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Effects of augmentative releases of eggs and larvae of the ladybird beetle, *Adalia bipunctata*, on the abundance of the rosy apple aphid, *Dysaphis plantaginea*, in organic apple orchards

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Abstract

The impact of augmentative releases of larvae and eggs of the indigenous ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) against the rosy apple aphid *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), a major pest insect on apple trees, was assessed in field experiments in Switzerland, during 1997. In a first experiment, eggs and larvae were released on 3-year old apple trees infested with five aphids at four different predator-prey ratios (0:5, 1:5, 1:1, 5:1). In a second experiment, eggs and larvae were released at a predator-prey ratio of 5:1 on branches of apple trees naturally infested with aphids. In both experiments, the interaction with ants was taken into account and the releases were done at two different times in spring. The results showed that an augmentative release of larvae significantly prevented the build-up of colonies of *D. plantaginea*. Significant reductions in aphid numbers were recorded at the two highest predator-prey ratios, 1:1 and 5:1. Larvae were efficient just before flowering of apple trees at a time when growers normally have to spray their trees. On trees where ants were present the larvae of *A. bipunctata* were significantly less efficient. Effects of eggs of *A. bipunctata*, however, were less reliable. At the first date of release (5 April), they did not hatch, probably as a consequence of bad weather conditions.

Introduction

In organic as well as in IPM apple orchards the rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), is the second most important pest after the codling moth. The common strategy to control *D. plantaginea* in IPM and organic apple orchards is to spray aphicides early in spring, before the trees start to bloom, when fundatrices are hatching. Apple growers base their decision for spraying on the presence of a single fundatrix in a sample of 50 buds (Anonymous, 1977). This low tolerance for *D. plantaginea* has resulted in intense spraying campaigns across Europe and favoured the appearance of resistance to pesticides (Delorme, 1998). As a consequence there is now a growing demand for alternative control measures.

In the past few years, indirect control strategies such as the planting of resistant apple varieties or the sowing of strips of flowering plants in apple orchards to enhance the number of aphid predators were tested (Wyss, 1995; 1997). The so-called weed strip-management contributes efficiently to the control of aphids in years when population densities are low. However, when aphid populations reach high levels, naturally occurring predators still reduce the number of aphids but they are not able to reduce the aphids' abundance below the economic threshold. The goal of the present study was to determine whether augmentative releases of an indigenous natural enemy could compensate for the lack of efficiency of naturally occurring predators of aphids.

Only few studies have been done on augmentative or inundative release of predators to control apple aphids. Contradictory results were found on releases of chrysopids and of a predatory cecidomyiid fly against the green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), in Canadian and American orchards (Bouchard et al., 1988; Hagley, 1989; Grasswitz & Burts, 1995). The first two studies reported successful results whereas the latter did not show any significant effect on the aphid population. Unfortunately, the reasons for the success or failure of these experiments were not analysed.

Preliminary trials to control *D. plantaginea* had already been conducted under laboratory and semi-field conditions to test the efficiency of different indigenous predators at different periods of time during spring and at different predator-prey ratios (E. Wyss, M. Villiger & H. Müller-Schärer, unpubl.). Larvae of the ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) significantly reduced the number of aphids in all experiments whereas larvae of the syrphid *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) and the cecidomyiid fly *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) gave positive results only just after the crucial period of blooming in spring. Thus, further research was focused on augmentative releases of *A. bipunctata* under field conditions.

Materials and methods

Orchards. Experiments were carried out in 1997 in two different types of orchards. The first type of orchard ('experimental orchard') consisted of 144 3-year old apple trees arranged in a square of 60 m $\times$ 60 m and was subdivided into three blocks. The two adjacent orchards of this first type located in Frick, Switzerland, only differed in the apple variety: one with cv. Glockenapfel and the other with cv. Rubinette. The second type of orchard ('commercial orchard') was a 20-year old commercial apple orchard located in Olsberg, Switzerland. Within this orchard 3 rows (cv. Idared) were subdivided into 7 blocks of 30 trees each. Within each block 18 branches of similar size were randomly selected among the 30 trees and allocated to the treatments.

