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Semiochemicals and habitat manipulation to support green lacewing activity to reduce aphid infestations in agroecosystems

Gunda Thöming*, Geir K. Knudsen

Norwegian Institute of Bioeconomy Research, Division of Biotechnology and Plant Health, PO Box 115, NO-1431 Ås
Norway

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Abstract

Conservation biological control (CBC) is a promising tool for ecological intensification that aims to establish resilient natural enemy populations that contribute to pest management with reduced use of pesticides and at the same time support native biodiversity in agroecosystems. Yet the impact of natural enemies in CBC is often limited due to missing resources such as food, habitat, and hibernation shelters. Here, we studied a CBC strategy that incorporates these essential resources combined with semiochemicals, focusing on how the common green lacewing can enhance biological control of aphids.

In a 4-year field study conducted at three locations in the region of East Norway, we developed a CBC strategy combining the three measures ATTRACT (a ternary attractant that increase lacewing egg laying), FOOD (floral buffer strips), and SHELTER (insect hotels for overwintering survival) to increase aphid biological control in spring barley. We recorded the number of lacewings, ladybirds, hoverflies, parasitized aphid mummies, and the two cereal aphid species *Sitobion avenae* and *Rhopalosiphum padi*. Our CBC strategy resulted in a significant increase in lacewing activity and significant aphid suppression. At all three locations and over the 4-year period, aphid infestation was below the economic damage threshold in the field plots using CBC measures. In contrast, during two of the years, the density of the aphid infestation in the control plots was significantly above the damage threshold. We found evidence that use of the ternary attractant supported green lacewings but led to loss of ladybirds, hoverflies, and parasitoids, even though flower strips were used as alternative resources.

Our study shows a promising increase in lacewing activity in the agricultural landscape and high biological control of aphids in barley. Long-term field studies are needed to evaluate the impact on non-target species and the agroecosystem before practical application of this approach can be considered.

Keywords: Conservation biological control; Agricultural landscape; Barley; *Chrysoperla carnea*; Ecological intensification; Ecosystem services; Floral buffer strips; Insect hotels; Natural enemies

Introduction

The continued decline of insects (Sánches-Bayo & Wyckhuys, 2019) underlines an urgent need to remedy this negative trend. One of the drivers of the decrease is habitat loss caused by current agricultural practices. Monoculture, use of

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*Corresponding author.
E-mail address: gunda.thoeming@nibio.no (G. Thöming).

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pesticides and fertilizers, and fragmentation lead to a loss of specialists living in semi-natural habitats, but also to an increase in a few generalist species of both insects (Sánches-Bayo & Wyckhuys, 2019) and plants (Aune et al., 2018). The current intensification of food production favours high yields, but the reliance on fertilizers and pesticides causes loss of biodiversity and ecosystem services such as biological control (Geiger et al., 2010). Thus, there is a need for ecological solutions in agriculture. Indeed, there is a great potential to manage the agricultural landscape in a manner that will better support natural predators and parasitoids of pest arthropods, and still provide high yields. It is in this context that conservation biological control (CBC) comes into force, establishing robust natural enemy populations that contribute to pest management with reduced use of pesticides (Eilenberg et al., 2001; Ramsden et al., 2017; Ramsden et al., 2016; Tscharntke et al., 2012; Tscharntke et al., 2005). However, the impact of beneficial insects is often limited by the lack of essential resources such as food, habitat and hibernation shelters in monocultures, because the diversity of the entomological fauna is always determined by the diversity of the vegetation.

Here, we studied a CBC strategy focused on supporting the common green lacewing *Chrysoperla carnea* s.l. Stephens (Neuroptera: Chrysopidae) to enhance biological control of aphids in cereals. Our aim was to gain knowledge on the mechanisms and interactions involved in this particular predator−aphid system in an agricultural landscape, and, in the long term, to develop a resilient tool for pest management using barley production as an example.

Chrysoperla carnea s.l. is an important insect predator feeding on aphids and other soft-bodied pest arthropods. Green lacewings are most often used for inoculation and inundation biological control (Eilenberg et al., 2001). However, efficient biological control of aphids by release of commercially available lacewings is not always successful in practice (Collier & Van Steenwyk, 2004; Van Lenteren, 2012), and therefore several efforts have been made to develop CBC approaches as an alternative in order to retain lacewings within the crop and increase their reproduction *in situ* (Jonsson et al., 2008; Khan et al., 2008; Rodriguez-Saona et al., 2011; Turlings & Erb, 2018). Conservation techniques to preserve adult lacewings and increase their offspring can entail use of the following (Senior & McEwen, 2001): attractants and food supplements, hibernation shelters and cropping patterns (e.g., strip harvesting, intercropping, or floral buffer strips), reduction of pesticide applications, and management of natural enemies of lacewings. Several semiochemicals affecting the behaviour of green lacewings have been identified (Aldrich & Zhang, 2016). Recent studies have shown that the use of common floral volatiles and herbivore-induced plant volatiles (HIPVs) can improve aphid biological control by *C. carnea* s.l. (Jones et al., 2016; Koczor et al., 2015; Pålsson et al., 2019). Aphids (Hemiptera: Aphididae) are important pests in Norwegian barley production. Currently, the control of pest insects in cereals in Norway relies on insecticides only and alternatives are demanded.

Based on this summarized knowledge, we hypothesized that a CBC strategy combining the three measures designated ATTRACT, FOOD, and SHELTER can increase biological control of aphids by establishing a resilient lacewing population in the agroecosystem. These measures involve the following: ATTRACT is the use of semiochemicals to attract lacewings and increase their egg laying; FOOD is establishment of floral buffer strips to enhance food and habitat sources for adult lacewings; SHELTER is the combined use of semiochemicals and insect hotels to increase the overwintering survival of adult lacewings.

Materials and methods

The investigation was conducted over a period of 4 years (2015−2018) in fields of spring barley (*Hordeum vulgare* L., cv. Helium) at three different locations in two counties (Akershus and Østfold) in East Norway. In this field study, we assessed the population level of common green lacewings (*C. carnea* s.l. eggs, larvae, and overwintering adults), ladybirds (Coccinellidae larvae), hoverflies (Syrphidae larvae), parasitized aphid mummies, and the two most common aphid species (nymphs and adults) in cereals in Norway, *Sitobion avenae* Fabricius and *Rhopalosiphum padi* L. (Hemiptera: Aphididae) while testing a CBC strategy including the three modules ATTRACT, FOOD, and SHELTER to increase aphid biological control by lacewings.

