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Endophytic colonization by *Beauveria bassiana* induces steroidal glycoalkaloids accumulation in tomato and suppresses *Tuta absoluta* performance

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Abstract

Beauveria bassiana is widely used as an entomopathogen in sustainable pest management, but whether its endophytic colonization can prime plant defence against herbivores remains unclear. Here we show that root drenching of tomato with *B. bassiana* substantially reduced the fitness of *Tuta absoluta*: larval development was prolonged by 2.1 days, fourth-instar larval weight dropped by 34%, pupal weight by 22%, and pupation rate, emergence rate and female fecundity all declined significantly. Foliar spray application produced weaker effects, affecting only pupal weight and emergence rate. No *B. bassiana* was re-isolated from insect bodies, ruling out direct infection and pointing to a plant-mediated mechanism. Transcriptomic profiling of *T. absoluta* larvae feeding on colonized plants revealed the upregulation of detoxification-related metabolic pathways, particularly cytochrome P450 genes. To test whether this response was triggered by steroidal glycoalkaloids (SGAs), we fed larvae on tomato mutants with reduced or elevated SGA content. Larvae feeding on SGA-knockout mutants showed no changes in detoxification enzyme activity or development relative to controls, whereas those feeding on SGA-overexpressing mutants exhibited strong developmental inhibition and a marked increase in cytochrome P450 activity. These results demonstrate that endophytic *B. bassiana* primes tomato defence against *T. absoluta* specifically through SGA induction. Our findings establish a mechanistic basis for integrating entomopathogenic fungi with plant innate immunity in pest management.

Keywords *Beauveria bassiana* · Tomato · *Tuta absoluta* · Growth and development · Steroidal glycoalkaloids

Introduction

T. absoluta is a high-risk invasive pest that has been recorded in more than 150 countries and regions worldwide (Guillemaud et al. 2015; Zhang et al. 2020). This polyphagous leafminer feeds on a broad range of host plants (Han et al. 2019). Larvae burrow within leaf tissues, causing desiccation, fruit malformation and stunted seedling growth; in severe infestations, complete host plant mortality can occur (Zhang et al. 2018). Without effective management, *T. absoluta* has been reported to cause yield losses of 80-100%, threatening the sustainability of tomato production across affected region (Desneux et al. 2011). Currently, chemical insecticides remain the primary control tactic (Han et al. 2018), yet heavy reliance on them has raised concerns about resistance, resurgence and residue, in addition to

risks to human health and the environment. The efficacy of chemical applications is further compromised by the pest's cryptic larval feeding behavior, high fecundity, strong dispersal capacity, and moderate-to-high resistance to several commonly used insecticides (Urbaneja et al. 2013; Biondi et al. 2018; Cherif et al. 2019; Guedes et al. 2019). Given these challenges, there is an urgent need to for sustainable alternatives that can effectively manage this high-risk invasive pest.

Entomopathogenic fungi, as natural enemies of insect pests, rank among the earliest biological control agents used in pest management (Kidanud et al. 2020; Srinivas et al. 2021). However, the field efficacy of conventional foliar applications is often inconsistent, limited by ultraviolet radiation, temperature extremes and humidity fluctuations (Alviti et al. 2025). Furthermore, direct spraying may pose unintended risks to non-target organisms (Bultman et al. 1997; Omacini et al. 2001). An alternative strategy has emerged from the recognition that many entomopathogenic fungi can adopt an endophytic lifestyle. These fungi colonise plant roots, stems, and leaves, either through natural infection or via deliberate inoculation methods such as seed soaking, root drenching, and foliar spraying, thereby establishing fungus-plant associations (Ahsan et al. 2024). Once endophytically established, these entomopathogenic fungi can suppress pest survival, development, and reproduction (Ramakuwela et al. 2020; Shrivastava et al. 2015; Jaber et al. 2018). Beyond this direct antagonism, endophytic colonization can also modify the host plant's secondary metabolite profiles, including alkaloids, phenolic compounds and flavonoids, changes that may further reduce pest fitness (Wang et al. 2023; Giannoulakis et al. 2023).

Plant secondary metabolites (PSMs) are central to plant defense against biotic stress (Khare et al. 2020). Among the PSMs whose accumulation is modulated by endophytic colonization of entomopathogenic fungi, SGAs in tomato deserve particular attention. SGAs are specialized metabolites biosynthesized from cholesterol in Solanaceous plants and are known not only for their structural diversity and pharmacological relevance, but also for their capacity to reinforce plant resistance against pathogens and herbivores (Zhao et al. 2021). We previously showed that root drenching with *B. bassiana* significantly induces SGAs accumulation in tomato leaves after endophytic establishment (Wang et al. 2023). Given that SGAs are among the most abundant defensive metabolites in tomato, their induction by endophytic fungi may represent an important mechanism through which these fungi confer host resistance to insect herbivores.

Although entomopathogenic fungi such as *B. bassiana* have been shown to colonize tomato plants endophytically and to suppress *T. absoluta,* the mechanism underlying this suppression remains unclear — in particular, whether it is mediated by direct fungal infection of the pest or indirectly through the induction of host plant chemical defenses after endophytic establishment. To distinguish between these possibilities, we established a *B. bassiana*-tomato-*T. absoluta* tripartite system and applied root-drenching and foliar-spray inoculation to facilitate fungal colonization of tomato plants. We then assessed the phenotypic responses and fungal infection status of *T. absoluta* larvae fed on inoculated tomato tissues. By integrating transcriptomic profiling, enzyme activity assays, and phenotypic validation using SGAs mutants fed to *T. absoluta*, we tested whether endophytic colonisation suppresses the pest through direct infection or through plant-mediated chemical defence. This work provides mechanistic evidence for indirect chemical defence mediated by an endophytic entomopathogenic fungus, and establishes a framework for managing leafminers through the induction of plant innate immunity.

Materials and methods

Biological materials

The tomato variety used in this study was 'Hezuo 903'. Seeds were surface-sterilized and sown in autoclaved potting substrate for germination. Seedlings were maintained in a climate-controlled growth chamber at 26±1°C, 65 ± 5% relative humidity, and a 14 h light:10 h dark photoperiod. Approximately one week after emergence, seedlings were transplanted into individual pots and grown under the same conditions for subsequent experiments.

