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Nathalie Maluta

nathaliepradomaluta@gmail.com

University of São Paulo

Thiago Castro

Koppert (Brazil)

Carla Mariane Marassatto

University of São Paulo

João Roberto Spotti Lopes

University of São Paulo

Research Article

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Posted Date: November 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11613823/v1

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Additional Declarations: No competing interests reported.

Endophytic colonization by entomopathogenic fungi differentially affects the probing behavior of *Dalbulus maidis* in maize and *Bemisia tabaci* MEAM1 in soybean

Nathalie Maluta[1,3] *, Thiago Castro[2,3], Carla Mariane Marassatto[1], João Roberto Spotti Lopes[1,3]

[1]Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, C.P. 9, 13418-900, Piracicaba, SP, Brazil; nathaliepradomaluta@gmail.com; carla.marassatto@usp.br, jrslopes@usp.br

[2] Koppert Biological Systems, Rodovia Margarida da Graça Martins s/n - Km 17,5, 13400-970, Piracicaba, SP, Brazil tcastro@koppert.com.br

[3]Sao Paulo Advanced Research Center for Biological Control, SPARCBIO, Piracicaba, Brazil

*Corresponding author: Nathalie Maluta E-mail: nathaliepradomaluta@gmail.com ORCID: 0000-0003-3817-1123.

Abstract

Endophytic colonization by entomopathogenic fungi represents a promising preventive strategy for sustainable pest management through plant-mediated effects on herbivorous insects. This study investigated the ability of commercial formulations of *Beauveria bassiana* and *Cordyceps javanica* to establish endophytically in maize and soybean plants and their effects on the probing behavior of *Dalbulus maidis* and *Bemisia tabaci* MEAM1. Plants were foliar sprayed with the fungal formulations, and insect probing behavior was monitored by DC-electrical penetration graph (EPG) 72h after application. In soybean, *B. bassiana* successfully established endophytically but did not significantly alter the probing behavior of *B. tabaci*, whereas *C. javanica* did not colonize soybean under the tested conditions. In maize, both fungi established as endophytes and significantly altered *D. maidis* probing behavior, reducing phloem-related activities while increasing activities in non-phloem tissues. These responses demonstrate that plant-mediated effects of endophytic entomopathogenic fungi on insect probing behavior are context-dependent, varying with the fungus–plant–insect combination. The marked reduction in phloem activities of *D. maidis* suggests potential implications for pathogen transmission and highlights the potential of

endophytic entomopathogenic fungi as an additional preventive strategy for managing this important maize vector.

Keywords: *Beauveria bassiana; Cordyceps javanica;* Corn-leafhopper; Electrical penetration graph; Hypocreales; Whitefly.

Key message

- How plant colonization by entomopathogenic fungi affects insect feeding remains poorly understood.
- *Beauveria bassiana* and *Cordyceps javanica* colonized maize plants and altered *Dalbulus maidis* probing behavior.
- Both fungi reduced phloem ingestion by *Dalbulus maidis* on maize.
- *Beauveria bassiana* colonized soybean but had limited effects on *Bemisia tabaci* MEAM1.
- These contrasting responses highlight the potential for targeted biological pest management.

Author contributions

N.M., T.C., C.M.M. and J.R.S.L. conceived and designed the research. N.M. conducted all experiments, processed, and analyzed the data. All authors contributed to the writing and approved the manuscript.

Introduction

Entomopathogenic fungi constitute an important group of biological control agents of arthropod pests and have received increasing attention as tools in integrated pest management (IPM) programs. Species belonging to genera such as *Beauveria, Metarhizium*, and *Cordyceps* (order Hypocreales) have broad host ranges and can infect different insect orders. Unlike many other microbial control agents, entomopathogenic fungi do not need to be ingested to initiate infection, as their conidia can adhere to the host surface, germinate, and penetrate directly through the cuticle. Following penetration, the fungus colonizes the hemocoel and proliferates within the host, ultimately leading to insect death and, under favorable conditions, subsequent sporulation (Vega et al. 2009).

Although historically studied primarily for their ability to infect and kill target pests, entomopathogenic fungi are now recognized as having much broader and more complex ecological roles. Several species can occur as rhizosphere colonizers, antagonists of plant pathogens, plant growth

promoters, and, particularly, as plant endophytes (Vega et al. 2009; Jaber and Ownley 2018; Panwar e Szczepaniec 2024; Marassatto et al. 2026). This ecological versatility considerably expands the potential use of these microorganisms in IPM because their effects may involve not only direct infection of insect hosts but also plant-mediated mechanisms.

From an evolutionary perspective, the association of some known entomopathogenic fungi with plants appears to predate the evolution of their insect-pathogenic lifestyle. Phylogenomic evidence suggests that *Metarhizium*, for example, diverged from plant-associated clavicipitaceous endophytes, such as *Epichloë* and *Claviceps*, whereas insect pathogenicity represents a more recently acquired trait. Consistent with this evolutionary history, both generalist and specialist insect-pathogenic *Metarhizium* species retain the ancestral capacity to colonize and associate with plant roots, suggesting that plant association is a fundamental component of the ecological history of this fungal lineage (Moonjely and Bidochka 2019). This ancestral association with plants is also supported by field observations, as diverse *Metarhizium* species have been recovered from plant-associated compartments, including sugarcane roots under commercial cropping conditions (Iwanicki et al. 2019).

Endophytic fungi are microorganisms capable of internally colonizing plant tissues for at least part of their life cycle without causing apparent disease symptoms (Wilson 1995). Several fungi traditionally recognized as entomopathogens can establish such associations, either naturally or following artificial inoculation. Among them, *Beauveria bassiana* (Bals. -Criv.) Vuill., 1912 (Hypocreales: Cordycipitaceae) is one of the most extensively studied species. Endophytic colonization by *B. bassiana* has been demonstrated in numerous plant species and can be achieved through different inoculation methods, including seed treatment, root or soil inoculation, stem injection, and foliar spraying (Ownley et al. 2008; Jaber and Ownley 2018; Silva et al. 2020; Marassatto et al. 2026). The efficiency, distribution, and persistence of colonization may vary according to fungal isolate, plant species and developmental stage, plant tissue, inoculation method, inoculum concentration, and environmental conditions.

