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Effects of augmentative releases of the coccinellid, *Adalia bipunctata*, and of insecticide treatments in autumn on the spring population of aphids of the genus *Dysaphis* in apple orchards

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Abstract

The impact of augmentative releases of indigenous predators and insecticide applications to control the autumn aphid forms of the genus *Dysaphis* (Homoptera: Aphididae), major pest insects on apple trees, was assessed in one-year field experiments in Switzerland. Eggs and larvae of the two-spot ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) were released on 4-year old apple trees in various numbers at five different dates in autumn 1998 when sexuales of the aphids were present. Additionally, Pyrethrum HP was sprayed at the same five dates to compare the effectiveness of these augmentative releases to a commonly applied insecticide. Augmentative releases of larvae before mid-October significantly prevented the deposition of overwintering eggs by aphids of the genus *Dysaphis* and consequently reduced the number of hatched fundatrices in spring, 1999. There was a significant negative functional response among the number of released coccinellid larvae in autumn 1998 and the number of observed fundatrices on apple trees in spring 1999. Applications of Pyrethrum HP before mid-October were more effective than augmentative releases of larvae of *A. bipunctata*. The release of coccinellid eggs had no impact on the number of fundatrices of the genus *Dysaphis* in the next spring because they did not hatch due to bad weather conditions. The weather conditions in autumn seemed to have an impact on the autumn migration of the winged aphids back to their primary host. The prevention of egg deposition of aphids in autumn is a promising control strategy and deserves further exploration for practical use.

Introduction

In Switzerland most insect damage in apple orchards is caused by the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and aphids of the genus *Dysaphis* (Homoptera: Aphididae) (Wildbolz & Stäubli, 1984). The polyphagous and holocyclic aphids of the genus *Dysaphis* have a complex biology. At the end of spring the different species (*Dysaphis plantaginea* Pass., *D. anthrisci* Börner, *D. brancoi* Börner, *D. chaerophylli* Börner and *D. radicola Mordv*.) leave their primary host (apple) and migrate to their summer host plants until autumn when the winged forms return to the primary host. There, sexual reproduction takes place and the overwintering eggs are laid. In the next spring the eggs hatch and new populations are built up. Populations of the rosy apple aphid, *D. plantaginea*, are most harmful often causing irreversible damage to the leaves, branches and fruits and severe yield losses (Graf, 1984).

The common strategy to control aphids of the genus *Dysaphis* is to spray aphicides in early spring when fundatrices hatch (Wyss et al., 1999a). However, intensive spraying of insecticides results in the appearance of resistance (Delorme, 1998) and has negative effects on beneficial insects. Therefore, alternative control strategies have been developed in recent years. The three major alternatives encompass the growing and cultivation of resistant apple varieties (Alston & Briggs, 1970), the sowing of weed strips to enhance the number of aphidophagous predators (Wyss, 1995) and the augmentative release of indigenous natural enemies (Wyss et al., 1999a). However, weed-strip management kept the aphids' abundance below the economic threshold only when aphid populations did not reach high levels (Wyss, 1994). To compensate for the lack of efficacy of the naturally occurring predators of aphids, Wyss et al. (1999b) tested augmentative releases of three predator species in field cages (semi-field conditions): the cecidomyiid fly, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), the syrphid fly, *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) and the two-spot ladybird beetle, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). *A. bipunctata* was the most effective predator. Moreover, Wyss et al. (1999a) confirmed that *A. bipunctata* significantly prevented the build-up of colonies of *D. plantaginea* on apple trees in the next spring.

