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Response of coccinellid community to the dimethoate application in olive groves in northeastern Portugal

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Abstract

In this work we assessed the effects of the application of dimethoate on the coccinellid community. The field work was carried out on a weekly basis, in two different olive groves, from April to November of 2002 and 2003 and captured coccinellids were identified to species level. Principal response curves (PRC) method was used to analyse the effect of the dimethoate application on the abundance of coccinellid species. A total of 23 species were identified from the two olive groves. Nine species occurred in both olive groves and in the two years of the study. *Scymnus interruptus* was the dominant species in the control grove with 46.4% of the total Coccinellidae recovered while in the grove treated with dimethoate, *Rhyzobius chrysomeloides* represented 35.7% of the total number captured. PCR showed that the main effect of the treatment was a significant reduction of the abundance of the most common species of the coccinellid community (*S. interruptus* and *Chilocorus bipustulatus*) in the treated grove. This can also have implications on the preservation of ecological functions associated with coccinellids, namely their role as control agents of olive pests.

Additional key words: Coccinellidae, integrated pest management, *Olea europaea,* organic farming, principal response curves, species abundance.

Resumen

Respuesta de la comunidad de coccinélidos a la aplicación de dimetoato en olivares del nordeste de Portugal

En este trabajo se analizan los efectos de la aplicación de dimetoato en la comunidad de coccinélidos. El trabajo de campo se ha realizado en dos olivares, semanalmente, de abril a noviembre en 2002 y 2003, y los coccinélidos capturados se han identificado hasta el nivel de especie. Se han utilizado las principales curvas de respuesta (PRC) para analizar el efecto que produce la aplicación de dimetoato en la abundancia de especies de coccinélidos. Se encontraron un total de 23 especies de coccinélidos en los dos olivares, nueve de ellas comunes en los dos olivares y en los dos años de estudio. *Scymnus interruptus* fue la especie dominante en el olivar no tratado con dimetoato (46,4% del total de coccinélidos capturados), mientras que en el olivar tratado *Rhyzobius chrysomeloides* representó el 35,7% del total de las capturas. Las PRC demuestran que el principal efecto producido al aplicar dimetoato ha sido una reducción significativa de la abundancia de las especies más comunes de la comunidad de coccinélidos (*S. interruptus* y *Chilocorus bipustulatus*) en el olivar tratado. Esto puede tener implicaciones en la conservación de las funciones ecológicas asociadas a los coccinélidos como, por ejemplo, su papel como agentes de control de las plagas del olivo.

Palabras clave adicionales: abundancia de especies, agricultura ecológica, Coccinellidae, curvas de respuesta principal, manejo integrado de plagas, *Olea europaea.*

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Abbreviations used: Cdt (canonical coefficient), IPM (integrated pest management), OF (organic farming), PRC (principal response curves), RDA (redundancy analysis).

Introduction

Generalist insect predators are common in agro-ecosystems. In the olive tree (*Olea europaea* L.) canopy, coccinellids are among the most abundant groups of predators where they can have a potential function on the natural control of pests which often cause some economic losses in the crop yield (Morris *et al.*, 1999; Soares *et al.*, 2005; Santos *et al.*, 2007).

The disturbance of the agro-ecosystems by several factors can change the abundance and the diversity of coccinellids influencing their ability to suppress the development of pest populations (Obrycki and Kring, 1998; Altieri, 1999). Among the most frequent disturbances occurring in an agro-ecosystem is the use of synthetic pesticides to control pests or diseases that can also be toxic to beneficial arthropods (Rodríguez *et al.*, 2003; Ruano *et al.*, 2004; Cardenas *et al.*, 2006; Santos *et al.*, 2007). After the application of a pesticide, the natural control exerted by predators over the pests is broken up either by direct pesticide-induced mortality (Walker *et al.*, 1996) or by lowering the number of preys (Obrycki and Kring, 1998).

