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ORIGINAL PAPER

# Contrasting olfactory responses of two egg parasitoids to buckwheat floral scent are reflected in field parasitism rates

**Maria Cristina Foti[1,5] · Ezio Peri[1] · Eric Wajnberg[3,4] · Stefano Colazza[1] · Michael Rostás[2]**

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## Abstract

Conservation biological control programs advocate the planting of flower strips alongside crops to improve the survival and effectiveness of parasitoids. Ideally, the provided food plants are highly attractive and benefit the targeted biocontrol agents without promoting pests or intraguild competition. Previous laboratory studies showed that *Trissolcus basalis*, an egg parasitoid of the stink bug *Nezara viridula*, is highly attracted to floral odors of buckwheat and that its nectar increases the wasp's fecundity. In the field, *T. basalis* competes with the co-occurring parasitoid *Ooencyrtus telenomicida* for host eggs. Therefore, in the present study, we explored whether *O. telenomicida* shows similar attraction to buckwheat volatiles. We then carried out a 2-year field experiment to assess whether the laboratory-observed olfactory behaviors of both species are reflected in changed parasitism rates of stink bug eggs. Parasitism by naturally occurring egg parasitoids was measured in tomato plots with and without a margin of flowering buckwheat. Our results showed that, unlike *T. basalis*, *O. telenomicida* was repelled by the odor of buckwheat flowers. In both years, *T. basalis* found more egg batches and parasitized more stinkbug eggs when flower margins were present. Egg parasitism correlated positively with proximity to the buckwheat margin. In accordance with our prediction, egg parasitism by *O. telenomicida* occurred almost exclusively in the control plots and for a shorter period during the season. We conclude that buckwheat strips can influence intraguild competition and hypothesize that the effect was mediated by floral volatiles.

**Keywords** Buckwheat · Conservation biological control · Ecosystem services · *Nezara viridula* · *Ooencyrtus telenomicida* · *Trissolcus basalis*

## Key Message

- Floral odor from buckwheat plants is known to attract the egg parasitoid *Trissolcus basalis* in the laboratory; however, we demonstrate that it repels the egg parasitoid *Ooencyrtus telenomicida.*
- Egg parasitism by *T. basalis* appears to be positively correlated with proximity to buckwheat plants.
- In the field, we find that *T. basalis*, but not *O. telenomicida*, parasitizes more host eggs in tomato plots in the presence of buckwheat margins.
- The same floral resource used as field margin can lead to asymmetric effects in the interaction of competing biocontrol agents.

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✉ Maria Cristina Foti
mariacristina.foti15@gmail.com

[1] University of Palermo, Department of Agricultural, Food and Forest Sciences, Viale delle scienze, 90128 Palermo, Italy

[2] Bio-Protection Research Centre, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand

[3] INRA, 400 Route des Chappes, 06410 Sophia Antipolis Cedex, France

[4] INRIA, Sophia Antipolis, Projet Hephaistos, 2004 Route des Lucioles, BP 93, 06902 Sophia Antipolis Cedex, France

[5] Fondazione Angelo e Salvatore Lima Mancuso, Piazza Marina 61, 90100 Palermo, Italy

## Introduction

An essential element of conservation biological control programs is the incorporation of flowering non-crop vegetation within or around the borders of agricultural fields (Rush et al. 2017). The measure can positively influence behavior and performance of natural enemies (parasitoids and predators) and thus their impact on pest insects by providing a range of ecological services, including food in the form of nectar and pollen, but also refuges and oviposition sites (Bianchi et al. 2013; Gurr et al. 2017). In simplified agricultural landscapes, these resources are often lacking and consequently biological pest suppression by natural enemies may be below its full potential.

Non-crop habitats designed to provide the necessary resources to natural enemies have not always achieved the desired effects. Recently, several hypotheses have been proposed to explain why this may be the case (Tscharntke et al. 2016). One of these stipulates that the composition of flower strips should play an important role because not all plant species are equally suitable. Natural enemies that are supposed to act as biological control agents may not be able to exploit the plant species sown in flower strips for various reasons. Furthermore, non-crop plants may also be a resource for the pests.

A possible approach to improving the effectiveness of floral resources in enhancing the biocontrol potential of parasitoids involves the identification of suitable flower species. Such plants should not only increase parasitoid fitness but should also be highly attractive and receive frequent visits by them (Wäckers 2005; Belz et al. 2013; Géneau et al. 2013). The latter aspect can be quite crucial because plants offering accessible nectar may not be the ones that are olfactorily the most alluring for parasitoids. Simulations of parasitoid behavior suggest that access to strongly scented flowers with reduced nectar availability leads to higher parasitoid longevity, nectar feeding, and parasitism levels of hosts compared to weakly scented plants with high nectar availability. Although ideally, companion flowers should offer both a strong scent and a good supply of nectar (Bianchi and Wäckers 2008).

