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Assessment of *Beauveria bassiana* for the biological control of corn borer, *Ostrinia furnacalis*, in sweet maize by irrigation application

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Abstract Kernels of sweet maize are directly consumed by humans. This high value crop is grown in arid and semi-arid regions of western Jilin Province, China where trickle irrigation is widely used and larvae of the corn borer, *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae), can cause significant kernel damage. Low humidity in arid regions is less conducive to the efficacy of the biological control agent, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae). Simulated semi-arid conditions in greenhouse experiments were conducted comparing *B. bassiana* application on a granule carrier or in aqueous suspension to sweet maize. Applications of *B. bassiana* adhered to granules and in suspension reduced *O. furnacalis* leaf feeding damage, number of boreholes and tunneling length. Treatments with a granular carrier showed the most significant reductions in maize damage when applied once at whorl stage and in combination with a second application at the ear. The greatest reductions in boring and tunneling attributed to these treatments occurred at internodes around the ear. Although reduced damage was greatest following granular compared to aqueous applications, the latter also provided significant reductions in feeding damage compared to controls. This study demonstrates the utility of *B. bassiana* as a biological control agent for the reduction in damage caused by second-generation corn borer to sweet maize and existing irrigation equipment could be adapted for efficacious aqueous treatments by growers.

Keywords *Beauveria bassiana* · *Ostrinia furnacalis* · Entomopathogenic fungi · Sweet maize

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Introduction

Sweet maize is a high value vegetable crop where fresh kernels are eaten by humans. Consumer preferences demand little to no insect feeding damage to kernels of sweet maize, but meeting these demands often involves intensive insect control methods with multiple chemical insecticide applications. However, these control methods pose risks to non-target and beneficial organisms (Musser and Shelton 2003) and to human health through consumption of insecticide residues (Lu et al. 2018).

Entomopathogenic fungi are extensively used in biological control tactics aimed to control pest feeding damage to crop plants. These practices can increase pest mortality and crop yield while being cost-effective, and less harmful to non-target beneficial organisms and humans compared to chemical insecticides (Charnley and Collins 2007; Zimmermann 2007; Mantzoukas and Eliopoulos 2020). Entomopathogenic fungi in endophytic associations tend to have greater persistence due to shelter from environmental factors that would otherwise decrease spore viability and rates of pest insect infestation (Kim et al. 2014; Maina et al. 2018). Regardless of their relatively prevalent use as biological agents, there is a dearth of studies reporting the efficacy of entomopathogenic fungi for control of insect pest damage compared to studies of chemical insecticides (Bing and Lewis 1991; Russo et al. 2019; Daud et al. 2020). This is especially true for the control of insect damage to sweet maize. Applications of the entomopathogenic biological control agent, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae), adhered to dry granular carrier materials are widely used (Lewis et al. 2002; Kim et al. 2014), but feasibility of this method in large-scale field studies or commercial setting remains limited (Chen and Xue 2016; Lian et al. 2011; Maniania 1993; Wang et al. 1992; Zhang et al. 1990).

Larval Asian corn borer (ACB), *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae), causes serious injury to cultivated maize throughout eastern Asia (Huang et al. 2020; Nafus and Schreiner 1991). This pest inflicts severe yield losses through physiological plant damage that can cause ear droppage prior to mechanical harvest. An univoltine *O. furnacalis* biotype undergo obligatory diapause and produce one mating generation per year, whereas facultative diapause among *O. furnacalis* in multivoltine populations produce one to many annual mating generations (Lu et al. 1995; Wang et al. 2021). Populations with two generations per year predominate across most of the dry regions of Jilin Province in northeast China where irrigated sweet maize is grown.

In Jilin Province first-generation multivoltine *O. furnacalis* females lay eggs in mid- and late-June when maize is in whorl stage, and the subsequent second generation lay eggs in early- to mid-August on maize in ear stage (Lu et al. 2015a, b). Yield loss results from leaf feeding damage and stalk tunneling by larvae of the first-generation, especially by 3rd instars that feed on the mesophyll of whorl stage leaves, which is a period when chemical insecticide applications are most effective (Nafus and Schreiner 1987). In contrast, 1st instars in the second generation mainly feed on the tassel, and sometimes beneath the husks or between the ear and stalk. Third instars in the second generation bore into and feed on stalks (Areekul et al. 1964; Patanakamjorn 1975; Nafus and Schreiner 1987). The short duration that second-generation larvae are exposed prior to boring into stalks leads to a correspondingly narrow time during which applications of contact chemical insecticides are effective. Therefore, few control measures are implemented to control damage by second-generation larvae (Lewis et al. 1996; Nafus and Schreiner 1991).

