Evaluation of hoverfly releases for the biological control of woolly apple aphid in combination with flower strips in an apple orchard

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Abstract

Aphids are key pests threatening apple production. The woolly apple aphid (WAA), Eriosoma lanigerum (Hausmann) (Hemiptera: Aphididae), is the aphid currently causing significant issues in its management for apple growers and, especially in organic farming, there is a lack of efficient control methods. Given the limited number of products available for pest control and their low efficiency when it comes to the woolly aphid, biological control can be a valuable strategy to counteract the pest and reduce insecticide use. To test the efficacy of hoverflies (Diptera: Syrphidae) as biological control agent of woolly aphid, a field experiment was conducted in 2023 in South Tyrol, Italy, in an apple (Malus domestica Borkh.) 'Fuji' orchard where a flower strip mixture was sown in autumn 2022. The objectives of the study were the evaluation of the flower mixture attractiveness to syrphid adults (i), an evaluation of the influence of flower strips on natural enemy promotion (ii), and the investigation of the impact of syrphids on WAA colonies (iii). Analyses, conducted with Generalised Linear Models (GLMs), revealed a higher presence of natural enemies and adult hoverflies in proximity of flower strips. Small but significant effects were observed for WAA attack severity and for the number of WAA colonies, but unfortunately with a slightly higher incidence in the apple trees near the flower strips.

Keywords: biological control; woolly apple aphid; hoverfly; flower strip; beneficial insect.

Introduction

Eriosoma lanigerum (Hausmann) is one of the most detrimental pests to apple orchards, causing remarkable damages to apple production worldwide. The absence of an effective control method significantly contributes to the importance of this aphid, which causes swelling and deformation of woody tissues, and the overall reduction of the plant health status. The application of biological control to contrast this pest is possible thanks to the presence of several natural enemies from different families of insects (Asante et al., 1993), among which hoverflies. Hoverflies (Diptera: Syrphidae) are one of the largest families of Diptera Brachycera counting a great number of aphid predator species, especially in the subfamily Syrphinae (Burgio et al., 2015). In their larval stages, hoverflies from aphidophagous species are known to be efficient aphid predators, with a single larva of Episyrphus balteatus (De Geer) estimated to feed on approximately 400 aphids under field conditions (Tenhumberg, 1995). Pollen and nectar provided by flowers are important for syrphid females to develop the ovary and produce viable eggs (Schneider, 1948; Gilbert, 1981). Hence, the presence of flowers in proximity of the orchard is fundamental when planning biological control using syrphids as aphid predators. In this context, flower strips i.e., a mixture of flowering herbaceous species, either sown or naturally settled on the field margin or the orchard interrow, are a valuable agronomic practice to provide food sources, like pollen and nectar, and shelter against adverse conditions for beneficial insects.

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The aim of the present work is the evaluation of a biological control strategy based on the combination of syrphid releases and flower strips for the control of this pest, by conducting an open field experiment. Only a few studies have investigated the use of the insects from this family as biological control agents against the woolly apple aphid, and particularly in combination with flower strips. Flowers are known to be extremely important to produce viable eggs for syrphids and the absence of this food source can strongly compromise the result from the actuation of any attempt of biological control. Consequently, the objectives of the study are i) the evaluation of a flower mixture attractiveness for syrphid adults compared to conventionally managed interrows, ii) an evaluation of the influence of flower strips on natural enemies abundance on the apple trees, and iii) the investigation of the impact of this biological control strategy on *E. lanigerum* colonies.

Material and Methods

The experiment occurred at Laimburg Research Centre in Vadena, Italy (46°22'49.2"N, 11°17'19.4"E), in an orchard of 'Fuji' apple grafted onto M9, planted in 2003, and trained to a central leader with a spacing of 1 m and 3 m width in the interrow. The field was organised with two sown flower strips planted in autumn 2022 on the western side, with a conventionally managed interrow (repeated mulching throughout the season) in between. The flower seed mixture comprises 15 species from 5 botanical families with the majority of the species presenting flowers with open corollas and accessible nectaries, which are favoured by hoverflies (Branquart *et al.*, 2000). Two different species of hoverflies were released during the season: *Sphaerophoria rueppellii* (Wiedemann) and *Episyrphus balteatus* (De Geer), to exploit their difference temperature adaptation along the season. The hoverflies were released both in the form of pupae and larvae, in four and three different release dates respectively, from mid-May to the end of June 2023. Evaluations were conducted according to the objectives of the study considering three categories:

<u>Syrphid adults visual observation:</u> as proposed by Burgio *et al.*, (2015), syrphid adults' presence was evaluated by applying the *belt method*. By walking in line at a steady pace of 10 m per minute from one end to the other of the interrow twice, all adult hoverflies encountered flying or lying on flowers up to 1 m in front and to either side of the observer were counted.