Buckwheat, *Fagopyrum esculentum* (Polygonaceae), is one of the most frequently studied plant species in habitat management for conservation biological control (Fiedler et al. 2008). It is an annual, herbaceous plant and an Old World crop believed to have originated from China (Ohnishi and Matsuoka 1996). It produces flowers that are distylous and self-incompatible, and can be pollinated by wind or insects (Sasaki and Wagatsuma 2007). Previous laboratory tests, linking parasitoid fitness with an assessment of olfactory attractiveness, suggested that flowering buckwheat is a very promising candidate for habitat management and conservation biological control approach that may provide enhanced control of the stink bug *Nezara viridula* (Hemiptera: Pentatomidae) (Foti et al. 2017). *Nezara viridula* is a cosmopolitan and worldwide serious pest commonly infesting tomato crops where it feeds directly on the fruit inducing discoloration around the feeding spots and fruit malformation, which causes economic problems to both fresh and processing tomatoes (Wakil et al. 2017). In Italy, Sicily is the main region for tomato production with about 50% of the Italian greenhouse tomato cultivation and about 40% of fresh market production (Incrocci et al. 2002). In this respect, *N. viridula* is one of the major pests, which causes significant harvest reduction. The study by Foti et al. (2017) emphasized the role of buckwheat floral nectar in increasing the parasitism rates of the main egg parasitoid of *N. viridula*, *Trissolcus basalis* (Hymenoptera: Platygastridae). In fact, it was shown that flowers of buckwheat were more attractive to *T. basalis* than other tested companion plants, and this preference was attributed to characteristic compounds in the buckwheat floral scent. The possibility to control *N. viridula* populations with the help of non-crop flowering plants is one of the more promising avenues, considering that the mass production of *T. basalis* for augmentative release is still a challenge. In the field, *T. basalis* can co-occur with *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae), a competing egg parasitoid of *N. viridula*. However, both wasps differ in their competitiveness, with the former showing parasitism rates of 79% of *N. viridula* eggs and the latter about 12% (Peri et al. 2011, 2014). Earlier studies revealed that *T. basalis* is superior in finding and parasitizing host eggs compared with *O. telenomicida* (Cusumano et al. 2016) but suffers from interspecific within-host larval competition in cases where both species oviposit into the same host egg (Cusumano et al. 2015). The outcome of larval competition is generally in favor of *O. telenomicida* and does not depend on the sequence of arrival (Cusumano et al. 2011, 2012). Providing tailored floral resources may shift such competitive interactions if natural enemy species differ in their responses to the offered resource, with potential consequences for biological control success.

Taking into account *T. basalis'* known preference for buckwheat odor and its co-occurrence with *O. telenomicida*, we first conducted laboratory assays to explore whether *O. telenomicida* shows likewise attraction to the floral volatiles of buckwheat. In a second step, we planted flowering buckwheat along the margin of a crop field to find out whether the observed olfactory responses of both species are reflected in changes in egg parasitism. The attraction of *T. basalis* and *O. telenomicida* to floral volatiles of buckwheat and the effect of flowering buckwheat plants on field stink bug parasitism rates are discussed.

## Materials and methods

### Insect rearing

A colony of *N. viridula* was established from insects collected in various crops around Palermo, Sicily, Italy, and reared in a climate chamber at 25 ± 1 °C, 60–65% r.h. and L16:D8 photoperiod. Immatures and adults were reared in different cages (47.5 × 47.5 × 47.5 cm, BugDorm-44545, and 24.5 × 24.5 × 24.5 cm, BugDorm-4S2222, for adults and immatures, respectively; MegaView Science Co. Ltd, Taichung, Taiwan) and fed with a diet of seasonal organic vegetables, soybeans, and sunflower seeds. Food was replaced every 2–3 days. Water was supplied using soaked cotton wool. Inside cages with adult bugs, paper towels were hung from the top as oviposition substrate. The obtained egg masses were collected daily to prevent cannibalism by adults. Eggs were used to maintain the colony and to carry out experiments.

An *O. telenomicida* colony was established from wasps emerging from *N. viridula* sentinel egg masses placed in the area of the experimental fields. Adult parasitoids were reared in 85-ml glass tubes, fed with a honey–water solution (80:20 v/v), and kept in an incubator at 24 ± 1 °C, 55% r.h. and L16:D8 photoperiod. Twice a week, 1- to 2-day-old egg masses (70–80 eggs) of *N. viridula* were exposed to seven mated *O. telenomicida* females for 48 h for parasitization and then stored for wasp development under the same climatic conditions.

A colony of *T. basalis* was not established since field experiments were based on naturally occurring wasp species.

### Laboratory tests

The olfactory response of *O. telenomicida* to the floral scent of buckwheat plants was tested in laboratory conditions using a still-air, four-chamber olfactometer, made of acrylic glass, consisting of a cylinder (4 cm high, 20 cm diameter) divided by vertical plates into four chambers. Odor samples were placed in chambers and covered with gauze to avoid the influence of visual cues. On the top of the cylinder, a removable walking arena (1.5 cm high, 20 cm diameter) consisting of a plastic gauze mesh was placed and covered with a glass plate (Steidle and Schöller 1997; Foti et al. 2017). A single, mated, 5- to 6-day-old female parasitoid was released in the center of the walking arena. The time the wasp spent above each odor chamber was observed for 5 min, and the residence time, i.e., the time the wasp spent over each odor sample, was computed using the software JWatcher (Blumstein and Daniel 2007). The positions of the samples were changed after each observation to avoid any bias due to eventual side preferences by the parasitoids. After every bioassay, the device was cleaned using fragrance-free soap, rinsed with demineralized water and dried. Odor sources and wasp females were used only once. Each treatment was replicated 20 times. The experiments were carried out in a dark room to avoid directional light, and the olfactometer was situated inside a white cardboard basket and illuminated from above by two cool white fluorescent tubes (Philips, TLD 58 W/640). The temperature in the bioassay room was 24 ± 1 °C. Wasps were allowed to acclimatize for at least 1 h in the room before the experiment. Two olfactometer tests were carried out to test responses to: (1) buckwheat flowering plants and (2) floral versus vegetative volatiles of buckwheat plants. In the first test, stems with leaves and inflorescences were excised; the cut end of the stem was wrapped in wet cotton wool and sealed with parafilm to prevent wilting and the emission of wound-related volatiles. The plants were placed in two opposing chambers of the four-chamber olfactometer, leaving the other two empty (control). The amount of biomass was visually estimated and kept constant throughout treatments. All plants were used within half an hour after cutting and replaced after each wasp was tested. In the second test, plant material was treated as above but stems with inflorescence were tested against non-flowering shoots.