In order to improve the impact of *A. bipunctata* on aphids of the genus *Dysaphis* we decided to test augmentative releases of this predator during the time of sexual reproduction of the aphids on the apple trees. A study of Hemptinne et al. (1994) showed that the abundance of *D. plantaginea* in spring is correlated with the mean temperatures between October and December of the previous year. Cold weather conditions between October and December could determine the success of the recolonization of the apple trees and subsequent survival of the overwintering eggs. The reduction of the number of sexuales of *D. plantaginea* in autumn could, therefore, have a direct effect on the population in the next year. Already Hough (1963) reported that insecticide applications against the egg-laying females of the rosy apple aphid before deposition of overwintering eggs strongly inhibited an outbreak in the next spring. This information triggered our study to release *A. bipunctata* for the control of aphids of the genus *Dysaphis* in autumn. The aims were 1) to determine the ideal release date, 2) to compare the effect of released eggs versus larvae, 3) to define the optimal number of predators, and 4) to compare the effectiveness of augmentative releases of *A. bipunctata* to Pyrethrum HP.

Materials and methods

Experimental orchards. Experiments were conducted in three experimental apple orchards at Frick, in the northwestern part of Switzerland in autumn 1998. Two orchards consisted of 144 4-year old cv. Rubinette apple trees and one of 144 4-year old cv. Glockenapfel apple trees. The trees were approximately 2 m high and had $\pm$ 50 flower buds each. All orchards were arranged in 12 rows of 12 trees each and subdivided in three blocks, each containing 48 trees. Treatments were replicated three times and assigned randomly to the 48 trees within each block.

Insects. *Adalia bipunctata* was reared on green peach aphids, *Myzus persicae* (Sulzer) (Homoptera: Aphididae) under laboratory conditions (temperature 20 °C, photoperiod L16:D8). The coccinellids were introduced into the orchards as eggs or as second instar larvae. Egg clutches, deposited on filterpaper, were divided into pieces with the required number of eggs and attached to the stems just below the branching point. Larvae were placed randomly on the trees using a soft paintbrush.

Winged gynoparae of *Dysaphis plantaginea, D. anthrisci, D. brancoi, D. chaerophylli* and *D. Radicola* (all species of the genus *Dysaphis* are together indicated as 'Dysaphis') as well as *Rhopalosiphum insertum* Walk. (Homoptera: Aphididae) migrate back to the apple trees from the surrounding vegetation in autumn. These gynoparae give birth to unwinged females ('females') on their primary host. Afterwards, they are fertilised by winged males. In addition to the gynoparae, females and males of the earlier mentioned aphid species, the monophagous and holocyclic green apple aphid, *Aphis pomi* DeGeer (Homoptera: Aphididae), also occurs on apple trees in autumn. The fertilised females of all these aphid species lay eggs which will overwinter. Unfortunately, the winged and unwinged aphid forms could not be identified at the species level. Only females of the genus *Dysaphis* could be distinguished from other aphid species. In contrast, fundatrices of the various aphid species can easily be identified and counted on the apple trees in spring.

The interaction of ants and aphids was not included in this study because there were only a few ants on the apple trees during the time of autumn migration of the aphids.

Measurements. The number of aphids and the developmental stage of *A. bipunctata* were monitored weekly on ten trees ('weekly observed trees') from 22 September–21 December 1998. The winged aphids ('alatae') were sampled in yellow water traps (surface area 0.038 m^2) during the same period. Traps were checked weekly and the number of alatae was recorded. From 19–24 November 1998 and from 19–22 April 1999 the numbers of aphids were monitored on every apple tree in the three orchards to determine the efficiency of the control measures in the preceding autumn. Temperature and precipitation were recorded from 15 September–21 December 1998.

Augmentative releases of A. bipunctata. The aim of the first trial, performed in autumn 1998, was to quantify the influence of the release date and two developmental stages of *A. bipunctata* (eggs versus second instar larvae) on the autumn forms of *Dysaphis* (gynoparae, females and males) and consequently on its spring population. Twenty eggs or larvae were released in three experimental blocks of a cv. Rubinette orchard on one or more of the following dates: 29 September, 9, 14 and 27 October and 9 November. Releases were made on individual trees in five combinations of these dates: all five, the last four, the last three, the last two or only the last date. To make a complete set of eight trees needed for analysis of the block design, three additional trees served as no-release controls for each set of release date combinations. Full combinations of factors resulted in 16 treatments and consequently the first trial consisted of a completely randomised 8×2×3 design. Each of the 16 release or control treatments was replicated three times in each of three orchard blocks. This graduated release allowed a determination of the latest effective release date of *A. bipunctata* to control the spring population of aphids.