Biological control agents, including the entomopathogenic fungus *B. bassiana*, offer sustainable season-long control of *O. furnacalis* feeding damage (Feng et al. 2017; Feng et al. 2017; Batool et al. 2020; Daud et al. 2020). Despite this, the efficacy of endophytic entomopathogenic fungi as a pest control agent remains vague, with prior reports over durations from only days to weeks (Bing and Lewis 1991; Pilz et al. 2011). In the arid and sem-iarid maize growing regions in the western Jilin Province, China, trickle irrigation systems are extensively used to apply pesticides. Prior studies indicate that low humidity field conditions decrease the efficacy of broadcast applied *B. bassiana* in aqueous suspensions against *O. furnacalis* at field locations where this insect has two generations per year (Luz and Fargues 1997; Lin et al. 1998; Shipp et al. 2003; Cui et al. 2012). In this study, we simulated dry to semi-arid conditions in greenhouse experiments to compare the effect of different *B. bassiana* application methods (granules *vs.* aqueous suspensions) for the control of feeding damage by second-generation *O. furnacalis*. This study is important for optimizing biological control tactics with a larger goal to reduce environmental impacts and human exposures to toxic insecticidal agents.

Method and materials

Ostrinia furnacalis egg mass collection

Ostrinia furnacalis egg masses were obtained from a laboratory colony maintained at the Institute of Plant Protection, Jilin Academy of Agricultural Sciences, Jilin, China (JAAS). Colony larvae were fed artificial diet and incubated at 26 ± 1 °C, 60–70% RH with a L:D 18:8 photoperiod) as described previously (Song et al. 1999). Moths were kept at constant temperature 26 °C with RH of 60–80% RH and a L:D 14:10 photoperiod, and egg masses laid on wax paper. Egg masses used in this study were laid by females the first day after caging. Furthermore, only egg masses with 60 eggs each were selected, allowed to develop to blackhead stage, and transported to the greenhouse in 2.0 ml air-permeable centrifuge tubes for infestations.

Preparation of *B. bassiana* suspensions and granules

The *B. bassiana* strain GZ01 (preservation number 12471; China General Microbiological Culture Collection Center, Beijing, China) was maintained by JAAS as described in Online Resource (Supplementary material 1a). GZ01 was previously applied to control *O. furnacalis* on a large scale in maize production fields (Li 2015). Strain GZ01 was prepared as (1) pore suspensions at 1×10^8 conidia ml^{-1} (Online Resource Supplementary material 1b) or (2) conidia on ground maize stover granules at 1×10^8 conidia g^{-1} (Online Resource Supplementary material 1c). (2019).

Endophytic colonization of maize plants by *B. bassiana* and control of *O. furnacalis*

Sweet maize seeds were sown in 30 cm diameter × 37 cm high pots with 20 kg of a 1:1 mix of local nutrient soil and placed in a greenhouse at JAAS (25 °C ± 2 °C during the day, 20 °C ± 2 °C during the night, RH 50–60%, L:D 12:12 photoperiod). Blastospore suspensions at 1×10^8 conidia ml^{-1} were prepared as described in Online Resource Supplementary material 1d. Fourteen plants were assigned to each inoculation of 20 ml of *B. bassiana* aerial conidia suspensions at 1×10^8 conidia ml^{-1} (I1); 20 ml of *B. bassiana* blastospore suspensions at 1×10^8 conidia ml^{-1} (I2); or 10 ml of aerial conidia suspensions and 10 ml of blastospore suspensions (I3). A 20 ml application of 0.05% Tween 80 was used as a control. For each treatment group, *B. bassiana* was applied to soil as seeds that were sown, and then poured onto soil every three days until the ear stage. Leaves were collected at Bundesanstalt, Bundessortenamt und Chemical Industrie (BBCH) defined growth stage 15 (five leaves unfolded) (Bleiholder et al. 2001) and surface sterilized, and transferred into potato dextrose agar (PDA) medium containing streptomycin sulphate (100 mg^{-1}) and incubated at 26 °C in the dark for seven days. Fungal growth was recorded daily for 15 days (Online Resource Supplementary material 1e). At BBCH growth stage 15 two 2nd instar *O. furnacalis* larvae were placed into the whorl leaves of each plant across all treatment groups using feather-tipped forceps. The leaf feeding ratings were recorded seven days after infestation.