### Field experiments

The effect of buckwheat plants on *N. viridula* egg parasitism by *T. basalis* and *O. telenomicida* was tested under field conditions. Experiments were carried out on an organic farm located in Ciminna (37°54′0″N,13°33′0″W), province of Palermo, Sicily, Italy, during the growing seasons (from June to October) of 2015 and 2016. Vegetables, olive trees, and grapevine were the main crops of the farm. Two plots (15 × 15 m) with tomato plants were established (Fig. 1). A margin of buckwheat plants (15 × 1 m) was established along one of the borders of a plot (test field). The other plot consisted of tomato plants only without buckwheat margin (control field). In the second year, the position of the buckwheat margin was reversed and was established alongside the 2015 control field. The 2015 test field was left without buckwheat plants and served as the control plot in 2016. Both plots were separated by 150 m of ground and were surrounded by 2 m of bare soil. The area was regularly mowed to prevent weeds.

Seedlings of tomato plants (*Solanum lycopersicum* cv "Costoluto Genovese") were transplanted in early April at a distance of 0.5 m within each row and 1.5 m between rows. Buckwheat (*Fagopyrum esculentum* cv "Kaitowase") was grown from seed sown into ten-cell plug trays filled with

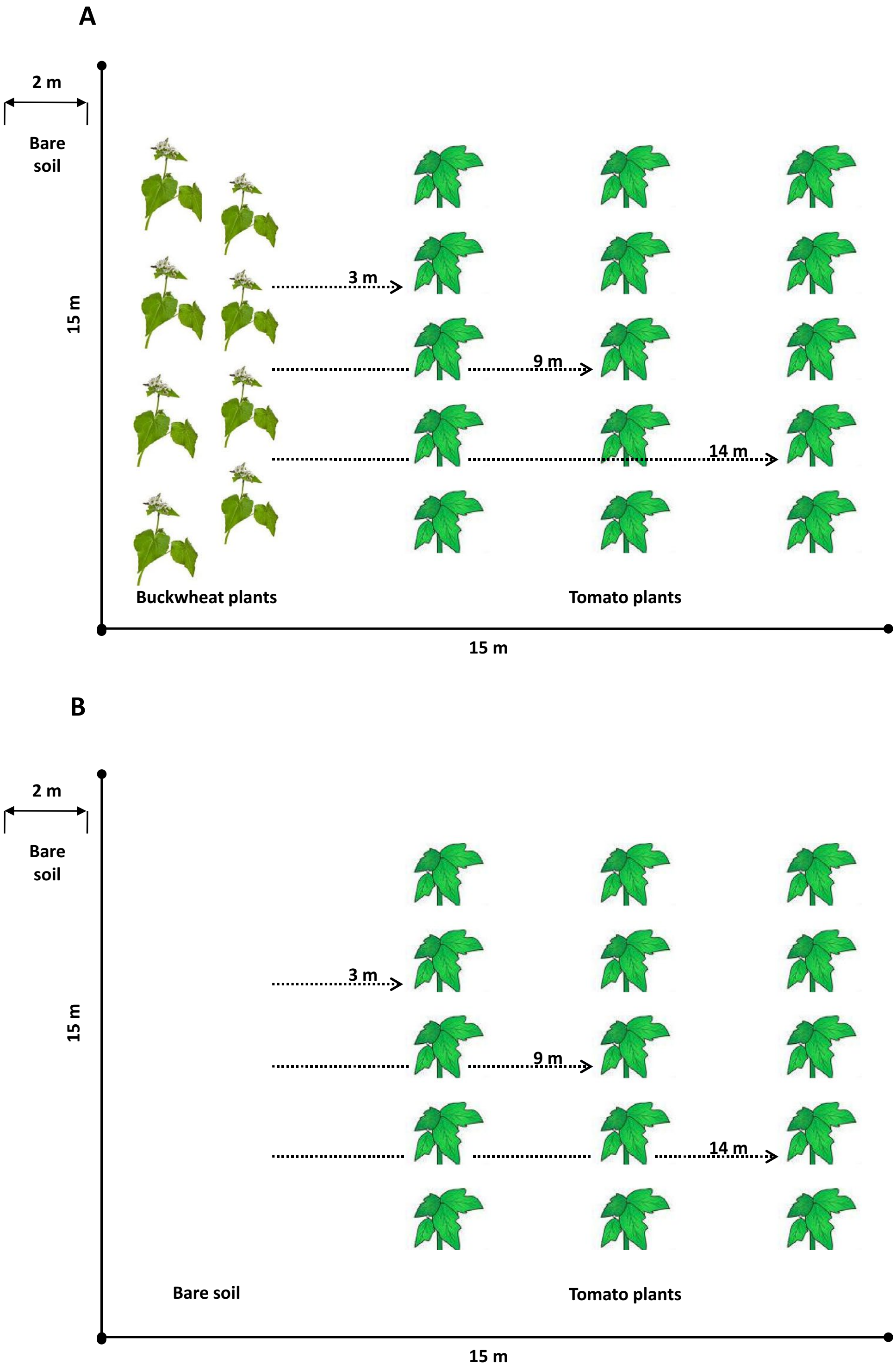

**Fig. 1** Experimental design of field experiments with tomato plants. Each field measured 15 × 15 m. In test field (**A**) a buckwheat plants border was established along one of the borders of a plot, while in control field (**B**) only tomato plants were established. Both plots were surrounded by 2 m of bare soil. Sentinel egg masses were placed in three rows at 3, 9, and 14 m distance from the buckwheat plants border in the test field and the equivalent border of bare soil in the control field

standard potting mix containing slow-release fertilizer. A row of buckwheat seedlings (3 weeks old) was transplanted in front of the first row of tomato plants in the test field in mid-April each year. A second row of buckwheat was transplanted in mid-June in front of the first row, in order to ensure the presence of plants in bloom during the entire experiments. The buckwheat seedlings were arranged at a distance of 0.5 m within a row and 0.5 m between both rows. Both plots were covered with plastic film (BioTelo Agri; Protema Agri, Milan, Italy) to suppress weeds, and water was provided by a drip irrigation system. During the growing seasons, both plots were left unsprayed.

