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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 among the 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 the lack of an efficient control method. Given the limited number of products available for pest control and the low efficiency of most of them 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 for syrphid adults (i), the investigation of the impact of syrphid on WAA colonies (ii), and an evaluation of the influence of flower strips on natural enemies promotion (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 attacks and for the number of WAA colonies, but with a slightly higher incidence in the apple trees near the flower strips.

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

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Introduction

1.1 Woolly apple aphid, *Eriosoma lanigerum* (Hausmann)

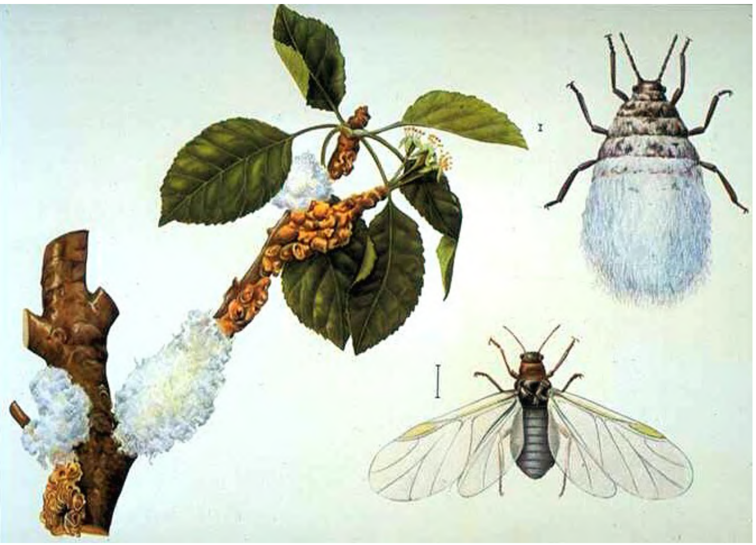


Figure 1.1: Woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae): life stages of apterous virginiparae and alate virginiparae (right), infestation on apple, causing woolly colonies and galls on stems (left) (Centre for Agriculture and Bioscience International, CABI).

Eriosoma lanigerum (Hausmann) is one of the most detrimental pests for apple orchards, causing remarkable damages to apple production worldwide. The absence of an effective control method significantly contributes to the importance of this aphid. Probably native to North America, the first European account appears to date back to the first description by Hausmann in 1802 (Baker, 1915).

Thus, woolly aphid is not of recent introduction in the European context; although, its importance in recent years is due to the removal of broad-spectrum insecticides, making it one of the most problematic pests for apple crop.

1.1.1 Biology and life cycle

Eriosoma lanigerum is a pest of both apple *Malus domestica* (Borkhausen), and the American elm *Ulmus americana* (L.). In its native region in North America, the American elm is the primary host of this pest. This is true especially in the eastern United States, where the American elm is particularly abundant (Baker, 1915; Beers et al., 2010). In the rest of the world where the primary host is absent, *E. lanigerum* can develop on apple throughout the entire year (Beers et al., 2010; Theobald, 1921). This is an important aspect to consider as it influences the biology and life cycle of the pest. Commonly known as woolly apple aphid (WAA), this species gets its name from the white wax filaments formed as a protective coat by adults (see fig. 1.1, Baker, 1915). It is found on both the root system and canopy of apple trees, although it does not directly feed on the leaves. The trophic activity on the trunk and branches results in swelling and deformation of woody tissues, with the formation of galls (Blackman and Eastop, 1984). WAA is primarily an indirect pest; despite that, direct damages on fruits may occur from colonisation of fruit core (Essig, 1942) or by the development of sooty blotch promoted by the presence of honeydew.

WAA reproduce both parthenogenetically and sexually (Baker, 1915; Sandanayaka and Bus, 2005). In its native region, migration between the primary and secondary host is historically recorded (Baker, 1915), with sexual reproduction and the presence of overwintering eggs on the American elm. However, with the exception of rare oviparous sexual morphs (Sandanayaka and Bus, 2005), WAA is mainly parthenogenically viviparous in most apple regions worldwide, where the primary host is absent. Only two forms are present in this context, apterous virginiparae and alate virginiparae (see fig. 1.1). This means that, despite other aphids, *E. lanigerum* is not presenting host-alternation and migration to other plants.

Colonisation of the canopy is done by overwintering nymphs, known as crawlers, which are present in the root apparatus. The migration from roots to the apple tree canopy typically occurs from early May to late July, displaying high variability due to environmental conditions. The migration peak, often observed around early June (Beers et al., 2010), can also have multiple peaks as indicated by Lordan et al. (2015) in a study in the Mediterranean region, due to temperature fluctuations. After July, WAA infestation faces a decline which could be followed by a new increase in September/October, as reported by Brown and Schmitt (1994) in West Virginia. New shoots and branches are colonised by crawlers (Lordan et al., 2015), although overwintering colonies might already be present in pruning cuts or wounds in the trunk. In recent years, warmer temperatures in winter may be the reason for the survival of more colonies in the canopy and, therefore, for a rapid re-establishment of the pest after the overwintering (Beers et al., 2007; Lordan et al., 2015). Woolly aphid colonies are enveloped in wool and contain animals of all stages, causing problems in discerning the different stages within a colony. In summer, migrant alate forms are responsible for colonising other apple trees and forming new colonies. WAA infestation varies between years and orchards. Additionally, the spatial distribution within an orchard is irregular, characterised by elevated infestation levels on specific trees or isolated rows (Asante et al., 1993).

1.1.2 Control methods

Since the withdrawal or restricted use of certain broad-spectrum insecticides, such as organophosphates, *E. lanigerum* has become one of the most severe pests in apple growing areas (Bangels et al., 2021; Beers et al., 2007; Gontijo et al., 2012). To date, there is no efficient synthetic insecticide that controls WAA.

Selection of resistant rootstocks appears to be a promising strategy to control woolly aphid infestation, as the root apparatus is a part of the tree colonised by the pest and, more importantly, the overwintering site. Rootstocks from the Cornell-Geneva apple rootstock series (for instance GENEVA® G41 and GENEVA® G202) have been identified as more tolerant compared to the most utilised rootstocks from the Malling-Merton series as M9 (Beers et al., 2007; Fazio and Beers, 2010; Robinson et al., 2003). Resistant rootstocks can facilitate the management of the pest and can be effective in controlling the pest if coupled with biological control. The latter appears to be a suitable strategy for WAA management as well, due to the presence of both predators and a specialised parasitoid, and thanks to the absence of residues compared to the use of synthetic insecticides. Natural enemies of woolly apple aphid are widely described in the review by Asante (1997), which has identified a total of 73 species of predatory insects, five species of hymenopterous parasitoids, two species of Acarina, and a fungal pathogen. Predators include insects from the families Coccinellidae, Syrphidae, Chrysopidae, and Forficulidae. Ladybirds are described by Asante (1997) as the most important family of WAA natural enemies, followed by syrphids. Biological control thanks to the predatory activity by ladybird, appears to have been reported already by Baker (1915). The use of the ladybird *Coccinella septempunctata* (L.) provided promising results in the control of WAA in a field experiment in Egypt (Mangoud, 2007). The role of syrphids in controlling WAA colonies is reported in several studies (Beers et al., 2007; Gontijo et al., 2012). The study by Gontijo et al. (2013) identifies syrphids as the more abundant predator present in an orchard with flower strips of sweet alyssum in which a reduction in WAA population was recorded. Earwigs, and in particular *Forficula auricularia* (L.), are accounted among the natural enemies able to suppress woolly aphid infestation by Mueller et al. (1988).

Among the hymenopterous parasitoids, *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) is the most important host-specific natural enemy of *E. lanigerum*. This parasitoid, native to North America, is now widespread and has been introduced into many apple-growing regions as a biological control agent (Clausen, 1978). *A. mali* is considered to be highly efficient in suppressing WAA infestations. However, several factors compromise its effectiveness. The most important factor is found probably in cold temperatures (Clausen, 1978), which reduce its impact at the start of the new season in spring. The developmental temperature threshold of WAA is probably lower compared to that of *A. mali* and the greater number of generations of the aphid compared to the parasitoid might be responsible for the lack of control in some contexts. In addition, *A. mali* can only contrast above-ground colonies, and chemical pesticides commonly used in horticulture are detrimental to its presence in apple orchards.

1.2 Syrphid flies (Diptera: Syrphidae)

Syrphids, commonly known as hoverflies in Europe or flowerflies in the USA (Omkar and Mishra, 2016), are one of the largest families of Diptera Brachycera, counting nearly 6000 species identified in all continents, except Antarctica (Burgio et al., 2015; Sommaggio, 1999). Interest in these insects originates from the ecosystem services provided. In fact, components of this family are known to be efficient pollinators and aphid predators, both traits of interest in the agricultural landscape. Furthermore, Burgio et al. (2015) and Sommaggio (1999) have examined their function as bioindicators for the evaluation of agricultural pollution and habitat disturbance, providing further evidence of their ecological importance.

1.2.1 Biology and life cycle

Syrphid adults have a size ranging from 4 to 35 mm in length (Sommaggio, 1999), with a brightly coloured abdomen with bands in black, brown, yellow or white resembling that of insects from the order Hymenoptera as a consequence of Batesian mimicry, a well-investigated trait in several studies for this family (Howarth and Edmunds, 2000; Omkar and Mishra, 2016). Together with their pleasant and colourful appearance, flying behaviour is one of the traits identifying the family, due to their strong attitude to hovering from which comes the common name 'hoverfly' (Omkar and Mishra, 2016). In fact, syrphids can be easily distinguished from other flies thanks to their stationary flight, as well as their ability to fly sideways. As for all other insects of the Diptera order, hoverflies present halteres and use only a single pair of forewings. The latter represents an identification key for the family thanks to the presence of the so-called 'vena spuria', a false vein in the central position with a less pronounced colour and that does not reach the end of the margin of the wing (Burgio et al., 2015). Sexual dimorphism is present in adults as a difference at the level of the eyes in most of syrphid species. Adult males usually present a holoptic arrangement, with eyes meeting at the level of the forehead, whereas females present a dichoptic arrangement, in which eyes are well divided and distinguishable (Burgio et al., 2015). The mouthparts are sponge-like, with a proboscis that varies in length between different species and from which enzymes are secreted to facilitate sugar acquisition (Burgio et al., 2015; Gilbert, 1981).

Adults dietary requirements are mainly based on pollen and nectar, but also aphid honeydew can also be part of it. While pollen is the main source of protein for syrphids, nectar is the source of carbohydrates. Pollen and nectar play a key role in the syrphids' life cycle. In fact, the availability of pollen and the development of the female reproductive system are strongly related. The production of mature ovaries requires the acquisition of pollen by females (Schneider, 1948) since their reproductive system is synovigenic *i.e.,* immature at emergence (Omkar and Mishra, 2016), and pollen is essential for its maturation. Male requirements for amino acids and carbohydrates are lower compared to females, which needs them for the production of eggs. The role of carbohydrates, on the other hand, is linked to the provision of the energy needed to allow the hovering

(Gilbert, 1981). The importance of pollen and nectar makes flower selection a critical element in syrphids foraging behaviour, a trait well investigated in numerous studies. Hoverfly adults have no particular flower preferences, despite the fact that large inflorescences and flat corollae are features favoured when selecting the source of pollen and nectar. Thus, plants from the Apiaceae, Asteraceae, Ranunculaceae, and Rosaceae families seem to be more suitable as a food source (Branquart et al., 2000). The explanation for this behaviour is found in insect morphology. The length of the proboscis and the width of the head are the two main features that affect hoverflies accessibility to nectaries (Pinheiro et al., 2013). As a consequence, the combination of insect morphology and floral anatomy determines the range of plants exploited by hoverflies. The study of Pinheiro et al. (2013) provides an example of how *Episyrphus balteatus* (De Geer) access to nectaries of *Echium plantagineum* (L.) is limited by its short proboscis, ensuing in a reduced adult longevity when adults from this species have access only to this plant. Mouthparts morphology determines not only flower selection, but also diet composition in hoverflies. Longer and thinner probosces are associated with syrphids having diet with a greater proportion of nectar, while shorter probosces are typical of pollen-feeding syrphids (Gilbert, 1981).

Mating occurs between 1-13 days after emergence (Omkar and Mishra, 2016), after a complex courtship that varies from species to species (Burgio et al., 2015). Females lay solitary oblong eggs, in number that can vary from 100 to more than 4000 depending on the species (Burgio et al., 2015). Eggs hatch after 2-3 days, releasing small larvae with morphological traits that differ in relation to the feeding habit and the habitat. In fact, a distinction can be made between phytophagous, mycophagous, saprophagous, and zoophagous larvae. The latter exemplify one of the reasons for syrphids ecological importance and their possible role as bio-indicators, being present in different ecosystems and having different environmental requirements (Sommaggio, 1999). The selection of a suitable surface to lay the eggs by the female is based on the dietary needs of the larvae, to ensure the survival of the progeny (Burgio et al., 2015). As an example, aphidophagous species are used to lay eggs close to aphid colonies, while saprophagous species lay eggs in micro-habitats in forest environments where larvae can easily access to food. All species belonging to the Syrphidae family undergo three instars prior to their transition to the pupal stage (Rotheray, 1993). Neonate larvae have poor stored resources, and thus they are obliged to search for food quickly after emergence (Gilbert, 2005). Syrphid larvae, in common with larvae belonging to the Diptera order, present the absence of segmented legs and a distinct head capsule. Despite being legless, they are characterised by a moderate level of mobility and larvae from aphidophagous species can cover large surfaces during predation. A characteristic that serves as a distinctive trait for hoverfly larvae within the Diptera order is the presence of two conjoined breathing tubes, resembling a posterior ’tail.’ This feature sets them apart from other larvae in the same insect order, with only a few rare exceptions (Rotheray, 1993; Sommaggio, 1999).