The *B bassiana* strain ARSEF 252 was obtained from the USDA ARS Collection of Entomopathogenic Fungal Cultures. After single-spore isolation, the strain was maintained as cryopreserved stocks at the Biotechnology Center of Southwest University. For experimental use, spores cryopreserved in glycerol at -80°C were thawed and spread onto potato dextrose agar (PDA) plates, which were incubated at 26 ± 1°C. After 14 days, freshly produced conidia were harvested by scraping into 0.05% Tween 80 solution, resuspended and filtered. The resulting suspension was adjusted to 1×10^8 conidia/mL and used within 24 h.

T. absoluta individuals were collected from tomato greenhouses in Bishan District, Chongqing, in 2023. The colony was reared on fresh tomato plants in cages under controlled conditions of 27±1°C, 70±10% relative humidity, and 14:10 h light:dark photoperiod.

Inoculation of tomato seedlings

Tomato seedlings at the five-leaf stage with uniform growth were selected for inoculation with the *B. bassiana* spore suspension (1×10^8 conidia/mL) by either root drenching or localized foliar spray. For root drenching, 10 mL of the suspension was drawn into a sterile syringe and uniformly applied to the soil around the root zone of each seedling. For foliar spray, 1 mL of the suspension was aspirated with a sterile syringe, transferred into a 30 mL hand-held sprayer, and evenly evenly applied to a single leaf per plant, always at the same nodal position to ensure consistency. Control plants received 0.05% Tween 80 solution by the same procedures.

Verification of endophytic colonisation

Leaf samples were collected at 7, 14, and 21 days post inoculation. At each time point, one leaf was randomly excised from each plant, placed into a 1.5 mL microcentrifuge tube and immediately ground to a fine powder in liquid nitrogen. *B. bassiana* spores served as the positive control; leaves from with Tween 80-inoculated plants served as the negative control. Total genomic DNA was extracted using a fungal DNA extraction kit (Ezup Column Fungal Genomic DNA Extraction Kit, Sangon Biotech). PCR was performed with the universal primer pair ITS1-F (5'-CTTGTTCGCTATCGGTCTC-3') and ITS4-R (5'-TCCGTAGGTGAACCTGCGG-3'). PCR products were sent to a commercial sequencing facility for Sanger sequencing, and the obtained sequences were compared against reference databases.

Insect performance assays

At 7 days post inoculation, ten neonatal *T. absoluta* larvae were introduced onto each tomato plant, and individual plants were numbered accordingly. Each treatment group comprised eight plants. Larval developmental duration and survival at each instar were recorded at 12 h intervals. Upon adult emergence, male and female moths were paired at a 1:1 ratio (ten replicate pairs per treatment). Each pair was transferred to a plastic container supplied with a cotton ball soaked in 10% honey water and with untreated tomato leaves as oviposition substrates. Leaves were replaced every 24 h, and the number of eggs deposited was recorded together with the oviposition period .

In a separate experiment, neonatal *T. absoluta* larvae were introduced onto tomato plants at a density of ten larvae per plant, with four plants per treatment group. Observations were conducted daily at the same time. After each molt, larvae were allowed to feed for an additional 24 h before being sacrificed with 75% ethanol. Head capsule width and body length were measured using a zoom stereomicroscope; body weight was determined with an electronic balance. Measurements were taken for third-instar larvae, fourth-instar larvae and pupae.

Detection of *B. bassiana* in insects tissues

To determine whether *B. bassiana* had colonized larval tissues, a re-isolation experiment was conducted. Larvae fed on tomato seedlings treated with 0.05% Tween 80 solution via root drenching served as the control group. At 7 days after root drenching inoculation with *B. bassiana* , ten larvae were introduced onto each tomato plant, with six plants per treatment group. After 9 days of feeding, larvae were collected and divided into three subgroups for different analyses.

For the first subgroup, whole larvae were surface-sterilized and homogenized at a ratio of three larvae per 200 μL of 1 × PBS; the homogenate was spread onto PDA plates. For the second subgroup, surface-sterilized larvae were dissected in sterile water to isolate the intestinal tracts, which were then homogenized at a ratio of ten intestines per 200 μL of 1 × PBS and spread onto PDA plates. All plates were incubated for 14 days and examined for the presence of *B. bassiana* colonies; PDA plates inoculated with *B. bassiana* spores served as positive controls.

For the third subgroup, three larvae were placed into each 1.5 mL microcentrifuge tube (four replicates per treatment), rapidly ground in liquid nitrogen, and then processed for fungal genomic DNA was extraction and PCR detection of *B. bassiana*-specific sequences.

RNA-Seq and transcriptomic analysis

Eight tomato seedlings were divided into two groups: four were inoculated with *B. bassiana* via root drenching, and four remained untreated as controls. At 7 days post inoculation, six neonatal *T. absoluta* larvae were introduced onto each seedling. After 9 days, by which time all larvae had reached the fourth instar, larvae were collected for RNA extraction. One larva from each of the four seedlings within the same treatment groupwas pooled into a single 1.5 mL nuclease-free microcentrifuge tube, yielding four biological replicate tubes per treatment group. All samples were flash-frozen in liquid nitrogen before total RNA extraction. RNA was sent to Biomarker Technologies Co., Ltd. for sequencing. Differentially expressed genes were identified withDESeq2 using thresholds of $|\log_2$ fold change$| \geq 1$ and adjusted P-value < 0.05. Functional annotation of the differentially expressed genes was performed against the Gene Ontology (GO) database.

Cytochrome P450 activity assay

Fourth-instar *T. absoluta* larvae were collected from tomato plants that had received a root drench with *B. bassiana* and from untreated control plants, following the same collection protocol as for transcriptome sequencing. Four biological replicates were established per treatment, each containing three larvae. Cytochrome P450 activity was determined using a commercial assay kit (Insect Cytochrome P450 ELISA Kit, Shanghai Fantai Biotechnology Co., Ltd), absorbance was measured at 450 nm.

SGAs mutant generation and validation

Generation of GAME9 mutants. To investigate the role of SGAs in tomato defense, we targeted GAME9, a key transcription factor involved in SGAs biosynthesis. A stable *GAME9* knockout mutant was generated by a commercial service provider, with wild-type plants serving as the control. For transient overexpression, the full-length CDS of *GAME9* was amplified from tomato cDNA and inserted into the pBI121 vector linearized by double digestion with *Sac*I and *Bam*HI, using homologous recombination to generate the recombinant plasmid pBI121-*GAME9*. This plasmid was then transformed into *Agrobacterium tumefaciens* and infiltrated into tomato leaves to achieve transient *GAME9* overexpression.