During each week of the entire experiment, ten sentinel egg masses of *N. viridula*, obtained from our laboratory rearing, were placed inside the experimental plots (five sentinel egg masses per plot). Egg masses were observed in the laboratory under a microscope to exclude eggs that were collapsed or damaged. Each sentinel egg mass (24 h old) was standardized to three rows of four eggs. The 12 eggs were glued (Henkel, Düsseldorf, Germany) onto a 2.5 × 2.0 cm white cardboard and attached to the adaxial leaf surface of a tomato plant with a paperclip (Peri et al. 2014). To analyze the spatial pattern of naturally occurring egg parasitism, the sentinel egg masses were placed in three rows at 3, 9, and 14 m distance from the buckwheat margin in the test field and the equivalent border of the control field. Either one or two sentinel egg masses were distributed per row, whereby the number of egg masses (one or two) attributed to each row was chosen randomly each week. After 5 days, the sentinel egg masses were returned to the laboratory. All eggs were examined under a microscope, and the number of intact eggs and eggs damaged by either chewing predators (broken) or sucking predators (collapsed) was recorded. The sentinel egg masses were then placed individually in 85-ml glass tubes and stored in an incubator (25 ± 1 °C, 60–65% r.h.) until host or parasitoid emergence. Emerging adult parasitoids were placed in vials with 95% ethanol for species identification. Unhatched eggs were dissected to check the presence of undeveloped larvae or fully developed parasitoids that failed to emerge. Dead host nymphs as well as partially and fully developed parasitoids were recorded.

The effect of buckwheat margins on egg parasitism was quantified using two complementary indices as defined by Bin and Vinson (1991). The host location index assesses a parasitoid's efficiency to find egg masses and is defined as the number of sentinel egg masses from which at least one parasitoid emerges divided by the total number of deployed egg masses. The host impact index defines the parasitoid's overall efficiency as a biological control agent and corresponds to the total number of parasitized eggs divided by the total number of sampled eggs. Both indices were multiplied by 100 and expressed as percentage.

The experiment started simultaneously in test and control fields when at least 60% of buckwheat plants were in bloom (beginning of June) and finished when all sentinel egg masses were left unparasitized for two consecutive weeks in at least one plot (mid-October).

### Statistical analysis

Residence time of *O. telenomicida* in the still-air four-chamber olfactometer was compared by using Student's *t* tests for paired samples. To analyze the host location index of the two egg parasitoid species, data from 2015 and 2016 were pooled and input into a mixed generalized linear model (GLMM) on binomial data. Effects of treatment (buckwheat + tomato and tomato), distance (3, 9, and 14 m), and the interaction between both main effects were tested with sampling date as random effect. Post hoc comparisons were performed with $\chi^2$ tests, using Bonferroni correction to find significant differences. Host impact index was analyzed separately for each year and for each egg parasitoid species with $\chi^2$ tests and Bonferroni correction for multiple comparisons. All computations were carried out with the R 2.14.1 software (R Development Core Team 2011).

## Results

### Laboratory tests

Testing the olfactory behavior of *O. telenomicida* toward buckwheat floral odors (Experiment 1), the parasitoid spent significantly more time in the areas above the control chambers (no odor source) than in the areas above the test chambers (stems with leaves and inflorescences) ($t = 3.982$, $df = 19$, $p < 0.001$; Fig. 2). In the bioassays testing buckwheat inflorescences against non-flowering buckwheat shoots (Experiment 2), wasps significantly preferred the areas above non-flowering shoots over inflorescences ($t = 7.611$, $df = 19$, $p < 0.001$; Fig. 2).

### Field experiments

All sentinel eggs ($N = 4800$) were recovered and had either hatched or were parasitized. None of the eggs were predated. The only egg parasitoids recovered were *T. basalis* and *O. telenomicida*.

The host location index was significantly affected by the factors treatment (GLMM $\chi^2 = 44.431$, $df = 1$, $p < 0.001$), distance (GLMM $\chi^2 = 13.25$, $df = 2$, $p = 0.001$) and the interaction treatment × distance (GLMM $\chi^2 = 20.504$, $df = 2$, $p < 0.001$). A clear difference between *T. basalis* and *O. telenomicida* was found in both years. Overall, *T. basalis* occurred earlier than *O. telenomicida*, was present for a longer period of time, and parasitized more egg masses in tomato fields with buckwheat margins than in control fields (Fig. 3A, C). Specifically, in 2015 the first egg masses parasitized by *T. basalis* were found 1 week earlier in the test than in the control field. In 2016, they were found 2 weeks earlier. During the 2015 season, *T. basalis* located 61% of sentinel egg masses in the test field and 29% in the control field. Similarly, in 2016, 61% and 25% parasitized egg masses were found in test and control, respectively. The egg parasitoid *O. telenomicida*, on the other hand, occurred almost exclusively in the control field and parasitized eggs during a shorter period (Fig. 3B, D). In 2015, *O. telenomicida* did not locate any sentinel egg masses in the test field but parasitized 16% of the egg masses in the control field. In 2016, similar host location results were obtained: 21% of

