Entomology, Ornithology & Herpetology: Current Research

Almeida, et al., Entomol Ornithol Herpetol 2017, 6:3
DOI: 10.4172/2161-0983.1000199

Research Article

Open Access

Effect of *Achillea millefolium* Strips and Essential Oil on the European Apple Sawfly, *Hoplocampa testudinea (Hymenoptera: Tenthredinidea)*

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Received date: August 15, 2017; **Accepted date**: September 05, 2017; **Published date:** September 12, 2017

Abstract

The European apple sawfly *Hoplocampa testudinea* (Klug) (Hymenoptera: Tenthredinidae) is a pest in numerous apple orchards in eastern North America. In Quebec, Canada, the European apple sawfly can damage up to 14% of apples and growers use phosphate insecticide during the petal fall stage to control the pest. Since the insecticide is toxic for beneficial insects, we established flower strips of *Achillea millefolium* L. (Asterales: Asteraceae) to interfere with the oviposition of the insect as an alternative. Populations of European apple sawfly adults were significantly reduced in areas close to flower strips compared to unmanaged areas (natural groundcover). In order to understand the mechanism of the flower strip effect, we tested the repellent effect of *A. millefolium* essential oil on populations of *H. testudinea*. Twenty flowers per apple tree were sprayed during full bloom, the oviposition period of the pest. Sprayed and control flowers were then collected and dissected to note the presence of oviposition scars and eggs of the European apple sawfly. Significantly less scars and eggs were observed on sprayed flowers than on control flowers, which means that *A. millefolium* had a repellent effect on the insect.

Keywords: Habitat management; Flower strip; Groundcover; Repellency

Introduction

The European apple sawfly (EAS), *Hoplocampa testudinea* (Klug) (Hymenoptera: Tenthredinidae), a Palearctic pest present in most apple orchards in North America, was first recorded in Quebec in 1979 [1-3]. Adults emerge from the soil at the tight cluster stage and oviposition in the receptacle of apple flowers during full bloom can damage up to 14% of apples in Quebec orchards [3-5]. Eight to 15 days later, at the petal fall stage, larvae hatch and develop in young fruits, causing damage by digging galleries under the epidermis while feeding on the pulp [6]. These early larval feedings leave typical brown spiral scars on apples, which depreciate the fruit [6,7]. Later, larvae enter in other fruits and burrow a 3 mm diameter tunnel. This more serious damage, known as secondary damage, affects fruit development, causing it to fall in June. Larvae overwinter in the ground in a cocoon [6,8]. Due to pollinator activity, no pesticides can be used to control this pest during full bloom, the oviposition period of *H. testudinea.* Thus, apple growers use mainly an organophosphate insecticide during the petal fall stage to kill young larvae. However, the treatment is toxic to wildlife and the environment [9,10].

Habitat management may thus constitute an alternative to chemical control since it can enhance insect diversity and reduce phytophagous insect populations in crops [11-13]. A review of 209 studies showed that 52% of pests were less abundant in more diversified crops than in monoculture, 9% of pests were more abundant and 39% of pests were not affected by habitat management [11,14]. Directly, habitat management increases the difficulty for pests to locate their host plant because of the different chemical stimuli produced by the diversity of plants, around or inside the crops, and because of the different strata and physical borders [12]. Habitat management may reduce residence time of pests in the crop and their oviposition [15,16]. Habitat management can affect pests indirectly by attracting natural enemies, predators and parasitoids by providing them with pollen, nectar, alternative preys or hosts and also refuges, oviposition sites and breeding sites [12,17,23].

As habitat management techniques, flower strips and groundcovers have been tested in apple orchards. However, the literature provides contrasting results: a lower abundance of herbivores and a higher abundance of parasitoids and predators [24-27], a higher abundance of herbivorous insects [28], or no differences between managed and control habitats [29,30].