The toxicity of insecticides such as carbaryl, endosulfan and esfenvalerate (Yardim and Edwards, 1998), deltamethrin (Rodríguez *et al.*, 2003) and dimethoate (Santos *et al.*, 2007) to the total abundance of coccinellids has been reported, showing a high susceptibility of this group to chemical products. But, in a community, different species have different sensitivities and respond differently to stress in what concerns, for instance, the likeliness of exposure and the capacity of recovery (Walker *et al.*, 1996). Thus, the sensitivity of each coccinellid species to insecticides might be a serious constrain to the successful conservation of the coccinellid biodiversity that can help to promote the maintenance of ecological functions such as the regulation of pests providing a long-term stability in the agro-ecosystems (Altieri, 1999; Philpott and Armbrecht, 2006). On the other hand, when selecting test organisms for laboratorial toxicity bioassays, the investigator should consider species that are sensitive to local pesticides and representative of functional roles developed by natural organisms (Markwiese *et al.*, 2001).

The study of the factors driving the variations in the coccinellid community structure in terms of species composition, relative abundance of each species and population dynamics is essential for the development of rational strategies to protect the olive grove. It is necessary to increase the knowledge about the effect of insecticides on the coccinellid community structure. Moreover, comparable measurements of abundance and species richness from different places or periods of time are used to study the effects of pollutants on communities and can help us to identify when the stability of the ecosystem is endangered (Walker *et al.*, 1996). The aim of this study was to evaluate the effect of dimethoate application on the structure of the coccinellid community in the olive tree canopy.

Material and methods

Study sites

Field studies were conducted in two olive groves near Mirandela (Portugal): Paradela and Valbom-dos-Figos groves between 2002 and 2003. Both olive groves occupied an area of three ha and were 10 km from one another. Valbom-dos-Figos grove (41° 33' 4'' N, 7° 8' 43'' W) has been following the organic growing guidelines since 1991 [Council Regulation (EEC) no. 2092/91 of 24 June 1991], no phytosanitary treatments were done and the soil was fertilized with organic nutrients two to four times a year. Paradela grove (41° 32' 38" N, 7° 7' 29" W) has been following the IPM guidelines since 2001 (Gomes and Cavaco, 2003) and according to information given by the farmer, a dimethoate spray [1.5 mL hL^{-1} of the formulation at 42.8% (w/v)] was applied on 13 June 2002 and on 16 June 2003 against the anthophagous generation of the olive moth, *Prays oleae* (Bernard). The soil was fertilized with organic and mineral nutrients two to four times a year.

The planting density was 10 × 10 m in Valbom-dos-Figos and 9 × 9 m in Paradela. In both olive groves, soil was ploughed superficially with a scarifier two to four times a year to control weeds and was not irrigated. In this work, Valbom-dos-Figos is referred as control and Paradela as treated grove.

Survey of coccinellids

From April to November of 2002 and 2003, the coccinellid community was sampled by using the beating technique on an approximately weekly basis. Five samples were collected weekly per olive grove, being each sample compounds by 10 beating branches from 10 different trees. The branches were randomly selected from a total number of 50 trees per olive grove. All captured individuals were frozen, sorted, counted and identified to species level using a binocular microscope. Species identification was based on external characteristics but extraction and observation of genitals of some species was needed to confirm the morphological identification. Coccinellid species were identified according Raimundo and Alves (1986) and Raimundo (1992).

Data analysis

Abundance data were evaluated for normality and homogeneity of variances with Kolmogorov-Smirnov test and Bartlett's test, respectively and when necessary, the transformation log $(x + 1)$ was used to normalize the data. Total abundance of coccinellids and the abundance of the five most common coccinellid species were compared using the following nested design: Treatment + Year + Treatment × Year + Week (year), using the General Linear Model module of Minitab Statistical Software, release 14 (Minitab Inc., 2003). Thus, the model includes two groves (control and dimenthoate treatment), two years (2002 and 2003), the interaction between groves and year, and also the 26 sampling dates each year (nested within years) as sources of variation. The main purpose of this model was to detect differences associated with each one of the two factors (treatment and year) while taking into consideration the variability associated with the temporal cycles (obtained by nesting the random factor, sampling date, within year). Significance levels for all analyses were set at $P \leq 0.05$.

