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Effect of faba bean nectar on longevity and fecundity of *Plutella xylostella* and its parasitoid *Cotesia vestalis*

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Abstract

Intercropping faba bean with vegetables provides a possibility to promote pest control and better nutrient cycling in sustainable agriculture. Faba bean produces extrafloral nectar which supports parasitoid wasps that play a role in the biological control of pest insects. However, adult lepidopteran pests also benefit from nectar, increasing their lifespan and the number of offspring they produce. Here, a laboratory-based study was conducted to assess the role of faba bean on the components of a *Brassica*-based host-parasitoid system. We measured how access to faba bean affected the longevity and fecundity of the brassica pest *Plutella xylostella* and its parasitoid *Cotesia vestalis*. It was also studied if odors of flowering faba bean disrupt host finding by *C. vestalis* in Y-tube bioassays and volatile organic compounds were analyzed to explain the olfaction-based choices made by the parasitoids. The longevity of *C. vestalis* was 6.6 times longer and the number of pupae produced almost 10 times greater when they had access to faba bean. Meanwhile, the longevity of *P. xylostella* was 3.6 times longer and it laid 4.6 times more eggs when provided access to faba bean. In Y-tube bioassays, *C. vestalis* females also oriented toward host-related odors of the damaged cabbage more than intact cabbage when odors of faba bean were mixed with both of them. In conclusion, faba bean provided sustenance to both pest insects and their natural enemies that prolonged their lifespans and their reproductive capacity.

Keywords *Cotesia vestalis* · Fecundity · Longevity · Sugar resources · *Vicia faba* · VOCs

Introduction

Parasitoid wasps provide ecosystem services that are fundamental to the biological control of insect pests (Bale et al 2008; Wang et al. 2019). However, resources for parasitoid wasps, such as available nectar for nutrition, are often limited in agricultural landscapes, especially in large monocultured fields (Tscharntke et al. 2005). Access to good quality nutrition improves parasitoid longevity and fecundity (Benelli et al. 2017; Chen et al. 2020; Jamont et al. 2013) and memory (Farahani et al. 2021), which can then result in better biological control of insect pests.

Floral (Albrecht et al. 2020) and extrafloral (Rogers 1985; Jones et al. 2017) nectar and honeydew (Tena et al. 2016) are the main sugar sources for insects in agroecosystems. Morphology of flowers (such as those characterized by long tubular petals) may limit the availability of nectar to parasitoids because they typically cannot access nectar from deeper parts of flowers due to their short mouthparts (Russell 2015). Extrafloral nectar (EFN), however, is easily accessible to insects with a range of different mouthparts, thus cultivating intercrops that produce EFN is a way to add resources for natural enemies to the vicinity of crop plants (Jones et al. 2017).

While nectar supports predators and parasitoids, it can also increase longevity and fecundity of herbivorous pest insects (Wäckers et al. 2007). For instance, Lepidopteran pests have a sucking proboscis to access nectar deeper in flowers, and the longevity and fecundity of the diamondback moth (*Plutella xylostella,* Lepidoptera: Plutellidae) has been shown to increase when given access to nectar-producing buckwheat (*Fagopyrum esculentum*: Polygonaceae) in a laboratory study (Chen et al. 2020). Diverse strips of

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flowering plants are considered as resources for beneficial insects in diversified cropping systems (Albrecht et al. 2020) but selectively cultivating flowers that support parasitoids can enhance biological control (Géneau et al. 2012). The same sugar source can have different effects on the longevity of different parasitoid species (Lavandero et al. 2005), so assessment of multiple parasitoid-nectar provider systems is needed.

Parasitoid females forage for their hosts by first locating the host habitat, from where they can locate the host itself and finally accept the suitable host (Vinson 1976). Olfactory cues are important in host location and selection processes because they are a component of plant indirect defense whereby plants emit volatile organic compounds (VOC) after herbivore damage to attract natural enemies of herbivores (De Moraes 2000). Background VOCs can mask resource-indicating odors (Schröder and Hilker 2008). Therefore, plant community structure and its VOC emissions and profiles need to be considered when selecting intercrops for efficient biological control. In terms of biocontrol, it is important that plants with accessible nectar are attractive to parasitoids (Wäckers 2004).

Plants of the Fabaceae represent interesting intercrop options due to their capability to improve soil properties by fixing atmospheric nitrogen (Sabagh et al. 2020). Faba bean (*Vicia faba*: Fabaceae) produces EFN on dark spots on stipules and sepals of developing flowers (Köpke and Nemecek 2010) and parasitoids can visit them (Bugg et al. 1989). It starts production of EFN before flowering and continues until pods are developed. Intercropping white cabbage (*Brassica oleracea*) with faba bean has recently been studied in strip crop (Lepse et al. 2017) and intercrop (Shanmugam et al. 2022) systems, but the effect of faba bean on natural enemies of *P. xylostella* in cabbage fields is not well known. Faba been EFN increases longevity and fecundity of female *Diaeretiella rapae* parasitoids on the cabbage aphid, *Brevicoryne brassicae* (Jamont et al. 2013).

In this laboratory study we used a model system comprising white cabbage and faba bean as the experimental plants, the diamondback moth as the main pest of white cabbage, and *Cotesia vestalis* (Haliday) [=*C. plutellae* (Kurdjumov), (Hymenoptera: Braconidae)] a solitary, koinobiont endoparasitoid and biocontrol agent of *P. xylostella*. We tested how access to flowering faba bean with flowers and extrafloral nectaries affects the longevity and fecundity of both *C. vestalis* and *P. xylostella* and tested if odors of faba bean disrupt orientation of *C. vestalis* toward host-damaged cabbage plants in Y-tube olfactometry tests. In addition, volatile organic compound (VOC) emissions were analyzed to determine if they explain the orientation of *C. vestalis*.

We hypothesized that 1) access to faba bean would improve longevity and fecundity of *C. vestalis* and *P. xylostella* and 2) *C. vestalis* orientates in a Y-tube olfactometer

toward the odor of host-damaged cabbage plants rather than intact plants, but that the odor of non-host faba bean may reduce the efficiency of orientation.

Materials and methods

Plant growth and insect-rearing protocols

White cabbage seeds (*Brassica oleracea* var. capitata cv. Lennox F1) were purchased from Puutarhaliike Helle Oy, Finland, and landrace faba bean (*Vicia faba* cv. Aunus) seeds were purchased from Maatiainen—The Finnish Landrace Association, Finland. Faba bean and cabbage were grown in one-liter pots in a 3:1:1 mixture of peat, soil, and sand. For the longevity and fecundity test of *P. xylostella*, cabbage was sown in $5 \times 5 \times 5$ cm pots. Plants were reared in a climate-controlled room (L16/D8). All plants were fertilized twice per week with 0.2% Taimi Superex NPK 19-4-20 fertilizer (Kekkilä Oyj, Finland).