Attract

Based on the results of preliminary studies (Koczor et al., 2015; Tóth et al., 2009), the three components methyl salicylate, phenylacetaldehyde, and acetic acid (> 95% chemical purity, Sigma Aldrich) were applied at a ratio of 1:1:1 (100 mg of each compound), unless otherwise indicated, and formulated on experimental or commercial dispensers. For the experimental approach, the three components were diluted in mineral oil and pipetted on dispensers consisting of 1-cm Parotisroll size 5 cotton wicks (Roeko, Langenau, Germany) inserted into 1.5-mL Easy-Fit polypropylene microtubes (closed microtubes with approx. 0.2 mm hole in the lid; Treff, Degersheim, Switzerland). The total load of active ingredients in each dispenser was always 300 mg, if not otherwise stated. Commercial dispensers used (Csalomon®, Plant Protection Institute, MTA ATK, Budapest, Hungary) have been described by Koczor et al. (2015). The rates of release of the compounds from these dispensers over time in the field have been reported by Pålsson et al. (2019). All dispensers were stored at −18 °C until used.

Food

Annual floral buffer strips were sown at field edges at the end of April each year at a density of 10 g seeds/m^2, with 30% flower and 70% grass seeds. The flower seed mix was based on a commercial product containing 33 different species (Blomstereng, Nelson Garden AS, Bergen, Norway; 10% flower seeds). The species composition of the seed mix is specified in Appendix A. The floral buffer strips and the barley were sown at the same time. Under the experimental conditions in our study, the applied flower seed mix provided flowering vegetation as food and habitat resources for insects in all 4 years (2015−2018) from the end of May until the first frost in autumn (September/October), when the lacewing adults started to search for overwintering shelter.

Shelter

Red wooden overwintering chambers developed for lacewings (insect hotel, box for green lacewings from Windhager Handelsgesellschaft m.b.H, Thalgau, Austria; 20 × 19 × 19 cm) were installed in August 2015 in the hedge banks or forest edges at the borders of the experimental fields (Fig. 1). The chambers were filled with corrugated cardboard rolls to provide shelter for overwintering lacewings. Corrugated cardboard was used to create similar conditions in all chambers. The overwintering chambers were installed on wooden posts at a height of 1.6 m and with a distance of 10 m between them. Six chambers were placed near the field block with floral buffer strips, and six were positioned close to the block without such strips. Every second chamber was equipped with a Csalomon® dispenser as lacewing attractant (Fig. 1), which was attached to the outside of the chamber in September. In early spring (> 5°C mean day temperature), the chambers were searched for green lacewings. As we did not want to remove the overwintering lacewings from the system, the cardboard rolls were taken out of the boxes and carefully uncoiled, and lacewings were counted, and thereafter the cardboard rolls were rewound and reinstalled to the insect hotels.

Experiment 1: Dose-dependent effect of ATTRACT on natural enemies and aphids

A preliminary experiment was conducted to study the number and distribution of lacewing eggs laid and larvae hatched, as well as the suppression of aphids and occurrence of other natural enemies in the field with and without ternary attractant depending on dose. This trial was performed in a spring barley field (0.5 ha) in Ås, Akershus County, in June/July 2015. In the field 24 plots (to enable six treatments with four replicates each) were created with an area of 25 m^2

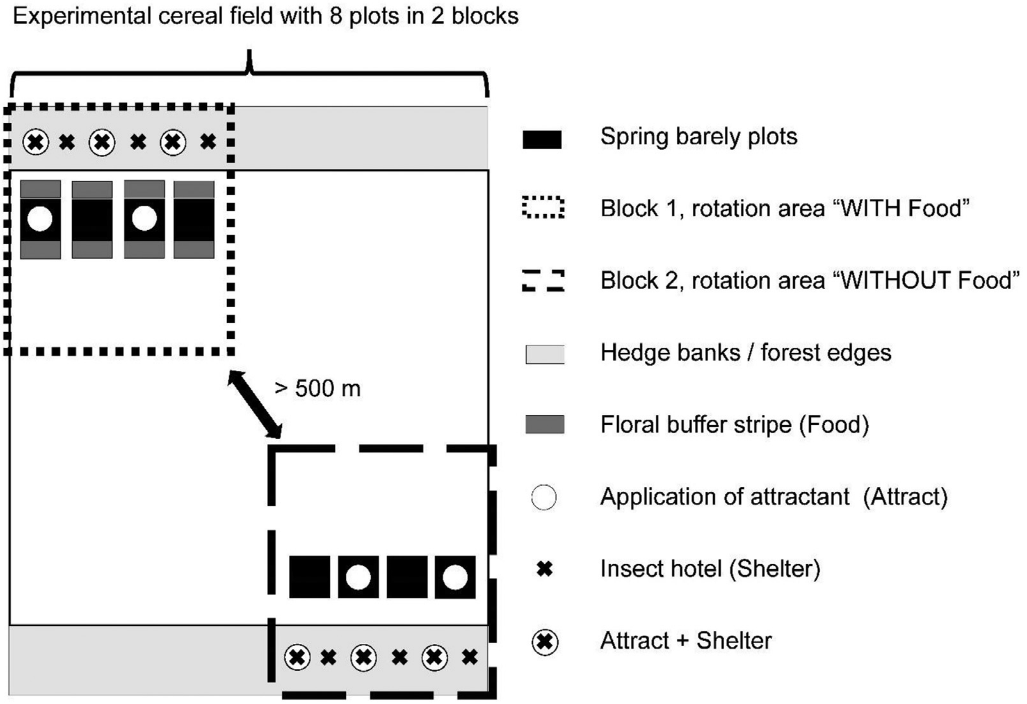

Fig. 1. Schematic representation of the field set up in Experiment 2 used to test a CBC strategy including the three modules ATTRACT, FOOD, and SHELTER to increase biological control of aphids by green lacewing.

each and at least 5 m distance between them. A ternary attractant was placed in the middle of each plot. The ternary blend consisted of methyl salicylate, phenylacetaldehyde, and acetic acid at a 1:1:1 ratio, and four different doses were tested: 3, 10, 30, and 100 mg of each compound formulated on experimental dispensers (i.e., 9, 30, 90, and 300 mg as total load). In addition, Csalomon® dispensers (CD: 100 mg of each compound, 300 mg total load) and a control without volatile treatment (C) were tested.

