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The potential of three native insect predators to control the rosy apple aphid, *Dysaphis plantaginea*

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Abstract. The potential of three aphidophagous predators, *Adalia bipunctata*, *Aphidoletes aphidimyza*, and *Episyrphus balteatus* to control the rosy apple aphid, *Dysaphis plantaginea* Pass., a major pest on apple in Europe, was assessed by means of laboratory and field cage experiments in Northern Switzerland. Under laboratory conditions, all three predators efficiently preyed upon *D. plantaginea* on apple seedlings. The searching success of larvae of *A. bipunctata* for individual aphids was not dependent on the size of branches of apple trees varying in leaf surface area from 150 cm^2 to 960 cm^2. Fifty and 70% of individual aphids were found and killed 6 hours and 48 hours, respectively, after release of single second instar larva of *A. bipunctata*. In a first field cage experiment in 1996, *A. bipunctata*, and to a lesser extent *E. balteatus*, proved to be effective and consistent predators of *D. plantaginea* during spring conditions, being little affected by cool temperatures and wet weather. In a subsequent field cage experiment in 1997, larvae of *A. bipunctata* and *E. balteatus* were released singly and in combination on aphid infested apple seedlings to study interactions between these two promising control agents. Both species had a significant negative effect on aphid population increase. The two species did not significantly interact and thus, their joint effect is best explained by an additive model. Combined releases of the two predator species reduced aphid densities to 5% of the control. This indicates the potential for augmentative releases of these native aphid predators to control *D. plantaginea*.

Key words: *Adalia bipunctata*, *Aphidoletes aphidimyza*, apple seedling, augmentative release, biocontrol, *Episyrphus balteatus*, field cage trials, laboratory trials

Introduction

In Switzerland, the rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), is a major pest, of growing importance, in apple orchards (Graf et al., 1998). This species of aphid often causes irreversible damage to leaves, branches, and fruits and is responsible for severe losses of yield (Graf, 1984).

In recent years, increasing levels of resistance to certain insecticides has stimulated an increased interest in new control strategies. As an indirect control strategy, naturally occurring predators of aphids can be encouraged by sowing strips of flowering plants in apple orchards (Wyss, 1995). This so-called weed strip-management contributes to the control of aphids in years of low or moderate aphid abundance. However, in years of high aphid density, these naturally occurring predators fail to reduce aphid abundance below the economic threshold of a single fundatrix per 50 buds (Anonymous, 1977). The objective of our study was to identify which of the indigenous species of predator could be used for augmentative releases to complement the impact of the naturally occuring predators of aphids.

A large number of predators have been recorded in association with apple aphids (Carroll and Hoyt, 1984; Bouchard et al., 1986; Hagley and Allen, 1990), but only a few of them have been assessed for augmentative or inundative release. Chrysopids and a predatory cecidomyiid fly released against the green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), in Canadian and American orchards have given contradictory results (Bouchard et al., 1988; Hagley, 1989; Grasswitz and Burts, 1995). The first two studies reported successful control whereas these predators had no effect on the abundance of the aphids in the third study. Unfortunately, the reasons for success or failure in these experiments were not analysed in detail.

In order to standardize environmental effects, we first performed release trials on apple seedlings under laboratory and semi-field conditions. The effectiveness of three native predators, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), and *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) in capturing and eating *D. plantaginea* and in reducing the number of aphids under spring climatic conditions was assessed. In 1996, the following questions were addressed: (1) Do larvae of *A. bipunctata*, *A. aphidimyza*, and *E. balteatus* prey on *D. plantaginea* under laboratory conditions? (2) How efficiently do the three predators control *D. plantaginea* under semi-field spring conditions? In 1997, subsequent trials under laboratory and semi-field conditions were carried out to answer the following additional questions: (1) Does the predatory success of the most effective predator, *A. bipunctata*, depend on the total leaf area and the initial distance between larva and aphid, when searching for a single individual of *D. plantaginea*? (2) Do larvae of *A. bipunctata* and *E. balteatus* differentially affect the build-up of populations of *D. plantaginea*? (3) Do larvae of *A. bipunctata* and *E. balteatus* interact in controlling colonies of *D. plantaginea* when released together or is their joint effect additive?

