Seasonal synchrony between *Saissetia oleae* and coccinellid species in Portuguese olive groves in different management systems

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Abstract

The black scale, *Saissetia oleae* (Olivier) (Hemiptera Coccidae), is one of the main olive pests and coccinellids are among the principal predators that feed on scales. The relationships between the temporal abundance of five common coccinellid species [*Chilocorus bipustulatus* L., *Scymnus subvillosus* (Goeze), *Scymnus mediterraneus* Iablokoff-Khnzorian, *Scymnus interruptus* (Goeze), *Rhyzobius chrysomeloides* (Herbst)] and the different phenological stages of *S. oleae* were investigated in two olive groves managed under two different systems (Integrated Pest Management and Organic Farming) during 2002 and 2003. Coccinellids and black scale were randomly sampled on a fortnightly basis and correlation analyses between the abundance of the coccinellid species and the different stages of the pest were carried out. Results showed that the abundance of *S. oleae* was similar between olive groves and years. However, significant differences were found for adult and larvae of coccinellid species. In both years and groves, the greatest abundance of coccinellids occurred between June and November, also corresponding to the period of greatest abundance of the first and second instar nymphs of *S. oleae*. Significant positive correlations were obtained between the second instar nymph and four out of five coccinellid species, being potentially the most predated stage of the pest. On the contrary, no significant positive correlations were found between the third instar nymph and the coccinellid species. *S. subvillosus* and *S. interruptus* were the coccinellid species that showed a higher number of significant positive correlations with the different stages of the pest indicating their potential as biological agents of *S. oleae*.

Key words: coccinellids, integrated pest management, organic farming, predator-prey interactions, *Saissetia oleae*.

Introduction

The black scale, *Saissetia oleae* (Olivier) (Hemiptera Coccidae), is a cosmopolitan plant-sucking pest that damages branches and leaves of the olive tree (*Olea europaea*, L.) and causes high losses in yield (Civantos, 1999). In Portugal, the black scale is considered one of the main pests of the olive grove (Pereira, 2004).

Natural enemies can have an important role in biological control of the black scale and their survey and identification are primary steps in recognizing their potential for the regulation of the pest. In the olive grove, parasitoids and coccinellids are among the most referred natural enemies of *S. oleae* (Argyriou and Katsoyannos, 1977; Velimirovic, 1994; Ba M'Hamed and Chemseddine, 2001; Ba M'Hamed and Chemseddine, 2002). Parasitoids have been largely studied and inclusively used to control *S. oleae* in successful projects (Orphanides, 1993). However, few studies have been done concerning coccidophagous coccinellids. Species like *Chilocorus bipustulatus* L., *Scymnus mediterraneus* Iablokoff-Khnzorian, *Scymnus subvillosus* (Goeze) and *Scymnus interruptus* (Goeze) are common in olive and citrus groves of the Mediterranean region and have been referred as potential predators of coccids, in particular *S. oleae* (Argyriou and Katsoyannos, 1977; Uygun and Elekçioğlu, 1998; Magro and Hemptinne, 1999; Ba M'Hamed and Chemseddine, 2001; Ba M'Hamed and Chemseddine, 2002). In Portuguese olive groves, coc-

cinellid communities showed a high abundance and diversity with more than 20 species identified as being potential predators of *S. oleae* (Santos, unpublished data).

Field surveys are useful methods in studying the relationships between natural enemies and their preys (Powell et al. 1996). In general, predators are in contact with their preys for only a short time and few or no remains are left, making it difficult to determine predator-prey interactions (Mills, 1997). Therefore, only a small number of surveys have been performed to identify predatory coccinellids of *S. oleae*. To overcome this difficulty, a valuable study of the role of predators can be made by statistically correlating their numbers against those of the pest (Kidd and Jervis, 1996). This indirect approach provides an idea about the interaction between the community of coccinellids and the pest, and consequently about which species are potential predators of *S. oleae*. Thus, the objective of this investigation was to study the synchrony between the abundance of five coccinellid species [*C. bipustulatus*, *S. mediterraneus*, *S. interruptus*, *S. subvillosus* and *Rhyzobius chrysomeloides* (Herbst)] and the abundance of each phenological stage of *S. oleae* in two olive groves under different management systems, organic farming and integrated pest management, during two consecutive years, 2002 and 2003. The choice of these five species was based on their representativity in the olive grove where they are very frequent and abundant, representing 84-97% of the overall community of coccinellids (Santos, unpublished data).