P. xylostella and *C. vestalis* were reared in polycarbonate cages ($33 \times 33x60cm$) with meshed fabric sides with a L16/D8 photoperiod at 21 ± 2 °C. Plants for insect rearing were grown as described above. *P. xylostella* were reared on broccoli plants. *C. vestalis* were collected from Maaninka research station of the Natural Resources Institute Finland in Kuopio, Finland (27°19′E, 63°8′N) 1 year prior to the experiment from an unsprayed brassica field. Parasitoids were offered *Brassica* plants, mainly broccoli, (*Brassica oleracea* var. Italica) infested with second to third instar larvae of *P. xylostella*. Adult diamondback moths and wasps were provided approximately 30% honey solution diluted in water and soaked into cotton wool while rearing.

C. vestalis used in experiments were collected from rearing cages as pupae and placed into empty cages until emergence. *P. xylostella* females and males were identified at the larval stage and reared on broccoli plants in separate cages until adult emergence. Adult parasitoids and moths were offered water soaked in cotton wool, but no additional food sources were offered prior to or during experiments. *C. vestalis* females were kept together with males so that they had an opportunity to mate before Y-tube tests, but mating status was not further verified.

Effect of faba bean on the longevity and fecundity of *P. xylostella*

Experiments were conducted in $33 \times 33 \times 60$ cm polycarbonate plastic cages with meshed sides, in a laboratory with conditions of 20 ± 2 °C and 50–60% relative humidity. Cages were illuminated from above with fluorescent lamps with a 16:8 L/D cycle. Two cages were used per replicate: one cage with one 4–6-week-old faba bean plant and the second

without faba bean. At the beginning of the experiment, maximum 12-h-old *P. xylostella* males and females were introduced to both cages with one male and one female per cage. A 2-week-old cabbage plant was placed in each cage. Cabbage plants were replaced every 48 h and eggs laid on them were counted. Eggs laid on the walls of the cage were also counted and then removed. During the experiment, we ran out of 2-week-old cabbage plants and utilized 3- to 4-week-old cabbage plants after *P. xylostella* had ceased laying eggs. Changing of the cabbage plants was continued for as long as one of the adults was alive. Faba bean was watered every 1 to 2 days and the plant was replaced with a new one once or twice during the experiment at the point that flowers started to wither. The presence of extrafloral nectaries and the condition of flowers was checked daily. The average lifespan of moths in each cage was recorded because the sex of the moths was not determined at the adult stage.

Effect of faba bean on the longevity and fecundity of *C. vestalis*

The experiment was conducted in $33 \times 33 \times 60$ cm polycarbonate plastic cages with meshed sides in a laboratory with conditions of 20 ± 2 °C and 50–60% relative humidity. Cages were illuminated from above with LED lamps with a 16:8 L/D cycle. For each replicate, there were three cages: one to test for the longevity and fecundity of *C. vestalis* with access to one faba bean plant and the second without a faba bean plants. The third cage was established to monitor larval mortality without parasitoids.

At the beginning of the experiment, a cabbage leaf—infested with 30 s- to third-instar *P. xylostella* larvae—was placed into each test cage and maximum 12-h-old parasitoids (one male and one female) were introduced to the cages. Cabbage leaves were prepared by placing a leaf from a 4- to 5-week-old cabbage plant into a 50-ml plastic tube filled with tap water. The leaf stem was sealed with parafilm to the opening of the tube. Larvae of *P. xylostella* were introduced onto the leaves with a paintbrush, 0.5–1 h prior to placing the leaf in the parasitoid cage. Faba bean plants were watered every 1 to 2 days and replaced twice during the experiment at the point that flowers started to wither. The presence of flowers and extrafloral nectaries were checked daily.

Parasitoids were monitored daily and the dates when the male and female parasitoids died were recorded. As long as the female parasitoid was alive, cabbage leaves and larvae were removed daily and replaced with fresh leaves infested with new larvae. Larval mortality without parasitoids was treated similarly for as long as the female parasitoid in that replicate was alive. Larvae removed from the test cages were reared in plastic Petri dishes (diameter 9 cm) with 25-ml 1.5% micro agar (Duchefa Biochemie, Haarlem,

the Netherlands) prepared with water, without additives. A round (diameter 8 cm) cabbage leaf disk was placed on top of agar for the larvae to feed on (agar was used to keep the cabbage leaves moist). Larval frass was removed every other day and new cabbage leaves were added. Larvae removed from the test cages were reared until *P. xylostella* and *C. vestalis* had pupated and the number of pupae of each was recorded.

Olfactometry tests with *C. vestalis*

Orientation of *C. vestalis* females in Y-tube olfactometer tests was observed for the following odor pair combinations: 1) intact cabbage plants (iCA) vs. *P. xylostella*-damaged cabbage (dCA); 2) *P. xylostella*-damaged cabbage with faba bean (dCA + FB) vs. *P. xylostella*-damaged cabbage (dCA); 3) intact cabbage with faba bean (iCA + FB) vs. *P. xylostella*-damaged cabbage alone (dCA); and 4) *P. xylostella*-damaged cabbage with faba bean (dCA + FB) and intact cabbage with faba bean (iCA + FB). Five plant pairs were used for each odor pair combination with ten *C. vestalis* females assayed per pair, giving a total of 50 *C. vestalis* tested for each odor pair combination. Faba bean and cabbage plants were 4 to 6 weeks old, and faba bean had flowers and extrafloral nectaries. To elicit damage to the cabbage plants, 20 *P. xylostella* larvae of the third or early fourth instars were added to the cabbage leaves, left to feed for 24 h, and then removed just before the olfactory tests.

This olfactory experiment was conducted with a Y-tube olfactometer of the following dimensions; main arm 10.5 cm, other arms 10 cm, inner diameter 1.6 cm, and angle between the two arms ~ 90°. The Y-tube was placed in a light green plastic container and was illuminated from above with a lamp (LIVAL Shuttle Plus Finland, Max 24 W/230 V). Plants were placed in 22-L glass desiccator chambers with two inlets. Purified air (AADCO 474-30 Ultra High-Purity Zero Air Generator (ZAG)) was pumped into and through the chambers containing the odor sources and then into one of the Y-tube arms. The air flow arriving at a Y-tube arm was set to 360 ml/min with a maximum difference of 10 ml/min per test between arms. The air flow rates were determined for each plant combination with a mini-Buck calibrator (M-5, A.P. Buck Inc., Orlando, Florida, USA). Before starting a new test pair, desiccator chambers and Y-tubes were cleaned with 70% ethanol and left to dry, and Y-tubes were heated to 120 °C for 1 h. The Y-tube was rotated 180° after each parasitoid was assayed and replaced after every 10 insects. The olfactometer treatments were alternated between left and right olfactometer arm after every ten insects to prevent locational bias. Prior to the experiment starting, a test was conducted with 20 *C.*

vestalis females and two empty desiccators as odor sources to determine if there was a bias in the system.

