

Short term response of ants to the removal of ground cover in organic olive orchards

MERCEDES CAMPOS¹, LUISA FERNÁNDEZ¹, FRANCISCA RUANO³, BELÉN COTES¹, MANUEL CÁRDENAS¹
and JUAN CASTRO²

¹Department of Environmental Protection, Estación Experimental del Zaidín, (CSIC) C/Profesor Albareda n° 1, 18008 – Granada, Spain; e-mail: mercedes.campos@eez.csic.es

²IFAPA Centro Camino de Purchil, CAP (Junta de Andalucía), P.O. Box 2027, 18080 – Granada, Spain

³Department of Animal Biology, University of Granada, 18071 – Granada, Spain

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Abstract. Ants are the most abundant group of soil arthropods in olive groves where they are involved in various trophic relationships of great importance for crops. The system of soil management is one agricultural practice that has a great effect on ants, so the objective of this study was to compare ant populations in organic olive orchards with a ground cover of natural vegetation and others where this natural vegetation is mechanically removed at the beginning of June. Ants were sampled using pitfall traps at 14, 30, 70 and 90 days after the removal of the ground vegetation. Overall, ant biodiversity did not change. However, changes were observed in the abundance of ant species, in particular, in those species that build shallow nests in the soil, both between the rows of trees and under the canopy of olive trees. In contrast, deep nesting species, such as *Messor barbarus*, were not affected. The response also differed between the various genera: the abundance of *Cataglyphis* increased, due to there being more of the species *C. rosenhaueri*, while there was a significant fall in *Aphaenogaster*, due to the decline in abundance of *A. senilis*. Thirty days after the removal of vegetation, the response of most of the genera was clearly noticeable, due to the increased activity of workers, and in some cases there were still differences after 90 days.

INTRODUCTION

Ants make up a large proportion of the arthropod fauna in many land ecosystems, where they play a key role in determining the structure and functioning of local communities (Hölldobler & Wilson, 1990). They are often used as indicators, because they show a rapid response to environmental changes, are abundant and diverse in many environments and easily sampled, and serve a wide variety of ecosystem functions (Peck et al., 1998; Andersen, 1990; Alonso & Agosti, 2000).

Olive orchard agroecosystems are inhabited by a rich and varied arthropod fauna and ants are one of the most abundant and diverse groups (Morris et al., 1999; Morris & Campos, 1999; Redolfi et al., 1999; Santos et al., 2007a). Most species of ants build their nests in the ground either under the tree canopy or between the rows of olive trees, although some species such as *Crematogaster scutellaris* (Hymenoptera: Formicidae) build their nests under bark (Redolfi et al., 1999). Ants play an important role within the arthropod community in olive orchards as they consume larvae of pests, such as *Prays oleae* (Lepidoptera: Plutellidae) (Arambourg, 1986; Morris et al., 2002; Pereira et al., 2002), but can also have harmful effects on natural enemies because ants eat the eggs of the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae) (Morris et al., 1998) and the parasitoid *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) used for the biological control of *Prays oleae* (Pereira et al., 2004). According to Altieri (1999), the

maintenance of the functional biodiversity in agroecosystems is a key ecological strategy for sustaining production. In general, the degree of biodiversity in these systems depends on four main characteristics of the agroecosystem: the diversity of vegetation within and around the system, the permanence of the different crops within the system, the intensity of management and the extent of the separation of the system from natural vegetation (Southwood & Way, 1970). In relation to the intensity of management in conventional olive farming, in which synthetic pesticides and fertilizer are allowed and the soil is frequently deeply ploughed, the abundance and richness of ant species is lower than on land organically farmed (Redolfi et al., 1999; Santos et al., 2007b). Research has demonstrated that the timing of spraying is of utmost importance if negative side effects are to be minimized (Santos et al., 2007b), but in the case of soil management the effect of different strategies on beneficial arthropods is less well known. If available, this type of information could be used to plan any necessary disturbances (Gliessman, 2007).