Among the plant species commonly tested in flower strips management, *Achillea millefolium* L. (Asteraceae) was very attractive to natural enemies, such as Syrphidae, predatory Coccinellidae and Ichneumonidae parasitoids, and for prey such as aphids [16,24,31]. In the laboratory, plant-derived compounds and extracts of *A. millefolium* constituted strong repellent for numerous species including the corn leaf aphid *Rhopalosiphum maidis* (Fitch) [32], the mosquito *Aedes aegypti L.* [33], the bean weevil *Acanthoscelides obtectus* (Say) [34] but also the braconids *Heterospilus prosopidis* Viereck and *Cotesia glomerata L.* [35]. The repellent effect was mainly due to two aromatic compounds, camphor and 1, 8-cineole [32,36,37]. The concentrations of these compounds vary with a wide range depending on the native area of the plant, and *A. millefolium* demonstrates a stronger potential than many other aromatic plant [35,38].

At this time, no research has been conducted to evaluate the effect of vegetation habitat management on EAS populations in orchards. Furthermore, no research has been carried out to test the effect of aromatic plants on EAS populations. The aims of the present research project were 1) to test the effect of flower strips of *A. millefolium* on *H. testudinea* populations, and 2) to evaluate in the field the potential repellent effect of essential oil extracts on the pest. We predicted that flower strips would reduce EAS populations close to the managed area, and consequently reduce damage to apples. As a potential mechanism, we proposed that *A. millefolium* would have a repulsive effect on apple sawfly females.

Materials and Methods

Apple orchards

The experiments took place in three commercial apple orchards (Compton (45.238356°N; 71.854711°W), Stanstead (45.075421°N; 72.069612°W) and Magog (45.213273°N; 72.135468° W) (Southern Quebec, Canada)). All apple trees were on dwarf or semidwarf rootstock. The orchard cultivars were McIntosh, Paulared, Lobo, Cortland and Spartan. The orchards were managed through application of pesticides. Azinphos-methyl was applied annually at petal fall against *H. testudinea* and phosalone or phosmet was applied at the end of July against *Cydia pomonella* (L.). Deltamethrin was applied in Compton at the beginning of May against bugs and phosmet was applied in Stanstead at the end of July to control *Rhagoletis pomonella* (Walsh). Acaricide (superior oil) was used at the beginning of May in Magog and Compton against spider mites.

Flower strip management

At the border of three apple orchards, composite flower strips were established in 2006 perpendicularly to apple tree rows. Flower strips (2 m × 20 m) were composed of *Solidago canadensis* and *A. millefolium* sowed respectively at 1 and 0.5 g/m^2. These plants were selected because they do not belong to the same family as apple trees, would not attract the apple tree pests, and their flowering period starts after that of apple trees (to avoid interfering with pollinators). During the sampling period of the study, *S. canadensis* had not emerged so the flower strip was only composed of *A. millefolium*. Each orchard contained flower strip areas paired with natural groundcover areas (2 m × 20 m) for a total of eight flower strips and eight controls for the three orchards (Compton (two), Stanstead (two) and Magog (four)). Paired areas had similar apple tree age, size and variety. Buffer zones of 10 m to 15 m composed of apple tree rows were established on each side of the sampling orchard areas to minimize edge effect.

Sampling pest (experiment 1)

Hoplocampa testudinea populations were monitored weekly from mid-May until the end of June, with sticky white traps hung in apple trees at 150 cm from the soil surface. On the two apple tree rows facing each flower strip and each control, one tree per row and per distance was chosen, located at 0 m, 10 m and 30 m. Zero meter was the first apple tree of the row, corresponding to a mean distance of 10 m from the managed stand. On each tree, one sticky white trap was hung and replaced weekly, and the number of adults was counted. *Hoplocampa testudinea* populations were also monitored within each flower strip and control treatments, weekly from mid-May until the end of June, with sticky white traps placed at 50 cm from the soil surface (height of *A. millefolium* in the flower strip), to verify if the pest was present in the treatment plots.

Fruit injury (experiment 1)

At harvest, at the end of August, 50 randomly selected fruits on each of the sampling trees in the orchards were examined for pest injury, for a total of 100 apples per distance in each treatment. The fruits were randomly selected around the tree, at different heights, and observed directly in the orchards. All damage by *H. testudinea* (typical spiral scars on apples and oviposition scars near the calyx) or other pests were identified and recorded. All the observations were made the same day in the same order for the three orchards.