Plants were placed into the desiccator chambers for 10 min prior to Y-tube tests starting to enable the system to stabilize. Maximum 2-day-old female *C. vestalis* adults that had been offered only water soaked into cotton wool were used in experiments. Parasitoids were introduced individually into the main arm of the Y-tube via two-cm long pieces of plastic tube with a fabric mesh covering one end. The tubes fit to the main arm of the Y-tube and the mesh cover allows air to flow through the system. Parasitoids were monitored for 300 s, starting from the moment the tube was placed into the main arm. The time spent by each insect in the left and right arm of the Y-tube were recorded and a choice was recorded for the arm in which the insect spent the longer time. An arm entry was considered if the entire body length of the parasitoid was completely within the arm and clearly beyond the junction between the two arms. Parasitoids that did not move into either of the arms were recorded as non-decisive. Each parasitoid was tested once.

Olfactometry test: collection and analysis of volatile organic compounds

VOC samples were collected from the Y-tube system to get information about the total plant emissions and VOC profiles during the olfactory choice tests. In the course of Y-tube tests, five randomly selected VOC samples were collected to represent each odor pair type ($n = 5$ for each odor source). VOC samples were collected by attaching a Stainless steel tube filled with Tenax TA adsorbent (150 mg) to the outlet of the desiccator chamber and pulling air through the tube with a vacuum pump for 30 min. Sampling of VOCs started after conducting Y-tube tests for 10 insects, when the plants had been in the desiccator chambers for 1 h. Total VOC emission of bean plants alone in desiccators were measured similarly to the odor sources used in Y-tube tests.

Samples were analyzed by gas chromatography–mass spectrometry (GC-MS-QP2020 and TD-30R, Shimadzu Company, Kyoto, Japan). Compounds were thermally desorbed from the sample tube at 300 °C for 10 min. Compounds were cryofocused in a cold trap at −20 °C and subsequently injected onto an ZB-5MS plus capillary column (60.0 m × 0.25 mm i.d. × 0.25 µm film thickness, Phenomenex, Torrance, California, USA). The carrier gas was helium. The column temperature was first held at 40 °C for 1 min. Thereafter, the temperature was programmed to increase from 40 to 125 °C at 5 °C min^{-1} and finally to 250 °C at 10 °C min^{-1}.

Compounds were identified with external standards, one series for terpenoids and one for green leaf volatiles (GLVs) (Sigma-Aldrich, USA) and the Wiley library (see Appendix Tables 2 and 3). Detected compounds that did not have an available standard were quantified with chemically similar standards. For example, 1,8-cineole was used for oxygenated monoterpenes, caryophyllene for sesquiterpenes, and α-pinene for monoterpenes and other compounds. For the compounds which were not possible to identify with standards and library, retention indices RI were calculated based on a series of alkanes C8-C20 injected as an external standard.

VOC emission rates (E) at the outlet of each desiccator chamber were calculated as follows: $E = (X \times A_i)/(t \times A_o)$, where E is the emission rate expressed in ng h^{-1}, X is the mass of volatile compound in the sampling tube (ng), t is the sampling time (h), and A_i and A_o are the incoming and outgoing air flows through the desiccator chamber.

Statistical analysis

SPSS software (version 27, IBM SPSS Statistics; Chicago, IL, USA) was used to analyze longevity, fecundity, and mortality data. Longevity and fecundity data were analyzed with independent sample T test and Welch's T test, while mortality data were analyzed with Welch ANOVA followed by the Dunnett T3 test for pairwise comparisons. Longevity data of *C. vestalis* and *P. xylostella* were analyzed as averages of male and female longevity per replicate. Fecundity of *C. vestalis* was analyzed as the total number of pupae produced per female, and the fecundity of *P. xylostella* was analyzed as the total number of eggs deposited per female. Mortality of *P. xylostella* larvae was analyzed as averages per treatment per insect replicate. The normality was tested using the Shapiro–Wilk test and homogeneity of variances was tested using the Levene's test and log$_{10}$ transformation was performed to data on the number of eggs and pupae to meet the assumptions of the *T* test and Welch's *T* tests.

Results of Y-tube bioassays were analyzed with a generalized linear mixed model (GLMM) with a binomial distribution and logit link function, using the lme4 package (Bates et al. 2015) in R version 4.3.2 (R Core Team 2023) and RStudio (Posit Team 2023). Insects nested within each plant pair were included in the model as random effects to avoid pseudoreplication (parasitoid females tested with the same plant pair). Wald test was used to extract the P-value of the intercept. Statistical analysis of the test for biases in the Y-tube system with two empty desiccators was conducted with a generalized linear model (GLM) with a binominal distribution and logit link function. Back-transformed estimated margin means for the standard errors were extracted with the 'emmeans' package (Lenth 2021).

VOC data were analyzed with SPSS software for each odor presented in the Y-tube, both as a full profile of all compounds in a VOC blend and as groupings of compounds, including the total volatile emission, green leaf volatiles, monoterpenes, and sesquiterpenes. Total emission

and emission by compound groups were analyzed with Welch ANOVA followed by Dunnett T3 test for pairwise comparisons. Individual compounds were analyzed with Kruskal–Wallis test followed by the Bonferroni test due to assumptions for data normality not being met for several compounds.

Principal Component Analysis (PCA) was performed on VOC data with SIMCA 17.0.1 (Umetrics, Umeå, Sweden) to visualize differences between VOC blends emitted by odor sources used in the Y-tube test and to determine which VOCs are important for the separation of the different odor sources. The loadings of the PCA model were identified to determine which compounds contributed most to the variation among the odor sources. The PCA was run separately for the VOC data representing the odor sources used in Y-tube tests and for the same data with the inclusion of faba bean (which was not used as an odor source alone). The aim was to determine the contribution of faba bean to the odors of the iCA + FB and dCA + FB plant mixtures. One outlier was removed from the dCA treatment for both models because the values were exceptional and separated strongly in the score plots. To see if odor sources were significantly separated, the PCA scores were tested with SPSS software for the first two PCs with one-way ANOVA, using odor source as a fixed factor.

Results

The effects of faba bean on the longevity and fecundity of *P. xylostella* and *C. vestalis* adults

Access to faba bean significantly increased the adult longevity and fecundity of *P. xylostella* and *C. vestalis* compared to a water-only control. The average adult longevity of *P. xylostella* was almost 4 times greater with faba bean compared with access to water only (*T* test: $t_{10} = 7.217$, $P < 0.001$) (Fig. 1a), while the mean longevity of *C. vestalis* adults with faba bean access was almost 7 times greater

compared to the control with access only to water (Welch *T* test: $t_{5.339} = 7.050$, $P = 0.001$) (Fig. 1b).