Currently in organic olive orchards the use of natural or planted ground cover is considered to be an important factor for improving soil water balance and preventing soil erosion (Pastor et al., 1997). However, in areas with a Mediterranean climate, ground cover is removed in order to reduce competition and associated loss of production. Ground cover is controlled by mechanical methods, either ploughing or clearing it with a mower, or by livestock grazing, mainly using sheep. At present, the effects of

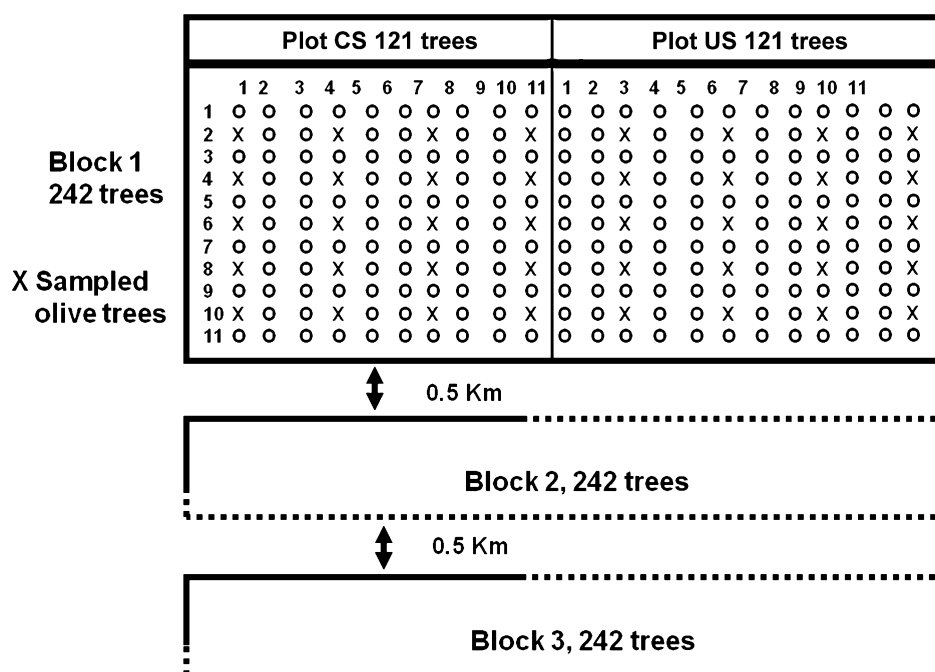


Fig. 1. Experimental design.

these methods on soil biological activity are not well known, so it is difficult to provide specific practical recommendations. In particular, to improve organic cultivation it is important to determine the timing and method of the removal ground cover as it can have an effect on the biological and physicochemical characteristics of the soil (Guzmán Casado & Alonso Mielgo, 2004; Cárdenas et al., 2006).

This study is part of a wider project whose objective is to measure the short term changes in the abundance and diversity of epigeal arthropod populations caused by the removal of ground cover of natural vegetation in organic olive orchards. It has already revealed that family richness, dominance and abundance of epigeal beetles are greater in uncovered soil, and that the family Silvanidae can be used as an indicator of the effect of this type of agricultural practice (Cotes et al., 2009). In the case of spiders, the removal ground cover has a positive effect on *Zodarium styliferum* (Simon, 1870) but diversity and dominance are not affected (Cárdenas, 2008). This survey seeks to determine how weed cover and weed-management practices (mowing and removal) influence abundance and composition of ant species.

MATERIAL AND METHODS

Ground cover management

Two soil treatments were compared: covered (CS) and uncovered soil (US) management. In the covered soil-treatment management, no plants were sown, the ground was spontaneously colonized by native annual Gramineae (e.g. *Bromus* sp., *Hordeum* sp., and *Diplotaxis* sp.) and no herbicide was applied. In the uncovered soil treatment, the pre-sampling intervention, during the first week of June 2005, to manage the ground cover was as follows: (1) a mower was used to cut any vegetation, then (2) a tine harrow to remove the coarser material, and, finally, (3) the remaining organic matter was shredded and

tilled into the soil to a depth of a few centimetres, i.e., the litter and seed remained in the soil, though partially buried. Under the canopy of the trees, vegetation and plant litter were cleared by hand.