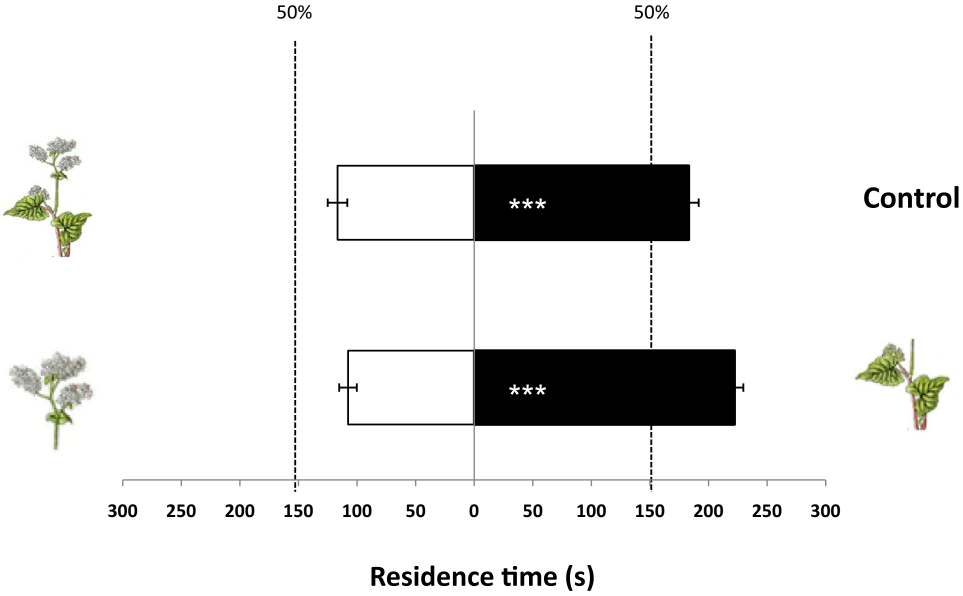

**Fig. 2** Olfactory responses in four-chamber olfactometer of *Ooencyrtus telenomicida* females to buckwheat volatiles. The upper bar represents the insect's response to combined floral and vegetative volatiles versus control (empty chamber). The lower bar shows the response to combined floral and vegetative volatiles versus vegetative volatiles of buckwheat plants. Dotted vertical lines indicate 50% of the observational time. Bars show residence time (mean ± SE) of female wasps in the sector above the odor source. $N = 20$ wasps tested per treatment. ***$p < 0.001$

egg masses were parasitized in the control field and 0.07% in the field with buckwheat margin.

In both years, the host location index for *T. basalis* decreased significantly with distance from the buckwheat margin (3 m versus 9 m $\chi^2 = 10.625$, $df = 1$, $p = 0.001$; 9 m versus 14 m $\chi^2 = 12.117$, $df = 1$, $p < 0.001$; 3 m versus 14 m $\chi^2 = 43.776$, $df = 1$, $p < 0.001$; Fig. 4A). However, no distance effect was found in the control fields (3 m versus 9 m $\chi^2 = 4.16$, $df = 1$, $p = 0.05$; 9 m versus 14 m $\chi^2 = 0.04$, $df = 1$, $p = 0.85$; 3 m versus 14 m $\chi^2 = 3.60$, $df = 1$, $p = 0.06$; Fig. 4A). In the case of *O. telenomicida*, a significant difference between 3 and 9 m was observed but with a higher host location index at 9 m rather than 3 m distance from the buckwheat margin (3 m versus 9 m $\chi^2 = 14.032$, $df = 1$, $p < 0.001$; 9 m versus 14 m $\chi^2 = 0.597$, $df = 1$, $p = 0.439$; 3 m versus 14 m $\chi^2 = 0$, $df = 1$, $p = 1$; Fig. 4B). Again, no significant distance effects were found in the control fields (3 m versus 9 m $\chi^2 = 0.441$, $df = 1$, $p = 0.50$; 9 m versus 14 m $\chi^2 = 0.610$, $df = 1$, $p = 0.44$; 3 m versus 14 m $\chi^2 = 2.694$, $df = 1$, $p = 0.10$; Fig. 4B).

The host impact index showed that the presence of buckwheat plants had significant but contrasting effects on both parasitoids over the 2 years. Host impact of *T. basalis* was significantly higher in buckwheat-treated plots ($\chi^2 = 881.51$, $df = 1$, $p < 0.0001$), while the impact of *O. telenomicida* was significantly higher in control plots ($\chi^2 = 204.26$, $df = 1$, $p < 0.0001$; Fig. 5).

Distance from buckwheat plants affected the host impact index in both years. The number of eggs parasitized by *T. basalis* decreased significantly with distance (in 2015: 3 m versus 9 m, $\chi^2 = 153.48$, $df = 1$, $p < 0.0001$; 9 m versus 14 m, $\chi^2 = 12.745$, $df = 1$, $p < 0.0001$; 3 m versus 14 m, $\chi^2 = 207.73$, $df = 1$, $p < 0.0001$; in 2016: 3 m versus 9 m, $\chi^2 = 31.271$ $df = 1$, $p < 0.0001$; 9 m versus 14 m, $\chi^2 = 28.547$, $df = 1$, $p < 0.0001$; 3 m versus 14 m, $\chi^2 = 102.2$, $df = 1$, $p < 0.001$ Fig. 6A, B).

Parasitism by *O. telenomicida* was not strongly affected by distance from the field margin, even in the control field. In 2015 (Fig. 6c), the wasps were not recorded in the test field, while in the control field statistical differences were found between distance 3 and 9 m ($\chi^2 = 16.56$, $df = 1$, $p < 0.001$) and between distance 3 and 14 m ($\chi^2 = 14.50$, $df = 1$, $p < 0.001$). No statistical difference was found between distance 9 and 14 m ($\chi^2 = 0.001$, $df = 1$, $p = 0.93$). In 2016 (Fig. 6d), the host impact index of *O. telenomicida* in the test field showed statistical differences only between 3 and 9 m ($\chi^2 = 8.815$, $df = 1$, $p = 0.003$). In the control field, no differences were found between 3 and 9 m, while differences were apparent between 9 and 14 m ($\chi^2 = 34.44$, $df = 1$, $p < 0.001$) and between 3 and 14 m ($\chi^2 = 55.27$, $df = 1$, $p < 0.001$).