Endophytic colonization of maize by *B. bassiana* strains has been consistently demonstrated following foliar application, seed treatment and root inoculation, with fungal recovery from leaves, stems, roots and, in some cases, newly developed tissues, although colonization frequency and persistence vary according to the fungal isolate, inoculation method, plant tissue and time after inoculation (Wagner and Lewis 2000; Russo et al. 2015; Renuka et al. 2016; Mahmood et al. 2019; Kropf and Gassmann 2026)

Endophytic colonization by entomopathogenic fungi may provide several benefits to plants, including growth promotion, enhanced nutrient acquisition, antagonism against plant pathogens, and protection against herbivores (Vega et al. 2009; Jaber and Ownley 2018; Wang et al. 2023). Endophytic entomopathogenic fungi may also contribute to plant nutrition by mediating nutrient transfer across trophic levels. Insect-derived nitrogen can be translocated to host plants through endophytic fungal associations, a phenomenon first demonstrated for *Metarhizium robertsii* Bisch., S.A. Rehner and Humber 2009 (Hypocreales: Clavicipitaceae) (Behie et al. 2012) and subsequently reported for other *Metarhizium* species and *B. bassiana* (Behie and Bidochka 2014).

The mechanisms underlying protection against insects are not yet fully understood and likely involve multiple processes acting independently or in combination. Consequently, effects on herbivores may occur even in the absence of direct fungal infection and may manifest as changes in host preference, feeding, development, survival, fecundity, and population growth.

These effects are particularly relevant for sap-sucking insects that act as vectors of plant pathogens. The economic importance of these insects arises not only from nutrient removal from plants but, more importantly, from their ability to transmit phytopathogenic agents during specific stylet activities. Thus, changes in probing behavior may have epidemiological consequences even when they do not immediately result in vector mortality. For instance, reductions in the frequency or duration of phloem-associated activities could potentially decrease opportunities for the acquisition and/or inoculation of phloem-limited pathogens.

Experimental evidence has demonstrated that entomopathogenic fungi can alter the probing behavior of insect vectors before fungal infection results in mortality. Using the Electrical Penetration Graph (EPG) technique, Maluta, Castro and Lopes (2022) demonstrated that exposure of *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae), to the entomopathogenic fungus *Cordyceps fumosorosea* [currently *Cordyceps javanica*] (Bally) Kepler, B. Shrestha and Spatafora, 2017 (Hypocreales: Cordycipitaceae) significantly altered phloem-associated activities. Similar effects were subsequently observed on *Dalbulus maidis* (DeLong and Wolcott 1923) (Hemiptera: Cicadellidae), demonstrating that fungal infection can modify relevant stylet activities before insect death (Maluta, Castro and Lopes 2023). These findings indicate that the benefits provided by entomopathogenic fungi may precede their lethal effects and emphasize the importance of considering sublethal and behavioral effects when evaluating these biological control agents.

118 More recently, evidence has shown that changes in insect probing behavior may also occur when
119 the fungus acts indirectly through endophytic colonization of the host plant. In tomato plants endophytically
120 colonized by *B. bassiana*, the whitefly *Bemisia tabaci* (Gennadius 1889) (Hemiptera: Aleyrodidae) MED
121 (Mediterranean) exhibited significant changes in biological and feeding parameters, including reduced
122 phloem salivation and shorter periods of phloem sap ingestion, together with a reduction in reproductive
123 rate (Wang et al. 2024).
124 Endophytic colonization by *B. bassiana* can also induce substantial changes in plant metabolism.
125 In tomato, fungal colonization altered the expression of genes and the accumulation of specialized
126 metabolites associated with plant defense. These metabolic changes increased plant resistance to *B. tabaci*,
127 with α-solanine playing an important role in suppressing whitefly reproduction. Collectively, these findings
128 provide evidence that endophytic associations can modify plant–insect interactions and affect feeding
129 activities that are particularly important for sap-sucking insects (Wang et al. 2023).
130 The corn leafhopper, *D. maidis*, is an important maize pest in the Americas, primarily because it
131 transmits several economically important plant pathogens, including *Spiroplasma kunkelii* Whitcomb et al.,
132 1986, maize bushy stunt phytoplasma (MBSP; 'Candidatus Phytoplasma zeae'), maize rayado fino virus
133 (MRFV) (Tymoviridae: Marafivirus), and maize striate mosaic virus (MSMV) (Geminiviridae:
134 Mastrevirus), recently confirmed to be transmitted by this leafhopper (Oliveira et al. 2004; Santana et al.
135 2020; Vilanova et al. 2022). Because pathogen transmission is closely associated with stylet activities,
136 particularly those occurring in the phloem, strategies that disrupt salivation and phloem sap ingestion may
137 potentially interfere with pathogen transmission.
138 Another economically important insect vector is *B. tabaci*, a cryptic species complex with
139 worldwide distribution. Among its members, Middle East–Asia Minor 1 (MEAM1), formerly known as
140 biotype B, is a highly polyphagous and invasive species and remains the predominant *B. tabaci* species in
141 major open-field crops in Brazil, including cotton, tomato and soybean (Pozebon et al. 2020; Fernandes et
142 al. 2024). In soybean (*Glycine max* [L.] Merr.), *B. tabaci* MEAM1 causes damage mainly through phloem
143 sap ingestion and honeydew excretion, which favors sooty mold development. High infestations have been
144 associated with reductions in soybean yield and quality under field conditions in Brazil (Schutze et al.
145 2022), highlighting the importance of developing sustainable strategies for its management.
146 The phloem-feeding habits of *D. maidis* and *B. tabaci* make them suitable models for investigating
147 plant-mediated effects of endophytic entomopathogenic fungi. Endophytic colonization may alter plant

traits that influence insect probing, particularly phloem-associated activities. These effects can be assessed using EPG technique, which enables real-time monitoring of stylet activities within plant tissues. However, whether and how endophytic entomopathogenic fungi alter the probing behavior of economically important insect vectors remains poorly understood, particularly across different fungus–plant–insect systems.