The dispensers were mounted with wires on wooden sticks (total length 60 cm) at the prevailing height of the vegetation. The wire mounting enabled weekly adjustment of the height of the dispenser position to correspond with the height of the growing barley plants. A marker point (i.e., 60-cm-long wooden stick) was installed in the middle of each control plot. The four plots for each of the six treatments were positioned randomly in the field.

The natural enemies and aphids were recorded in five sectors. The sectors for this registration were dispensers or marker points (= Centre; CTR) and all others were positions at a distance of 30 cm in the directions north (N), south (S), west (W), and east (E) of the dispensers or marker points. The insect counts were performed on the three plants closest to the five inspection points (CTR, N, S, W, and E), respectively. At BBCH stage 13 (leaf development, three leaves unfolded) of barley plants (Meier, 2001), the sectors were checked for natural enemies and aphids (first registration), and the dispensers and marker points were placed in the experimental fields. Over an experimental period of 8 weeks, the sectors were checked weekly for natural enemies and aphids as described above. The dispensers were changed once after 4 weeks. Since the attraction, the oviposition, and the development of offspring of *C. carnea* s.l., as well as the suppression of aphids and the occurrence of other natural enemies were evaluated in the same field experiment, we did not trap adult lacewings nor remove counted individuals. Instead, we marked counted eggs and mummies in order to avoid double counting. Considering that other stages of the recorded insects (i.e., larvae, nymphs, and adults) are mobile, double counting may have occurred. Before and after the study, i.e. in 2015 and 2019, we identified the green lacewing species that are attracted to the ternary blend in the three experimental locations in East Norway. At each location, samples of lacewings were collected using CSALOMON® VARs funnel traps (Csalomon, Plant Protection Institute, MTA ATK, Budapest, Hungary) containing the ternary attractant (Csalomon® dispenser). These lacewings were transferred to the laboratory and taxonomically identified. Similar to other studies (Koczor et al., 2015), this field trapping using the ternary blend revealed individuals of the species complex *Chrysoperla carnea* s.l. only, i.e. mainly *Chrysoperla carnea* Stephens and only single individuals of *C. lucasina* Lacroix.

Experiment 2: Impact of ATTRACT + FOOD + SHELTER on natural enemies and aphids

In our 4-year field study (2015-2018), we tested the hypothesis of whether a CBC strategy including the modules ATTRACT, FOOD, and SHELTER can establish a robust lacewing population in an agroecosystem to reduce aphid infestations in cereals. Each year, one experimental field of approximately 4 ha was established at three different locations in East Norway (= three experimental fields). At each location, overwintering chambers for lacewings were installed at the field borders as described above. Eight plots (each 15 × 15 m) divided into two blocks were established in each of the three experimental fields (Fig. 1). Four treatments (with two plots per treatment) were tested: (1) as a control (C), SHELTER (insect hotels) only, without any additional measures; (2) ATTRACT + SHELTER, application of the lacewing attractant in the field and insect hotels; (3) FOOD + SHELTER, floral buffer strips and insect hotels; (4) ATTRACT + FOOD + SHELTER, floral buffer strips combined with lacewing attractants in the field and insect hotels. The plots without floral buffer strips (treatments 1 + 2, block 1) were located on one side of the experimental field, and the plots with floral buffer strips (treatments 3 + 4, block 2) were on the other side of the field (Fig. 1). The isolation distance was at least 15 m between the plots with and without volatile treatment, and at least 500 m between the two blocks with and without floral buffer strips. The plots in the block with floral buffer strips (treatments 3 + 4) were bordered by a 0.7 m flower strip on two sides. Csalomon® dispensers were used as lacewing attractant and were installed at vegetation height as described above.

The barley and the floral buffer strips were planted each year according to the crop rotation practises, i.e. the plots were established at a different location each year, although within the same block (= rotation area) without (block 1) or with (block 2) floral buffer strips and always near the insect hotels (Fig. 1). No pesticides were used during the 4-year period in any of the locations. Data on the insects were recorded in early spring (overwintering *C. carnea* s.l.) and June/July (experimental period of 8 weeks) each year as described above.

Statistical analyses

The numbers of lacewing eggs, larvae, and overwintering adults (Experiment 2 only), aphids, and other natural enemies were analysed using generalized linear mixed models (GLMMs) with a Poisson or a negative binomial distribution and a log link (PROC GLIMMIX, SAS 9.4). For Experiment 1, the total numbers counted for each of these groups were used as the response variables, with treatment, sector and interaction between treatment and sector as fixed factors and plot as a random effect. For Experiment 2, the cumulative counts of each of these groups were used as the response variables, with treatment, year, interaction between treatment and year as fixed factors and location as a random effect. The choice of the most fitting distribution was based on a test for overdispersion. After establishing the significance of the fixed factors, Tukey's tests were performed for pairwise comparisons between levels of each factor when necessary.

A generalized estimated equation (GEE) regression analysis dealing with the repeated measures in a time sequence was used to analyse correlation between the overwintering lacewing adults recorded in early spring in the two field blocks with and without floral buffer strips and the lacewing eggs recorded the following summer in the respective barley field plots for the four treatments: (1) Control, insect hotels only, without any additional measures; (2) ATTRACT + SHELTER, application of the lacewing attractant in the field and insect hotels; (3) FOOD + SHELTER, floral buffer strips and insect hotels; (4) ATTRACT + FOOD + SHELTER, floral buffer strips combined with lacewing attractants in the field and insect hotels (PROC GENMOD, SAS 9.4). A significance level of $\alpha = 0.05$ was selected in all analyses.

Results

Experiment 1: Dose-dependent effect of ATTRACT on natural enemies and aphids

A total of 1612 lacewing eggs and 439 lacewing larvae were recorded in the barley field. Over the 8-week period of the ATTRACT dose-response experiment, very few lacewing eggs and larvae were recorded in the untreated control. In general, in all five registered sectors, all four of the different doses of the ternary blend tested in experimental dispensers, as well as the commercial dispenser, resulted in a significantly higher number of lacewing eggs and larvae compared to the control not treated with volatiles. Highest numbers of individuals were always found close to the volatile source in the central sector, and a clear increase in number of individuals with rising dose was recorded. No significant differences were found between use of the commercial dispenser and the appropriate test dispenser loaded with 100 mg of each compound (Fig. 2A, Table 1).

