Fitness Consequences of Oviposition Choice by an Herbivorous Insect on a Host Plant Colonized by an Endophytic Entomopathogenic Fungus

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Abstract

Entomopathogenic fungi (EPF), often considered as a bioinsecticide, are also able to colonize and establish a symbiotic relationship with plants as an endophyte. Recent studies demonstrated that endophytic EPF can enhance plant growth and are antagonistic to fungal pathogens. These newly emerging, but not yet fully understood, ecological roles suggest the possibility that EPF may further mediate oviposition preferences and offspring performance of an herbivorous insect. However, such EPF-mediated effects and underlying mechanisms are largely unexplored. Here, we examined the hypothesis that the endophytic EPF *Beauveria bassiana* can modulate oviposition behavior of the Asian corn borer *Ostrinia furnacalis*. We observed that *O. furnacalis* females preferred to lay eggs on *B. bassiana* inoculated maize plants. This is likely attributed to the net effects of plant volatile profiles induced by *B. bassiana*, with an increase in emitted amount of insect-attractive compounds 2-ethyl-1-hexanol and 3-hexen-1-ol and a decrease in insect-repellent compounds β-caryophyllene, naphthalene, and α-pinene. This finding provides an example of EPF-induced plant volatile-mediated interaction between plants and insects. However, fewer *O. furnacalis* larvae, pupae, and adults survived on the oviposition-preferred maize possibly due to lower plant nitrogen content. These results indicated that oviposition selection by *O. furnacalis* did not reflect the maximization of offspring fitness following *B. bassiana* inoculation. We suggest that fitness consequences of oviposition preferences should be considered when incorporating EPF as a biopesticide and as a potential biofertilizer within an integrated pest management programs.

Introduction

Plants interact with multiple organisms, including herbivorous insects (Awmack and Leather 2002), as well as microbes associated with plants or insects (Behie et al. 2012; Pineda et al. 2013; Cosme et al. 2016) in natural and managed ecosystems. Microorganisms, such as plant pathogens or endophytic microbes can affect plant abundance, nutritional quality and defense (Hartley and Gange 2009), which subsequently influence plant-insect interactions at the individual, population, and community levels (Shikano et al. 2017; Noman et al. 2020). Microbe-mediated interactions are thus important for structuring plant traits and influencing insect behaviour (Schausberger et al. 2012; Biere and Bennett 2013). This highlights the importance of considering how microbes influence the interactions between plants and insects when evaluating the ecological and evolutionary consequences of this tripartite interaction. The effects also extend to the application of microbes in controlling insect pests and plant pathogen in natural and agricultural ecosystems.

Entomopathogenic fungi (EPF), often solely considered as insect pathogens, have been well studied over a hundred years as effective biological control agents (Vega et al. 2009). However, recent studies demonstrated that some EPF have an alternate lifestyle as endophytes (endophytic entomopathogenic fungi) (Behie et al. 2017; Hu and Bidochka 2021). These fungi are able to colonize plant tissues under natural settings and after plant artificial inoculation (Vega 2018; González-Mas et al. 2019). The newly emerging, but not yet understood, discovery provides opportunities for understanding microbe-plant-

insect interactions and improving EPF efficacy in direct and indirect defense against insect pests and plant pathogens in integrated pest management systems (Vidal 2015; Jaber and Ownley 2018).

EPF can mediate the interactions of insects with their host plants in several ways, such as mediating herbivory (Rondot and Reineke 2018; Russo et al. 2020) and oviposition behavior (Jaber and Araj 2018). For example, EPF can alleviate feeding of aphids for inoculated maize plants (Mahmood et al. 2019), but benefiting parasitoids (González-Mas et al. 2019). Oviposition is also an important behavior, because for the majority of herbivorous insects, particularly relatively immobile larvae, the choice of an oviposition site is critical for both the fitness of the offspring and inclusive fitness (Gripenberg et al. 2010; Xiang et al. 2019). Endophytic EPF have the ability to promote plant growth, nutrition, and induce secondary metabolites (Sui et al. 2019; Rasool et al. 2021), which likely further shape oviposition behaviour of insects by influencing oviposition cues, and consequently influencing the fitness of the offspring through altered food quality. However, the effects of EPF on insect oviposition preference and offspring performance are poorly understood.

When selecting the “most suitable” plants for oviposition, female insects use plant volatiles as important cues (Gadenne et al. 2016; Webster and Cardé 2017). However, plant volatiles are influenced by a variety of biotic and abiotic factors (Islam et al. 2017; Turlings and Erb 2018). Plant volatiles serve multiple ecological functions and their production can be modified after plants are exposed to microbes, such as plant pathogenic fungi or endophytic fungi (Sharifi et al. 2018). These microbe-induced plant volatiles (MIPVs) have been shown to modify feeding behaviour of insects (Rostás et al. 2015) and mites (Schausberger et al. 2012). However, whether and how EPF that associate with host plants influence insect oviposition behaviour by inducing plant volatiles remains poorly understood (Tasin et al. 2018).

Generally, female insects will maximize their fitness by ovipositing on host plants on which their offspring perform best (Gripenberg et al. 2010; Kohandani et al. 2017). Such oviposition selection of female insects is often mediated by intrinsic factors of the insect themselves (such as experience of larvae or adults, Proffit et al. 2015) and extrinsic factors (such as water stress, Duan et al. 2021). Microbes associating with plants can influence insect performance after eggs are laid on the host plants (Fernandez-Conradi et al. 2018; Li et al. 2021). This is likely due to a change in food quality or a defense response induced by plant-associated fungi (Cory and Hoover 2006; Saikkonen et al. 2013). EPF introduced as endophytes onto plants could alter aphid development and abundance (Mahmood et al. 2019; Qin et al. 2021; Rasool et al. 2021). However, there is a paucity of information on whether and how EPF affect insect offspring performance following oviposition on host plants inoculated by EPF.

Here, we used an important global food crop, maize (*Zea mays*), an insect pest, Asian corn borer (*Ostrinia furnacalis*), and the EPF *Beauveria bassiana* as a model system. We tested whether and how endophytic entomopathogenic fungi mediate oviposition preference and offspring performance of herbivorous insects. Specifically, we address the following questions: (1) Does *B. bassiana* association with maize alter volatile compound profiles? (2) Do these changes in volatile compound profiles alter oviposition

selection of *O. furnacalis*? (3) Does *B. bassiana* association with maize affect offspring fitness of *O. furnacalis* by changing quality of host plants after laying eggs?

Materials And Methods

Study organisms

Maize (*Zea mays*) is one of the main crops for human consumption and animal fodder in the world (Fig. S1a), and accounts for more than one-third of China's cereal production (FAO 2016). We chose sweet maize single hybrid Kennian 1 in this study because it is a major cultivar in China's commercial production, and our previous study has shown that this cultivar is colonized by *B. bassiana* (Sui et al. 2020).

Beauveria bassiana [BbHOSD1 (A3)] was isolated from a dead grub (*Holotrichia oblita*) at the Institute of Plant Protection, Jilin Academy of Agricultural Sciences in 2010 (Fig. S1b). The strain was deposited in the China General Microbiological Culture Collection Center (CGMCCC No. 19373). The fungus was cultured and grown on potato dextrose agar (PDA, Hopebio Spectrum Instruments Co., Ltd., Shanghai, China) for 15-20 days at 26 ± 0.7°C in the dark, and conidia were harvested by scraping with a sterile spatula, and then kept at 4°C in dark storage before use.

Asian corn borer (*Ostrinia furnacalis*, Fig. S1c) is one of the most serious insect pests of corn in China and causes ca. 30% yield losses (Wang et al. 2014). The eggs of *O. furnacalis* were obtained from maize stands in the field, and an *O. furnacalis* colony was established in the laboratory (air temperature 26.4 ± 1°C, 70-75% relative humidity, L16:D8) using artificial diet. Adults were used to oviposition trial, and larvae were utilized in survivability studies.

