Early-season predation on aphids by winter-active spiders in apple orchards revealed by diagnostic PCR

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Abstract

Aphids are major pests in apple orchards, debilitating the crop and spreading disease. We investigated whether early-season predation by canopy spiders may be effectively controlling aphid numbers in three organic orchards. For this purpose, we monitored the aphid population dynamics from the winter eggs to colony stages and compared this to spider abundances and rates of predation on aphids detected by diagnostic polymerase chain reaction. For the latter, we applied existing general aphid primers. We found that spiders ate colony fundatrices and that aphid numbers were negatively related to spider abundance. Spiders were the main active predators within the orchards when the first colony fundatrices were present, indicating their importance in the early control of aphid populations.

Keywords: *Aphis pomi*, conservation biological control, diagnostic PCR, *Dysaphis plantaginea*, generalist predator, gut content analysis

Introduction

In European apple orchards, the rosy apple aphid *Dysaphis plantaginea* (Passerini), the green apple aphid *Aphis pomi* (de Geer) and the woolly apple aphid *Eriosoma lanigerum* (Hausmann) are considered to be the three main aphid pests of economic importance (Marc & Canard, 1997). In particular, *D. plantaginea* can be the most detrimental, causing yield reductions from 30% (Deberardinis *et al.*, 1994; Blommers *et al.*, 2004) to 80% (Qubbaj *et al.*, 2005). Moreover, several other aphid species attack the apple trees, such as *Aphis spiraecola*

(Pagenstecher), *Rhopalosiphum insertum* (Walker), *Myzus varians* (Davidson) and *Myzus ornatus* (Laing). Only *A. pomi* has the apple tree *Malus domestica* (Borkh) (Rosales: Rosaceae) as a unique host, but *E. lanigerum* and *A. spiraecola* are also permanently present on apple trees because they are anholocyclic in Europe (no sexual reproduction). The other species include the apple tree in their life cycle as a primary host in spring. Their alate migrants lay eggs in autumn, close to apple shoots, and start to hatch in late March. The migration of *D. plantaginea* winged adults to their secondary host, the plantain herb *Plantago* spp. (Lamiales: Plantaginaceae), begins in May and lasts until the end of June (Bonnemaison, 1959; Carroll & Hoyt, 1984). Due to the exponential growth rate of these aphids, and their high damage risk, insecticide control is usually initiated at very low population levels. For example, insecticide treatment is recommended when a single

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D. plantaginea fundatrix is observed on a sample of 100 shoots (Minarro *et al.*, 2005).

The need to control these aphids by non-chemical methods has stimulated research into the potential of natural enemies to suppress these pests in apple orchards (Carroll & Hoyt, 1984; Mueller *et al.*, 1988; Brown, 2004; Minarro *et al.*, 2005; Fréchette *et al.*, 2008; Dib *et al.*, 2010). Two phases of control, involving different groups of predators, can be distinguished. Early in the season, before the aphid colonies build-up, biological control of aphids requires resident and actively foraging predators that are efficient at finding aphids even at very low density. These predators can have low predation rates because of the large impact that removal of small numbers of individuals can have on subsequent prey densities (Murdoch *et al.*, 1985; Chang & Kareiva, 1999). At that time, the main natural enemies are usually generalist predators because they are able to remain present in absence of a target pest, sustained on alternative prey (Symondson *et al.*, 2002). After colonies have established and the exponential growth phase has begun, biological control of aphids requires more specialised natural enemies that have a high predation rate and/or growth rate, and are actively searching for colonies (Murdoch *et al.*, 1985).