Opposite effects were observed for the aphids: in 2015, *R. padi* was the dominant aphid in the barley field, and by comparison, only few individuals of *S. avenae* were found. In all of the studied sectors, all tested volatile treatments achieved significant aphid suppression (*R. padi* and *S. avenae)* compared to the untreated control. A clear decrease in *R. padi* with increasing dose and no significant differences between the commercial dispenser and the respective test dispenser (100 mg) were noted (Fig. 2B, Table 1).

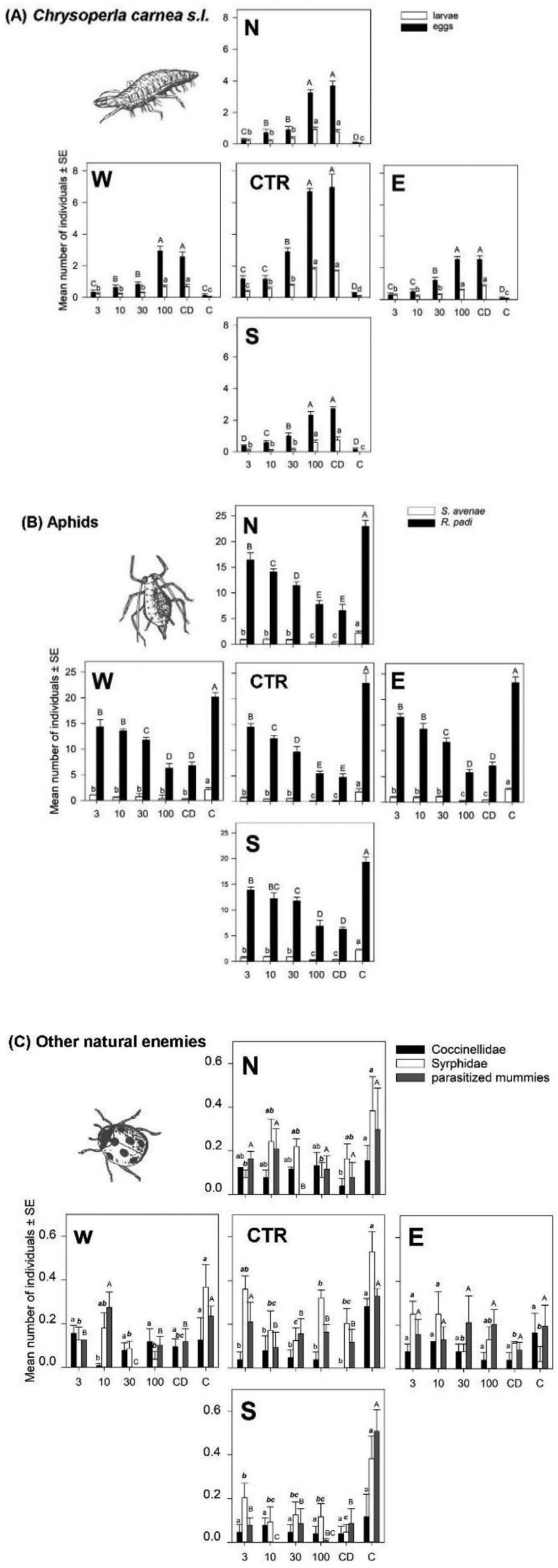

Fig. 2. Numbers of individuals (± SE) of natural enemies and aphids found on three barley plants in the central (CTR) sector of the plot and on plants located 30 cm north (N), south (S), east (E),

Other natural enemies were also observed in the field, where we found a total of 77 Coccinellidae larvae, 183 Syrphidae larvae, and 141 parasitized aphid mummies. These natural enemies occurred in lower numbers than lacewing eggs and larvae in all volatile treatments (Fig. 2A, C). There was a trend towards higher numbers of other natural enemies in the untreated control compared to the volatile treatments, but no dose effect (Fig. 2C, Table 1).

Experiment 2: Effects of ATTRACT + FOOD + SHELTER on natural enemies and aphids

Over the 4-year experimental period, the general occurrence of insects in East Norway varied considerably. Incidences of insects were particularly high in 2016 and were very low in 2017 throughout the region and for most insect species. This trend was mirrored in our study (Figs 3-4).

In all, 1424, 3390, 66, and 1138 lacewing eggs and 374, 3543, 2, and 700 lacewing larvae were recorded in the barley fields in June/July of the years 2015−2018, respectively. In 2015, 2016, and 2018, there were clear increases in lacewing occurrence in the plots with semiochemicals with and without floral buffer strips (ATTRACT + SHELTER, ATTRACT + FOOD + SHELTER) compared to the control plots (C) with only insect hotels. Higher numbers of lacewing eggs and larvae were recorded in the plots with FOOD + SHELTER compared to the control plots (statistically significant for lacewing larvae in 2016 and lacewing eggs in 2018). The plots with ATTRACT + SHELTER showed a significant increase in lacewing larvae (2015 and 2016) and eggs (2015, 2016, and 2018) compared to the plots with FOOD + SHELTER. The significantly highest numbers of lacewing larvae and eggs were recorded in the plots with all three measures ATTRACT + FOOD + SHELTER compared to all the other treatments in all 3 years (Fig. 3A, Table 1).

The opposite effect was found regarding the aphids. In 2015, 2016, and 2018, a clear suppression of aphids was noted in the plots with ATTRACT + SHELTER and ATTRACT + FOOD + SHELTER compared to the control plots (C). In 2015, *R. padi* was the dominant aphid species in our fields, and we observed significant aphid suppression with both semiochemical treatments (ATTRACT + SHELTER and ATTRACT + FOOD + SHELTER) compared to the other two treatments (C and FOOD + SHELTER). In 2016 and 2018, *S. avenae* was the dominant aphid species. The highest numbers of aphids were recorded in the control plots (C), and significant aphid suppression (both species) was found for the other three treatments. In 2016, plots with FOOD + SHELTER exhibited significantly greater numbers of aphids (both species) compared to the ATTRACT + SHELTER and ATTRACT + FOOD + SHELTER plots, where almost no aphids were found. However, in 2018, no differences in aphid suppression were noted between FOOD + SHELTER, ATTRACT + SHELTER, and ATTRACT+ FOOD + SHELTER (Fig. 3B, Table 1).

and west (W) of the central sector. Letters above the bars indicate significant differences (GLMM, Tukey's test, $p < 0.05$) between four different doses of the ternary blend (with 3, 10, 30, and 100 mg of each of the three compounds), a commercial dispenser (CD: 100 mg of each compound), and a control (C) without volatile treatment in each sector for the following: (A) lacewing eggs (uppercase) and larvae (lowercase); (B) *R. padi* (uppercase) and *S. avenae* (lowercase); (C) ladybirds (lowercase), hoverflies (bold italic lowercase), and parasitoid mummies (uppercase).