The effect of the dimethoate application on the community of coccinellids was evaluated by the principal response curves (PRC) method using Canoco for Windows, Version 4.5 (Ter Braak and Šmilauer, 2002). The PRC analysis is derived from the multivariate ordination technique redundancy analysis (RDA) (Van den Brink and Ter Braak, 1999; Van den Brink *et al.,* 2003). The result of a PRC analysis is a graph that summarizes the effect of the insecticide application on the community (y-axis) over time (x-axis) in which the grove treated with dimethoate is related to the control grove. By definition, the control is zero in every date and the effect of the dimethoate application is quantified, in the y-axis, by the basic response pattern or canonical coefficient (Cdt) at each sampling period (t). Thus, the deviation of the treatment curve (d) compared to the control is proportional to the effect of insecticide spraying. The Cdt graph is combined with the species weight (b_k) that represents the affinity of individual species (k) with the overall-community response (Cdt). In this case, a positive species weight value indicates a reduced abundance of the species in the grove treated with dimethoate, compared to the control, while a species with a negative weight is expected to increase in abundance, relative to the control. Species with weights between 0.5 and –0.5 responded differently to the insecticide application, either by showing no response or by showing a response unrelated to the general pattern. The PRC analysis was followed by a Monte Carlo permutation test to test whether the PRC diagram showed a significant part of the variance explained by the application of the insecticide (Van den Brink and Ter Braak, 1999). Only those species which were caught in both olive groves and occurred in both years were used in the PRC analysis.

Community composition was investigated by plotting the rank-abundance curves of each grove, with the relative abundance of each species as its index of abundance. Thus, the relative abundance for the most common species is plotted first, then the next most common and so on until the array is completed by the rarest species of all (Magurran, 2004).

Results

Coccinellid abundance and species richness

A total of 2,946 coccinellids belonging to 23 species were collected in the control and in the treated groves during the two years (Table 1). Significantly more coccinellids were collected in the control grove (2,402 individuals) than in the treated grove (544 individuals) ($F = 243.29$; df = 1, 466; $p < 0.001$), but the overall abundance was similar for both years ($F = 0.46$; df = 1, 466; $p = 0.5$) (Table 2).

Nine of the most abundant coccinellids species were found in both groves during the two years of the study and represented 98.0% of the total species collected: *Chilocorus bipustulatus* (L.), *Exochomus nigromaculatus* (Goeze), *Scymnus subvillosus* (Goeze), *Scymnus mediterraneus* Iablokoff-Khnzorian, *Scymnus interruptus* (Goeze), *Scymnus apetzi* Mulsant, *Nephus bisignatus* (Boheman), *Rhyzobius litura* (Fabricius) and *Rhyzobius chrysomeloides* (Herbst). Four species [*Scymnus rufipes* (Fabricius), *Rhyzobius lophantae* (Blaisdell), *Coccinella*

Table 1. Relative (and total abundance) of each coccinellid species captured in the control and in the treated olive groves during 2002 and 2003, n = 130

Species	Control		Dimethoate	
	2002	2003	2002	2003
Platynaspis luteorubra (Goeze, 1777)	0.4% (4)	0.1% (1)	1.3% (4)	
Chilocorus bipustulatus (L., 1758)	40.0% (298)	14.1% (203)	3.3% (10)	3.8% (9)
Exochomus quadripustulatus (L., 1758)	0.4% (4)	0.1% (1)	0.3% (1)	
Exochomus nigromaculatus (Goeze, 1777)	0.1% (1)	0.9% (13)	0.7% (2)	3.0% (7)
Stethorus punctillum Weise, 1891		0.2% (3)	7.2% (22)	
Scymnus subvillosus (Goeze, 1777)	11.8% (114)	7.4% (106)	11.1% (34)	11.8% (28)
Scymnus mediterraneus Khnz., 1972	7.9% (76)	2.5% (36)	18.6% (57)	24.5% (58)
Scymnus interruptus (Goeze, 1777)	21.4% (206)	63.1% (908)	6.8% (21)	20.3% (48)
Scymnus rufipes (Fabr., 1798)		0.1% (1)		
Scymnus apetzi Muls., 1846	0.9% (9)	1.2% (17)	2.2% (7)	7.2% (17)
Scymnus coenosp. *apetzoides* Capra/Fürsch, 1967				0.8% (2)
Nephus bisignatus (Boheman, 1850)	0.6% (6)	0.3% (4)	0.7% (2)	0.8% (2)
Nephus semirufus Weise, 1885			1.0% (3)	
Nephus heikei Fürsch, 1965				0.8% (2)
Nephus helgae Fürsch, 1965		0.1% (1)		0.4% (1)
Rhyzobius lophantae (Blaisd., 1892)		0.1% (1)		
Rhyzobius litura Fabr., 1837	0.9% (9)	0.4% (5)	2.3% (7)	0.4% (1)
Rhyzobius chrysomeloides (Herbst, 1792)	24.3% (234)	9.5% (137)	44.0% (135)	25.0% (59)
Adalia decempunctata (L., 1758)	0.1% (1)		0.3% (1)	0.8% (2)
Coccinella setempunctata (L., 1758)	0.1% (1)	0.1% (1)		
Oenopia doublieri (Muls., 1846)				0.4% (1)
Propylaea quatuordecimpuntacta (L., 1758)		0.1% (1)		
Subcoccinella vigintiquatuorpunctata (L., 1758)			0.3% (1)	
Total abundance	963	1,439	307	237
Richness	13	17	15	14