In the second trial, the influence of the release date (9 and 27 October versus 9 and 14 November 1998), developmental stage (eggs versus second instar larvae) and number of *A. bipunctata* (treatments of 0, 5, 10 or 25 predators per tree) on the autumn forms and consequently on the spring population of *Dysaphis* was quantified. Full combinations of factors resulted in sixteen treatments which were replicated three times within each block of the second cv. Rubinette orchard. Thus, the second trial consisted of a completely randomised 2×2×4×3 block design.

In the third trial, the influence of release date (9, 14 or 27 October 1998) and the number of *A. bipunctata* larvae (0, 1, 2, 3, ... 19, 20, 25, 30 or 35 predators per tree) in preventing the deposition of eggs by female *Dysaphis* was quantified. This trial was conducted to determine the optimal number of predators necessary to suppress the aphid below the economic threshold value of a single fundatrix in a sample of 50 buds (Anonymous, 1977). The third trial consisted of a functional response design and treatments were randomly assigned within the cv. Glockenapfel orchard.

Insecticide applications. The fourth trial in autumn 1998 quantified the influence of spraying date (29 September, 9, 14 and 27 October and 9 November) with the insecticide Pyrethrum HP (Andermatt Biocontrol AG, conc. 4% with pyrethrine as active ingredient) on the autumn forms (gynoparae, females and males) and consequently on the spring population of *Dysaphis*. Apple trees of the cv. Glockenapfel orchard were sprayed with a 0.2% solution of the insecticide at five date combinations: sprays at all five, the last four, the last three, the last two or only the last date. To make a complete set of eight trees needed for analysis of the block design, three additional trees served as no-spray controls for each set of application date combinations. There were eight treatments which were replicated three times within each block. A completely randomised 8×3 block design resulted. This trial allowed a comparison of the effectiveness of augmentative releases of *A. bipunctata* to an usual insecticide to prevent the deposition of overwintering eggs by females.

Data analysis. The observed number of fundatrices of *D. plantaginea, D. anthrisci, D. brancoi, D. chaerophylli and D. radicola* was pooled for analysis because the number of *D. plantaginea* was too small in both orchards with cv. Rubinette. The number of aphids was ln-transformed [$\ln(x + 0.1)$] and treated as a dependent variable. Block, developmental stage and date of application were treated as nominal independent variables and the number of predators as a continuous independent variable. The trials for augmentative releases of *A. bipunctata* were analysed by a two-way ANOVA and the insecticide trial by a one-way ANOVA.

Results

Precipitation, temperature and frequencies of aphids in autumn. The daily mean temperature was 9 °C during the releases which corresponds to the theoretical thermal threshold for development of *A. bipunctata* (Obrycki & Tauber, 1981). Moreover, there was no longlasting rain-free period in autumn 1998 (Figure 1). Therefore, the weather conditions were not favourable for the development of eggs and larvae of *A. bipunctata* in the apple orchards. Eggs were often washed off and pupae were never observed in autumn 1998.

Most aphids were caught between 6 and 28 October 1998 in the yellow traps and the females of *Dysaphis* were observed for the first time on 19 October 1998 on the weekly observed trees just after the appearance of alatae (Figure 2). Unfortunately, winged males and gynoparae could not be distinguished. That is why the moment of the occurrence of males could not be determined.