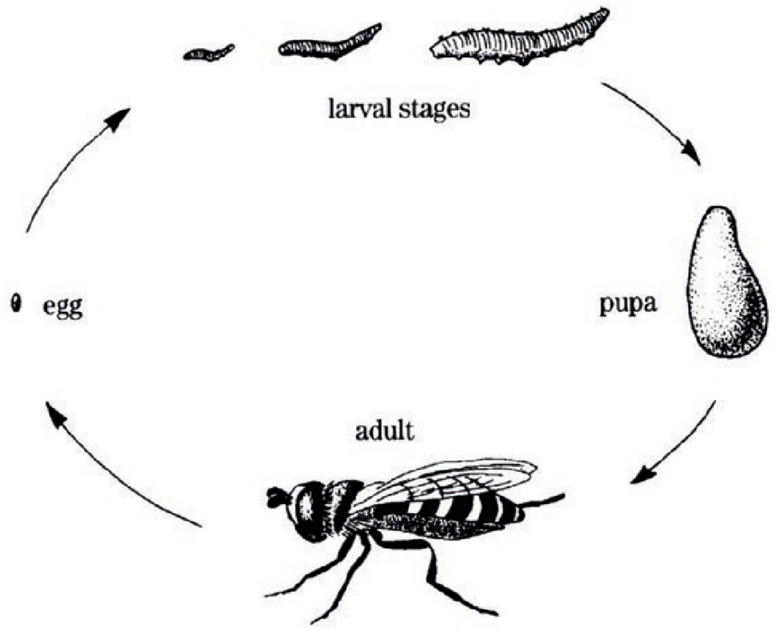


Figure 1.2: Syrphid (Diptera: Syrphidae) complete life cycle, with the stage of egg, the three instar larvae, the pupa and the adult stage (Burgio et al., 2015).

The pupae are derived from the hardened skin of the third larval stage, giving rise to a teardrop-shaped pupal case. According to the environmental conditions, adults typically emerge after a timespan of 1 to 2 weeks. The number of generations per year varies according to species and climatic conditions, being however around 5-7 generations per year for the majority of syrphid species (Omkar and Mishra, 2016). Syrphids can be categorised into three distinct reproductive patterns: univoltine, bivoltine, or multivoltine. The latter often exhibit a migratory behaviour (Wotton et al., 2019), with overwintering pupae and larvae (Burgio et al., 2015). Figure 1.2 exemplifies the complete life cycle of hoverflies.

1.2.2 Role as biological control agent

Syrphids are known to be efficient aphid predators. The use of biological control as a measure of pest suppression is receiving increasing interest due to the growing demand for more sustainable crop protection practices. Considering that aphids are among the most impacting pests for several crops, hoverflies are a suitable alternative to reduce insecticide applications. Components of the subfamily *Syrphinae* are known to be aphid predators and count some of the most well-known species of relevance for biological control (Burgio et al., 2015).

During the larval stages, aphids are the basic dietary requirement for aphidophagous hoverfly species. The suitability of syrphids as good candidates for biological control is determined by three features, namely adult's flight ability, number of eggs laid, and prey consumption efficacy. Syrphids are known to be good fliers and to be able to cover wide areas during their lifetime, with some species such as *E. balteatus* even performing long-range migrations during spring and autumn (Wotton et al., 2019). Along with their flight ability, the high number of eggs laid by a single female provides the possibility to widely impact on aphid infestation by addressing each colony spread in the field. Females of *E. balteatus* are known to lay approximately 4000 eggs (Omkar and Mishra, 2016), which results in a considerable number of larvae that can directly start feeding within aphid colonies upon hatching. In this context, oviposition is an important process and is generally dependent on the density of the prey, with small and young colonies preferred over larger colonies (Burgio et al., 2015; Omkar and Mishra, 2016). Different stimuli determine the selection of the oviposition site, and tritrophic interactions between the host plant, prey, and the natural enemy are an important aspect to consider (Amiri-Jami et al., 2016). The process is described by Omkar and Mishra (2016), and involves the use of long- and short-range cues for the assessment of vegetation and aphid colonies, respectively, and the utilisation of olfactory and gustatory stimuli. The latter are notably known to be important for the location of aphid colonies to lay eggs, with aphid honeydew being a stimulus for oviposition (Budenberg and Powell, 1992). Semiochemicals emitted by the prey or the association of host plant and prey are among the factors triggering the selection of oviposition site as well (Amiri-Jami et al., 2016; Verheggen and Haubruge, 2009). In addition, the presence of conspecific eggs or larvae is a deterrent for oviposition site selection for some species (Amiri-Jami et al., 2016). Eggs are laid directly within the colony and larvae can begin feeding after emergence. Aphidophagous syrphid larvae are characterised by a foraging behaviour that involves the elevation of the anterior part of the body, followed by movements - like strikes - in all directions to locate by contact the prey. Once the prey is identified, it is lifted and the content of the aphid is sucked (Bhatia, 1939). Hoverfly larvae are voracious aphid predators, with a single larva of *E. balteatus* estimated to feed on approximately 400 aphids under field conditions (Tenhumberg, 1995). The third instar larva is the most voracious, and its voracity increases with aphid population density (Dunn et al., 2020). This attribute is well-suited for syrphid utilisation in the context of biological control.

Syrphids can be commonly categorised as either specialised or generalised aphid predators. Even in this case, specialisation is linked to oviposition, and thus host selection by syrphid females. Although syrphid larvae are quite mobile and in the last larval stages they can feed covering large surfaces, at emergence their dispersal ability is limited, and factors like accessibility and proximity to the first prey are extremely important for offspring survival. Hence, polyphagous hoverfly larvae prey preference is a trait that should still be further investigated since it plays a central role in the context of biological control (Gilbert, 2005). In fact, an aphid species might be preferred to the one that is the target when applying augmentative biological control using hoverflies.

As for any other strategy of biological control, the use of hoverflies implies considering a complex system. The article from Gilbert (2005) discusses the ecological interactions that are part of this system and that are summarised in figure 1.3. The different trophic levels can influence the potential biological control performance of hoverflies as consequence of predation from other arthropods and competition with other aphidophages. The latter phenomenon is particularly important and is called intra-guild predation *i.e.,* predation within the same trophic level, involving mainly other aphid predators like ladybirds and lacewings (Burgio et al., 2015). In addition, syrphid species that share the same aphid prey can take part in inter-specific predation of eggs and larvae as well (Amiri-Jami et al., 2016; Verheggen and Haubruge, 2009). Furthermore, ants are known to protect aphid colonies and, during the first instar, hoverfly larvae are particularly vulnerable to their predation.

Environmental suitability is another important aspect to consider. The presence of flowering plants is a limiting factor for adults development and, in the agricultural landscape, the application of insecticides for pest control can be a threat to hoverflies. For this reason, a proper landscape management is required and a particular attention should be given to this aspect.

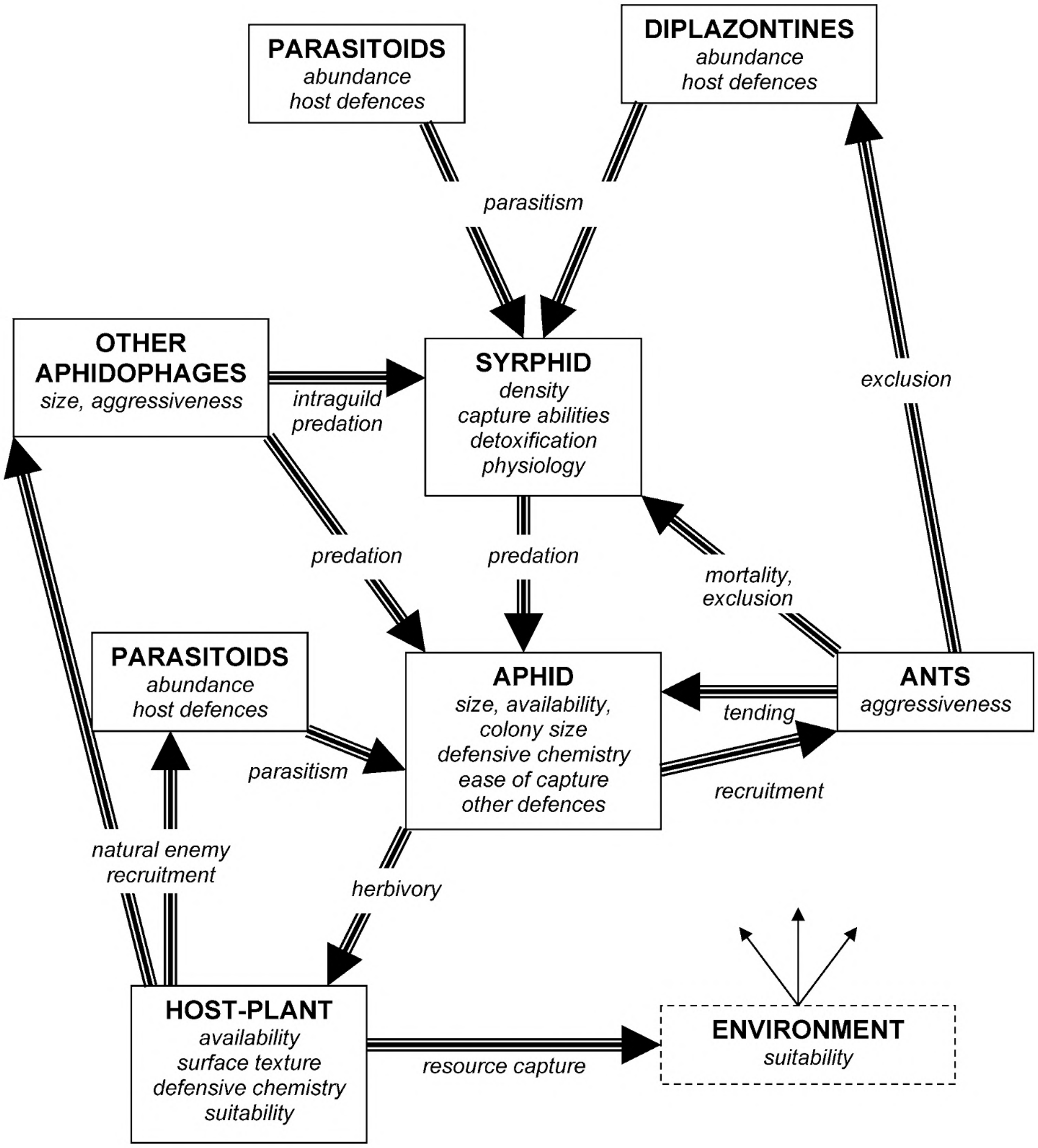


Figure 1.3: Schematic representation of ecological interactions involving aphidophagous syrphids (Gilbert, 2005).

1.2.3 Aphid predator species

1.2.3.1 *Episyrphus balteatus* (De Geer)

Subfamily: *Syrphinae*

Tribe: *Syrphini*

Figure 1.4: Adult of *Epysirphus balteatus* (De Geer) (Diptera: Syrphidae) (Karwath, 2005).

Episyrphus balteatus (De Geer) is probably the most studied and common aphidophagous hoverfly (Gilbert, 2005). It is a generalised aphid predator found in a variety of habitats, ranging from agricultural land to urban areas. It is distributed in Europe, Asia, and North Africa (Burgio et al., 2015). In Europe, *E. balteatus* is particularly abundant in central and northern countries (Tenhumberg, 1995). Adults present a banded abdomen in black and yellow with a double black band on the third and fourth tergum (Burgio et al., 2015). The proboscis is short and thick and pollen from Umbrelliferae and Asteraceae flowers seems to be preferred (Gilbert, 1981). The thermal requirements for this species favour temperate conditions, with high mortality rates occurring at temperatures above 25°C (Hart et al., 1997). Due to its large size and frequent hovering, *E. balteatus* has a high energy requirement (Gilbert, 1981). Under laboratory conditions, females lay approximately 2000–4500 eggs (Omkar and Mishra, 2016), while the larvae are proven to consume up to 400 aphids during their lifetime (Tenhumberg, 1995). *E. balteatus* is a polyphagous aphid predator, with more than 200 aphid species known to be suitable as prey (Burgio et al., 2015). The larvae are characterised by white stripes beside the digestive system and visible red malpighian tubules, both traits that make *E. balteatus* larvae easy distinguishable from other in the same family (Burgio et al., 2015). The migratory behaviour of the adults has been well investigated by Hlaváček et al.

(2022) and Wotton et al. (2019), and migratory routes are known from South to North Europe.

1.2.3.2 *Sphaerophoria rueppellii* (Wiedemann)

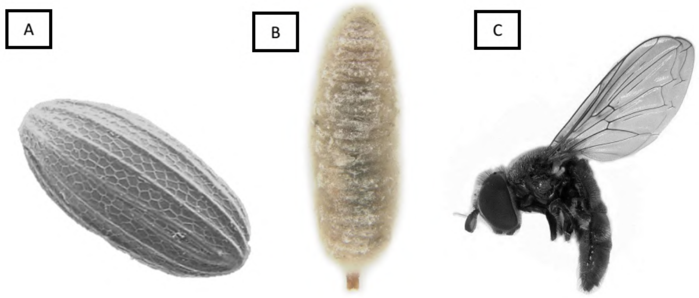
Subfamily: *Syrphinae*

Tribe: *Syrphini*

Figure 1.5: Adult of *Sphaerophoria rueppellii* (Wiedemann) (Diptera: Syrphidae) feeding on a flower of coriander (credit Francesco Panzeri, 2023)

Sphaerophoria rueppellii (Wiedemann) is an aphidophagous hoverfly present in the Mediterranean area, adapted to conditions of high temperature and humidity (Amorós-Jiménez et al., 2012). Adults present a banded abdomen in black and yellow, longer and thinner compared to other species. The larvae are bright green with light dorsal stripes, like other species from the genus *Sphaerophoria* (Burgio et al., 2015). Due to its adaptation to high temperatures, this species is well suited for the implementation of biological control for those crops grown in greenhouses and in regions with warm climates (Amorós-Jiménez et al., 2012).

1.2.3.3 *Heringia calcarata* (Loew), a woolly apple aphid specialized predator



Subfamily: *Pipizinae*

Tribe: *Pipizini*

Figure 1.6: Egg (A), 2nd instar larva (B), and adult (C) of *Heringia calcarata* (Loew) (Diptera: Syrphidae) (Short, 2003).