Materials and methods

Study areas

Field studies were carried out in two olive groves near Mirandela (Portugal). One grove, Valbom-dos-Figos (41°33'4"N 7°8'43"W), has been following organic growing guidelines since 1991, no pesticides were applied and soil was fertilized with organic nutrients two to four times a year. The other grove, Paradela (41°32'38"N 7°7'29"W), has been following integrated pest management (IPM) guidelines since 2001. According to the information obtained from the farmer, a dimethoate spray (150 ml/hl of the formulation at 42.8% W/V) against the anthophagous generation of *Prays oleae* (Bernard), the main olive pest in the region, was applied annually in June. In 2002 treatment was applied on 13 June and in 2003 on 16 June. 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. Both olive groves occupied an area of 3 ha and were 10 km from one another. The soil was ploughed superficially with a scarifier two to four times a year and was not irrigated and the trees were pruned every two or three years.

From now on, each olive grove will be referred to according to its management system either as an organic grove or an IPM grove.

Sampling procedure

Sampling was carried out on a fortnightly basis, between April and November of two consecutive years: 2002 and 2003.

For *S. oleae* sampling, five samples were randomly collected in both olive groves. Each sample derived from ten randomly selected trees where four branches (one branch per orientation) with four leaves was sampled giving a total of 160 leaves per sample. All the phenological stages which were found on those branches (first, second, third and fourth instar nymphs and mature females) were sorted and counted using a binocular microscope.

Coccinellids were collected by the beating technique where the shoots were beaten over a rectangular cloth (0.6 m × 0.5 m) between 09-10 in the morning. In each sampling period, five samples per olive grove were collected. Each sample has the beatings of the ten randomly selected branches. Both adults and larval stages captured at each sampling period were sorted and counted using a binocular microscope. Adult coccinellid species were identified according to Raimundo and Alves (1986) and Raimundo (1992).

Data analysis

Univariate statistical analyses were performed using the Statistica Statistical package, version 7.0 (StatSoft, 2004). Data were evaluated for normality and homogeneity of variances with the Kolmogorov-Smirnov and Bartlett's tests, respectively and the transformation $\log_{10}$ $(x + 1)$ was used to normalise the data. The abundance of *S. oleae* and the abundance of adults and larvae of coccinellids, caught in both olive groves over different times, were compared by using the repeated measures analysis of variance (Zar, 1998). One-way ANOVA was used to compare the average number between years in each olive grove.

Pearson's correlation coefficients for the abundance of each coccinellid species and the abundance of phenological stages of *S. oleae* were determined in order to investigate the interaction between predators and pest fluctuations. This was carried out for each olive grove for the two years studied.

Correspondence analyses (CA) were performed with Canoco for Windows, Version 4.5 (Ter Braak and Šmilauer, 2002). CA is an unconstrained multivariate method adapted to species abundance data (Legendre and Legendre, 1998). It was used to look for similarity in distributions of relative abundance of the species, measured by their Chi-square distance. The species' point in the CA diagram is at the centroid of the samples where it occurs and, consequently points in proximity correspond to species often occurring together.

Results

Fluctuations of *S. oleae* population

A total of 52851 and 54645 individuals of the different phenological stages of *S. oleae* were collected, respectively, in the organic and IPM groves during the two years of the study. Repeated measures ANOVA showed that the total abundance of *S. oleae* was similar in both olive groves in 2002 ($F_{1, 8} = 1.25$, $P > 0.05$) and in 2003 ($F_{1, 8} = 0.02$, $P > 0.05$). Considering year-to-year data, one way-ANOVA showed that there were no significant differences within the organic ($F_{1, 143} = 1.01$, $P > 0.05$) and the IPM groves ($F_{1, 138} = 0.37$, $P > 0.05$).

