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Spiders as potential biological controllers in apple orchards infested by *Cydia* spp. (Lepidoptera: Tortricidae)

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Abstract

Spiders have been commonly considered as polyphagous predators. For this reason, it has been argued that spiders may not be efficient in controlling pests. However, in recent years it has been demonstrated that they are able to significantly decrease the damage caused by insects to harvest. In this paper we present some preliminary results of a field experiment that has taken place in 2007, in a biological apple orchard at Caraglio (Cuneo, North-Western Italy). The aim of the experiment was to study the potential reduction of the damage caused by *Cydia* spp. (Lepidoptera: Tortricidae) to apples by increasing the population size of spiders living on trees by offering them additional shelter space through the provision of artificial shelters (polyethylene bark-traps) during wintertime. The spider assemblage was found to be strongly dominated by *Anyphaena accentuata* (43%), followed by *Dictyna arundinacea* (20%) and *Philodromus* spp. (8%). Compared to control, the total number of spiders increased significantly in trees provided with artificial shelter and trees with such device showed lower frequency of damaged apples. Results from this preliminary study suggest that habitat manipulation in apple orchards may increase the population size of bark dwelling spiders and thus increase their potential preying efficiency.

INTRODUCTION

Spiders have been commonly considered as polyphagous predators (Bristowe 1941). For this reason, in spite of their abundance and diversity in agroecosystems, it has been argued that spiders may not be efficient in controlling pests (Debach & Rosen 1991). However, due to their high abundance and predominantly insectivorous feeding habits, spiders are suspected to play a fundamental predatory role in agroecosystems, woodlands and other terrestrial ecosystems (Marc et al. 1999). As an example, in China spiders have been actively preserved in order to combat particular pests (Zhao 1993).

In a review on this subject, Marc et al. (1999) redefined the role of spiders as biocontrollers in the agroecosystems, specifying that *"their huge differences in hunting strategies, habitat preferences and active periods combine to make the group potentially efficient. Thus, it should be possible to pinpoint one or several spe-*

cies in a community that are able to fight against a particular pest".

It appears that, at least in some cases (see Sunderland et al. 1986; Mansour 1987; Agnew & Smith 1989; Hooks et al. 2003), spiders are efficient in limiting pests in agroecosystems. However, not all spider species are necessarily efficient in every agroecosystem or against all types of prey but, in general terms, an increase of their density could exert a higher predatory pressure on pests. As demonstrated by Marc (1993b, cited in Marc et al. 1999) in French apple orchards, this can best be done by habitat manipulations, which offer additional shelter to spiders and could lead indirectly to a reduction of the fruit damage caused by several species of apple skin eating pests (including *Cydia pomonella*).

Polyethylene bubble wrap bark traps represent an efficient method to sample spiders living on trees (Roberts & Roberts 1988; Isaia et al. 2006). Since these traps function by providing a shelter for overwintering and for night active spiders, in a previous work (Isaia et al. 2006) we pointed out that additional research may be worthwhile to evaluate the possible role of bark traps as artificial shelters for spiders, especially referring to species involved in biological control of insect pests in perennial orchards. In this sense our paper presents the results of a field experiment that has taken place in 2007 in a certified biological apple orchard, sited in Caraglio (Cuneo, North-Western Italy). The here presented trap system is primarily offering additional shelter space. In this way it offers spiders the opportunity to increase their density during wintertime.

Aim of the work

The key questions of our work are:

1: Does the provision of artificial shelters in the cold season increase the total number of spiders living on apple trees?

2: Does the provision of artificial shelters reduce significantly the damage caused to apples by pests (esp. *Cydia* spp., Lepidoptera: Tortricidae)?

MATERIAL AND METHODS

Study area

The study was carried out in a certified biological apple orchard of 0.98 ha sited in Caraglio (Cuneo, North-Western Italy), ca. 120 km south of Torino and 30 km west of Cuneo. The plantation consists of the cultivars "Goldrush" and "Golden Orange", planted in 1990 at a distance of 6 x 3 m, in 12 rows of 43 trees each. The orchard is surrounded by agricultural fields (mais and raspberry) and poplar stands. The alleys, between tree rows, were grassy, mowed two or three times a year. TIOVIT sulphur (32 kg/ha), proteinised sulphur (12 kg/ha), polisulphur (41500 ml/ha) acting as fungicides and Neem oil (2400 ml/ha), acting against soft bodied insects like aphids and whiteflies, were sprayed during the experiment, mainly in spring and summer from April to August. For defence against *Cydia*, sexual confusion technique was used in the orchard from April to September 2008.