Insects. *Adalia bipunctata* was reared on pea aphids, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), in the insectary of the Faculté universitaire des Sciences agronomiques de Gembloux (Belgium) and delivered to Switzerland by express mail. They were introduced into the orchards as eggs or as second instar larvae ready to moult into the third instar.

In order to get fundatrices of *Dysaphis plantaginea* of a known age, cuttings of apple tree branches were incubated in the laboratory at room temperature and hatched aphids were transferred to apple tree saplings and reared on them.

Treatments. In the experimental orchards, trials were carried out to quantify the effect of the two developmental stages of *A. bipunctata* (eggs or second instar larvae) on the abundance of *D. plantaginea* at four predator-prey ratios (1:5, 1:1, 5:1, and 0:5 as a control) and with the presence or absence of ants. A first release of predators was carried out in April in one orchard (cv. Glockenapfel) and a second one in May in the second orchard (cv. Rubinette). The release dates (5 April and 7 May 1997) were related to the time of applications of insecticides against the aphid population in spring.

Before releases were started, the orchards were sprayed with an organic pyrethrine insecticide to kill all insects. Two days later, five fundatrices of *D. plantaginea* reared in the laboratory were placed on the trees, and after another 2 days the *A. bipunctata* were released. Treatments with ants excluded were obtained by spraying a ring of glue at the base of the stem of apple trees.

The number of aphids and predators were monitored at weekly intervals and the efficiency of the control measures was determined 3 weeks after the release of predators.

The aim of the experiment performed in the commercial orchard was to quantify the influence of the date of release (3 April 1997, before blooming; 24 April 1997, just after blooming) and the two developmental stages (as above) of *A. bipunctata* on its efficiency to control *D. plantaginea* at a predator-prey ratio of 5:1. There were two controls: a spray with NeemAzal-T/S (Trifolio-M GmbH, conc.: 0.3%, an azadirachtine based insecticide) and the absence of ladybirds.

In the autumn of 1996, the year before the experiment, *D. plantaginea* laid many eggs in the commercial orchard. Therefore, there was no need for artificial infestation of the branches as the number of fundatrices per branch varied from 1 to 3. Based on the observed numbers of fundatrices, eggs and larvae of *A. bipunctata* were added in such numbers that a 5:1 predator-prey ratio was obtained.

Table 1. Nested ANOVA table on the influence of the treatment factors on build-up of the colonies of *D. plantaginea* in the experimental orchards for first and second repetition. Data were transformed prior to analysis ($\ln(x + 0.5)$); only significant interaction terms are given

Source of variation	df	Sum of squares	Sign. of F[1]
Location of the orchards (L)	1	16.25	*
Block within location (Error 1)			
Block within location	4	5.80	ns
Developmental stage (DS)	1	267.77	***
Predator-Prey Ratio (PPR)	2	173.53	***
Presence or absence of ants (PAA)	1	22.17	*
DS × PPR	3	84.14	***
DS × L	1	19.72	*
Tree number (Error 2)			
Tree number	251	1082.30	***
Date of control (DC)	1	48.43	**
PAA × DC	1	7.13	*
Within + Residuals (Error 3)	271	399.80	
Total	568	4360.00	

[1](ns) not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

All branches involved in the experiment had a barrier of glue at their base to stop ants. The trees were lightly pruned to make sure that there was no contact between experimental branches.

The numbers of aphids and predators were counted weekly. The efficiency of the control measures was determined 2 weeks after the second release of predators.

Data analysis. The observed numbers of aphids were ln-transformed ($\ln(x + 0.5)$), prior to the analysis in order to satisfy statistical assumptions. This transformation is meaningful as the variable describes an exponentially growing number of insects. Ln-transformed numbers were considered as a dependent variable analysed by a nested ANOVA (experimental orchards) or a two-way ANOVA (commercial orchard).

Results

Trials in two experimental orchards. There was a significant interaction between the ladybird developmental stage and the date of release (Table 1) because the eggs were destroyed by rain and frost in April. However, the two releases of larvae had a strong impact on the numbers of aphids (Table 1, Figure 1) mainly at the two highest predator-prey ratios: 1:1 and 5:1 (Table 1, Figure 3).

When considering the date of release, it is clear that significant control of aphids was already achieved in April (Figure 2). In addition, ants interfered with the *A. bipunctata* larvae especially during the second release and limited the impact of the predators on the number of aphids (Table 1, Figures 1 and 2), as ant-attended trees hosted 30% more aphids than ant-free ones.