When provided access to faba bean, the mean fecundity of *P. xylostella* females was almost 5 times higher than the control with access only to water (*T* test: $t_{10} = 2.544$, $P = 0.029$) (Fig. 2a), and the mean fecundity of *C. vestalis* was almost 10 times greater than the control resulting in production of an average 222 pupae per female (*T* test: $t_{10} = 4.005$, $P = 0.002$) (Fig. 2b). There were no statistically significant differences in the mortality of *P. xylostella* larvae between the *C. vestalis* treatments with and without bean, and the larva control without *C. vestalis* (Welch ANOVA: $F_{2, 9.176} = 0.940$, $P = 0.425$) (Fig. 2c).

Behavioral responses of female *C. vestalis* to odors of faba bean and white cabbage

To test for biases in the system, two empty desiccators were used as odor sources with 20 *C. vestalis* females assayed. There were no significant differences in choices between the Y-tube arms (GLM: $Z = -0.800$, $P = 0.4231$). Two parasitoids selected the left side of the olfactometer and four selected the right side, while 14 did not make a choice.

Y-tube tests showed that parasitoid females were significantly attracted to odors of damaged cabbage over intact cabbage both with (dCA + FB vs. iCA + FB, GLMM: $Z = 2.450$, $P = 0.0143$) (Fig. 3) and without faba bean (dCA vs. iCA, GLMM: $Z = 2.131$, $P = 0.0331$). Also, an odor mixture of herbivore-damaged cabbage and faba bean attracted significantly more parasitoids than herbivore-damaged cabbage alone (dCA + FB vs. dCA, GLMM: $Z = 2.190$, $P = 0.0285$), but an odor mixture of intact cabbage and faba bean did not attract significantly more parasitoids than herbivore-damaged cabbage (iCA + FB vs. dCA, GLMM: $Z = 1.873$, $P = 0.0611$) (Fig. 3).

Fig. 1 Average lifetime (longevity) of **a** *P. xylostella* and **b** *C. vestalis*. Values presented are means ± SE calculated from mean of male and female lifetimes per replicate (*n* = 6 per treatment). Different letters indicate differences between the treatments (Independent samples *T* test, *P* < 0.05)
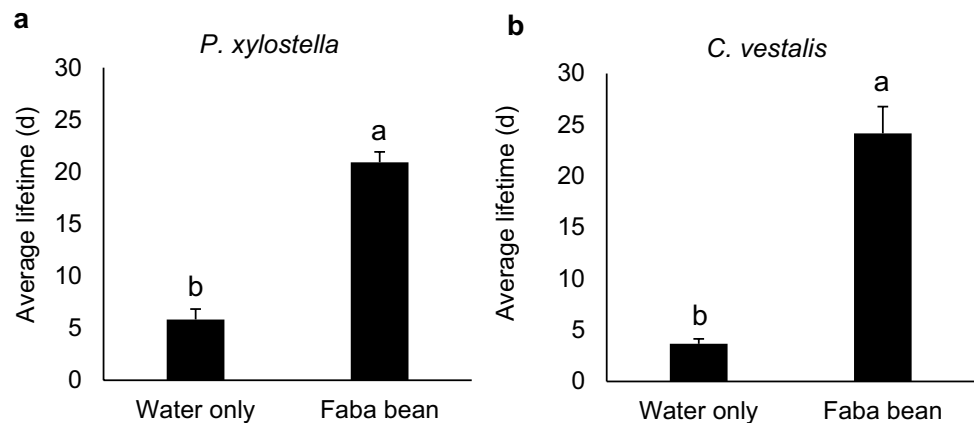
Fig. 2 Average total number of **a** *P. xylostella* eggs. **b** *C. vestalis* pupae produced by each female with either faba bean or water only. **c** Mortality of *P. xylostella* larvae in the fecundity experiment (larvae that did not develop into *C. vestalis* or *P. xylostella* pupae). Values presented are means ± SE ($n = 6$ per treatment). Different letters indicate difference between the treatments (**a** and **b**: Independent sample *T* test, **c**: Dunnett T3 after Welch ANOVA, $P < 0.05$, *n.s.* n-significant)
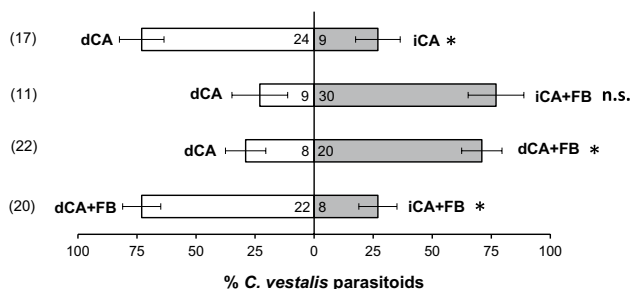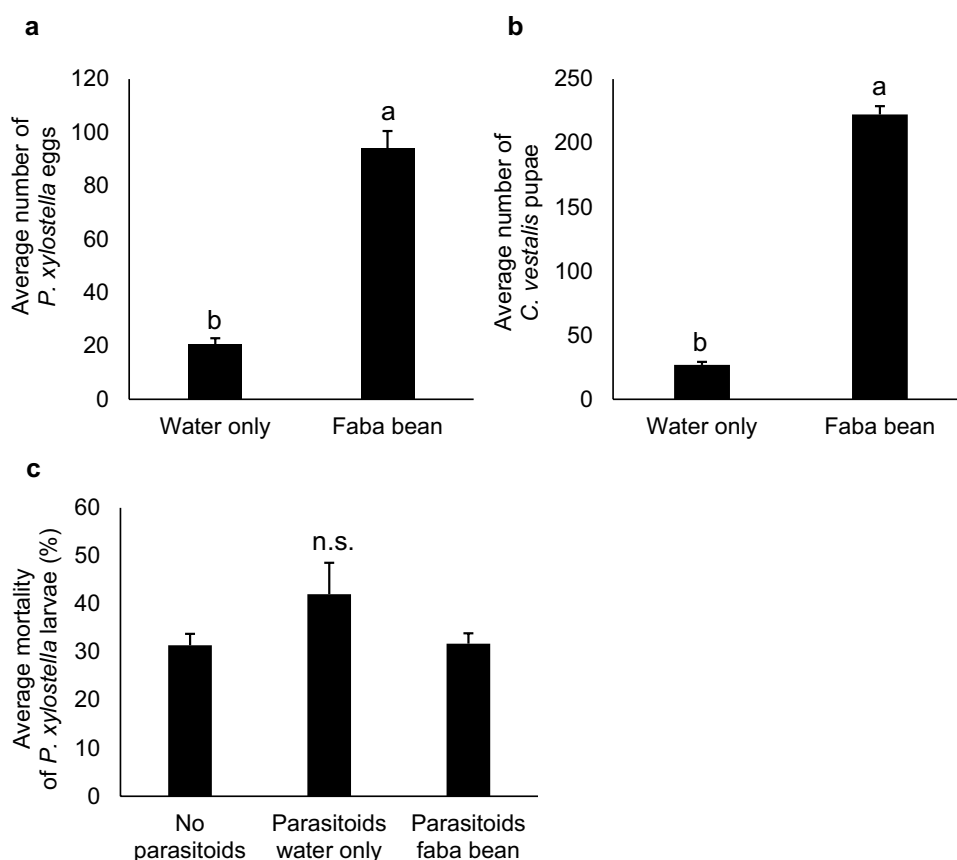
Fig. 3 Orientation of *Cotesia vestalis* females to odor pairs presented in a Y-tube olfactometer (% of parasitoids making a selection). The odors presented were intact white cabbage (*Brassica oleracea* var. *capitata*) (iCA), *Plutella xylostella*-damaged white cabbage (dCA), and selected combinations of white cabbage and faba bean (*Vicia faba*) (FB). Fifty parasitoids were tested per combination, and the number on the bar indicates the number of parasitoids that selected that odor source. The number in parentheses is the number of individuals not making a selection. Error bars represent the standard error of the estimated marginal mean. Asterisks indicate significant differences based on generalized linear mixed models (GLMMs) with a Wald test ($P < 0.05$; ns, $P \geq 0.05$)
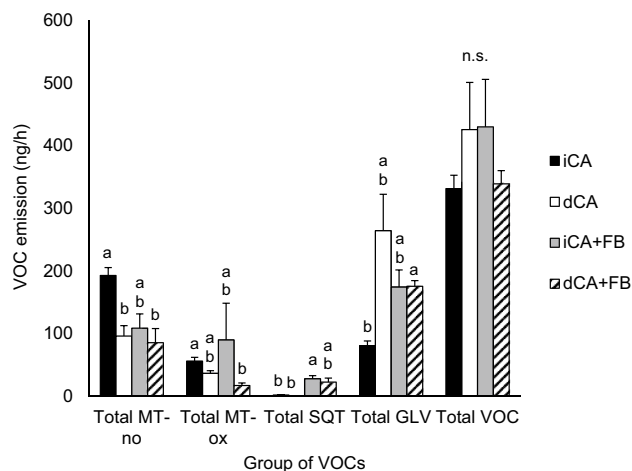
Fig. 4 Total VOC emissions of non-oxygenated monoterpenes (MT-no), oxygenated monoterpenes (MT-ox), sesquiterpenes (SQT), green leaf volatiles (GLV), and total VOC emissions (ng/h) measured from Y-tube system. *iCA* intact cabbage, *dCA* damaged cabbage, *FB* faba bean. Values presented are means ± SE ($n = 4$–5 per treatment). Different letters indicate difference between the treatments (Dunnett T3 post hoc test after Welch ANOVA, $P < 0.05$)