Material and methods

Laboratory trial (1996)

In 1996, the efficiency of *A. bipunctata*, *A. aphidimyza*, and *E. balteatus* as predators of the rosy apple aphid *D. plantaginea* was assessed under controlled conditions (temperature 20 °C, humidity 70 to 80%, light cycle 16L:8D) on 6 week old apple seedlings (cv. Golden Delicious) in the laboratory. *Aphidoletes aphidimyza* was provided by Andermatt Biocontrol AG and *A. bipunctata* and *E. balteatus* by the insectary of the Faculté Universitaire des Sciences agronomiques of Gembloux, Belgium. Larvae of the three predators were reared and fed 'ad libitum' with pea aphids, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), before using them for the tests. The tests were carried out on individual apple seedlings growing in automatically irrigated plastic containers. Each seedling was placed in an acrylic cylinder (0.25 m in diameter, 0.4 m height). Apple seedlings were infested with two adult fundatrices of *D. plantaginea*. After 24 hours one of the following 7 treatments was applied: 3 days old eggs laid on paper or plant material (separately for each of the three predators) were glued to a leaf of each of the seedlings (three treatments), second instar larvae (again, separately for each predator) were transferred to each seedling using a paint brush (three treatments), or the aphids were left without predators (control treatment). Both eggs and larvae were supplied in sufficient numbers to give a predator–prey ratio of 5:1 (i.e. 10 predators: 2 aphids). The 7 treatments were each repeated four times on two occasions in 1996. The number of aphids was counted at two day intervals for 14 days after the release of the predators. In addition, the hatching rate of 100 eggs from each batch of predators was determined to assess their quality.

Laboratory trial (1997)

In a laboratory trial in 1997, the searching success of the second instar larva of *A. bipunctata* (which was the most efficient treatment in the 1996 trials) was assessed in relation to various distances between predator and prey and to total surface area available under controlled conditions (temperature 18 °C, humiditiy 70 to 80%, light cycle 16L:8D). Branches of apple (cv. Glockenapfel and cv. Gloster) of different lengths (0.25 m, 0.5 m, and 1 m) and leaf surfaces (150 ± 5 cm^2, 500 ± 10 cm^2, 960 ± 20 cm^2), respectively, were used. The cut ends of 20 branches of each of the three categories of branch were each placed into flasks containing water. One adult fundatrix of *D. plantaginea* was released at the tip of each branch and after 2 hours one satiated second instar larva of *A. bipunctata* was placed at the base of each branch. The success of each larva in finding and killing an aphid was recorded after 1, 2, 6, 24, and 48 hours. The objective was to simulate conditions in spring when temperature during the day reaches 15 °C and only a few fundatrices are present on apple trees.

Field cage trial (1996)

In 1996, the effectiveness of the three predators was studied under semi-field conditions in 1 m × 1 m × 1 m field cages, which consisted of a framework of aluminium covered with a synthetic white muslin (mesh width 0.8 mm) open at the bottom. One pot (0.35 m in diameter) containing 10 apple seedlings (cv. Golden Delicious) was placed in an automatically irrigated plastic basin in the centre of each cage. The water served both to irrigate the seedlings and as a barrier to ants. Naturally occuring aphidophagous predators were prevented from entering the cages by the muslin. The cages were placed 1m apart on a plastic mulch.

Before the start of the experiment each pot of apple seedlings was infested with 5 adult fundatrices of *D. plantaginea.* Two days later the experiment was started by adding 25 eggs of either *A. bipunctata* (a), *A. aphidimyza* (b), *E. balteatus* (c), or no eggs (d) as control. Where fundatrices already had offspring, the young aphids were removed to get a 5:1 predator–prey ratio. The 4 treatments were arranged in 8 blocks each containing one replicate of the four treatments, resulting in 32 cages. The experiment was repeated three times: 4 April, 19 April, and 31 May 1996.