Experimental design

The experimental design consisted of two parts, one concerning spiders (key question 1) and one concerning apples (key question 2) and has been arranged as follows:

Spiders (key question 1)

In the treated row, three artificial shelters were provided on each of the 43 trees composing the row: one was tied around the main trunk (circa 50 cm from ground) and two around two main branches of the tree (circa 100 cm from ground), using a tape. Artificial shelters were made with a double strip of material (polyethylene bubble wrap) 20-25 cm wide and 40 cm long. A similar-sized strip of black polyethylene was placed on the outer side of each trap to exclude light. Once removed, artificial shelters were put in a bag saturated with ether to kill specimens and replaced with fresh ones at the same positions on the trees. Artificial shelters were dissected and carefully examined under a stereomicroscope to remove spiders.

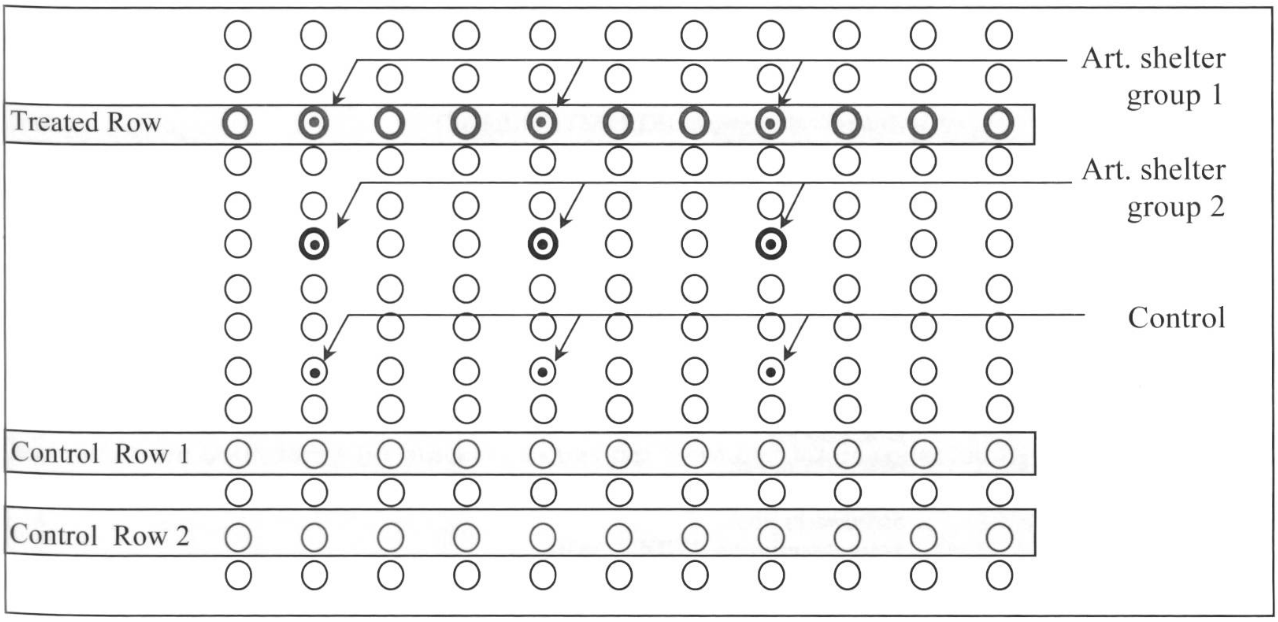

Fig. 1. Experimental design. Circles represent the trees in the apple orchard, bold black circles refer to trees provided with artificial shelters, black dots indicates trees chosen for spider sampling. Rows chosen for the evaluation of insect damage are indicated in shaded grey boxes. Distances and number of trees are not true to scale, see text for details.