Achillea millefolium essential oil (experiment 2)

A second experiment was carried out with essential oil of *A. millefolium* in the most infested orchard by the EAS (Stanstead orchard). We randomly selected 40 apple trees, 20 treated apple trees and 20 controls. Each treated tree was paired with a control tree of the same cultivar, age and size, at a distance of six meters. The cultivars were Spartan, Empire and Cortland. On each tree, at about 150 cm from the soil surface, one branch with a minimum of four floral bouquets was randomly selected and flagged. During the five days of the full bloom period, the flagged branch of each of the 20 treated apple trees received one application of *A. millefolium* essential oil (Union nature aroma-phyto inc. Québec) at 9:00 AM each morning. The essential oil was diluted in water at a concentration of 4% (based on pre-tests). For each application, ten sprayings of 2 ml each were applied per branch, for a total of 20 ml of solution per branch containing 0.8 ml of essential oil. At the same time, the control branches of the 20 other apple trees received one application of water (ten sprayings of 2 ml per branch for a total of 20 ml of water).

On the sixth day of the experiment, 20 flowers were collected per branch on each control and treated trees. We also randomly collected 20 other flowers within a radius of 30 cm from the treated branch. These 20 peripheral flowers were collected to verify if the flowers near the treated branch received the same repulsive effect of essential oil as the sprayed flowers. Flowers were put in bags, placed in a cooler, and observed in the laboratory during the following 24 h. We noted the presence and the number of oviposition scars made by EAS females on each flower. To verify which proportion of scars corresponded to an egg of *H. testudinea* (because of unsuccessful oviposition), all flowers with an oviposition scar were put individually in an identified petri dish with a moisturized blotting paper and sealed up with paraffin wax. Flowers were then placed in an incubator at 25°C during three days and then dissected and observed under a binocular microscope to detect the presence of EAS eggs.

Data analysis

In order to evaluate the impact of the flower strip on the EAS populations, the mean number of insects trapped by sticky white trap was calculated, based on the average number of insects trapped each week for each distance in the managed and control plots of the three orchards. Paired T-test analyses were then performed for each of the measured distances on the average captures per trap and for the overall amount of trapped sawflies. Data were calculated from the mean numbers of trapped apple sawflies from the whole collection period (six weeks). Since multiple tests were done, the Holm-Bonferonni procedure was applied to obtain a more conservative p value (α=0.05) [39].

The same design was used to evaluate the effect of flower strip presence on apples damaged by the European apple sawfly by comparing the mean numbers of damaged apples. Paired T-tests (managed and unmanaged areas) were also performed with corrected p values.

In order to evaluate the proportion of flowers with oviposition scars and eggs between the three treatments (sprayed, peripheral and control), we compared the mean numbers of oviposition scars and eggs per branch with a univariate analysis of variance (ANOVA), with Tukey-Kramer post-hoc tests [40].

All statistical analyses were performed with SPSS for Windows, version 17.0 [41] and JMP IN 4 [40].

Results

Experiment 1: Flower strip effect

During the sample season, 3-441 EAS adults were captured with sticky white traps in the three orchards. The maximum number of insects captured in one week on one trap was 109 (observed in an apple tree from an unmanaged area). The EAS population was significantly less abundant in managed areas of the orchard than in control areas (T=3.27; df=7; P=0.018) (Figure 1). Regarding the distances, the EAS populations were significantly less abundant at 0 m and 10 m in managed areas than in control areas (T=3.65; df=7; P=0.016 and T=2.41; df=7; P=0.046, respectively). No statistical analyses were done within the flowers strips and natural groundcover since very few individuals were captured.

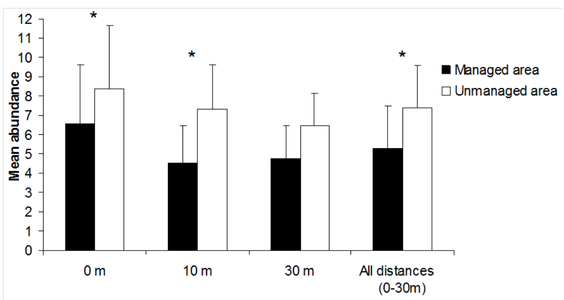

Figure 1: Mean abundance of European apple sawfly adults per sticky white trap per week (± SE) during the sample season in three apple orchards, relative to the treatment (managed or unmanaged) and the distance to the treatment * α=0.05.