Samples were collected 14, 30, 75 and 90 days after the removal of natural vegetation.

Field-experiment

The study was conducted in a large olive orchard in southern Spain, located at 3°38'36"W, 37°51'38"N. The organically managed orchard consisted of single-trunk olive trees (Picual cultivar), with a crown diameter of 1.5–3 m, each planted in 8 × 8 m grid. A randomized complete block design was adopted with two treatments, covered (CS) and uncovered soils (US) grouped into three blocks. Within each separate block, the conditions were as uniform as possible, but between blocks, there were marked differences. In the in-line array of blocks the distance between them was 0.5 km (Fig. 1).

Each block, with 242 trees (88 × 176 m), consisted of two plots representing the two treatments. In each plot of 121 trees (88 × 88 m), a total of 20 pitfall traps were set under the canopy of 20 selected trees. The sampling unit consisted of a row of five trees separated by one unsampled row, and each sampling unit was separated from others by two unsampled rows of trees (Fig. 1).

Ant collection

The ants were collected in pitfall traps, which were set on the north-facing side of each tree. The traps consisted of plastic cups, 11 cm in diameter, filled with Scheerpeltz liquid (60% ethanol 97°, 38% distilled water, 1% pure acetic acid, 1% glyc-erine) and were left in the soil for 48 h.

Captured arthropods were separated from the plant and inorganic matter, and the ants identified to species under a stereomicroscope (Stemi SV8, Zeiss). For the assessment of ant species diversity, the following measures were calculated: dominance, Hurlbert's PIE (probability of interspecific encounters) index and the Shannon index. Dominance is one of the most commonly used measures of biodiversity, Hurlbert's PIE index indicates the probability of two individuals randomly sampled from

TABLE 1. Number of individuals of the different species of ants captured.

Formicidae	Ground cover present						Ground cover removed				
	Days	14	30	70	90	Total	14	30	70	90	Total
Subfamily Myrmicinae											
<i>Aphaenogaster senilis</i>		802	468	574	438	2282	235	294	163	102	694
<i>A. gibbosa</i>		32	4	5	7	48	3	37	0	11	51
<i>Messor barbarus</i>		194	114	130	24	462	192	244	135	172	743
<i>Pheidole pallidula</i>		97	66	91	58	312	196	327	121	215	859
<i>Tetramorium semilaeve</i>		10	10	2	6	28	49	67	42	42	200
<i>Crematogaster scutellaris</i>		41	0	4	8	53	0	2	0	0	2
Subfamily Formicinae											
<i>Cataglyphis rosenhaueri</i>		555	820	103	57	1535	829	2150	67	39	3085
<i>C. velox</i>		0	2	0	0	2	0	0	0	0	0
<i>Camponotus pilicornis</i>		74	92	20	14	202	234	340	12	30	616
<i>C. micans</i>		2	0	0	0	2	0	0	0	0	0
Subfamily Dolichoderinae											
<i>Tapinoma nigerrimum</i>		50	8	3	7	68	187	121	73	50	431
	Total	1857	1584	932	619	4992	1925	3482	613	661	6681

the same population belonging to two different species, and, finally, the Shannon index provides a measure of both species numbers and the evenness of their abundance. The indices were calculated using Ecosim software, version 7.72 (Gotelli & Entsminger, 2010).

Statistical analysis

With five trees per row as the unit sample size, a comparison was made between CS and US in terms of total abundance and abundance of different genera, as well as the different diversity measures calculated for each 48-h period sampled. As tests revealed that the data did not have a normal distribution, non-parametric statistical tests were used (Kruskal-Wallis test) for independent samples using the Statgraphic Plus programme.