To study the cumulative effect of vicinity we sorted the trees provided with artificial shelters into two groups of three trees each: Artificial shelter group 1 and Artificial shelter group 2. Artificial shelter group 1 is included in the treated row; Artificial shelter group 2 consists of three trees that are part of a row without any other artificial shelters (see Fig. 1).

Beating tray sampling was performed immediately after taking away the artificial shelters on the same trees (both Artificial shelter group 1 and Artificial shelter group 2) and on control group, i.e. 3 trees without artificial shelters. We used a rectangular canvas 2 x 2 m placed under each tree. The trees were shaken sharply four to five times and dislodged spiders were collected from the canvas with an aspirator and preserved in 70% alcohol.

Artificial shelter sampling was performed on Artificial shelter group 1 and Artificial shelter group 2, for a total of 3 + 3 trees x 12 sampling events (monthly from October 2006 to October 2007) = 72 sampling events. Beating tray was performed on Artificial shelter group 1, Artificial shelter group 2 and control, for a total of 3 +3 +3 trees x 12 sampling events = 108 sampling events.

Spiders collected by means of the two methods have been examined under a stereoscope up to 40x and identified whenever possible at species level. Voucher specimens are stored at Dipartimento di Biologia Animale e dell'Uomo of Turin University. Nomenclature follows Platnick (2008).

We applied a two-way ANOVA with LSD post-hoc test to test the differences of average abundances of spiders living on each tree among groups and in relation to sampling period. Analyses were carried out both for beating tray and artificial shelters samplings. The null hypothesis was that abundances were equivalent in all groups and that the period of sampling did not affect them differentially.

We tested differences in terms of abundance of spiders on:

(1) Artificial shelter group 1 VS Artificial shelter group 2 (artificial shelter samplings)
(2) Artificial shelter group 1 VS Artificial shelter group 2 VS Control group (beating tray).

Family	Species	Total Number
Anyphaenidae	*Anyphaena accentuata* (WALCKENAER, 1802)	1321
Araneidae	*Araneus* juv.	5
	Araniella opistographa (KULCZYNSKI, 1905)	61
	Atea juv.	7
	Cyclosa conica (PALLAS, 1772)	104
	Gibbaranea bitubercolata (WALCKENAER, 1802)	2
	Mangora acalypha (WALCKENAER, 1802)	42
	Nuctenea umbratica (CLERCK, 1757)	13
	Zilla diodia (WALCKENAER, 1802)	2
Clubionidae	*Clubiona pallidula* (CLERCK, 1757)	2
	Clubiona juv.	32
Corinnidae	*Phrurolithus* juv.	3
Dictynidae	*Dictyna arundinacea* (LINNEAUS, 1758)	636
Gnaphosidae	*Drassodes* juv.	6
	Haplodrassus juv.	4
	Micaria pulicaria (SUNDEVALL, 1831)	1
	Scotophaeus juv.	6
Linyphiidae	*Erigone dentipalpis* (WIDER, 1834)	2
	Lepthyphantes mengei (KULCZYNSKI, 1887)	2
	Lepthyphantes zimmermanni (BERTKAU, 1890)	2
	Lepthyphantes juv.	5
	Meioneta rurestris (C.L. KOCH, 1836)	1
	Microlinyphia pusilla (SUNDEVALL, 1829)	1
	Walckenaeria clavicornis (EMERTON, 1882)	1
	Walckenaeria vigilax (BLACKWALL, 1853)	2
Lycosidae	*Pardosa agrestis* (WESTRING, 1861)	1
	Pardosa lugubris (WALCKENAER, 1802)	3
	Pardosa juv.	6
Miturgidae	*Cheiracanthium mildei* (L. KOCH, 1864)	3
	Cheiracanthium virescens (SUNDEVALL, 1833)	1
	Cheiracanthium juv.	14
Oxyopidae	*Oxyopes* juv.	10
Philodromidae	*Philodromus aureolus* (CLERCK, 1757)	3
	Philodromus cespitum (WALCKENAER, 1802)	4
	Philodromus juv.	227
Salticidae	*Ballus chalybeius* (WALCKENAER, 1802)	37
	Macaroeris nidicolens (WALCKENAER, 1802)	1
	Pseudicius encarpatus (WALCKENAER, 1802)	14
	Salticus scenicus (CLERCK, 1757)	26
	Salticus zebraneus (C.L. KOCH, 1837)	4
	Salticus juv.	93
Tetragnathidae	*Pachygnatha* juv.	1
	Tetragnatha juv.	5
Theridiidae	*Achaearanea tepidariorum* (C.L. KOCH, 1841)	8
	Dipoena melanogaster (C.L. KOCH, 1845)	2
	Enoplognatha juv.	13
	Keijia tincta (WALCKENAER, 1802)	1
	Robertus neglectus (O.P.-CAMBRIDGE, 1871)	1
	Simitidion simile (C.L. KOCH, 1836)	1
	Theridion nigrovariegatum (SIMON, 1873)	3
	Theridion varians (HAHN, 1831)	3
	Theridion juv.	78
Thomisidae	*Diaea dorsata* (FABRICIUS, 1777)	5
	Misumena vatia (CLERCK, 1757)	9
	Misumenops tricuspidatus (FABRICIUS, 1775)	21
	Ozyptila praticola (C.L. KOCH, 1837)	1
	Pistius truncatus (PALLAS, 1772)	2
	Synaema globosum (FABRICIUS, 1775)	16
	Xysticus juv.	10
Uloboridae	*Hyptiotes paradoxus* (C.L. KOCH, 1834)	1
Not identified (spiderlings)		195
Total		**3086**