## Discussion

Following on from our previous study, which demonstrated that the floral scent of buckwheat plants attracts *T. basalis* (Foti et al. 2017), we tested in an olfactometer whether such odors would have similar effects on the co-occurring egg parasitoid *O. telenomicida*. Surprisingly, our results showed that the odor of buckwheat flowers did not attract but repelled female *O. telenomicida*. Taking these contrasting observations into account, we hypothesized that flowering buckwheat margins alongside tomato plots should increase the parasitism rate of the vegetable pest *N. viridula* by *T. basalis* but not *O. telenomicida*. This notion was confirmed

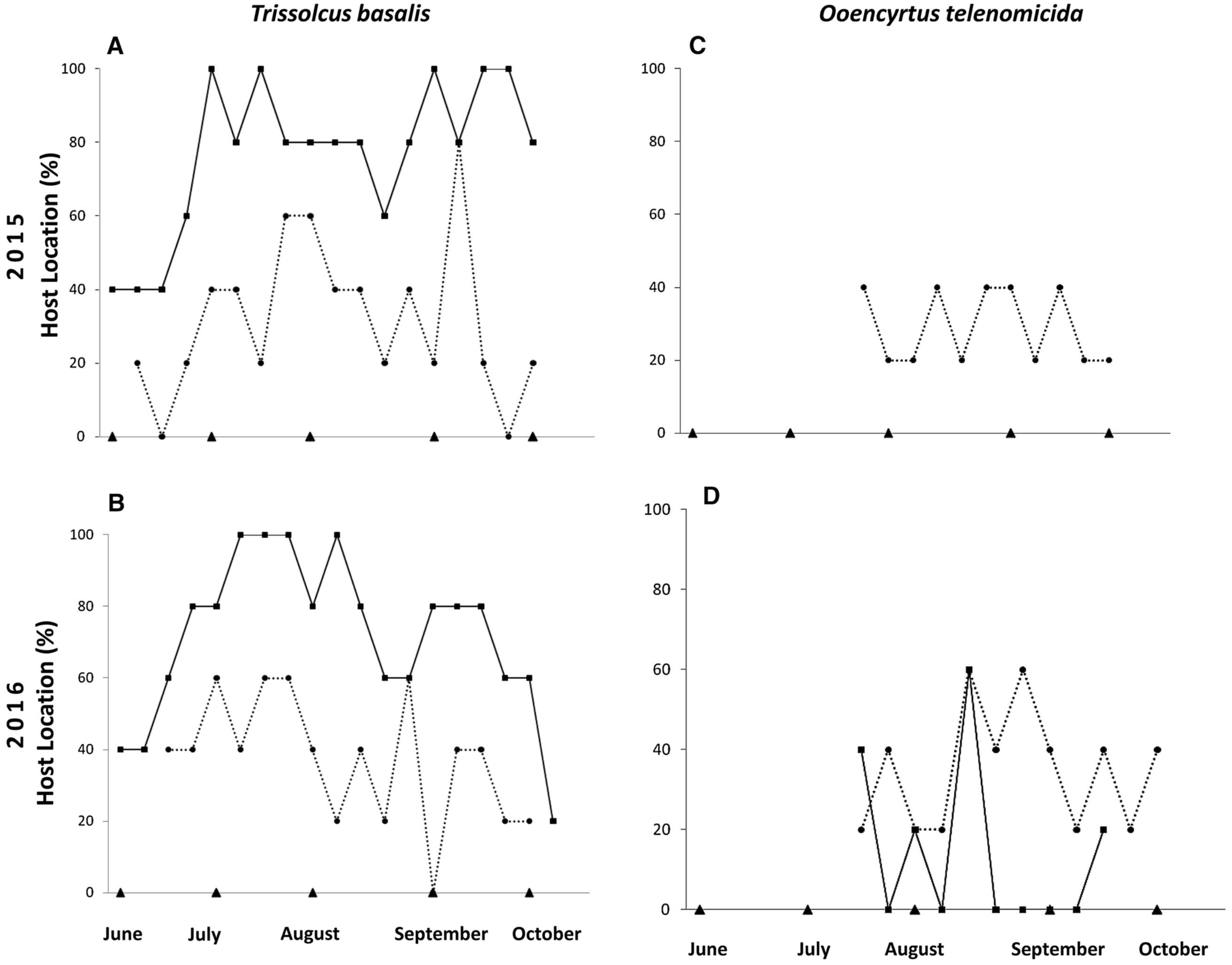

**Fig. 3** Parasitism rates of *Nezara viridula* eggs expressed as host location indices (%), i.e., number of egg masses from which at least one parasitoid emerged divided by the total number of egg masses. Solid lines indicate host location indices in test plots (with buckwheat margin); dotted lines show host location indices in control plots (without buckwheat). Results are presented separately for *Trissolcus basalis* (**A**, **B**) and *Ooencyrtus telenomicida* (**C**, **D**)

in a 2-year field experiment. The assessment of sentinel eggs placed inside the crop at various distances revealed that *T. basalis* was able to find more stink bug egg masses and subsequently to parasitize more eggs. The findings corroborate previous experiments conducted under laboratory conditions that demonstrated positive effects of access to buckwheat on egg parasitism rates (Foti et al. 2017). Jacometti et al. (2010) reported similar findings in a system consisting of buckwheat for promoting the control of *Acyrthosiphon pisum* (Harris) by the lacewing *Micromus tasmaniae* (Walker). The authors showed that buckwheat increased predator populations and decreased pest numbers in an alfalfa field. Interestingly, the presence of buckwheat plants also induced an earlier recruitment of *T. basalis*. Egg masses were available at the same time in both tomato fields but the first parasitized eggs were recorded in the buckwheat treatment 3 weeks before they occurred in the control plots. This can be an important advantage in successful pest management since biological control is generally most effective when pest populations are still low (Gurr et al. 2000).