Multivariate analysis

Differences in ant species composition between treatments were assessed using a multivariate nonparametric test of differences between groups (blocked multi-response permutation procedure, blocked MRPP). Group differences are reported in terms of the A statistic, the “chance-corrected within-group agreement” ($A = 0$ is the expectation under the null model; $A = 1$ when all members of each group are identical within groups; and positive A -values indicate groups are more different than would be expected by chance). The Bray-Curtis distance was used as a dissimilarity measure. The design in blocks was defined as the blocking variable and the treatment as the grouping variable, and compared for each of the periods sampled.

Finally, plot scores were compared using non-metric multidimensional scaling (NMS) ordinations of the four periods sampled in order to determine whether ant species assemblages had changed as a function of treatment and during the course of the period monitored. NMS ordination is an iterative search for the positions of species and plots in relatively few dimensions (axes) that minimizes the departure from monotonicity in the association between distance (dissimilarity) in the original data and the ordination space. Dissimilarity matrices were constructed using the Sørensen distance and ant species abundances were arcsine transformed before analysis.

RESULTS

Families, genera and species found

A total of 11,673 individuals were captured, belonging to three subfamilies (Myrmicinae, Formicinae and Doli-

choderinae), eight genera and 11 species. In June and July, total captures were higher than in September (Table 1).

Effect of removing the ground cover on ant abundance and population dynamics

A total of 4,992 ants were captured in CS, among which the subfamily Myrmicinae (63.8%) was the most abundant, followed by Formicinae (34.8%) and then Dolichoderinae (1.4%). After removal of the ground vegetation, the total number captured rose to 6,681, a substantial increase with respect to the initial, undisturbed situation. The subfamilies present were the same as in CS, although the relative abundances had changed, for example, the presence of the subfamily Myrmicinae fell by 20%, captures of Formicinae doubled and of Dolichoderinae increased six-fold. These changes were mostly observed 14 and 30 days after removal of the ground cover, and at these sampling periods the most abundant taxa were Myrmicinae and Formicinae in CS and US, respectively. However, 70 and 90 days after the disturbance, the numbers of the subfamily Formicinae caught decreased in both treatments, with the result that the subfamily Myrmicinae became the most abundant (Table 1).

Two weeks after the removal of the ground cover, the mean number of ants captured was similar in the two treatments. However, after 30 days, in CS the number caught were similar to those in the first sample, while in US the numbers caught increased significantly compared to both the previous sample ($P = 0.0024$) and CS ($P = 0.0001$), where the captures were stable (Fig. 2). After 70 and 90 days, the number of captures diminished and no significant differences were detected between treatments (Fig. 2).

Subfamily Myrmicinae

The six species of the subfamily Myrmicinae responded differently to the removal of ground cover. Specifically, captures of *Aphaenogaster senilis* and *Crematogaster scutellaris* fell by 1,588 and 51 individuals, respectively, while those of the other three species, *Messor barbarus*,

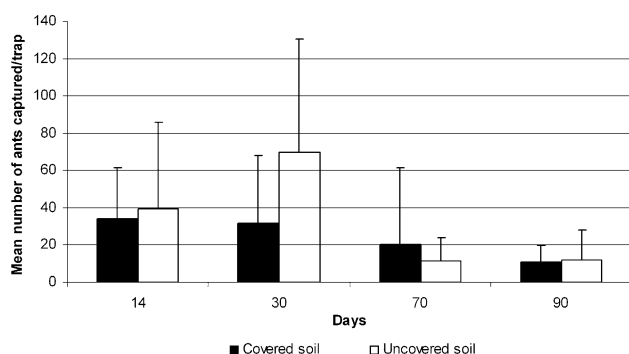


Fig. 2. Mean (\pm S.E.) number of ants captured in the experimental and control plots 14, 30, 70 and 90 days after the ground vegetation was removed from the experimental plot.

Tetramorium semilaeve and *Pheidole pallidula*, increased by 281, 172 and 547 individuals, respectively (Table 1).