Counting of other natural enemies in the fields resulted in the following total numbers over the 4 consecutive years (2015−2018), respectively: 114, 267, 3, and 127 Coccinellidae larvae; 208, 105, 2, and 184 Syrphidae larvae; 181, 335, 16, and 357 parasitized aphid mummies. These natural enemies occurred in lower numbers than lacewing eggs and larvae in all treatments (Fig. 3A, C). In 2018, ladybird larvae, hoverfly larvae, and parasitized aphid mummies were significantly more abundant in the FOOD + SHELTER plots than in the rest of the treatments. In 2016, hoverfly larvae and parasitized aphid mummies, and in 2015, hoverfly larvae only were recorded in significantly higher numbers in the FOOD + SHELTER plots than in the other treatments (Fig. 3C, Table 1).

In 2017, very few insects occurred in our experimental fields, and we noted no significant differences between treatments regarding either natural enemies or aphids (Fig. 3, Table 1).

In all, we found 429, 129, and 246 overwintering lacewing adults in the insect hotels in early spring in the years 2016, 2017, and 2018, respectively, and this overwintering survival of adults matched the abundance of lacewing eggs and larvae in the barley fields in the subsequent summer seasons (Figs. 3−5). The significantly highest numbers of overwintering lacewings were found in the field blocks with floral buffer strips and in insect hotels equipped with volatiles (+ FOOD, + ATTRACT) compared to the other treatments in 2016 and 2018, respectively. After the first winter season (2016), overwintering adults were significantly more abundant in the field blocks with floral buffer strips and in insect hotels without dispensers (+ FOOD, Control) than in the field blocks without floral buffer strips (- FOOD, + ATTRACT and Control). No significant differences between treatments were observed in 2017 (Fig. 4, Table 1).

For the treatments ATTRACT + FOOD + SHELTER ($R^2 = 0.90$, $p < 0.0001$) and FOOD + SHELTER ($R^2 = 0.81$, $p = 0.0304$), we noted a significant correlation between overwintering survival of adult lacewings and oviposition rate in the following summer, with more adults in spring resulting in more eggs in summer; no such correlation was found for the two other treatments (ATTRACT + SHELTER and Control) (Fig. 5).

Table 1. Results of generalized linear mixed models for Experiment 1, testing the dose-dependent effect of ATTRACT on natural enemies and aphids, and Experiment 2, testing effects of ATTRACT + FOOD + SHELTER on natural enemies and aphids.

Experiment	Response variable	Fixed factor’s	*F*	DF	*p*-Value
1	Lacewing eggs	Treatment	300.16	5	< 0.0001
		Sector	123.05	4	< 0.0001
		Treatment x Sector	15.09	20	< 0.0001
	Lacewing larvae	Treatment	87.10	5	< 0.0001
		Sector	46.73	4	< 0.0001
		Treatment x Sector	4.77	20	< 0.0001
	R. padi	Treatment	164.08	5	< 0.0001
		Sector	3.81	4	0.0066
		Treatment x Sector	1.00	20	0.4730
	S. avenae	Treatment	56.60	5	< 0.0001
		Sector	1.35	4	0.2589
		Treatment x Sector	0.26	20	0.9993
	Coccinellidae	Treatment	2.99	5	0.0151
		Sector	0.76	4	0.5533
		Treatment x Sector	0.95	20	0.5313
	Syrphidae	Treatment	13.32	5	< 0.0001
		Sector	3.08	4	0.0199
		Treatment x Sector	1.01	20	0.4641
	parasitized aphid mummies	Treatment	5.74	5	0.0001
		Sector	0.80	4	0.5267
		Treatment x Sector	0.95	20	0.5267
2	Lacewing eggs	Treatment	469.89	3	< 0.0001
		Year	444.84	3	< 0.0001
		Treatment x Year	140.26	9	< 0.0001
	Lacewing larvae	Treatment	517.26	3	< 0.0001
		Year	1156.77	3	< 0.0001
		Treatment x Year	298.74	9	< 0.0001
	Overwintering lacewing adults	Treatment	32.02	3	< 0.0001
		Year	18.96	2	< 0.0001
		Treatment x Year	5.74	6	0.0008
	R. padi	Treatment	137.74	3	< 0.0001
		Year	797.19	3	< 0.0001
		Treatment x Year	46.52	9	< 0.0001
	S. avenae	Treatment	326.92	3	< 0.0001
		Year	207.81	3	< 0.0001
		Treatment x Year	101.69	9	< 0.0001
	Coccinellidae	Treatment	56.18	3	< 0.0001
		Year	142.03	3	< 0.0001
		Treatment x Year	16.84	9	< 0.0001
	Syrphidae	Treatment	124.84	3	< 0.0001
		Year	103.28	3	< 0.0001
		Treatment x Year	20.44	9	< 0.0001
	parasitized aphid mummies	Treatment	74.77	3	< 0.0001
		Year	64.63	3	< 0.0001
		Treatment x Year	19.59	9	< 0.0001

Discussion

Our results show that it is possible to avoid aphid infestations in spring barley by combining different natural mechanisms as pest management tools in a deliberately compiled CBC strategy. We used a ternary blend of semiochemicals aimed at attracting adult green lacewings to the fields and stimulating their egg laying (ATTRACT), combined with floral buffer strips established at field edges to enhance food and habitat resources for adult lacewings throughout the field season (FOOD), and insect hotels equipped with the ternary attractant as hibernation shelters to support the overwintering survival of adult lacewings (SHELTER). By providing food, habitat, and hibernation shelter resources, we