As anticipated from previous studies in Sicily, Italy (Peri et al. 2014), eggs of *N. viridula* were parasitized by the co-occurring parasitoid *O. telenomicida*. In this species, the presence of buckwheat margins had quite the opposite effect since in both years *O. telenomicida* parasitism rates were lower in tomato fields with buckwheat margins compared with control fields. The role of buckwheat in determining natural enemy behavior seems to be further supported by the fact that parasitism rates by *T. basalis* decreased significantly with distance from the flower margin. The results corroborate a simulation model by Bianchi and Wäckers (2008), which the authors

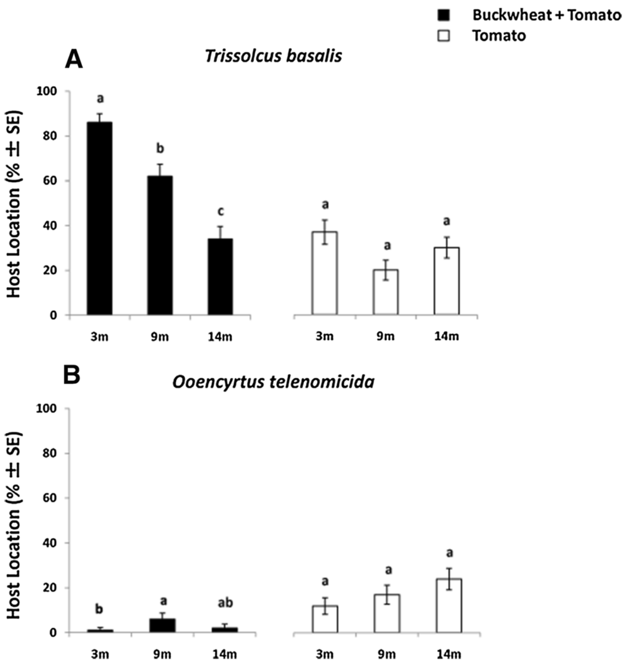

**Fig. 4** Parasitism rates of *Nezara viridula* eggs expressed as host location indices and in dependence of distance from the field margin (*x*-axis). Results for 2015 and 2016 are pooled and presented separately for *Trissolcus basalis* (**A**) and *Ooencyrtus telenomicida* (**B**). Black and white bars indicate means (± SE) for test (with buckwheat margin) and control fields (without buckwheat margin), respectively. Different letters indicate significant differences ($p < 0.05$) between distances within the same treatment (test or control)

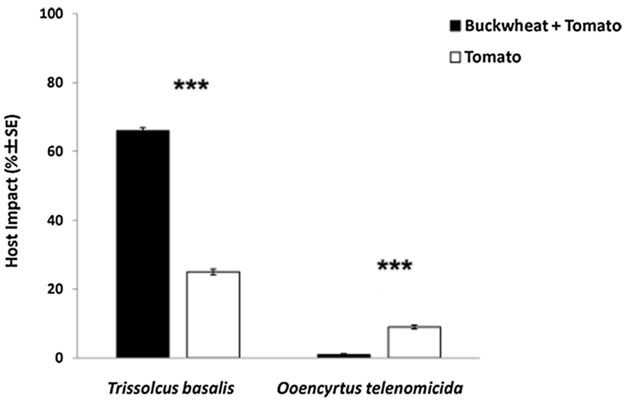

**Fig. 5** Parasitism rates of *Nezara viridula* eggs expressed as host impact indices, i.e., number of parasitized eggs divided by the total number of sampled eggs. Bars show pooled means (± SE) of total host impact in 2015 and 2016. Black and white bars indicate test (buckwheat + tomato) and control (tomato) field, respectively. ***$p < 0.001$

validated with spatial distribution data of the parasitoid *Cotesia glomerata* (L.). The model explores various scenarios with differences in "flower scent attractiveness" as well as in "nectar and host availability." As derived from the model, *T. basalis* parasitism was highest at 3 m from the flower strip and declined gradually with distance.

The presence of buckwheat odors could explain the low rate, or complete absence, of parasitization by *O. telenomicida* in plots with flower strips, because we demonstrated that this species avoids buckwheat floral volatiles. The floral scent composition of the plant is characterized by a range of short chain carboxylic acids and 1,4-benzoquinone (Foti et al. 2017). The latter is a known insect repellent and a rather uncommon floral compound. Its function may be to act as a filter to exclude folivorous insects (Burger et al. 2012; Hassemer et al. 2015). Nevertheless, some flower visitors can tolerate 1,4-benzoquinone such as the bee *Hoplitis adunca* (Hymenoptera: Megachilidae), a specialized pollinator of blueweed, *Echium vulgare*, which uses the compound as host recognition cue (Burger et al. 2012). In our case, *T. basalis* is not a specialist of buckwheat but it may have evolved a higher tolerance for specific volatile compounds than *O. telenomicida*. Furthermore, both wasps differ more than in their behavioral responses to floral semiochemicals. Females of *O. telenomicida*, unlike *T. basalis* for instance, do not locate their hosts by following herbivore-induced plant volatiles or non-volatile chemical footprints left by the hosts on the plant surface (Peri et al. 2011, Colazza et al. 2017). Both parasitoids, however, are attracted by the sex pheromone emitted by male *N. viridula* (Colazza et al. 1999, 2009; Peri et al. 2011). Visual cues are also known to play a role in parasitoid foraging behavior (Kugimiya et al. 2010; Lucchetta et al. 2008), and it is conceivable that *T. basalis* may have a stronger preference for the white buckwheat flowers than *O. telenomicida*, thus enforcing *T. basalis* olfactory choice. Another reason for the differential effects of buckwheat could be the accessibility or suitability of the nectar. Nectar can contain toxic and repellent secondary metabolites (Stevenson et al. 2017) to which *O. telenomicida* may not be adapted.