Maize inoculation with *Beauveria* conidia on a granular carrier

Sweet maize seeds were germinated (Online Resource Supplementary material 1f) and sown in pots as described above. Eighty plants were distributed across 20 groups with four plants in each group, and a distance of 30 cm was maintained between plants within groups such that the leaves did not contact adjacent plants. Plants were grown in a greenhouse at JAAS (25 ℃ ± 2 ℃ during the day, 20 °C ± 2 °C during the night, RH 50–60%, and a L:D 12:12 photoperiod). To avoid cross-contamination by splashing of soil during watering, an automatic dripping irrigation system was used for 1 h once every two days to replicate dry area conditions.

Each of the plants within groups was subjected to a different *B. bassiana* application and *O. furnacalis* infestation regime. Infestations consisted of pinning two black headed *O. furnacalis* egg masses on wax paper substrate to each plant, at one or two separate times to simulate *O. furnacalis* first-generation oviposition by placing the two egg masses into whorls, and second-generation oviposition by sticking the two eggs on the back side of leaves above the ears. Two treatments with five grams of *B. bassiana* granules with 1×10^8 conidia g^{-1} were applied five days post-*O. furnacalis* infestation: Treatment 1 (T1) a single application to the whorl of each plant at BBCH growth stage 15 (after first-generation *O. furnacalis* infestation); or Treatment 2 (T2) to whorls and on the ear at BBCH growth stages 15 and 65, respectively. Uninoculated controls infested at whorl (C1) or ear stage (C2) were included. Leaf feeding was rated seven days after egg infestation on whorl stage maize according to a Chinese national grading standard NY/T 1248.5–2006 (Supplementary Table S1; Wang et al. 2006). In the autumn, the entire stalk of the sweet maize from all treatments were dissected, stem internodes marked, then the number of living *O. furnacalis* larvae, the number of damaged plants, quantity location of corn borer entry holes per plant, and the length of tunneling were recorded.

Root irrigation with *Beauveria* conidia in aqueous suspension

Maize was grown in a greenhouse in the same replicated design as described above, except that five plants were included per group across 20 groups. Also, 20 ml of *B. bassiana* conidial suspension at 1×10^8 conidia ml^{-1} was poured onto soil at time of sowing, followed by three additional inoculations to plants at 3rd leaf stage, early whorl stage and early ear stage as described previously (Li 2015), and corresponding to BBCH growth stages 13, 39, and 65, respectively. Plants were infested with *O. furnacalis* egg masses at whorl and ear stages (Treatment 3; T3), whorl stage (Treatment 4; T4) or ear stage (Treatment 5; T5). Controls C1 and C2 were included as described above. Plants were infested with egg masses and rating of *O. furnacalis* damage was recorded as described in our methods above.

Data analysis

Differences in the efficacy of *B. bassiana* treatments were made independently within and between granule and suspension experiments for all measures: leaf feeding ratings, number boreholes, tunnel length, and number of dead and surviving *O. furnacalis* larvae. Significance in variation was assessed by one-way ANOVA. Due to uneven variance of empirical data, a square root transformation was used before ANOVA for tunnel length and average number of boreholes in respective plant nodes among the granule treatments. *Post-hoc* Tukey's Honest Significant Difference (HSD) tests were then performed on leaf feeding ratings, number boreholes, tunnel length, and number of dead and surviving *O. furnacalis* larvae within and between granule and suspension experiments. All statistical analyses used the multcomp package (http://multcomp.r-forge.r-project.org; Bretz et al. 2011) in R 4.4.2 (R Core Team 2022) via the integrated development environment, Rstudio 2022. 07.2 + 576 (RStudio Team, 2019).

Results

Endophytic colonization of maize plants by *B. bassiana* and control of *O. furnacalis*

We detected *B. bassiana* in maize leaves treated with *B. bassiana* spore suspensions (Fig. S1), with colonization rates for blastospores and aerial conidia treatment (50%), blastospores treatment (50%) and aerial conidia suspensions treatment (57.14%). No *B. bassiana* colonies were formed by samples from un-inoculated controls (Supplementary Fig. S1). The leaf feeding ratings for first-generation of *O. furnacalis* damage (Table 1) were significantly different ($F_{3,52} = 5.141$, $p = 0.0035$), with subsequent Tukey's HSD tests showing significance only between control (C) and aerial conidia & blastospore treatment (I3; $p = 0.0019$; Table 1).

