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Perennial flower strips in apple orchards promote natural enemies in their proximity

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ABSTRACT

Apple production across Europe is experiencing major yield losses due to pest damage, and the need for alternative production methods are growing. Ecological infrastructures are important to create resilient production systems and increase functional biodiversity. This study investigated perennial flower strips as a means of promoting natural enemies, thereby reducing infestation by the rosy apple aphid, Dysaphis plantaginea, and fruit damage caused by it. In 2016 and 2017, effects of perennial flower strips were assessed in eight organic apple orchards with and without flower strips. In orchards with flower strips, assessments were done in tree rows at two distances: 1st and 3rd row from the flower strip. Assessments included field samples of arthropods, visual observations of D. plantaginea symptoms and observations of predators, observations of predators directly associated with aphid colonies, and fruit damage. In 2016, significantly more symptoms of D. plantaginea were observed in the 3rd row from the flower strip compared to the 1st row from the flower strip. Both years, more predators were observed in the 1st row from the flower strip than in the 3rd row from the flower strip. In 2017, more predators were observed in orchards with a flower strip than in orchards with no flower strip, and more predators were observed inside the aphid colonies in trees in the 1st row from the flower strip than in the 3rd row from the flower strip. Predator diversity in apple trees was also positively affected by proximity to a flower strip. In conclusion, perennial flower strips consistently promote the occurrence of natural enemies in their proximity, while the effect decreases with distance. The correlation between aphid abundance and predators was not always consistent, likely because the majority of the observed predators were generalists and therefore contributing to the natural regulation of the entire herbivore complex in the apple orchards.

1. Introduction

Major yield losses due to pest damage and lack of control options in organic apple production have prompted the development of alternative pest control strategies. Strategies promoting functional agrobiodiversity can enhance ecosystem services such as natural pest regulation (Bianchi et al., 2013; Wood et al., 2015). The strategy encourages the use of ecological infrastructures such as hedgerows and flower strips to promote natural enemies, by providing food and shelter for reproduction and overwintering habitat (Landis et al., 2005). Flower strips can be excellent sources of pollen, nectar and alternative prey for beneficial arthropods such as generalist predators, and are therefore a potentially resourceful and efficient strategy for pest control (Landis et al., 2000; Wåkers and Van Rijn, 2012). Ecological infrastructure such as flower strips benefit natural enemies as shown in several studies (Landis et al., 2000; Simon et al., 2011; Gontijo et al., 2013; Tschumi et al., 2015; Balzan et al., 2016; Herz et al., 2019). Potentially, such infrastructures can boost or at least maintain yield levels at reduced levels of inputs of mechanical or chemical plant protection measures (Letourneau et al., 2009; Demestithas et al., 2017). Applied successfully, preventative strategies can lead to a more robust agroecosystem and more predictable yields, though few studies have included yield (Bostanian et al., 2004; Holland et al., 2016; Herz et al., 2019; Cahenzli et al., 2019).

The rosy apple aphid, Dysaphis plantaginea Passerini (Hemiptera: Aphididae) is a worldwide pest, and a key pest across Europe (GBIF, 2020; Blommers et al., 2004). Its feeding causes fruit deformation, leaf curling, distorted shoots and reduced tree growth (Guillemaud et al., 2011) and even at low pest densities can cause economical losses (Cross et al., 2007). Insecticide resistance in D. plantaginea is widespread (Delorme et al., 1999; Foster et al., 2007), so alternatives to chemical...
control of this pest has high relevance for the industry across Europe. This is especially so for organic producers. Currently there are no control options against *D. plantaginea* for organic growers in Denmark (Mid
deldatabasen, 2019), and more than 20 percent of the area with apple production in Denmark is grown organic (Statistics Denmark, 2019; Sigsgaard and Jacobsen, 2017). Apple orchards are perennial production systems, which increases the risk of pest population build-up, but it also provides the opportunity to create resilient and sustainable systems over several years, by suitable preventive measures (Simon et al., 2011).

There is a range of generalist and specialized natural enemies that contribute to the natural regulation of *D. plantaginea* in apple orchards. Predatory bugs (Anthocoridae, Miridae), spiders, earwigs, coccinellids, syrphid larvae, chrysopid larvae, and gall midge larvae (*Aphidoletes* sp.) are the primary natural enemies (Wyss, 1995; Mitarro et al., 2005; Dib et al., 2016).

Heterogeneity of agroecological infrastructures is a key issue for a successful pest control strategy (Tscharntke et al., 2006), making decisions about orchard structure and management procedures critical. For example, more natural enemies have been found in the vicinity of flower strips compared to further away, when distance is a variable (Sigsgaard, 2014; Tschumi et al., 2015; Albert et al., 2017), while other studies have found no effect of distance to flower strip (Tschumi et al., 2016). Several factors influence the beneficial effects of such ecological infrastructures; one of them being whether it is an annual or perennial vegetation. Perennial flower strips can offer undisturbed areas of structurally varied resources, with the potential for a long-term impact (Tschumi et al., 2016). However, there is still a lack of knowledge of ecological infrastructures and their local and area wide impact on arthropod dynamics (Letourneau and Bothwell, 2008; Schellhorn et al., 2015; Holland et al., 2016; Albrecht et al., 2020).