Augmentative releases of A. bipunctata. There was a significant interaction between the release date and the developmental stage of coccinellids in the first trial (Table 1). This is due to the cold and rainy weather in autumn 1998 where most eggs did not hatch. However, the release of larvae of *A. bipunctata* in autumn 1998 had a significant impact on the number of fundatrices of *Dysaphis* in spring (Table 1). Because of the lack of efficiency of eggs only larvae were considered for analyses. The analysis showed that the augmentative releases of larvae of *A. bipunctata* later than 14 October 1998 had no significant effect on aphids (Figure 3) even if the effect of the treatment with releases at all five dates was clearly not significant (P = 0.07).

Also in the second trial, only treatments with larvae of *A. bipunctata* were considered for analysis. There was no difference between the effect of the two releases on fundatrices of *Dysaphis* in spring (Table 1). However, treatments with a higher number of released coccinellids had significantly fewer fundatrices in spring (Table 1).

In the third trial, the number of released larvae also had a significant influence on the number of *Dysaphis* in spring (Table 1). The release on 14 October 1998 had the highest negative effect whereas the last release on 27 October 1998 revealed no impact on the number of fundatrices in spring 1999. The evaluation of the optimal number of larvae was therefore calculated for the first two releases. Fifty-six larvae of *A. bipunctata* were found to be the optimal number of predators to release in autumn to reduce the number of fundatrices of *Dysaphis* below the economic threshold value in spring (Figure 4).

Table 1. ANOVA table on the influence of treatment factors on the number of fundatrices of the genus *Dysaphis* in spring in the four trials. Data were transformed prior to analysis ($\ln(x + 0.1)$). In the second trial only data of treatments with larvae of *A. bipunctata* were considered for analysis

Source of variation	df	Sum of squares	Sign of F[a]
First trial			
Block within orchards	2	8.67	ns
Release date (RD)	5	41.15	**
Developmental stage (DS)	1	32.42	***
RD × DS	5	73.40	***
Error	123	315.87	
Total	136	453.72	
Second trial			
Block within orchards	2	13.01	*
Release date	1	0.74	ns
Number of released larvae	1	12.18	*
Error	67	137.21	
Total	71	163.14	
Third trial			
Block within orchards	2	0.57	ns
Release date	2	18.35	***
Number of released larvae	1	6.91	*
Error	66	73.83	
Total	71	100.94	
Fourth trial			
Block within orchards	2	4.44	ns
Application date	5	268.36	***
Error	64	56.09	
Total	71	328.89	

[a]ns: not significant; *P < 0.05; **P < 0.01; *** P < 0.001.

Applications of insecticide. The application of Pyrethrum HP in autumn had a strong impact on fundatrices of *Dysaphis* in spring (Table 1, Figure 5). The application of the insecticide on 28 October 1998 significantly reduced *Dysaphis* populations, but not to the extent of the previous three applications (Figure 5).

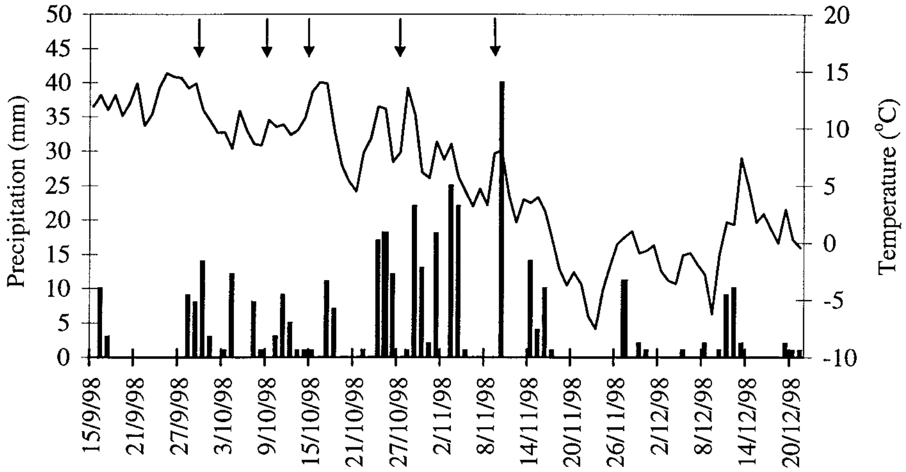

Figure 1. Total daily precipitation in mm (bars) and daily mean temperatures in °C (line) from 15 September to 21 December 1998 at Frick, Switzerland. Arrows indicate the release dates of *A. bipunctata* and the insecticide treatments.