Characterization of mutant lines. The expression levels of SGAs biosynthesis-related genes were examined in leaves of both mutant types.Leaves from the *GAME9* knockout mutant were submitted to Metware Biotechnology Co., Ltd. for untargeted metabolomics profiling. Total alkaloid content in leaves of the *GAME9* transient overexpression line was determined using a total alkaloid content assay kit (Plant Total Alkaloid Content Assay Kit, Shanghai Enzyme-linked Biotechnology Co., Ltd).

Bioassays with *T. absoluta*. Neonatal *T. absoluta* larvae were introduced onto leaves from three tomato types: wild-type control, *GAME9* knockout mutant, and *GAME9* transient overexpression line. Phenotypic observations followed the same protocol as described for larvae feeding on *B. bassiana*-colonized tomato plants, except that the observation interval for developmental duration was extended from 12 h to 24 h. Fourth-instar larvae were also collected from each treatment to gene expression analysis and cytochrome P450 activity measurement.

Statistical analysis

All statistical analyses were performed with GraphPad Prism (version 8.0.2). For growth and development data, normality was assessed with Shapiro-Wilk test, and homogeneity of variances with Levene's test. Pairwise comparisons were made with the independent samples *t*-test when both normality and variance homogeneity assumptions were met, with Welch's *t*-test when variances were unequal, and with the Mann–Whitney *U* test when data deviated from normality. Quantitative data, including enzyme activity measurements, were analyzed with the independent samples *t*-test. All data are presented as means ± standard error (SE). Statistical significance was designated as *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

Results

Effects of *B. bassiana*-Colonized tomato on *T. absoluta* development, survival, and reproduction

After confirmation successful endophytic colonization of *B. bassiana* in tomato leaves (Fig. S1; Fig. S2), we assessed the developmental duration of *T. absoluta* larvae fed on tomato plants subjected to each treatments. Relative to controls, larvae fed on root- drenched, *B. bassiana* showed significantly prolonged development at the first instar ($U = 823.5$, $P < 0.001$; Fig. 1A), fourth instar ($U = 1853$, $P = 0.0072$; Fig. 1D), and total larval stage ($U = 357.5$, $P < 0.001$; Fig. 1E). By contrast, larvae fed on tomato plants that received a localized foliar spray of *B. bassiana* showed no significant difference in developmental duration at any stage except the first instar ($U = 1121$, $P = 0.0030$; Fig. 1A).

To determine whether endophytic colonization of tomato by *B. bassiana* also affected *T. absoluta* growth, we measured head capsule width, body length of third- and fourth-instar larvae, fourth-instar larval weight and pupal weight. In the root-drench group, fourth-instar larval weight ($t = 2.984$, df = 47, $P = 0.0045$; Fig. 2E) and pupal weight ($t = 2.957$, df = 92, $P = 0.0039$; Fig. 2F) were significantly

lower than in control. Among the morphometric measures, only third-instar head capsule width showed a significant decrease (t = 3.056, df = 62, P = 0.0033; Fig. 2A). In the foliar-spray group, only pupal weight was significantly reduced (t = 2.909, df = 93, P = 0.0045; Fig. 2F), with no significant changes in the remaining parameters (Fig. 2).

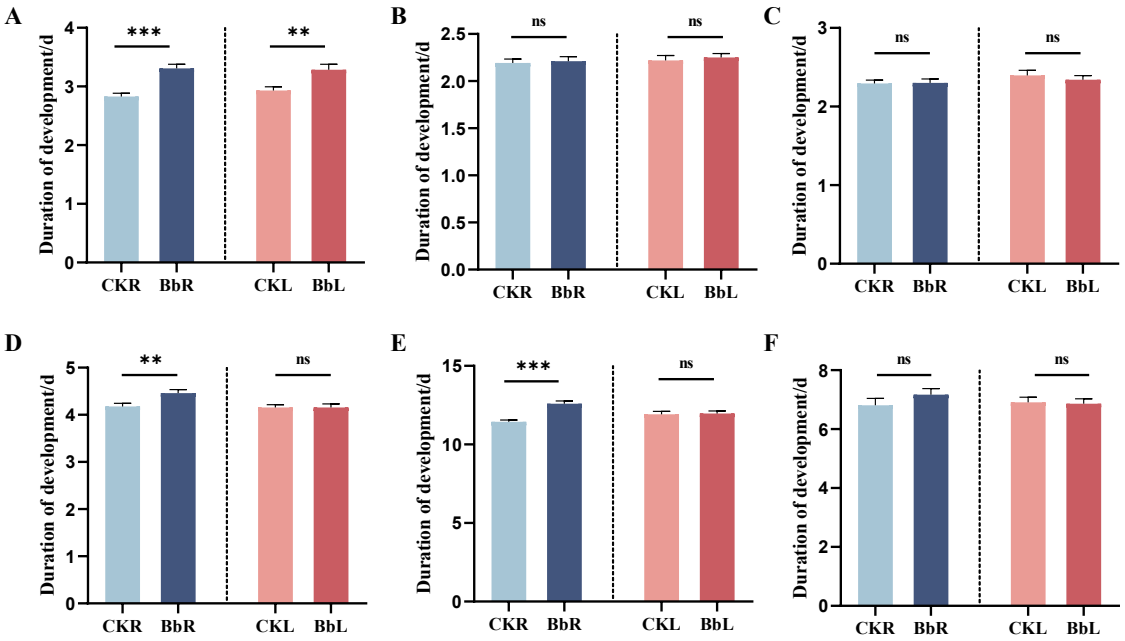


Fig. 1 Developmental duration of *T. absoluta* at different stages after feeding on *B. bassiana*-colonized tomato plants: A. First instar, B. Second instar, C. Third instar, D. Fourth instar, E. Total larval stage, F. Pupa. CKR: control, root drench with 0.05% Tween 80; BbR: *B. bassiana* root drench; CKL: control, foliar spray with 0.05% Tween 80; BbL: *B. bassiana* foliar spray. All data are means ± SE, *represents significant difference (*P < 0.05, **P < 0.01，***P < 0.001) ; ns, not significant. Except for the analysis of pupal development time in *T. absoluta* feeding on tomatoes with localized leaf colonization by *B. bassiana*, which was performed using Independent Samples *t* Test, all other development time analyses were conducted using the Mann-Whitney *U* test.