Aphaenogaster senilis was the most abundant species in CS making up 45.7% of the total captured. The removal of the ground vegetation resulted in a significant decline ($P = 0.0001$) in number captured after 14 days (58.9%) and differences continued to be recorded between treatments throughout the sampling period ($P = 0.0029$, $P = 0.0001$, $P = 0.0001$) (Table 1).

The removal of ground cover did not seem to affect the *Messor barbarus* populations, although an increase in the total number captured was recorded, the differences between treatments were not significant at any of the sampling periods (Table 1).

The mean number of *Pheidole pallidula* captured during the entire study was higher in US, although the differences were only significant at 30 and 90 days ($P = 0.0134$ and $P = 0.0374$) after the removal of ground cover (Table 1).

Only a few individuals of *Tetramorium semilaeve* were captured in CS, but the removal of ground cover resulted in a greater number captured in US, although the difference between treatments was only significant at 30 days ($P = 0.0072$) (Table 1).

It was difficult to determine the effect of the removal of ground cover on *Crematogaster scutellaris* as very few individuals were captured. This may be attributed to the fact that this ant nests under the bark and in crevices in olive tree trunks (Morris et al., 1999; Pereira et al., 2002), so the sampling method employed was not suitable for this species.

Subfamily Formicinae

The two genera recorded in this study were favoured by the removal of ground cover, as number of individuals of the genus *Cataglyphis* captured doubled and of *Camponotus* trebled (Table 1).

Cataglyphis rosenhaueri was the most abundant in US, where it accounted for 46.2% of the total number captured (Table 1). While 14 days after the removal of the ground cover, captures of this species increased, significant differences were recorded only at 30 days in CS ($P = 0.0001$) (Table 1).

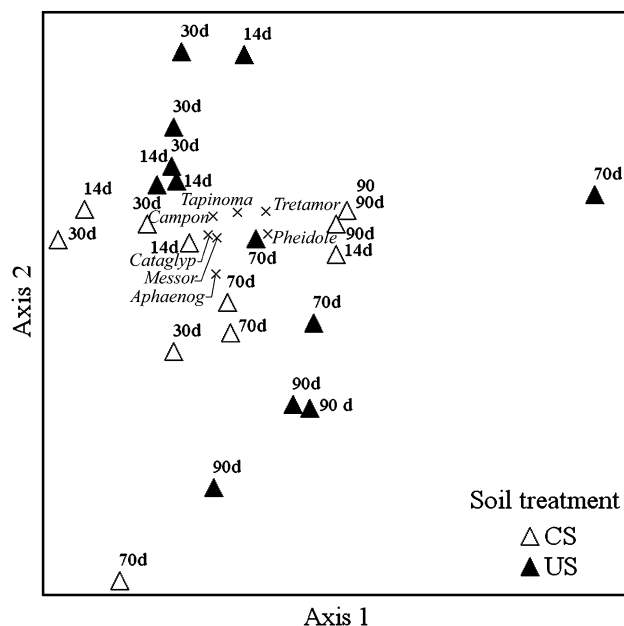


Fig. 3. NMDS ordination plot of ant genus abundance (logarithmically transformed) in the three blocks sampled at 14, 30, 70 and 90 days after the ground vegetation was removed from the experimental plot. Each triangle represents an individual plot. All of the NMDS ordinations were based on the Sørensen distance measure.

The abundance of *Camponotus pilicornis* in the study area was low, especially in CS. As a consequence of removing the ground cover, the numbers of this ant caught increased in the first two samples, but there were no significant differences between treatments (Table 1).

Subfamily Dolichoderinae

Tapinoma nigerrimum is the only species from the subfamily Dolichoderinae represented and the numbers caught were six-fold higher in plots without ground cover. In all the four samples the captures were higher in US and the differences were significant after 30, 70 and 90 days ($P = 0.0008$, $P = 0.0001$ and $P = 0.0270$) (Table 1).