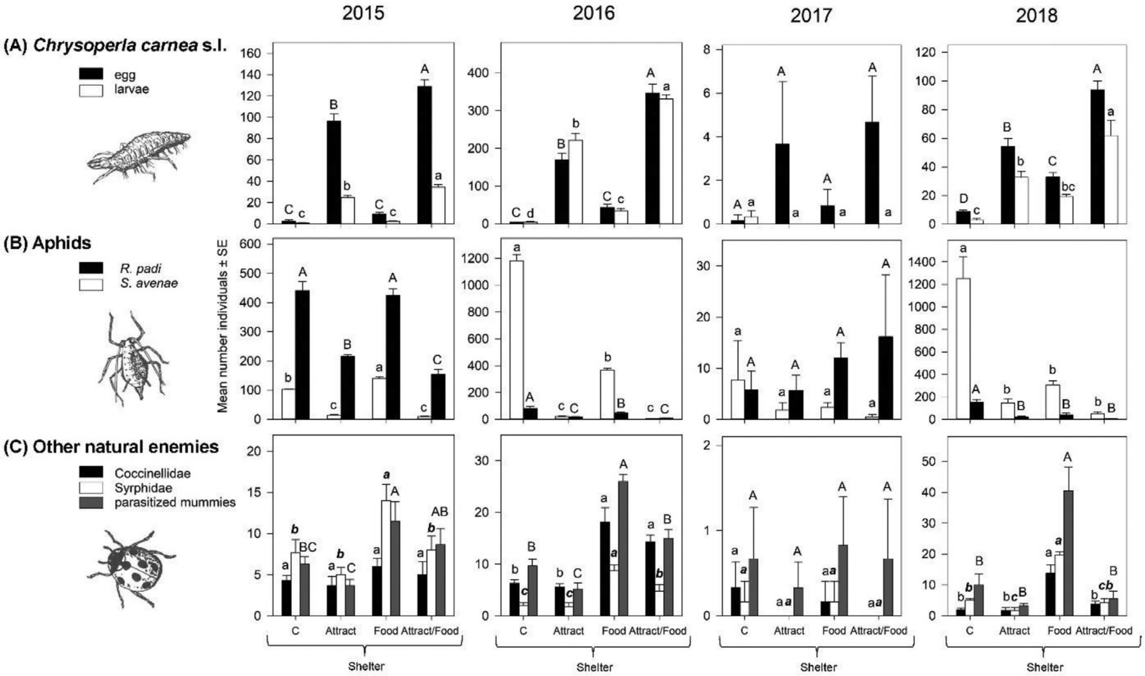

Fig. 3. Numbers of individuals (± SE) of natural enemies and aphids recorded over 4 years (2015−2018) in barley fields with the following four biological control regimes: insect hotels only without any additional measures, as control fields (C); lacewing attractants in the field and insect hotels (ATTRACT); floral buffer strips and insect hotels (FOOD); floral buffer strips combined with lacewing attractants in the field and insect hotels (ATTRACT/FOOD). Letters above the bars indicate significant differences between the four control regimes (GLMM, Tukey's test, $p < 0.05$): (A) lacewing eggs (uppercase) and larvae (lowercase); (B) *R. padi* (uppercase) and *S. avenae* (lowercase); (C) ladybirds (lowercase), hoverflies (bold italic lowercase), and parasitoid mummies (uppercase).

achieved all-season support for green lacewings and other natural enemies of pest arthropods. Concurrently, we boosted the existing lacewing population in the agricultural landscape by applying a ternary attractant and oviposition stimulator consisting of common floral volatiles and HIPVs that are naturally used by plants; methyl salicylate, phenylacetaldehyde, and acetic acid. The classical HIPV methyl salicylate is emitted by plants when they are attacked by pest arthropods in order to recruit natural enemies of those pests (Gadino et al., 2012; Molleman et al., 1997); acetic acid is used to signal that nectar (sugar) is available as food source (Knight et al., 2014; Landolt & Alfaro, 2000; Tóth et al., 2009); and phenylacetaldehyde acts both as food and as a SOS signal (El-Sayed et al., 2016; Jones et al., 2016; Tóth et al., 2006). In the present 4-year field study, we observed that a CBC strategy comprising ATTRACT, FOOD, and SHELTER facilitated a significant increase in biological control of aphids by establishing a resilient lacewing population in the agroecosystem. We were able to keep the aphid infestations below the damage threshold set for aphids in cereal crops in Norway (Andersen, 2003; Heggen et al., 2005) at all three locations in East Norway and over all years that the field experiments were conducted. In contrast, the aphid (*S. avenae*) infestations in the control plots were notably above the economic damage threshold at all locations in 2016 and 2018. In commercial barley production this implies that it would be necessary to apply pesticides to avoid economic loss due to aphid infestation. These findings show that by employing a multi-approach CBC strategy, the aphid pest population can be kept below the damage threshold and the use of synthetic pesticides can be avoided.

When considering the effect of field application of the ternary attractant to boost lacewing activity in the crop, we observed that the impact of the semiochemicals was strongest close to the dispenser, with regard to both increased egg laying by lacewings and suppression of aphids. Similar effects have been found in experiments testing the same ternary blend in cherry, apricot, walnut, and apple orchards (Jones et al., 2016; Koczor et al., 2010; Koczor et al., 2015; Pålsson et al., 2019; Tóth et al., 2009). In addition, the strong dose-response we found in both lacewing activity and aphid suppression supports the hypothesis that the ternary blend applied in a carefully selected dose can concentrate and regulate lacewing activity to a certain degree. This opens possibilities to fine-tune the application of volatiles (by both dose and lure density) in a crop as required and to adjust this to the respective agricultural landscape with its particular entomological resources and vegetation diversity.

It seems that non-target beneficials, specifically ladybirds, hoverflies, and parasitoids, were affected by application of the ternary attractant, although we found no dose-related effects. As shown and discussed in previous experiments