Experimental design

The *B. bassiana* inoculation experiment was conducted ca. May 2017. Maize plants were treated by one of two treatments: (1) maize inoculated with sterilized water containing 0.05% Tween-80 (Dingguo, Beijing, China) solution (Uninfected control), (2) maize inoculated with *B. bassiana* suspension containing 0.05% Tween-80 solution (Infected). To establish *B. bassiana* as an endophyte in *Z. mays*, we used two inoculation methods (Sui et al. 2020): seed immersing and soil drench inoculation. Seeds of maize were surface-sterilized (dipped in 70% ethanol 5 min and then immersed in 2% NaOCl for 3 min). Half of the sterilized maize seeds were immersed in *B. bassiana* conidial suspension (1×10^8 conidia ml^{-1} containing 0.05% Tween-80) for 12 hours, and then were sown 6 cm below the surface of 23 g autoclaved peat soil (Humin substrate, Fenghong Co., Jilin, China, 121°C for 2 h, 0.1MPa) in a plastic pot (35 cm in diameter and 45 cm in height). For the soil drench inoculations, 200 ml of a *B. bassiana* conidial suspension (1×10^8 conidia ml^{-1} containing 0.05% Tween-80) was applied on day 7, 12, 17, and 22 after sowing respectively to ensure colonization. For the control treatments, other half of the sterilized seeds were immersed of a sterile 0.05% Tween-80 solution and the soil was drenched with 200 ml of sterile 0.05% Tween-80 solution. Each treatment had 20 pots with three maize seeds per pot. The plants

were grown in the greenhouse (air temperature 27.5 ± 0.8°C, and relative humidity 63.5 ± 14.2%) with 14L:10D light cycle.

Endophytism

Maize leaf colonization by *B. bassiana* was assessed by plating sterilized leaf segments on PDA 20 day after seedling emergence (Tefera and Vidal 2009). A portion of the fourth entirely fully developed leaflet from each plant was sampled, surface-sterilized by 100% ethanol, and then placed on sterile tissue paper in a laminar flow cabinet (Sui et al. 2019). Nine 1 cm^2 leaf pieces of each leaf per plant were placed onto PDA, and incubated for 20 days at 26°C in the dark. Identification of *B. bassiana* outgrowth from the leaves was based on colony and conidial morphology (Fernandes et al. 2006, Fig. S1d), all plants from each treatment were tested. Colonization rates were calculated as follows: colonization rate (%) = (the number of *B. bassiana* colonized plants/total number of plants) × 100. In this study, we observed natural *B. bassiana* endophytism at a rate of 3.7% in uninfected maize, and the endophytism rate was 43.3 % in *B. bassiana*-inoculated treatments (Fig. S2). Thus, the colonized plants in the infected treatments were utilized for further experiments, and colonized plants in the uninfected treatment were removed. In total, each treatment had 20 pots with one plants per pot, with 10 pots for oviposition selection trials of *O. furnacalis*, and the other 10 pots for the collection of volatile compounds.

Oviposition selection of *O. furnacalis* for uninfected and infected maize

Oviposition preferences of *O. furnacalis* for host plants inoculated by *B. bassiana* were examined by two-choice tests (De Moraes et al. 2001, Rizvi et al. 2016). Ten mated *O. furnacalis* females were released into a pyramidal screen cages (120 cm × 60 cm × 120 cm), which contained two plants (one plant as an uninfected control and the other as an infected treatment) at the 7-8 plant leaf stage when *O. furnacalis* often oviposit their eggs in the field. Females were released into these cages at 19:00, considering the nocturnal activity of *O. furnacalis*. After 72 h, these females were removed from these experimental cages. The number of egg mass and eggs they have laid were counted. These oviposition cages were placed in the greenhouse with at an air temperature of 26.5 ± 0.8°C and relative humidity of 63.5 ± 14.2%. The position of each pot in each cage was randomly determined, and the distance between pots was 35 cm. The cages were separated from each other by at least 1.2 m. Plants and insects were used once, and 10 replicates (cages) were performed.

Collection and measurement of maize volatile

When conducting the oviposition experiment, we collected samples of volatile compounds from treated maize leaves using solid phase microextraction (SPME filed sampler 100-μm polydimethylsiloxane, Supelco [Sigma-Aldrich] Bellefonte, PA, USA), and analyzed and identified these compounds using gas chromatography linked to mass spectroscopy (Agilent 5975, Agilent Technologies, Madrid, Spain). Volatiles were collected using SPME techniques at ambient temperature (26.4 ± 0.6 °C). Three entire young leaves per plant were sampled because *O. furnacalis* preferred to lay eggs on these leaves (Zhu et al. Observation), and then were placed into a Teflon sampling bag with polyperfluoroethylene propylene

(E-Switch, Du Pont Co, USA). We analyzed 23 samples in total (18 plants and 5 ambient controls). A detailed description of volatile collection and chemical analysis is available in Appendix S1. Relative amounts of each chemical compound from these tested plants in each replicate were calculated by the proportion of peak area of each chemical compound to total peak area of all chemical compounds, and then used for further analyses.

Electronantennogram responses of *O. furnacalis* to chemical compounds

The electronantennographic (EAG) responses of gravid *O. furnacalis* females were recorded using the EAG instrument with a data acquisition interface board (Type IDAC-02) and a universal single-ended probe (Type PRS-1) and related software (PC-EAG version 2.4) from Syntech (Hilversum, Netherlands). The solutions of tested chemical stimuli (with liquid paraffin as the solvent) at different concentrations (0.001, 0.01, and 0.1μg/μl), and the antennae of live gravid moths were used for the EAG recordings. Sixteen chemical compounds were used to examine the EAG responses of gravid *O. furnacalis*, and yet 1-penten-3-one, 3-carene, 1-penten-3-ol, azulene, and 2-ethyl furan were not included since commercial standards were not available (for a detailed list of compounds see Table S1). EAG values were recorded by using a standard method (Zhu *et al*., 2016). Solutions were applied (10 μl) to a filter paper strip (5 mm × 60 mm), and the solvent was allowed to evaporate for 30 s before the strip was placed inside a glass Pasteur pipette. Ten microliters of liquid paraffin was used as the control. Test stimulations were carried out by applying puffs of air for 2 s through a Pasteur pipette containing the filter paper as the stimulus. Puffs of the test stimuli were applied at 30 s intervals in randomized order of each chemical. Puffs with liquid paraffin were applied at the beginning and at the end of each tested compounds for monitoring relatively-stable baseline during the antennal preparation. These chemical compounds were tested from low to high dosage. Mated females (15-20 individuals) were tested for each compound and concentration. EAG responses were normalized with respect to the solvent control (Sun et al. 2014). To calculate the relative EAG values, the mean response to the sample minus the mean response to the control was divided by the mean response to the control.

Oviposition bioassays of *O. furnacalis* with chemical compounds

For *O. furnacalis* adult oviposition bioassays in the presence of individual volatile compounds, a transparent polythylene cage (15 cm × 8 cm × 8 cm) was used, modified from De Moraes et al. (2001) and Huang et al. (2009). The oviposition cage consisted of an oviposition container and a lid (17 cm × 10 cm). Two holes (3 cm in diameter) were cut in the lid, and two glass cuvette tubes (3 cm in diameter and 8 cm in height) containing filter paper with 10μl of either a test chemical or liquid paraffin (the solvent) covered the two holes. One hole received the tested compounds or distilled water, and the other the solvent (the control). The distance between the two holes was 5 cm. A piece of wax paper was adhered to the inside of the container because females will lay eggs on wax paper. The opening of the glass tubes were covered with wax paper that been perforated ten times with a needle to allow volatiles to pass through into the oviposition cage. Experiments were performed using mated females (temperature 26.0 ± 0.9°C, RH 71.3 ± 12.4%, photoperiod 14L:10D). Six compounds, 3-hexen-1-ol, 2-ethyl-1-hexanol, α-pinene, β-

caryophyllene, naphthalene, and caproaldehyde at a concentration of 0.1 ug/ul for each chemical compound were used to examine oviposition bioassays of gravid *O. furnacalis* due to higher EAG responses of *O. furnacalis* for them (Table S1). Eggs laid on the wax paper were counted after 72 h. Mated females (18-26 individuals) were tested for each chemical compound. Oviposition stimulation index (OSI) was calculated using the following formula to determine whether the compounds repelled or attracted females to oviposit (Huang *et al*. 2009):

$$OSI = \frac{T - C}{T + C} \times 100$$

In this equation, T is the number of eggs on wax paper in the presence of the tested compound, and C is the number on wax paper of the control. The OSI ranges from -100 to 100, and when OSI = 0, this indicates that oviposition on the tested compound was equal to that of the control, when OSI < 0, this indicates that the tested compound acted as repellent, when OSI > 0, the chemical tested was an attractant.