Volatile Organic Compounds

According to Welch ANOVA, total VOC emission (ng h⁻¹) did not differ significantly between the odor sources

$(F_{3, 7.288} = 0.625, P = 0.620)$, but there were statistically significant differences in total non-oxygenated $(F_{3, 7.991} = 7.235$ $P = 0.011)$ and oxygenated monoterpenes $(F_{3, 8.041} = 7.265$ $P = 0.011)$, sesquiterpenes $(F_{3, 6.840} = 10.838$ $P = 0.005)$, and GLVs $(F_{3, 7.154} = 16.740$ $P = 0.001)$ (Fig. 4). According to Dunnett T3 post hoc test, odors of iCA had significantly more oxygenated and non-oxygenated monoterpenes and less green leaf volatiles than dCA + FB. There were also statistically significant differences in emissions of several individual compounds according to Kruskal–Wallis test (Table 1). VOC emission of faba bean (ng h^{-1}) is shown in Appendix Table 4.

Odor sources used in the Y-tube test (iCA, dCA, iCA + FB, dCA + FB) separated statistically significantly along Principal Components 1 and 2 (One-way ANOVA: $F_{3, 15} = 18.597$, $P < 0.001$ and $F_{3, 15} = 8.168$, $P = 0.002$, respectively). According to Tukey's post hoc test along PC1, odors of iCA separated clearly from all the other odor sources and along PC2, odor of dCA separated from iCA + FB and dCA + FB (Fig. 5). The first two PCs together explained 45.9% of the total variation in the dataset (Fig. 5). PCA loadings reveal that eight monoterpenoids contribute the most to the separation of the VOC blend of cabbage from the other odor sources (Fig. 5).

Principal Component Analysis was also conducted with VOC data of faba bean included (Appendix Fig. 1.). In this case, the PCA scores for the odor sources were significantly separated along PCs 1 and 2 (Welch ANOVA: $F_{4, 9.188} = 19.778$, $P < 0.001$ and: $F_{4, 8.801} = 5.772$, $P = 0.015$, respectively). According to Dunnett T3 post hoc test along PC 1, the clearest separation was between FB and iCA, which both separated from all the other odor sources (dCA, dCA + FB, and iCA + FB), which grouped close to each other. Along PC 2, iCA and dCA separated from dCA + FB.

Discussion

Faba bean increases longevity of pests and parasitoids

Faba bean increased the longevity and fecundity of both the cabbage pest and its natural enemy in our model system in the laboratory. Faba bean proved to be a good nutritional source for *C. vestalis*, resulting in improved survival and reproductive capacity. Parasitoids were observed to visit and feed regularly from EFNs located at stipules, which confirms that EFN was an important energy source for them, while it was not confirmed if parasitoids visited faba bean flowers. The average longevity of *C. vestalis* females was higher than in laboratory-based tests in the literature, with earlier reports documenting a 12.5-day lifespan with access to floral nectar of alyssum (Chen et al. 2020) and a maximum 5-day lifespan

with selected flowers, such as cosmos (Chau et al. 2019). On the other hand, Shimoda et al. (2014) found that *C. vestalis* females lived in greenhouses for over 30 days with artificial food providing supplementary glucose and fructose.

In this test, *C. vestalis* females without access to faba bean started to oviposit in *P. xylostella* larvae immediately after they were transferred to the test cage. Meanwhile, parasitoids with access to faba bean took longer to start ovipositing and they were observed to visit EFNs of faba bean. Lee and Heimpel (2007) observed a similar behavior for *Diadegma insulare* whereby sugar-fed females approached patches of *P. xylostella* less actively in the short term. The reason for this behavior could be that parasitoids with access to food avoided unnecessary oviposition. Mortality of *P. xylostella* larvae was slightly higher when *C. vestalis* was offered only water, which could be explained by parasitoids over-attacking larvae and consequently increasing mortality.