The subsequent numbers of aphids and predators on the apple seedlings were recorded on 3 occasions at intervals of four to seven days. Records were, at latest, stopped 4 weeks after the start of the experiments.

Field cage trial (1997)

Single and joint effects of the two most efficient aphidophagous predators in the 1996 field cage trials (i.e.*A. bipunctata* and *E. balteatus*) were evaluated in 1997. An experiment similar to that used in 1996 was set up by placing ten apple seedlings (cv. McIntosh) in each of 32 cages arranged in a randomized block design with 8 blocks. Before the start of the experiment, each apple seedling was infested with 2 adult fundatrices of *D. plantaginea*. Two days later, the experiment was started by adding either (1) two satiated larvae of *A. bipunctata*, (2) two satiated larvae of *E. balteatus*, (3) two larvae of each species, or (4) no predators (control). The resulting predator–prey ratio was 1:1 or 2:1 (for joint releases), respectively. This experiment was repeated three times: 5 April, 7 May, and 25 June 1997.

Aphid numbers were counted in the same intervals as in the 1996 experiment and records were again stopped 4 weeks after the start of the experiments.

Temperature and humidity data were recorded from 21 April to 10 May, 1995. The former was measured at 0.1 m, 0.5 m, and 0.8 m above ground using Lite^{TM} data loggers, while humidity was measured at 0.5 m above ground with StowAway^{TM} data loggers. Measurements were taken inside and outside two cages at 15 minute intervals to assess environmental effects of caging.

Data analysis

The number of aphids were ln-transformed (ln(x + 0.5)) prior to analysis. This transformation is biologically meaningful as population growth is exponential.

Searching success was analyzed using general log-linear analysis with time (1, 2, 6, 24, and 48 hours after start), branch category (small, medium, large), and aphid consumption (success and failure) as factors.

The results of the three replicates of the 1996 field cage experiments were analyzed using one-way ANOVA, while the three timings of the 1997 field cage experiments were treated as factor and were analyzed using a nested ANOVA model. Temperature and humidity data were analyzed using a repeated measures ANOVA.

Results

Laboratory trials

In the 1996 trial, *Adalia bipunctata*, *A. aphidimyza*, and *E. balteatus* had a similar impact on the number of aphids two weeks after their release (Figure 1) whether introduced as eggs or larvae. The number of aphids was reduced more quickly after the introduction of larvae than eggs. The mean hatching rate in the laboratory of eggs of *A. bipunctata* was 78.5%, of *A. aphidimyza* 81.2%, and of *E. balteatus* 75.9%, indicating high quality of the predator material.

Searching success of larvae of *A. bipunctata* increased significantly with time, but was independent of branch length or total leaf surface area (Figure 2). Fifty percent of the aphids were, on average, found and killed within 6 hours, and 70% within 48 hours.

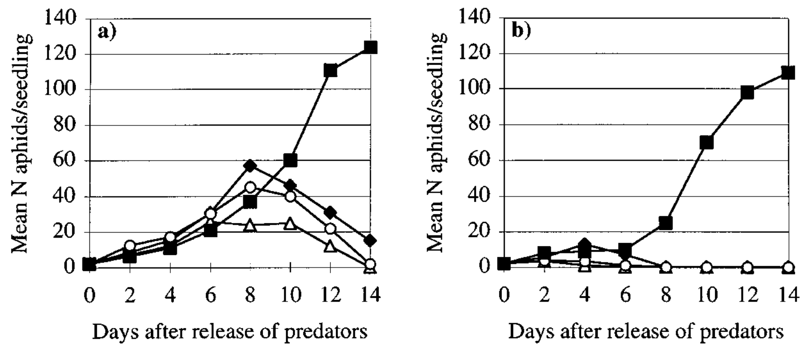

Figure 1. Predator effects on the development of the mean number of *D. plantaginea* per seedling, when *A. bipunctata* (white triangles), *A. aphidimyza* (black diamonds), and *E. balteatus* (white circles) were released as eggs (a) or larvae (b) at an initial predator-prey ratio of 5:1 (1996 laboratory trial; the shown means were back-transformed after analysis). All treatments were significantly different from control (black squares) at the end of the experiment ($p < 0.05$; Tukey–Kramer Test).