The patterns of abundance of the different phenological stages of *S. oleae* were similar in both olive groves. The first instar nymphs were abundant from early July to middle of August, reaching the peak of abundance at the end of July of both years. The second instar nymphs occurred mainly from the end of July to the end of the sampling period. The peak of abundance was reached at the end of August in both years. In 2002, the third instar nymphs were abundant mainly in April and again in November, in the organic grove. In the IPM grove, abundance peaked essentially in November. In 2003, the pattern of abundance of this stage was slightly different. In the organic grove, it peaked in the middle of April and then at the end of July. In the IPM grove, it peaked at the end of July 2003. In 2002, the fourth instar nymphs occurred during spring and at the end of October and early November, reaching their maximum abundance in November in both groves. In 2003, this stage was abundant in April and May. In 2002, mature females reached their maximum abundance in the middle of June in both groves. In 2003, the peak in both groves was reached in the middle of May (figure 1).

Fluctuations of coccinellid species

A total of 1302 adult coccinellids and 269 larvae were collected in the organic grove and 223 adults and 125 larvae in the IPM grove during the two years of study.
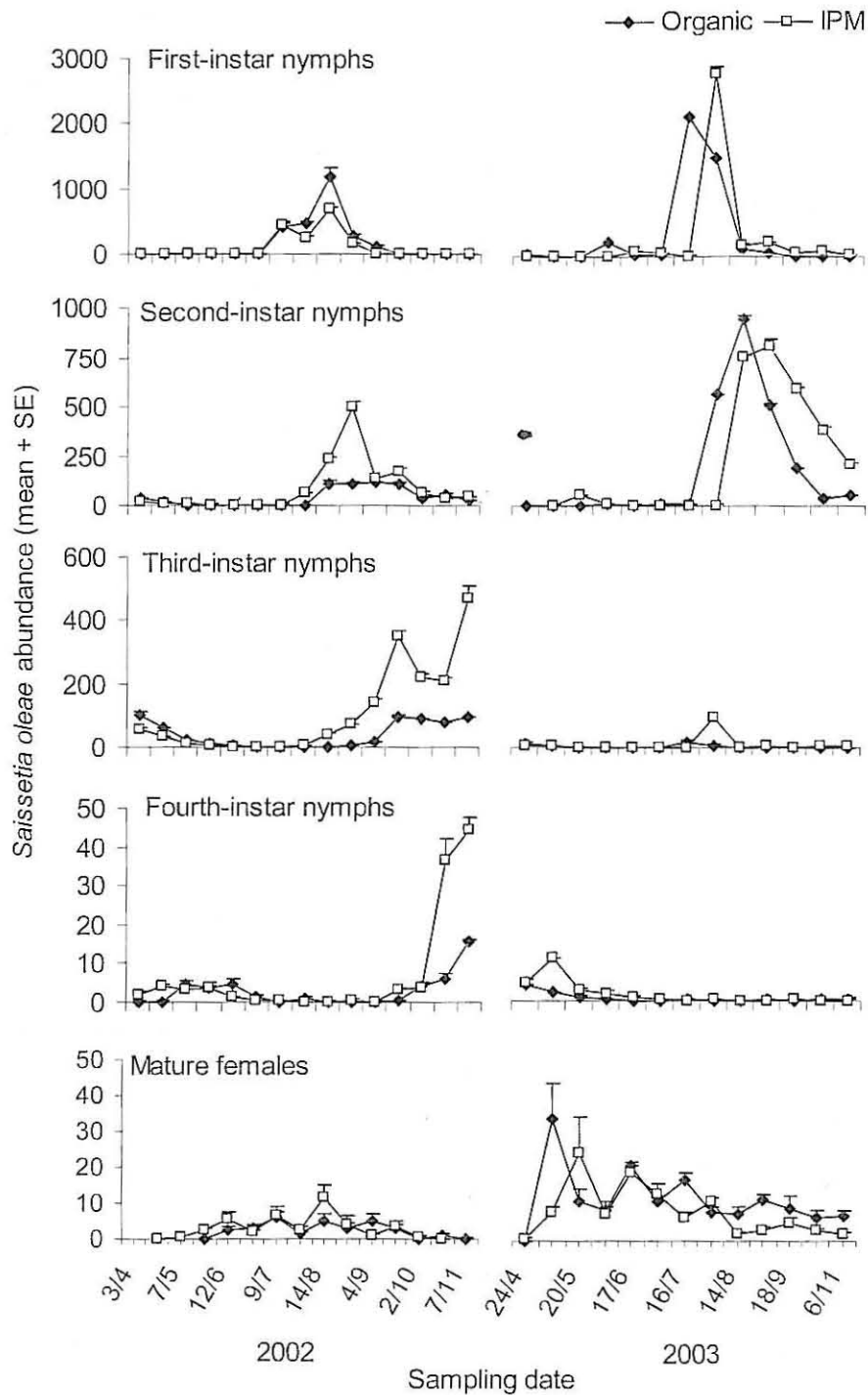
Figure 1. Fluctuations (mean + SE) of the different phenological stages of *S. oleae* during 2002 and 2003, in the organic and IPM groves (n = 5). Note different scale of y-axis.