Beauveria granule inoculation effects on control of second-generation of *O. furnacalis* (ear stage of corn)

Number of surviving larvae and measures of damage to maize among controls (C1 and C2) and treatments (T1 and T2: Table 2) showed that *O. furnacalis* can be controlled when *B. bassiana* granules were applied to maize whorl based on measurements taken seven days after *O. furnacalis* infestation. Specifically, significant variation was detected among C1, C2, T1 and T2 for all measures ($F_{3,76} \geq 3.873$, $p \leq 0.0124$, Supplementary Table S2a). Significantly lower levels of *O. furnacalis* damage in maize treated with granules

Table 1 Effect of inoculation with *Beauveria bassiana* strain GZ01 spore suspensions on leaf feeding damage by first-generation *Ostrinia furnacalis* to sweet maize at growth stage 15 (5 leaves unfolded) (Bleiholder et al. 2001)

ID	Treatment description	% col	LFR Mean ± SE	Tukey's HSD (Q-statistic\p-value) C	I1	I2	I3
C	Control: 0.05% Tween 80	0.00	4.21 ± 0.58	–	0.1250	0.5032	**0.0019**
I1	Aerial conidia suspensions	57.14	2.50 ± 0.49	3.1735	–	0.8140	0.3946
I2	Blastospore suspensions	50.00	3.14 ± 0.65	1.9835	1.1901	–	0.0838
I3	Aerial conidia & blastospore	50.00	1.29 ± 0.41	5.4214	2.2479	3.4380	–

The percent colonization (%col) was calculated from 14 leaf samples with *B. bassiana* growth after 15 d on potato dextrose agar (PDA) medium. Results for leaf feeding rating (LFR) shown as mean values (± SE). Following significant one-way ANOVA, subsequent Tukey's Honest Significant Difference (HSD) test results are indicated (p-values less than 0.05 are highlighted)

Table 2 Effect of *Beauveria bassiana* granular applications on *Ostrinia furnacalis* damage to sweet maize

Treatment (ID)	*B. bassiana* inoculation	*O. furnacalis* infestation	Leaf feeding rating[1]	Boreholes	Tunnel length (cm)	Surviving larvae	Dead larvae
Control 1 (C1)	NA	Whorl	5.10 ± 0.39a	4.65 ± 0.41a	10.70 ± 1.49ab	1.25 ± 0.26ab	0.20 ± 0.12a
Control 2 (C2)	NA	Ear	3.78 ± 0.34b	5.60 ± 0.79a	14.99 ± 3.39a	1.70 ± 0.36a	0.20 ± 0.09a
Treatment 1 (T1)	Whorl	Whorl & ear	1.72 ± 0.17c	2.55 ± 0.39b	5.17 ± 1.05b	0.60 ± 0.21b	0.10 ± 0.07a
Treatment 2 (T2)	Whorl & ear	Whorl & ear	2.70 ± 0.26bc	2.15 ± 0.26b	5.91 ± 0.86b	0.70 ± 0.18b	0.50 ± 0.17a

Results are shown for leaf feeding rating, borehole number, tunnel length, surviving larvae number and number of dead *O. furnacalis* larvae from treatment and control maize plants (NA indicates no application; control). Values (± SE) with same letter within the column are not significantly different based on Tukey's Honest Significant Difference (HSD) test at $p < 0.05$

[1]Chinese national grading standard NY/T 1248.5–2006 (Wang et al. 2006; Supplementary Table S1)

at whorl stage and those infested at whorl and ear stages (T1) compared to controls infested at whorl stage (C1), as detected by Tukey's HSD tests for leaf feeding ratings ($Q_{k=4,v=76} = 11.260$, $p = 0.0010$, Supplementary Table S2a), and borehole number ($Q_{4,76} = 4.174$, $p = 0.0215$, Supplementary Table S2b). Significant differences were also detected between T1 and C2 (infested at ear stage) for leaf feeding rating ($Q_{4,76} = 6.878$, $p = 0.0010$, Supplementary Table S2a), the borehole number ($Q_{4,76} = 6.061$, $p = 0.0010$, Supplementary Table S2b), the tunnel lengths ($Q_{4,76} = 4.977$, $p = 0.0040$, Supplementary Table S2c) and the number of surviving larvae ($Q_{4,76} = 4.276$, $p = 0.0193$, Supplementary Table S2d), wherein all rating were lower for treatments compared to control groups. Compared to uninoculated C1 (infested at whorl stage), T2 (granular *B. bassiana* applications and *O. furnacalis* infestations at both whorl and ear stages) showed significantly lower leaf feeding ratings ($Q_{4,76} = 7.987$, $p = 0.0010$, Supplementary Table S2a), and borehole number ($Q_{4,76} = 4.968$, $p < 0.0041$, Supplementary Table S2b). Analogously, T2 was significantly lower than C2 plants in the number of boreholes ($Q_{4,76} = 6.856$, $p = 0.0010$, Supplementary Table S2b), tunnel lengths ($Q_{4,76} = 4.602$, $p = 0.0090$, Supplementary Table S2c), and the number of alive larvae ($Q_{4,76} = 3.842$, $p = 0.0399$, Supplementary Table S2d). C1 and C2 showed significant differences only in leaf feeding ratings ($Q_{4,76} = 4.282$, $p = 0.0142$, Supplementary Table S2; Fig. 1a). No differences in levels of damage were observed between T1 and T2. These results revealed the relative equivalence of *B. bassiana* granule treatments at whorl stage and at both whorl and ear stages for reducing *O. furnacalis* leaf feeding ratings (Fig. 1a, Table 2) as well as decreasing borehole number, tunnel length, and number of surviving larvae (Fig. 1b, Table 2).