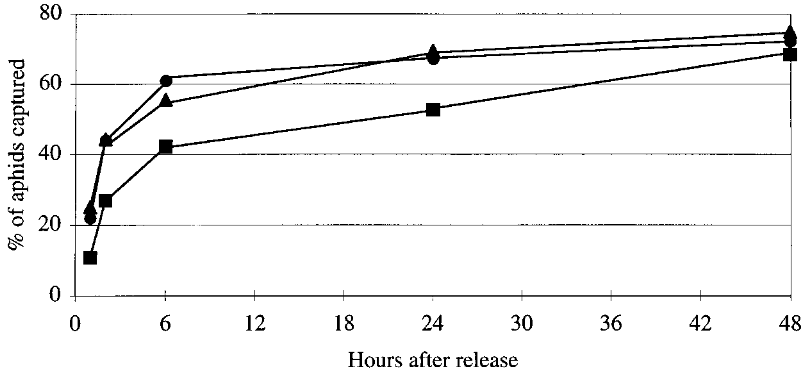

Figure 2. Cumulative percentage of *D. plantaginea* fundatrices consumed over time by larvae of *A. bipunctata* on small (triangles), medium (squares), and large (circles) branches in the 1997 laboratory experiment. Branch size did not significantly affect the percentage captured ($p > 0.05$; loglinear analysis).

Field cage trials

Overall, *A. bipunctata* was the most efficient and consistent predator in controlling aphids in 1996 (Figure 3). The eggs of *A. aphidimyza* and *E. balteatus* were more affected by cold temperatures (release 4 April 1996) and disease due to wet weather (release 31 May 1996) than those of *A. bipunctata* (data not shown).

In 1997, the larvae of both *A. bipunctata* and *E. balteatus* had a significant negative effect on aphid populations (Table 1, Figure 4). There was no signi-