Table 2. Statistical output for the nested analysis of variance of the total abundance of coccinellids and of the five species considered to be the most important in the PRC analysis

	Treatment	Year	Treatment × Year	Week (Year)
Total abundance	$F_{1,466} = 234.29$ $P < 0.001$	$F_{1,466} = 0.46$ $P = 0.500$	$F_{1,466} = 6.84$ $P = 0.009$	$F_{50,466} = 5.18$ $P < 0.001$
Chilocorus bipustulatus	$F_{1,466} = 268.36$ $P < 0.001$	$F_{1,466} = 1.28$ $P = 0.263$	$F_{1,466} = 2.74$ $P = 0.098$	$F_{50,466} = 2.27$ $P < 0.001$
Scymnus subvillosus	$F_{1,466} = 38.58$ $P < 0.001$	$F_{1,466} = 1.02$ $P = 0.318$	$F_{1,466} = 3.09$ $P = 0.079$	$F_{50,466} = 2.62$ $P < 0.001$
Scymnus mediterraneus	$F_{1,466} = 0.78$ $P = 0.379$	$F_{1,466} = 0.07$ $P = 0.785$	$F_{1,466} = 2.80$ $P = 0.095$	$F_{50,466} = 4.08$ $P < 0.001$
Scymnus interruptus	$F_{1,466} = 170.61$ $P < 0.001$	$F_{1,466} = 12.93$ $P = 0.001$	$F_{1,466} = 41.65$ $P < 0.001$	$F_{50,466} = 5.97$ $P < 0.001$
Rhyzobius chrysomeloides	$F_{1,466} = 34.60$ $P < 0.001$	$F_{1,466} = 11.54$ $P = 0.001$	$F_{1,466} = 0.26$ $P = 0.609$	$F_{50,466} = 1.93$ $P < 0.001$

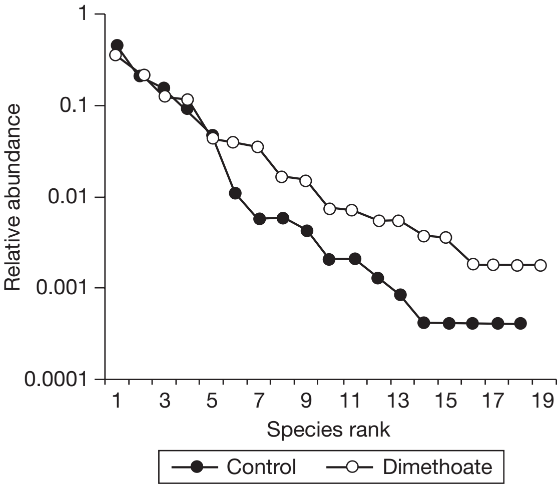

Figure 1. Rank abundance curves for the control and for the olive grove treated with dimethoate.

setempunctata L. and *Propylaea quatuordecimpunctata* (L.)] were found only in the control while five species, *Scymnus* coenosp. *apetzoides* Capra/ Fürsch, *Nephus semirufus* Weise, *Nephus hiekei* Fürsch, *Oenopia doublieri* (Mulsant) and *Subcoccinella vigintiquatuorpunctata* (L.), were collected only in the treated grove. These species comprised the remaining 2.0% of the total coccinellids captured. Except for *S. vigintiquatuorpunctata* all the coccinellid species captured were predators.