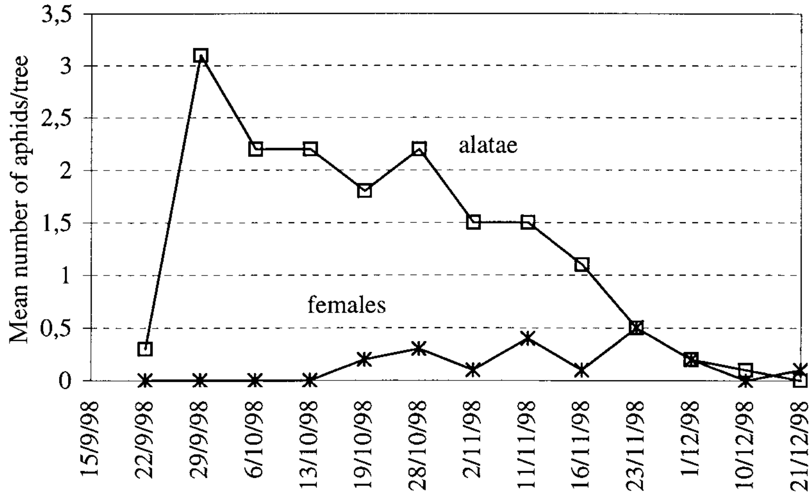

Figure 2. Mean number of females of the genus *Dysaphis* and alatae per weekly observed tree from 22 September to 21 December 1998 in the apple orchards at Frick, Switzerland.

Discussion

Dysaphis plantaginea has recently gained importance as a pest of apple trees (Graf et al., 1998). Because of the appearance of resistance (Delorme, 1998; Höhn et al., 1995) there is high demand for alternative control strategies such as augmentative releases of natural enemies. The challenge for the released biocontrol agent is to hold populations of the rosy apple aphid below a very low economic threshold value of one aphid per 50 buds (Anonymous, 1977). Hemptinne and Dixon (1997) and Dixon et al. (1997) demonstrated that aphidophagous coccinellids generally were ineffective as biocontrol agents because aphids were unstable food resources and coccinellids developed slower than aphids. Nevertheless, these two facts may not be relevant for the release of coccinellids in autumn because there are no propagating aphid colonies on apple trees, besides a few gynoparae and sexuparae. The goal of a successful release of coccinellids in autumn is to prevent the deposition of overwintering eggs. Unwinged females of *D. plantaginea* should therefore be eliminated before eggs are deposited.

For an exact timing of the releases of *Adalia bipunctata* and the application of insecticides, trees were monitored at weekly intervals for the presence of different forms of aphids during autumn 1998. In addition, samples of yellow traps were checked