In conclusion, our study confirms the beneficial effects of buckwheat on *T. basalis* in controlling *N. viridula*, while the contrasting effects on *O. telenomicida* highlight the importance of selecting appropriate companion plants (Rahat et al. 2005; Tschumi et al. 2015). The data furthermore suggest that olfactory responses play an important role for both parasitoid species, and we hypothesize that chemical orientation toward or away from floral buckwheat odors contributed to the spatial pattern of parasitism observed in the field. To our knowledge, this is the first study to show that a flowering plant used for conservation biological control has opposite effects on two natural enemy species targeting the same host. Nevertheless, planting buckwheat along field margins is a promising option for farmers in Sicily to reduce crop damage caused by *N. viridula* and should be further explored in larger scale on-farm trials. Further research should also explore

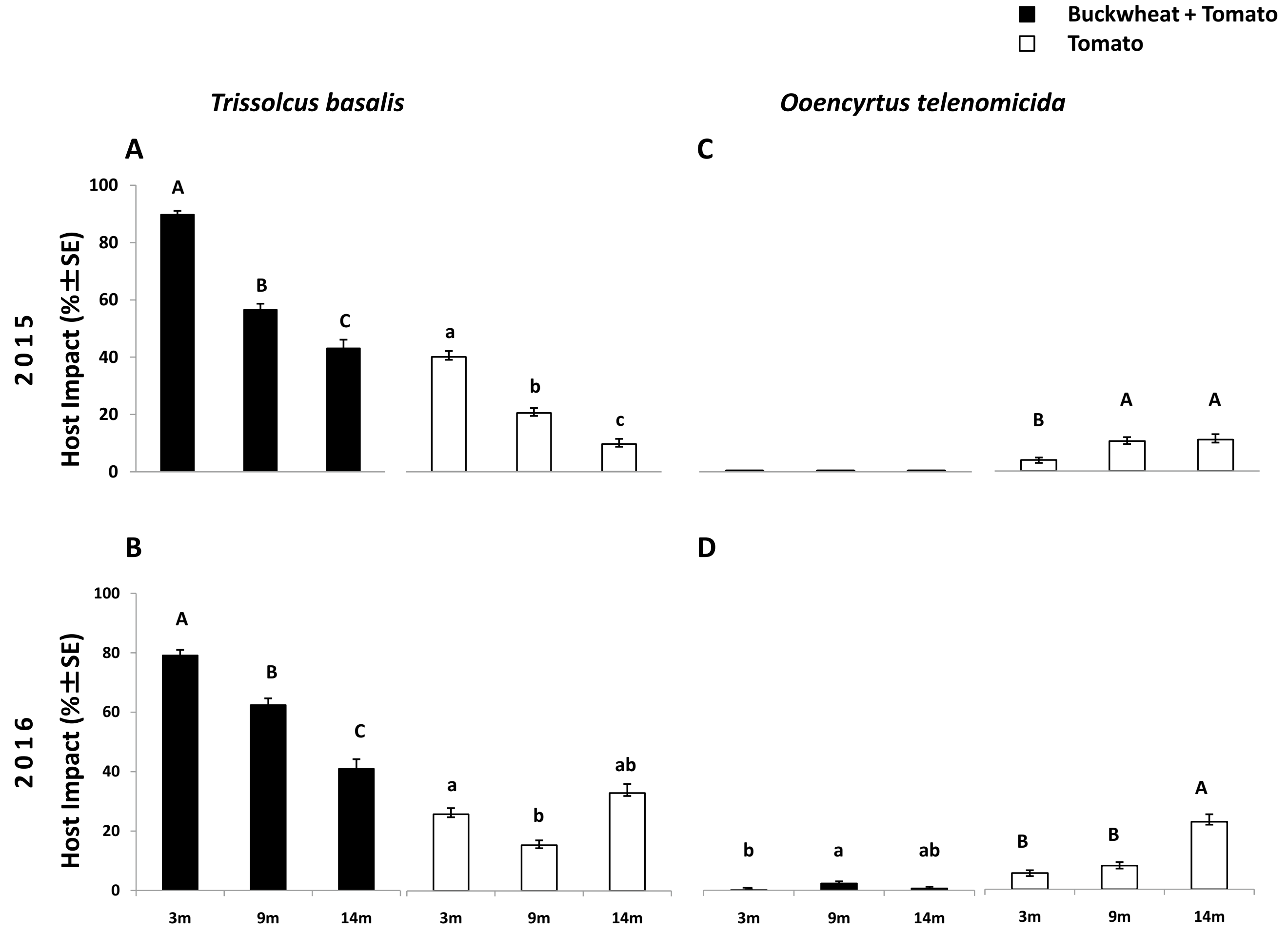

**Fig. 6** Parasitism rates of *Nezara viridula* eggs expressed as host impact index and in dependence of distance from the field margin (*x*-axis). Results are presented separately for *Trissolcus basalis* (**A**, **B**) and *Ooencyrtus telenomicida* (**C**, **D**). Black and white bars indicate means (± SE) for test (with buckwheat margin) and control fields (without buckwheat margin), respectively. Different letters indicate significant differences ($p < 0.05$) between distances within the same treatment (test or control)

the practical and economic aspects of planting rows of buckwheat within the crop to overcome the distance effects that we have seen in this study.

**Author Contributions** MCF, EP, SC, and MR contributed to the design and implementation of the research; MCF acquired field data; MCF and EW analyzed the results. All authors discussed the results and wrote the final manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interests regarding this research.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Human and animal rights** This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

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