The present study includes assessments of pest abundance and resulting fruit damage, and natural enemy abundance, activity and diversity, in relation to distance to flower strips, and in comparison with orchards without flower strips. The aim of this study was to investigate whether i) perennial flower strips can increase the abundance and diversity of natural enemies in apple orchards, ii) distance to flower strip influences predator abundance and regulation of *D. plantaginea*, and iii) the presence of flower strips can suppress pests and resulting fruit damage.

2. Materials and methods

2.1. Apple orchards

The field study was conducted in eight organic apple orchards in 2016 and 2017; five orchards with perennial flower strips and three orchards with no flower strips, which served as control orchards (Table 1). All orchards were located on Zealand, Denmark. In the orchards with flower strips, all assessments were done in the 1st row and in the 3rd row from the flower strip, representing a distance of 2 m and 10 m from the flower strip, respectively. The width of the flower strips varied between orchards (Table 1). Assessments in the control orchards were also done in two rows, both located at least three rows from a non-crop area to exclude potential effects of other types of vegetation in similar distances. The non-crop areas of the control orchards were trees and/or spontaneous herbal vegetation. In the orchards with no flower strip, no plant protection products were applied. In two out of five orchards with flower strip, pheromone disruption was present, and in one orchard with flower strip, four Sulphur treatments were done per year, from April to June. The organic orchards are certified and follow the required EU and Danish legislations for organic produce (EU Regulative 2018/848/EU, Danish Consolidation Act 2015/1765 and 2017/1773), and are managed according to standard procedures of pruning, fertilization and soil rotation.

2.2. Flower strips

Flower strips were established in five organic apple orchards in May 2015, in all cases these strips replaced ca. five year old flower strips with fewer species. Within each orchard, flower strips either were positioned so they replaced a row of apple trees or placed adjacent to the outer row of the apple orchard. Minimum five apple tree rows consecutively. Assessments were conducted in the 1st row (2 m from flowers strip) and in the 3rd row (10 m from the flower strip) (in black) from the flower strip.

Fig. 1. Graphical overview of the orchard design with a flower strip. The strip either positioned replacing a row of apple trees or adjacent to the outer row of the apple orchard. Minimum five apple tree rows consecutively. Assessments were conducted in the 1st row (2 m from flowers strip) and in the 3rd row (10 m from the flower strip) (in black) from the flower strip.

Table 1

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Altitude (m)</th>
<th>Closest town</th>
<th>Orchard size (ha)</th>
<th>Tree age (years)</th>
<th>Orchard system</th>
<th>FAB structure</th>
<th>Flower strip width (m)</th>
<th>Location of FAB</th>
<th>Number of rows assessed</th>
<th>Planting distances: between trees (m)</th>
<th>between rows (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55.5201</td>
<td>LL. Skensved</td>
<td>5.4</td>
<td>15</td>
<td>tall spindle</td>
<td>Flower strip</td>
<td>1.5</td>
<td>replacing row</td>
<td>4</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>55.1674</td>
<td>Store Elmue</td>
<td>1.0</td>
<td>14</td>
<td>tall spindle</td>
<td>Flower strip</td>
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<td>replacing row</td>
<td>4</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>55.2894</td>
<td>Kyse</td>
<td>0.0</td>
<td>10</td>
<td>tall spindle</td>
<td>Flower strip</td>
<td>3.0</td>
<td>replacing row</td>
<td>4</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>55.6733</td>
<td>Taastrup</td>
<td>4.5</td>
<td>8</td>
<td>tall spindle</td>
<td>Flower strip</td>
<td>3.2</td>
<td>edge of orchard</td>
<td>2</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>55.6338</td>
<td>Kagerup</td>
<td>1.9</td>
<td>7</td>
<td>tall spindle</td>
<td>Flower strip</td>
<td>1.0</td>
<td>edge of orchard</td>
<td>2</td>
<td>1.0</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td>55.7671</td>
<td>Løserup</td>
<td>1.3</td>
<td>12</td>
<td>individual trees</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>7</td>
<td>55.6041</td>
<td>Lejre Streby</td>
<td>3.5</td>
<td>9</td>
<td>tall spindle</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>0.8</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>55.3660</td>
<td>Streby</td>
<td>2.0</td>
<td>18</td>
<td>individual trees</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>3.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>
of the apple orchard (approximately 2 m from the apple row) (Fig. 1). Flower strip seedbeds were initiated by first using a rotocultivator, followed by 3–4 times harrowing with weekly to biweekly intervals to control weeds. Thereafter, the seed mixture, consisting of seeds mixed with vermiculite (50:50) was sown in spring 2015 (4 g per m², in a weight ratio of 18%: 82% dicotyledons to grasses). After sowing, a land roller was used to enable a good ground connection of the seeds. In 2016, flower strips were mulched in spring, in mid-June and in August before harvest, at 8–10 cm cutting height. Based on experiences from 2016, flower strips were mulched twice in 2017, in spring before flowering, and again before harvest.