Performance measurement of *O. furnacalis*

To evaluate the performance of *O. furnacalis* after they oviposit their eggs on uninfected and infected maize, we conducted a no-choice rearing experiment that mimicked the situation in which the larvae have no possibility of switching to another plant after hatching. The second-instar *O. furnacalis* larvae were placed into a container (35 cm × 20 cm × 15 cm in size) for rearing with fresh maize leaf and stalk and had participated in the oviposition selection experiment. The larvae were reared in full-sib groups of forty individuals in one container for one replicate, with ten replicates for each treatment, and were allowed to develop until pupation. Every two days, the treated maize food in these containers was replaced by fresh food, and the number of larvae, pupae, adults, and individual infected by *B. bassiana* were recorded.

Measurements of maize characteristics

Plant morphological variables including height of each maize plant, leaf length and leaf width for the third to fifth leaf in the middle stratum of each maize plant were measured (Duan et al. 2021). All ten maize plants per treatment were measured. The average of the three leaves of each plant was used for further analysis. Total nitrogen and total carbon of the maize was measured. Ten plants (including aboveground leaf and stalk) per treatment were collected, and were dried in an oven at 80°C for 48 h. The dried maize plants were then grounded in Willey mill equipped with a 1 mm mesh screen before chemical analyses. Five samples of 2 mg per plant were measured using an element analyzer (vario EL cube, ELEMENTAR). The average of five samples per plant and each replicate was further analyzed.

Data analyses

For the oviposition and performance data (number of egg masses, number of eggs, and number of surviving larvae, pupal, adults, and infected cadavers), we used generalized linear mixed models with a

Poisson distribution and a log link function to examine the difference in oviposition selection and offspring fitness between uninfected and infected maize plants by *B. bassiana*. For *B. bassiana* inoculation, the amount of plant volatile emission data (inoculation rate, relative amount of each compound), and characteristics of maize plants, we used a generalized linear mixed models with Gaussian distribution and identity link function to test the inoculation rate of *B. bassiana*, and the effects of *B. bassiana* on the emission of plant volatiles. We used the nlme package for these above analyses. To examine the differences in EAG responses among the four concentrations for each compound, and the relationships between insect performance and chemical properties of maize we used linear mixed models (LMMs) with the lm-function of the vegan package. For post hoc analysis, we used Tukey's post hoc tests with the mulcomp package. For the oviposition bioassay data (number of eggs), chi-squared goodness of fit test was used to examine oviposition preferences of *O. furnacalis* for distilled water, liquid paraffin, and chemical compounds. All analyses were carried out in R (version 3.6.0 × 64, 2019, The R Foundation for Statistical Computing Platform).

Results

Oviposition selection

Gravid *O. furnacalis* females alter their oviposition selection for host plants infected by *B. bassiana* (χ^2 = 3.89, p = 0.049 for egg mass, χ^2= 103.77, p < 0.001 for number of eggs). *O. furnacalis* laid significantly more egg masses by 175.9% and greater number of eggs by 213.7% on *B. bassiana*-infected maize compared with uninfected maize (Fig. 1).

Emission of plant volatile compounds

Forty compounds were identified from uninfected and *B. bassiana*-infected maize. Some of these compounds, such as β-terpineol, 1-penten-3-ol, n-dodecane, eicosane, heptacosane, α-pinene, 3-carene, 2-ethyl furan, m-xylene, and azulene, were not detected in infected plants, and caproaldehyde, 1-penten-3-one, nonadecane, and hexacosane were not detected in uninfected plants (Table 1). The relative amount of (*Z*)-3-hexen-1-ol and 2-ethyl-1-hexanol significantly increased in *B. bassiana*-treated plants, but the relative amount of (*E*)-2-hexenal, heneicosane, β-caryophyllene, and naphthalene significantly decreased (Table 1).

EAG responses of gravid *O. furnacalis* to different compounds

Results found that the EAG responses of gravid *O. furnacalis* females to seven compounds, including (*Z*)-3-hexen-1-ol, 2-ethyl-1-hexanol, (*E*)-2-hexenal, caproaldehyde, β-caryophyllene, α-pinene, and naphthalene, were more sensitive, compared to the solvent control, and further, their EAG response values increased with the increasing concentration gradient of each chemical compound (Table 2).

Oviposition bioassays

The baseline of paraffin (solvent) compared to paraffin and a blank control (distilled water) in the oviposition cages were tested, and there was no difference in number of eggs laid by gravid *O. furnacalis* on the solvent control film (88.45 ± 4.68, mean ± S.E) and blank control film (77.8 ± 7.14, mean ± S.E, χ^2 = 0.68224, p = 0.4088, Fig. S3).

Results from the oviposition bioassay found that the number of eggs laid by gravid *O. furnacalis* on the films containing (*Z*)-3-hexen-1-ol and 2-ethyl-1-hexanol were higher than that of the solvent, but lower for (*E*)-2-hexenal, α-pinene, β-caryophyllene, and naphthalene (Fig. S3). Furthermore, positive OSI values for 2-ethyl-1-hexanol and (*Z*)-3-hexen-1-ol was recorded, and negative OSI values for (*E*)-2-hexenal, α-pinene, β-caryophyllene, and naphthalene (Fig. 2). There was no difference in oviposition between caproaldehyde and the solvent.

Performance of *O. furnacalis*

The number of larvae, pupae, and adults was significantly lower in *B. bassiana*-infected maize compared with uninfected maize, but there was no difference in number of infected cadavers between uninfected and *B. bassiana*-infected maize (Fig. 3).

Characteristics of maize plants

Plant height, leaf length, and leaf width did not change between uninfected and *B. bassiana*-infected (Fig. 4). Nitrogen content in *B. bassiana*-infected plants was significantly lower by17.3% than that of uninfected plants, and there was no change in carbon content between the two *B. bassiana*-treatment plants (Fig. 4).

Discussions

Plants, insects, and microbes interact using chemical cues more complex than previously thought (Shikano et al. 2017; Noman et al. 2020). Microbial associations with plants can result in the alteration of plant volatile compound profiles which can have the effects of attracting or repelling insects from feeding or ovipositing on that plant (Shapiro et al. 2012; Pineda et al. 2013; Rostás et al. 2015; Contreras-Cornejo et al. 2021). Here we provide evidence that the endophytic EPF, *B. bassiana*, can affect the interactions of plants with an herbivorous insect by mediating oviposition preferences and consequently offspring fitness.

Many studies have shown that the association of plants by microbes, such as pathogens and endophytes, can affect insect oviposition behavior (Wilson and Faeth 2001; Pineda et al. 2013; Rizvi et al. 2016). Similarly, our study found that plants colonized by EPF *B. bassiana* were considered as attractive oviposition sites for gravid *O. furnacalis* (Fig. 1). Insects often locate their oviposition site based on morphological characteristics of hosts and the detection of volatile compounds emitted by plants (Bruce et al. 2005; Jürgens et al. 2013). Previous studies have demonstrated that some herbivorous insects, such as *O. furnacalis* prefer ovipositing on taller host plants (Duan et al. 2021). However, unchanged

maize height and leaf traits after inoculation by EPF may possibly be considered as unacceptable or neutral signals for *O. furnacalis* (Fig. S5). Therefore, plant volatiles may play an important role in the "selection decision". Here we found that *B. bassiana* colonized maize tissues, and could also induce and/or modify the emission of volatile compounds from maize leaves, that is, maize infected with *B. bassiana* emitted less relative amounts of β-caryophyllene and naphthalene, but greater relative amounts of 2-ethyl-1-hexanol and 3-hexen-1-ol. Furthermore, bioassays showed that gravid *O. furnacalis* were more sensitive to oviposition based on alterations of plant volatile profiles where β-caryophyllene, naphthalene, and α-pinene were repellents, but 2-ethyl-1-hexanol and 3-hexen-1-ol were attractants in this study. Thus, the synthetic effects of these attractive and repellent compounds result in *O. furnacalis* females preferentially lay on EPF infected plants. This finding indicated that EPF could mediate plant volatile emission, and consequently influence oviposition selection of gravid *O. furnacalis.* However, metabolic pathways of these plant volatiles induced by EPF are still unclear, and need further exploration.