At harvest, 4440 apples were observed, with a damage of 16.3% in managed areas and 17.8% in control areas. Damage from mainly five insects was identified: the European apple sawfly, 41.6% of all apples damaged, followed by the tarnished plant bug Lygus lineolaris (Palisot de Beauvois) (39%) (Miridae), other mirids (7.8%), spring budworms (4.7%) and summer budworms (2.1%) (Tortricidae). The percentage of apples damaged was similar in managed and unmanaged areas (Figure 2).

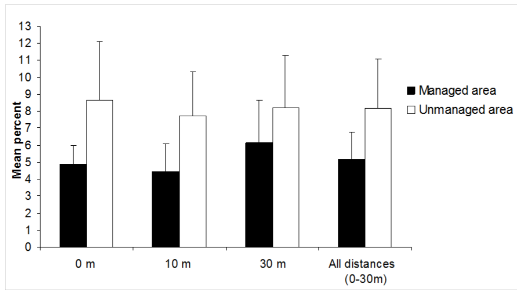

Figure 2: Mean percent of apples damaged by the European apple sawfly (± SE), relative to the treatment (managed or unmanaged) and the distance from the treatment.

Experiment 2: *Achillea millefolium* essential oil effect

Of the 969 collected flowers, 12.6% presented oviposition scars and 7.4% were bearing an EAS egg. A maximum of eight oviposition scars and five eggs was observed on control branches (20 flowers). There were significantly more oviposition scars on control flowers (23.4%) than on sprayed flowers (9.1%) and peripheral flowers (9.5%) (F=12.98; df=2, 15; P<0.0001) (Figure 3). There was no difference between sprayed and peripheral flowers. The same pattern was observed for eggs of European apple sawfly with 12.9% of control flowers bearing an egg compared to 5.5% on sprayed flowers and 5.2% on peripheral flowers (F=5.08; df=2, 15; P=0.0125). There was no difference between sprayed and peripheral flowers.

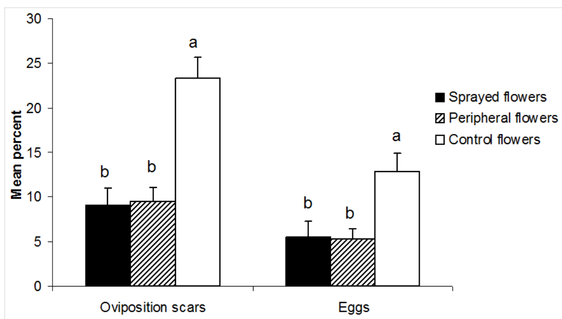

Figure 3: Mean percent of flowers per branch (± SE) with oviposition scars and eggs of the European apple sawfly, relative to the treatment (control not sprayed, sprayed focal flowers and peripheral flowers).

Discussion

As predicted, *A. millefolium* flower strips significantly reduced the population of European apple sawfly adults in the managed areas. As a method, *A. millefolium* essential oil had a deterrent effect on *H. testudinea* females. Nevertheless, apple damage in the presence/absence of flower strips was not different.

EAS populations were less abundant in areas close to flower strips. In another study conducted in Quebec apple orchards for five years [24], a flower strip composed of four Asteraceae including *A.*

millefolium did not reduce EAS populations and damage. The influence of *A. millefolium* may have been masked by the other companion plants: *Tanacetum vulgare* L., *Chrysanthemum maximum Ramond, Aster tongolensis Franchet* (Asteraceae).

Regarding the distance, the reducing effect on *H. testudinea* was observed at 0 m and 10 m of the flower strip, but was no longer observed over 30 m in the managed area. Such limitation suggests that flower strips should be managed within the apple orchard in order to obtain an efficient system. Landscape, (geomorphology, configuration, composition) and local characteristics (orientations of the tree rows, densities, cultivars, tree height, distance between flower strips and rows) may clearly influence the effect of flower strip management on the focal insects [42,43].