Table 1. Species listed in alphabetical order (nomenclature follows Platnick 2008).

	Tot	Av/tree	SD	Date of Max	Date of Min
Beating tray					
Control group	558	15,50	13,25	21/09/2007 (42)	14/12/2007 (0)
Artificial shelter group 1	835	23,19	18,29	20/06/2007 (61)	23/11/2007 (4)
Artificial shelter group 2	627	17,42	13,09	30/10/2007 (46)	14/12/2007 (1)
Artificial Shelters					
Artificial shelter group 1	485	13,47	14,11	23/11/2007 (61)	17/08/2007 (4)
Artificial shelter group 2	588	16,33	24,29	22/11/2007 (121)	21/09/2007 (0)

Table 2. Abundance (Tot), average per tree (Av/tree), standard deviation (SD), date of maximum and minimum abundance (in brackets values per tree) of spiders collected by means of the two methods, beating tray and artificial shelters.

ANOVA was performed using the software SPSS version 12.0.1 (SPSS Inc. 2004). To attain normality data were log transformed. A level of significance of alpha = 0.05 was used for all analyses.

Apples (key question 2)

To test the effectiveness of the provision of the artificial shelters against pests we considered the frequency of damaged apples on the trees, easily distinguishable by the presence of circular holes caused by *Cydia* spp. on apple peels. The damage has been evaluated in three different rows of trees (see Fig. 1): Treated row (all trees provided with artificial shelters), Control row 1 and Control row 2. A total of 60 apples (random counts per tree) x 12 trees (randomly chosen in the row) x 3 groups x 5 sampling events = 21 600 have been counted. The evaluation of damage started on 25/05/2007 together with the observation of the first damaged apple and went on monthly up to 21/09/2007.

We applied two-way ANOVA with LSD post-hoc test to test differences of average frequencies of damaged apples on each tree among groups and in relation to sampling period. The null hypothesis was that frequencies of damages were equivalent in all groups and that the period did not affect them differentially. ANOVA was performed using the software SPSS version 12.0.1 (SPSS Inc. 2004). To attain normality data were arcsin transformed. A level of significance of alpha = 0.05 was used for all analyses.

RESULTS

Spiders

We sampled 3086 spiders, belonging to 58 species and 16 families (Table 1). The spider community was found to be strongly dominated by the bark dwelling night-active hunter *Anyphaena accentuata* (43%), followed by the mesh web spinner *Dictyna arundinacea* (20%).