Significant differences between groves were found for adults ($F_{1,8}$ = 76.86, P < 0.001 in 2002 and $F_{1,8}$ = 178.71, P < 0.001 in 2003) and larvae ($F_{1,8}$ = 8.42, P < 0.05 in 2002 and $F_{1,8}$ = 35.2, P < 0.001 in 2003). But, there were no significant differences between years in the organic ($F_{1,143}$ = 0.79, P > 0.05 for adults and $F_{1,143}$ = 2.56, P > 0.05 for larvae) and in the IPM grove ($F_{1,138}$ = 0.23, P > 0.05 for adults and $F_{1,138}$ = 0.03, P > 0.05 for larvae).

In the organic grove, the total of adults peaked from the end of August to October of 2002, but in 2003 the peaked abundance was reached earlier, from the middle

of June to the end of July. Larvae had two main periods of abundance, the first occurred in April and the second occurred during August and September of both years (figure 2). In the IPM, the first peak of abundance of adult coccinellids was reached in April and May 2002. However, after the insecticide application in June, a decrease in abundance was observed. Although a slight recovery occurred from the middle of August to the end of the sampling period, abundance remained low in comparison to the organic grove. Larvae abundance was high in April and May 2002, but decreased thereafter
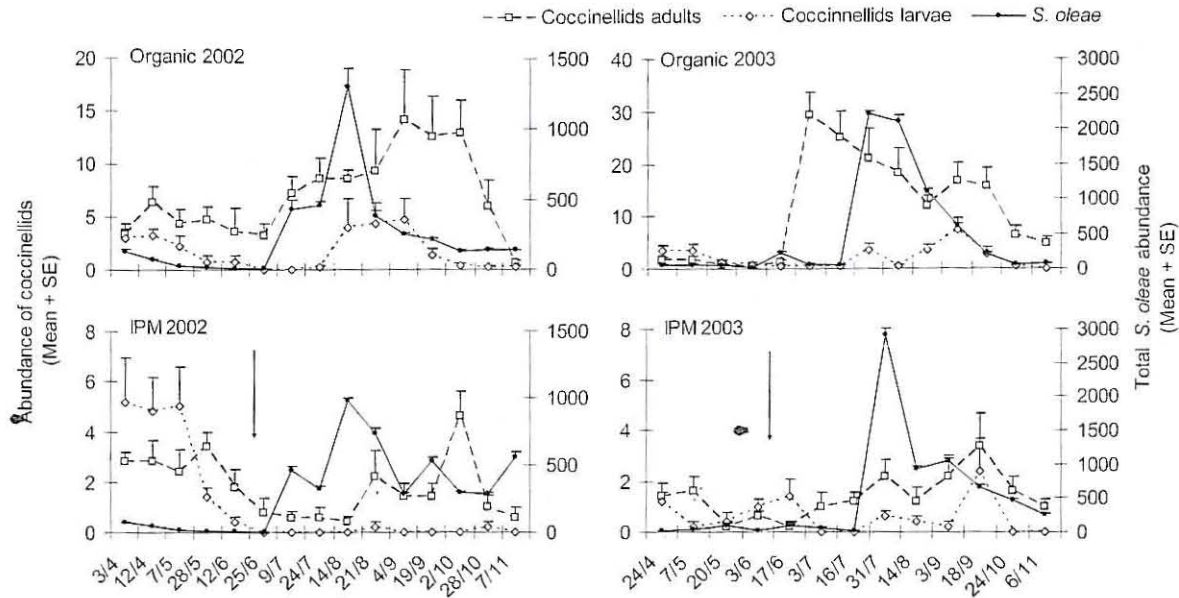
--□-- Coccinellids adults   ···◇··· Coccinnellids larvae   —●— S. oleae