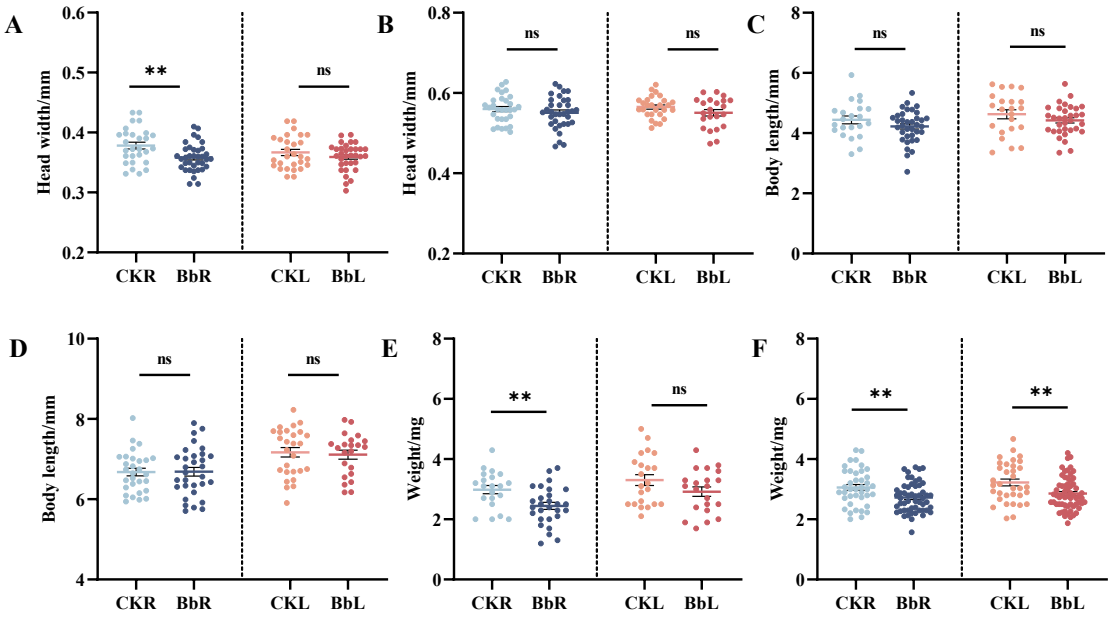


Fig. 2 Body length, head width and body weight of *T. absoluta* larvae on tomato plants treated with *B. bassiana*.

(A) Body length, third instar; (B) Head width, third instar; (C) Body length, fourth instar; (D) Head width, fourth instar; (E) fourth-instar larval weight; (F) pupal weight. Treatment labels as in Fig. 1 Data are means ± SE. *P < 0.05, **P < 0.01; ns, not significant. All analyses were conducted using the Independent-samples t test.

Stage-specific survival did not differ significantly between root-drench and foliar-spray trearments. However, larvae fed on root-drenched plants showed significant reductions in both pupation rate (U = 13, P = 0.044; Fig. 3B) and emergence rate (t = 3.629, df = 4, P = 0.022; Fig. 3C). In the foliar-spray group, only emergence rate was significantly reduced (t = 4.321, df = 4, P = 0.012; Fig. 3C). Female adults that developed on root-drenched plants displayed no significant change in oviposition period, but their fecundity was significantly reduced (t = 4.170, df = 18, P < 0.001; Fig. 3E).

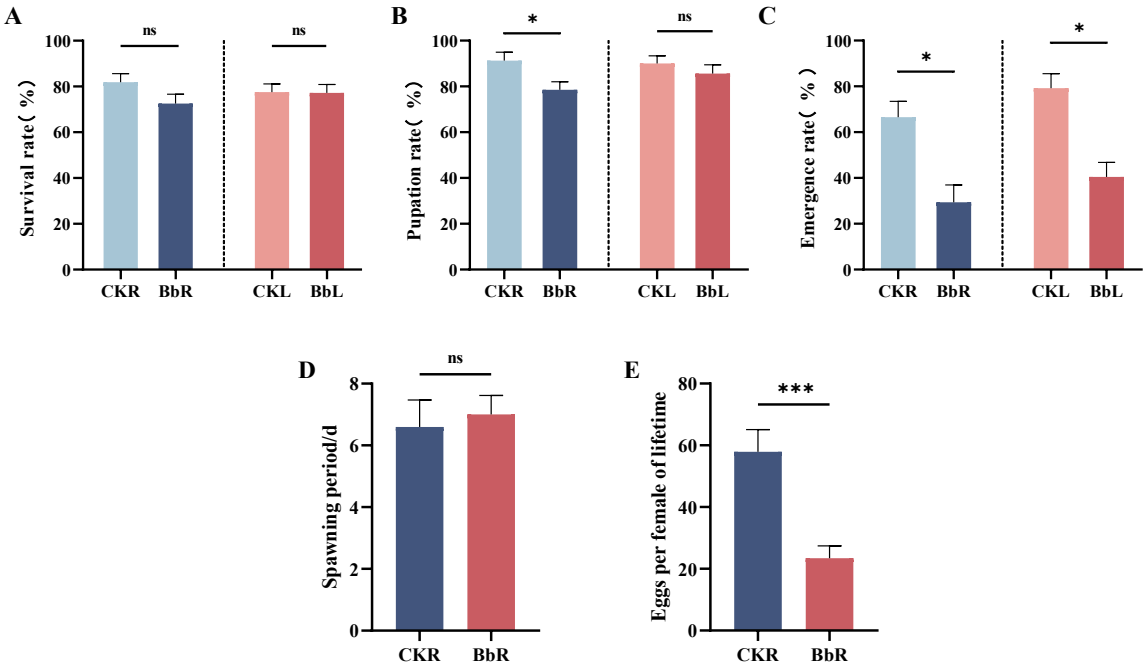


Fig. 3 Survival and Egg-laying behavior of *T. absoluta* on *B. bassiana*-colonized tomato plants. (A) Larval survival rate, (B) Pupation rate, (C) Emergence rate, (D) oviposition period, (E) fecundity (eggs per female). Treatment labels as in Fig. 1. Data are means ± SE. *P < 0.05, ***P < 0.001; ns, not significant. Larval survival rate and pupation rate in the root-drench treatment were analyzed with the Mann-Whitney U test; remaining comparisons used the Independent Samples t Test.