Starting from early 2000, a new species of hoverfly has been identified as a candidate biological control agent of WAA in the United States. In apple fields in the Virginia state, larvae of the syrphid *Heringia calcarata* (Loew) have been recorded as the most abundant hoverfly species present as larvae and eggs in an apple orchard highly infested by WAA (Bergh and Louque, 2000). Few studies have been published to introduce the characteristics that make this syrphid a candidate for biological control. *H. calcarata* adults are black coloured and of small dimensions, being approximately 7 mm long (Bergh and Short, 2008). As for the other hoverflies, predation is performed by the trophic activity of the larvae. The study by Short and Bergh (2006) provides preliminary data on *H. calcarata* prey preference for WAA over the apple aphids *Dysaphis plantaginea* (Passerini) and *Aphis spiraecola* (Patch) in laboratory conditions. Furthermore, the survival of *H. calcarata* larvae was greater on a diet based only on WAA compared to other aphids. The total aphid consumption was recorded to be approximately 100 aphids/larva, value lower than that for other aphid species. To date, there is a lack of published studies on *H. calcarata* in the European context.

1.3 Flower strips

Flower strips consist of a mixture of flowering herbaceous species, either sown or naturally settled on the field margin or the orchard interrow, with the aim of achieving agricultural and ecological benefits (Kowalska et al., 2022). The loss of biodiversity in agricultural landscapes has been a main issue in recent and past years due to the increasing adoption of agronomic practices encouraging monocolture. Many strategies have been designed to counteract this trend and promote the delivery of ecosystem services, such as pollination and pest control. Flower strips are one of those. Flowers are known to be of high ecological importance for insects as source of food, in the forms of pollen, nectar, and alternative preys. In addition, the presence of a grass cover contributes to provide a shelter, which allows for the maintenance of insect populations and the production of offspring (Kowalska et al., 2022). Orchards are suitable environments for the promotion of this practice due to their perennial character and their diversified structure, which entails the presence of fruit trees and also herbaceous plants in the alleyways and field margins. These features provide space for the utilisation of the non-crop vegetation to implement strategies to improve biodiversity. Furthermore, the perennial character allows for a certain stability and resilience over time, and to build-up arthropods communities.

An incentive for the adoption and the utilization of flower strips as an alternative for the preservation of biodiversity in Europe comes also fromthe European Commission in the form of the agri-environmental schemes, adopted already since 1998 to convince farmers to reduce the environmental risks and the impact of modern agriculture. Nowadays, this attention is still present in the EU biodiversity strategy for 2030 within the framework of the Common Agricultural Policy (CAP) for 2023-2027. The second pillar is entirely dedicated to the ”restoration of Nature in Europe”, proposing actions such as the improvement of pollinator habitats and the reduction in the use of chemical pesticides (Directorate General for Environment, European Commission, 2021).

The interest in flower strips is not just emerging from the agricultural sector. In fact, another positive side effect derives from the enhancement of landscape attractiveness, particularly for tourism. The presence of flowers contributes to a visually pleasant panorama, which improves the overall appeal of the countryside for activities such as hiking, cycling, and various forms of slow tourism.

1.3.1 Flower strip composition

The composition of flower strips and the selection of plant species play a crucial role in determining the benefits derived from the implementation of this agronomic practice. Each plant species produces flowers with unique characteristics, establishing the attractiveness and suitability for various types of insects based on their morphology and behaviour. Attributes such as flower morphology and accessibility to nectaries are related to the range of insects capable of exploiting the resources provided by flowers. Therefore, species selection differs depending on whether the aim is to promote pollinators or natural enemies. According to Campbell et al. (2017), pollinators - and especially bees - are found mainly in proximity to concealed-nectar plants i.e, plants holding the nectar in deep corollae, while natural enemies are more abundant when plant species present open-nectar flowers *i.e.,* flowers presenting nectar in shallow or open structures and in external nectaries. The reasoning behind this behaviour is found in insect morphology, such as proboscis size, and behavioural adaptation of some insects to certain types of flowers. If the aim of implementing flower strips is the promotion of natural enemies, the selection of plant species should be directed towards plants possessing open nectaries, which are more suitable for the main family of predators. The plants selected to support pest control should not only be accessible, but should also improve the fitness of the natural enemies population. This implies the improvement of longevity and fecundity, to increase the size of the population and consequently their role as predators (Campbell et al., 2017). Thus, attractiveness, flower accessibility, and quality of the pollen and nectar are all important factors that determine the suitability of a plant as food source for the promotion of natural enemies (van Rijn and Wäckers, 2016; Wäckers, 2004). These types of plants are called insectary plants and are defined by Parolin et al. (2012) as 'flowering plant which attracts and possibly maintains, with its nectar and pollen resources, a population of natural enemies that contribute to the management of biological pests in crops'.

A mixture of species is always preferred to a single species as it improves plant biodiversity and provides diverse food source for insects. An aspect to consider when using a mixture of seeds is the absence in the field of some of the species in the immediate year following sowing, owing to several factors such as competition with weed seeds or unfavourable site conditions for germination (Uyttenbroeck et al., 2015). This can lead to the development of a vegetation that does not present all the desired traits that were expected when designing the seed mixture. Consequently, the implementation of flower strips may not include all the necessary plants required to achieve the objectives, e.g., promotion of pollinators or natural enemies. The efficacy of implementing flower strips is substantially influenced by this factor, as their composition undergoes changes over time, reaching its full potential following the initial year (Jacobsen et al., 2022).

Flower strips remain a practice with significant potential for refinement, allowing customisation according to the specific needs of the farmer. This is true both in terms of species utilised and spatial location of flowers in the field. Flower strips can be implemented in both field margins and orchard alleyways or crop interrow (Cahenzli et al., 2019; Rodríguez-Gasol et al., 2019). The study from Brennan (2013) shows an example of how flower strips of sweet alyssum, *Lobularia maritima* (Desv.), arrangement can be optimized in a lettuce field to have an efficient intercropping, which maximizes flower production by alyssum and pest control, without reducing lettuce yield, and minimizing in the meanwhile the ground covering by the non-cropping plants. Agronomic aspects of flower strip implementation are still poorly studied, although more knowledge is required to have the most efficient land utilisation.

Plant selection should also consider the potential role that a plant can play as a weed. Additionally, phenology and blooming time are important factors, particularly when considering the potential overlapping period with the crop in the case of promotion of pollinators. Ideally, the best flower strip is the one that provides flowers for a longer period, from early to late in the season (Fiedler et al., 2007).

1.3.2 Functional biodiversity

Among the objectives of planting flower strips is the promotion of the so-called functional biodiversity, intended as the sum of the multitrophic interactions between plants, prey, and predator/parasitoid aiming to control pest population below the economic injury level (Burgio et al., 2015). The tool to achieve this objective is an accurate landscape management, through the selection of an appropriate plant community and the creation of a favourable habitat for predators, with the aim of improving natural enemies performance, their fitness, enhancing their ability to find the pest, providing alternative preys as well as creating shelter against unfavourable climatic conditions or place to overwinter (Burgio et al., 2015).

The presence of flowers can be beneficial for many natural enemies and their impact on pest populations is reported in several studies. For instance, in a study by Tschumi et al. (2015) the population of the cereal leaf beetle, *Oulema* sp., was found to be 53% lower in fields adjacent to flower strips compared to the control, leading to a corresponding 61% reduction in pest damage. This outcome, coupled with the observed increased population of predatory bugs and lacewings, suggests that the presence of predators may keep the pest population below the economic injury level. The study by Cahenzli et al. (2019) shows comparable findings regarding the abundance of natural enemies, but with less pronounced outcomes considering pest management. In the study by Markó et al. (2013), instead, no evidence was found in the improvement of aphid population management with the presence of flowering plants compared to a conventional grass cover. In fact, there is a notable variability observed among different locations and across different years. This variability may be attributed to environmental conditions, which play a crucial role in determining the effectiveness of pest management strategies. The presence of predators is most beneficial in spring when pest populations are increasing (Haaland et al., 2011). Nevertheless, colder springs with low temperatures slow down natural enemies development and thus impact on early control of pests (Jacobsen et al., 2022). Hence, timing and environmental conditions are important factors when applying biological control. Additionally, consideration must be given to the application of insecticides, as this practice can have detrimental effects on the population of natural enemies (Cahenzli et al., 2019).

Chapter 2

Aim of the thesis

The impact of *E. lanigerum* as a pest for apple production and the notable scarcity of effective control methods are the primary motivations for the initiation of this study and the associated field experiment conducted. Nowadays, a more sustainable approach to pest control is demanded by many stakeholders in the agricultural sector, and biological control offers a viable alternative to the widespread use of synthetic insecticides.

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. As deeply discussed in the introduction, syrphids are known to be efficient aphid predators and their role in the control of *E. lanigerum* has been poorly investigated in the literature. 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 for the production of 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 using these insects. Furthermore, no articles related to an open field experiment on this topic have been found.

Hence, the aim of this study is to conduct an open field experiment to acquire additional knowledge regarding the implementation of biological control against WAA using hoverflies combined with sown flower strips in an organic apple orchard. The objectives of the study are the evaluation of the flower mixture attractiveness for syrphid adults compared to conventionally managed interrows, the investigation of the impact of this biological control strategy on WAA colonies, and an evaluation of the influence of flower strips on natural enemies abundance on the apple trees.

Chapter 3

Materials and methods

3.1 Experimental field

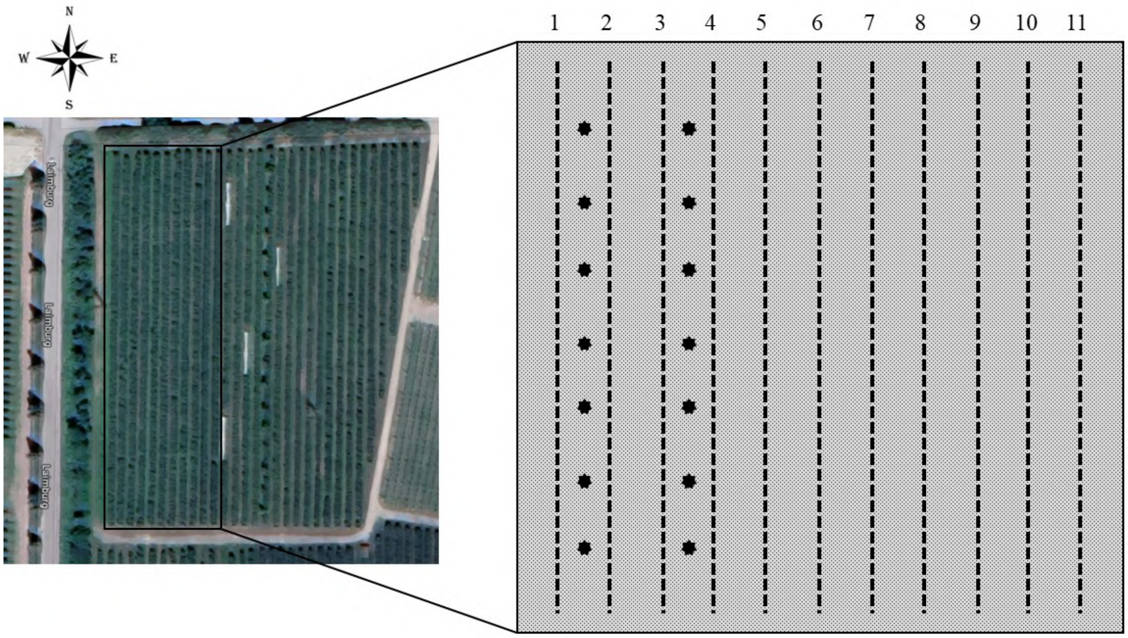


Figure 3.1: Representation of the experimental field, consisting of an orchard with 11 rows (identified by the solid lines and an identification number) of ’Fuji’ apple grafted onto M9 and trained to a central leader located at Laimburg Research Centre, in Vadena (South Tyrol, Italy). Two flower strips were sown in autumn 2022 in the western part of the field, and are identified by the stars. The interrows without the flowers are conventionally managed, performing repeated mulching throughout the season.

The experimental field consists of 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 is located at Laimburg Research Centre in Vadena, Italy (46°22’49.2”N, 11°17’19.4”E). Due to prior year’s severe WAA attack and the resulting high pest population density, the field was a good candidate for the experiment. Except for a single application in April of Neem extract (*Azadirachta indica*) in half of the field, due to a miscommunication between the employees, no pesticide treatments were carried out during the trial. The impact of this treatment on the experiment can be considered marginal, as it occurred prior to the migration of WAA nymphs in the canopy and, thus, only a limited number of overwintering colonies already present on the trunk might have been affected. Fungicide applications and other field activities were performed in accordance with the traditional management approach applied in South Tyrol’s organic apple farming. Overhead irrigation was applied in spring, at the beginning of April, at four different times as a preventive measure against frost damage.

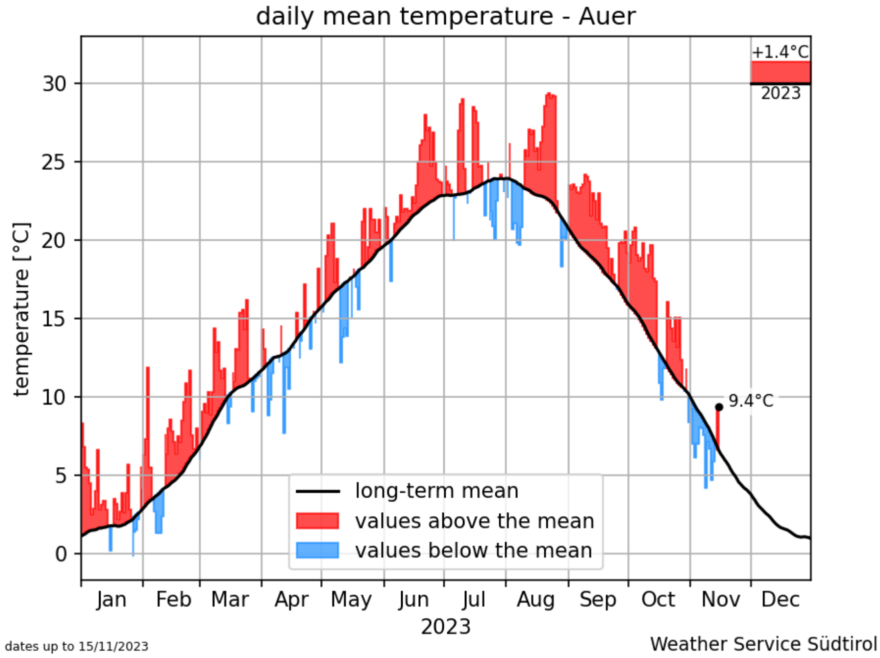
The field is organised with two sown flower strips on the western side, with a conventionally managed interrow in between, as shown in figure 3.1. The management of the grass cover has been carried out in all other interrows of the field, including the ones utilised as a control, in accordance with the protocol followed at the research centre, which entails repeated mulching throughout the season.