As a potential mechanism to explain the lower abundance of the pest in managed areas, the results confirmed that *A. millefolium* has a repellent effect on the European apple sawfly. The repellent effect of *A. millefolium* is mainly attributed to two main aromatic compounds, camphor and 1, 8-cineole, possessed by most of Asteraceae and aromatic plants [44]. The number of flowers with scars and eggs of EAS was significantly lower on branches treated by essential oil of *A. millefolium* or peripheral flowers. Our results demonstrated that essential oil of *A. millefolium* can repel adults of *H. testudinea* and reduce oviposition on trees near the managed stand. The repellent effect of extracts of *A. millefolium* has been already documented on numerous pests but not on Tenthredinidae, and more specifically not in the fields [32-35].

Under laboratory conditions [35], *A. millefolium* had a repellent effect on hymenopterous parasitoids (*Cotesia glomerata* (L.), *Heterospilus prosopidis* (Viereck) (Braconidae), and *Pimpla turionellae* (L.) (Ichneumonidae)), which may constitute a significant non-target effect in the field. The potential impact on pollinators, more specifically hymenopterous pollinators, also needs to be taken into account.

Our prediction about the reduction of apples damaged was not confirmed. The decreasing effect of the flower strip on apple sawfly populations was not associated with a diminution of apples damaged. The mean percentages of apples damaged ranged from 5.1% in managed areas to 8.2% in unmanaged areas. Despite the significant reduction of apple sawfly numbers, the pest population was still higher than the economic threshold. The threshold is evaluated from 4 to 6 EAS captured per trap from the tight cluster stage to the calyx, whereas in our study, we noted a mean cumulative capture of 17.8 and 21.7 EAS per trap for the managed and unmanaged areas, respectively.

To avoid intraspecific competition for a limiting resource, numerous phytophagous species have developed mechanisms such as the use of visual and chemical clues to discriminate previous attacks by competitors [45]. Under laboratory conditions, *H. testudinea* adults, oviposited significantly less frequently in blossoms containing oviposition wounds and eggs by conspecifics than in healthy, uninfected ones [46]. As a result, oviposition will not be crowded but regularly distributed in the field. Therefore, very abundant populations should exploit the oviposition sites uniformly, and this may have masked a possible effect of the flower strips at a small scale (the managed areas). This means that, at a larger scale, for example in an orchard completely managed with flower strips, a significant decrease of the pest populations may lead to a significant decrease of the damages.

Furthermore, the scent, the abundance of pollen and nectar of apple cultivars influence the degree of attraction by *H. testudinea* and the rate of apples damaged [6,47] and may consequently modulate the success of the management of a flower strip in the orchard.

Conclusion

The present research demonstrated that a flower strip composed of *A. millefolium* reduces EAS populations, and that *A. millefolium* essential oil has a repellent effect on *H. testudinea* adults and reduces its oviposition on apples. Nevertheless, the efficacy of the flower strips was limited to a few meters and did not significantly reduce damage to apples; and the efficacy of essential oils was limited to a few hours. Therefore, we suggest to study flower strips at larger scales (one orchard=one unit), and to study carefully the impact on the other pests and on their guilds of natural enemies. We also suggest studying *A. millefolium* as one component of composite multi specific flower strips.

Acknowledgments

We especially thank the three anonymous apple growers. We are very grateful to Spencer Mason, Alessandro Dieni, Annick Pannetier Leboeuf, Benjamin Carrara, Claudia Roberge, David Beauregard, Francine Pelletier, Franz Vanoosthuyse, Geneviève Labrie, Jacinthe Tremblay, Louise Voynaud, Noémie Bourdon-Charest, Olivier Morrisset and Sylvain Hutchison for their help during the sampling. Thanks to Bertrand Fournier, Maxime Paquet and Olivier Lalonde for their appreciated advices in statistics. Thanks to Yves Mauffette and Conrad Cloutier for their valuable comments on a previous version of the manuscript. We also thank Mr. and Mrs. Mason as well as Martin Hutchison for their support during the experiments. Finally, this research was supported Horticulture Indigo and the Club Agroenvironnemental de l'Estrie.

The funding of this project was provided by the regional industry council of Quebec that execute the Canadian Agricultural Adaptation Program for Agriculture and Agri-Food Canada [6799].

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