Access to faba bean benefitted not only the parasitoid but also its host *P. xylostella* which had increased longevity and fecundity. This means that *P. xylostella* could potentially benefit from a faba bean intercrop under field conditions and cause more damage to Brassicaceous crops. It is recognized that nectar resources in the field can benefit lepidopteran pests (Wäckers et al. 2007), but the effect varies between different species of flowering plants (Chen et al. 2020; Lavandero et al. 2005). Winkler et al. (2005) also found that access to a sugar source increased the lifespan of both *P. xylostella* and its parasitoid *D. semiclausum.*

In field conditions, herbivore damage by aphids can increase (Jaber and Vidal 2009) or reduce (Yoshida et al. 2018) extrafloral nectar secretion rate. At the same time, honeydew produced by aphids is a nutrition source for some parasitoids (Tena et al. 2016; Luquet et al. 2021). Mechanical damage and soil nutrient status can also affect the number of extrafloral nectaries (Mondor and Addicot 2003; Mondor et al. 2006), but the secretion rate of extrafloral nectar does not necessarily change (Mondor et al. 2013). EFN also supports other natural enemies of *P. xylostella*, like lacewings (Limburg and Rosenheim 2001), spiders, wasps, and predatory beetles (Heil 2015), which can aid biological control. In the field, parasitism rates have been reported to be higher closer to nectar-providing plants (Jamont et al. 2014), therefore intercropping nectar-providing crop plants could be space- and resource effective ways to promote pest control.

Faba bean affects the orientation of *C. vestalis* toward host-related odor cues from damaged cabbage

Naïve *C. vestalis* females oriented toward the odor of damaged cabbage significantly more frequently than intact cabbage. This is a well-documented phenomenon (Pinto et al.

Table 1 Medians and Interquartile Ranges (IQR) of Volatile Organic Compound emissions (4–5 randomly selected samples) measured from air incoming to arm of Y-tube

Emission (ng h⁻¹)	iCA (n=5)		dCA (n=4)		iCA+FB (n=5)		dCA+FB (n=5)		H value	P value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR		
Non-oxygenated monoterpenes										
α-Thujene	22.9 a	(20.4–23.4)	9.8 ab	(7.4–12.4)	9.3 ab	(6.8–10.1)	4.7 b	(3.9–8.9)	10.789	**0.013**
α-Pinene	8.9	(8.1–10.6)	5.0	(4–6.2)	4.5	(3.6–5.6)	2.6	(2.5–9.9)	3.905	0.272
Camphene	n.d		n.d		n.d		*		0.923	0.820
Sabinene	81.6 a	(68.4–88.5)	33.1 ab	(26.1–43.6)	31.1 ab	(28.7–41.8)	27.2 b	(18.6–29.2)	10.377	**0.016**
β-Pinene	5.8 a	(4.1–6.7)	3.6 ab	(3.2–5)	3.0 ab	(2.6–3)	2.1 b	(1.3–4.6)	5.331	0.149
β-myrcene	27.7 a	(27.6–28.5)	11.5 ab	(9.2–15.6)	10.7 ab	(8.5–12.3)	6.1 b	(5.6–8.7)	12.688	**0.005**
3-Carene	n.d		0.0	(0–0.8)	n.d		1.0	(0–1)	4.625	0.201
α-Terpinene	2.0 a	(1.7–2)	1.1 ab	(0.8–1.1)	1.3 ab	(1.1–1.3)	0.0 b	(0–1)	12.561	**0.006**
Limonene	46.3 a	(44–47.5)	21.1 ab	(16.6–27.5)	20.9 ab	(18.7–24.7)	14.8 b	(7.8–29.2)	9.468	**0.024**
(E)-β-Ocimene	1.3	(1.2–1.6)	0.0	(0–0.5)	4.4	(3.3–7.8)	4.6	(2.1–5)	6.970	0.073
γ-Terpinene	2.8 a	(2.6–3.6)	1.3 b	(1.2–1.5)	1.7 ab	(1.6–1.8)	0.0 b	(0–1.9)	11.664	**0.009**
Oxygenated monoterpenes										
1,8-Cineole	37.6 a	(37.2–49.4)	16.7 ab	(14.4–20.8)	19.8 ab	(18.4–20.2)	9.3 b	(5.8–9.7)	12.670	**0.005**
(Z)-Sabinene hydrate	7.2 a	(6.8–7.6)	3.3 ab	(3.1–3.7)	2.1 ab	(1.9–3.6)	0.8 b	(0–1.4)	15.615	**0.001**
Linalool	n.d		n.d		*		*		1.911	0.591
Oxygenated mt RI 1281	0.0	(0–1.7)	2.9	(2.2–5.7)	n.d		n.d		6.423	0.093
Oxygenated mt RI 1306.5	n.d. a		2.5 a	(1.7–3.5)	n.d. a		n.d. a		8.097	**0.044**
Oxygenated mt RI 1317.5	n.d		0.4	(0–0.9)	0	(0–0.5)	0.9	(0.8–0.9)	5.762	0.124
Menthol	3.6	(2–5)	5.5	(3.5–7.7)	2.3	(2.1–2.4)	1.4	(1.4–1.5)	6.924	0.074
Terpinen-4-ol	1.5 a	(1.1–1.5)	1.1 ab	(1–1.2)	1.3 ab	(1.2–1.4)	0.7 b	(0.6–0.7)	10.102	**0.018**
Homoterpenes										
(E)-DMNT	n.d. b		4.3 a	(3.9–4.8)	n.d.b		2.6 ab	(0–3.5)	13.328	**0.004**
Sesquiterpenes										
Sesquiterpene RI 1407.5	n.d b		n.d.b		9 a	(7.7–11.6)	8.2 ab	(1.9–8.4)	13.274	**0.004**
Sesquiterpene RI 1475	n.d. b		n.d. b		5.2 a	(4.7–6.4)	4.1 ab	(0.9–6.4)	13.119	**0.004**
Sesquiterpene RI 1449.5	1.5 a	(1.5–1.9)	0.6 b	(0–1.2)	1.8 a	(1.6–1.9)	1.3 ab	(1.1–1.3)	11.660	**0.009**
(E)-Caryophyllene	n.d. b		n.d. b		6.8 a	(6.4–8.2)	5.0 a	(3.8–8.6)	15.120	**0.002**
α-Humulene	n.d. b		n.d. bc		0.0 a	(0–4.8)	0.0 ac	(0–2.4)	4.270	0.234
Green leaf volatiles										
(Z)-3-hexen-1-ol	n.d b		8.6 a	(8.3–13.7)	15.7 a	(12.8–18.3)	6.3 ab	(5.8–7.4)	13.142	**0.004**
1-hexanol	n.d a		2.5 a	(1.8–3)	0 a	(0–0)	0.0 a	(0–0)	8.690	**0.034**
(E)-2,4-hexadienal	n.d		0.0	(0–0)	0	(0–0)	0.0	(0–0)	2.800	0.423
1-Octen-3-ol	n.d ab		0.0 ab	(0–0.7)	1.7 ab	(0–2.1)	1.7 a	(1.4–3)	8.304	**0.040**
2-Methyl-2-hepten-6-one	3.9 ab	(3.2–4.4)	7.2 a	(4.6–10)	2.4 b	(2.1–2.6)	2.5 ab	(2.3–3.1)	11.617	**0.009**