Data on abundances of spider per group (Artificial shelter group 1, Artificial shelter group 2 and control) referring to beating tray and to artificial shelter samplings are illustrated in Table 2. As confirmed by statistical analysis (see Tables 3 and 4), no significant differences were found by comparing abundance of spiders sampled by artificial shelter samplings in Artificial shelter group 1 VS Artificial shelter group 2. All were significantly affected by period of sampling (see also Table 2, date of max and min). Beating tray performed on Artificial shelter group 1 and Artificial shelter group 2 showed higher means compared to control, attesting the role of artificial shelters in increasing abundance of spider populations. The temporal trend of occupancy of artificial shelters by spiders is illustrated in Fig. 2, with peaks of occupancy in the cold season and peaks of abundance in beating tray samples in spring and summer.

	SS	df	MS	F	P value
Dep. Var: artificial shelters only					
Groups	0.059	1	0.059	0.863	0.373
Period	0.750	11	0.068	23.173	0.000**
Groups x Period	0.750	11	0.068	1.571	0.138
Error	2.083	48	0.043		
Dep. Var: beating tray only					
Groups	0.764	2	0.382	14.301	0.000**
Period	0.587	11	1.483	55.543	0.000**
Groups x Period	1.301	22	0.027	1.477	0.111
Error	1.301	72	0.018		
Dep. Var: frequency of damaged apples					
Groups	0.055	2	0.028	8.102	0.012*
Period	0.168	4	0.042	12.279	0.002*
Groups x Period	0.027	8	0.003	3.185	0.002*
Error	0.177	165	0.001		

Table 3. Summary statistics for ANOVA (spider abundance and frequency of damaged apples). (SS: sum of squares, df: degrees of freedom; MS: mean square).

Apples

Statistical analysis confirmed significant differences among groups in the frequency of damaged apples and, in this case also, a relevant significant influence of the period of sampling (Table 3) was observed. Post-hoc test highlighted that the treated row showed significantly lower average frequencies of damaged apples in respect to control, attesting the possible role of artificial shelters in decreasing the fruit damages (Table 4).

DISCUSSION

Spiders

The great number of species found (56) is comparable with species richness listed for untreated apple orchards by Marc & Canard (1997), ranging from 34 to 77. The same authors give a list of species that is definitely comparable to what we found. Also the dominance of bark-dwelling spiders described by Szinetàr & Horvàth (2006) are largely confirmed by our study. In particular, artificial shelters are entirely dominated by anyphaenids and dictynids, followed by salticids and philodromids. Beating tray samplings are characterised by a more diverse assemblage, consisting of several families of web weavers like araneids, theridiids or ambush spiders, such as thomisids. The abundance of bark dwelling spiders, like anyphaenids, dictynids and clubionids *sensu latu* (including miturgids) increases in trees provided with artificial shelters, attesting their role in offering additional shelter space. This is furthermore interesting if we consider that anyphaenids, clubiondids *s.l.* and dictynids are considered as potential predators of harmful pests by Marc & Canard (1997) (larvae of Lepidoptera and non-flying aphids for anyphaenids and clubionids s.l. and winged aphids for dictynids). Seasonal trends of variations of spider abundance (see Fig. 2) are comparable with those observed in our previous work on bark traps (Isaia et al. 2006), with high abundances of spiders in artificial shelters in the cold season, decreasing in spring and summer. It is important to note that despite the use of two sampling methods during one entire year sampling,