B. bassiana colonized tomato plants but dose not enter *T. absoluta* larvae

To test whether the observed effects on *T. absoluta* fitness resulted from direct fungal infection or from plant-mediated mechanisms, we conducted two complementary assays on fourth-instar larvae that had fed on root-drenched, colonizedplants. First, whole-larval homogenates and intestinal homogenates were plated separately on PDA medium. After 14 days of incubation, no *B. bassiana* colonies were detected from either homogenate type (Fig. 4B and 4C). Second, PCR analysis of the same samples failed to amplify *B. bassiana*-specific sequences (Fig. 4D).

Transcriptomic and physiological responses of *T. absoluta* to colonised tomato plants

Quality control and global differential expression analysis confirmed the reliability of the transcriptome

data and revealed substantial transcriptional changes in larvae fed on root-drenched, colonized plants (Table S1; Fig. S3; Fig. S4). GO enrichment analysis of down-regulated DEGs identified significant enrichment in several biological processes related to insect development, with cuticle development and ecdysis (chitin-based cuticle) exhibited the strongest signal (Fig. 5A). Among up-regulated DEGs, the most significantly enriched molecular functions included oxygenase activity, flavin adenine dinucleotide binding, monooxygenase activity, and triglyceride lipase activity (Fig. 5B).

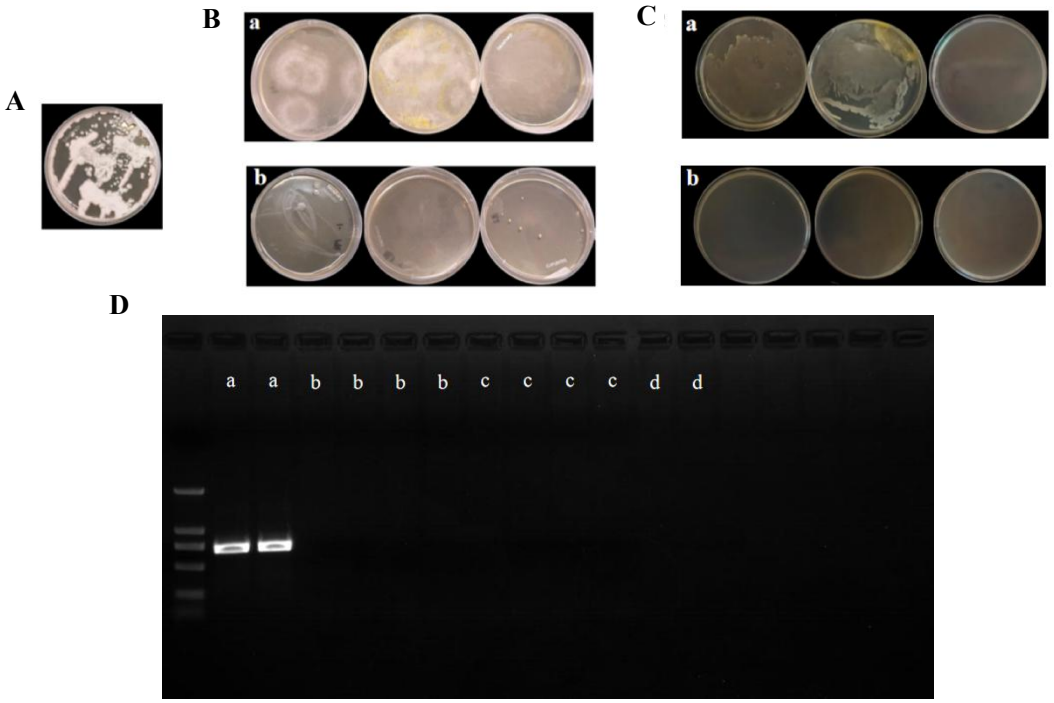


Fig 4 Detection of *B. bassiana* in *T. absoluta* larvae. (A) *B. bassiana* colony (positive control), (B) Intestinal homogenate culture, (C) whole-body homogenate culture (a: larvae fed on Tween-80-drenched plants; b: larvae fed on *B. bassiana*-drenched plants). (D) PCR detection of *B. bassiana* sequences in larvae (a: *B. bassiana* conidia, b: larvae fed on Tween-80-drenched plants, c: larvae fed on *B. bassiana*-drenched plants, d: nuclease-free water).

Because our earlier work showed that *B. bassiana* colonization promotes the accumulation of secondary metabolites in tomato leaves, we selected seven DEGs enriched in oxidoreductase activity and monooxygenase activities for validation via qRT-PCR. All seven selected genes were significantly up-regulated in larvae fed on root-drench, fungus-colonized tomato plants (Fig. 5C). Pfam domain annotation further identified the GMC oxidoreductase family and the cytochrome P450 family among the products of these genes. Given the central tole of cytochrome P450 in insect detoxification, we measured its enzymatic activity directly. Cytochrome P450 activity was significantly elevated in *T. absoluta* larvae fed on *B. bassiana*-colonized tomato plants ($t = 3.113$, df = 6, $P = 0.0208$; Fig. 5D).

Generation and Validation of *GAME9* Knockout and Overexpression Tomato lines

Our earlier work demonstrated that *GAME9*, a key transcription factor governing the initiation of the SGAs biosynthetic pathway, is significantly up-regulated in tomato leaves after *B. bassiana* root drench. To validate the role of *GAME9* in SGAs regulation, we examined two *GAME9*-modified lines. In the *GAME9* knockout line, the expression of most SGAs synthesis-related genes was substantially reduced relative to wild-type, and *GAME9* itself was barely detectable (Fig. 6A). In the transient overexpression line, *GAME9* transcript levels were significantly elevated at 3 and 6 days post infiltration (dpi),

although *GAME9* expression had begun to decline by 6 dpi and was nearly lost by 9 days post infiltration (Fig. 6C). Because *GAME9* expression remained relatively high at 6 dpi, this time point was selected for further analysis. Notably, the expression patterns of SGAs synthesis-related genes in the overexpression line were generally opposite to those in the knockout line (Fig. 6B).