We hypothesized that the establishment and effects of endophytic entomopathogenic fungi are context-dependent, varying according to the fungal species, host plant, and insect vector involved. Specifically, we expected endophytic colonization to alter the probing behavior of sap-sucking insects, particularly phloem-associated activities, with the magnitude and nature of these effects depending on the specific fungus–plant–insect interaction. To test this hypothesis, we investigated the ability of *B. bassiana* and *C. javanica* to establish endophytically in maize and soybean plants and assessed their effects on the probing behavior and stylet activities of *D. maidis* on maize and *B. tabaci* MEAM1 on soybean using EPG technique.

Material and Methods

Dalbulus maidis colony and maize plants.

A laboratory colony of healthy *D. maidis* was established from adults collected from maize fields in the municipality of Jardinópolis, São Paulo State, Brazil (20.912931° S, 47.896399° W). The colony was maintained in screened aluminum-framed cages (35 × 35 × 53 cm) with an acrylic access door containing healthy maize plants, inside a climate-controlled greenhouse at the Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP), Piracicaba, SP, Brazil (25 ± 2 °C, 60–70% RH, natural photoperiod). Throughout the study, all experimental plants were grown from commercial maize seeds experimental hybrid FS710 PW (previously codified as LP-2020, LongPing High-Tech Seeds and Biotechnology, Jardinópolis, Brazil).

Bemisia tabaci MEAM1 colony and soybean plants.

A healthy colony of *B. tabaci* MEAM1 was originally obtained from the Agronomic Institute of Campinas (IAC), São Paulo State, Brazil, and maintained under greenhouse conditions (25 ± 10 °C) on cabbage plants (*Brassica oleracea* L. var. acephala DC., cv. Manteiga). The identity of the whitefly population was confirmed as *B. tabaci* MEAM1 by sequencing the mitochondrial cytochrome oxidase I (mtCOI) gene following the protocol described by De Barro et al. (2011). Throughout colony maintenance,

178 no chemical or biological insecticides were applied. Soybean plants cv. IAC-Foscarin 31, a conventional
179 early-maturing cultivar developed by IAC) was used in the experiments.
180
181 **Endophytic colonization of *Beauveria bassiana* and *Cordyceps javanica* in maize and soybean plants**
182 Prior to the EPG assays, preliminary experiments were conducted to assess the ability of
183 commercial formulations of *B. bassiana* strain ESALQ-PL63 and *C. javanica* strain ESALQ-1296 to
184 establish endophytically in maize and soybean plants and to determine the onset and persistence of
185 colonization following foliar application. In maize, Boveril Evo® (minimum of 2.0×10^9 viable conidia/g)
186 was applied at 0.32 g per 100 mL of water (off-label rate), whereas Octane® (minimum of 2.5×10^9 viable
187 conidia/mL) was applied at 0.32 mL per 100 mL of water, corresponding to the manufacturer-recommended
188 rate for *D. maidis*. In soybean, Boveril Evo® was applied at 1.2 g per 100 mL of water, whereas Octane®
189 was applied at 0.32 mL per 100 mL (off-label rate). Conidia viability had a germination rate of 86% in the
190 experiments.
191 Maize plants at the V2-V3 growth stage (2–3 fully expanded true leaves) and soybean plants at V4
192 (three fully expanded trifoliate leaves) were sprayed until runoff using a compression sprayer (Vonder, 1.5
193 L, Curitiba, Paraná, Brazil). Immediately after spraying, each plant was enclosed in a plastic bag for 24 h
194 to maintain high relative humidity. Endophytic colonization by the fungi was assessed at 1, 3, 5, 10, and 15
195 days after spraying. The same plants were sampled throughout all post-application periods. To assess
196 endophytic colonization, 32 maize plants and 32 soybean plants were evaluated for *B. bassiana*, whereas
197 28 maize plants and 20 soybean plants were evaluated for *C. javanica*.
198 To confirm endophytic colonization by *B. bassiana* and *C. javanica*, 1 g of plant tissue was
199 collected from each plant at each time point. Leaf fragments were surface sterilized by sequential immersion
200 in 70% ethanol for 30s and 0.5% sodium hypochlorite for 1 min, followed by three rinses in sterile distilled
201 water. The samples were dried for 1 min on sterile filter paper in a laminar-flow cabinet (McKinnon et al.
202 2017; Marassatto et al. 2026). The final wash water was plated (100 µL) to confirm the effectiveness of
203 surface sterilization. Subsequently, each 1g plant tissue sample was transferred to a mortar and macerated
204 with 2 mL of phosphate-buffered saline (PBS) (NaCl, 8 g/L; KCl, 0.2 g/L; Na_2HPO_4, 1.44 g/L; KH_2PO_4,
205 0.24 g/L). The resulting homogenates were then placed on an orbital shaker at 350 rpm for 50 min at room
206 temperature. One hundred and fifty microliters of each plant extract were plated onto a modified semi-

selective culture medium based on Strasser et al. (1996), as described by Marassatto et al. (2026). Samples were plated in replicate at each time point.

Plates were incubated in a B.O.D. chamber at 25 ± 2 °C under a 12:12 h light (L) photoperiod for 15 days. Endophytic colonization was assessed qualitatively based on fungal re-isolation from surface-sterilized plant tissues. A plant was considered successfully colonized when the inoculated fungus was recovered from at least one of the plated tissue fragments. The number of fungal colonies per plate was not quantified, because the purpose of the assay was to determine the presence or absence of endophytic colonization rather than fungal abundance within plant tissues.

EPG analysis of *Dalbulus maidis* and *Bemisia tabaci* stylet activities on endophytically colonized plants.

The probing behavior of *D. maidis* and *B. tabaci* was evaluated using the DC-electrical penetration graph (EPG) technique, which enables real-time monitoring of insect stylet activities within plant tissues and allows recorded waveform patterns to be associated with specific biological activities performed during probing.

Maize plants and *Dalbulus maidis:*

To evaluate the effects of endophytic colonization by *B. bassiana* and *C. javanica* in maize on the probing behavior of *D. maidis*, V3-stage maize plants sprayed with the products and rates described above were used.