The spatial distribution of *O. furnacalis* damage was biased towards the middle part of the plant at internodes around the ear. Within treatments and

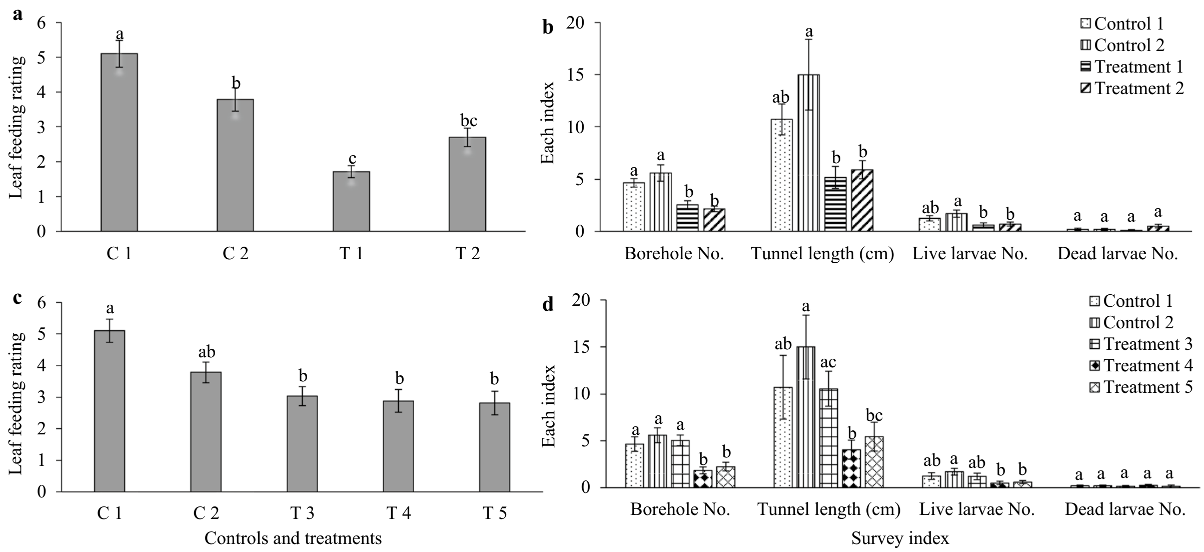

Fig. 1 Effects of *Beauveria bassiana* treatments on *Ostrinia furnacalis* leaf feeding ratings, borehole number, tunnel length (cm), number of alive larvae and number of dead larvae on sweet maize control and treatment plants. Corresponding results shown for granular (**a**; **b**) and irrigated aqueous suspension treatments (**c**; **d**). Control (C) and treatment (T) conditions for granular and aqueous suspension conditions are described in Tables 2 and 3, respectively. Bars (±SE) with different lower-case letters indicate significant differences among treatments ($p < 0.05$)

controls there were significant differences in the number of boreholes and tunnel length between upper, middle and lower internodes (Fig. 2; $p < 0.05$; remaining data not shown).

Control of *O. furnacalis* on sweet maize using *Beauveria* conidial suspensions

The second *B. bassiana* application method used suspensions that were sprayed on soil and vegetative

