

## Colonization of apple orchards by predators of *Dysaphis plantaginea*: sequential arrival, response to prey abundance and consequences for biological control

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**Abstract.** *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae), *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) and *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) are the three most abundant natural enemies of *Dysaphis plantaginea* Passerini (Homoptera: Aphididae) in Asturian (NW Spain) apple orchards. They attack this aphid in sequence: *E. balteatus* arrived first, followed by *A. bipunctata* and then by *A. aphidimyza*. The cecidomyiids arrived too late to have a regulating effect. The syrphids laid an average of  $2.3 \pm 1.7$  eggs per aphid colony and the coccinellids  $18.4 \pm 9.9$  regardless of the degree of the infestation rates of the apple shoots. This value corresponds to the size of an egg batch laid by one female. Therefore, these aphid predators did not respond numerically to the abundance of the pest. The results of this study indicate that natural populations of syrphids and ladybird beetles are unable to control *D. plantaginea*, and therefore a more complex strategy than waiting for natural enemies is required.

**Key words:** *Adalia bipunctata*, aphids, apple, biological control, Coccinellidae, *Dysaphis plantaginea*, *Episyrphus balteatus*, resource partitioning, Syrphidae

### Introduction

The rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), is a major apple pest in Western Europe as well as in Asturias (NW Spain) (Bonnemaison, 1959; Bassino, 1982; Pasqualini and Briolini, 1982; Cruz de Boelpaeppe et al., 1987; Gendrier et al., 1989; Savini, 2000; Dapena and Miñarro, 2001). It is a dioecious species with a strict holocyclic life cycle (Bonnemaison, 1959). Apple trees, the primary host, suffer leaf-rolls and shoots and fruit deformations in the spring (Forrest and Dixon, 1975; De Berardinis et al., 1994). In case of an outbreak,

yield can be reduced by as much as 30% (De Berardinis et al., 1994). In early summer, this aphid leaves apple trees and migrates to the secondary host, *Plantago* spp.

*Dysaphis plantaginea* populations are generally controlled by insecticides, which are applied as soon as one fundatrix is observed on a sample of 100 shoots. Such an extremely low threshold is probably due to the fear of heavy crop damage. However, to our knowledge, this threshold has never been determined experimentally. As a result of frequent use of insecticides, *D. plantaginea* is beginning to show resistance to most registered insecticides (Delorme et al., 1998). Consequently, farmers are spraying more frequently and losing control over this pest (Dedieu, 1998; Graf et al., 1999). Therefore, effective alternatives for the control of *D. plantaginea* are required. Biological control appears as a possible alternative in fruit production that does not pose the health- and environment-damaging risks of pesticides.

To assess the potential for biological control in apple trees, observations were made during 2 years in two apple orchards. The goals of this study were to: (1) identify the most important natural enemies of *D. plantaginea* and (2) measure the response of these natural enemies to the abundance of *D. plantaginea*.

## Material and methods

### *Study orchards*

Trials were carried out in 1999 and 2001 in an experimental and commercial apple orchard in Asturias, Spain. The 0.3-ha experimental orchard was planted in 1995 with 17 different local cider-apple cultivars growing on Pajam 2 rootstocks. No pesticides were sprayed in this orchard. The 0.4-ha commercial orchard was planted in 1992 with 2 local dessert-apple cultivars grafted on M.9 rootstocks. This orchard was managed following organic guidelines and no synthetic pesticides or fertilizers were used. The only insecticides applied were summer white oil and Rotenone to control aphids, and granulosis virus to control codling moths. Copper and sulphur preparations were used as a means to control fungal diseases.

### *Sampling method*

In 1999, four trees of each of the 17 cultivars in the experimental orchard were sampled for the presence of *D. plantaginea*. Five shoots of

each tree were randomly selected and marked with coloured plastic strips before *D. plantaginea* egg hatching. From mid-April to the moment when all *D. plantaginea* had migrated to a secondary host, the 340 selected shoots were examined on a weekly basis for the presence of aphid colonies. In 2001, the number of cultivars sampled was reduced to 10, and consequently the number of shoots examined was reduced to 200. The same sampling method was followed in the commercial orchard, but only 20 trees (10 of each cultivar) were selected. In this orchard in 2001, aphid sampling extended just from the beginning of shoot colonization, May 2, to May 23, when apple trees were sprayed with insecticide. The abundance of *D. plantaginea* was expressed as the percentage of shoots infested by aphids.