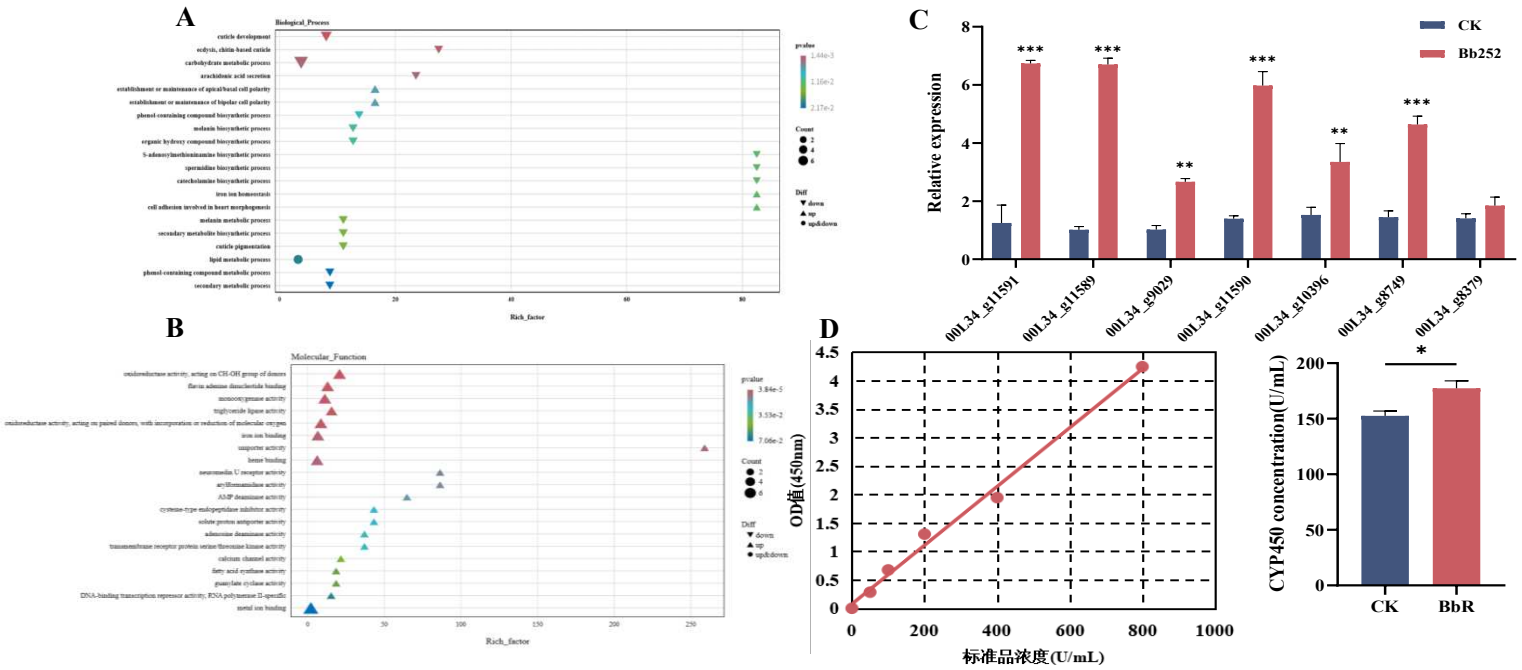


Fig. 5 Gene expression and detoxification responses of *T. absoluta* larvae after feeding on root-drenched, *B. bassiana*-colonised tomato plants. (A) GO enrichment (biological processes) of down-regulated DEGs, (B) GO enrichment (molecular functions) of up-regulated DEGs, (C) expression levels of peroxidase and monooxygenase-related genes, (D) cytochrome P450 activity. Data are means ± SE. *P < 0.05.

Untargeted metabolomics analysis of *GAME9* knockout leaves identified two SGAs—tomatine and dehydrotomatine—that were significantly reduced relative to wild-type, with dehydrotomatine present at extremely low levels (Fig. 6D). Tomatine is the predominant SGAs in tomato, dehydrotomatine is its oxidative derivative. Knocking out *GAME9* therefore suppressed the accumulation of both compounds, leading to a marked reduction in total SGAs content. To determine whether transient overexpression of *GAME9* also affects alkaloid accumulation, we measured total alkaloid content at 6 dpi and found it significantly elevated relative to controls(t = 6.133, df = 6, P = 0.0009; Fig. 6E), confirming the suitability of this line for subsequent bioassays.