The seed mixture consisted of 36 plant species, eight monocotyledons (grasses) and 29 dicotyledons (Table S1). Monocotyledons were included to stabilise the plant community and minimize invasion by local pioneer plants (Pfiffner et al., 2019). Except for Trifolium pratense L., which has been widely cultivated for centuries, only biannual or perennial native, wild plant species were sown, to ensure the flower strips longevity (Bischoff et al., 2010). The plant species are ecotypes selected based on their nutritious and structural value as food and shelter for natural enemies, flowering times that ensure a long flowering period of the flower strip, and plants’ ability to establish and compete with other native plants in the plant community; with plant sizes ranging from low rosette plants to tall flowering plants. Plants with a short corolla with easily accessible nectar and pollen for aphid natural enemies were selected (Kühn et al., 2004; L. Pfiffner, pers. comm.).

2.3. Entomological assessment methods

A range of entomological assessment methods was conducted in the apple orchards, to investigate the effects of flower strips on the arthropod community. Visual observations assess D. plantaginea symptoms and predators on the same flower cluster (FC) or long shoot (LS) of the tree, and observations of predators in aphid colonies are conducted to show presence and specificity of predator visitors in the colonies. Beating samples provide a more global assessment of the arthropod community in the orchard, and shows the potential contribution of flower strips to biodiversity in the orchard.

2.3.1. Visual observations of D. plantaginea colonies and arthropod predators

Visual observations were conducted four times in the growing season of 2016 and 2017, timing based on the phenological development of the trees (the BBCH-scale); before flowering (BBCH 59), after flowering (BBCH 69–70), after June drop (BBCH 74), and soon before harvest (BBCH 87); in the 1st row from the flower strip, in the 3rd row from the flower strip and in the orchards with no flower strips. At each assessment time, in 10–20 trees per row (depending on the number of assessment rows), six flower clusters (before and after flowering) or 6 long shoots (by June drop and before harvest) per tree, were randomly chosen from branches in all orientations. Each flower cluster/long shoot was observed for symptoms of D. plantaginea (curled leaves) confirmed by presence of an existing aphid colony or remains of a colony (like cast skins), and presence of natural enemies, in particular: syrphid larvae, chrysopid larvae, Aphidoletes larvae, coccinellid larvae and adults, antlions, mirids, spiders and earwigs.

2.3.2. Colony associated natural enemies

An investigation of the specific predators associated with D. plantaginea colonies was conducted by inspection of naturally established colonies in the 1st and 3rd row from the flower strip. Ten colonies per row, in the same rows as the visual observations of D. plantaginea colonies and natural enemies, were assessed two times in the growing season of 2017; after flowering and after June drop. The colonies were randomly selected along the row, and visually inspected by carefully opening the curled leaves and recording presence of D. plantaginea and predator species.

2.3.3. Natural enemy diversity

The arthropod species community in the apple trees were identified from beating samples conducted three times during the growing season, on the same days but always after the visual observations, at the time after flowering (BBCH 69–70), after June drop (BBCH 74), and soon before harvest (BBCH 87), in the same rows as the visual observations. Each sample consisted of three firm beatings on one branch per tree, on 33 trees (Sigsgaard, 2010). Depending on the length of the row, two to three samples were taken per row. Each sample were collected into a funnel (40 × 60 cm) of fine mesh, ending in a plastic bag. The bag containing the arthropods was closed, placed in a cooling box during transport, and stored in a freezer at −20 °C for subsequent taxonomical identification. Arthropods were morphologically identified to species level when possible, by the use of taxonomical keys (Danmarks Fauna, 1907; Lissner, 2011; Roberts, 1985; Skipper, 2013; Southwood and Leston, 2005). Based on these samplings, natural enemy diversity was analyzed by the Shannon-Wiener Index and Pielou’s evenness Index.

2.4. Fruit damage

Fruit damage was assessed twice each year: after June drop and soon before harvest. The first assessment was before growers’ pruning of damaged fruits, to minimize bias in the damage assessment. Ten apples per tree were randomly selected on ten trees per row, and assessed for symptoms of D. plantaginea, i.e. under-developed, malformed fruits. In 2017, a late frost at the pink tip stage (BBCH 56) caused a major decline in bud development and subsequent fruit yield. Yield was much lower than normal and varied hugely between orchards. The results of fruit damage in 2017 are therefore excluded from further analysis.