Trial in a commercial orchard. By selecting the branches before starting, eggs and larvae were always released on branches with similar numbers of aphids.

There appears to be a strong treatment effect (Table 2) due to the action of the Neem and some of the developmental stages of *A. bipunctata*. When placed on the trees in April, eggs did not produce a significant decrease in the numbers of aphids thus confirming the results obtained in the experimental orchards. They nevertheless hatched normally in May and the larvae then had a negative impact on *D. plantaginea*. Larvae released as second instar were significantly more efficient than the eggs (Figure 4) confirming again the previous experiments. In addition, they gave a comparable control of aphids to spraying with Neem.

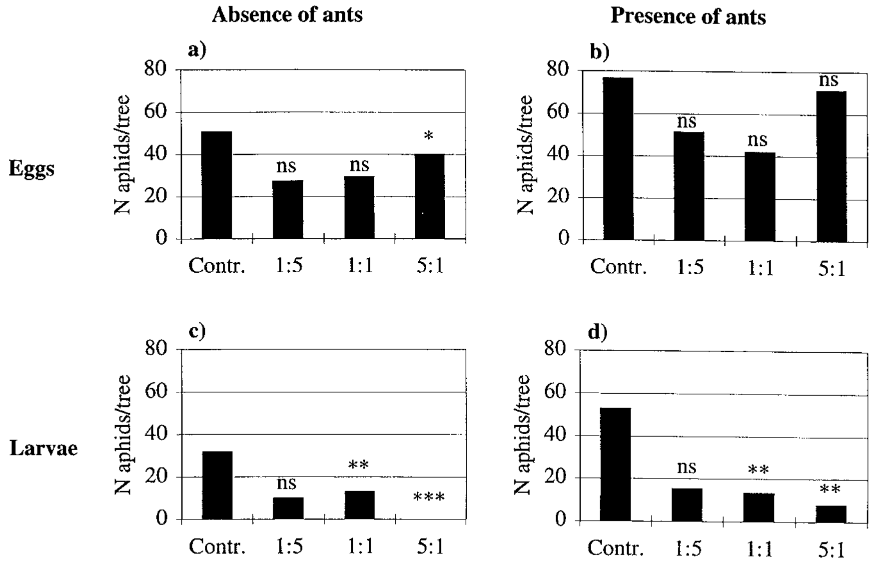

Figure 1. Effects of releases of *A. bipunctata* at 3 different predator-prey ratios on the mean number of *D. plantaginea* per tree, averaged over the first and second repetition. *A. bipunctata* were released as eggs (a, b) or larvae (c, d) and ants were either excluded (a, c) or allowed free access to the trees (b, d) in the experimental orchards. Comparison of treatments with control (no *A. bipunctata* released) using a simple contrast method with ln-transformed data. (ns) not significant; (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

Table 2. ANOVA table on the influence of the treatment factors on the build-up of the colonies of *D. plantaginea* in the commercial apple orchard six weeks after the first and three weeks after the second date of release. Data were transformed prior to analysis ($\ln(x + 0.5)$)

Source of variation	df	Sum of squares	Sign. of F[1]
Block	6	39.97	**
Treatment	5	126.49	****
Developmental stage (DS)	1	38.28	****
Date of release(DR)	1	4.51	ns
DS × DR	1	2.80	ns
Within + Residuals	120	273.09	
Total	125	399.59	

[1]ns: not significant; ** $P < 0.01$; **** $P < 0.0001$.

Naturally occurring predators of aphids were recorded on the experimental trees. They were equally abundant on the control branches as on those treated with eggs or second instar larvae so that they did not bias the results of the trials.

Discussion

D. plantaginea has a rather low temperature threshold for development, 4.5 °C (Graf et al., 1985), and achieves rapid growth early in spring. As economic damage to apple trees is caused even at low densities, it is important to reduce the population as early as possible. This could in theory be done by a natural enemy that is active at low temperatures and displays a good functional response at low densities of prey. Dixon et al. (1997) showed that naturally occurring aphidophagous coccinellids are not very efficient biocontrol agents because they develop significantly slower than their prey. The only reliable strategy is to introduce the voracious instars of indigenous predators. Experiments on apple tree saplings kept in muslin cages showed that eggs and larvae of *A. bipunctata* control *D. plantaginea* more successfully than *Episyrphus balteatus* and *Aphidoletes aphidimyza* (E. Wyss, M. Villiger & H. Müller-Schärer, unpubl.).