Adult females of *D. maidis* (5–15 days old) were placed individually in capped glass tubes and immobilized on crushed ice for 2–3 min. Insects were then positioned under a stereomicroscope and immobilized using a vacuum-assisted system, with the dorsal side facing upward. A gold wire (2 cm long and 18 µm in diameter; EPG Systems, Wageningen, The Netherlands) was attached to the insect pronotum using conductive silver glue (Colloidal Silver Liquid; Ted Pella), and the opposite end of the wire was connected to a copper electrode. After 1 h of starvation, wired insects were connected to the EPG system and placed on the abaxial surface of the youngest fully expanded leaf of maize plants that had been sprayed with the fungal formulations 72 h earlier.

Soybean plants and *Bemisia tabaci* MEAM1

To evaluate the effects of fungal endophytic colonization in soybean on the probing behavior of *B. tabaci*, soybean plants with three fully expanded trifoliate leaves were sprayed at the rates described above and used for EPG assays.

Adult females of *B. tabaci* MEAM1 (1–10 days old) were placed individually in capped glass tubes and immobilized on crushed ice for 2–3 min. The immobilized whiteflies were then transferred to a Petri dish containing crushed ice and positioned under a stereomicroscope with the dorsal side facing upward. A gold wire (1.5 cm long and 12.5 µm in diameter; EPG Systems, Wageningen, The Netherlands) was attached to the pronotum using conductive silver glue (Colloidal Silver Liquid; Ted Pella), and the opposite end of the wire was connected to a copper electrode. After 1 h of starvation, the wired insects were connected to the EPG system and placed on the abaxial surface of a trifoliate leaf of soybean plants sprayed with the fungal formulations 72 h before the beginning of the recordings.

EPG recording and data analysis

EPG recordings were obtained using an eight-channel direct-current (DC) EPG monitor (Giga-8d) connected to a computer running Stylet+ software for Windows (EPG Systems, Wageningen, The Netherlands) (Tjallingii 1978; 1988). The EPG system, insects, and plants were enclosed within a Faraday cage to minimize external electrical interference. Recordings were conducted in a climate-controlled room maintained at 25 ± 1 °C under light conditions.

Each insect was monitored continuously for 8h, and at least 15 valid recordings were obtained for each treatment (control, *B. bassiana*, and *C. javanica*) and insect species. After EPG recording, insects were removed and plant tissue samples were collected 96 h after fungal application to confirm endophytic colonization by fungal re-isolation, following the procedure described above. Only EPG recordings from insects that exhibited stylet probing activity during the recording period and were recorded on plants in which endophytic colonization by *B. bassiana* or *C. javanica* was confirmed by fungal re-isolation were included in the analyses.

EPG waveforms and their biological meanings for *D. maidis* using the DC-EPG system were previously characterized by Carpane et al. (2022) and Maluta, Castro and Lopes (2023) as follows: np: non-probing; Dm1: stylet movement through the intercellular spaces of the epidermis and parenchyma; Dm2: putative active ingestion of mesophyll cell contents or xylem sap; Dm3: an activity of uncertain biological

function, possibly associated with mechanical stylet movements; Dm4: phloem conditioning, characterized by salivation into sieve elements; and Dm5: phloem sap ingestion.

For *B. tabaci*, EPG waveforms were previously characterized by Janssen et al. (1989) and Jiang et al. (1999) as follows: np: non-probing; C: intercellular apoplastic stylet pathway; pd: intracellular punctures during the stylet pathway phase; E1: salivation into phloem sieve elements; E2: passive phloem sap ingestion from sieve elements; and G: active xylem sap ingestion.

EPG recordings were processed using the EPG-Excel Data Workbook 5.0 developed by Sarria et al. (2009). For each insect, sequential and non-sequential EPG variables were extracted and subsequently summarized for each treatment. Following the terminology proposed by Backus et al. (2007), the non-sequential number of waveform events per insect (NWEI), and total waveform duration per insect (WDI). Sequential variables included the 'time to 1st probe from start of EPG', 'time from start of EPG to 1st phloem' and 'Time from 1st probe to 1st phloem'.

Data distributions and homogeneity of variances were assessed before statistical analysis. Continuous EPG variables were compared between treatments using Student's t-test when parametric assumptions were met and the Mann–Whitney U test otherwise. Differences in the proportion of individuals producing each waveform were evaluated using a Fisher´s test. For each fungus–plant–insect combination, EPG variables were compared with the corresponding untreated control using predefined pairwise comparisons. Statistical analyses were performed using IBM SPSS Statistics version 22.0.

Results

Endophytic colonization of *Beauveria bassiana* and *Cordyceps javanica* in maize and soybean plants

Both *B. bassiana* ESALQ-PL63 and *C. javanica* ESALQ-1296 successfully established as endophytes in maize plants within 24 h after foliar application. *Beauveria bassiana* was recovered from 68.7% of plants at 1, 3, and 5 days after application, after which its recovery markedly declined to 12.5% at 10 days and was no longer detected at 15 days. A similar temporal pattern was observed for *C. javanica*, which was recovered from 75.0% of plants at 1, 3, and 5 days, followed by a decline to 32.1% at 10 days and 3.6% at 15 days. These results indicate that both fungal species rapidly established endophytically in maize, but their recovery progressively declined over time.

In soybean plants, *B. bassiana* was recovered from 84.4% of plants at 1, 3, and 5 days after spraying, decreasing to 33.3% in 10 days, with no recovery at 15 days. In contrast, *C. javanica* was not recovered from soybean plants at any of the evaluated sampling times at the applied rate (Fig. 1).