The rank abundance curves showed that the coccinellid community of the treated grove was dominated by four species. The fifth most abundant species, ranked above the curve for the control grove (Fig. 1). Therefore, the treated grove showed higher species evenness than the control grove, where the community was dominated by five species. After the two years, a total of eighteen species were collected in the control and nineteen in the treated grove. The species composition of the coccinellid community varied with the dimethoate application and year. In 2002, *C. bipustulatus* dominated the community in the control grove representing 30.9% of total abundance, followed by *R. chrysomeloides* (24.3%) and *S. interruptus* (21.4%). In the treated grove, the community was dominated by *R. chrysomeloides* (44.0%) followed by *S. mediterraneus* (18.6%) and *S. subvillosus* (11.1%). In 2003, *S. interruptus* largely dominated the community in the control grove representing 63.0% of total abundance, followed by *C. bipustulatus* (14.1%) and *R. chrysomeloides* (9.5%). In the treated grove, three species with similar abundances dominated the community: *R. chrysomeloides* (24.9%), *S. mediterraneus* (24.5%) and *S. interruptus* (20.3%).

Response of coccinellid species to the dimethoate application

PRC diagram shows changes through the two years of study in the variability of coccinellid community. Of the total variance, 29% is explained by the sampling date and 32% by the dimethoate application. The Monte Carlo permutation test showed a significant difference between groves and 73% of that difference is displayed by the first PRC axis (Fig. 2). The PRC diagram shows small differences between the control and the curve of the treated grove on the sampling dates immediately before each spray. After spraying, the negative values of *Cdt* for the grove treated with dimethoate indicate that the total of captures were lower in this grove than in the control. This effect was even more evident in 2003. Only in October of both years, the values of *Cdt* for the treated grove approached the control. *S. interruptus* and *C. bipustulatus* obtained high species weights in the diagram, indicating a reduced abundance in the treated grove. On the contrary, *S. mediterraneus* obtained a lower negative value, indicating a slight increase in abundance in the treatment compared with the control.

The changes in the abundance of the five most abundant species are shown in Figure 3 and the results of the correspondent nested ANOVAs are summarized in Table 2. In general, abundance increased from July till the end of the sampling period in the control grove. However, in the treated grove, only the abundance of *S. mediterraneus* increased after spraying with dimethoate. Moreover, during the summer months of 2003, the treated grove reached higher abundances than the control. The abundance of *C. bipustulatus*, *S. subvillosus*, *S. interruptus* and *R. chrysomeloides* over the two years was significantly higher in the control than in the treated grove, while for *S. mediterraneus* there were no significant differences between olive groves in any year. Only for *S. interruptus* a significant interaction between olive groves and years was observed.

Discussion

Among the 23 coccinellid species identified, nine species were represented in both olive groves in the