Our studies showed that augmentative releases of second and third instar larvae of *A. bipunctata* on apple trees are an efficient method of controlling the build-up of the spring population of *D. plantaginea*. Larvae introduced in April controlled the aphids mainly at 1:1 and 5:1 predator-prey ratios. An early release is a decisive pre-requisite if biological control is to be successful in apple orchards. The predator-prey ratios of this study are quite favourable compared to the ratios used by Hagley (1989) to control *Aphis pomi* on apple trees with chrysopids (1:10 and 1:19). Here the larvae of *A. bipunctata* were released in rather cold

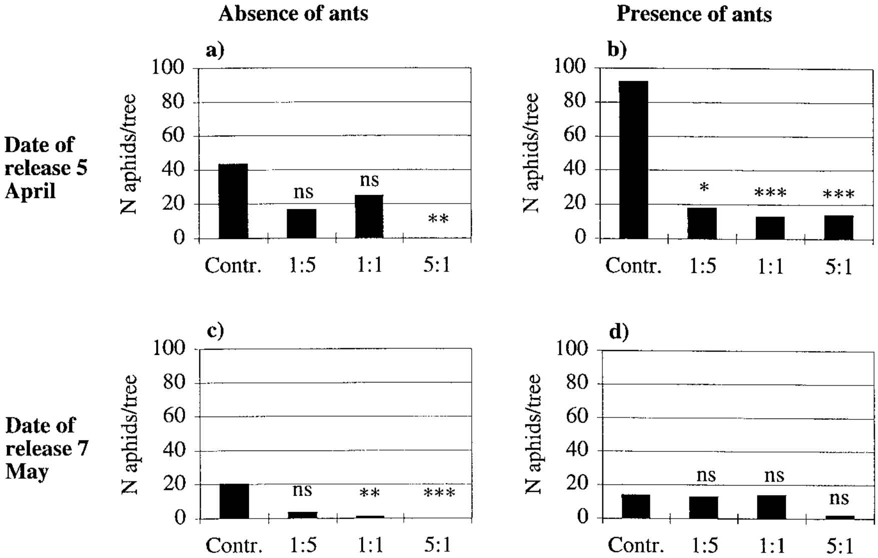

Figure 2. Effects of releases of *A. bipunctata* on the mean number of *D. plantaginea* per tree when larvae of *A. bipunctata* were released in the first (a, b) and the second (c, d) repetition and ants were either excluded (a, c) or allowed free access to the trees (b, d) in the experimental orchards. Comparison of treatments with control (no *A. bipunctata* released) using simple contrast method with ln-transformed data. (ns) not significant; (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

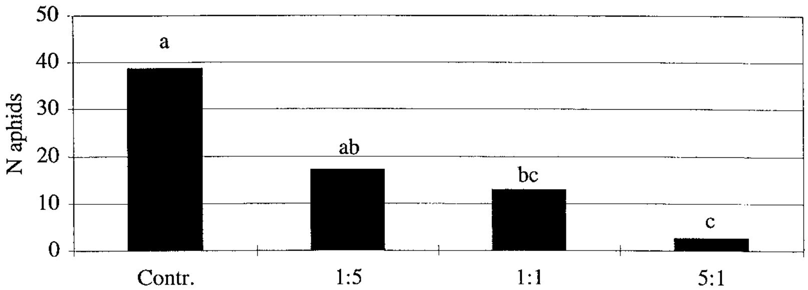

Figure 3. Effects of releases of larvae of *A. bipunctata* at the different predator-prey ratios on the mean number of *D. plantaginea* per tree averaged over the two repetitions and pooled for the factor presence or absence of ants. Comparison of predator-prey ratios by one-way ANOVA; predator-prey ratio with different letters are significantly different ($P < 0.05$).