<u>Beneficial insects presence on apple trees:</u> recorded close to WAA colonies on 50 selected apple branches in six different dates, considering 5 categories: *Aphelinus mali*, ladybird adults, ladybird larvae, syrphid larvae, syrphid excrements. The latter category was included since it is unlikely to observe active syrphid larvae in the colony given their nocturnal predatory behaviour. Thus, meconium and excrement, appearing as a black lucid smear on apple leaves, may be stronger indicators of hoverfly larval activity and presence.

Impact on WAA colonies: Evaluated considering the number of colonies and the severity of the attack, both after the release of larvae and pupae. The measurements were made by counting and estimating the size of the active colonies visible on the branches. Colony size was preferred as a parameter to estimate attack severity while preserving the integrity of the colonies. The size was estimated after the release of the larvae on 50 apple branches using severity classes, with a value ranging from 0 (absence of colonies) to 5 (high infestation). The evaluation after the release of the pupae was still done using size as parameter but comparing the colonies to known surface area, as an attempt to standardize the sampling effort and reduce the subjectiveness in the evaluation. In this case, the evaluation was done on 200 one-year-old branches and shoots.

The data gathered were analysed using the software R (R Core Team, 2022). The analyses were conducted using Generalized Linear Model (GLM) for the effect of flowers in

influencing the abundance of natural enemies, while Generalized Linear Mixed Effect Model (GLMM) was used to analyse the effect of flowers in influencing syrphids adults abundance and the effect of flowers in influencing WAA number of colonies and attack severity after the release of both syrphid larvae and pupae.

Results

Flower attractiveness for hoverflies

The presence of the flowers influenced significantly the number of syrphid adults observed in the field (Estimate = 1.794, p < 0.001). This suggests a significant positive association between the presence of flowers and the abundance of syrphid adults, indicating that the presence of the treatment 'flower' is linked to an increase in the expected count of hoverflies (Fig. 1).

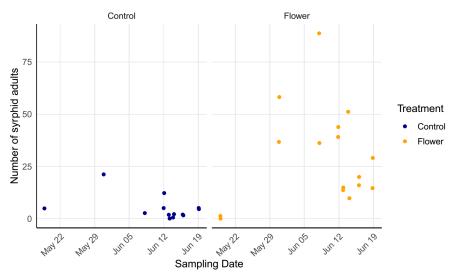


Figure 1: Hoverfly (Diptera: Syrphidae) adults abundance detected by visual observation in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of syrphid adults observed in the field (p < 0.001). Each dot represents a single evaluation.

Beneficial insects presence on apple trees

The presence of the flower strips significantly influenced the sum of all the categories of natural enemies (Estimate = 1.151, p < 0.001) and also each of the categories selected. Overall, natural enemies were more abundant in the apple rows close to the flower strip compared to the control. The implementation of the flower strips positively influenced the presence of syrphid larvae, with more larvae recorded on branches in proximity to the flower strips (Estimate = 2.944, p = 0.004). Simultaneously, a higher feeding activity was observed in the 'flower' treatment compared to the control, with a significant impact of the flower strips on the number of syrphid larvae excrement, indicating a higher predatory activity (Estimate = 2.773, p < 0.001).

Effect on woolly apple aphid colonies

Contrary to expectations, the presence of flowers unexpectedly led to no differences in WAA attack severity in proximity to the flower strips compared to the control after the release of the larvae (Estimate = 0.216, p = 0.260), and to a small but higher incidence after the release of the pupae (Estimate = 0.431, p = 0.030).

Discussion

Studies on the effectiveness of syrphids as biological control agents of WAA, combined with the implementation of flower strips, are lacking. The attractiveness of flower strips to hoverflies and the ability of syrphids to suppress WAA colonies were investigated, together with the influence of flower strips on the promotion of natural enemies on apple trees. In the trial performed, the flower strips had significantly more syrphid adults than the conventionally managed interrow used as a control, suggesting greater attractiveness to the selected flowering species. Flower strips influenced also significantly the abundance of natural enemies on apple trees. For what concerns the effectiveness of syrphids combined with flower strips in suppressing WAA attack, the findings did not meet the expectations. No significant differences or small significant differences were observed close to flower strips compared to the control for the evaluations, both after larvae and pupae releases. The observed results might have been affected by the spatial separation between the flower strips and the control. The close proximity of the flower strips to the control might have impacted the results. According to Cahenzli et al. (2019), it is recommended to place flower strips and control plots at a greater distance, such as in separate orchards with similar microclimates. Progressing in this direction was not feasible for various reasons. Firstly, there were no other fields with analogous characteristics available to extend the experiment. Secondly, utilising different fields would introduce diverse environmental and agronomic conditions for both treatments, making comparisons challenging.

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