Number and types of predators was recorded weekly during the spring by visual examination on 20 apple shoots randomly selected among those infested with *D. plantaginea*. Eggs and larvae were brought back to the laboratory and reared on *D. plantaginea* or *Aphis pomi* in order to determine their species.

#### *Statistical analysis*

The infestation rate of *D. plantaginea* was calculated each week at each orchard as the percentage of shoots with aphid colonies. The correlation between the infestation rate and respectively the percentage of aphid colonies with ladybird beetle eggs, syrphid eggs, ladybird beetle larvae and syrphid larvae were calculated. Only the data corresponding to the beginning of egg laying to the maximum infestation rate were considered. The correlation between the infestation rate and respectively the number of ladybird beetle eggs and the number of syrphid eggs were also calculated. The frequencies of aphid colonies with only eggs of coccinellids or syrphids or with eggs of both were compared for significance by  $\chi^2$ .

A preliminary analysis of these data showed that the frequencies did not significantly vary between orchards and years; therefore, these data were pooled together.

## **Results**

*Dysaphis plantaginea* infestations differed in intensity and in the time of occurrence among years and orchards (Figure 1). Aphids occurred from mid-April to early July, and the peak of infestation varied from the 3rd week of May to mid-June. At that time 15–55% of the shoots were

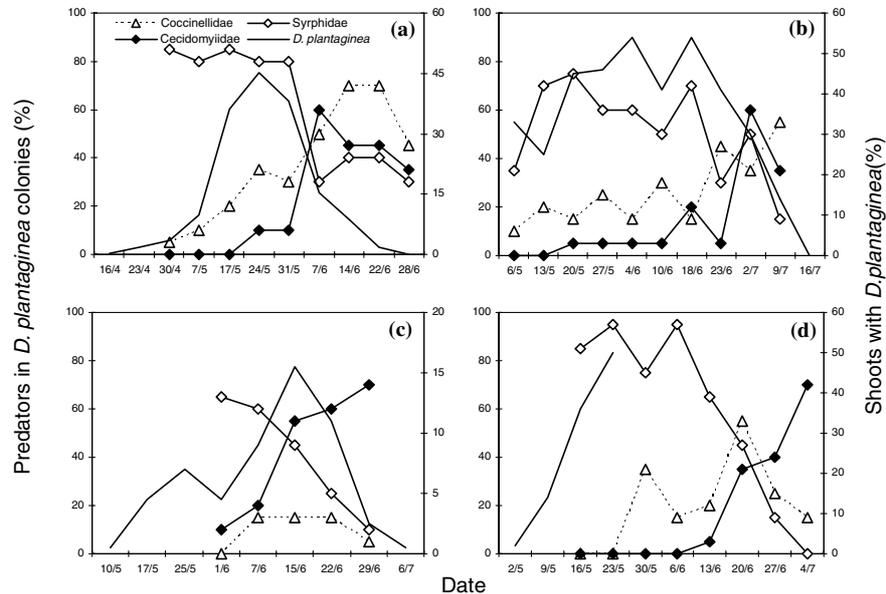


Figure 1. Occurrence of aphidophagous predators in *D. plantaginea* colonies in: (a) the experimental orchard in 1999; (b) the commercial orchard in 1999; (c) the experimental orchard in 2001; and (d) the commercial orchard in 2001. (Note: Sample of aphids ceased in the commercial orchard in 2001 (d) because many of the colonies in the marked shoots were affected by the insecticide application. Sampling of predators continued on unaffected colonies).

infested by aphids. Differences in the infestation rate were observed among cultivars (Miñarro and Dapena, in prep).