Effect of removing ground cover on the diversity and assemblages of ant species

With regard to the diversity measures, the Shannon index indicated a higher diversity in CS and Hurlbert's PIE a higher homogeneity of ant species composition in US, but the differences between treatments were not significant (Table 2).

The blocked MRPP indicated that the ant species assemblages differed in composition between the treatments ($A = 0.214$, $p = 0.005$). Consequently subsequent analyses were performed separately for each sample. However, the highest heterogeneity between treatments was recorded 30 and 70 days after the ground cover was removed (30 days: $A = -0.008$; 70 days: $A = -0.026$), and was less in the first and last samples, with the lowest heterogeneity recorded at 14 days ($A = 0.028$) and 90 days ($A = 0.026$), although none of these differences were statistically significant.

TABLE 2. Diversity measures of the total numbers of ants captured in the two treatments.

	Ground cover present	Ground cover removed
Hurlbert's PIE index	0.59 ± 0.13	0.65 ± 0.01
Dominance	0.42 ± 0.02	0.41 ± 0.02
Shannon index	1.08 ± 0.01	0.95 ± 0.28

An ordination solution was obtained using NMS and a random starting configuration of a maximum of six axes, a stability criterion = 0.00001, based on 50 permutations using real data and Monte Carlo tests based on 50 permutations. The final ordination solution was determined after examining all possible solutions from Monte Carlo tests. The solution with the minimum number of dimensions (three axes) that provided the lowest stress (10.386) and lowest instability (0.00001) over 500 iterations was chosen. Examination of the NMS ordination plot revealed that in the four samples CS and US differ (Fig. 3) because each point is a two-dimensional representation of ant species composition in each plot (covered or uncovered). Points that are close together have ant assemblages that are more similar in genus composition compared to those that are further apart. In general, the CS plots occur close together, however the US plots are clearly separated into two groups. In the upper left corner the first and second samples are close to each other, while the last two samples occur together in the lower part of the plot.

DISCUSSION

The major effects of habitat disturbance are often indirect and stress related influencing habitat structure, microclimate and food supplies (Andersen, 2000). The most obvious effect of ploughing on soil inhabiting ants is the destruction of their nests and, while this initially results in a change in the composition of the ant community, after a period of several months, if there is no further ploughing, the community returns to its original composition (Perfecto & Castiñeiras, 1998). In this study, the removal of ground cover from olive orchards temporarily modified the abundances of the ants and of the subfamilies. At 14 and 30 days after the disturbance, the most abundant subfamily was the Formicinae, which occurred to the detriment of the Myrmicinae. However, from 70 days onwards, the subfamily Myrmicinae was the most abundant. In relation to this, it is worth noting that although ploughing does not permanently eliminate ants, changes in the composition of the community can be important for biological control (Perfecto & Castiñeiras, 1998).

The fact that the heterogeneity of the ant genus assemblages was highest just after the removal of the ground cover indicated that the ants responded quickly, while in the following periods the composition of genera became more homogeneous. The stress value obtained indicated that the ordination provided a good fit to the data, which were distributed in two main groups (CS and US).

The response of the various species to the removal of ground cover was most evident at 30 days. This may be

because this is a time of great activity, possibly due to the fact that some nests were destroyed and these had to be rebuilt in other places or the new resources in the rows between the olive trees required more and longer foraging.

Importance of nesting strategy

Ants in olive groves have a broad range of nesting habits and accordingly tend to respond in different ways to disturbances caused by farming practices (Redolfi et al., 2004).