Among generalist predators of aphids, spiders possess characteristics that make them well-adapted for control of aphids early in the year. They are present on the canopy at the right time (Pekar & Kocourek, 2004; Simon *et al.*, 2009), and some species actively hunt in winter (Marc & Canard, 1997; Miliczky *et al.*, 2008; Korenko & Pekar, 2010). In many instances, spiders kill more prey than they consume (Riechert & Lockley, 1984; Greenstone, 1999). Wyss *et al.* (1995), looking at aphid remains in spider webs, found that web-building spiders contribute to the biological control of the *D. plantaginea* alate migrants in autumn. However, other studies, looking at communities of aphid predators (Brown, 2004; Minarro *et al.*, 2005; Dib *et al.*, 2010), concluded that spiders should not be considered as biocontrol agents of aphids because of their low abundance and predation rates compared to other groups, such as earwigs, ladybirds, syrphid and cecidomyiid flies, lacewings and true bugs. As these studies were looking at predation on well-established aphid colonies, their conclusions neglected the potential impact of spiders during the early phase of colony establishment.

Here, we hypothesised that spiders are significant predators that contribute to the early-season control of aphid pests in apple orchards. Two supplementary hypotheses were tested in support of this primary question: (i) early-season spiders eat aphid fundatrices, (ii) later appearance of colonies is negatively correlated with spider abundance early in the season. Other more specialised natural enemies (e.g. parasitoids) arrive when colonies are already established and growing exponentially, but any delay to the onset of this growth phase will improve the ability of these specialists to limit aphid population growth (Murdoch *et al.*, 1985; Chiverton, 1986). To address this question of early season predation, the numbers of aphids and canopy-dwelling spiders were followed from mid-March until the end of April in three organic orchards. Predation on aphids by spiders was studied by gut content analyses with diagnostic polymerase chain reaction (PCR) using aphid-specific primers. Gut content analysis of predation by spiders on aphids has been widely used in cereal crops (see references in Harwood & Obrycki, 2005; Kuusk *et al.*, 2008). This has been shown to be an effective method for analysing predation by fluid-feeders in the field without disrupting the system under study (Greenstone, 1999; Symondson, 2002; King *et al.*, 2008).

Materials and methods

Evaluation of the aphid specific primers

The aphid primers (Aphid F/Aphid R), originally designed by Chen *et al.* (2000) for detection of predation on cereal aphids, were tested for their ability to amplify DNA from the apple aphids *D. plantaginea*, *A. pomi*, *A. spiraecola* and *E. lanigerum* and the stonefruit aphids *Myzus cerasi* (Fabricius), *Myzus persicae* (Sulzer) and *M. varians* all collected locally. PCR was carried out in 10 μl volume reactions using 1 μl DNA template and the PCR protocol was adapted from Chen *et al.* (2000). PCR reactions contained 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl$_2$, 1.0 μM of each primer, 50 mM KCl, 0.1 mM of each dNTP, 0.05 U μl^{-1} of Taq DNA polymerase (Promega) and were performed in a PTC-200 thermocycler (MJ Research, Watertown, MA, USA). After an initial denaturing step of 3 min at 94°C, a cycle of 94°C for 30 s, 52°C for 30 s and 72°C for 1 min, was repeated 34 times, and then a last extension period at 72°C for 2 min was performed. PCR products were separated by electrophoresis in a 1.5% agarose gel with TBE buffer and visualised by ethidium bromide staining and photographed under UV light.