Figure 2. Abundance (mean + SE) of total specimens of *S. oleae* and coccinellids (larvae and adults) collected in each sampling date in the organic and IPM olive groves during 2002 and 2003 (n = 5). Arrows indicate the date of the spray application in the IPM grove. Note different scale of y-axis.

and no recovery was observed. During 2003, four small peaks of abundance were observed. The first occurred in April, the second in June, the third in August and the last in September.

The patterns of abundance of coccinellid species were slightly different for the groves studied. In the organic grove, three main peaks of abundance were registered during the year, with a variable dominance of each species in the community (figure 3). The first peak oc-

curred from April to the end of May and *R. chrysomeloides* dominated the community, followed by *C. bipustulatus*. During this period, the other species were less abundant. The second peak occurred from the middle of June to the middle of August with the dominance of *S. interruptus*. The last period was dominated by *C. bipustulatus* and occurred from the end of August to the end of October.

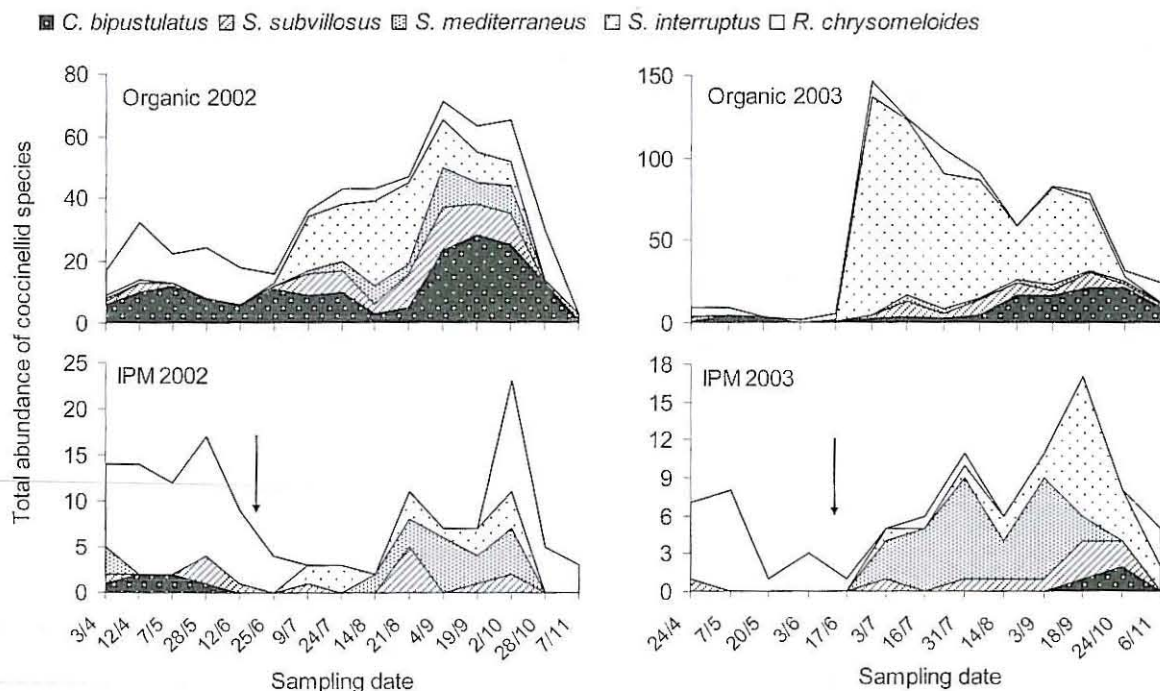In the IPM grove, the dominance and the fluctuations