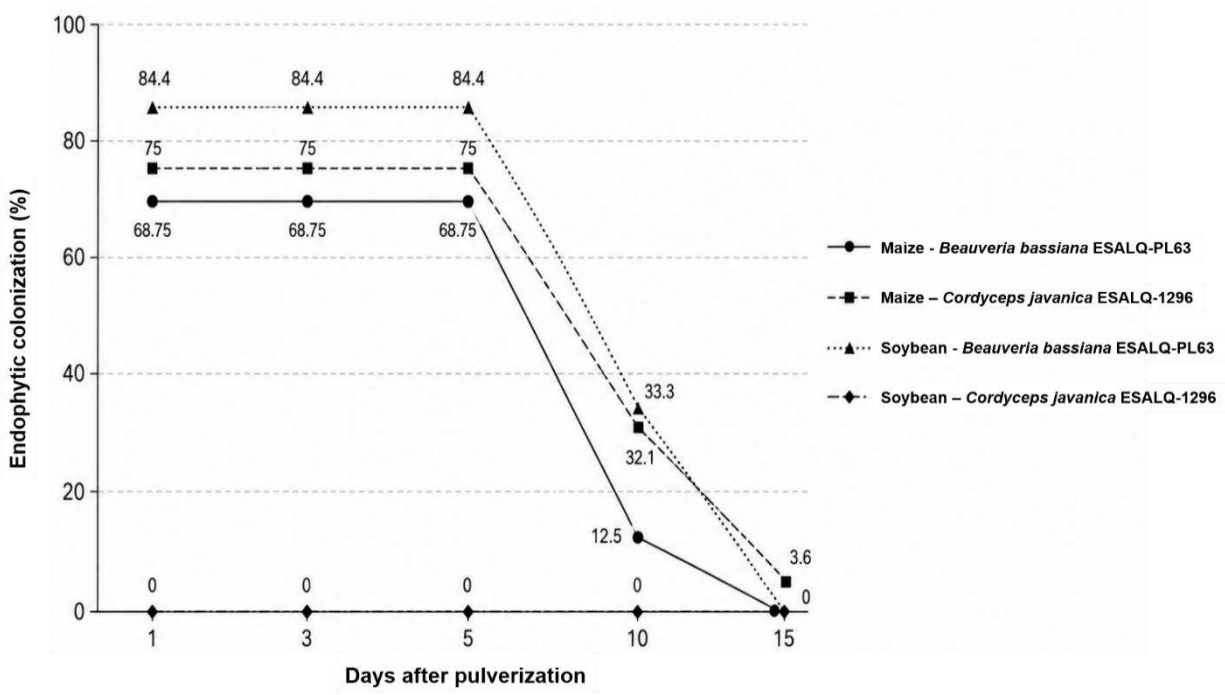

Figure 1. Endophytic colonization percentage of maize and soybean plants over time after fungal application, as determined by fungal re-isolation on culture medium.

Based on these preliminary colonization results, EPG assays were conducted 72 h after spraying, when fungal recovery remained high. Following the 8 h EPG recordings, plant tissues were sampled at 96 h (4 days) after spraying to confirm fungal endophytic colonization. Because *C. javanica* was not recovered from soybean plants under the tested conditions, this fungus–plant combination was not included in the EPG assays.

The same plants were monitored over time to assess the persistence of endophytic colonization. This longitudinal assessment showed that fungi initially recovered from colonized plants became progressively less detectable over time, with fungal recovery declining at later sampling intervals and eventually becoming undetectable by culture-based re-isolation.

Dalbulus maidis* on maize colonized by *Beauveria bassiana

The probing behavior of *D. maidis* was moderately affected when insects fed on maize plants endophytically colonized by *B. bassiana*. Among the EPG variables affected, the number of probes was significantly higher on *B. bassiana*-colonized plants than on control plants (Fig. 2B). Probes comprised all stylet activities performed within plant tissues during the 8-h recording period, including both non-phloem- and phloem-associated activities. In contrast, total probing duration was slightly but significantly higher on control plants (Fig. 2E).

The number of Dm1 events, associated with the pre-phloem phase, was significantly higher on *B. bassiana*-colonized plants than on control plants (Fig. 2B), whereas the total duration of Dm1 did not differ significantly between treatments (Fig. 2F). Non-probing (np) duration was significantly affected by endophytic colonization, with *D. maidis* spending more time with their stylets withdrawn from plant tissues on *B. bassiana*-colonized plants than on control plants (Fig. 2D). However, the number of non-probing events did not differ significantly between treatments (Fig. 2A).

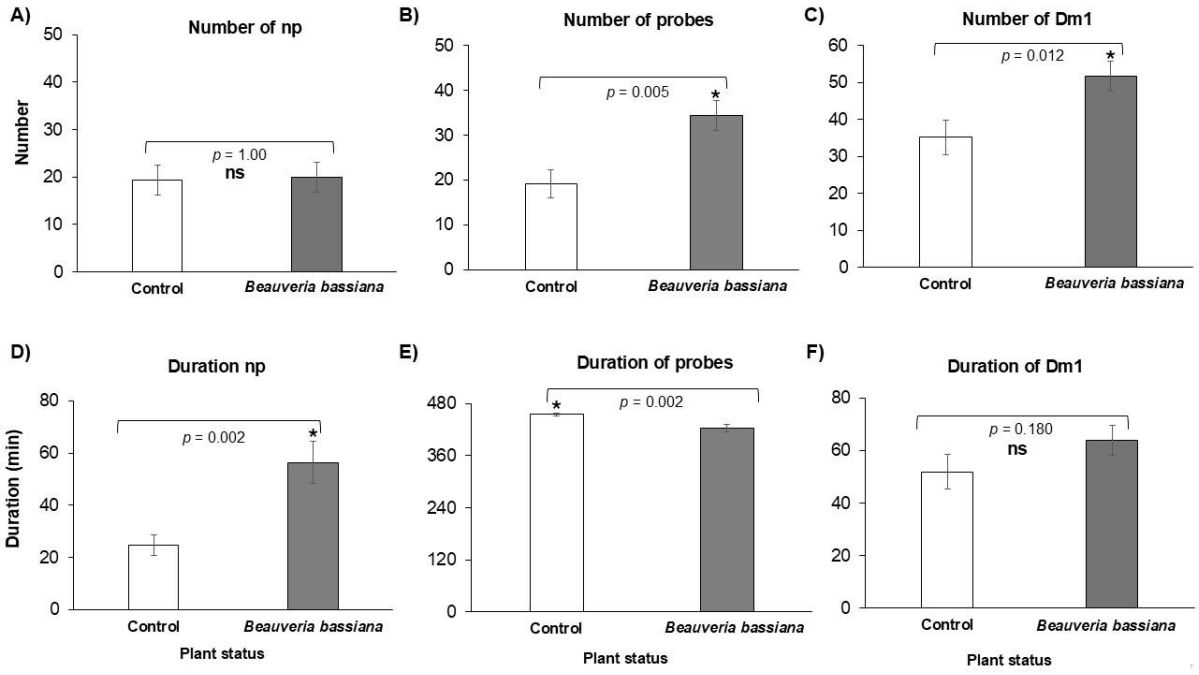

Figure 2. Effects of endophytic *Beauveria bassiana* strain ESALQ-PL63 on the probing behavior of *Dalbulus maidis* on maize plants during 8-h recordings. Dm1: pre-phloem phase, during intercellular penetration of the stylets. Bars represent (mean ± SE). Asterisks (*) indicate significant differences between control and *B. bassiana*-colonized plants ($P < 0.05$), **ns**: non-significant.