Spider collection and aphid monitoring

The three organic apple orchards were situated within a 5-km^2 area near Avignon in south-eastern France. In early March 2010, shoots, on which at least one aphid egg was observed, were marked with coloured plastic strips. For statistical independence, each marked shoot was on a different tree. Respectively, 17, 14 and 30 shoots were marked in orchards 1, 2 and 3. To collect spiders within all canopy habitats, two cardboard traps (10-cm wide bands, secured with elastic bands) that mimic tree bark were installed on each sampled tree, one wrapped around the branch next to the marked shoot and the other around the main trunk (1 m above the ground). This trapping method is considered the best technique to sample spiders that use tree bark as a shelter (Mizell & Schiffhauer, 1987), as well as web-building spiders when the foliage is absent (Marc & Canard, 1997). The shoot was considered the sampling unit for aphid numbers and the two cardboard traps the sampling unit for the spider community. The marked shoots were observed weekly for fundatrices and the presence of colonies on six occasions in early spring (22 and 31 March; 7, 14, 22 and 27 April). On 6 May, trees corresponding to the marked shoots were carefully examined, and the number of colonies and aphids per colonies were assessed. As *D. plantaginea* is easy to recognize by its grey colouration, we distinguished the *D. plantaginea* colonies from the other green aphid colonies (*A. pomi*, *R. insertum*, *A. spiraecola*, *M. varians*, *M. ornatus*) which cannot be separated to species in the field. Spiders were collected at the same time as the aphid monitoring except on the last date (27 April) for all orchards and on 22 March in orchard 2. In order to avoid local population depletion in orchard 3, where trees were young, spiders were retrieved in trees marked even (15 trees) one week and in trees marked odd (15 trees) the following week. At each sampling time, the cardboard traps were unwrapped
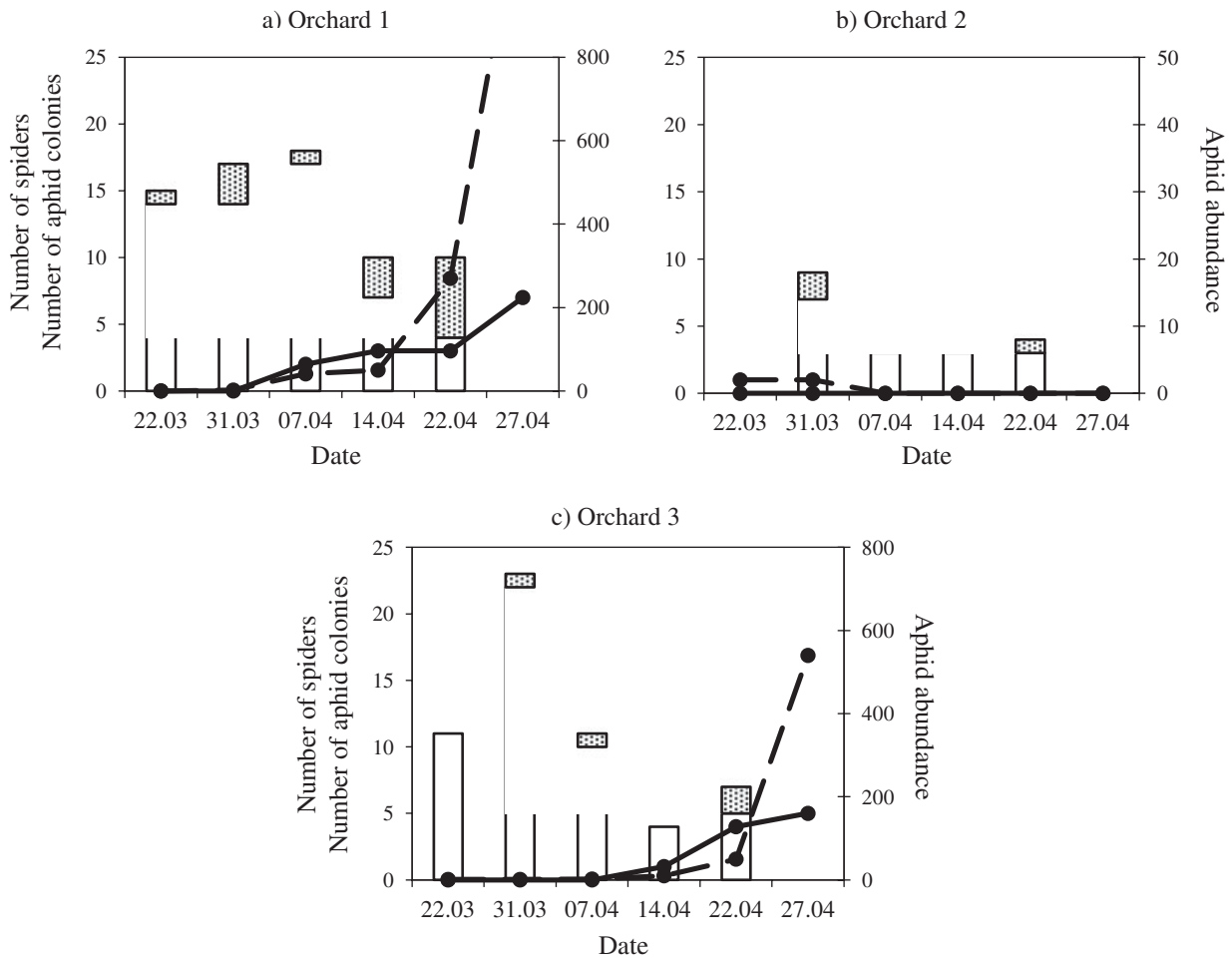
Fig. 1. Numbers of spiders (bars, Y1-axis), aphid colonies (black line, Y1-axis) and aphids (dashed line, Y2-axis) in three apple orchards (a) orchard 1, (b) orchard 2 and (c) orchard 3. Spiders detected positive by the aphid-specific diagnostic PCR are represented by the shading on the bars.