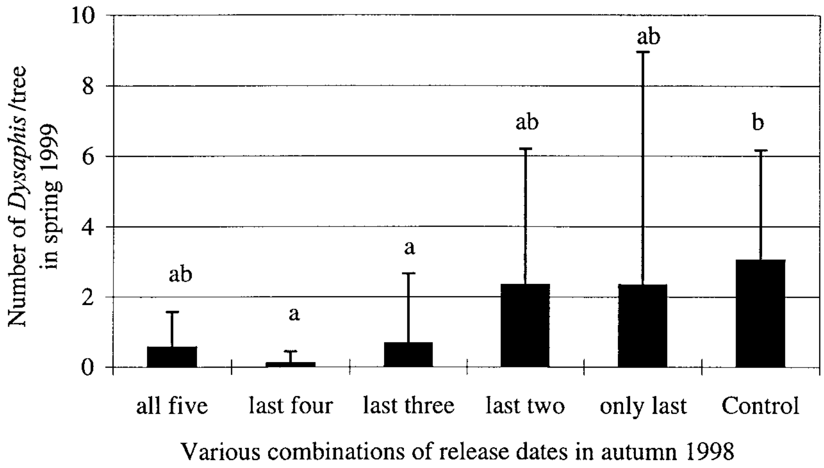

Figure 3. Effects of the release dates of *A. bipunctata* larvae on the mean number of fundatrices of the genus *Dysaphis* per tree in spring 1999 for the first trial. Dates of releases were 29 September, 9, 14, and 27 October, and 9 November 1998. Comparison of the release dates by a one-way ANOVA; release dates with different letters are significantly different ($P < 0.01$).

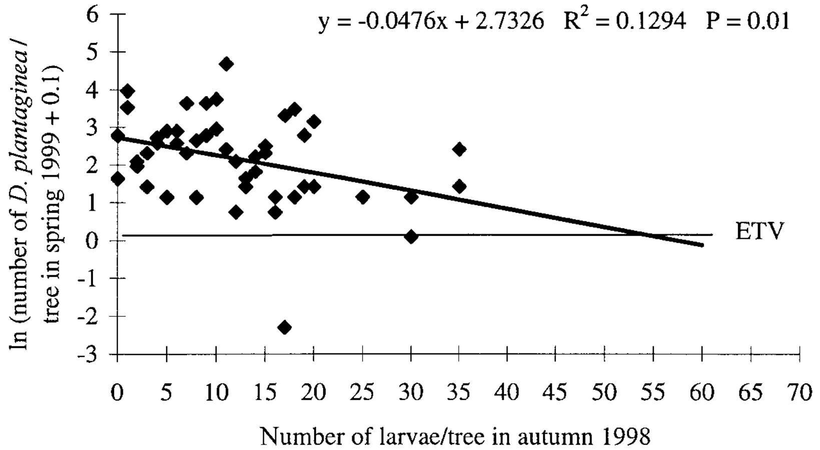

Figure 4. Linear regression and evaluation of optimal predator-prey ratio for the release of larvae of *A. bipunctata* in the third trial. Data were pooled for the releases of 9 and 14 October 1998. ETV: economic threshold value (ln-transformed).

for alatae of *D. plantaginea*. Unfortunately, it was not possible to determine the alatae to species level within a reasonable time. Therefore, important data are lacking to determine phenology and main migration time of the different forms of *D. plantaginea*. Müller (1954) pointed out that migration of alatae depends on weather conditions, especially temperature, wind and rain. Temperature and precipitation were recorded during autumn 1998 but only trends between weather conditions and migration of alatae were visible. However, there was a significant correlation between the number of aphids per tree in November 1998 and the number of fundatrices of *Dysaphis* in April 1999 in two of the four trials. Future research will have to pay more attention to the presence of different forms of *Dysaphis* on apple trees and the correlation with weather conditions in autumn for an exact timing of releases of *A. bipunctata* and application of insecticides.

Nevertheless, this one-year study shows that augmentative releases of second instar larvae of *A. bipunctata* on apple trees in autumn reduce the number of fundatrices of *Dysaphis* in spring. Larvae released before mid-October 1998 partially prevented the deposition of overwintering eggs by aphid females. Furthermore, there was a significant negative functional response between the number of released larvae in autumn 1998 and the number of observed fundatrices on apple trees in spring 1999. Fifty-six larvae per 50 buds were calculated to be necessary to reduce the num-