3.2 Meteorological data

Meteorological data were accessible for the whole duration of the experiment, obtained from the meteorological station of the Autonomous Province of Bozen/Bolzano, situated in Ora near the Laimburg Research Centre. Charts representing the daily mean temperature and cumulative daily precipitation, provided by the station, are included below (see figure 3.2 and 3.3).

3.3 Flower strips

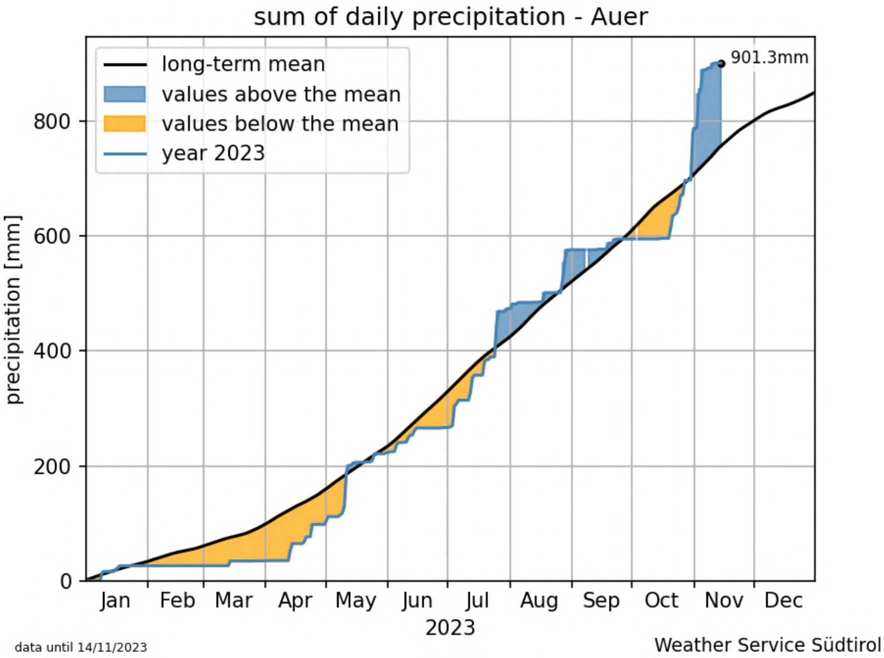
Flower seed mixture was supplied by the Italian company Gea Smart srl, and it is produced by the French company Nova Flore, comprising 15 species from 5 botanical families (commercial name



dates up to 15/11/2023

Weather Service Südtirol

Figure 3.2: Trend of daily mean temperature in Auer/Ora in 2023. Data collected by the meteorological station near Laimburg Research Centre (chart provided by the Autonomous Province of Bozen/Bolzano).



data until 14/11/2023

Weather Service Südtirol

Figure 3.3: Trend of the sum of daily precipitation in Auer/Ora in 2023. Data collected by meteorological station, near Laimburg Research Centre (chart provided by the Autonomous Province of Bozen/Bolzano).

of the mix is ’Inter-rang biodiviersitè, flowers mix’). The complete list of the species was not provided by the producing company. Only 7 of the 15 species are known and mentioned on the label and website. The known species in the mixture are listed below:

- Yarrow, *Achillea millefolium* (L.)
- Golden marguerite, *Anthemis tinctoria* (L.) J.Gay
- Marigold, *Calendula arvensis* (Vaill.) L.
- Caraway, *Carum carvi* (L.)
- Purple viper’s-bugloss, *Echium plantagineum* (L.)
- Wild candytuft, *Iberis amara* (L.)
- Sweet alyssum, *Lobularia maritima* (Desv.)

For the unknown species of the mixture, identification of seeds, leaves, and flowers was performed. Two guidebooks were utilised for the identification (Cappers and Bekker, 2013; Hanf, 1990). The complete list of known and identified species is not reported to respect Nova Flore’s intention of not sharing the results of their work. Despite that, the majority of the species in the mix present flowers with open corollas and accessible nectaries, which are favoured by hoverflies (Branquart et al., 2000).

Table 3.1: Technical details of flower strips sowing for the field experiment at Laimburg Research Centre in Autumn 2022. Two interrows were sown. The seed dose used of 4 g/m^2 is the one proposed by the French company ’Nova Flore’, producing the seed mixture.

Seed Dose (g/m^2)	**Width (m)**	**Length (m)**	**N. of interrows**
4	0.5	90	2

Sowing was performed at the end of October 2022. A rotary tiller working at a depth of 15 cm was used to prepare the seedbed, performing the operation twice, a week apart. The planting method was by hand, with the addition of sand to the seed mixture to increase uniformity and facilitate the planting procedure. The seed dose used for the experiment was 4 g/m^2, according to the protocol provided by the producing company. Two different interrows, separated by a row in between (figure 3.1), were sown for their entire length with a width of 0,5 m (table 3.1 summarises the technical details). Following the sowing, a roller was used and no irrigation was applied in autumn. As already stated while introducing the experimental field, overhead irrigation was applied in April as preventive measure against frost damage. Weed removal was performed twice by hand (11th and 14th April 2023) to reduce competition for newborn seedlings.

3.4 Hoverflies release

Two different species of hoverflies were released during the season: *Sphaerophoria rueppellii* (Wiedemann) and *Episyrphus balteatus* (De Geer). The hoverflies used in this study were reared and supplied by the Spanish company Biocom Sirfidos, in the forms of pupae and larvae. The timing of their release was determined based on two main factors: the results of the monitoring of WAA crawlers migration on tree trunks, and the growth status of flower strips. The latter factor is of particular importance since flowers provide pollen and nectar that are necessary to sustain females to produce mature ovaries, and thus for the production of viable eggs (Omkar and Mishra, 2016; Schneider, 1948; Sommaggio, 1999). A total of 4 release dates for pupae (17th and 31st May 2023, 14th and 28th June 2023) and 3 release dates for larvae (17th and 31st May 2023, 14th June 2023) were performed.

3.4.1 Mode of application

Both pupae and larvae were released in the apple orchard rows situated near the flower strips, as shown in the scheme of the field (figure 3.7). The mode of application differed between pupae and larvae. The larvae were supplied by ’Biocom sirfidos’ in plastic bottles, each bottle containing 500 individuals. The commercial names for the products containing *Sphaerophoria rueppellii* and *Episyrphus balteatus* larvae are ’Ruecombat’ and ’Baltecombat’ respectively. The pupae were provided by ’Biocom sirfidos’ in paper boxes, each containing 125 individuals in a mix of both species, with the commercial name ’Sirficombat’ (figure 3.4 and 3.5). For every release date, a total of 1000 larvae and 250 pupae were distributed across the field.

Syrphid larvae are highly mobile and have the ability to independently access aphid colonies. To

Figure 3.4: An example of biocontrol agents formulation supplied by the company ’Biocom Sirfidos’ (Alicante, Spain). The picture shows the pupal mix (a) with the commercial name ’Sirficombat’, the larvae of *Episyrphus balteatus* (Diptera: Syrphidae) (b) with the commercial name ’Baltecombat’, and the larvae of *Sphaerophoria rueppellii* (Diptera: Syrphidae) (c) with the commercial name ’Ruecombat’ (credit Francesco Panzeri 2023).

Figure 3.5: Device used to release syrphid larvae in the field, after the placement on an apple branch or shoot, supplied by the producing company ’Biocom sirfidos’ (Alicante, Spain). The paper hook facilitates the larvae to reach the infested branches and shoots, where the woolly aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colonies are present (credit Francesco Panzeri 2023).

facilitate this process, a paper box with a hook was hung on tree branches that already presented active WAA colonies. Given that the larvae have limited stored resources, it is crucial for them to start feeding promptly during their initial stage after release. Consequently, the positioning of the boxes in proximity of the prey is an important step to consider. The presence of the hook facilitates the larvae's ability to climb up from the box, reach the infested branches and shoots, and initiate feeding within the aphid colonies.

The distribution of pupae requires less time and labour, as it is applied simply by releasing the contents of the boxes inside a polystyrene box hung to a tree in the row between flower strips. After emergence, hoverflies can fly out of the box, feed on flowers, and complete their biological cycle. Table 3.2 summarises the number of pupae and larvae delivered for each bottle/box and the number of syrphids for each release date.

Table 3.2: Summary of the number of syrphid (Diptera: Syrphidae) pupae and larvae released during the field experiment at Laimburg Research Centre. Syrphids were supplied in the forms of pupae and larvae by the company 'Biocom sirfidos' (Alicante, Spain) using boxes for pupae and bottles for larvae. Quantity of pupae and larvae released in the field was defined according to the protocol proposed by the producing company. The numbers in the table refer to the contents of the bottles and boxes for the first two columns, and for a single date of release for the last two columns.

Syrphid species	**N. pupae/box**	**N. larvae/bottle**	**N. pupae/field**	**N. larvae/field**
Episyrphus balteatus (De Geer)	125	500	250	1000
Sphaerophoria rueppellii (Wiedemann)	125	500	250	1000

3.5 Sampling methodology

3.5.1 Crawlers migration on the tree

WAA is known to overwinter primarily in the root apparatus, although overwintering colonies are also occasionally observed on the upper part of the plant. At the beginning of the new season, in spring, WAA nymphs - commonly referred to as crawlers - start their migration from the roots to the canopy to colonise branches and new shoots and complete their biological cycle. According to the methodology used by Beers et al. (2010), WAA nymph migration on the tree was identified by positioning sticky bands, made of a double-face sticky tape, which completely encircle the trunk of 20 apple trees, selected from two different rows (see fig. 3.6). Bands were set out starting from 21st April 2023 and replaced every week until 22nd June 2023. The evaluation was done by rating each individual band with a value from 0 to 9, according to the percentage of nymphs covering its surface. Since different insects of small dimensions were present, WAA nymphs were distinguished from other insects using a magnifying lens.

Figure 3.6: Adhesive band, made of a double-sided sticky tape, on apple tree trunk to monitor woolly apple aphid nymphs, *Eriosoma lanigerum* (Hempitera: Aphididae), migration from the bottom to the top of the canopy (credit Francesco Panzeri, 2023).

3.5.2 Syrphid adults visual observation

Syrphid adults' presence in the proximity of flower strips is a good indicator of flower attractiveness for these insects. As proposed in the guidebook on syrphid flies written by Burgio et al. (2015), syrphid adults' presence was measured by applying the *belt method*. By walking in line at a steady pace from one end to the other of the interrow, all adult hoverflies encountered flying or lying on flowers up to 1 m in front and to either side of the observer were counted thanks to a tally counter. No distinction between syrphid species was made, as it was not possible to accurately determine the species while they were in flight. According to the author of the guidebook, the length of the walked line should be of 200 m and with a velocity of 10 m per minute. However, due to constraints imposed by the field dimensions, the length of the line walked was of 100 m. Nevertheless, two repetitions were performed for each interrow. The two interrows with sown flower strips served as treatment, while the other two interrows 20 m apart were used as control.

3.5.3 Evaluations after syrphid release

3.5.3.1 Woolly apple aphid colonies evaluation for predation after release of syrphid larvae

The approach used to assess the status of WAA colonies was similar to the one proposed by the European and Mediterranean Plant Protection Organization (EPPO), for the assessment of pesticide efficacy. The measurements were made by counting and estimating the size of the active colonies visible on the branches. WAA colonies are enveloped by "wool", and contain aphids of all stages, precluding accurate determination of the number of aphids by non-destructive methodologies. To address this limitation, colony size was preferred as a parameter to estimate attack severity while preserving the integrity of the colonies. The size was estimated by assigning to each colony a value corresponding to different severity classes, with a value ranging from 0 (absence of colonies) to 5 (indicative of large colonies with high infestation). A total of 50 infested branches per row, divided evenly between both sides of the rows, were examined to determine the effectiveness of the predation by the larvae, both for treatment area (in proximity of flower strips) and control area (in proximity of conventionally managed interrow), represented in figure 3.7. On each sampling day, evaluations were conducted always on designated branches, previously marked with plastic tape and labelled with a progressive number. The assessment of WAA colonies began in the middle of

May and was extended until the end of June. Initially, the colonies were present on the branches and mainly on the pruning cuts and cracks of the tree trunk. Consequently, the branches selected for the evaluation were those that already had colonies.