from the tree and allowed to fall into a 4 cm × 25 cm tray. Predators were shaken out and the same trap secured again in the tree. In addition to spiders, small numbers of overwintering tenebrionids, ladybirds and earwigs were found, but as they are inactive at this time, they were not sampled or tested for predation on aphids. Spiders were collected individually into Eppendorf tubes then stored in a cool box (4°C) for a maximum of 3 h and frozen at –20°C in the laboratory. They were identified before DNA extraction. Identification was to genus level only for species for which juveniles cannot be determined to species. The DNA was extracted with the Qiagen DNeasy® Blood & Tissue extraction kit, resuspended in 200 µl of manufacturer's elution buffer and stored at −20°C. Finally, extraction success was verified by amplification of the spider DNA with the universal arthropod primers LCO1490 and HCO2198 (Folmer *et al.*, 1994), and any possibility of cross-over contamination within batches of samples was excluded by the use of negative controls (water) (King *et al.*, 2008).

Data analysis

Factors influencing aphid predation by spiders

The effects of orchard, spider identity (species or genus level), canopy habitat (branch or trunk), aphid population (number of aphids per shoot) and date were tested against the probability of detecting aphid DNA in the spider guts, using a general linear model with binomial family errors and logit link function (R Development Core Team, 2011).

Effect of spiders on the emergence of aphid colonies

The binary variable "presence or absence of aphid colonies on the shoot on 22 April" was set up from the aphid records. The abundances of spiders collected from 22 March to 22 April in the corresponding tree were summed. And so, the number of aphid-positive spiders was calculated as the sum of all positive spiders for aphid predation collected from 22 March to 22 April in the corresponding tree. The effects of orchard, spider abundance and number of aphid-positive spiders on

Table 1. Composition of the spider assemblage and their predation on aphids detected by the aphid-specific diagnostic PCR in three orchards.

		Orchard 1		Orchard 2		Orchard 3	
		Spiders collected	Aphid positive spiders	Spiders collected	Aphid positive spiders	Spiders collected	Aphid positive spiders
Foliage-active spiders							
Anyphaenidae	*Anyphaena accentutata* (Walckenaer)	16	3	1	0	–	–
Clubionidae	*Clubiona* spp.	16	6	4	1	4	0
Gnaphosidae	*Zelotes* spp.	2	0	–	–	1	0
	Aphantaulax trifasciata (Simon)	0		3	0	3	0
Miturgidae	*Cheiracanthium mildei* (Cambridge)	3	1	–	–	–	–
Philodromidae	*Philodromus* spp.	14	1	5	0	35	1
Salticidae	*Ballus chalybeius* (Walckenaer)	–	–	1	0	1	0
	Heliophanus auratus (Koch)	6	1	5	1	3	0
	Pseudeuophrys lanigera (Simon)	7	2	–	–	2	1
Sit-and-wait spiders							
Thomisidae	*Runcinia grammica* (Koch)	–	–	–	–	1	0
	Synema globosum (Fabricius)	–	–	–	–	3	1
	Thomisus onustus (Walckenaer)	–	–	–	–	1	0
	Xysticus bifasciatus (Koch)	1	0	–	–	–	–
Web-builder spiders							
Amaurobiidae	*Amaurobius* spp.	2	0	–	–	2	1
Linyphiidae	*Meioneta* spp.	1	0	–	–	1	0
Theridiidae	*Anelosimus* spp.	4	0	–	–	–	–
	Theridion spp.	–	–	1	1	–	–
	Total	72	14	20	3	57	4

the presence/absence of aphid colonies were tested using a generalised linear model with binomial family errors and logit link function.