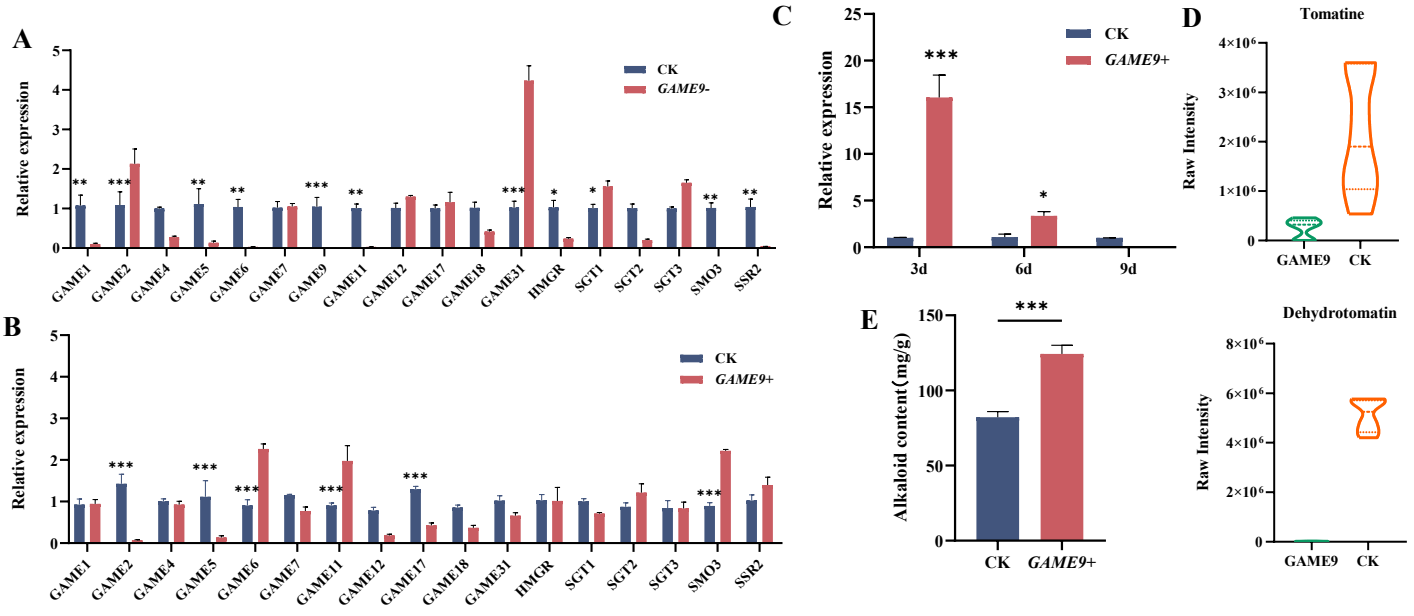


Fig. 6 SGAs biosynthesis-related gene expression and total SGAs content in leaves of *GAME9* mutant tomato plants. (A) Expression of SGAs biosynthesis genes in *GAME9* knockout leaves, (B) Expression of SGAs biosynthesis genes in *GAME9* overexpressing leaves, (C) time course of *GAME9* expression after transient overexpression (3, 6, 9 dpi), (D) Violin plots of differentially accumulated SGAs in *GAME9* knockout leaves, (E) total alkaloid content in *GAME9* transiently overexpressing leaves at 6 dpi. Panels A-C, E: data are means ± SE, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; ns, not significant. All analyses were conducted using the Independent-samples t test.

SGAs content determines detoxification response and fitness of *T. absoluta*

To determine whether the detoxification response induced by *B. bassiana* root drench colonization is mediated by altered SGAs content, we measured the expression of oxidoreductases and monooxygenases genes, as well as cytochrome P450 activity, in fourth-instar *T. absoluta* larvae fed on *GAME9*-modified tomato lines. In larvae fed on *GAME9* knockout plants, most detoxification-related genes showed no significant changes in expression relative to the control group (Fig. 7A), whereas cytochrome P450 activity was significantly reduced ($t = 4.824$, df = 4, $P = 0.0085$; Fig. 7B). In larvae fed on *GAME9* transient overexpression plants, most detoxification-related genes were significantly up-regulated (Fig. 7A), a pattern closely resembling that observed in larvae fed on *B. bassiana*-colonized plants. Cytochrome P450 activity was also significantly elevated in this group ($t = 3.657$, df = 4, $P = 0.0216$; Fig. 7B). These results link variation in SGAs content to the induction of detoxification responses in *T. absoluta*.