The colonies of *D. plantaginea* were exploited by a diverse guild of natural enemies. However, Cecidomyiidae, Syrphidae and Coccinellidae were the most abundant (Table 1). The cecidomyiid fly was *Aphidoletes aphidimyza* (Rondani). *Episyrphus balteatus* (DeGeer) (69.8%) was the most abundant syrphid, although *Scaeva pyrastris* (L.) (13.3%), *Syrphus ribesii* (L.) (11.5%) and *Epistrophe* sp. (5.4%) larvae were also recorded. *Adalia bipunctata* (L.) constituted 86.1% of the coccinellids. *Coccinella septempunctata* L. (8.3%), *Propylea quatuordecimpunctata* (L.) (2.8%) and *A. decempunctata* (L.) (2.8%) were also identified.

There appeared to be a sequential colonization of apple trees by the specialist predators. Syrphids tended to arrive first, followed by ladybird beetles, and then cecidomyiids (Figure 1). This pattern is even clearer if one considers the succession of eggs (Figures 2 and 3). Generalist predators belonging to Anthocoridae (Heteroptera), Miridae (Heteroptera), Chrysopidae (Neuroptera), Cantharidae (Coleoptera), Forficulidae

Table 1. Predator abundance in *D. plantaginea* colonies

		Aphidophagous 1999		2001	
		instars		Experimental	Commercial
Coccinellidae	Adults + larvae	171	67	25	88
Syrphidae	Larvae	173	129	53	135
Chrysopidae	Larvae	1	1	4	0
Cecidomyiidae	Larvae	346	124	253	240
Anthocoridae	Adults + nymphs	51	6	44	6
Miridae	Adults + nymphs	24	1	23	4
Forficulidae	Adults	0	2	0	0
Cantharidae	Adults	2	5	0	1
Spiders		11	4	1	2
Number of sample dates		9	10	5	8

Total number of aphidophagous instars in each orchard and year.

(Dermaptera) and Araneae appeared late in the development of aphid colonies and in low numbers (Table 1).

As a consequence of their early arrival, syrphids tended to lay eggs earlier in the development of aphid colonies than ladybird beetles. That is, syrphid eggs were mostly counted when the infestation rate of the shoots was low. This gets translated as a marginally significant negative relationship between the infestation rate of the shoots and the percentage of colonies of aphids with syrphid eggs ( $r = -0.507$ ;  $p = 0.064$ ) (Figure 4). Syrphid eggs progressively hatched following the increase of the aphid infestation rate giving rise to a significant positive relationship between the infestation rate of the shoots and the percentage of colonies of aphids with syrphid larvae later in the season ( $r = 0.602$ ;  $p < 0.001$ ) (Figure 5). In the case of ladybird beetles, the proportion of colonies of aphids with eggs or larvae was never correlated with the infestation rate (for eggs  $r = 0.468$ ;  $p = 0.091$  and for larvae  $r = -0.231$ ;  $p = 0.248$ ) (Figures 4 and 5).

Although they arrived successively, there was some overlap between the occurrence of the last syrphids and the first coccinellids (Figure 6). Therefore, both predators might have laid eggs in the same colonies of prey. Ladybirds laid their eggs as frequently in the presence of syrphid