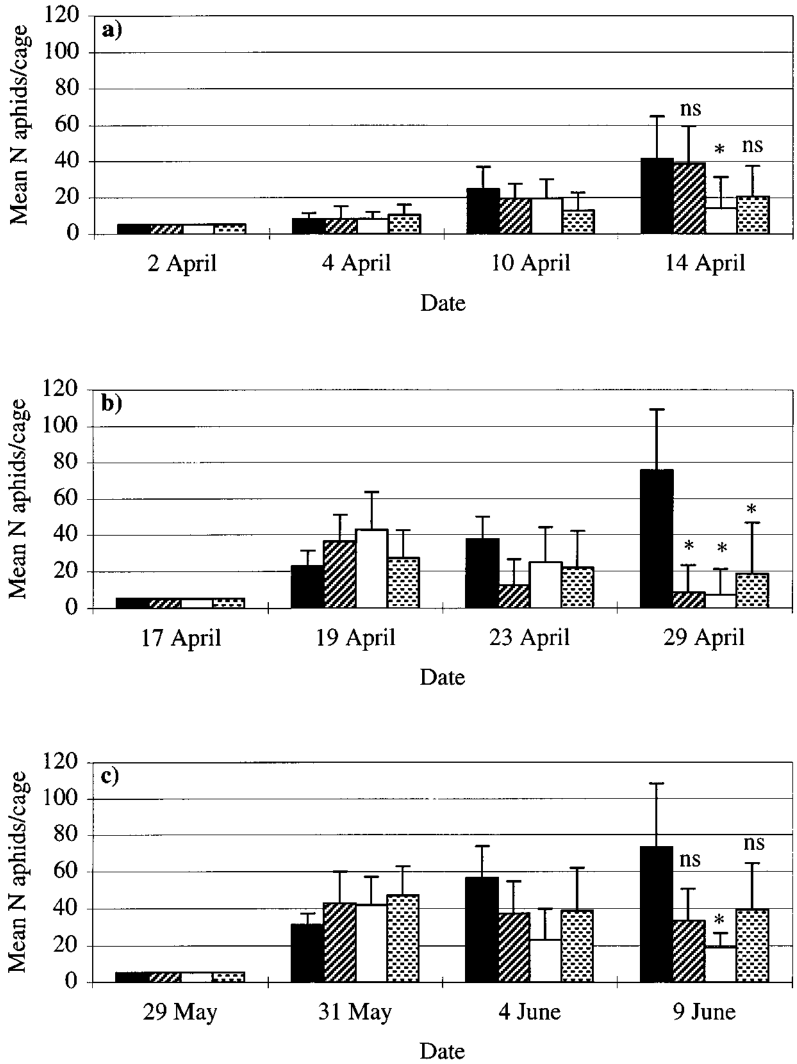

Figure 3. Predator effects on the mean number (+ SD) of *D. plantaginea* when *A. bipunctata* (white bars), *A. aphidimyza* (hatched bars), and *E. balteatus* (dotted bars) were released 4 April 1996 (a), 19 April 1996 (b), and 31 May 1996 (c) in the field cages. Comparison of treatments with control (black bars; no predators released) at the latest date of control using simple contrast method: (ns) not significant; $^{*}p < 0.05$.

Table 1. Nested ANOVA table on the influence of two aphidophagous predators on the build-up of colonies of *D. plantaginea* in the field cages. Data were transformed prior to analysis (ln(x + 0.5))

Source of variation	df	Sum of squares	Sig. of F[1]
Repetition (R)	2	102.69	***
Block within repetition (Error 1)	21	64.00	ns
Larvae of *A. bipunctata* (A)	1	109.31	*
Larvae of *E. balteatus* (E)	1	167.81	*
A × E	1	16.36	ns
A × R	2	11.44	ns
E × R	2	46.18	ns
Cage (Error 2)	3	17.02	ns
Date of control (DC)	1	0	ns
A × DC	1	16.61	*
E × DC	1	6.66	ns
A × E × DC	1	2.83	ns
Within + Residuals (Error 3)	152	435.92	
Total	191	1004.24	

[1]ns = not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

ficant interaction between the two species and thus, their joint effect is best explained by an additive model. Overall, the effect of the syrphid larvae was greater than that of the coccinellid larvae (Table 1, Figure 4). The combined effect of the two species reduced aphid densities to 5% of the control. The number of aphids differed significantly in the 3 repetitions (Table 1), being most abundant at the end of the second repetition in May.

Overall, temperature and relative humidity did not differ between the inside and outside of the field cages (repeated measures ANOVA) (representative data at 0.5 m above ground are shown in Figure 5), although temperature tended to be higher inside the cages on warm days.

Discussion

The three predators proved to be efficient control agents of *D. plantaginea* under laboratory conditions, whether released as eggs or larvae. The three

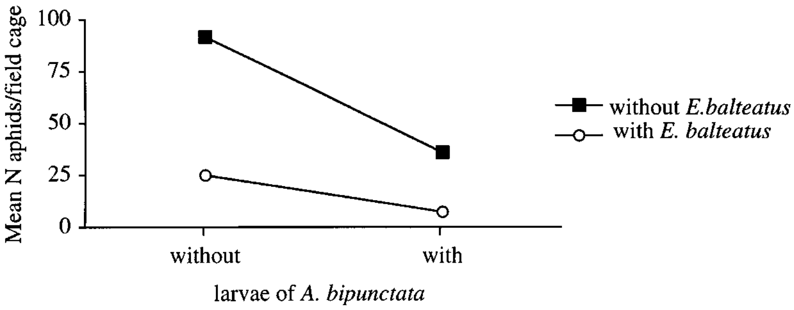

Figure 4. Single and combined effects of the larvae of *A. bipunctata* and *E. balteatus* in the field cage experiment in 1997 (mean of three repetitions; shown means were back-transformed after analysis). With/without refers to two/no larvae of predators applied.