2.5. Statistical analysis

All statistical analyses were conducted in R, version 3.6.3 (R Development Core Team, 2020). Generalized and Gaussian linear and linear mixed models were used as specified below, and each year (2016 and 2017) was analyzed separately when nothing else is mentioned. Visual observations of proportion of flower clusters/long shoots (FC/LS) with symptoms of D. plantaginea, other herbivores, and predators, were analyzed with logistic regression, with treatment (1st row/3rd row/-control), time (before flowering/after flowering/June drop/before harvest) and their interaction as random effects. Pairwise comparisons of treatments were done for the main predator groups (groups with >1% presence on apple branches) in the visual observations, and for the beating samples (groups with >3% presence on apple branches) (years combined), allowing for overdispersion and with Tukey adjustment for multiple comparisons. The number of predators observed per colony (colony associated predators) in 2017 were analyzed separately for the two time periods after flowering and at June drop; using logistic regression with treatment as fixed effect, and orchard and physical row in field (each row considered, to account for potential correlation between observations of FC/LS from the same row) as random effects. All statistical analyses were conducted in R, version 3.6.3 (R Development Core Team, 2020). Generalized and Gaussian linear and linear mixed models were used as specified below, and each year (2016 and 2017) was analyzed separately when nothing else is mentioned. Visual observations of proportion of flower clusters/long shoots (FC/LS) with symptoms of D. plantaginea, other herbivores, and predators, were analyzed with logistic regression, with treatment (1st row/3rd row/-control), time (before flowering/after flowering/June drop/before harvest) and their interaction as random effects. Pairwise comparisons of treatments were done for the main predator groups (groups with >1% presence on apple branches) in the visual observations, and for the beating samples (groups with >3% presence on apple branches) (years combined), allowing for overdispersion and with Tukey adjustment for multiple comparisons. The number of predators observed per colony (colony associated predators) in 2017 were analyzed separately for the two time periods after flowering and at June drop; using logistic regression with treatment as fixed effect, and orchard and physical row in field as random effects. The same model was used for fruit damage (proportion of damaged apples) assessed in August before harvest. A Gaussian linear mixed model was applied to analyze the proportion of eggs eaten (predation activity), with treatment, time and their interaction as fixed effects, and orchard as random effect. Treatment groups were compared with estimated marginal means averaging effects. More specifically, treatment effects were evaluated over time, when nothing else is mentioned, by orchards with and without flower strips, and near (1st row) and far (3rd row) from the flower strips. Natural enemy diversity was assessed by the Shannon-Wiener diversity index (H') to account for both abundance and evenness (R package vegan, function diversity). The analyses were done separately for each year, and included time (after flowering/June drop/before harvest), and treatment (1st row/3rd row/control). Due to uneven number of samples, analyses were based on proportions of...
families/genera/species identified. Based on $H'$, Pielou's evenness index ($J'$) was calculated to describe the evenness of the species sampled (Oksanen, 2013). The values of the diversity analyses were compared by a two-way ANOVA, with treatment (1st row/3rd row/control) and time (after flowering/June drop/before harvest) as fixed effects. All models were validated by residual plots.

3. Results

3.1. Visual observations

The visual observations of *D. plantaginea* colonies on flower clusters and long shoots was lower in 2016 compared to 2017. In 2016, there was an effect of distance to flower strip; significantly more symptoms of *D. plantaginea* were observed in the 3rd apple row from the flower strip compared to the 1st row from the flower strip ($p = 0.002$). No such differences were found between the orchards with flower strips and the orchards without flower strips ($p = 0.11$). In 2017, no differences in symptoms of *D. plantaginea* were found either in distances to flower strip or between orchards with and without flower strips (Control vs. flower strip orchard: $p = 0.26$; 1st vs. 3rd row from flower strip: $p = 0.82$) (Fig. 2).

A total of 838 predators were visually observed on flower cluster and long shoots in 2016, and 1300 predators were visually observed in 2017. Primarily spiders, anthocorids, mirids and forficulids were observed (at >1% of the flower clusters/long shoots observed) (Table 2), and less frequently syrphid larvae, chrysopid larvae, and coccinellid larvae and adults (at <1% of the flower clusters/long shoots observed). Both years, a significant interaction between treatment and time ($p < 0.0001$) and overall effect of treatments (average over the year) (2016: $p = 0.03$; 2017: $p = 0.03$) was found. Both years, more predators were observed in the 1st row from the flower strip than in the 3rd row from the flower strip (2016: $p = 0.003$; 2017: $p = 0.01$) (Fig. 3). In 2017, more predators were observed in orchards with flower strip than in orchards with no flower strip ($p = 0.02$). In 2016, there were more predators found in the 1st row from the flower strip than in the 3rd row, before flowering ($p < 0.01$). In addition, more predators were observed by June drop in the 1st row from the flower strip than in the 3rd row ($p = 0.01$), and more predators were found in orchards with flower strips than in orchards without flower strips ($p < 0.0002$) (Fig. 3). Specifically for each of the observed predator groups, more Anthocoridae ($p = 0.04$) were observed in the 1st row from the flower strip compared to control orchards, in 2016 (Table 2). No differences were observed for Araneae, Miridae and Forficula, and no differences were observed between the 1st and the 3rd row from the flower strip. In 2017, more Araneae were observed in the 1st row compared to both the 3rd row ($p < 0.001$) and the control orchards ($p = 0.03$), and more Anthocoridae were observed in the 1st row than in the 3rd row ($p = 0.04$), while no differences were observed between treatments for Miridae and Forficula. Other herbivores observed during the visual observations were primarily Lepidopteran

### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Row</th>
<th>Araneae</th>
<th>Miridae</th>
<th>Anthocoridae</th>
<th>Forficula</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>1st row</td>
<td>6.9 ± 1.4$^a$</td>
<td>2.9 ± 0.5$^a$</td>
<td>1.5 ± 0.3$^b$</td>
<td>&lt;1.0$^b$</td>
</tr>
<tr>
<td></td>
<td>3rd row</td>
<td>4.8 ± 1.0$^a$</td>
<td>1.9 ± 0.4$^a$</td>
<td>1.2 ± 0.3$^{ab}$</td>
<td>&lt;1.0$^b$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.0 ± 1.4$^a$</td>
<td>1.5 ± 0.3$^a$</td>
<td>&lt;1.0$^b$</td>
<td>&lt;1.0$^b$</td>
</tr>
<tr>
<td>2017</td>
<td>1st row</td>
<td>11.5 ± 2.3$^a$</td>
<td>7.0 ± 1.4$^a$</td>
<td>2.9 ± 0.6$^a$</td>
<td>2.1 ± 0.4$^a$</td>
</tr>
<tr>
<td></td>
<td>3rd row</td>
<td>7.7 ± 1.5$^b$</td>
<td>6.8 ± 1.3$^a$</td>
<td>1.5 ± 0.3$^{ab}$</td>
<td>1.1 ± 0.2$^a$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.4 ± 1.1$^b$</td>
<td>6.0 ± 1.2$^a$</td>
<td>2.0 ± 0.4$^{ab}$</td>
<td>1.2 ± 0.3$^a$</td>
</tr>
</tbody>
</table>

Fig. 2. Percentage of flower clusters/long shoots (FC/LS) with symptoms of *D. plantaginea*. The values display the means from 2016 (left) and 2017 (right), with standard errors, of the visual observations of *D. plantaginea* at each of the four assessment times: pre-flowering, post-flowering, at June drop, and before harvest, in the control orchards, and in the 1st and 3rd row from the flower strip. Statistically significant results were, in 2016 more symptoms of *D. plantaginea* observed in the 3rd row than in the 1st row from the flower strip ($p = 0.002$).
larvae such as geometrids (e.g., *Operophtera brumata* L.) and tortricids (eg. *Cydia pomonella* L.). Both years, an interaction between treatment and time of season was found for other herbivores (2016: p = 0.02, 2017: p = 0.006). In 2016, more herbivores were observed in the orchards with no flower strips compared to orchards with flower strips (p = 0.03), and more herbivores were observed in the 3rd row from the flower strip than in the 1st row (p = 0.04). No such difference was found in 2017.

### 3.2. Colony associated natural enemies

Overall, 66% ± 14.2 of the aphid colonies investigated contained predators, disregarding treatments. The most frequently occurring predator groups observed inside the colonies were Forficulidae, Aranea, Miridae and Anthocoridae (Table 3). At the first assessment, after flowering, the proportion of *D. plantaginea* colonies that contained predators was similar between all treatments (61.9% ± 12.4 of colonies contained predators) (p = 0.26). By June drop, significantly more colonies were observed with predators in the 1st tree row from the flower strip (78.8% ± 16.0 of colonies contained predators) than in the 3rd row from the flower strip (62.5% ± 12.9 of colonies contained predators) (p = 0.03). There was no difference in the proportion of colonies with predators between the orchards with flower strips (70.6% ± 14.3) and the orchards with no flower strips (70.0% ± 14.6) (p = 0.87).

### 3.3. Natural enemy diversity

A total of 79 groups of families, genera or species were identified from the beating samples (Table 4). Both years combined, the 120 samples from the 1st row from the flower strip contained 5856 individuals, in the 3rd row from the flower strips, the 117 samples contained 5882 individuals, and the 50 samples from the control orchards, contained 1517 individuals. Considering the sample size (33 branches per sample) this converts into an average of 1.48 predators per branch in the 1st row from the flower strip, 1.52 predators per branch in the 3rd row from the flower strip, and 0.92 predators per branch in the control orchards. Mainly predators but also arthropods considered omnivores were included in the diversity analysis, considering their potential contribution to aphid predation (Orpet et al., 2019; Jacobsen et al., in prep.). The pairwise comparisons of treatments on main predator groups,