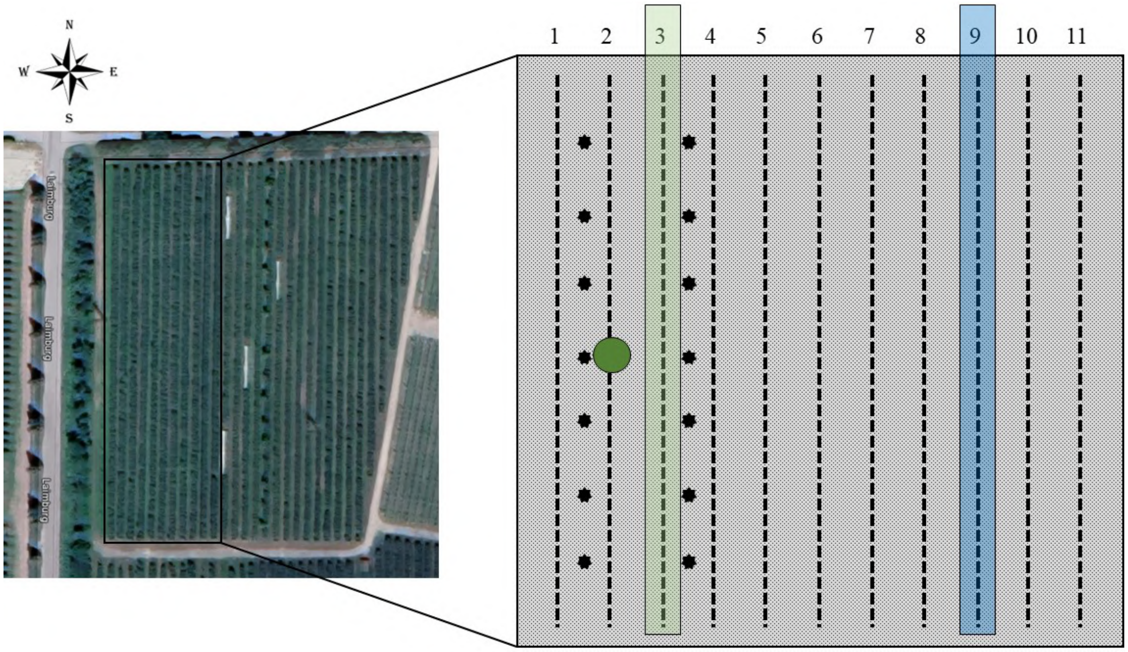


Figure 3.7: The field scheme illustrates the release points for the two species of syrphids (*Sphaerophoria rueppellii* (Diptera: Syrphidae) and *Episyrphus balteatus* (Diptera: Syrphidae) and the apple rows utilised for the evaluation. The green circle indicates the location where the box with pupae was hung on a tree (apple row n. 2) for the release. The green rectangle (apple row n. 3) represents both the row chosen for syrphid larval release on 50 apple branches and the row where 200 one-year-old branches were selected for evaluating woolly apple aphid colonies following the pupal release. The blue rectangle (apple row n. 9) indicates the row used for the control. Consequently, the green rectangle identifies the treatment area named 'flower', while the blue rectangle denotes the control area.

As the season progressed, it became evident that this methodology was not detecting subtle changes and was not accurate enough to be applied on a week-by-week basis. In fact, WAA colonies tend to merge as they expand, and it became difficult to distinguish the number of colonies and assess the severity of the attack, which was estimated using five classes. Therefore, a new approach was introduced starting from the middle of June. Both methodologies were then used together, to ensure the possibility of using the data collected with the former approach in the first part of the season. However, the data from the new methodology were not used for the evaluation of WAA colonies after the release of syrphid larvae, due to the lack of data from the first part of the season, but just for the evaluation after the release of syrphid pupae

The newly adopted methodology was suggested by Ewald Lardschneider, a colleague in the Organic Farming Group at Laimburg Research Centre. The new approach maintained the concept of estimating attack severity based on colony size, but introduced a new technique. In fact, each colony size was determined not by classifying it in predefined categories from 0 to 5, but by comparing the colonies to rectangles with known surface areas, which were printed on a paper. Figure 3.8 provides the visual representation of the paper with the surfaces used in the field for the evaluation. Each rectangle is identified with a numerical value placed above it, while within the brackets below the respective rectangle is reported the known surface in cm^2. Surfaces were represented using rectangles rather than squares, as the distribution of aphid colonies followed the branches, resulting in a more linear expansion than in other dimensions. The implementation of this new methodology was an attempt to reduce the subjective part of the evaluation, with the purpose of making it more objective and more independent from the operator taking the measurement.

3.5.3.2 Woolly apple aphid colonies evaluation for predation after release of syrphid pupae

The assessment of WAA colonies, conducted to evaluate predation following the release of pupae, was carried out using the newly introduced methodology described in the previous paragraph. This evaluation was intentionally scheduled later compared to the larval evaluation. This decision was based on the time required for the complete life cycle of syrphids, including: the emergence of adults from pupae, their feeding on flowers, mating, egg-laying, and subsequently, the emergence of larvae and the start of predation. This entire life cycle, influenced by environmental conditions, typically has an approximately 14-day timespan. Thus, the limitations associated with the initial methodology for the evaluation were already being addressed, making the new methodology a more suitable approach to adopt.

In this case, colonies located on 1-year-old branches and on shoots were evaluated. A total of 200 infested branches and shoots per row, divided evenly between both sides of the rows, were considered for both the treatment area (in proximity of flower strips) and the control area (in proximity of conventionally managed interrow), represented in figure 3.7. Evaluations were performed on three dates, from the beginning to the end of June. The evaluation sheet in figure 3.8 is the same used for this evaluation as well.

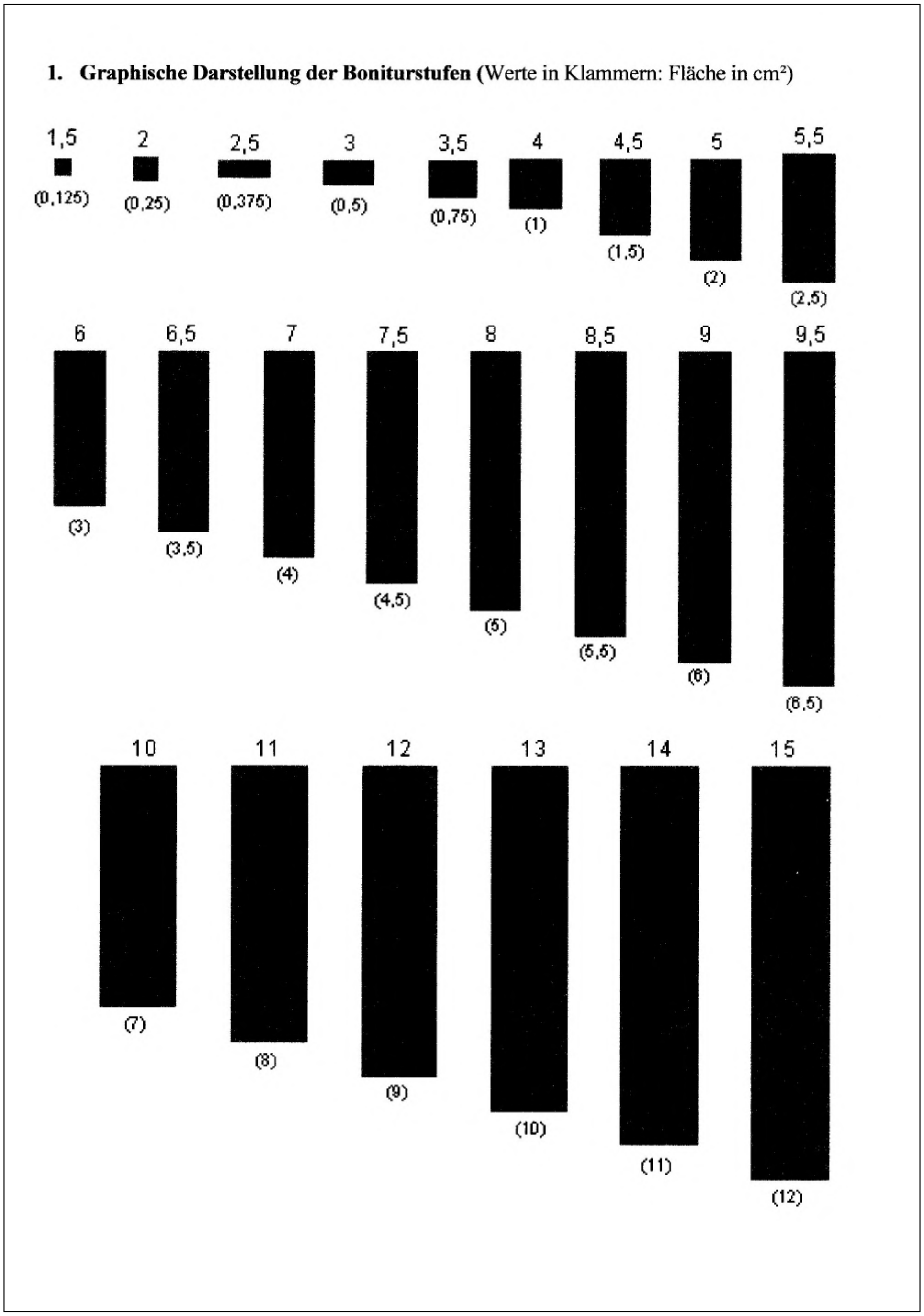


Figure 3.8: Graphical representation with rectangles of known surfaces used for the estimation of woolly apple aphid, *Eriosoma lanigerum* (Hempitera: Aphididae), colonies attack severity using the size as the parameter for the evaluation. Each colony on apple tree branches and shoots was compared to the rectangles, whose surface area is known, to determine their size. Rectangles rather than squares were used since the distribution of the colonies over time follows the length of the branches and shoots. The numbers above the rectangles are just identifiers for the known surfaces (credit Ewald Lardschneider 2023).

3.5.4 Beneficial insects presence close to woolly apple aphid colonies

For the same shoots and branches sampled for WAA colonies number and severity, hoverflies and ladybirds larvae on or in the proximity of each colony were counted. Additionally, the presence/absence of the parasitoid *Aphelinus mali* was noted. The identification of *A. mali* was established by detecting the presence of active adults in the colonies, using a magnifying glass, and by observing the presence of mummies within the colonies. Assessment of ladybirds was conducted for both adults and larvae, taking into account their predatory behaviours in both life stages. The presence of hoverflies was noted not only as larvae, but also as pupae and excrement. Indeed, it is unlikely to see active syrphid larvae in the colony given their nocturnal predatory behaviour (Burgio et al., 2015). Thus, as proposed by the researchers at Biocom Sirfidos, meconium and excrement may be stronger indicators of hoverfly larval activity and presence, leading to the choice of include them as a parameter for the assessment of hoverflies presence in WAA colonies. Syrphid larvae excrement appear as a black lucid smear, visible on the apple leaves (figure 3.9).

Figure 3.9: Syrphid (Diptera: Syrphidae) larvae excrement, recognisable as a black lucid smear, are a good indicator of hoverfly larvae predatory activity (credit Francesco Panzeri 2023).

To summarise and conclude, five categories were recorded to evaluate natural enemies presence and are reported hereafter:

- *Aphelinus mali*, a WAA specialised parasitoid

- Ladybird adults
- Ladybird larvae
- Syrphid larvae
- Syrphid larvae excrement

3.6 Statistical analysis

The data were analysed using the software R (R Core Team, 2022). Henceforth, when using the term ’treatment,’ it is intended to refer to : the treatment named ’control,’ intended as the conventionally managed interrows, and the treatment named ’flower,’ denoting flower strips. The location of the ’control’ and ’flower’ treatments in the field is illustrated in the scheme in figure 3.7. None of the datasets from data collection are normally distributed. Thus, the analysis were conducted as follow:

- The effect of flowers in influencing syrphids adults abundance, detected by a in-field visual observation, was analysed with a Generalized Linear Mixed Effect Model (GLMM) from the lme4 package (Bates et al., 2015). The number of syrphid adults observed was considered as response variable, while the treatments and the date were addressed as explanatory variables. The two different interrows per treatment used as replicates for the evaluation were accounted as random variable to control for the variability.
- The effect of flowers in influencing the abundance of natural enemies was analysed with a Generalized Linear Model (GLM) for each single natural enemy class (ladybird adult, ladybird larva, syrphid larva, syrphid excrement, *A. mali*) and a Generalized Linear Mixed Effect Model for negative binomial family (Negative Binomial GLMM), from the MASS package (Venables and Ripley, 2002), for the sum of natural enemies. The count for each natural enemy category recorded was considered as the response variable, while the treatment and the date were addressed as explanatory variables. Same approach was used for the sum of natural enemies, although accounting the interrows used as replicates for the evaluation as random variable for the variability in the Negative Binomial GLMM.

- The effect of flowers in influencing WAA number of colonies and attack severity after the release of both syrphid larvae and pupae was analysed with a GLMM (Bates et al., 2015) and Negative Binomial GLMM (Venables and Ripley, 2002) respectively. The number of WAA colonies and the severity of the attack was considered as the response variable, while the interaction treatment-date was addressed as explanatory variable. The different apple trees sampled within the apple row were accounted as random variable for the variability.

The package ggplot2 (Wickham, 2016) was used for produce the charts to graphically display the results. All the values are reported as average ± standard deviation. Significance level is set at 0.05.

Chapter 4

Results

4.1 Flower development

Flowers have been particularly abundant in both the strips compared to the previous experiments conducted at Laimburg Research Centre. Following the sowing in autumn 2022, the first seedlings were visible after germination in the second half of March. The first flowers were present since the beginning of May, with a significant blooming in both the strips at the end of May. The first flowers to be present in the field were those of sweet alyssum (*L. maritima*) and marigold (*C. arvensis*), although only few flowering plants per strip were present. Flowering of cornflower (*C. cyanus*) started since 15th May, followed by chamomile (*M. chamomilla*), wild candytuft (*I. amara*), coriander (*C. sativum*), and golden marguerite (*A. tinctoria*).

Hence, not all the species present in the mixture were present in the field. Only 7 out of the 15 species produced flowers, while other species, such as yarrow (*A. millefolium*), were present in other phenological stages, but not flowering. Figure 4.1 shows flowers development throughout the season. From the image, it is evident that a conspicuous number of flowers were present at the end of May, despite the fact that chamomile flowers were the more abundant. The climax of blooming occurred in the second half of June, with the higher diversity of species flowering.

Figure 4.1: Development of one of the flower strips throughout the season in the experimental field at Laimburg Research Centre (South Tyrol, Italy). A conspicuous number of flowers were present since the end of May. The peak of blooming occurred in the second half of June. Only 7 out of the 15 species in the mixture used produced flowers during the first year (credit Francesco Panzeri 2023).