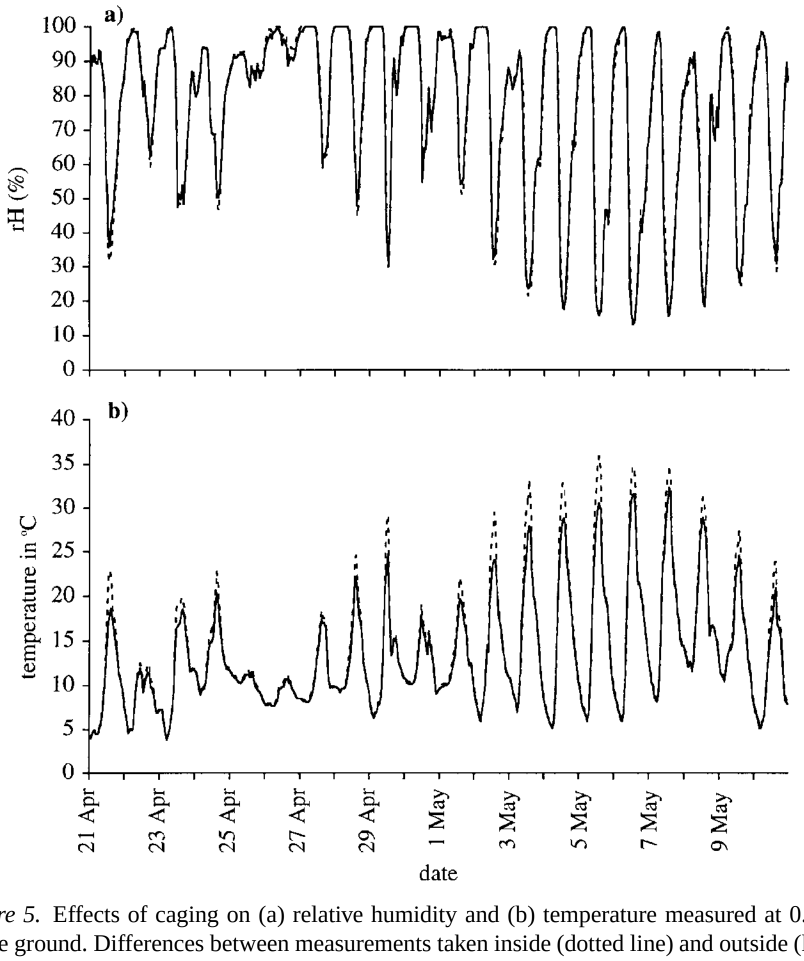

Figure 5. Effects of caging on (a) relative humidity and (b) temperature measured at 0.5 m above ground. Differences between measurements taken inside (dotted line) and outside (line) of the field cages are not significant (repeated measures ANOVA, $p > 0.05$).

predators are described as non-specific aphid predators (Hodek, 1993) and associated with the green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae) (Carroll and Hoyt, 1984; Bouchard et al., 1986; Hagley and Allen, 1990). It is important to know whether laboratory reared control agents behave normally. This simple and well defined testing procedure allows a first judgement of predators before they are used in more complex and expensive field trials (cf. Grasswitz and Burts, 1995).

The quality of laboratory reared predators also needs to be assessed before they are released in the field. Compared to the hatching rates reported in the literature (Hämäläinen, 1976; Geusen-Pfister, 1987; Havelka and Zemek, 1988), those observed in this study for the eggs of the three predators were above 75%, which indicated high quality.