Regarding phloem-associated variables, the number of Dm5 events was significantly lower in insects feeding on *B. bassiana*-colonized plants than in those feeding on control plants (Fig. 3B), indicating fewer phloem ingestion events. In contrast, no significant differences were observed in the number of Dm4 events (Fig. 3A), Dm4 duration (Fig. 3C), or Dm5 duration (Fig. 3D). Total phloem-phase duration was also numerically lower in insects feeding on *B. bassiana*-colonized plants (176.2 ± 27.1 min) than in those feeding on control plants (236.2 ± 26.4 min), although this difference was not statistically significant (P = 0.12).

The proportion of individuals producing waveform Dm4 did not differ significantly between the control (100%; 18/18) and the *B. bassiana* plants (83.3%; 15/18; Fisher's exact test, P = 0.23). In contrast, the proportion of individuals producing waveform Dm5 was significantly lower in the *B. bassiana* plants (72.2%; 13/18) than in the control (100%; 18/18; Fisher's exact test, P = 0.04). No significant differences were detected for the remaining EPG variables evaluated, which are presented in the Supplementary Information (SI1).

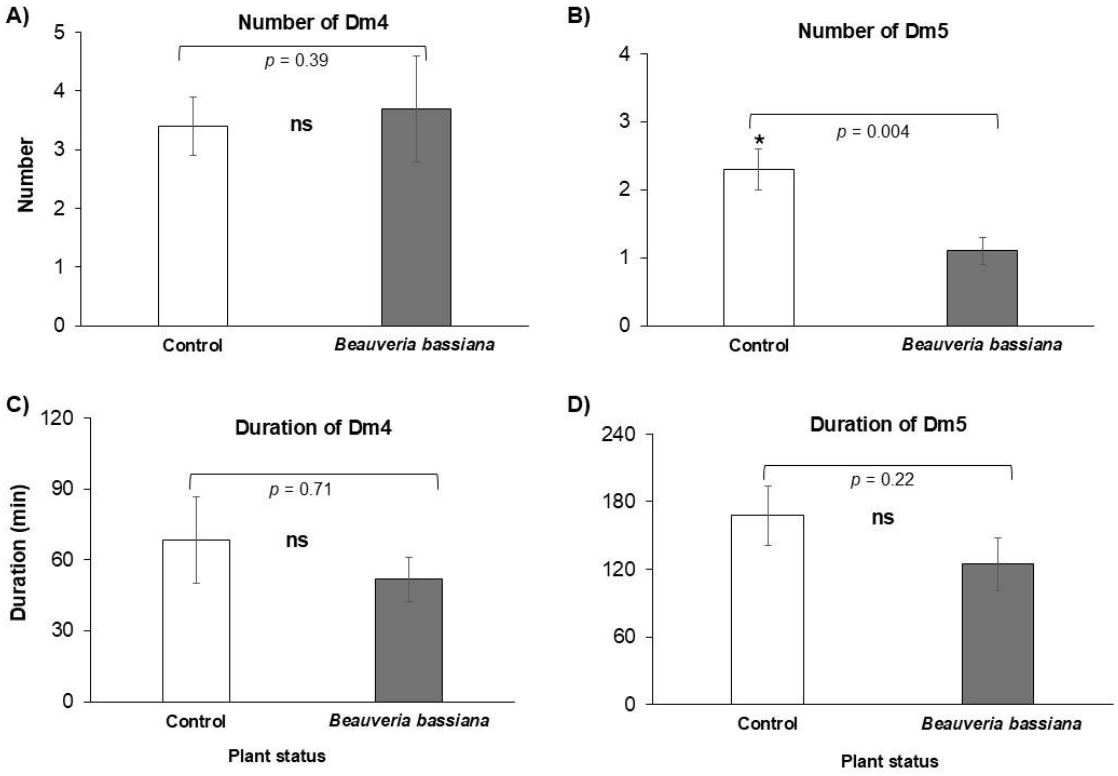

Figure 3. Effects of endophytic *Beauveria bassiana* strain ESALQ-PL63 on phloem activities of *Dalbulus maidis* on maize plants during 8-h recordings. Bars represent (mean ± SE). Asterisks (*) indicate significant differences between control and *B. bassiana*-colonized plants (P < 0.05), **ns**: non-significant.