4.2 Crawlers migration from roots to canopy

The chart in figure 4.2 presents the results obtained from monitoring crawlers migration. A slight increase in crawlers migration was observed during the first half of May, with a more pronounced increase noted in the latter part of the month. The relatively lower temperatures during the first half of May, in comparison to the regional average (see fig. 3.2), may have affected the migration, with a potential slowdown, and have contributed to the observed trend. The migration peak occurred around the beginning of June (sampling date: June 8th), with a sharp decline in migration during the following weeks, eventually reaching values close to zero.

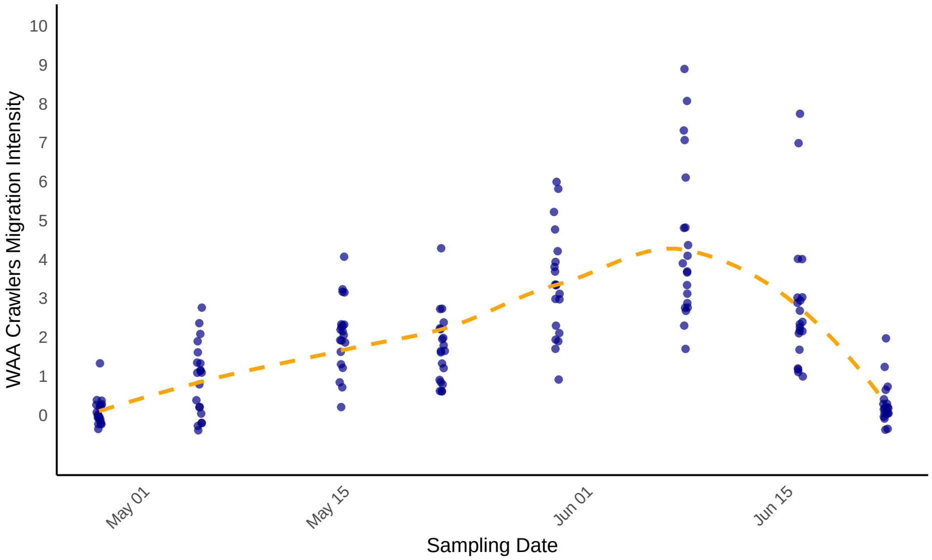


Figure 4.2: Results from the monitoring of woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), nymphs - commonly called crawlers - migration from the root apparatus to the canopy of apple trees. Crawlers migration revealed a typical pattern characterised by an increase, followed by a peak, and then a subsequent decline. The monitoring of crawlers migration was used to define when to release syrphid (Diptera: Syrphidae) pupae as part of the biological control strategy against *E. lanigerum*. Each dot represents the evaluation of the adhesive band for a single tree, the line represents the average.

4.3 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

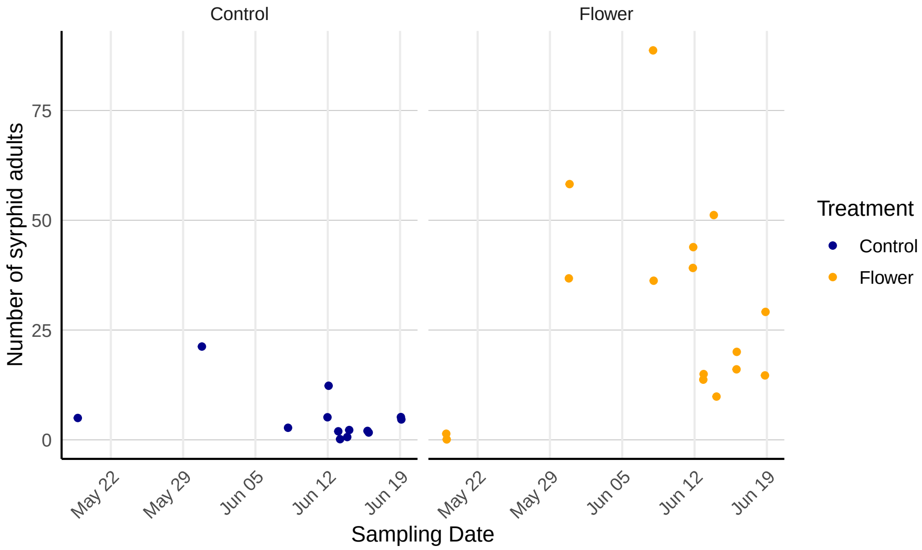


Figure 4.3: Hoverfly (Diptera: Syrphidae) adults abundance detected by visual observation in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of syrphid adults observed in the field ($p < 0.001$). Each dot represents a single evaluation.

treatment 'flower' is linked to an increase in the expected count of hoverflies. The average number of syrphid adults in the flower strips was 29.6 ± 20.0 syrphid adults/interrow, value higher compared to the average of the conventionally managed interrow 5.9 ± 6.6 syrphid adults/interrow, both displaying however a high variability within the treatment. More hoverflies adults were observed in the middle of June, coinciding with the peak of blooming of the flower strips (see fig. 4.3).

Table 4.1: Summary of Poisson Generalized Linear Mixed Model (GLMM) for the evaluation of syrphid (Diptera: Syrphidae) adults visual observations.

Variable	Estimate	Std. Error	P-value
Intercept	-108.9	93.07	0.242
Flower	1.794	0.210	< 0.001
Date	0.006	0.005	0.235

4.4 Beneficial insect presence close to woolly apple aphid colonies

Beneficial insects were categorised according to five categories: *Aphelinus mali* (the WAA specialised parasitoid), ladybird adults, ladybird larvae, syrphid larvae, and syrphid larvae excrement. Results for these categories are reported following this order.

Table 4.2: Summary of Binomial Generalized Linear Model (GLM) for the evaluation of *Aphelinus mali* presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Fixed effects	**Estimate**	**Std. Error**	**P-value**
Intercept	-19.33	1070	0.986
'Flower'	-0.5113	0.3229	0.113
2023-05-30	1.661×10^{-10}	1514	1.000
2023-06-06	16.62	1070	0.988
2023-06-14	19.22	1070	0.986
2023-06-20	21.61	1070	0.984

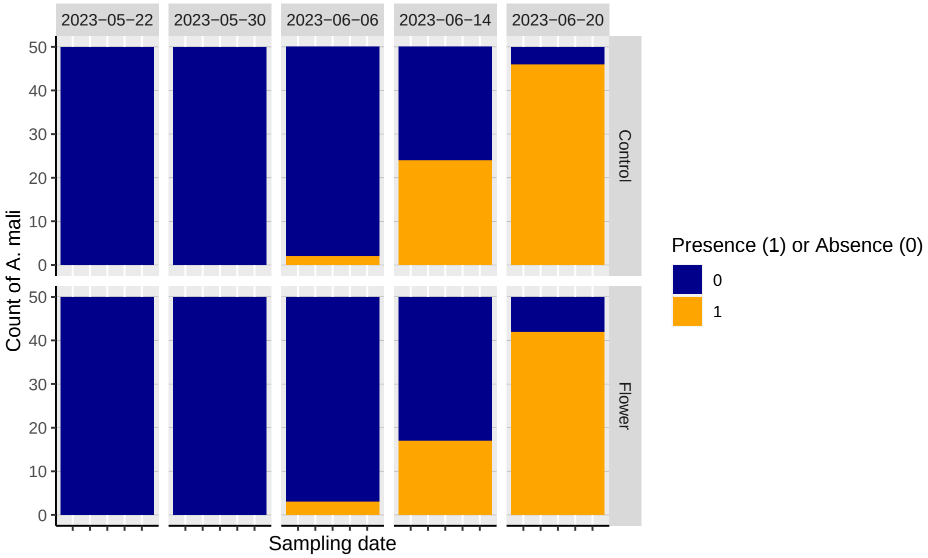


Figure 4.4: Comparison of the count of woolly aphid, *Eriosoma lanigerum* (Hempitera: Aphididae), colonies presenting *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). No significant differences are present between control and flower strips ($p = 0.113$).

The results of the logistic regression analysis to examine the relationship between *Aphelinus mali* presence and the predictor variables, treatment and date, showed that there are no significant differences between the count of *A. mali* in the control and the flower strips $(Estimate = -0.5113, p = 0.113)$. *A. mali* started to be present from the beginning of June and showed an increase in its recorded presence in both the control and the flower strips throughout the month, reaching levels of presence close to the totality of *Eriosoma lanigerum* (Hempitera: Aphididae) colonies presenting the parasitoid at the end of June (see fig. 4.4).

Table 4.3: Summary of Poisson Generalized Linear Model (GLM) for the evaluation of ladybird adult presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Variable	**Estimate**	**Std. Error**	**P-value**
Intercept	-22.46	2444	0.993
'Flower'	1.792	0.764	0.019
2023-05-30	5.684×10^{-10}	3457	1.000
2023-06-06	5.689×10^{-10}	3457	1.000
2023-06-14	19.00	2444	0.994
2023-06-20	17.70	2444	0.994

Table 4.4: Summary of Poisson Generalized Linear Model (GLM) for the evaluation of ladybird larva presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Variable	**Estimate**	**Std. Error**	**P-value**
Intercept	-5.858	1.0142	< 0.001
'Flower'	1.792	0.197	< 0.001
2023-05-30	3.932	1.010	< 0.001
2023-06-06	3.401	1.017	< 0.001
2023-06-14	4.533	1.005	< 0.001
2023-06-20	3.555	1.0142	< 0.001

Both ladybird (Coleoptera: Coccinellidae) adults and larvae resulted to be more present on the apple branches in proximity to the flower strips (figure 4.5 and 4.6). The analysis revealed a significant

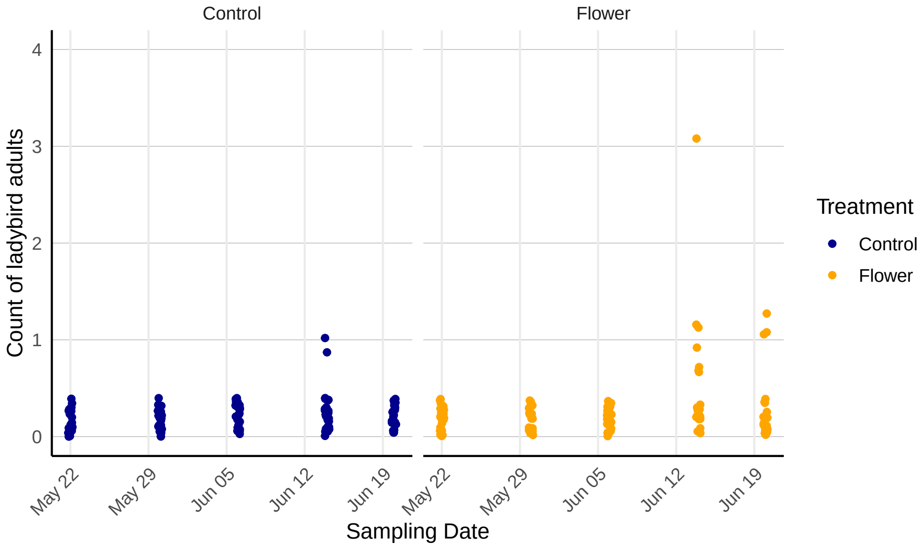


Figure 4.5: Comparison of the count of ladybird (Coleoptera:Coccinellidae) adults presence for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of ladybird adults observed ($p = 0.019$). Each dot represents the count for a single apple branch.

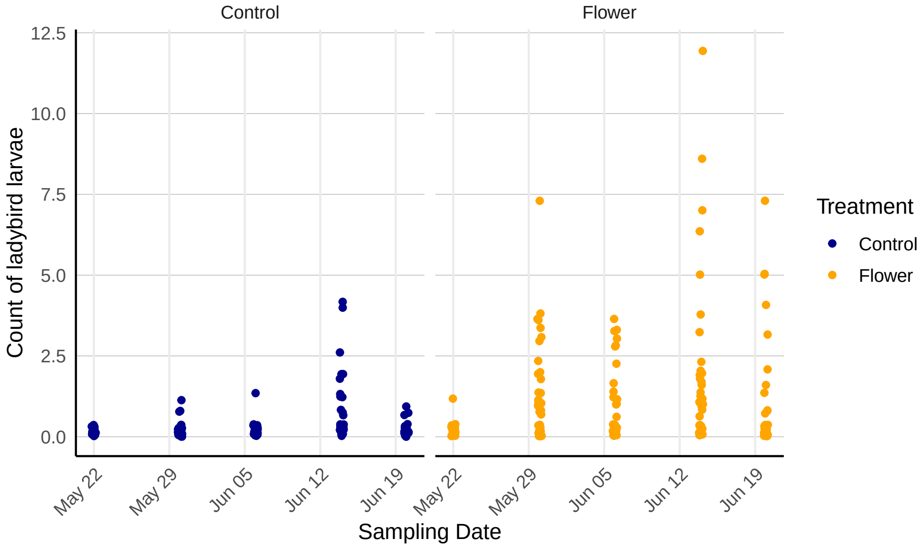


Figure 4.6: Comparison of the count of ladybird (Coleoptera: Coccinellidae) larvae presence for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of ladybird larvae observed ($p < 0.001$). Each dot represents the count for a single apple branch.

Figure 4.7: *Eriosoma lanigerum* (Hemiptera: Aphididae) colony with three ladybird (Coleoptera: Coccinellidae) larvae in its proximity, probably feeding. A number of ladybirds higher than expected were found close to flower strips, compared to the control (credit Francesco Panzeri 2023).

impact of the predictor variable on the response variable, for both ladybird adults $(Estimate = 1.792, p = 0.019)$ and especially for ladybird larvae $(Estimate = 1.792, p < 0.001)$. The observed response from ladybirds was unexpected, as insects from the Coccinellidae family are not typically considered among those benefiting significantly from the implementation of flower strips. Notably, in proximity to certain WAA colonies within the ’flower’ treatment, more than one ladybird larva was observed for each WAA colony (figure 4.7).

The implementation of the flower strips also positively influenced the presence of syrphid larvae, with more larvae recorded on branches in proximity to the flower strips $(Estimate = 2.944, p = 0.004)$ (see fig. 4.8 and fig. 4.11). Simultaneously, a higher feeding activity was observed in the 'flower' treatment compared to the control. In fact, flower strips had a significant impact on the number of syrphid larvae excrement recorded on the labelled apple branches, indicating a higher predatory activity $(Estimate = 2.773, p < 0.001)$ (see fig. 4.9).