The fact that the searching success of the larvae of *A. bipunctata* was independent of the initial distance between the larva and aphid may be related to: (1) the total surface area of the branches not differing sufficiently, or (2) their upright position or simply linear structure helping the larvae locate aphids through negative geotaxis and positive phototaxis (Majerus and Kearns, 1989). Nevertheless, this study showed that larvae of *A. bipunctata* are able to find single aphids in a short time period on branches of apple trees. This situation corresponds to field conditions in spring, when few fundatrices occur on trees.

The subsequent studies under semi-field conditions showed that *A. bipunctata* is able to withstand the climatic conditions prevailing in spring, while *A. aphidimyza* and *E. balteatus* were adversely affected by low temperatures and night frosts in the early experiment. The thermal threshold for egg development of *A. aphidimyza* and *E. balteatus* is above 10 °C (Havelka, 1980; Geusen-Pfister, 1987). During the third release rainfall is thought to have caused the development of unidentified diseases in larvae and eggs of *A. aphidimyza* and *E. balteatus*. In contrast, *A. bipunctata* was little affected by either low temperature or disease, and was the most effective predator in controlling *D. plantaginea*. Based on these results, *A. bipunctata* was released under open field conditions (Wyss et al., 1999).

To determine if the potential of these predators to control aphids could be enhanced, the very mobile larvae of *A. bipunctata* were jointly released with the slower larvae of *E. balteatus*. The previously observed negative effect of both species alone was confirmed, but since no significant interaction was found between the two species, the two predators are likely to have an additive rather than a synergistic effect on aphid abundance. In a series of field exclosure/enclosure experiments, Rosenheim et al. (1993) found that predation by generalist hemipteran predators caused substantial mortality of larvae of the chrysopid, *Chrysoperla carnea* (Stephens), used to control *Aphis gossypii*.

Moreover, these predators significantly reduced the overall control effect on aphids below single species effects. These findings are opposite to ours, as we found lowest aphid numbers, when predators were released in combination. Observations revealed that larvae of *E. balteatus* often reduced aphid colonies to zero. In contrast, the larvae of *A. bipunctata*, although more mobile, do not find all the aphids in a colony. This may be because of the avoidance behaviour shown by some aphids, as reported for sessile, flat shaped species of birch aphid, *Betulaphis brevipilosa* Börner, by Hajek and Dahlsten (1987), which escape predation by larvae of *A. bipunctata*. Syrphid larvae, in contrast, move rather slowly and once they have found prey, they show a very strong area restricted searching behaviour (Chandler, 1969). This supports the combined use of these two predators.

Caging had only a negligible effect on temperature and relative humidity, and so allowed controlled experiments to be conducted under conditions close to that of the natural environment. The three-step screening, from laboratory to field cages (reported here) to open field studies (Wyss et al., 1999) turned out to be an efficient and economic way to evaluate the biological control potential of aphidophagous predators.

The present study showed the potential of indigenous aphidophagous predators for the control of the rosy apple aphid. Further studies are planned to determine the exact timing of predator releases to minimize aphid damage to apple crops, and to develop a cheap method for rearing these predators.

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References

Anonymous, 1977. Pommier III. Contrôles, seuils et indicateurs pour la lutte. *ACTA Lutte intégrée*, Association des Coordinations Techniques Agricoles, Paris. 79 pp.

Bouchard, D., J.G. Pilon and J.C. Tourneur, 1986. Role of entomophagous insects in controlling the apple aphid, *Aphis pomi*, in Southwestern Quebec. In: I. Hodek (ed), *Ecology of Aphidophaga*. Academia and Dr. W. Junk, Prague and Dordrecht. pp. 369–374.

Bouchard, D., S.B. Hill and J.G. Pilon, 1988. Control of the green apple aphid populations in an orchard achieved by releasing adults of *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). In: E. Niemczyk and A.F.G. Dixon (eds), *Ecology and Effectiveness of Aphidophaga*. SPB Academic Publishing, The Hague. pp. 257–260.