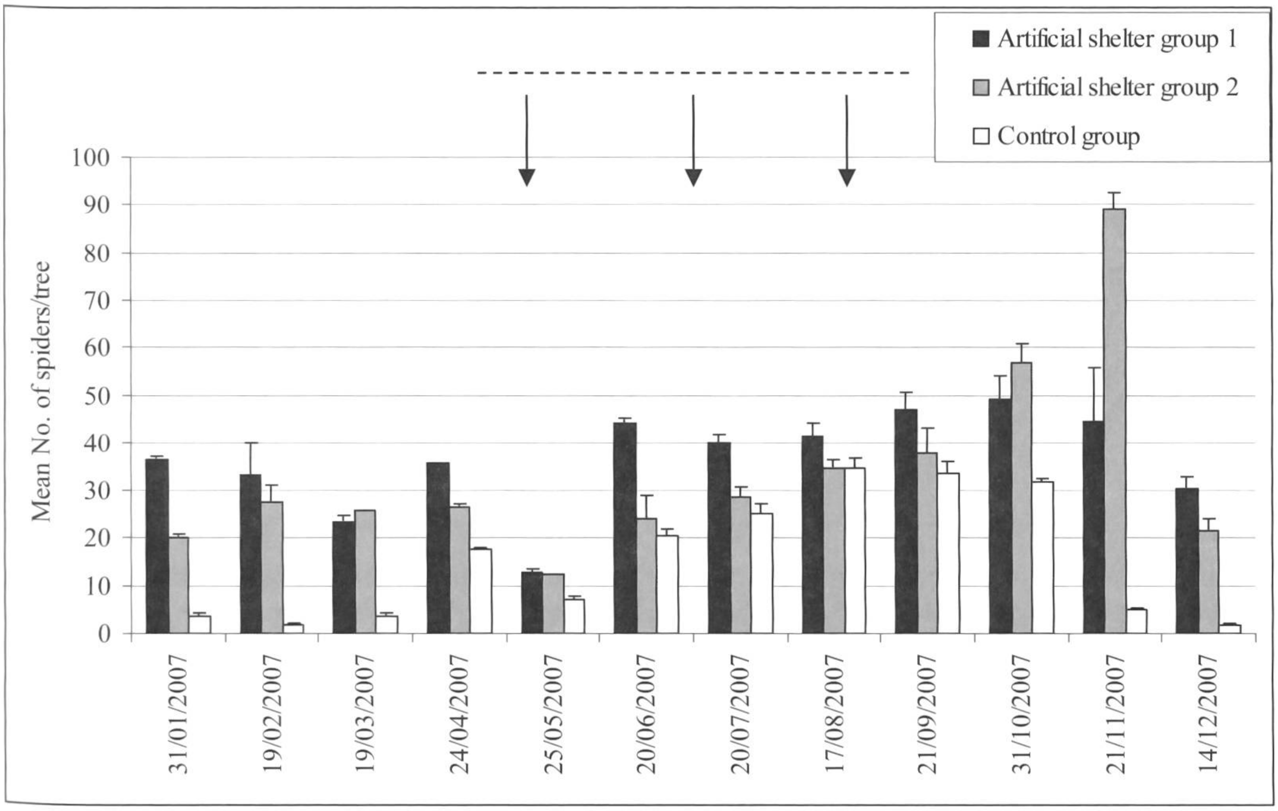

Fig. 2. Temporal trends of total spider abundance per tree in relation to the biological cycle of *Cydia* spp. Flights of first, second and third generation of *Cydia* are indicated by arrows. Dashed lines indicate the period of activity of *Cydia*. Values refer to artificial shelter group 1, artificial shelter group 2 and control group considering artificial shelters + beating tray samples. Values are mean spider number per tree, bars are standard errors.

the number of spiders was still high, attesting the strong migration potential of spiders in this kind of agroecosystem.

Concerning artificial shelter samplings, significant differences were found among periods but not between artificial shelters group 1 vs artificial shelters group 2. No cumulative effect related to the vicinity of other artificial shelters was thus seen. On the other hand, significant differences were observed by considering beating tray samples only. Post-hoc test (see Table 4) revealed significant differences among control group and artificial shelters group 1 and 2. According to this, we could hypothesize that artificial shelters widen the niche of tree dwelling spiders by increasing the carrying capacity of the system. Such devices consequently act as a source for spider populations, determining higher abundances of spiders on trees.

Apples

On the basis of the model experiments of Mansour (1987) and Mansour et al. (1980, 1981) and by the field experiments of Marc (1990 and 1993a, b), Marc & Canard (1997) argue that spiders are able to decrease significantly insect damage in apple orchards. Mansour et al.'s experiments (1980 and 1981) demonstrated that this effect was due to direct predation and to a disturbing effect of *Cheiracanthium mildei* (Araneae: Miturgidae) on newly hatched larvae of *Spodoptera littoralis* (Lepidoptera: Tortricidae), a serious pest in commercial apple orchards in Israel. Moreover, Marc (1990 and 1993a, b), in his field experiments of habitat manipulation, demonstrated that spiders could induce a significant reduction of the damage caused to apples by *Anthonomus pomorum* (Coleoptera: Curculionidae) and by some Lepidopterans Tortricidae, including *Cydia pomonella*.