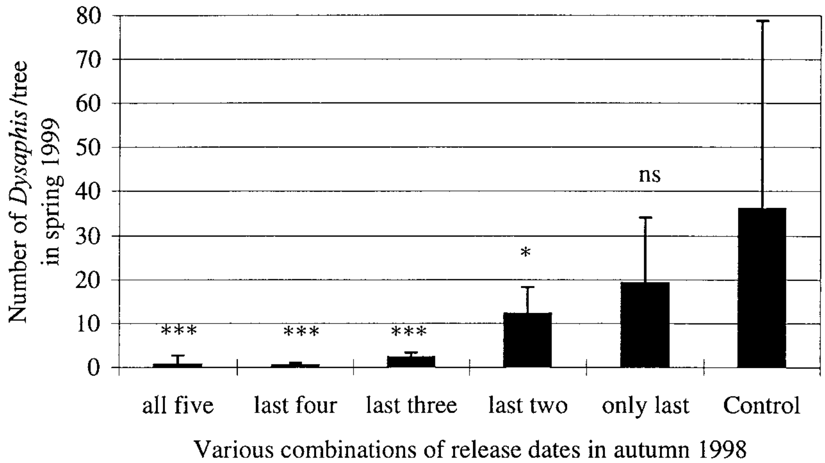

Figure 5. Effects of the application dates of the insecticide Pyrethrum HP on the mean number of fundatrices of the genus *Dysaphis* per tree in spring 1999 in the fourth trial. Application dates were 29 September, 8, 13, and 28 October, and 9 November 1998. Comparison of the application dates with the control (no application of insecticide) using simple contrast method with ln-transformed data. ns: not significant; * $P < 0.05$; ***$P < 0.001$.

ber of fundatrices of *D. plantaginea* in spring 1999 below the economic threshold. Such a great number of larvae required for a successful control can be explained by the scarce food resources and bad weather conditions during the development of the coccinellids. Therefore, only single larvae could be found on apple trees 3 to 4 days after release. This might be because of cannibalism and foraging activities of the larvae.

The rearing of coccinellid larvae is expensive and the release by a soft paintbrush needs much time. A possible way to reduce expenses could be the release of coccinellid eggs. Eggs are cheaper to rear and new spraying techniques enable simple, quick and cheap application (Löchte, 1995). Unfortunately, the released eggs in our study did not hatch in autumn 1998. In general, larvae have the outstanding advantage that they are active immediately after release (Wyss et al., 1999a). Also contact insecticides like Pyrethrum work immediately after application. Our study shows that the applications of Pyrethrum until mid-October 1998 prevented the deposition of overwintering eggs by *Dysaphis* and, consequently, nearly no fundatrices could be observed in spring 1999. Previous dates of application reduced the number of fundatrices below the economic threshold value. Overall, the insecticide Pyrethrum had an effectiveness which was never obtained by augmentative releases of coccinellids.

The similar results of the different trials with applications of coccinellids and Pyrethrum as well as the short period of egg deposition by aphid females do not point to a numerical response because there was no increased efficacy with increasing number of applications. Therefore, this one-year study indicates that mid-October would be the optimal date to control rosy apple aphid in autumn. Otherwise, it is hard to compare the different trials of this study and make general conclusions. Especially the different preferences of *Dysaphis* for the two varieties Rubinette and Glockenapfel complicated the data set. A similar preference was reported by Schmid (1993) who observed that the apple variety cv. Glockenapfel was more susceptible to rosy apple aphid than the variety cv. Rubinette.

Our results confirmed Hough's report (1963) that the control of rosy apple aphid, before overwintering eggs are deposited, reduces the threat of an outbreak next year. Therefore, control of aphids of the genus *Dysaphis* in autumn is a promising strategy and deserves further exploration. Further studies should also monitor the direct effect of autumnal control measures on the sexuals and the influence of weather conditions on the predators to understand the complex interactions. Yet, augmentative releases of beneficials have to be more effective and cheaper, to be competitive with insecticides. This will only be possible if rearing becomes cheaper and if the methods of release are improved.

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