Table 4.5: Summary of Poisson Generalized Linear Model (GLM) for the evaluation of syrphid larvae presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Variable	**Estimate**	**Std. Error**	**P-value**
Intercept	-4.269	1.011	< 0.001
'Flower'	2.944	1.026	0.004
2023-05-30	-2.639	1.035	0.0108
2023-06-06	-1.253	0.567	0.0271
2023-06-14	-18.138	1407	0.990
2023-06-20	-2.639	1.035	0.0108

Table 4.6: Summary of Poisson Generalized Linear Model (GLM) for the evaluation of syrphid excrement presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Variable	**Estimate**	**Std. Error**	**P-value**
Intercept	-4.347	0.571	< 0.001
'Flower'	2.773	0.515	< 0.001
2023-05-30	0.435	0.387	0.261
2023-06-06	-0.095	0.437	0.827
2023-06-14	0.087	0.417	0.835
2023-06-20	0.493	0.383	0.198

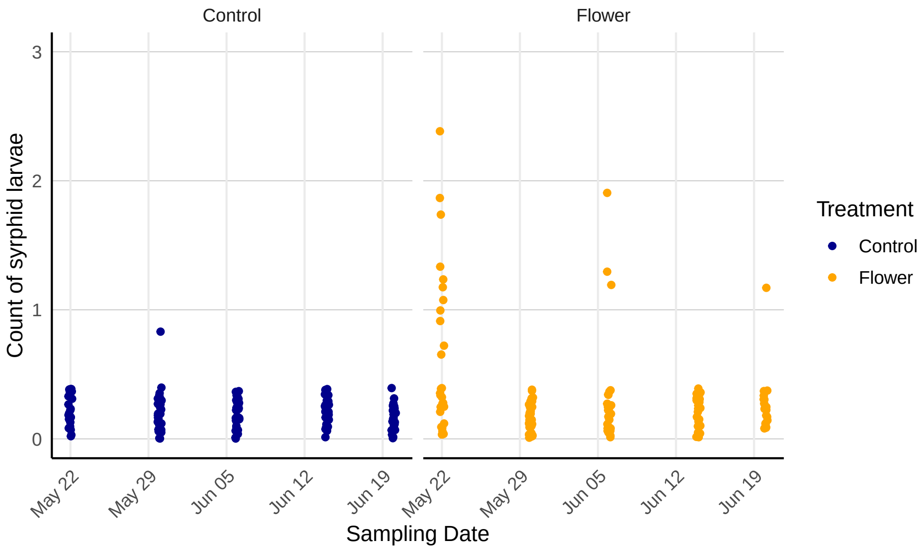


Figure 4.8: Comparison of the count of syrphid (Diptera: Syrphidae) larvae presence for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of syrphid larvae observed ($p < 0.001$). Each dot represents the count for a single apple branch.

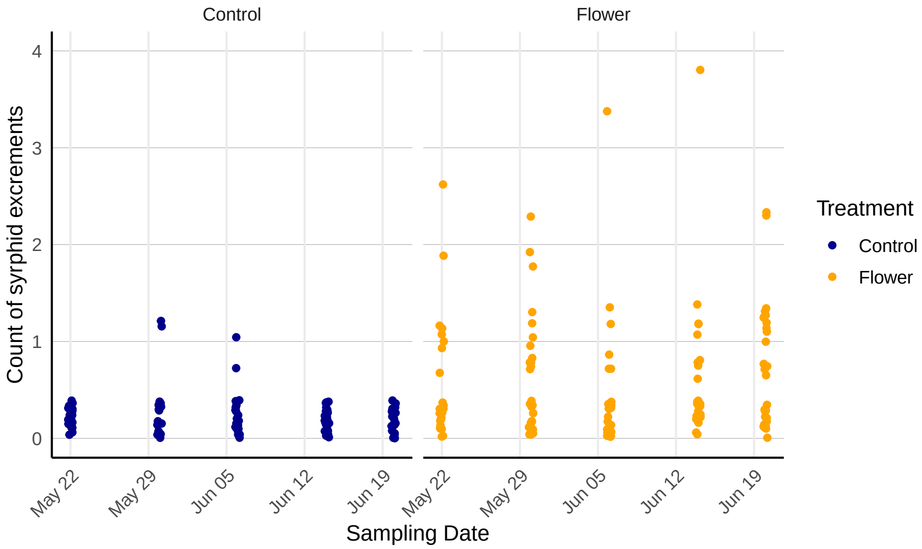


Figure 4.9: Comparison of the count of syrphid (Diptera: Syrphidae) larvae excrement presence for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of syrphid larvae excrement observed ($p < 0.001$). Each dot represents the count for a single apple branch.

The presence of the flower strips significantly influenced also the sum of all the categories of natural enemies ($Estimate = 1.151, p < 0.001$). Overall, natural enemies were more abundant in

Table 4.7: Summary of Negative Binomial Generalized Linear Mixed Model (GLMM) for the evaluation of the sum of all the categories of natural enemies presence in the two treatments: conventionally managed interrow ('control') and flower strips ('flower').

Fixed Effects	Estimate	Std. Error	P-value
Intercept	-2.202	0.243	< 0.001
'Flower'	1.151	0.161	< 0.001
2023-05-30	0.951	0.253	< 0.001
2023-06-06	0.636	0.265	0.016
2023-06-14	1.815	0.237	< 0.001
2023-06-20	1.793	0.238	< 0.001

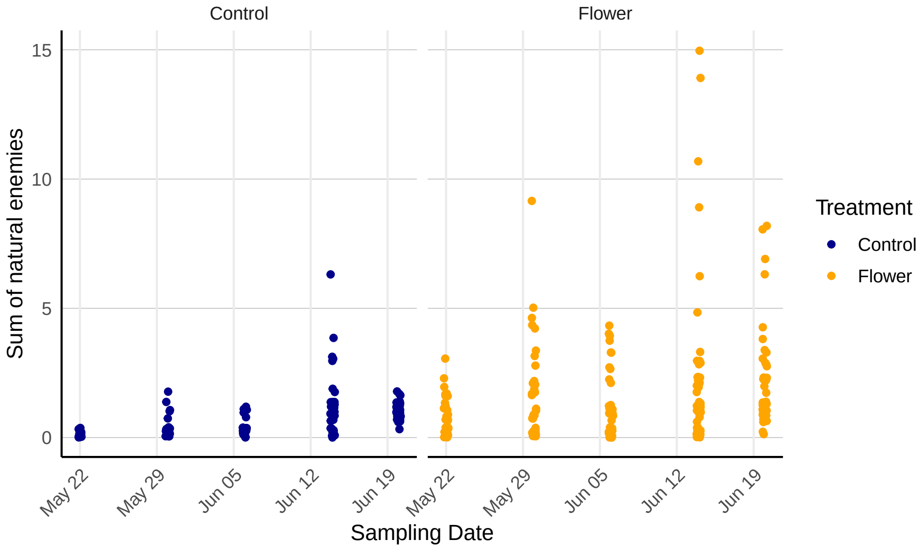


Figure 4.10: Comparison of the sum of all the natural enemies categories for each sampling date on 50 selected apple branches in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). The presence of the flowers influenced significantly the number of natural enemies observed ($p < 0.001$). Each dot represents the count for a single apple branch.

the apple rows close to flower strip compared to the control (see fig. 4.10).

Figure 4.11: Larva of *Episyrphus balteatus* (Diptera: Syrphidae) within a woolly apple aphid colony, *Eriosoma lanigerum* (Hemiptera: Aphididae), on one of the apple branches used for the evaluation during the experiment (credit Francesco Panzeri 2023).

4.5 Effect on woolly apple aphid colonies

4.5.1 Evaluation after syrphid larvae release

Contrary to expectations, the presence of flowers unexpectedly led to a higher incidence of WAA in proximity to the flower strips $(Estimate = 0.581, p = 0.011)$. Although the effect was small, the number of WAA colonies observed in the field was significantly influenced by the presence of flowers. Less colonies of woolly aphid were present in the control throughout the season, with the treatment 'flower' displaying a higher number of colonies per tree from the beginning to the end of the season (see fig. 4.12).

Table 4.8: Summary of Poisson Generalized Linear Mixed Model (GLMM) for the evaluation of the number of woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colonies on 50 selected apple branches after the release of syrphid (Diptera: Syrphidae) larvae.

Fixed Effects	**Estimate**	**Std. Error**	**P-value**
Intercept	1.025	0.163	< 0.001
’Flower’	0.581	0.229	0.011
2023-05-22	0.315	0.097	0.001
2023-05-30	0.508	0.094	< 0.001
2023-06-06	0.474	0.094	< 0.001
2023-06-16	-0.590	0.124	< 0.001
2023-06-20	-1.854	0.201	< 0.001
’Flower’x2023-05-22	-0.168	0.118	0.156
’Flower’x2023-05-30	-0.305	0.115	0.008
’Flower’x2023-06-06	-0.457	0.117	< 0.001
’Flower’x2023-06-16	0.325	0.145	0.025
’Flower’x2023-06-20	0.886	0.222	< 0.001

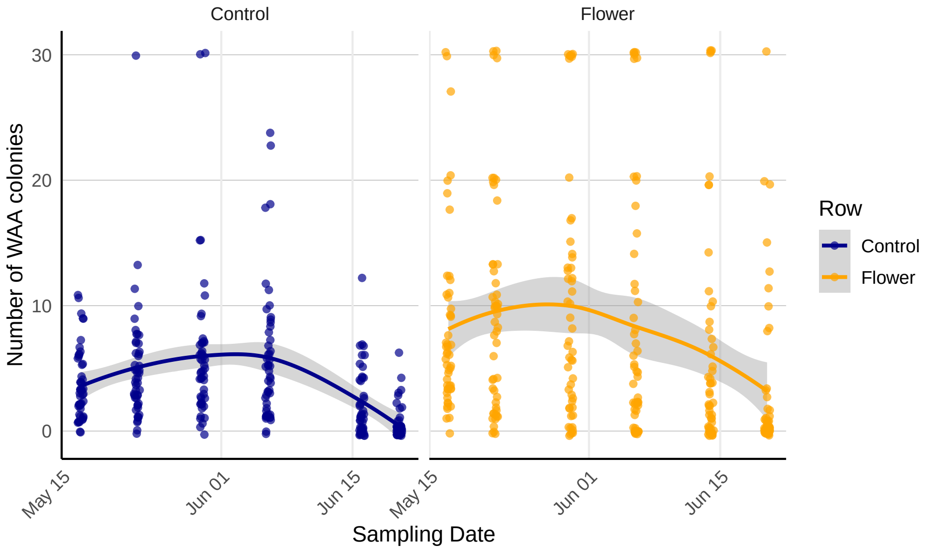


Figure 4.12: Comparison of the number of woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) colonies on 50 selected apple branches after the release of syrphid larvae in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). Flower strips had a small but significant effect on the number of *E. lanigerum* colonies observed in the field $(p = 0.011)$, with a higher number of colonies in the treatment 'flower' compared to the control. Each dot represents the evaluation done on each one of the 50 selected apple branches. The line represents the average.

Results for the evaluation of severity of the attack after the release of syrphid larvae show no significant effect on the presence of the flowers in influencing WAA attack severity $(Estimate = 0.216, p = 0.260)$ (see fig. 4.14). Despite that, predation of WAA colonies by hoverfly larvae was recorded in some of the evaluated branches, captured through both photographs and videos (see fig. 4.13).

Table 4.9: Summary of Negative Binomial Generalized Linear Mixed Model (GLMM) for the evaluation of the severity of the attack of woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colonies on 50 selected apple branches after the release of syrphid (Diptera: Syrphidae) larvae.

Fixed Effects	**Estimate**	**Std. Error**	**P-value**
Intercept	0.241	0.139	0.083
’Flower’	0.216	0.191	0.260
2023-05-22	0.343	0.150	0.022
2023-05-30	0.454	0.146	0.002
2023-06-06	0.574	0.144	< 0.001
2023-06-16	0.110	0.159	0.489
2023-06-20	-0.989	0.217	< 0.001
’Flower’x2023-05-22	-0.190	0.204	0.352
’Flower’x2023-05-30	-0.264	0.201	0.188
’Flower’x2023-06-06	-0.421	0.199	0.035
’Flower’x2023-06-16	-0.036	0.214	0.866
’Flower’x2023-06-20	0.262	0.278	0.346

Figure 4.13: Reduction over time of a woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colony after the release of syrphid (Diptera: Syrphidae) larvae. The box hung with a paper hook is the device used to release the larvae and facilitate them in reaching the colony on the apple branch. The black smear on one of the side of the box in the picture of 18th June is an excrement of syrphid larvae, indicating predatory activity (credit Francesco Panzeri 2023).

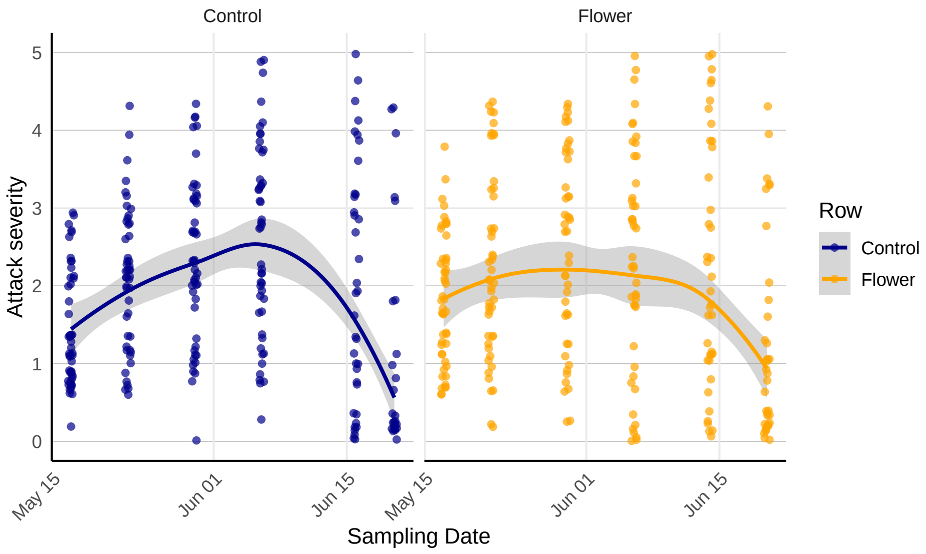


Figure 4.14: Comparison of the severity of woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) attack on 50 selected apple branches after the release of syrphid larvae in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). Flower strips had no significant effect on *E. lanigerum* attack severity $(p = 0.260)$. Each dot represents the evaluation done on each one of the 50 selected apple branches. The line represents the average.