Host plants that are colonized by microbes can have substantial effects on insects that interact with these plants (Fernandez-Conradi et al. 2018; Rondot and Reineke 2018). For example, endophytic establishment of *B. bassiana* reduced survival and fecundity of aphids in a range of plants (González-Mas et al. 2019), and fall armyworm and European corn borer on corn (Bing and Lewis 1991; Russo et al. 2020). Our results provide evidence of the negative effects of the presence of *B. bassiana*, as an endophyte on the performance of *Ostrinia furnacalis* feeding on leaves and stems of colonized corn plants. Thus, these results show a unique phenomenon in reporting the decline in fecundity of some insect on host plants inoculated with the endophytic entomopathogenic fungus *B. bassiana*. The feeding trials suggested that the fungal propagules were not in direct contact with the insects, and endophytic EPF could influence plants in an analogous way with plant pathogens and endophytes, which may alter insect performance by changing plant quality and other traits (Gange et al. 2019; Rasool et al. 2021). For plant-feeding insects, food quality is a key determinant in influencing insect performance, and plant tissues that have a higher nitrogen content usually enhance insect growth and development (Franzke & Reinhold 2011). In our study, the poor quality of host maize, i.e. the reduced nitrogen content in EPF-inoculated plants was correlated to a decrease in insect survival and reproduction. Furthermore, Pieterse et al. (2014) reviewed that induced systemic resistance is generally the main mechanism by which plants defend against pests and is triggered by biological inducers. A reduction in the number of larvae, pupae, and adults of *O. furnacalis* larvae and adults may thus be explained as an induced systemic resistance response of inoculated maize plants caused by endophytic *B. bassiana* (Jaber and Araj 2018). Indeed, the underlying mechanisms responsible for the *in planta* decreased *O. furnacalis* fitness are unknown, and require further elucidation.

Generally, oviposition selection of female insects maximizes offspring performance (Gripenberg et al. 2010). However, a "bad" mother selecting plants that are poorer for their offspring fitness is not without precedent (Proffit et al. 2015; Duan et al. 2021). Our results also confirmed this phenomenon, that is, female *O. furnacalis* prefer laying eggs on a host plants inoculated with EPF, but the offspring perform worse than on an uninoculated plant. In an herbivorous insect with limited larval mobility, particularly Lepidoptera, oviposition selection of females is crucial because egg-laying sites provide food sources for

their offspring. However, when the host plant, which is preferred by an herbivorous insect, is influenced by changes in biotic and abiotic parameters, female insects could make wrong oviposition decisions (Garvey et al. 2020). Fernandez-Conradi et al. (2018) suggested that the response differences between an herbivore preference and performance on host plants did vary following microbe inoculation. Insect offspring are mainly affected by changes in nutritional quality and defense of host plants (Tack and Dicke 2013), while oviposition selection is frequently modified by host cues, such as colors or odors (Carrasco et al. 2015). Clearly, the effects of microbes on plants and their volatile profiles and the concomitant effects on herbivorous insects are a complex and multifaceted interaction that requires further exploration.

Our finding demonstrated that maize plant inoculated with an EPF can result in opposing effects on insect behavior and life history. There is a strong oviposition preference by *O. furnacalis* females to EPF inoculated plants, yet this preference appears to be detrimental to offspring fitness. The observed effects of EPF on oviposition preference of *O. furnacalis* are mainly to be linked to synthetic effects of attractive and repellent compounds induced by EPF, and responses of *O. furnacalis* performance are closely related with changes in plant quality and induced systemic resistance. Regardless, there is accumulating evidence to suggest that plant volatile compounds are key factors in understanding interactions between plants, microbes and insects. We highlight that the relationships between oviposition preference and offspring fitness in *O. furnacalis* should be considered in biocontrol efforts using EPF within integrated pest management programs.

Declarations

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Author Contribution Statement HZ, BR and QL conceived and designed the research. JF, HW, MD, WX, LS and ZZ conducted this experiment. HZ, MB, MD analyzed data. HZ and MB wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors have declared that they have no conflict interest.

Ethical approval This article does not contain any studies with human participants or vertebrates performed by any of the authors.

References

1. Anastasaki E, Drizou F, Milonas PG (2018) Electrophysiological and oviposition responses of *Tuta absoluta* females to herbivore-induced volatiles in tomato plants. J Chem Ecol 44:288–298. https://doi.org/10.1007/s10886-018-0929-1
2. Awmack CS, Leather S (2002) Host plant quality and fecundity in herbivorous insects. Ann Rev Entomo 47:817-844. https://doi.org/10.1146/ANNUREV.ENTO.47.091201.145300
3. Behie SW, Moreira CC, Sementchoukova I, Barelli L, Zelisko PM, Bidochka MJ (2017) Carbon translocation from a plant to an insect-pathogenic endophytic fungus. Nature Commun 8:14245. https://doi.org/10.1038/ncomms14245
4. Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577. https://doi.org/10.1126/science.1222289
5. Biere A, Bennett AE (2013) Three-way interactions between plants, microbes and insects. Funct Ecol 27:567–573. https://doi.org/ 10.1111/1365-2435.12100
6. Bing LA, Lewis LC (1991) Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Environ Entomo 20:1207–1211. https://doi.org/ 10.1093/ee/20.4.1207
7. Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274. https://doi.org/ 10.1016/j.tplants.2005.04.003
8. Contreras-Cornejo HA, Biveros-Bremauntz F, del-Val E, Macías-Rodríguez L, López-Carmona DA, Alarcón A, González-Esquivel CE, Larsen J (2021) Alterations of foliar arthropod communities in a maize agroecosystem induced by the root-associated fungus *Trichoderma harzianum*. J Pest Sci 94:363–374. https://doi.org/ 10.1007/s10340-020-01261-3
9. Cory JS, Hoover K (2006) Plant-mediated effects in insect-pathogen interactions. Trends Ecol Evol 21:278–286. https://doi.org/ 10.1007/s10340-020-01261-3
10. Cosme M, Lu J, Erb M, Stout MJ, Franken P, Wurst S (2016) A fungal endophyte helps plants to tolerate root herbivory through changes in gibberellin and jasmonate signaling. New Phytol 211:1065–1076. https://doi.org/ 10.1111/nph.13957
11. Carrasco D, Larsson MC, Anderson P (2015) Insect host plant selection in complex environment. Curr Opin Insect Sci 8:1–7. https://doi.org/ 10.1016/j.cois.2015.01.014
12. De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410:577–580. https://doi.org/ 10.1038/35069058
13. Duan MY, Zhu H, Wang H, Guo SY, Li H, Jiang LL, Li XT, Xie G, Ren BZ (2021) Effects of water deficiency on preference and performance of an insect herbivore *Ostrinia furnacalis*. https://doi.org/10.1017/s0007485321000407
14. Fernandes EKK, Costa GL, Moraes ÁML, Zahner V, Bittencourt VREP (2006) Study on morphology, pathogenicity, and genetic variability of *Beauveria bassiana* isolates obtained from *Boophilus microplus* tick. Parasitol Res 98:324–332. https://doi.org/ 10.1007/s00436-005-0058-y
15. Fernandez-Conradi P, Jactel H, Robin C, Tack AJM, Castagneyrol B (2018) Fungi reduce preference and performance of insect herbivores on challenged plants. Ecology 99:300–311. https://doi.org/