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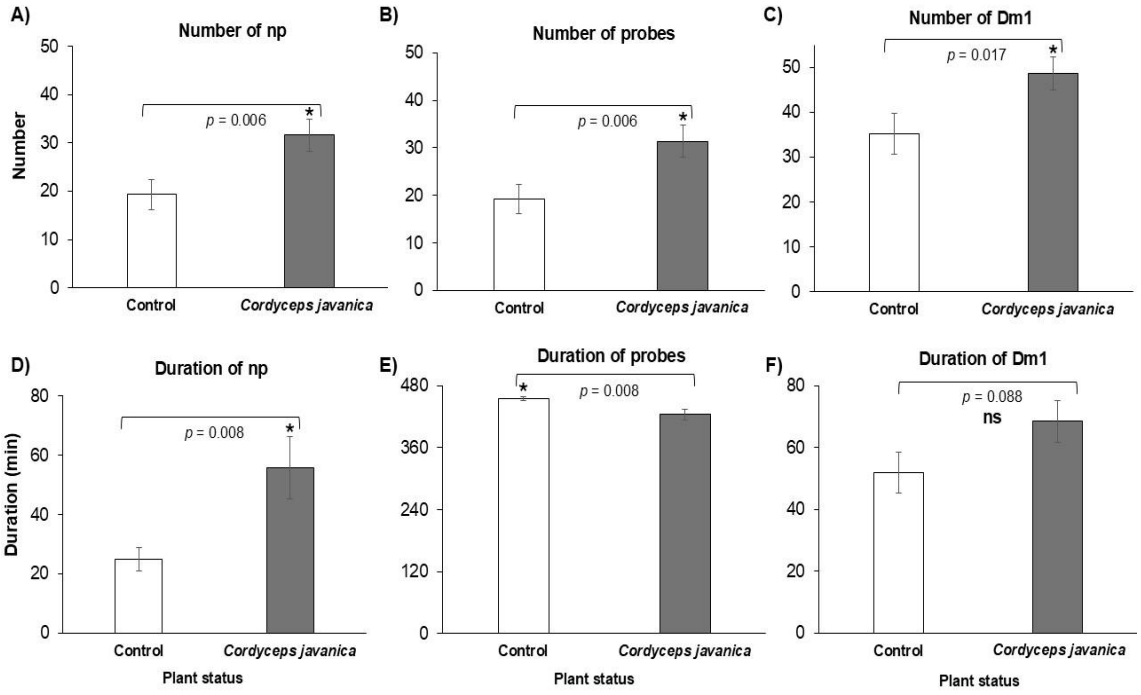
349 ***Dalbulus maidis* on maize colonized by *Cordyceps javanica***

350 Endophytic colonization of maize plants by *C. javanica* significantly affected the probing behavior
351 of *D. maidis*. During the non-phloem phase, insects feeding on *C. javanica*-colonized plants exhibited a
352 significantly higher number of non-probing (np) events and probes compared with insects on control plants
353 (Fig. 4A and B). Similarly, the number of Dm1 waveform events was significantly higher on fungus-
354 colonized plants (Fig. 4C). The total duration of non-probing was also significantly increased by fungal
355 colonization (Fig. 4D), whereas the total probing duration was significantly reduced (Fig. 4E). Although
356 the duration of Dm1 tended to be longer on *C. javanica*-colonized plants, this difference was not statistically
357 significant (Fig. 4F).



358

359 **Figure 4.** Effects of endophytic *Cordyceps javanica* strain ESALQ-1296 on the probing behavior of
360 *Dalbulus maidis* on maize plants during 8-h recordings. Dm1: pre-phloem phase, during intercellular
361 penetration of the stylets. Bars represent (mean ± SE). Asterisks (*) indicate significant differences between
362 control and *C. javanica*-colonized plants ($P < 0.05$), **ns**: non-significant.

363

364 Phloem-associated activities were also affected by endophytic colonization. The number of Dm4
365 events was significantly reduced in insects feeding on *C. javanica*-colonized plants compared with the

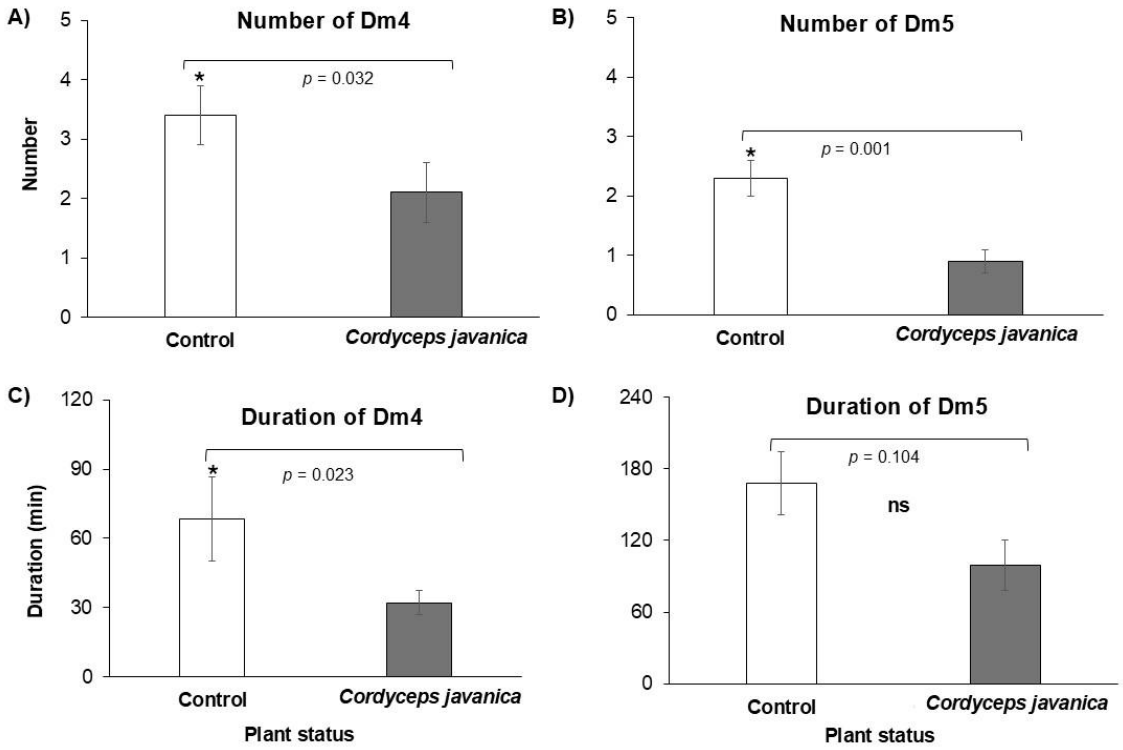
366 control (Fig. 5A). Similarly, a stronger reduction was observed for the number of Dm5 events (Fig. 5B).
367 Likewise, the total duration of Dm4 was significantly shorter on fungus-colonized plants (Fig. 5C).
368 Although the total duration of Dm5 was numerically lower in insects feeding on *C. javanica*-colonized
369 plants, this difference was not statistically significant (Fig. 5D).

370 Total phloem-phase duration (Dm4+Dm5) was also lower in insects feeding on *C. javanica*-
371 colonized plants (131.6 ± 26.1 min) than in those feeding on control plants (236.2 ± 26.4 min) (P = 0.030).