Results

Amplification of orchard aphid DNA

The general aphid primers only failed to amplify the DNA from *E. lanigerum*, which was not observed in the orchards.

Aphid dynamics

The mean initial number of eggs per shoot was 1.3 ± 0.1 (mean $\pm$ SE), 1.0 ± 0.0 and 1.3 ± 0.2 in orchards 1, 2 and 3, respectively, and was not different between orchards (Kruskal-Wallis $\chi^2 = 3.639$, df $= 2$, $P = 0.162$). The first funda-trices were recorded on 22 March and the first *D. plantaginea* colony was observed on 22 April, two weeks after the first appearance of other aphid colonies (7 April). *Dysaphis plantaginea* colonies represented 23.5% of the total number of colonies on 27 April (ten colonies) and 71% of the total number of colonies on 6 May (27 colonies). At the end of shoot monitoring (27 April), 23.6% of the marked shoots (i.e. for which at least one aphid egg was initially observed) were infested by an aphid colony in orchard 1, 13.3% in orchard 3 and none in orchard 2. On 27 April, the aphid population was at the beginning of its exponential growth phase (fig. 1).

Spider communities and other predators

In total, 149 spiders were collected in the three orchards (72, 20 and 57 in orchards 1, 2 and 3, respectively) (table 1). The mean numbers of spiders collected per tree and sampling occasion were, respectively, 0.85 ± 0.09, 0.34 ± 0.09 and 0.71 ± 0.22 (mean $\pm$ SE) in orchards 1, 2 and 3 and were significantly different between orchards (Kruskal-Wallis $\chi^2 = 6.379$, df $= 2$, $P = 0.041$). The spider communities differed between orchards. Orchard 1 and orchard 3 displayed species-rich communities; orchard 3 was dominated by *Philodromus* spp.; and orchard 2, particularly, lacked web-building and sit-and-wait spiders (table 1).

Predation by spiders on aphids

Spiders were found positive for aphid consumption from 22 March, which was two weeks before the first observation of colonies (fig. 1). In consequence, from 22 March to 7 April, it is likely that spiders attacked colony fundatrices. The probability of detecting aphid DNA in the spider guts increased with the number of aphids per shoot (F $= 7.52$, df $= 1$, $P = 0.007$) and depended on the date (F $= 4.00$, df $= 4$, $P = 0.004$) but was not explained by spider identity (F $= 1.37$, df $= 20$, $P = 0.15$), orchard (F $= 2.40$, df $= 2$, $P = 0.09$) and canopy habitat, i.e. branch or trunk, (F $= 0.16$, df $= 1$, $P = 0.69$). Temperature thresholds for most other potential natural enemies of aphids make it unlikely that these were active at this time (none were observed).

Effect of spiders on the presence of colonies

The presence of aphid colonies on 22 April was related to spider abundance (F $= 6.035$, df $= 1$, $P = 0.017$), number of aphid-positive spiders (F $= 10.023$, df $= 1$, $P = 0.003$) and orch-ard (F $= 3.167$, df $= 2$, $P = 0.049$) (fig. 1). The relationship was negative (parameter estimation $= -1.134$) for spider abundance only, showing that shoots from trees with more spiders were

less likely to harbour an aphid colony. The presence of aphid colonies was positively related (parameter estimation = 2.321) to the number of aphid-positive spiders, suggesting density dependent predation on aphids.

Discussion

The impact of the early-season predation by spiders on aphid populations was studied by analysing early-season aphid population dynamics, spider abundances and predation rates on aphids. Gut content analyses revealed predation events on aphid fundatrices. Spider identity, and their location within the canopy, had no significant effect on predation rates, although numbers were low, and therefore this result should be treated with caution. Aphid abundance had a positive effect on predation rates. Moreover, the presence of an aphid colony on a shoot was negatively correlated with early-season spider abundance in the corresponding tree.