10.1002/ecy.2044

16. Food Agriculture Organization (FAO) UN. 2016. Madagascar locus crisis. Food and Agricultural Organization of the United Nations.
17. Franzke A, Reinhold K (2011) Stressing food plants by altering water availability affects grasshopper performance. Ecosphere 2:85. https://doi.org/ 10.1890/ES11-00095.1
18. Gadenne C, Barrozo RB, Anton S (2016) Plasticity in insect olfaction: to smell or not to smell? Ann Rev Entomo 61:317–333. https://doi.org/ 10.1146/annurev-ento-010715-023523
19. Gange AC, Koricheva J, Currie AF, Jaber LR, Vidal S (2019) Meta-analysis of the role of entomopathogenic and unspecialized fungal endophytes as plant bodyguards. New Phytol 223:2002–2010. https://doi.org/ 10.1111/nph.15859
20. Garvey MA, Creighton JC, Kaplan I (2020) Tritrophic interactions reinforce a negative preference-performance relationship in the tobacco hornworm (*Manduca sexta*). Ecological Entomology 45:783–794. https://doi.org/ 10.1111/een.12852
21. González-Mas N, Cuenca-Medlna M, Gutlérrez-Sánchez F, Quesada-Moraga E (2019) Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. J Pest Sci 92:1271–1281. https://doi.org/ 10.1007/s10340-019-01098-5
22. Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. Ecol Lett 13:383-393. https://doi.org/ 10.1111/j.1461-0248.2009.01433.x
23. Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Ann Rev Entomo 54:323–342. https://doi.org/ 10.1146/annurev.ento.54.110807.090614
24. Hu S, Bidochka MJ (2021) Root colonization by endophytic insect-pathogenic fungi. J Appl Microbio130:570–581. https://doi.org/ 10.1111/jam.14503
25. Huang C, Yan F, Byers JA, Wang RJ, Xu C (2009) Volatiles induced by the larvae of the Asian corn borer (*Ostrinia furnacalis*) in maize plants affect behavior of conspecific larvae and female adults. Insect Sci 16:311–320. https://doi.org/ 10.1111/j.1744-7917.2009.01257.x
26. Islam MN, Hasanuzzaman ATM, Zhang Z, Zhang Y, Liu T (2017) High level of nitrogen makes tomato plants releasing less volatiles and attracting more *Bemisia tabaci* (Hemiptera: Aleyrodidae). Front Plant Sci 8:466. https://doi.org/ 10.3389/fpls.2017.00466
27. Jaber LR, Araj S (2018) Interactions among endophytic fungal entomopathogens (Ascomycota: Hypocreales), the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the aphid endoparasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). Biol Control 116:53–61.https://doi.org/ 10.1016/j.biocontrol.2017.04.005
28. Jaber LR, Ownley BH (2018) Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? Biol Control 116:36–45. https://doi.org/ 10.1016/j.biocontrol.2017.01.018

29. Jürgens A, Wee S, Shuttleworth A, Johnson SD (2013) Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. Ecol Lett 16:1157–1167. https://doi.org/ 10.1111/ele.12152
30. Kohandani F, Le Goff GJ Hance T (2017) Dose insect mother know under what conditions it will make their offspring live? Insect Sci 24:141–149. https://doi.org/ 10.1111/1744-7917.12300
31. Li Y, Duan T, Li Y (2021) Research progress in the interactions of fungal pathogens and insect pests during host plant colonization. J Plant Dis Protect http://doi.org/ 10.1007/s41348-021-00431-4
32. Mahmood Z, Steenberg T, Mahmood K, Labouriau R, Kristensen M (2019) Endophytic *Beauveria bassiana* in maize affects survival and fecundity of aphid *Sitobion avenae*. Biol Control 137:104017. https://doi.org/ 10.1016/j.biocontrol.2019.104017
33. Noman A, Aqeel M, Qasim M, Haider I, Lou Y (2020) Plant-insect-microbe interaction: a love triangle between enemies. Sci Total Environ 699:134181 https://doi.org/ 10.1016/j.scitotenv.2019.134181
34. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced systemic resistance by beneficial microbes. Ann Rev Phytopatho, 52:347–375. https://doi.org/ 10.1146/annurev-phyto-082712-102340
35. Pineda A, Dicke M, Pieterse CMJ, Pozo MJ (2013) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? Funct Ecol 27:574–586. https://doi.org/ 10.1111/1365-2435.12050
36. Proffit M, Khallaf MA, Carrasco D, Larsson MC, Anderson P (2015) 'Do you remember the first time?' Host plant preference in a moth is modulated by experienced during larval feeding and adult mating. Ecol Lett 18:365–374. https://doi.org/ 10.1111/ele.12419
37. Qin X, Zhao X, Huang S, Deng J, Li X, Luo Z, Zhang Y (2021) Pest management via endophytic colonization of tobacco seedlings by the insect fungal pathogen *Beuveria bassiana*. Pest Manag Sci 77:2007–2018. https://doi.org/ 10.1002/PS.6229
38. Rasool S, Vidkjær NH, Hooshmand K, Jensen B, Fomsgaard IS, Meyling NV (2021) Seed inoculations with entomopathogenic fungi affect aphid populations coinciding with modulation of plant secondary metabolite profiles across plant families. New Phytol 229:1715–1727. https://doi.org/ 10.1111/nph.16979
39. Rizvi SZ, Raman A, Wheatley WM, Cook G (2016) Oviposition preference and larval performance of *Epiphyas postvittana* (Lepidoptera: Tortridae) on *Botrytis cinerea* (Helotiales: Sclerotiniaceae) infected berries of *Vitis vinifera* (Vitales: Vitaceae). Insect Sci 23:313–325. https://doi.org/ 10.1111/1744-7917.12191
40. Rondot Y, Reineke A (2018) Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduces infestion with piercing-sucking insects. Biol Control 116:82–89. https://doi.org/ 10.1016/j.biocontrol.2016.10.006
41. Rostás M, Cripps MG, Silcock P (2015) Aboveground endophyte affects root volatile emission and host plant selection of a belowground insect. Oecologia 177:487–497. https://doi.org/ 10.1007/s00442-014-3104-6

42. Russo ML, Jaber LR., Scorsetti AC, Vlanna F, Cabello MN, Pelizza SA (2020) Effects of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*. J Pest Sci https://doi.org/10.1007/s10340-020-01302-x
43. Saikkonen K, Gundel PE, Helander M (2013) Chemical ecology mediated by fungal endophytes in grasses. J Chem Ecol 39:962–968. https://doi.org/ 10.1007/s10886-013-0310-3
44. Schausberger P, Peneder S, Jürschik S, Hoffmann D (2012) Mycorrhiza changes plant volatile to attract spider mite enemies. Funct Ecol 26:441–449. https://doi.org/ 10.1111/j.1365-2435.2011.01947.x
45. Shapiro L, De Moraes CM, Stephenson AG, Mescher MC (2012) Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. Ecol Lett 15:1430–1438. https://doi.org/ 10.1111/ele.12001
46. Sharifi R, Lee S, Ryu C (2018) Microbe-induced plant volatiles. New Phytol 220:684–691. https://doi.org/ 10.1111/nph.14955
47. Shikano I, Rosa C, Tan C, Felton GW (2017) Tritrophic interactions: microbe-mediated plant effects on insect herbivores. Ann Rev Phytopatho 55:313–331. https://doi.org/ 10.1146/annurev-phyto-080516-035319
48. Sui L, Zhu H, Xu W, Guo Q, Wang L, Zhang Z, Li Q, Wang D (2020) Elevated air temperature shifts the interactions between plants and endophytic fungal entomopathogen in an agroecosystem. Fungal Ecol 47:100940. https://doi.org/ 10.1016/j.funeco.2020.100940
49. Sun X, Liu Z, Zhang A, Dong HB, Zeng FF, Pan XY, Wang Y, Wang MQ (2014) Electrophysiological responses of the rice leaffolder, *Cnaphalocrocis medinalis*, to rice plant volatiles. J Insect Sci 14:article 70. https://doi.org/ 10.1673/031.014.70
50. Tack AJM, Dicke M (2013) Plant pathogens structure arthropod communities across multiple spatial and temporal scales. Funct Ecol 27:633–645. https://doi.org/ 10.1111/1365-2435.12087
51. Tasin M, Herrera SL, Knight AL, Barros-Parada W, Contreras FE, Pertot I (2018) Volatiles of grape inoculated with microorganisms: modulation of Grapevine moth oviposition and field attraction. Microb Ecol 76:751–761. https://doi.org/ 10.1007/s00248-018-1164-6
52. Tefera T, Vidal S (2009) Effect of inoculation method and plant growth medium on endophytic colonization of Sorghum by the entomopathogenic fungus *Beauveria bassiana*. BioControl 54:663–669. https://doi.org/ 10.1007/s10526-009-9216-y
53. Turlings TCJ, Erb M (2018) Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. Ann Rev Entomo 63:433–452. https://doi.org/ 10.1146/annurev-ento-020117-043507
54. Vega FE (2018) The use of fungal entomopathogens as endophytes in biological control: a review. Mycologia 110:4–30. https://doi.org/ 10.1080/00275514.2017.1418578
55. Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koile M, Maniania NK, Monzon A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: new insights on their