---

Table 3

<table>
<thead>
<tr>
<th>Percentage colonies with:</th>
<th>1st row</th>
<th>3rd row</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syrphidae larvae</td>
<td>19.4 ± 4.1</td>
<td>4.7 ± 1.3</td>
<td>8.3 ± 2.0</td>
</tr>
<tr>
<td>Chrysopidae larvae</td>
<td>3.1 ± 0.9</td>
<td>0.7 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Coccinellidae larvae</td>
<td>9.4 ± 2.2</td>
<td>6.0 ± 1.5</td>
<td>16.7 ± 3.7</td>
</tr>
<tr>
<td>adults</td>
<td>1.9 ± 0.7</td>
<td>1.3 ± 0.6</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td>Aphidoletes sp. larvae</td>
<td>3.8 ± 1.1</td>
<td>7.3 ± 1.8</td>
<td>11.7 ± 2.7</td>
</tr>
<tr>
<td>Aphidoletes sp. adults</td>
<td>23.1 ± 4.8</td>
<td>16.0 ± 3.5</td>
<td>19.2 ± 4.2</td>
</tr>
<tr>
<td>Miridae nymphs + adults</td>
<td>28.8 ± 4.8</td>
<td>41.3 ± 8.4</td>
<td>48.3 ± 9.9</td>
</tr>
<tr>
<td>Araneae immatures + adults</td>
<td>16.9 ± 8.4</td>
<td>23.3 ± 4.9</td>
<td>15.8 ± 3.5</td>
</tr>
<tr>
<td>Araneae adults</td>
<td>3.6 ± 3.6</td>
<td>16.9 ± 3.6</td>
<td>15.8 ± 3.5</td>
</tr>
<tr>
<td>Forficulidae nymphs + adults</td>
<td>40.6 ± 8.3</td>
<td>34.0 ± 7.0</td>
<td>9.2 ± 2.2</td>
</tr>
<tr>
<td>Predators total</td>
<td>68.1 ± 13.7</td>
<td>68.0 ± 13.7</td>
<td>61.7 ± 12.5</td>
</tr>
</tbody>
</table>

---

Fig. 3. Percentage of flower clusters/long shoots (FC/LS) with predators observed. The values display the means from 2016 (left) and 2017 (right), with standard errors, at each of the four assessment times; pre-flowering, post-flowering, at June drop, and before harvest, in the control orchards, and in the 1st and 3rd row from the flower strip. Statistically significant results for 2016 and 2017: overall more predators were observed in the 1st row than in the 3rd from the flower strip (p = 0.003, p = 0.01 respectively per year), and in 2017 more predators were observed in orchards with flower strip than in orchards without flower strip (p = 0.02).
Table 4

Arthropod predators (family/genus/species) identified from beating samples in apple orchards. Values represent proportion (%) of predator species per branch, in the 1st row from the flower strip, in the 3rd row from the flower strip, and in the control orchards with no flower strip (Control). Family/genus/species names in bold are predator groups represented on >3% of branches with small case letters for differences between treatments (1st row, 3rd row and Control).

<table>
<thead>
<tr>
<th>Order</th>
<th>Family/genus/species</th>
<th>1st row</th>
<th>3rd row</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneae</td>
<td>Araneidae</td>
<td>3.46</td>
<td>2.90*</td>
<td>2.79*</td>
</tr>
<tr>
<td></td>
<td>Araneiella spp.</td>
<td>0.63</td>
<td>0.34</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Tetragnathidae</td>
<td>2.65</td>
<td>2.23</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>Linyphiidae</td>
<td>13.31*</td>
<td>14.32*</td>
<td>6.42*</td>
</tr>
<tr>
<td></td>
<td>Theridiidae</td>
<td>12.40*</td>
<td>12.41*</td>
<td>5.94*</td>
</tr>
<tr>
<td></td>
<td>Thomisidae</td>
<td>1.06</td>
<td>0.85</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Philodromidae</td>
<td>15.03*</td>
<td>13.9</td>
<td>7.38*</td>
</tr>
<tr>
<td></td>
<td>Clabionidae</td>
<td>0.96</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Salticidae</td>
<td>0.15</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Lycosidae</td>
<td>0</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Dictyna</td>
<td>0.58</td>
<td>0.31</td>
<td>0.06</td>
</tr>
<tr>
<td>Opiliones</td>
<td>Opiliones</td>
<td>40.91*</td>
<td>46.06*</td>
<td>28.30*</td>
</tr>
<tr>
<td>Heteroptera</td>
<td>Heteroptera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forficula auricularia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroptera</td>
<td>Neuroptera</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 (continued)

<table>
<thead>
<tr>
<th>Order</th>
<th>Family/genus/species</th>
<th>1st row</th>
<th>3rd row</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Propylea quattuordecimpunctata</td>
<td>1.24</td>
<td>2.25</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Chlorisus renipustulatus</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

>3% of specific predator family per branch (combined for species within Miridae, Anthocoridae and Coccinellidae), showed a higher abundance of Philodromidae in both the 1st row and in the 3rd row from the flower strip compared to the control orchards (p = 0.011 and p = 0.030, respectively). A higher abundance was also found for the total number of species within Anthocoridae, in both the 1st and 3rd row from the flower strip compared to the control orchards (p = 0.05 and p = 0.002, respectively) (Table 4). No differences were observed between treatments for the remaining predator groups with >3% abundance; Araneidae, Linyphiidae, Theridiidae, Opiliones, Miridae total, Coccinellidae total and Forricula auricularia (Table 4).