	(I) group	(J) group	MD(I-J)	SE	P value
Dep. Var: beating tray only					
LSD	Control	Art. Sh1	-0.2060	0.0317	0.000**
		Art. Sh2	-0.1040	0.0317	0.002*
	Art. Sh1	Art. Sh2	0.1020	0.0317	0.002*
Dep. Var: beating tray + artificial shelters					
LSD	Control	Art. Sh1	-0.5232	0.3049	0.000**
		Art. Sh2	-0.4537	0.3049	0.000**
Dep. Var: frequency of damaged apples					
LSD	Treated Row	Cntr R1	-0.0139	0.0059	0.021*
		Cntr R2	-0.0422	0.0059	0.000**

Table 4. Summary statistics for Multiple comparisons (LSD Post-hoc test) (spider abundance). Art. Sh1 and Art. Sh2 = Artificial shelter groups 1 and 2, respectivley. Cntr R1 and Cntr R2 = Control Row 1 and 2 respectivley. MD= Mean Difference, SE= Standard Error. Only significant values are reported.

According to this author, the reduction (60%) of the buds infested by the larval stage of *Anthonomus pomorum* was due to predation by araneids, theridiids and amaurobiids and the decrease of the damage induced by *Cydia* spp. on the apples (from 25 to 40%) was due to the predation of the bark dwelling spiders *Clubiona corticalis*.

In our case (Table 4), we observed that numbers of damaged apples were significantly lower on trees provided with artificial shelters. We can thus hypothesize that this was due to the presence of a higher number of potential predators, and especially to the increase of bark dwelling spiders, that potentially prey upon the moth. This interpretations is in accordance with the hypothesis formulated by Marc & Canard (1997) in respect to *Anyphaena accentuata* (the dominant species in the studied orchard) on the role of the different species in pest control. Following the same approach of the above cited authors and by considering the temporal trend of spider abundance in relation to the biological cycle of *Cydia* (Fig. 2), it could be argued that spiders could act as biological controller in different ways, in relation to the different stages of the pest. In our case, increased populations of spiders overwintering in artificial shelters could colonise the trees abundantly and potentially act as predators on the different stages of development of the pest. Considering the list of the families found (16 families and 56 species), the predatory pressure on *Cydia* can be potentially seen as the action of the entire spider community on the pest population, starting with the predation on overwintering larval stages by winter-active spider species (mostly linyphiids) or overwintering spiders under barks (anyphaenids and philodromids); on flying adults of different generations by web builders (araneids, dictynids, linyphiids, theridiids, tetragnathids and uloborids) and ambushers (thomisids); on eggs (see the experiment of Nyffeler et al. 1990 and Hooks et al. 2006 for predation of arthropod eggs) and on larval stages by diurnal (oxyopids and salticids) and nocturnal (gnaphosids, philodromids, anyphaenids and clubionids) foliage dwellers.

CONCLUSIONS

The results from this study suggest that habitat manipulation in apple orchards may increase the population size of bark dwelling spiders and thus increase their potential preying efficiency. Taking into account our results and in accordance to Marc (1993b), we could infer that the provision of artificial shelters implies an increase in spider population living on trees (Table 3) and, possibly, a consequent decrease in terms of crop damage (Table 4) due to the increase of the spider community predatory efficiency. This increase must be seen by considering that artificial shelters offer additional shelter space, especially by giving the opportunity to spiders to increase their density during wintertime. Despite the fact that the most abundant species, *Anyphaena accentuata*, (a medium sized spider that preys possibly on several different stages of the moth) potentially represents a good biocontroller of *Cydia* spp. (see also Marc & Canard 1997), the real effectiveness of this species in crop protection still needs to be tested by means of model experiments aiming to prove the real predation potential in respect to such pests.

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