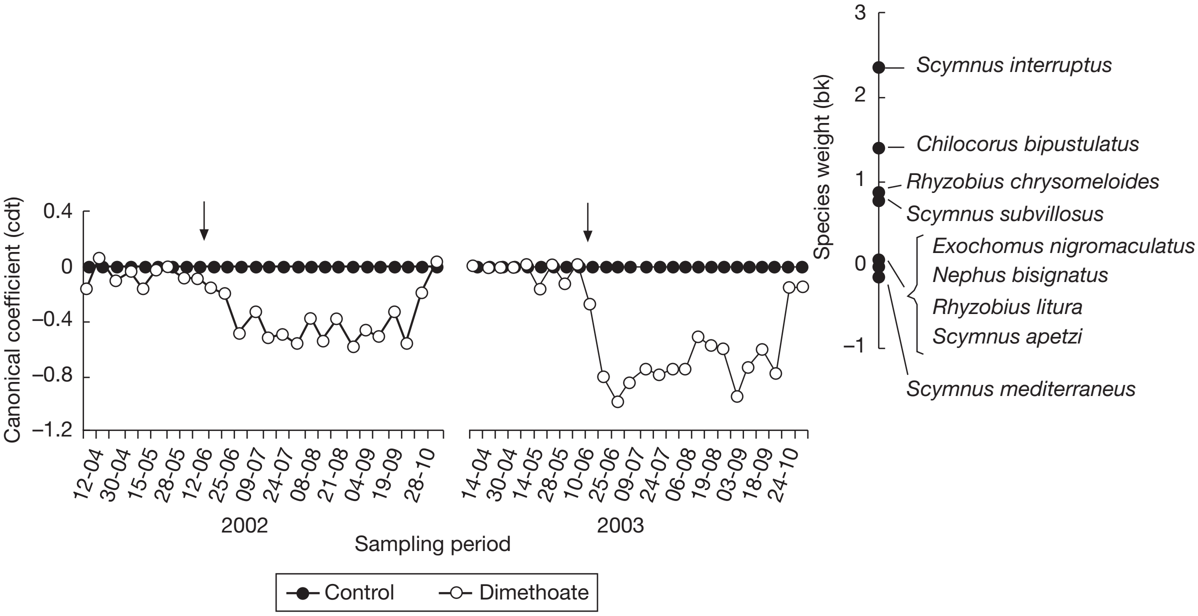

Figure 2. Principal response curves diagram and species weight for the most common coccinellid species, showing variation in abundance during 2002 and 2003. Arrows indicate spray dates in the grove treated with dimethoate.

two years. From these, *C. bipustulatus*, *S. subvillosus*, *S. mediterraneus*, *S. interruptus* and *R. chrysomeloides* were in general the most abundant species completing their life cycles. Argyriou and Katsoyannos (1977) referred *C. bipustulatus* as an abundant and widely distributed species in Greek olive groves, in a study where *Exochomus quadripustulatus* (L.), *S. subvillosus* and *S. apetzi* were also found. Ba M'hamed and Chemseddine (2002) reported *S. mediterraneus* as a common species in the olive groves of the Mediterranean region.

Coccinellid species represented by less than a total of 10 individuals appeared mostly in summer causing an increase in the species richness. These species can be considered occasional and probably do not use the olive tree as a typical habitat. Their appearance may be attributed to the migration from the surrounding field vegetation (weeds and shrubs) and, due to their lower abundance, they do not have a significant impact in the control of olive pests but are important to the species richness.

The community structure apparently changed as a consequence of the spraying, suggesting different susceptibilities of the coccinellid species to it. After the two years of study and considering only the five most abundant species, with the exception of *S. mediterraneus*, all the other species were less abundant in the treatment than in the control. *S. interruptus* and *C. bipustulatus* were the most affected species, followed by *R. chrysomeloides* and *S. subvillosus*. The significant reduction of these species, as was shown by PRC analysis, could be due to direct toxic effects such as the intrinsic susceptibility to the insecticide caused by different detoxifying capacities (Walker *et al.*, 1996). In addition, insecticides can affect predator species indirectly via the depletion of the prey population. This fact, associated with differences in the mobility of each predator to search for new habitats, may influence fecundity and longevity (Obrycki and Kring, 1998). Also, different feeding strategies like prey stage selection and the voracity of each species may induce various exposures to the insecticide (Singh *et al.*, 2004). As a consequence, large-sized species (e.g. *C. bipustulatus*) will ingest more preys and hence more insecticide than smaller species. In the treatment, the community was reduced to four dominant species, while in the control there were five, due to the significant decline of *C. bipustulatus*, which is one of the most important predators of *Saissetia oleae* (Olivier) either in the olive or citrus groves (Limón *et al.*, 1976; Argyriou and Katsoyannos, 1977; Santos *et al.*, 2009). Therefore, the significant reduction of this species from the treated grove can have negative consequences for the natural control of olive pests, which can increase rapidly resulting in pest outbreaks.

In the treated grove, the decline in the abundance of potential predators that occurred after the insecticide