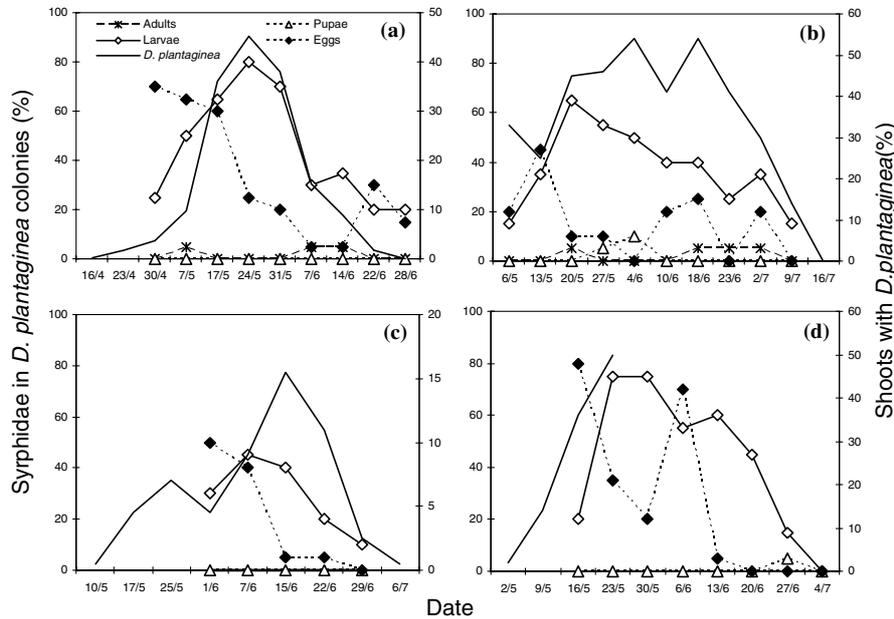


Figure 2. Evolution of the developmental stages of Syrphidae in colonies of *D. plantaginea* in: (a) the experimental orchard in 1999; (b) the commercial orchard in 1999; (c) the experimental orchard in 2001; and (d) the commercial orchard in 2001. (Note: Sample of aphids ceased in the commercial orchard in 2001 (d) because many of the colonies in the marked shoots were affected by the insecticide application. Sampling of predators continued on unaffected colonies).

eggs as in the absence of eggs ( $\chi^2 = 0.752$ ; 1 df;  $p = 0.386$ ). However, this was not the case for syrphids ( $\chi^2 = 113.470$ ; 1 df;  $p < 0.001$ ).

On average, syrphids laid  $2.3 \pm 1.7$  ( $n = 134$ ) eggs per aphid colony while coccinellids laid  $18.4 \pm 9.9$  ( $n = 30$ ) (Figure 7). In both cases, the numbers of eggs per aphid colony were independent on the infestation rate (for syrphids  $r = -0.018$ ;  $p = 0.839$  and for coccinellids  $r = 0.061$ ;  $p = 0.748$ ).

## Discussion

*Adalia bipunctata*, *E. balteatus* and *A. aphidimyza* were the three most abundant predators in the Asturian apple orchards as is often the case in Europe (Wyss et al., 1999b; Solomon et al., 2000). These three species of predators attacked the colonies of aphids in a sequence: first the syrphids, then the ladybird beetles and finally the cecidomyiid flies. The cecidomyiids arrived too late in the development of the colonies to have