The destruction of nests with the removal of ground cover had an effect on species such as *Aphaenogaster senilis* and *Cataglyphis rosenhaueri*. Both species build shallow nests in the ground in the open ground between rows of olive trees and under the canopies of the trees (Plaza & Tinaut, 1989; Redolfi et al., 1999). In the case of *A. senilis* fourteen days after the removal of ground cover, there was a 71.5% reduction in the numbers caught and it did not recover within the period of this study. According to Andersen (2000), disturbance is defined as the removal of biomass and for most soil animals this is synonymous with death. Ants, however, are modular organisms and many "modules" (individual ants) can be lost without necessarily threatening the reproductive unit (the colony). So, it seems likely that the removal of ground vegetation in the middle of June destroyed some nests, which caused a large decrease in the numbers caught and a sudden emigration to safer areas. An abrupt decrease in ant assemblages with this life strategy could be interpreted as a quick response to the effects of a short-term disturbance of the soil arthropods in US. Previous studies have proposed that the Silvanid (Coleoptera) be used as a bio-indicator group for evaluating the effect of soil agronomic practices in the organic farming of olives (Cotes et al., 2009). The workers of *C. rosenhaueri*, after deep ploughing in July, carry off eggs and immature ants and reorganise their nests at depths as shallow as 4 cm (Redolfi et al., 1999). This behaviour, namely the building of shallow nests and readily moving them to new locations, could account for the large number of individuals of this species that were captured in June and July after the removal of the ground cover. In September, the number caught declined and reached values close to those recorded in CS. Similar dynamics, with an initial apparent increase in numbers and decrease thereafter, is reported for other ant species subject to disturbances such as fires (García et al., 1995).

Species such as *M. barbarus* build their nests at a depth of 2 m or more (Ruano & Tinaut, 1993) and consequently and not surprisingly the removal of ground cover did not affect their abundance, as the mowing and tilling only breaks the surface crust of the soil.

Importance of feeding behaviour

Another important aspect that determines the movement of ants is the increased availability of new food resources. So, it is possible that the greater presence of *Pheidole pallidula* in US was due to its ability to exploit diverse food sources or because the scarcity of food forced them to

undertake longer journeys, making them more likely to fall into pitfall traps. This species is very aggressive and its ecological success is based on it being active day and night and its variety of foraging techniques (Detrain, 1990).

Tapinoma nigerrimum nests in the ground under the canopy of trees and forages in the olive trees. Only small numbers were captured in CS, which could be attributed to the sampling method used in this study. However, it is described as an opportunistic, a generalist and a colonizer, which occurs principally in seriously degraded environments (Cerdá & Retana, 1988). These characteristics could explain its greater presence in US.

Importance of environmental conditions

The level of activity of some species is also influenced by temperature and solar radiation (Retana et al., 1987; Palacios et al., 1999), so the greater presence of species, such as *C. pilicornis*, in US could be explained by their relocating their nests to areas with better environmental conditions, which in turn increased their risk of capture, rather than the availability of food sources.

Interactions between species

In nature, the relationships between insects within a community form an extensive food web composed of several trophic levels. So, the greater presence of *Cataglyphis rosenhaueri* in US could have resulted in an increase in the abundance of ant predators such as spiders of the families Zodariidae and Gnaphosidae (Cárdenas et al., 2006). In particular, the genus *Zodariion* (Araneae: Zodariidae) prefers to feed on ants of the subfamily Formicinae (e.g., *Cataglyphis* and *Camponotus*) rather than Myrmicinae (e.g., *Messor* and *Tetramorium*) (Pekár, 2005).

On the other hand, there are also interactions between the two ant species, *A. senilis* and *T. nigerrimum*. The first species usually appears later and displaces *T. nigerrimum*, either by attacking or killing it (Cerdá et al., 1988). So, the relatively low abundance of *Aphaenogaster senilis* in US may favour their presence.

These studies are of great importance because ants occupy various different trophic levels and so by studying these species it is possible to determine the effect of particular agricultural practices on the functioning of the olive grove agro-ecosystem. In this way potential bio-indicator species can be determined and the timing and the optimal method of removing ground cover specified.

CONCLUSIONS

Removal of ground cover in June resulted in particular changes in the abundance of those species of ants that nest in the soil, either between the rows or under the canopies of the olive trees. However, ant diversity did not change.

The response of the various species differed: in particular, the abundance of *Cataglyphis rosenhaueri* increased and that of *Aphaenogaster senilis* decreased significantly.

In the majority of species, the response was most evident in an increased activity of workers a month after the removal of the vegetation.

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