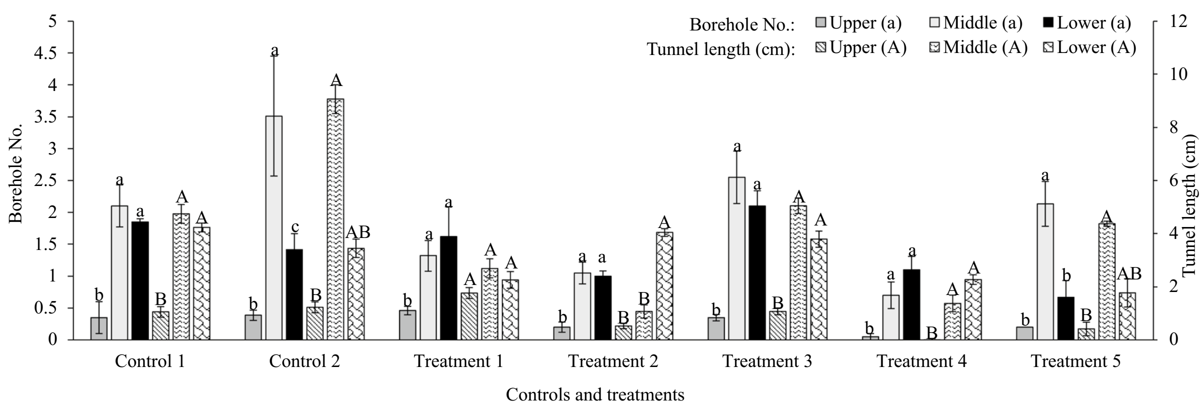

Fig. 2 Effect of *Beauveria bassiana* applications on spatial distribution of *O. furnacalis* damage to sweet maize upper, middle and lower parts of the same treatment for (a) borehole number and (A) tunnel length (cm). C: Control (C) and treatment (T) for granular and aqueous suspensions applications are described in Tables 2 and 3, respectively. Lower: 4–8 nodes under ear; Middle: 3 nodes under ear to 3 nodes above ear; Upper: 4–7 nodes above ear and male spike. Bars (±SE) with different letters denote significant differences of borehole number (lower case letters) and tunnel length (capital letters) among upper/middle/lower parts within same treatment ($P < 0.05$)

tissues to simulate repeated overhead field irrigations. These resulted in significant differences in 7-day post-infestation damage ratings between all treatments and control plants across all measures ($F_{4,95} \geq 3.290$, $p \leq 0.0143$; Supplementary Table S2a). Specifically, for infestations at whorl stage the ratings were significant lower in T4 compared to C1 for leaf feeding ($Q_{5,96} = 6.081$, $p = 0.0010$, Supplementary Table S2a), and borehole number ($Q_{5,96} = 5.183$, $p = 0.0037$, Table S2b). Plants treated with *Beauveria* in aqueous suspension and infested in ear stage in T5 showed significantly lower number of boreholes ($Q_{5,96} = 6.201$, $p = 0.0010$, Supplementary Table S2b), tunnel lengths ($Q_{5,96} = 4.707$, $p = 0.0107$, Supplementary Table S2c) and the number of surviving larvae ($Q_{5,96} = 4.010$, $p = 0.0434$, Supplementary Table S2d) compared to its corresponding C2. In contrast, borehole number and tunnel lengths of plants infested in whorl and ear stages (T3) did not differ significantly from either of the two controls, C1 or C2. These measures were significantly higher in T3 compared to T4 (infestation at whorl stage) ($Q_{5,96} = 7.556$, $p < 0.0010$, Supplementary Table S2b; $Q_{5,96} = 4.935$, $p = 0.0065$, Supplementary Table S2c). Only borehole number was different between T3 and T5 (infestation at ear stage) ($Q_{5,96} = 6.612$, $p < 0.0001$, Supplementary Table S2b). The number of dead larvae was not significantly different among the treatments or when compared to their controls (Fig. 1d).

Boreholes on maize treated with *B. bassiana* suspension were predominantly documented in the internodes around the ear node, whereas internodes above the ear had fewer than those below the ear. This was similarly shown for tunnel length. There were significant differences among the upper, middle and lower internodes of stalks in the same treatment for borehole number and the tunnel length (Fig. 2; $p < 0.05$; remaining data not shown).

Significant variation was detected among a combination of granular and suspension treatments for leaf feeding ratings ($F_{4,95} = 2.779$, $p = 0.0312$), borehole number ($F_{4,95} = 9.407$, $p < 0.0001$), and tunnel lengths ($F_{4,95} = 3.642$, $p = 0.0083$) (Supplementary Table S2), but not for number of surviving larvae ($F_{4,95} = 1.493$, $p = 0.2105$). Results of subsequent Tukey's HSD tests for leaf feeding ratings, borehole number, and tunnel length data indicated a total of four significant differences between treatments from granular (T1 and T2) and suspension treatments (T3, T4, and T5). Specifically, three of these significance estimates were a consequence of higher readings for T3 compared to T1 ($Q_{5,96} \geq 4.089$, $p \leq 0.0374$) (Supplementary Table S2).