Table 1 (continued)

Emission (ng h⁻¹) Non-oxygenated monoterpenes	iCA (n = 5) Median	IQR	dCA (n = 4) Median	IQR	iCA + FB (n = 5) Median	IQR	dCA + FB (n = 5) Median	IQR	H value	P value
(Z)-3-hexenyl acetate	n.d b		50.4 a	(45.9–99.2)	54 ab	(38.6–74)	0.0 ab	(0–24.2)	10.491	**0.015**
1-Hexyl acetate	n.d b		2.4 a	(2.2–4.4)	1.4 ab	(1.2–2.6)	n.d. b		13.899	**0.003**
2-Ethyl hexanol	16.3	(14.8–20.3)	17.0	(14.3–19.9)	15.2	(12.1–17.1)	14.1	(9.9–15.9)	1.074	0.783
Bicyclodihydropentadiene	n.d b		10.7 ab	(7.8–12.3)	n.d b		12.8 a	(8.7–13.7)	13.469	**0.004**
Nonanal	63.5 b	(41.5–67)	106.7 a	(96.4–115.1)	77.7 ab	(74.4–80.9)	79.3 ab	(78.6–131.6)	9.804	**0.020**
Isothiocyanates										
Allyl isothiocyanate	n.d. b		17.1 a	(1.5–37.7)	0.0 ab	(0–1.8)	2.0 ab	(1.2–2)	10.483	**0.015**
Phenethylisothiocyanate	n.d. a		0.9 a	(0–2.3)	n.d. a		n.d. a		7.917	**0.048**
Other nitrogen-containing compounds										
Estragole	n.d		n.d		0.0	(0–1.7)	1.2	(0–2.9)	6.091	0.107
8-nitro-3,5-methano-2,3,4,5-tetrahydro-1H-1-benzazepine	n.d		n.d		24.9	(24.3–29.6)	24.1	(0–24.3)	12.960	**0.005**
Other compounds										
Methylsalicylate	n.d		3.5	(2.7–5.9)	0.6	(0.5–0.8)	1.6	(1.4–3.1)	12.972	**0.005**
Geranyl acetone	5.1	(4.1–7.8)	5.4	(4.5–6.1)	3.5	(3.4–4.1)	5.6	(4.8–6.1)	4.322	0.229

RI indicates retention indices, which were calculated for unknown compounds based on a standard series of alkanes C8-C20

iCA intact cabbage, *dCA* damaged cabbage, *FB* faba bean

The *H*-values and *P*-values are from the Kruskal–Wallis test and the different letters indicate differences between the plot types (*P* < 0.05, Bonferroni post hoc test, marked with bold). *Compound was emitted only from very few replicates and not showing in the median
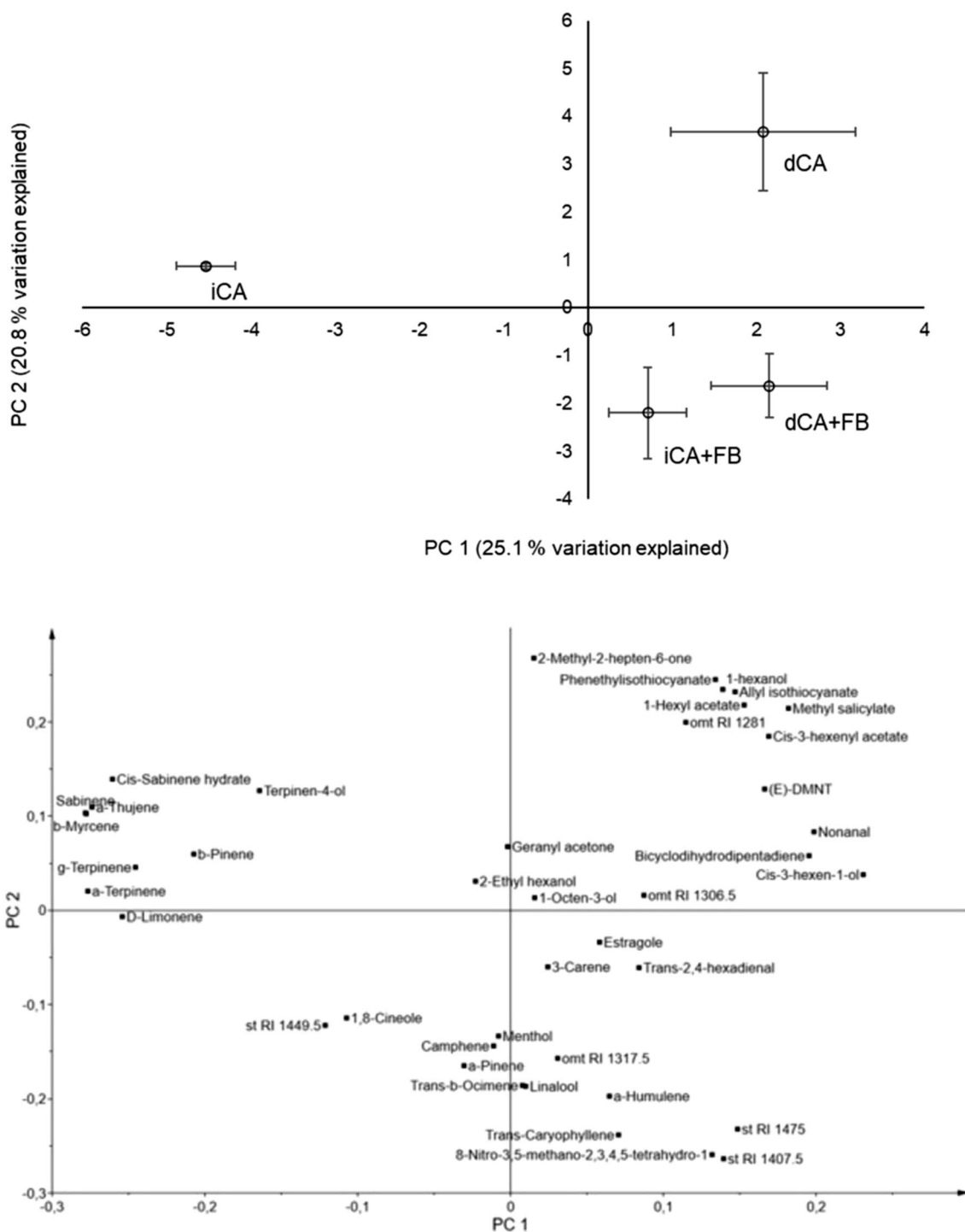
Fig. 5 Principal Component Analysis (PCA) of the VOC blends. Top panel: score plot of the samples showing the mean of each treatment score ± SE. Bottom panel: loading plot of the two components of the PCA, showing the contribution of each of the compounds toward the model

2007; Kugimiya et al. 2010; Girling et al. 2011; Uefune et al. 2017), whereby cabbage plants emit herbivore-induced plant volatiles (HIPV) that attract natural enemies of herbivores. In this study, parasitoids were also able to locate host-related plant odors when odors of faba bean were mixed with intact

and damaged cabbage. In addition, parasitoids oriented more toward odors of damaged cabbage mixed with faba bean than odors of damaged cabbage only. PCA revealed that the VOC blends emitted by intact cabbage differed significantly from all the other odor sources used in Y-tube tests, highlighting

induction of HIPVs from the damaged cabbage. Separation of odor combinations of intact and damaged cabbage with faba bean from the damaged cabbage was less clear in the PCA, which could explain orientation of *C. vestalis* toward the faba bean.