ecology. Fungal Ecol 2:149–159. https://doi.org/ 10.1016/j.funeco.2009.05.001

56. Vidal S (2015) Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 108:1–9.
57. Wang ZY, He L, Zhang F, Lu X, Babendreier D (2014) Mass rearing and release of Trichogramma for biological control of insect pests of corn in China. Biol Control 68:136–144. https://doi.org/ 10.1016/j.biocontrol.2013.06.015
58. Webster B, Cardé RT (2017) Use of habitat odour by host seeking insects. Biol Rev 92:1241–1249. https://doi.org/ 10.1111/brv.12281
59. Wilson D, Faeth SH (2001) Do fungal endophytes result in selection for leafminer oviposition preference? Ecology 82:1097–1111. https://doi.org/ 10.1890/0012-9658(2001)082[1097:DFERIS]2.0.CO,2
60. Xiang Y, Dong S, Liu C, Wang Z (2019) Physical and chemical traits affecting the oviposition preference of honeysuckle geometrid, *Heterolocha jinyinhuaphaga* Chu among honeysuckle varieties. Arthropod-Plant Inter 13:905–913. https://doi.org/ 10.1007/s11829-019-09707-x
61. Zhu G, Xu J, Cui Z, Dong X, Ye Z, Niu D, Huang Y, Dong S (2016) Functional characterization of SlitPBP3 in *Spodoptera litura* by CRISPR/Cas9 mediated genome editing. Insect Biochem Molec 75:1–9. https://doi.org/ 10.1016/j.ibmb.2016.05.006

Tables

Table 1 Relative emission of volatile from uninfected and infected maize plants by *Beauveria bassiana* by SPME. Values are means ± SE (n = 9). Different small letters indicate significant difference between uninfected and infected maize plants using generalized linear mixed models with nlme package at significance of $p < 0.05$.

[#]NA indicates that retention index was not identified in this study. [*]ND indicates the compound was not detected in this experiment.

Compounds	CAS	Retention index	Relative amount (%)		t value	p value
			Uninfected plants	Infected plants		
(*Z*)-3-hexene-1-ol	**928-96-1**	**953**	**7.85±1.51**	**22.03±5.7**	**2.403**	**0.033**
1-hexanol	111-27-3	963	2.8±2.21	0.31±0.31	-1.112	0.288
β-terpineol	8000-41-7	1440	2.21±1.32	ND*		
1-penten-3-ol	616-25-1	729	3.21±2.08	ND		
2-ethyl-1-hexanol	**104-76-7**	**1240**	**6.88±1.67**	**12.37±2.1**	**2.383**	**0.035**
2-butyl-1-octanol	3913-2-8	NA#	1.07±1.04	1.44±1.06	0.248	0.808
(*Z*)-4-hexen-1-ol	928-91-6	959	0.92±0.84	2.06±1.09	0.826	0.425
Trimethylacetaldehyde	630-19-3	NA	0.11±0.09	0.85±0.58	1.262	0.231
(Z)-3-hexenal	6789-80-6	846	4.89±1.2	10.33±3.19	1.594	0.137
3-methoxysalicylaldehyde	148-53-8	NA	0.74±0.57	0.27±0.21	-0.758	0.463
(*E*)-2-hexenal	**6728-26-3**	**940**	**10.85±2.43**	**5.33±0.61**	**-2.209**	**0.047**
2-hexena	505-57-7	933	1.6±1.07	2.16±1.22	0.345	0.736
caproaldehyde	66-25-1	814	ND	2.39±1.24		
3-pentanone	96-22-0	719	3.6±1.37	0.85±0.49	-1.892	0.083
1-penten-3-one	1629-58-9	984	ND	3.77±1.84		
(*Z*)-3-hexenyl acetate	3681-71-8	1134	2.92±1.45	4.3±1.84	0.588	0.568
undecane	1120-21-4	1100	2.83±1.38	0.65±0.35	-1.633	0.128
n-dodecane	112-40-3	1200	1.87±1.19	ND		

n-hexadecane	544-76-3	1600	1.84±0.83	1.59±0.99	-0.192	0.851
n-heptadecane	629-78-7	1700	0.93±0.49	1.17±0.72	0.274	0.788
octadecane	593-45-3	1800	0.92±0.6	0.5±0.37	-0.595	0.563
nonadecane	629-92-5	1900	ND	1.69±1.01		
eicosane	112-95-8	2000	1.89±1.17	ND		
heneicosane	**629-94-7**	**2100**	**2.68±1.42**	**2.03±1.4**	**-0.33**	**0.033**
hexacosane	630-01-3	2600	ND	3.73-1.54		
heptacosane	593-49-7	2700	0.98±0.57	ND		
3-ethyl-3-methylheptane	17302-01-1	NA	0.92±0.8	0.57±0.54	-0.37	0.835
(*Z*)-pinane	6876-13-7	NA	0.94±0.58	0.77±0.56	-0.212	0.212
β-caryophyllene	**87-44-5**	**1746**	**14.97±2.68**	**2.41±0.63**	**-4.561**	**<0.001**
α-pinene	**80-56-8**	**918**	**3.68±1.29**	ND		
β-pinene	127-91-3	998	7.22±3.1	4.81±1.93	-0.663	0.52
3-methylene-6-(1-methylethyl)-cyclohexene	555-10-2	NA	0.36±0.21	0.86±0.6	0.795	0.442
3-carene	13466-78-9	1136	1.1±0.63	ND	-1.754	0.105
α-muurolene	10208-80-7	1808	1.75±0.73	3.23±1.55	0.862	0.405
2-ethyl furan	3208-16-0	663	0.87±0.41	ND		
naphthalene	**91-20-3**	**1498**	**2.45±0.51**	**1.11±0.16**	**-2.292**	**0.041**
m-xylene	108-38-3	883	0.7±0.34	ND		
para-dichlorobenzene	106-46-7	NA	0.56±0.52	2.99±2.85	-1.066	0.307

azulene	275-51-4	1499	2.35±1.44	ND		
(R)-camphor	464-49-3	NA	0.75±0.36	1.66±0.83	1.012	0.332

Table 2 Electronantennographic (EAG) responses of gravid female *Ostrinia furnacalis* to different compounds at three concentration levels. Liquid paraffin was used as control (*). Values are means ± SE. At least 15 individuals were examined for each compound at each concentration level using linear mixed model with vegan package at significance of $p < 0.05$.