Discussion

Beauveria bassiana is a naturally occurring fungus that resides in soil and forms an endophytic relationship with maize (Bing and Lewis 1992; Lewis 2001; Russo et al. 2019) and other crop plants (Vega 2008). In accordance with prior observations, this study detected endophytic growth of *B. bassiana* strain GZ01in maize plants which resulted in a significant decrease in *O. furnacalis* leaf feeding damage compared to control (non-endophyte containing) plants (Table 1). Leaf feeding ratings in this study were highest among plants infested at whorl stage (C1), thus showing that first-generation *O. furnacalis* larvae mainly feed on leaves. In contrast, borehole number, tunnel length, and number of surviving larvae were highest in C2 that were infested at ear stage (Table 2), agreeing with prior results that second-generation *O. furnacalis* larvae mainly bore into stalks (Nafus and Schreiner 1987). Findings of this study further demonstrated that *B. bassiana* can effectively control damage to sweet maize by first- and second-generation *O. furnacalis*, when either adhered to granules or in aqueous suspension. These findings are in accordance with prior results that showed analogous control of damage by European corn borer, *O. nubilalis*, following application of *B. bassiana* granules to maize leaves (Berry et al. 1980; Bing and Lewis 1992). Granular applications also resulted in reduced damage by stem boring and leaf feeding by other insects (Ramos et al. 2020; Renuka et al. 2016; Russo et al. 2019). Our study is the first to investigate the efficacy of *B. bassiana*-based biological control programs in arid regions.

Prior studies indicate that the efficacy of *B. bassiana* control is negatively impacted by increasing temperature (Kryukov et al. 2012), ultraviolet radiation exposures (Acheampong et al. 2020), and low humidity conditions (Shipp et al. 2003). However, formulations including suspensions in oils are used to increase viability when exposed to UV (Inglis et al. 1995; Kaiser et al. 2019) and show utility in arid regions (Hoddle and Driesche 2009). Our greenhouse study showed significant reductions in all *O. furnacalis* maize damage ratings and measurements for all treatments compared to one or both controls, especially borehole number. These effects were observed despite no light or UV protection provided to granules outside of application within the whorl, where these exposures may have been minimized.

Ostrinia furnacalis larvae cause feeding damage to maize, which varies across instar and growth stages of the infested plant (Nafus and Schreiner 1987). The location of corn borer oviposition on maize shifts across the growing season, with first-generation female egg laying biased against the upper leaves (Shelton et al. 1986; Spangler and Calvin 2001) and second-generation preference for near the ear (Sorenson et al. 1993; Windels and Chiang 1975). Correspondingly, feeding damage from early instars occurs on centrally located leaves. Due to larval movements, damage also occurs on upper leaves in both generations and to tassel tissue in the second generation (Huber et al. 1928). Later instars of second-generation are concentrated around ears and silks (Zoerb et al. 2003), and bore into the pith tissue of stalks in final instars (Huber et al. 1928; Nafus and Schreiner 1987). Our study suggests that a single application of *B. bassiana* granules at whorl stage could provide significant season-long control for both generations of *O. furnacalis*, and dual application provided no significant comparative increase in efficacy (Table 2) except a reduction in leaf feeding (Fig. 2). This latter finding suggested that a "booster" inoculation at ear stage may provide additional control of leaf damage, but economic feasibility of additional application will need to be evaluated in future studies.

Beauveria bassiana applications via an initial root drench followed by repeated overhead applications simulating in-field treatments of maize using irrigation equipment also resulted in significantly reduced *O. furnacalis* damages. Interestingly, despite T4 and T5 being significantly different from one or both controls across leaf feeding rating, borehole number, tunnel length, and number of surviving larvae, T3 (infestation at whorl and ear) only showed a significant effect in borehole number (Supplementary Table S2; Table 3). Although not investigated further, these differences may be influenced by an increasing amount of larval feeding in T3 that received two *O. furnacalis* infestations compared to the single *O. furnacalis* infestation of T5. Additionally, between experiments, the borehole number for T3 (5.05 ± 2.50) was significantly higher compared to T1 (2.55 ± 1.76) or T2 (2.15 ± 1.18; Supplementary Table S2b). Similar results were shown for T3 compared to T1 for borehole number and tunnel length measures. These lines of evidence might suggest that aerial irrigation may not provide season-long control of first- and second-generation *O. furnacalis*. This contrasted with T1 and T2 which received granular treatments at whorl and both whorl and ear stages, respectively. Overall, this study indicated that although *B. bassiana* applications of aqueous suspensions may reduce damage from a single infestation, corresponding reductions may not

Table 3 Effect of *Beauveria bassiana* applications in aqueous suspensions on *Ostrinia furnacalis* damage to sweet maize