■ *C. bipustulatus* ▨ *S. subvillosus* ▦ *S. mediterraneus* ◻ *S. interruptus* ◻ *R. chrysomeloides*

Figure 3. Total abundance of coccinellids species during 2002 and 2003, in the organic and IPM olive groves. Arrows indicate the date of the spray application in the IPM grove. Note different scale of y-axis.

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of the coccinellid species were different when compared to the organic grove. *R. chrysomeloides* dominated the community in spring and in autumn. *C. bipustulatus* showed only one annual peak of abundance which occurred from April to the middle of May in 2002 and from the middle of September to the end of October in 2003. The other three species were more abundant from early July to the middle of October of both years and apparently, presented three short consecutive generations during summer when *M. mediterraneus* dominated the community (figure 3).

Interactions between coccinellids and *S. oleae*

In the organic grove, the increase of the total abundance of coccinellids at the end of June coincided with the increase in abundance of the first instar nymphs (figure 2). Significant positive correlations were found between first instar nymphs of *S. oleae* and both *S. subvillosus* and *S. interruptus*. In early September, the abundance of coccinellids reached a new peak, which coincided with the increase in the number of the second instar nymphs of the phytophagous. Significant positive correlations were observed between four species of coccinellids (which were in decreasing order of significance *S. subvillosus*, *C. bipustulatus*, *S. mediterraneus* and *S. interruptus*) and the second instar nymphs of *S. oleae*. A significant positive correlation was also found between coccinellid larvae and the second instar nymphs of the black scale. On the other hand, no significant positive correlations were found between coccinellid and the third and fourth instar nymphs of the pest (table 1).

In the IPM grove, the peak of the total abundance of both coccinellid adults and larvae occurred in April,

when the third and fourth instar nymphs of *S. oleae* were the dominant stages and significant positive correlations were found between *R. chrysomeloides* and the fourth instar nymph of the black scale (table 2). A second peak of coccinellid abundance was reached in September and October and coincided with the occurrence of the second instar nymphs of *S. oleae*. Significant positive correlations were observed, in decreasing order of significance, between *S. interruptus*, *S. subvillosus* and *S. mediterraneus* and that stage of the pest. As with the organic grove, the second instar nymphs of *S. oleae* was the stage that presented more significant positive correlations with three out of five coccinellid species (table 2).

The associations between coccinellid species and nymphal stages of *S. oleae* are shown in the ordination diagram (figure 4). For both olive groves, the first two axes of the CA explain 60% of the variance within species. The proximity among points displayed by the ordination diagram suggests an association between *S. interruptus*, *S. subvillosus*, *S. mediterraneus* and the first and second instars nymphs of *S. oleae* while *C. bipustulatus* shows a closer association between the second and the third instars nymphs in the organic grove than in the IPM grove.

Discussion

From this study, several significant positive correlations were found between some coccinellid species and *S. oleae*, indicating the existence of seasonal synchrony between predators and pest. Thus, *S. interruptus* presented the larger number of positive correlations with the black scale, namely with the first and second instar nymphs

Table 1. Values for Pearson's correlations (*r*) between coccinellid species and different phenological stages of *S. oleae* in the organic grove during 2002 and 2003 (n=29).