Compound	Relative EAG values				F	*p*
	Control*	10^{-3}ug/ul	10^{-2}ug/ul	10^{-1}ug/ul		
(*Z*)-3-hexen-1-ol	**1**	**1.24±0.11**	**1.53±0.11**	**2.58±0.23**	**37.04**	**<0.001**
β-terpineol	1	1.60±0.29	1.58±0.20	1.27±0.17	0.583	0.463
1-penten-3-ol	1	1.04±0.07	1.08±0.08	1.16±0.06	4.514	0.06
2-ethyl-1-hexanol	**1**	**1.20±0.06**	**1.51±0.11**	**2.21±0.20**	**46.78**	**<0.001**
(*E*)-2-hexenal	**1**	**1.51±0.13**	**2.16±0.12**	**2.84±0.23**	**0104.2**	**<0.001**
Caproaldehyde	**1**	**1.02±0.03**	**1.26±0.07**	**1.68±0.1**	**36.43**	**<0.001**
n-dodecane	1	1.02±0.12	1.09±0.34	1.12±0.22	0.782	0.397
nonadecane	1	1.16±0.13	0.99±0.18	1.08±0.18	0.023	0.884
eicosane	1	1.08±0.11	1.05±0.04	1.16±0.08	2.495	0.145
heneicosane	1	1.08±0.12	1.08±0.11	1.15±0.10	1.335	0.271
hexacosane	1	1.06±0.01	1.07±0.04	1.12±0.08	3.627	0.086
heptacosane	1	1.11±0.12	1.02±0.01	1.40±0.25	3.091	0.109
β-caryophyllene	**1**	**1.21±0.07**	**1.71±0.10**	**2.67±0.12**	**78.03**	**<0.001**
α-pinene	**1**	**1.19±0.14**	**1.71±0.08**	**2.46±0.24**	**49.37**	**<0.001**
naphthalene	**1**	**1.29±0.12**	**1.32±0.09**	**2.25±0.21**	**23.88**	**<0.001**
m-Xylene	1	1.07±0.22	0.91±0.10	1.10±0.11	0.071	0.795

Figures

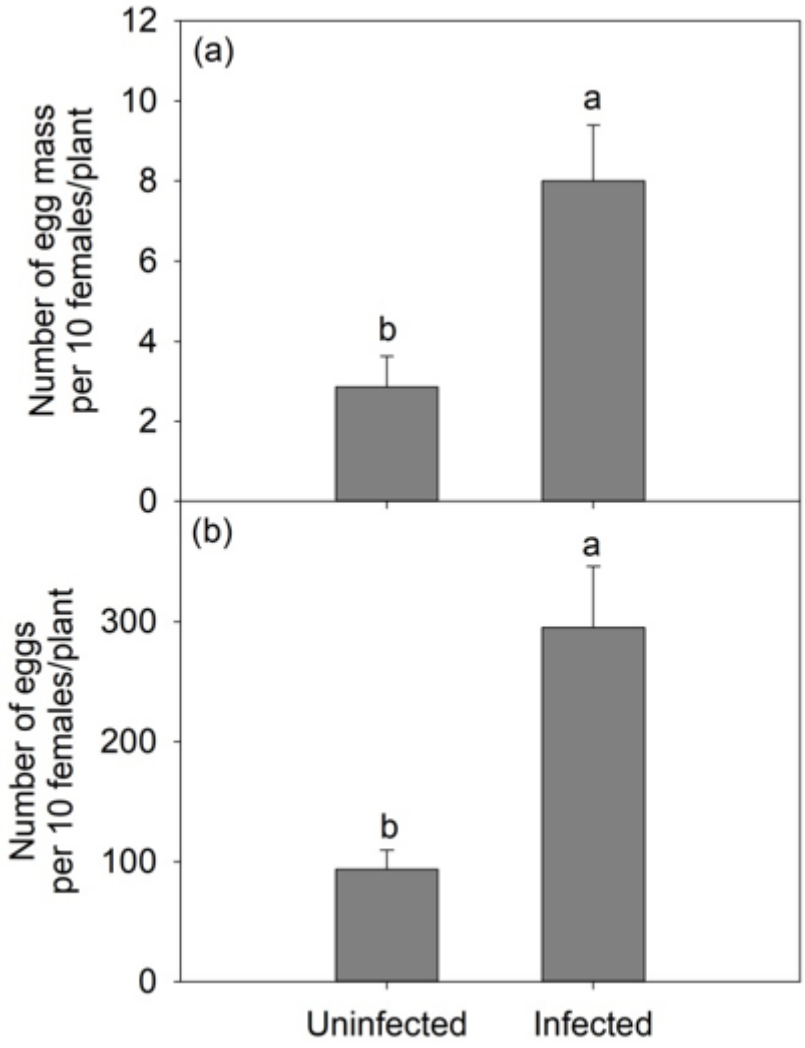

Figure 1

Effects of Beauveria bassiana on the number of egg masses (a) and the number of eggs of Ostrinia furnacalis female moth on each maize plant (b). Values are means ± SE. (n = 10). Different letters above bars indicate significant differences between uninfected and infected maize plants ($p < 0.05$).

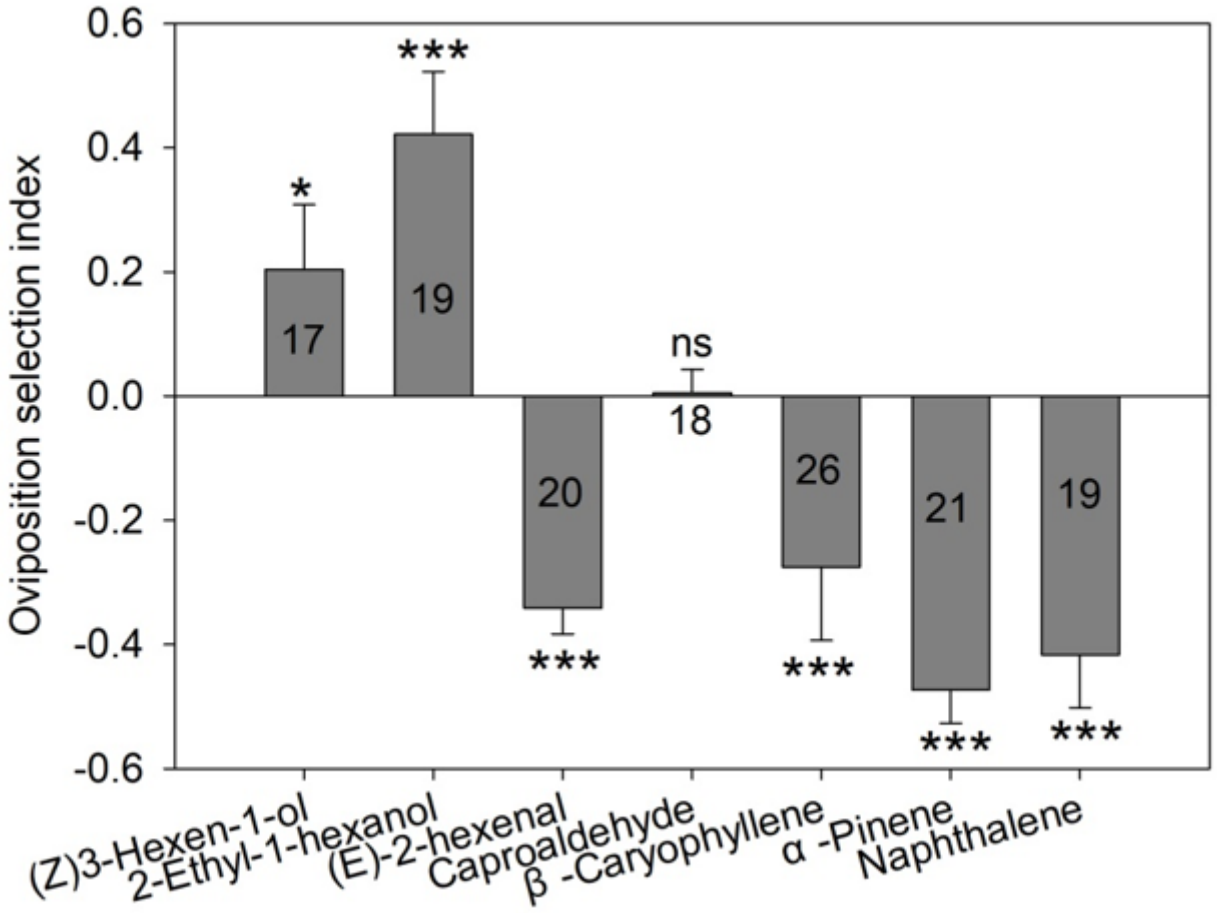

Figure 2

Oviposition stimulation index (OSI) of Ostrinia furnacalis females to different compounds. Values are means ± SE, and number is sample size. Asterisk indicates significant differences between the number of eggs on the treated (chemicals) and on the control (Paraffin) using χ2 tests ($p < 0.05$). * $0.05< p < 0.01$; *** $p < 0.001$; ns, no significance.