Carroll, D.P. and S.C. Hoyt, 1984. Natural enemies and their effects on apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), colonies on young apple trees in Central Washington. *Environ. Entomol*. 13: 469–481.

Chandler, A.E.F., 1969. Locomotory behaviour of first instar larvae of aphidophagous Syrphidae (Diptera) after contact with aphids. *Anim. Behav.* 17: 673–678.

Geusen-Pfister, H., 1987. Untersuchungen zur Biologie und zum Reproduktionsvermögen von *Episyrphus balteatus* Deg. (Dipt., Syrphidae) unter Gewächshausbedingungen. *J. Appl. Entomol.* 104: 261–270.

Graf, B., 1984. Analyse der Populationsdynamik von *Dysaphis plantaginea* (Pass.), *Rhopalosiphum insertum* (Walk.) und *Aphis pomi* (DeGeer) (Homoptera: Aphididae) auf dem Apfelbaum in einer ostschweizerischen Obstanlage. Ph.D. Thesis, ETH Zürich, Switzerland. 139 pp.

Graf, B., H. Höhn and H. Höpli, 1998. Sind Sommerbehandlungen gegen die Mehlige Apfelblattlaus gerechtfertigt? Ansatz für eine zeitbezogene Schadenschwelle. *Schweiz. Z. Obst- Weinbau* 134: 277–279.

Grasswitz, T.R. and E.C. Burts, 1995. Effect of native natural enemies and augmentative releases of *Chrysoperla rufilabris* Burmeister and *Aphidoletes aphidimyza* (Rondani) on the population dynamics of the green apple aphid, *Aphis pomi* DeGeer. *Intern. J. Pest Management* 41: 176–183.

Hagley, E.A.C., 1989. Release of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) for control of the green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae). *Can. Entomol.* 121: 309–314.

Hagley, E.A.C. and W.R. Allen, 1990. The green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), as prey of polyphagous arthropod predators in Ontario. *Can. Entomol.* 122: 1221–1228.

Hajek, A.E. and D.L. Dahlsten, 1987. Behavioural interactions between three birch aphid species and *Adalia bipunctata* larvae. *Entomol. Exp. Appl*. 45: 81–87.

Hämäläinen, M., 1976. Rearing the univoltine ladybeetles, *Coccinella septempunctata* and *Adalia bipunctata* (Col., Coccinellidae), all year around in the laboratory. *Ann. Agric. Fenn*. 15: 66–71.

Havelka, J., 1980. Effect of temperature on the developmental rate of preimaginal stages of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Entomol. Exp. Appl*. 27: 83–90.

Havelka, J. and R. Zemek, 1988. Intraspecific variability of aphidophagous gall midge *Aphidoletes aphidimyza* (Rondani) (Dipt., Cecidomyiidae) and its importance for biological control of aphids. *J. Appl. Entomol*. 105: 280–288.

Hodek, I., 1993. Habitat and food specificity in aphidophagous predators. *Biocontrol Sci. Techn*. 3: 91–100.

Majerus, M. and P. Kearns, 1989. *Ladybirds*. Richmond Publishing Co. Ltd., Slough.

Rosenheim, J.A., L.R. Wilhoit and C.A. Armer, 1993. Influence of intraguild predation among generalist insect predators on the suppression of a herbivore population. *Oecologia* 96: 439–449.

Wyss, E., 1995. The effects of weed strips on aphids and aphidophagous predators in an apple orchard. *Entomol. Exp. Appl.* 75: 43–49.

Wyss, E., M. Villiger, J.-L. Hemptinne and H. Müller-Schärer, 1999. Effects of augmentative releases of eggs and larvae of the two-spot ladybird beetle, *Adalia bipunctata*, on the abundance of the rosy apple aphid, *Dysaphis plantaginea*, in organic apple orchards. *Entomol. Exp. Appl.* 90: 167–173.