Frequent orientation of parasitoids toward odor sources with faba bean was an unexpected result. Faba bean plants emitted high amounts of GLVs such as (Z)-3-hexenyl acetate which was part of a synthetic odor blend shown to increase the number of *P. xylostella* parasitized by *C. vestalis* in field conditions (Uefune et al. 2012, 2021). On the other hand, (Z)-3-hexenyl acetate alone has not been shown to be attractive to *C. vestalis* in laboratory studies (Shiojiri et al. 2006; 2010). *Cotesia vestalis* has potential hosts other than *P. xylostella*, which could explain why it was attracted to odors of faba bean even though it is not reported to successfully parasitize lepidopteran pests feeding on fabaceous plants (Hiroyoshi et al. 2017). The nutritional status of parasitoid females could also have affected their orientation in the Y-tube. Parasitoids were not fed after emergence so they could have potentially oriented toward nectar-related odors of faba bean. In the field, parasitoids need to forage in complex environments, which can reduce their capacity to locate and attack hosts (Gols et al. 2005). Also, VOC emissions of plants may differ in the field compared to the laboratory, so it is important to study parasitoid foraging in field conditions in addition to laboratory experiments. These laboratory-based results show that *C. vestalis* might be attracted by odors of faba bean in addition to odors of host-damaged cabbage, potentially due to the nectar provided by the faba bean.

Conclusion

Faba bean has the potential to improve biological control by parasitoid wasps, which was emphasized by it significantly improving longevity and fecundity of *C. vestalis* in laboratory tests by providing sugar resources. Furthermore, odors of faba bean did not repel *C. vestalis*, which suggests that intercropping faba bean with cabbage could improve biological control of *P. xylostella*. However, *P. xylostella* also benefitted from access to faba bean as an increase in longevity and fecundity, and this should be considered when planning mixed cropping fields with nectar-rich companion plants. Parasitism of *P. xylostella* by *C. vestalis* should be studied in intercropped agricultural fields of cabbage and faba bean to understand if this method provides robust biological control.

Appendix

See Tables 2, 3, and 4.

Table 2 External standard series for terpenoids

Compound name	Purity (%)
α-Pinene	> 97
Camphene	95
β-Pinene	99
β-Myrcene	90
3-Carene	95
D-Limonene	95
Ɣ-Terpinene	95
Terpinolene	90
1,8-Cineole	99
Linalool	97
Camphor	96
Borneol	98
Terpinen-4-ol	97
α-Terpineol	98
Bornylacetate	97
(E)-β-Farnesene	> 90
α-Humulene	> 98
(E)-Caryophyllene	> 98
Aromadendrene	> 97
β-Elemene	> 98
α-Phellandrene	> 95

Table 3 External standard series for green leaf volatiles

Compound name	Purity (%)
(Z)-3-hexen-1-ol	98
1-hexanol	98
1-octen-3-ol	> 97
(E)-2-hexenal	98
Nonanal	95
(Z)-3-hexenyl acetate	> 98
(Z)-3-hexenyl butyrate	> 98
Methyl salicylate	> 99
(Z)-3-hexenyl isovalerate	> 98
(Z)-3-hexenyl tiglate	> 97

Table 4 The Medians and interquartile range (IQR) of volatile organic compound emission (ng h^{-1}) of faba bean measured from Y-tube test system ($n=5$)

Compound	Median	IQR
Non-oxygenated monoterpenes		
α-Thujene	n.d	
α-Pinene	9.5	(5.1–9.5)
Camphene	1.1	(0–1.1)
Sabinene	n.d	
β-Pinene	6.9	(0–6.9)
β-myrcene	1.6	(0–1.6)
3-Carene	4.7	(0–4.7)
α-Terpinene	n.d	
Limonene	9.2	(7.1–9.2)
(E)-β-Ocimene	2.9	(0–2.9)
γ-Terpinene	0.9	(0–0.9)
Total	28.2	(18.5–45.6)
Oxygenated monoterpenes		
1,8-Cineole	64.6	(3.1–64.6)
(Z)-Sabinene hydrate	n.d	
Linalool	3.8	(0–3.8)
Oxygenated mt RI 1281	8.1	(0–8.1)
Oxygenated mt RI 1306.5	4.8	(0–4.8)
Oxygenated mt RI 1317.5	0.7	(0–0.7)
Menthol	21.6	(7.1–21.6)
Terpinen-4-ol	n.d	
Total	93.2	(7.1–96.5)
Homoterpenes		
(E)-DMNT	n.d	
Sesquiterpenes		
Sesquiterpene RI 1407.5	7.2	(0–7.2)
Sesquiterpene RI 1475	3.9	(0.6–3.9)
Sesquiterpene RI 1449.5	2.6	(1.5–2.6)
(E)-Caryophyllene	2.6	(0–2.6)
α-Humulene	n.d	
Total	9.5	5.0–13.8
Green Leaf Volatiles		
(Z)-3-hexen-1-ol	339.8	(101.3–339.8)
1-hexanol	41.3	(8.6–41.3)
(E)-2,4-hexadienal	5.5	(0–5.5)
1-Octen-3-ol	9.9	(2.8–9.9)
2-Methyl-2-hepten-6-one	19.6	(6.1–19.6)
(Z)-3-hexenyl acetate	464.8	(27.9–464.8)
1-Hexyl acetate	13.4	(0–13.4)
2-Ethyl hexanol	37.9	(24.9–37.9)
Bicyclodihydropentadiene	n.d	
Nonanal	102.9	(57.6–102.9)
Total	958.2	(724.6–1206.0)
Other compounds		
Methylsalicylate	1.5	(0.8–1.5)
Geranyl acetone	11.1	(6.6–11.1)
Isothiocyanates		

Table 4 (continued)

Compound	Median	IQR
Allyl isothiocyanate	n.d	
Phenethylisothiocyanate	n.d	
Total isothiocyanates	n.d	
Other nitrogen-containing compounds		
Estragole	n.d	
8-Nitro-3,5-methano-2,3,4,5-tetrahydro-1H-1-benzazepine	20.7	(0–20.7)
Total VOC	1219.94	(811.3–1353.1)

RI indicates retention indices, which were calculated for unknown compounds based on a standard series of alkanes C8-C20

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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