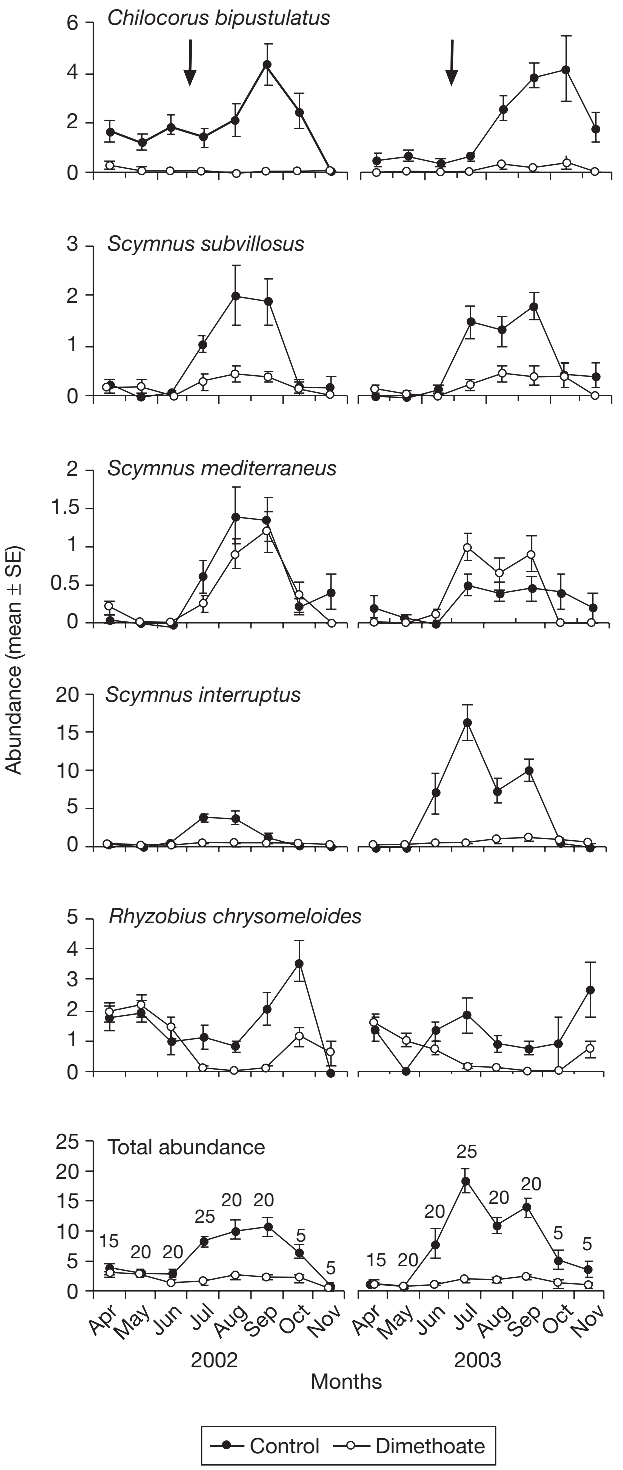

Figure 3. Monthly mean abundance (± standard error of the mean - SE) of the species considered to be the most important in the PRC analysis and total abundance from April to November of 2002 and 2003, in the control and treated groves. The numbers above bars represent the number of samples (n) on which the mean is based. Arrows indicate spray dates in the grove treated with dimethoate. Note different scales of the y-axis.

application may have favoured the increase of *S. mediterraneus* in the community. *S. mediterraneus* is a small-sized species (S. Santos, personal observation) that can be easily predated by other arthropods, including coccinellids, influencing the outcome of interactions between competitors (Rosenheim *et al.*, 1995). Eggs and young larvae, for instance, may be particularly vulnerable to predation. This interaction was already observed among the aphidophagous coccinellid species *Adalia bipunctata* (L.), *A. decempunctata* (L.), *Coccinella septempunctata* L. and *C. undecimpunctata* L. in a laboratory study (Agarwala and Dixon, 1992). However, the abundance of *S. mediterraneus* in the treated grove does not automatically mean that this species was insensitive to the insecticide. Ba M'hamed and Chemseddine (2002) observed that dimethoate showed moderate activity on *S. mediterraneus* adults at recommended doses. Probably, this species had a fast recovery rate either by immigration from the surrounding environment or due to its life history strategy.

The influence of the dimethote application in the remaining species showed in the PRC diagram is inconclusive because of the few numbers of individuals collected.

In summary, changes in the overall abundance observed in the treated grove were determined by changes of the most abundant species. The regular disturbances occurring in this grove may have changed the natural balance between species. In addition, the species composition of the community could be determined by direct or indirect effects of the insecticide. Consequently, more sensitive species will be progressively eliminated and the community will be dominated by resilient species. Contrarily, in the control where disturbances were minimal, species composition was determined by the species that were the most effective competitors.

The significant effects observed after the insecticide application on the abundance of the most common species, can endanger ecological functions like the control of olive pests. Therefore, it is necessary to implement or enhance plant protection practices that encourage the conservation of these natural enemies, which can be achieved by avoiding or significantly reducing insecticide use. In integrated pest management, pesticide applications must be scheduled according to the life history of the beneficial species (coccinellids) and the pest they intend to control. Thus, the conjugation of natural and chemical pest control will reduce harmful effects while pest control will be more effective.

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