	First-instar	Second-instar	Third-instar	Fourth-instar	Mature females
C. bipustulatus	−0.112 n.s.	0.454 *	−0.023 n.s.	−0.309 n.s.	0.013 n.s.
S. subvillosus	0.475 **	0.695 ***	−0.056 n.s.	−0.558 **	0.263 n.s.
S. mediterraneus	0.330 n.s.	0.595 ***	0.199 n.s.	−0.275 n.s.	0.048 n.s.
S. interruptus	0.708 ***	0.397 *	−0.359 n.s.	−0.604 ***	0.610 ***
R. chrysomeloides	−0.063 n.s.	−0.088 n.s.	0.325 n.s.	0.040 n.s.	−0.241 n.s.
Total coccinellids	0.487 **	0.600 **	−0.169 n.s.	−0.574 **	0.400 *
Larvae	0.274 n.s.	0.403 *	0.172 n.s.	−0.154 n.s.	0.111 n.s.

n.s.- non significant, * p < 0.05, ** p < 0.01, *** p < 0.001.

Table 2. Values for Pearson's correlations (*r*) between coccinellid species and different phenological stages of *S. oleae* in the IPM grove during 2002 and 2003 (n=28).

	First-instar	Second-instar	Third-instar	Fourth-instar	Mature females
C. bipustulatus	−0.031 n.s.	0.248 n.s.	−0.049 n.s.	−0.111 n.s.	−0.202 n.s.
S. subvillosus	0.354 n.s.	0.543 **	0.268 n.s.	−0.299 n.s.	0.118 n.s.
S. mediterraneus	0.433 *	0.439 *	0.304 n.s.	−0.400 *	0.173 n.s.
S. interruptus	0.487 **	0.578 **	−0.158 n.s.	−0.459 *	0.175 n.s.
R. chrysomeloides	−0.625 ***	−0.636 ***	−0.082 n.s.	0.407 *	−0.239 n.s.
Total coccinellids	0.017 n.s.	0.097 n.s.	0.084 n.s.	−0.018 n.s.	0.009 n.s.
Larvae	−0.332 n.s.	−0.334 n.s.	−0.214 n.s.	0.068 n.s.	0.023 n.s.

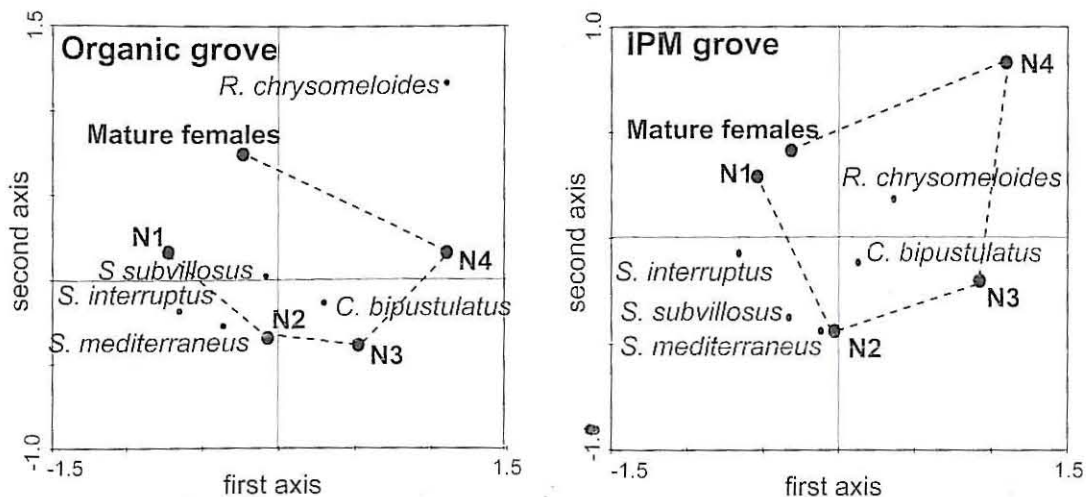n.s.- non significant, * p < 0.05, ** p < 0.01, *** p < 0.001.