The Shannon-Wiener diversity (H’) ranged from 1.9 to 2.5 in 2016, and from 1.9 to 2.7 in 2017 (Table 5). In 2016, the Shannon-Wiener diversity of predator species was significantly higher in the 1st row from the flower strip (p = 0.04) compared to the control orchards, while the 3rd row from the flower strip was not different from the control orchards (p = 0.055). No differences in H’ was found between the 1st and 3rd row, and between sampling times in 2016. In 2017, the diversity of predator species was lower in the 3rd row from the flower strip (p = 0.03) compared to the control orchards. No diversity was found between control orchards and the 1st row from the flower strip (p = 0.10). Regarding the time of sampling, diversity was higher after flowering and at June drop, compared to the time before harvest (p = 0.001).

The evenness by Piélot’s Index was stable at 0.7 throughout the season of 2016, and no differences were found between treatments or time points. In 2017, the index ranged from 0.6 to 0.8, and species evenness was found to be lower in the 3rd row from the flower strip compared to the control orchards (Table 5) (p = 0.03). No such difference was found between control orchards and the 1st row from the flower strip (p = 0.06). In addition, evenness was higher after flowering compared to later in the season before harvest (p = 0.006). In 2017 there was no difference in species evenness between the first two sampling times; after flowering and at June drop (p = 0.18).

3.4. Fruit damage

Fruit damage caused by D. plantaginea assessed by June drop in 2016 was 4.1% (±0.9) in the 1st row from the flower strip, 2.3% (±0.5) in the 3rd row from flower strip, and 7.2% (±1.5) in orchards with no flower strip. By the time before harvest in August, fruit damage was 2.6% (±0.6), 2.2% (±0.5), and 6.2% (±1.3) respectively. There was no significant difference in fruit damage between orchards with and without flower strips (neither at June drop: p = 0.16, nor before harvest: p = 0.08), and no difference in fruit damage between the 1st and 3rd row from the flower strip (at June drop: p = 0.71, before harvest: p = 0.55).

4. Discussion

Flower strips in crop production are in part implemented with the intention to promote natural enemies, and thereby decrease pest populations and resulting crop damage. In the present study, our visual
observations consistently found that trees next to flower strips had significantly more natural enemies, than trees further away. Additionally, we found significantly more natural enemies in orchards with flower strips than in orchards without flower strips. Other studies have found similar patterns (Tschumi et al., 2015; Albert et al., 2017; Cahenzli et al., 2019). Albert et al. (2017) found more natural enemies near flower strips and hedgerows, and Cahenzli et al. (2019) found that perennial flower strips established in the alley ways of apple tree rows in existing orchards promoted the abundance of aphid predators. These and similar studies show that species rich flower strips can promote the occurrence of natural enemies, while also emphasizing the importance of proximity to agro-ecological infrastructure.

*Dysaphis plantaginea* infestation was lower in 2016 than in 2017 (Fig. 2). Aphid infestations are affected by the natural variation in climatic conditions impacting yearly fluctuations in population dynamics (Albert et al., 2017; Marliac et al., 2015), and with lower temperature thresholds for development than their natural enemies, in cool springs like 2017 aphid populations can start developing before natural enemies. In 2017, no differences in *D. plantaginea* infestations or numbers of other herbivores were observed between the 1st and 3rd row from the flower strip. In addition, there was never a significant difference in the number of *D. plantaginea* observed in apple orchards with and without flower strips. In other studies, proximity to flower strips have been shown to have a direct impact on the pest density. Sigggaard (2014) found a higher mortality of codling moth in the 1st row from the flower strips than in the 3rd row, and Tschumi et al. (2016) found that perennial flower strips reduced the density of cereal leaf beetles in winter wheat. Both studies (Sigggaard 2014; Tschumi et al., 2016) consider similar distances as the present study. Predator and herbivore complexes in cropping systems are dynamic, and depend on factors like competition, predation, resource availability, and abiotic factors that vary between regions and years (Montoya et al., 2006). The majority of predators observed in the present study were generalists; spiders, anthocorids, mirids and earwigs (Table 2), while specialized predators such as syrphid and coccinellid larvae were less frequent. Especially spiders and anthocorids were more frequently present in the proximity of the flower strip (Table 2), confirmed and specified by the beating samples (Table 4). In Northern Europe we see a majority of predators with a more generalized feeding habit in spring, as was also the case in this study, and these generalists have likely contributed to the regulation of the entire herbivore complex. Albert et al. (2017) found a correlation between aphid and natural enemy abundances; in Northern France they found the more specialized syrphid larvae to be more abundant than in this study. More specialized predators often play a larger role in pest regulation in Southern European regions, than in Northern Europe. The fewer specialist predators found, probably explains why a direct correlation between *D. plantaginea* and predator abundance was not found in 2017.