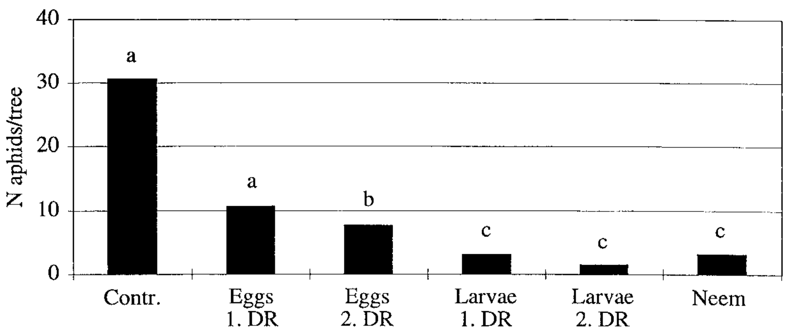

Figure 4. Treatment effects on the mean number of *D. plantaginea* on the observed branches of apple trees in the commercial orchard at Olsberg on 15 May, 6 weeks after the first, and 3 weeks after the second date of release (DR). Comparison of treatments by one-way ANOVA; treatments with different letters are significantly different ($P < 0.05$).

weather conditions and when aphid fundatrices were scarce. Further work would probably be needed to define an optimal predator-prey ratio taking into account efficiency and cost of control. Reinforcing the action of natural populations of enemies at a crucial time, as mentioned by Hughes (1989) for classical biocontrol agents, seemed to work well for the indigenous predators used in these trials.

Eggs of *A. bipunctata* were included in the experimental design because they are easier to handle and cheaper to rear than larvae. Unfortunately they did not hatch in April. On the contrary, larvae controlled the aphids before the trees set flowers. Although rearing and handling larvae pose more problems than eggs, second instar larvae have one outstanding advantage: they are active immediately, first as second then third and fourth instars, in killing and continue to do so for 10 to 12 days. During that time period their searching ability and consumption are at their maximum (Mills, 1979).

Ants often have a negative impact on the biological control of aphids because they sometimes move the beneficials or even kill them (Bradley, 1973; Nault et al., 1976). In fact, the access of ants resulted in 30% higher aphid abundance than on ant-free trees. Aphid populations did not benefit immediately from ant attendance, since their beneficial effect developed only after 2 weeks, as the interaction between date of control and ants suggests. It could not be clearly defined if this beneficial effect of ants on aphids could be explained with the ‘sanitation hypothesis’ or the ‘protection hypothesis’ (Reimer et al., 1993). Some casual observations of ants attacking larvae of *A. bipunctata* in our experimental orchards would confirm the second hypothesis. In addition, these observations support the findings of Kreiter & Iperti (1986) and Reimer et al. (1993), who stated that ants interfered in the biological control of aphids and coccids. But ants were not very troublesome when the augmentative release took place in April because they were not as active as later in the season. This period in April, before the apple trees start to bloom, is the crucial period to suppress aphid populations, because aphid damage at this time is still limited.

Reports on augmentative releases of coccinellids in open field fruit or other crops to control aphids are very rare. In Europe, *Harmonia axyridis* (Pallas), a ladybird introduced from the Far East, was successful against aphids on rose bushes (Ferran et al., 1996) and on hop (Trouvé et al., 1997). *H. axyridis* can be reared easily and cheaply and is, therefore, easily available (Schanderl et al., 1988). However, one should always keep in mind that introductions of any species are rarely neutral in their effects (Pimm, 1991). Considering only the case of ladybird beetles, the decline of several North American species is clearly linked to the introduction of the Palearctic *Coccinella septempunctata* L. from 1958 to 1973 (Schaefer & Dysart, 1988; Horn, 1991). The more recent release of *H. axyridis* seems already to have produced similar effects. This could be attributed to the fact that this species behaves like a super-predator that develops better when feeding on a mixture of aphids and ladybirds (Hironori & Katsuhiro, 1997). Therefore negative side effects following an intensive utilisation of *H. axyridis* in Europe are likely. In contrast, *A. bipunctata* is an indigenous and very common predator of aphids in orchards (e.g., Hemptinne, 1989) but a method of cheap rearing has to be developed to fulfil the requirements of a good biological control agent (van Lenteren, 1988). At the present stage, realistic estimations of the costs related to the suggested control strategy are impossible to make.

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