Previous studies hypothesized that spiders could play an important role in the control of pests during winter (Marc & Canard, 1997; Marc *et al.*, 1999; Korenko & Pekar, 2010) because some spiders are able to remain active in winter and hunt for prey at low temperatures (Korenko *et al.*, 2010). Indeed, our results clearly showed that spiders prey upon colony fundatrices. They also suggested that spiders were mobile within the canopy; as, within this limited sample of spiders, those collected on the trunk were equally positive for predation on aphids as spiders collected on the shoots where the eggs/fundatrices were present. Moreover, other potential predators of aphids were unlikely to have been active during March and early April, and activity was not observed. The temperature thresholds for development of *D. plantaginea* and *A. pomi* are 4.5°C and 5.9°C, respectively (Graf *et al.*, 1985), which means that predators must be active around this temperature threshold to prey upon fundatrices. Korenko *et al.* (2010) showed that *Philodromus* spp. and *Anyphaenea accuenta-ta* (Walckenaer), which were present in their study orchard, had lower temperature thresholds of predatory activity than the developmental thresholds of *D. plantaginea* and *A. pomi* (1.2°C and 3.5°C, respectively). In consequence, these winter-active spiders are above their temperature threshold of activity when the first *D. plantaginea* and *A. pomi* eggs hatch. By contrast, temperature thresholds for other predators on apple trees are higher than those for the aphids, with 6°C for the earwig *Forficula auricularia* (Helsen *et al.*, 1998) and the hoverfly *Episyrphus balteatus* (Hart *et al.*, 1997), 9.0°C for the ladybird *Adalia bipunctata* (Obrycki & Tauber, 1981) and 10.5°C for the cecidomyiid fly *Aphidoletes aphidimyza* (Morse & Croft, 1987). These predators were indeed reported to prey upon aphids later in the season when the first colonies are already established (Dib *et al.*, 2010). Thus, temporal niche partitioning (Finke & Snyder, 2010) for aphids as a prey resource results from this difference in phenology among predators, reducing predator interference and limiting temporal refugia for pests (Symondson *et al.*, 2002). Nevertheless, spiders are known to be intraguild predators (Hodge, 1999; Wise, 2006), and their impact on other natural enemies should be studied before assuming niche complementarity between predators.

There are indicators suggesting that spiders may have been efficient at controlling aphid numbers in the present study. First, the time lag between the presence of the first fundatrices and their predation by predators is a critical factor for the appearance of colonies and their subsequent damage (Wyss *et al.*, 1999; Brown, 2011). In particular, Brown (2011) concluded that limitation of *D. plantaginea* colony development is more efficient when predation occurs within the first week of colony establishment. Here, spiders tested positive for predation on aphids as soon as the first eggs hatched and were, thus, likely to have had a considerable impact on aphid population dynamics, for example delaying the aphid exponential growth phase (Birkhofer *et al.*, 2008). Second, control of colony development is improved by high initial predator abundance (Wyss *et al.*, 1999). Here, the presence of a colony was indeed negatively correlated to the initial spider abundance on a tree. Finally, although prey preferences (Agustí *et al.*, 2003) and functional specialisation (Marc & Canard, 1997) are common in spiders, this may not be the case in winter when prey are scarce (Marc *et al.*, 1999). Spider identity was not significant in explaining predation on aphids, but more work would be needed with larger sample sizes to confirm this. Results so far indicate that no specific taxa would need to be particularly encouraged for early-season control of aphids.

To conclude, our results support the hypothesis that spiders are important for the early-season control of aphids. Furthermore, the impact of spiders on aphid populations delays the exponential growth phase and, therefore, increases the effectiveness of other predators (and parasitoids) that are active later in the season. The high potential of spiders as intraguild predators, and positive and negative effects of spiders within a predator assemblage (Hodge, 1999), needs further study if their role in aphid suppression within conservation biological control programmes is to be fully understood.

Acknowledgements

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