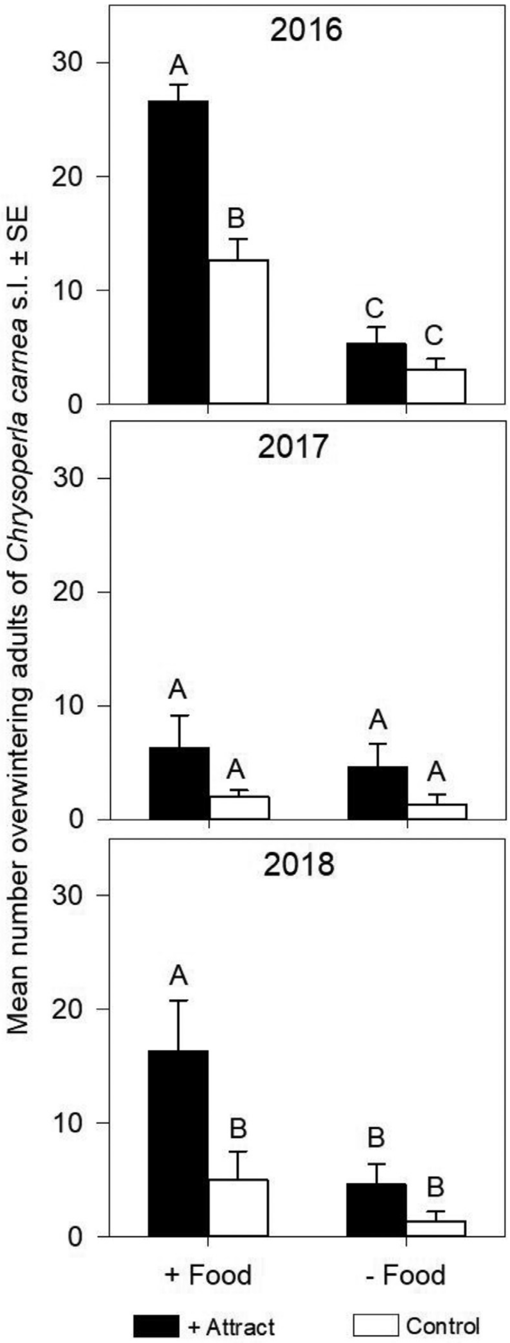

Fig. 4. Numbers of overwintering adults of *Chrysoperla carnea* s.l. (± SE) found in 2016, 2017, and 2018 in the insect hotels in early spring at the borders of barley fields for the following four treatments: insect hotel equipped with volatile dispensers and located near to the field block with floral buffer strips (+ Attract and + Food); insect hotel without volatile dispensers and located near to the field block with floral buffer strips (Control and + Food); insect hotel equipped with volatile dispensers and located near to the field block without floral buffer strips (+ Attract and - Food); insect hotel without volatile dispensers and located near to the field block without floral buffer strips (Control and - Food). Letters above the bars indicate significant differences between the four treatments (GLMM, Tukey's test, $p < 0.05$) for each of the three years.

(Pålsson et al., 2019), lacewings were the predominant beneficial insect species in our investigation, and they arrived earlier than ladybirds, hoverflies, and parasitoids in the studied fields (Pålsson et al., 2019). Such early arrival and predominance make the lacewings highly competitive with other beneficials, which might explain the composition and population dynamics of natural enemies we found in the current experiments. The type of early predation supported by the ternary attractant is crucial for avoiding aphid infestations that surpass the economic damage thresholds (Bianchi et al., 2006; Dedryer et al., 2010; Porcel et al., 2018; Zhang & Swinton, 2009).

In the 4-year period during which we performed this field study, there was marked variation in insect occurrence both in East Norway in general and in our experiments. In particular, we noted very high numbers of insects in 2016 followed by low numbers in 2017. Despite this, we observed a similar trend in the effects of our measures on lacewing activity in all four years (2015−2018) and indications of a robust lacewing population that recovered to a dense level in 2018. These population dynamics were observed for natural enemies and aphids in barley fields in the summer seasons, but also for overwintering lacewing adults at field borders in early spring. Pesticides can be excluded as cause for these variations in insect occurrence as no pesticides were applied in none of the experimental locations over the 4-year period. A lasting impact of the low population of aphids in 2017 on populations of lacewings and other natural enemies was not recorded. Starvation may have led to the lower numbers of natural enemies in 2017, but the populations of lacewings and other natural enemies recovered in parallel with aphids already in 2018 to a solid level. Although we have no data to explain these fluctuations in populations, these observations do constitute initial evidence that our CBC strategy can provide adequate food, habitat, and hibernation shelter resources to achieve year-round support for resilient green lacewing populations, thus implying that this approach can serve as a robust and effective tool for pest management. Nonetheless, more field studies with consecutive use of the CBC strategy at the same location must be performed over a greater number of years to achieve reliable assessment of this approach.

Using semiochemicals in the absence of associated herbivores to support natural enemies in an agricultural system, as we experienced in 2017, may lead to starvation with unpredictable consequences for the agroecosystem. Furthermore, boosting a particular species of beneficials in an agricultural system over time may influence the population dynamics of other species in the second and/or third tropic level in the ecosystem due to factors such as competition, cannibalism, and intraguild predation (Turlings & Erb, 2018). In any case, it is also necessary to consider the attraction of insects belonging to the fourth trophic level and the resulting interaction

