wildflower patches and field margin habitats. — *Agr. For. Entomol.* **3**: 57–64.
- SYMONDSON W.O.C., SUNDERLAND K.D. & GREENSTONE M.H. 2002: Can generalist predators be effective biocontrol agents? — *Annu. Rev. Entomol.* **47**: 561–594.
- TRAUGOTT M. 2003: The prey spectrum of larval and adult *Cantharis* species in arable land: An electrophoretic approach. — *Pedobiologia* **47**: 161–169.
- VILLEGAS C.M., VERDUGO J.A., GREZ A.A., TAPIA J. & LAVANDERO B. 2013: Movement between crops and weeds: temporal refuges for aphidophagous insects in Central Chile. — *Cien. Inv. Agr.* **40**: 317–326.
- WACKERS F.L. & VAN RIJN P.C.J. 2012: Pick and mix: Selecting flowering plants to meet the requirements of target biological control insects. In Gurr G.M., Wratten S.D., Snyder W.E. & Read D.M.Y. (eds): *Biodiversity and Insect Pests: Key Issues for Sustainable Management*. John Wiley, Chichester, pp. 139–166.

- WEBER D.C. & LUNDGREN J.G. 2009: Assessing the trophic ecology of the Coccinellidae: their roles as predators and prey. — *Biol. Contr.* **51**: 199–214.
- WIN S.S., MUHAMAD R., AHMAD Z.A.M. & ADAM N.A. 2011: Population fluctuations of brown planthopper *Nilaparvata*

*lugens* Stal and white backed planthopper *Sogatella furcifera* Horvath on rice. — *J. Entomol.* **8**: 183–190.

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