Besides specialization, timing is a critical factor in the natural regulation of pests. Predator abundance is in a state of flux, and often increasing, through a production season. In 2016, predators were present earlier than in 2017 (Fig. 3). Generalist predators are widely recognized for their importance early in the season to obtain a sufficient pest control level (Symondson et al., 2002). This, and the fact that spring 2016 was warmer than spring 2017, could be the determining reasons for the overall lower aphid infestation during the season of 2016.

Farm level studies are affected by a high variation in management procedures between orchards and variation in weather conditions between years (Jacobsen et al., 2019; Sigggaard et al., 2014). Based on the visual observations, further studies and larger sample sizes may be needed to assess stronger effects on the numbers of *D. plantaginea*. In addition, it is expected that a perennial flower strip in the first years after establishment do not provide its full potential of resilience that it is intended to deliver. On these grounds, it is reasonable to assume that the level of predators will continue to remain sufficient or grow in the following years, disregarding other major disturbances, increasing pest suppression in the proximity of the flower strip and in the orchard as such. The application of plant protection products can contribute to the variation, though with limited impact in the present study, considering the low number and frequency of use (section 2.1).

The higher abundance of predators visually observed in the 1st row by June drop, corresponded with more predators observed in the colonies of *D. plantaginea* in the 1st row, than in the 3rd row. *Dysaphis plantaginea* are leaf-curling aphids, meaning that the colonies are hidden inside the leaf. More predators per observation was recorded with the assessment method of investigating colonies; likely because a curled leaf is optimal for predators to hunt unseen, seek shelter, and being far less exposed compared to for example on a long shoot. Syrphid larvae especially were abundant in the colonies in the 1st row in the present study (Table 3). This emphasizes the beneficial impact of flower strips for the more specialized natural enemies such as these found here, as well as other studies, also finding more chrysopid and coccinellid larvae near or in the flower strip (Tschumi et al., 2015; Cahenzli et al., 2019). The differences in predators associated with aphid colonies between orchards with and without flower strips were insignificant. This is likely due to the fact that none of the orchards without flower strips had any application of plant protection measures otherwise, thereby favoring the arthropod community.

In 2016, diversity was higher, while evenness of predators was equal among treatments (1st row, 3rd row, and control orchards). This probably due to the generally lower number of arthropods this year, thus too low to display any differences. In 2017 a higher species diversity of predators, as well as a higher evenness in the 1st row from the flower strips compared to the 3rd row was found. This is similar to the findings of e.g. Campbell et al. (2017) who also found that plant diverse agro-ecological structures were able to promote species richness of natural enemies. A higher predator diversity was found in control orchards compared to the 3rd row in the orchards with flower strip. Higher
diversity may be partly a reflection of tree age, which was generally higher in control orchards (9–18 years as compared to 7–15 years) (see Table 1), i.e. allowing for a greater build-up of natural enemies over years. A greater diversity of natural enemies in the proximity of the flower strip emphasizes the importance of agroecological infrastructures and considerations of orchard design for natural pest regulation (Gurr et al., 2017; Snyder, 2019).

In 2016, neither distance to flower strip nor presence of flower strip affected the number of damaged fruits. Damage was variable and the overall abundance of *D. plantaginea* was low that year. The damage levels found are within the same range as other studies, which have overall abundance of fruit damage in apple orchards by June drop was significantly reduced in the flower strip plots compared to plots with no flower strips, in a study that included 14 orchards, comparing interrow flower strips to grass strips in a block design. A similar outcome may have been obtained in the present study in 2017 where aphid infestation was higher, but yield could not be reliably assessed because of severe frost damage.

In conclusion, perennial flower strips consistently across both study years promoted the occurrence of predators in organic apple orchards, assessed by visual observations. The generalist nature of the majority of the predators observed, likely contribute to an overall regulation of the herbivore community in the apple orchards, not only the natural regulation of *D. plantaginea*. Distance to flower strip was an influencing factor, which stresses the importance of design of agroecosystems, to allow for a sufficient integration from ecological infrastructures into the cropping area. However, it should be noted that some differences were found between orchards with flower strips and orchards without flower strips, showing an overall positive effect of a flower strip on orchard performance. Flower strips is a preventive pest control measure, and suitable to use in combination with other preventive and/or direct measures of plant protection. This study contributes to the growing knowledge of conservation biological pest control and encourages the implementation of perennial flower strips to build resilient and sustainable production systems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2022.105962.

References