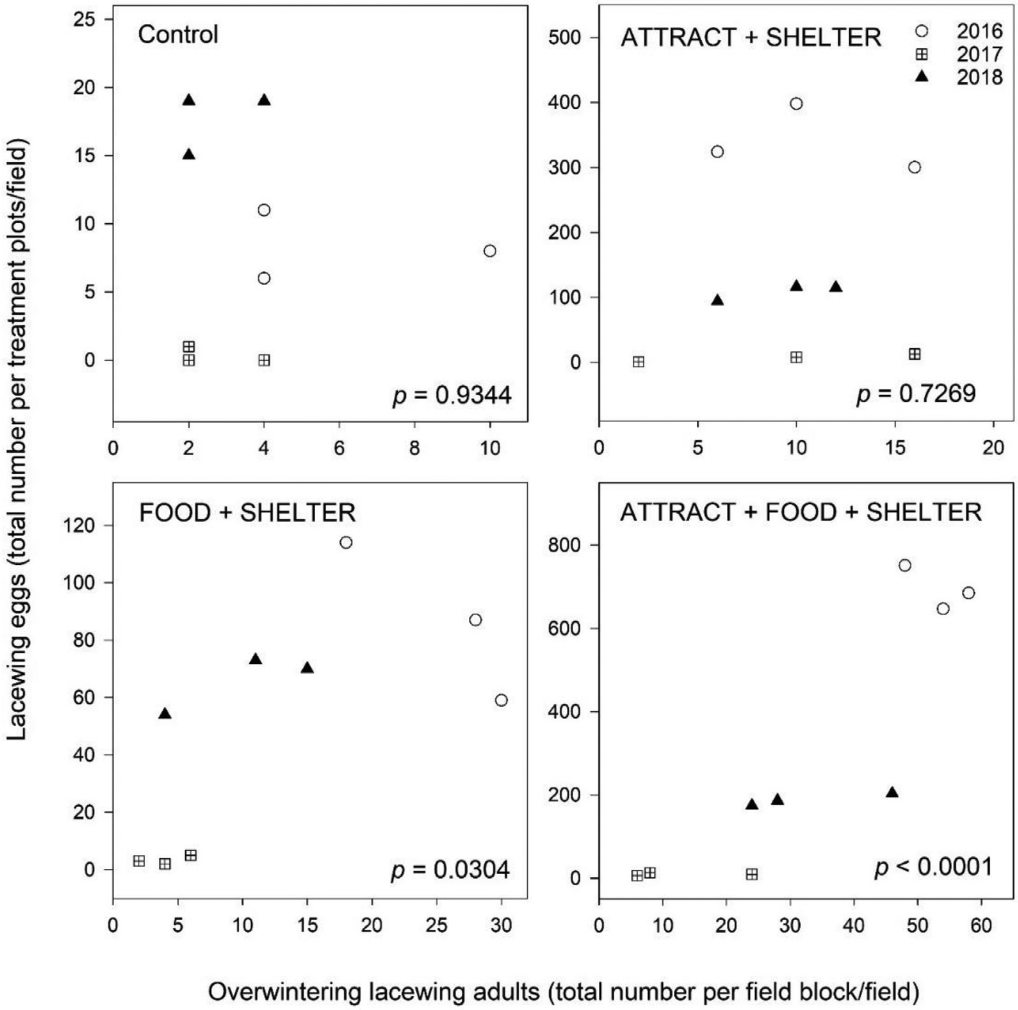

Fig. 5. Correlation of the overwintering lacewing adults found in the insect hotels in early spring (total numbers of overwintering adults per field block per field) with the lacewing eggs (total numbers of eggs per treatment plots per field) recorded in summer (2016-2018). The study was conducted in barley fields with the following four biological control regimes: Control, insect hotels only, without any additional measures; ATTRACT + SHELTER, application of the lacewing attractant in the field and insect hotels; FOOD + SHELTER, floral buffer strips and insect hotels; ATTRACT + FOOD + SHELTER, floral buffer strips combined with lacewing attractants in the field and insect hotels (GEE-based regression analyses, $p < 0.05$).

with the third level (Al Abassi et al., 2001; Orre et al., 2010).

Another issue to consider is the specificity of the semiochemicals used. The same compound or blend might have attractant or repellent effects on insects other than the target species. For example, methyl salicylate is known to be repellent to aphids in cereals such as *R. padi, S. avenae*, and *Metopolophium dirhodum* Walker (Pettersson et al., 1994). However, there is also evidence that methyl salicylate is attractive to pest insects, for example, the apple fruit moth *Argyresthia conjugella* Zeller (Lepidoptera: Yponomeutidae) (Bengtsson et al., 2006). It has been shown that methyl salicylate attracts not only lacewings but also other natural enemies, such as some ladybirds, hoverflies, and parasitoids (e.g., James & Price, 2004; Rodriguez-Saona et al., 2011). In contrast, the presence of methyl salicylate has been reported to have repellent effects on a parasitoid wasp of lepidopteran larvae feeding on Brassicaceae (Snoeren et al., 2010). Clearly, detailed studies of the effects of the applied semiochemicals on species other than the target species must be conducted to elucidate the population dynamics and consequences for the agroecosystem before full use of this strategy can be considered in pest management.

To reduce or even avoid undesired effects on non-target species in the agricultural ecosystem and at the same time support and stabilize the effects on the target species, semiochemicals can be combined with provision of alternative resources for prey, nectar, pollen, and habitat, such as the flower strips in our study (Hatt et al., 2018; Simpson et al., 2011). Our data clearly show that all beneficial species (i.e., the investigated target and non-target beneficials) were supported by the floral buffer strips. However, we also found evidence that applying the lacewing attractant boosted the green lacewings at the cost of ladybirds, hoverflies, and parasitized aphid mummies, even though flower strips were used as alternative resources for prey, nectar, pollen, and habitat. Our semiochemicals had no direct effect on the abundance of non-target natural enemies, if no additional food resources were provided (control with only SHELTER vs. ATTRACT + SHELTER). Still, it remains to be determined whether there will be effects on non-target species in the long term.

Conclusions

Our results show that the CBC strategy we applied here can lead to a promising increase in lacewing activity in the agricultural landscape and substantial biological control of aphids in barley that restricts the number of these pest insects to a level below the economic damage threshold. However, we found the first indication that the lacewing attractant might also influence the population dynamics of species other than the target species in the agroecosystem. A long-term field study testing application of the ternary attractant at the same location over many years is needed to evaluate the consequences for the species complex that exists in the agricultural landscape. Furthermore, we tested this CBC approach on barley, but the distribution of aphids, other pests, and natural enemies in other types of crops (e.g., perennials such as apples; Pålsson et al., 2019) can be much more uneven and unpredictable. Moreover, the susceptibility to pest infestations is much lower in crops other than barley, e.g., some vegetables with economic damage thresholds near zero. Thus, we recommend that additional experiments be conducted to develop CBC strategies similar to the one tested in our study, but specific to other crops and adapted to the respective agricultural landscapes with their particular entomological resources and vegetation diversity. Furthermore, additional research should support the feasibility of such a CBC approach both logistically and economically, including the acceptance by growers, for Norway and other countries. The use of a biodegradable formulation loaded with the ternary attractant such as the odour paste developed and tested by Pålsson et al. (2019) instead of using dispensers as described in this study, and the development of a mechanization of an application technique for such an odour paste might improve the feasibility of the overall CBC strategy. In addition, first preliminary studies in East Norway have indicated that maintenance and restoration of existing field edges might provide a similar or even better food and shelter source for lacewings and resulting biological control of aphids than annual floral buffer strips used in this study. Due to its topography and varied, often small, farm sizes, Norway still has more variation in semi-natural vegetation types, including numerous field edges, than most other countries in Western Europe. This offers a good opportunity to use already existing field edges as cost-effective food source and shelter habitat for natural enemies to increase biological pest management, and at the same time achieve higher acceptance by farmers and adoption of the overall strategy. However, further studies are needed to verify these preliminary findings.

Declaration of Competing Interest

None.

Authors' contributions

GT and GKK conceived ideas and designed methodology; GT and GKK collected the data; GT analysed the data; GT led the writing of the manuscript. Both authors contributed critically to the drafts and gave final approval for publication.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.baae.2021.01.004.

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