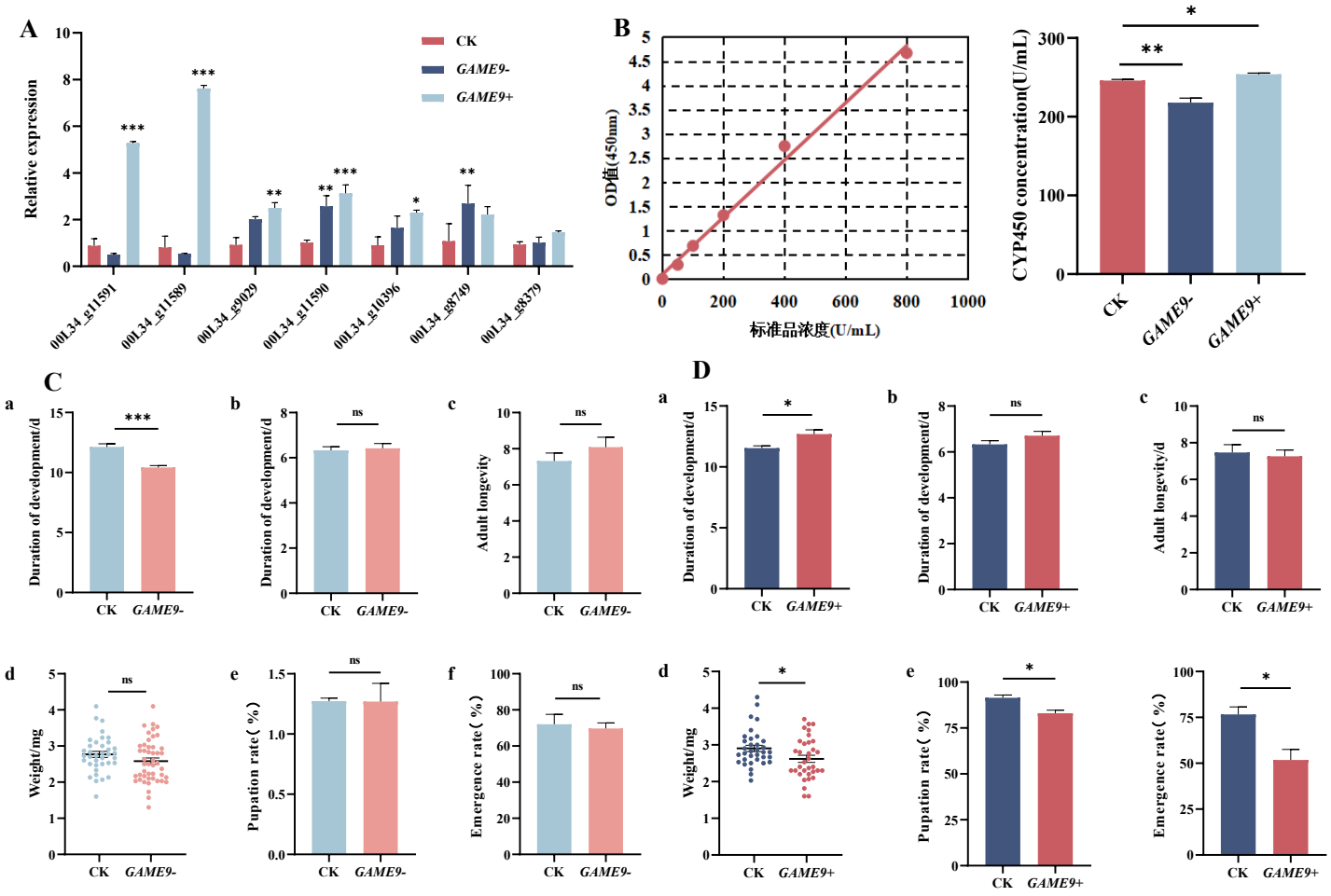


Fig. 7 Effects of feeding on *GAME9* mutant tomato plants on detoxification metabolism and development of *T. absoluta*: (A) expression of peroxidase and monooxygenase -related genes in *T. absoluta* feeding on mutant tomatoes, (B) cytochrome P450 activity, (C) life-history parameters on *GAME9* knockout leaves (a: larval development time, b: pupal development time, c: adult lifespan, d: pupal weight, e: pupation rate, f: emergence rate, (D) life-history parameters on *GAME9* overexpression leaves (panel labels as in C). CK: larvae fed on wild-type leaves; *GAME9*-: larvae fed on *GAME9*-knockout leaves; *GAME9*+: larvae fed on *GAME9*-overexpressing leaves. Data are means ± SE, **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns, not significant. Panels Ca- Cc,and Da-Dd used the Mann-Whitney *U* test ; all other comparisons used the Independent Samples *t* test.

To assess whether altered SGAs content directly affects *T. absoluta* fitness, we reared larvae on *GAME9*-modified lines and measured larval developmental duration, pupal duration, adult longevity, emergence rate and pupal weight. Larvae fed on *GAME9* knockout plants showed a significantly shortened larval developmental time relative to controls ($U = 612.5$, $P < 0.001$), with no significant differences in the other parameters (Fig. 7C). Thus, reduced SGAs content in tomato leaves did not adversely affect *T. absoluta* growth. By contrast, larvae fed on *GAME9* overexpression plants showed significantly prolonged larval developmental ($U = 442$, $P = 0.0289$), reducted pupal weight ($U = 408$, $P = 0.0366$), reduced pupation rate ($t = 3.762$, df = 4, $P = 0.0197$), and reduced emergence rate ($t = 5.539$, df = 4, $P = 0.0240$; Fig. 7D). Elevated SGAs content therefore imposes a significant fitness cost on *T. absoluta*, consistent with a causal role of SGAs in mediating the resistance induced by endophytic *B. bassiana*.

Discussion

The suppressive effects of endophytic *B. bassiana* on herbivorous insects have been documented across multiple plant–insect systems (Agbessenou et al., 2020; Zheng et al., 2023; Klieber et al., 2016), but the mechanistic basis has remained unclear. The present study demonstrates that *B. bassiana*, when colonizing tomato as an endophyte, suppresses *T. absoluta* primarily through a plant-mediated mechanism: the induction of SGAs accumulation. This indirect defence pathway impaired multiple life-history traits, including larval development, pupal weight, emergence rate and fecundity, and was activated independently of direct fungal infection of the insect.

Several lines of evidence support this conclusion. First, no *B. bassiana* was re-isolated from *T. absoluta* larvae or detected by PCR, ruling out direct parasitism under our experimental conditions. Direct infection has occasionally been reported in insects feeding on endophyte-colonized plants in other studies (Akello et al., 2008; Zheng et al., 2023). This discrepancy may reflect strain-specific variation in pathogenicity and sporulation capacity: highly virulent strains, once established endophytically, might be more likely to breach the cuticular or intestinal barriers of *T. absoluta*. Second, transcriptomic analysis of larvae feeding on colonized plants revealed the down-regulation of genes related to growth and developmental processes, together with the up-regulation of detoxification-associated genes, particularly those encoding cytochrome P450s and oxidoreductases. Our earlier work had identified SGAs as the most prominently altered class of insecticidal secondary metabolites in tomato leaves after B. bassiana colonization (Wang, 2024). The activation of detoxification pathways in *T. absoluta* thus appears to be a direct response to elevated SGAs in its dietary environment.

We tested this causal link directly using *GAME9* knockout and transient overexpression lines to manipulate SGAs content in tomato leaves. Larvae feeding on knockout plants (reduced SGAs) showed no significant changes in detoxification gene expression or cytochrome P450 activity relative to

controls, and their development was not adversely affected. By contrast, larvae feeding on overexpression plants (elevated SGAs) exhibited significant up-regulation of detoxification genes, increased P450 activity, and substantial developmental inhibition. These results identify SGAs as key chemical mediators of tomato resistance against *T. absoluta* and establish that endophytic *B. bassiana* enhances this resistance by inducing SGAs accumulation. Identifying specific plant metabolites that modulate insect enzyme activity is a critical step towards developing mechanism-based pest management strategies (Chamani et al., 2025), and the present study provides direct experimental evidence for such a metabolite–defense link.

Despite the activation of detoxification responses, larval growth and development remained significantly suppressed, indicating that the insect's capacity to neutralize SGAs was insufficient. Several factors may explain this phenomenon. First, SGAs may exert specific toxicity towards *T. absoluta* through mechanisms that operate outside conventional detoxification pathways (Pentzold et al., 2015). Second, detoxification is metabolically costly, and the allocation of resources to stress responses may divert energy away from growth and reproduction (Terblanche et al., 2026). Third, once SGAs concentrations exceed a certain threshold, the limited detoxification capacity of *T. absoluta* may be unable to fully neutralize their deleterious effects. These constraints underscore why the up-regulation of cytochrome P450 activity, though detectable, did not translate into rescued larval performance.

This study elucidates a causal pathway through which endophytic colonization by *B. bassiana* enhances tomato resistance against *T. absoluta*: the induction of SGAs in host leaves. The findings further establish mechanistic linkages among entomopathogenic fungal endophytism, plant secondary metabolite induction, and pest physiological responses. These results are specific to the *B. bassiana*–tomato–*T. absoluta* tripartite system, and the generalizability of the SGAs-mediated defence mechanism to other endophyte–plant–herbivore combinations remains to be tested. Future work examining the dose–response relationship between SGAs concentration and *T. absoluta* performance, as well as the long-term stability of SGAs induction under field conditions, would help determine the practical potential of leveraging endophyte-triggered plant immunity for pest management.

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