4.5.2 Evaluation after syrphid pupae release

The presence of flowers had a small but significant effect on the number of WAA colonies observed in the field $(Estimate = 0.309, p = 0.011)$. However, the presence of flowers revealed a higher presence of WAA in proximity to the flowers compared to the control, the opposite of the expected result. The monitoring of the number of WAA colonies throughout the season showed a decreasing trend, which led to values close to zero for both the flower strips and the control (see fig. 4.15).

Table 4.10: Summary of Poisson Generalized Linear Mixed Model (GLMM) for the evaluation of the number of woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colonies on 200 selected apple branches after the release of syrphid (Diptera: Syrphidae) pupae.

Fixed Effects	**Estimate**	**Std. Error**	**P-value**
Intercept	0.747	0.092	< 0.001
'Flower'	0.309	0.122	0.011
2023-06-12	-0.537	0.096	< 0.001
2023-06-26	-3.125	0.283	< 0.001
'Flower'x2023-06-12	-0.183	0.129	0.155
'Flower'x2023-06-26	0.046	0.368	0.900

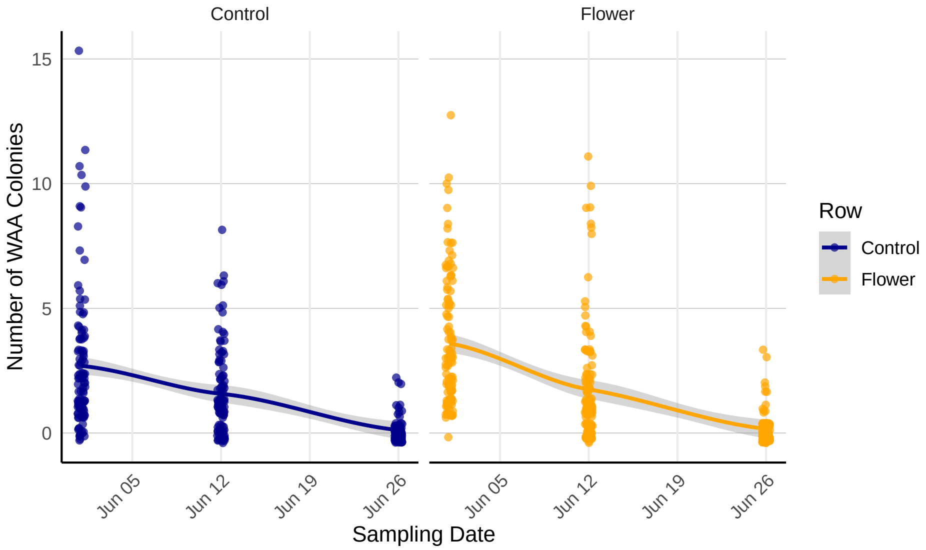


Figure 4.15: Comparison of the number of woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) colonies on 200 selected apple branches after the release of syrphid larvae in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). Flower strips had a small but significant effect on the number of *E. lanigerum* colonies observed in the field ($p = 0.011$), with a higher number of colonies in the treatment 'flower' compared to the control. Each dot represents the evaluation done on each one of the 200 selected apple branches. The line represents the average.

Table 4.11: Summary of Negative Binomial Generalized Linear Mixed Model (GLMM) for the evaluation of the severity of the attack of woolly apple aphid, *Eriosoma lanigerum* (Hemiptera: Aphididae), colonies on 200 selected apple branches after the release of syrphid (Diptera: Syrphidae) pupae.

Fixed Effects	**Estimate**	**Std. Error**	**P-value**
Intercept	-0.429	0.160	0.006
'Flower'	0.431	0.199	0.030
2023-06-12	-0.302	0.127	0.020
2023-06-26	-3.099	0.397	< 0.001
'Flower'x2023-06-12	-0.057	0.167	0.733
'Flower'x2023-06-26	-0.609	0.596	0.307

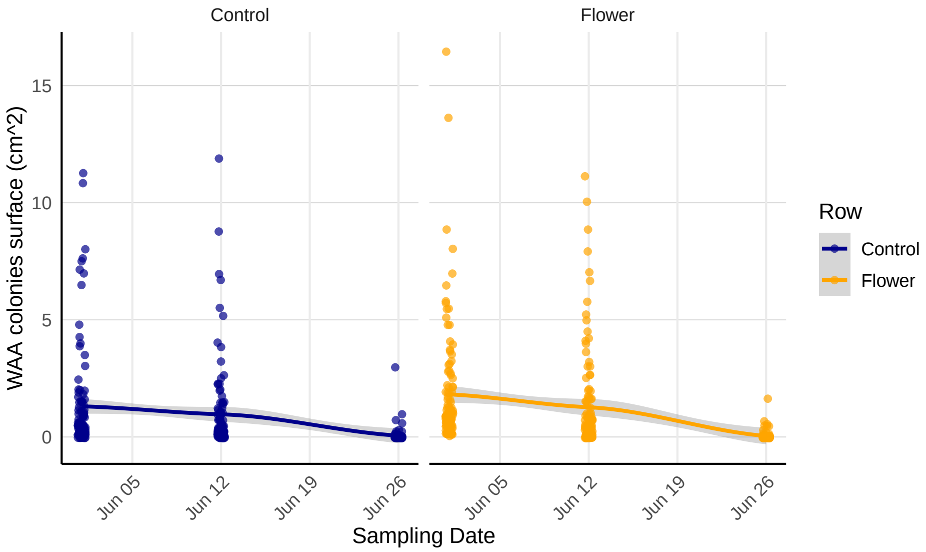


Figure 4.16: Comparison of the severity of woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae) attack on 200 selected apple branches after the release of syrphid pupae in the two treatments: conventionally managed interrow ('control') and flower strips ('flower'). Flower strips had a small but significant effect on *E. lanigerum* attack severity ($p = 0.260$), with a higher attack severity in the treatment 'flower' compared to the control. Each dot represents the evaluation done on each one of the 200 selected apple branches. The line represents the average.

A similar result was observed for the severity of the attack, estimated by evaluating WAA colonies surface. The presence of flowers showed a small but significant effect $(Estimate = 0.431, p = 0.030)$. Even in this case, WAA colonies showed a slightly higher attack in the treatment 'flower' compared to the control and a decreasing trend for both the treatments (see fig. 4.16).

Chapter 5

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. Results from the field experiment conducted, reported in the previous chapter, provided additional knowledge and also offer considerations for future studies aimed at implementing this biological control strategy.

The flower strips development resulted to be satisfying compared to previous experiences at Laimburg Research Centre, although not all the species of the mixture were observed flowering in the field. It is possible that, for some species, more time was needed for germination, that competition with weeds was too strong, or that the site conditions were not favourable enough for the establishment. In the first year flower strips are not expected to provide their full potential (Jacobsen et al., 2022). This result is in accordance with the experience of Uyttenbroeck et al. (2015), who highlights how the success of flower strips requires a longer timeframe to have a clear understanding of all the benefits. Species composition in the field for a flower strip mixture is a dynamic concept, subject to variation from year to year. The species composition and the attractiveness of the mixture to hoverflies in subsequent seasons are important parameters to monitor in future studies. 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. However, it is important to consider and improve other factors in the agronomic management of flower strips. Among these, blooming time is a critical factor to be considered. Timing determines the efficiency of the biological control of pests, and the availability of early flowering species can contribute to a faster establishment of syrphids population. Having flowers available earlier in the season allows to anticipate the release of hoverflies and, thereby, contrast WAA colonies when they are still developing. In this context, owing to the absence of flowers in early spring in the year of the experiment, the use of syrphid larvae can be seen as a ’curative method’ for trees presenting high level of attack or already presenting overwintering colonies in the canopy, while the main strategy should be a ’preventive method’ applied by releasing the pupae. Hence, anticipating the presence of flowers should allow to anticipate the release of pupae as a consequence. The aim is to maintain aphid populations below the economic injury level, and this can be achieved by suppressing colony development as early as possible in the season. Given the significance of flowers in the life cycle of syrphids (Schneider, 1948), their early presence is essential.

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. However, when significant, the results showed a higher presence of WAA in proximity of flower strips than in the control. The WAA attack at the end of June was recorded to be almost absent in both the treatment areas. This occurrence is in accordance with what was observed in other studies after the appearance of the parasitoid *A. mali*. However, as reported by the employees performing the ordinary orchard management for this field, WAA suppression appeared to be greatly anticipated compared to previous seasons. This aspect requires further investigation to determine whether its occurrence is solely due to environmental conditions or if the introduction of hoverflies or the presence of the flower strips may have influenced it. 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. A more aligned experimental design with the study’s objectives would have necessitated the incorporation of four distinct treatments, organised as follows:

- A control group with neither flowers nor syrphids.
- A treatment group with no flowers but with the release of syrphids.
- A treatment group with flowers but without the release of syrphids.
- A treatment group with both flowers and syrphid releases.

The adoption of the latter configuration would have facilitated the independent examination of the two factors, namely, flower strips and syrphids. This approach would have provided a clearer comprehension of their respective significance and influence on WAA colonies, as well as for the other parameters under investigation. Nevertheless, spatial constraints and the absence of a suitable field to perform this type of experiment were the main limitations to the implementation of the proposed arrangement. The constraints also included the requirement for a larger surface area and a higher uniformity in WAA attack. In fact, another problem was related to the selection of the apple rows used for the evaluation in the experiment. The control areas exhibited initial lower values regarding both the severity of attacks and the number of colonies, in contrast to the apple rows situated near the flower strips. This may have influenced the magnitude of syrphid effect on the colonies. The explanation for this observation can be found in the variability inherent in WAA attacks within the field, which manifests a high variability both within the same field and along the same row, as highlighted by Asante et al. (1993).The selection of rows for the evaluation of the two treatments was defined by the spatial separation between them, precluding the selection of a different row for the control. Despite the results were not meeting the expectations, predation of WAA colonies by hoverflies was recorded in some of the evaluated branches, captured through both photographs and videos. Figure 4.13 provides an example of the reduction of a WAA colony after the release of syrphid larvae. This effect can be attributed to the hoverflies due to the presence of syrphid excrements on the box surface in the picture taken on 18th June.

Natural enemies were significantly higher in apple rows close to flower strips compared to conventionally managed interrows. This result is in accordance with the findings reported in several studies (Cahenzli et al., 2019; Gontijo et al., 2013; Rodríguez-Gasol et al., 2019). For all the categories utilised in the study, values were higher in proximity of flower strips compared to the control. Syrphid excrements was confirmed as a good indicator to detect hoverfly larvae presence and their feeding activity. Syrphid larvae were accounted to be more present in proximity of flower strips. Despite that, an underestimation of the actual number of larvae present in the field could be done, as the assessment of natural enemies presence was conducted in the morning, while syrphid larvae feeding activities primarily occur during the nocturnal hours (Burgio et al., 2015). However, syrphid larvae was observed and their presence within the colonies was documented as shown in figure 4.11. The assessment of syrphid excrement values may have been underestimated too, as some of the black smears on the leaves were overlooked due to my limited experience. This occurrence was later pointed out by the technicians and researchers from Biocom Sirfidos during a field visit in June. Moreover, syrphid larvae may be subjected to predation by ants or birds, and phenomenon of intra-guild predation could have influenced the impact of syrphid, especially after the releases of the larvae. Ladybird larvae were abundant more than expected close to flower strips compared to the control. This aspect needs further investigation, considering that insects from this family are not typically considered to get particular benefits from the presence of flower strips.

Measurement of fruit damage, disease incidence or yield gain or loss should be added to this kind of study. These data are important for farmers to evaluate such biological control strategy and to convince growers about their adoption. Thus, future studies should include evaluations concerning also these parameters.

Chapter 6

Conclusion

Eriosoma lanigerum represents one of the main challenges in pest control in the apple production sector. Lack of efficient control methods with synthetic insecticides demand to propose different approaches to limit the impact of this pest. Biological control in this context is a valuable strategy, which could lead to the suppression of the pest, together with a overall higher sustainability of the production system. The aim of this study was the evaluation of a biological control strategy involving the use of syrphids as a biological control agent, coupled with flower strips for the provision of pollen and nectar, which are fundamental for syrphid development. The objectives of the study were the evaluation of the flower mixture attractiveness for syrphid adults, the investigation of the impact of syrphid on WAA colonies, and an evaluation of the influence of flower strips on natural enemies promotion. Agricultural habitats are facing a reduction of the overall biodiversity coupled, and the implementation of agronomic practices, like flower strips, that counteract this trend might be beneficial. The results of this study provide additional knowledge in this context. Flowers promoted the presence of natural enemies significantly compared to the usual management of the interrows, with repeated mulching along the season. However, their impact on the pest object of the study was not as expected. While flower strips enhanced the abundance of natural enemies and the presence of adult hoverflies, the effect of syrphids coupled with flower strips on WAA did not meet the expectations. No or small differences were noted in the assessment of both the number of WAA colonies and the severity of attack. When differences were present, they predominantly indicated a modest but yet higher presence of WAA in proximity to the flower strips. The experimental design may have played a role in these outcomes, with the spatial separation between the control and flower strips potentially insufficient to detect significant differences in WAA colonies. Despite that, a positive impact of flower strips on enhancing the presence of natural enemies and adult hoverflies was evident. This suggests that, at an ecosystem scale, the presence of flowers is beneficial.

Future studies should investigate the application of this biological control strategy by independently evaluating the role of hoverflies releases and flower strips, to understand if this two elements alone and combined are valuable and if they could reduce WAA infestations.

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