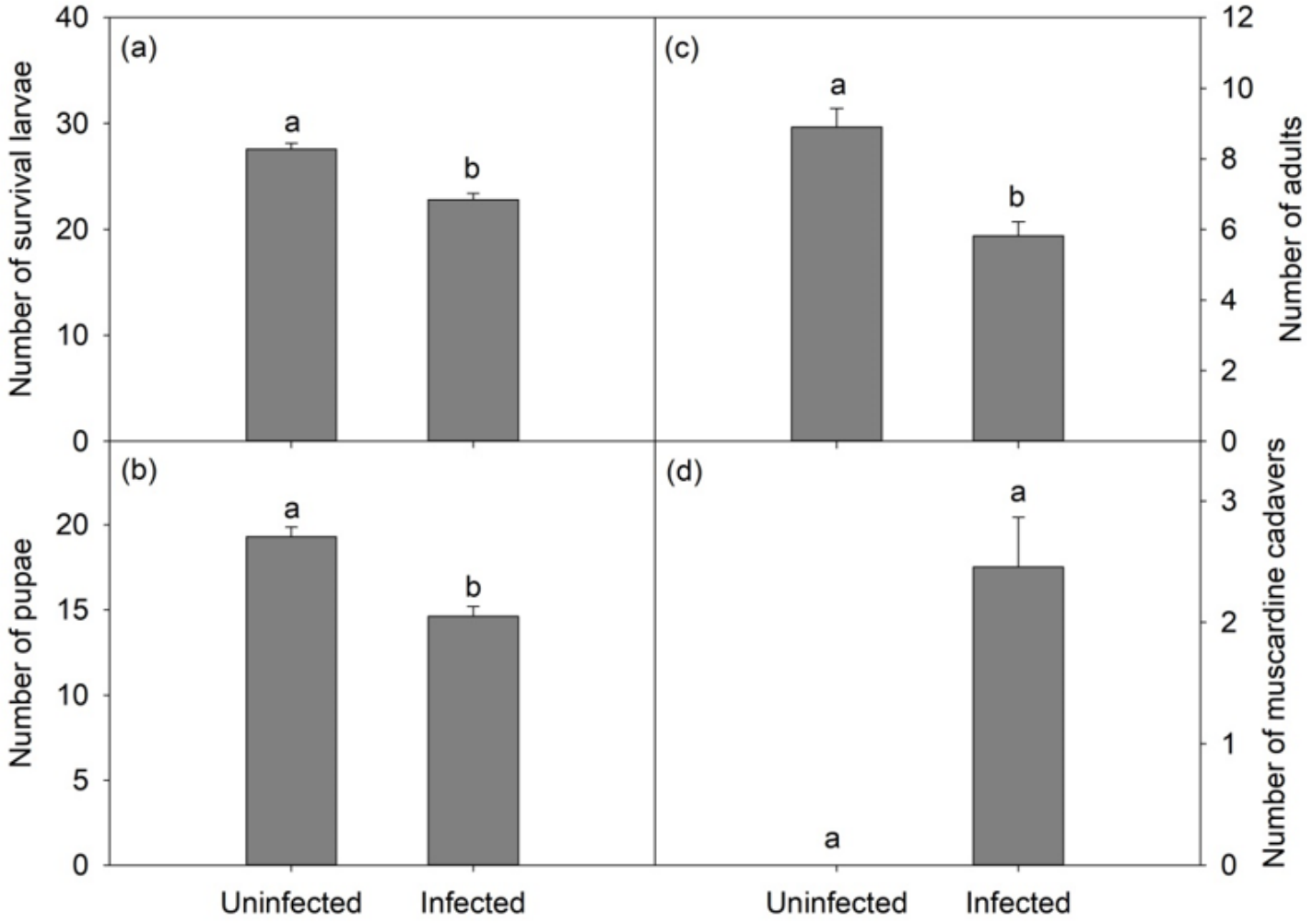

Figure 3

Changes in insect offspring performance between uninfected and infected treatment by Beauveria bassiana. (a) Number of survival larvae, (b) number of pupae, (c) number of adults, and (d) number of muscardine cadavers. Values are means ± SE. (n = 10). Different letters above bars indicate significant differences between uninfected and infected maize plants ($p < 0.05$).

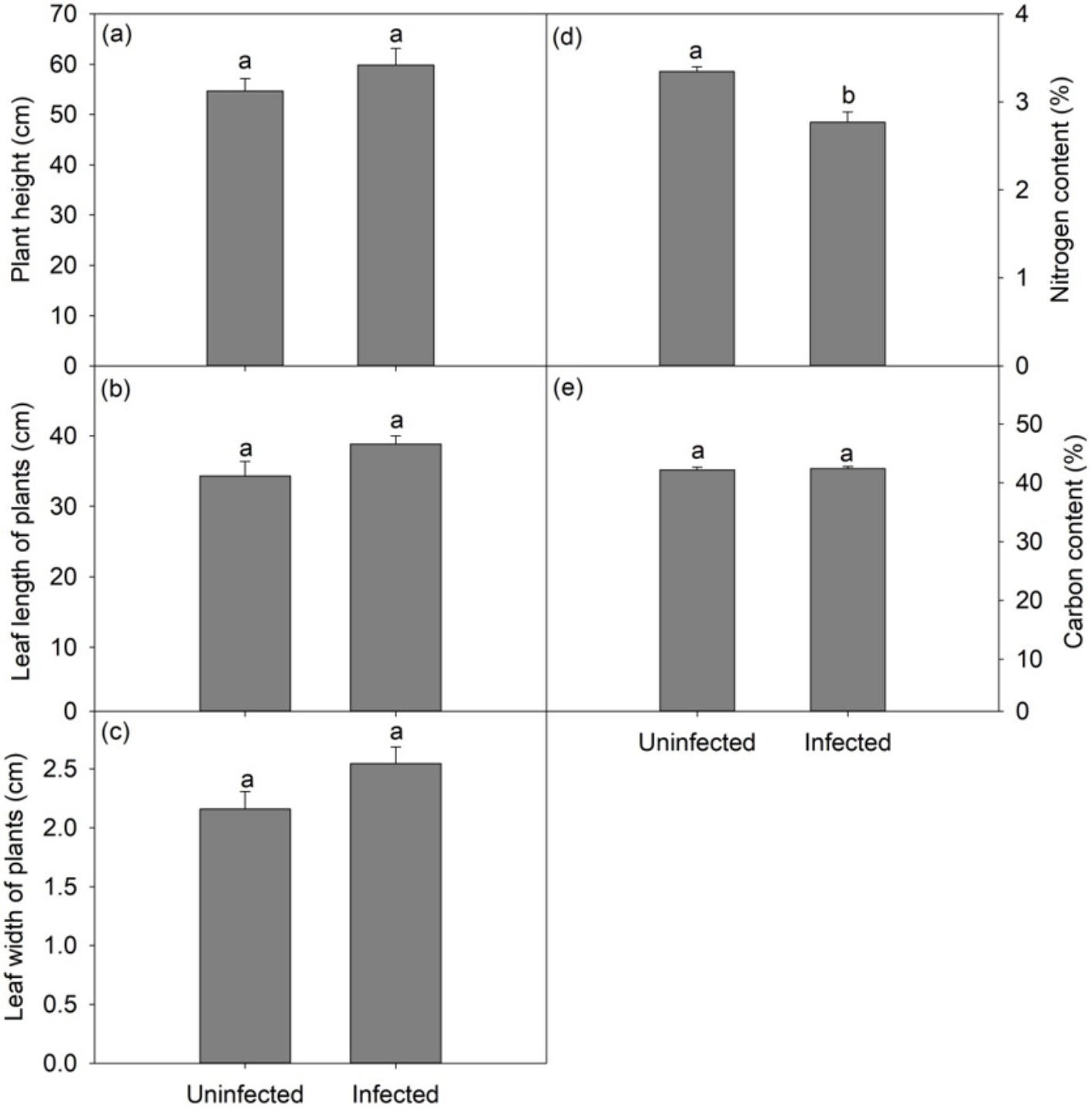

Figure 4

The effects of Beauveria bassiana on properties of maize plants after inoculation. (a) plant height, (b) leaf length of plants, (c) leaf width of plants, (d) nitrogen content, and (e) carbon content. Values are means ± SE. (n = 10). Different letters above bars indicate significant differences between uninfected and infected maize plants ($p < 0.05$).

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