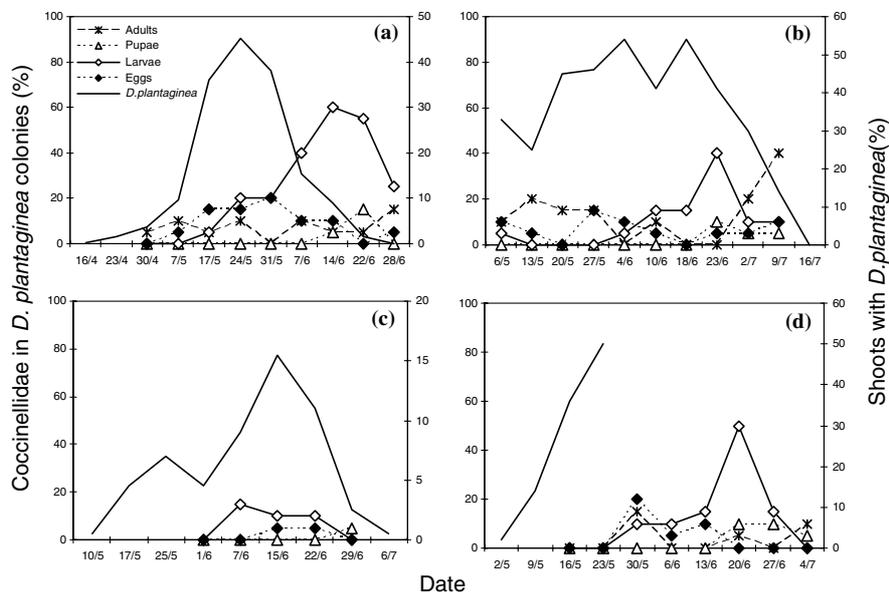


Figure 3. Evolution of the developmental stages of Coccinellidae in colonies of *D. plantaginea* in: (a) the experimental orchard in 1999; (b) the commercial orchard in 1999; (c) the experimental orchard in 2001; and (d) the commercial orchard in 2001. (Note: Sample of aphids ceased in the commercial orchard in 2001 (d) because many of the colonies in the marked shoots were affected by the insecticide application. Sampling of predators continued on unaffected colonies).

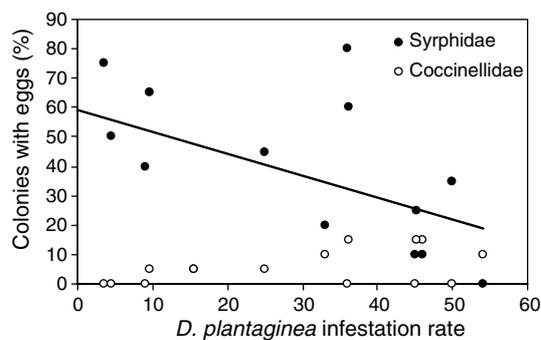


Figure 4. Relationship between oviposition of aphidophagous predators and *D. plantaginea* infestation. (For syrphids  $r = -0.507$ ;  $p = 0.064$  and for coccinellids  $r = 0.468$ ;  $p = 0.091$ ).

a significant impact on regulating the population of *D. plantaginea*. This pattern is not unique and has been also observed to some extent in other orchards (i.e. Brown, 1993; Brown and Lightner, 1997) where the

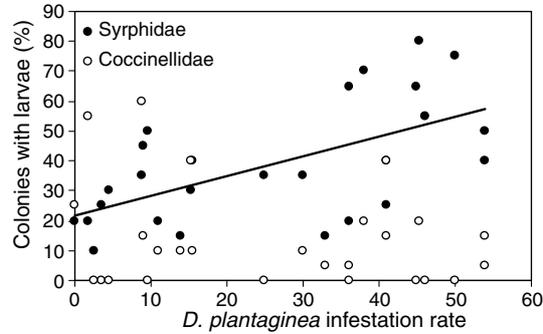


Figure 5. Relationship between *D. plantaginea* infestation and occurrence of larvae of aphidophagous predators in the aphid colonies. (For syrphids  $r = 0.602$ ;  $p < 0.001$  and for coccinellids  $r = -0.231$ ;  $p = 0.248$ ).

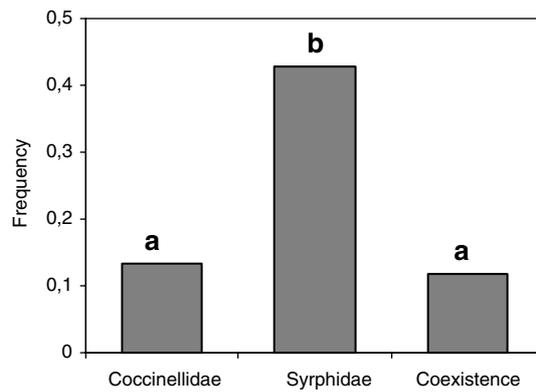


Figure 6. Coexistence of coccinellids and syrphids in *D. plantaginea* colonies. Columns with the same letter are not significantly different for  $\chi^2$  at the 0.05 level.