Figure 4. Correspondence analysis (CA) ordination diagram for coccinellids species and different phenological stages of *S. oleae* in organic grove (Total inertia = 0.759, axis 1 eigenvalue = 0.313, axis 2 eigenvalue = 0.137) and in the IPM grove (Total inertia = 0.904, axis 1 eigenvalue = 0.342, axis 2 eigenvalue = 0.197).

and the mature females. However, the correlation between *S. interruptus* and mature females of *S. oleae* does not reflect a clear functional association between them because the predation pressure on mature females with eggs is unlikely to occur due to the hardness of the female integument that protects the eggs. Instead, some predation might be exerted on recently emerged nymphs. The period of higher abundance of *S. mediterraneus* and *S. subvillosus* coincided with that of the first and second instar nymphs. Furthermore, *C. bipustulatus* abundance correlated well with the number of the second instar nymphs of *S. oleae* (table 1) and appears to be clearly associated with stages N2 and N3 in both groves (figure 4). Previously, Argyriou and Katsoyannos (1977) showed that *C. bipustulatus* and *S. subvillosus* were species that could be successfully reared on *S. oleae*, and Ba M'Hamed and Chemseddine (2001) showed that *S. mediterraneus* was able to complete its life cycle consuming a large number of *S. oleae* eggs in the laboratory. Our data also suggest that coccinellid larvae can be important predators principally of the second instar nymphs of the pest. Apparently, immature stages of *S. oleae*, with predominance of the first and second nymph were potentially the most predated. The prevalence of the second instar nymphs during the end of summer is of great interest for the natural control of this pest because (1) this stage is very sensitive to the action of predators (Passos-Carvalho *et al.*, 2003) and (2) at this time the abundance and diversity of coccinellids is high, making the suppression of *S. oleae* more efficient (Snyder *et al.*, 2006). On the contrary, the third and the fourth instar nymphs were probably the least predated stages in part because their peak abundances coincide with the winter period when the abundance of the predators is reduced.

The management practices, carried out in the studied groves, have apparently influenced the pest and the predators in different ways but more studies are needed to confirm this idea. It seems that *S. oleae* was not influenced by the management regime probably because

mature females, the dominant stage in June, were especially tolerant to insecticide sprays (Passos-Carvalho *et al.*, 2003). In addition, the fluctuations of the pest population were similar in both olive groves. However, it seems that the abundance of coccinellid species was influenced by the management regime and shorter generations apparently occurred during both years in the IPM than in the organic grove, which can be seen as the ability of different populations to rapidly increase in abundance and respond to a disturbance (Begon *et al.*, 1996). Also, the synchrony between the predators and the pest seems to be affected since the number of positive correlations obtained in the IPM grove was lower than in the organic grove.

The efficiency of predators will increase if seasonal synchrony with the pest exists. However, a significant positive correlation between their populations does not necessarily mean that the predators have an impact on the control of the pest. It may only indicate that the predators rapidly respond to the variation of the pest numbers and that a great coexistence between predators and pest exists, but the predation rate can be low and consequently, insufficient to control the pest. On the other hand, significant negative correlations may indicate that predators slowly respond to the variation of pest population, but the predation rate can be high. In this case, an asynchronism between predators and the pest can exist. Alternatively, the two populations may show no correlation over time, but this does not necessarily mean that predators have no impact because the combination of positive and negative associations between guilds of predators and their prey may result in the appearance of no correlation at all (Kidd and Jervis, 1996).

Conclusions

This study showed that the patterns of abundance of the pest were similar between groves. However, the patterns of abundance of coccinellids vary between groves. The

two groves were dominated by different species of coccinellids, particularly after pesticide application in the IPM regime.

In both olive groves, the coexistence between coccinellids and *S. oleae* is likely to reflect predator-prey relationships, already referred to by other researchers. This relationship is fairly consistent between groves: *S. interruptus*, *S. subvillosus*, *S. mediterraneus* are associated with stages N1 and N2 of *S. oleae*, while *C. bipustulatus* is associated with stages N2 and N3. However, further studies need to be carried out, under controlled conditions, to assess if the synchrony between the predator species and the different phenological stages of the prey imply predation or is merely coincidental.

Acknowledgements

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