Treatment (ID)	*B. bassiana* irrigation	*O. furnacalis* infestation	Leaf feeding rating[1]	Boreholes	Tunnel length (cm)	Surviving larvae	Dead larvae
Control 1 (C1)	NA	Whorl	5.10 ± 0.37a	4.65 ± 0.41a	10.70 ± 1.49ab	1.25 ± 0.26ab	0.20 ± 0.12a
Control 2 (C2)	NA	Ear	3.78 ± 0.32ab	5.60 ± 0.79a	14.99 ± 3.39a	1.70 ± 0.36a	0.20 ± 0.09a
Treatment 3 (T3)	Seeding to ear	Whorl & ear	3.03 ± 0.30b	5.05 ± 0.56a	10.56 ± 1.85ac	1.20 ± 0.34ab	0.15 ± 0.08a
Treatment 4 (T4)	Seeding to ear	Whorl	2.88 ± 0.36b	1.85 ± 0.37b	4.06 ± 1.02b	0.50 ± 0.18b	0.25 ± 0.10a
Treatment 5 (T5)	Seeding to ear	Ear	2.82 ± 0.37b	2.25 ± 0.47b	5.45 ± 1.53bc	0.60 ± 0.17b	0.15 ± 0.15a

Results are shown for leaf feeding rating, borehole number, tunnel length, surviving larvae number and number of dead *O. furnacalis* larvae from treatment and control plants (NA indicates no application; control). Values (± SE) with same letter within the column are not significantly different based on Tukey's Honest Significant Difference (HSD) test at $p < 0.05$

[1]Chinese national grading standard NY/T 1248.5–2006 (Wang et al. 2015)

be provided following dual infestations. Protection from such cumulative larval damage is needed in growing regions with two *O. furnacalis* generations (Lu et al. 2015a, b; Wang et al. 2021). Thus, granular applications may provide the greatest level of season-long control, which is especially important in areas of bivoltine *O. furnacalis* populations.

Since our methods did not use any protectants within aqueous suspensions (only in 0.05% Tween), the viability of *B. bassiana* and corresponding efficacy may be increased in aerial irrigations when mixed with oils that protect from UV damage. This warrants further study. Application of *B. bassiana* in aqueous suspensions through established irrigation equipment might prove to be more economically feasible compared to granular application that require specialized equipment (Bateman et al. 2007).

Experiments have used living plants to study the effect of endophytic fungi on the control of larval feeding damage (Li 2015; Abed and Saleh 2017; Qin et al. 2021), as opposed to detached leaves (Gurulingappa et al. 2010; Russo et al. 2019; Ramos et al. 2020). We encountered confounding factors in *on planta* experiments under greenhouse conditions. For instance, our study showed leaf feeding damage on C2 and T5, which were not infested with *O. furnacalis* egg masses in whorl stage. This may have occurred due to larval movement, which was previously shown to occur on and between maize plants (Goldstein et al. 2010). Regardless, the leaf feeding ratings in C2 (3.78) and T5 (2.82; Table 3) were lower than in C1 ($Q_{5,96}=3.612$, $p=0.0875$). Thus, this damage putatively due to larval movement had negligible impact on our results. One outlier measurement which resulted in significantly greater tunnel length in the middle compared to lower internodes in C2, was contained to one replication which had much higher measures compared to the others. This caused increased heterogeneity and overall variance.

In conclusion, our data from greenhouse experiments indicate that applications of *B. bassiana* on granules or in aqueous suspensions reduced damage to sweet maize compared to untreated controls in simulated arid conditions. These data show that *B. bassiana* can be an effective biocontrol agent in arid regions, but *B. bassiana* granular applications provided greater reduction of damage caused by *O. furnacalis* compared to aqueous suspension. Regardless, since there is no effective mechanized method to precisely apply granules into whorls in commercial-scale fields, the overall feasibility of this method remains in question. Our results suggest that *B. bassiana* in aqueous suspension provides a potential alternative for effective control of first- and second- generation *O. furnacalis* damage to sweet maize. Although not tested directly in our study, *B. bassiana* aqueous suspensions are likely amenable to field applications using existing grower-owned drip irrigation equipment. However, temperature, humidity, light and UV exposures are likely to be different in field compared to our simulated greenhouse conditions. Thus, future replicated field trials using oil-based UV protectants within aqueous suspension are warranted to demonstrate impact on efficacy effects under natural conditions.

Author contributions MF conducted the experiments, analyzed data, and wrote the draft manuscript; YZ helped the *Beauveria bassiana* suspension and granule preparation and inoculation; BSC was involved in experiment design, writing and revision of the manuscript; LL maintained the original strain of *B. bassiana* and rejuvenation of *Ostrinia furnacalis*; HY was involved in rejuvenation of *O. furnacalis*; QD and YG, WS, XC, SZ were involved in investigations and *O. furnacalis* rearing in the laboratory. YW secured funding, oversaw the research project, project design and writing of the manuscript.

Declarations

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

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