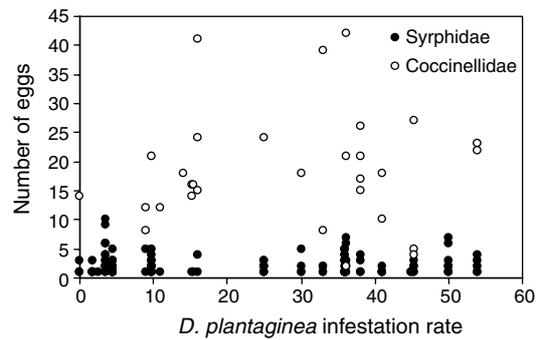


Figure 7. Number of eggs of Syrphidae and Coccinellidae per *D. plantaginea* colony at different infestation rates. The relationships were not significant in both cases. (For syrphids  $r = -0.018$ ;  $p = 0.839$  and for coccinellids  $r = 0.061$ ;  $p = 0.748$ ).

species arrived in the same order as in this study. *Adalia bipunctata*, *E. balteatus* and *A. aphidimyza* exclusively prey upon aphids during the reproductive period. Therefore as potent competitors, their fitness might be reduced significantly in the presence of each other. In such situations resource partitioning frequently evolved as a means of reducing the impact of interspecific competition (Begon et al., 1996). The three species of predators differ in their lower developmental threshold, 6.0, 9.0 and 10.5 °C for *E. balteatus* (Hart et al., 1997), *A. bipunctata* (Obrycki and Tauber, 1981) and *A. aphidimyza* (Morse and Croft, 1987), respectively. It is interesting to note that Brown and Lightner (1997) recorded the arrival of *A. aphidimyza* before *Coccinella septempunctata*, the lower developmental threshold of which is 12.1 °C (Obrycki and Tauber, 1981). It is therefore possible that thermal requirements for development evolved in order to reduce the impact of interspecific competition. Nevertheless, our results did not suggest antagonism between *E. balteatus* and *A. bipunctata*. Comparing the frequency of *D. plantaginea* colonies with coexistence of syrphids and coccinellids or with syrphids or coccinellids alone we recorded a significantly similar number (Figure 6). The presence of syrphids alone was significantly higher but probably was more the consequence of an earlier occurrence of syrphids than the result of a real competition between these predators. Wyss et al. (1999b) showed that these two predators did not compete but combine their effects to reduce *D. plantaginea* populations.

There were on average 2.3 eggs of *E. balteatus* and 18.4 eggs of *A. bipunctata* per aphid colony regardless of the value of the infestation rate. Concerning *A. bipunctata*, 18.4 is very similar to the number of ovarioles per ovary and corresponds to the number of eggs laid in each oviposition bout (Stewart et al., 1991a, b; Magro et al., unpublished results). These results suggest once again that the numerical response of these two natural enemies to the abundance of aphids is weak or absent (Hemptinne et al., 1992, 1994). They also suggest that only one predator laid eggs in each aphid colony. This is consistent with the oviposition behaviour of these predators. In order to maximize their fitness, it has been demonstrated that they have to lay few eggs rather early in the development of the aphid colonies (Kindlmann and Dixon, 1993). In addition, as soon as one female lays eggs in a colony, this colony is not suitable for other conspecifics (Kan, 1988a, b; Doumbia et al., 1998; Hemptinne et al., 2001). The observations made in the Asturian orchards are well explained in light of the oviposition behaviour of these two specialist predators and they tend to confirm the inability of natural populations of aphidophagous specialist predators in regulating aphid abundance (Kindlmann and Dixon, 1999; Dixon, 2000).

This field study does not rule out biological control of aphids with predators. It just shows that natural populations of specialist enemies are unable to control *D. plantaginea* abundance. The biological control of *D. plantaginea* requires a more sophisticated strategy than waiting for the natural populations of antagonists. It necessitates the combination of three complementary approaches. First, and probably most importantly, resistant varieties to aphids have to be used more frequently than is actually the case (Dapena and Miñarro, 2001). However, there always is the possibility that aphids break down the resistance. Therefore, and second, a forecasting model has to be implemented to determine when an outbreak is likely to occur (Hemptinne et al., 2004). Finally and if necessary, larvae of *A. bipunctata* have to be released in autumn or as soon as possible after fundatrices hatch (Wyss et al., 1999a, b; Kehrlí and Wyss, 2001). This strategy was efficient in experimental conditions. It remains to be seen if it is economically feasible.

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