372 The proportion of individuals producing waveform Dm4 did not differ significantly between
373 Control (100.0%; 18/18) and *C. javanica*-colonized plants (81.3%; 13/16; Fisher's exact test, P = 0.094).
374 In contrast, the proportion of individuals producing waveform Dm5 was significantly lower in *C. javanica*-
375 colonized plants (68.8%; 11/16) than in Control (100.0%; 18/18; Fisher's exact test, P = 0.016).

376 Overall, these results show that endophytic colonization by *C. javanica* altered both non-phloem
377 and phloem-related probing activities of *D. maidis*, characterized by more frequent interruptions and
378 probing attempts, increased stylet pathway activities, and reduced occurrence and duration of phloem-
379 associated activities. Additional details are provided in the Supplementary Information (SI2).

380



381

382 **Figure 5.** Effects of endophytic *Cordyceps javanica* strain ESALQ-1296 on phloem activities of *Dalbulus*
383 *maidis* on maize plants during 8-h recordings. Bars represent (mean ± SE). Asterisks (*****) indicate significant
384 differences between control and *C. javanica*-colonized plants ($P < 0.05$), **ns**: non-significant.

385

386 ***Bemisia tabaci* on soybean colonized by *Beauveria bassiana***

387 The probing behavior of *B. tabaci* was generally unaffected by *B. bassiana*-colonized soybean
388 plants. No significant differences were detected between insects feeding on control and *B. bassiana*-
389 colonized plants in the number of non-probing (np) events, total duration of np, number of probes, total
390 probing time, number or total duration of stylet pathway (C) events, or number of xylem ingestion (G) (P
391 > 0.05). However, the total duration of xylem sap ingestion was significantly reduced on *B. bassiana*-
392 colonized plants compared with control plants (Control: 89.7 ± 12.5 min, *B. bassiana* plants: 45.6 ± 10.2
393 min; $P = 0.012$).

394 Endophytic colonization by *B. bassiana* did not significantly affect the phloem-associated
395 activities of *B. tabaci* (Fig. 7). No differences were detected in the number or total duration of E1 (Fig.7A,
396 C), or E2 (Fig.7B, D), or in the total duration of phloem activities (E1+E2) between insects feeding on
397 control and colonized plants (control: 37.5 ± 14.6 min, *B. bassiana* colonized plants 39.3 ± 16.9 min; $P =$
398 0.611).

399 The proportion of individuals producing waveform E1 (phloem salivation) did not differ
400 significantly between control (75.0%; 12/16) and *B. bassiana*-colonized plants (61.1%; 11/18; Fisher's
401 exact test, $P = 0.477$). Similarly, the proportion of individuals exhibiting E2 (phloem ingestion) was 56.3%
402 (9/16) on control plants and 55.6% (10/18) on colonized plants (Fisher's exact test, $P = 1.000$).

No significant differences were observed in the sequential EPG variables, including the 'Time from the start of the EPG recording to the first probe', 'The time from the start of the recording to the first phloem phase (E)', and 'The time from the first probe to the first E' ($P > 0.05$). Overall, endophytic colonization of soybean by *B. bassiana* had a limited effect on the probing behavior of *B. tabaci*, with the only significant change detected being a reduction in the total duration of xylem ingestion (G). Additional details are provided in the Supplementary Information (SI3).

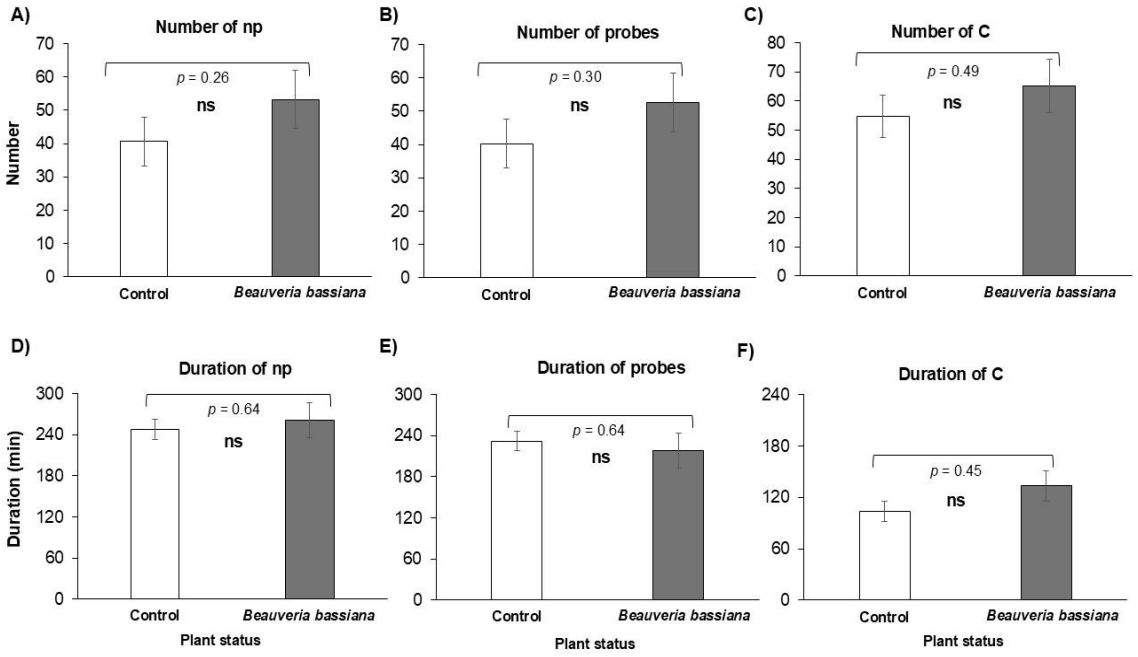


Figure 6. Effects of endophytic *Beauveria bassiana* strain ESALQ PL63 on the probing behavior of *Bemisia tabaci* MEAM1 on soybean plants during 8-h recordings. C: pre-phloem phase, stylet pathway during intercellular penetration of the stylets. Bars represent (mean ± SE). Asterisks (*) indicate significant differences between control and *B. bassiana*-colonized plants ($P < 0.05$), **ns**: non-significant.

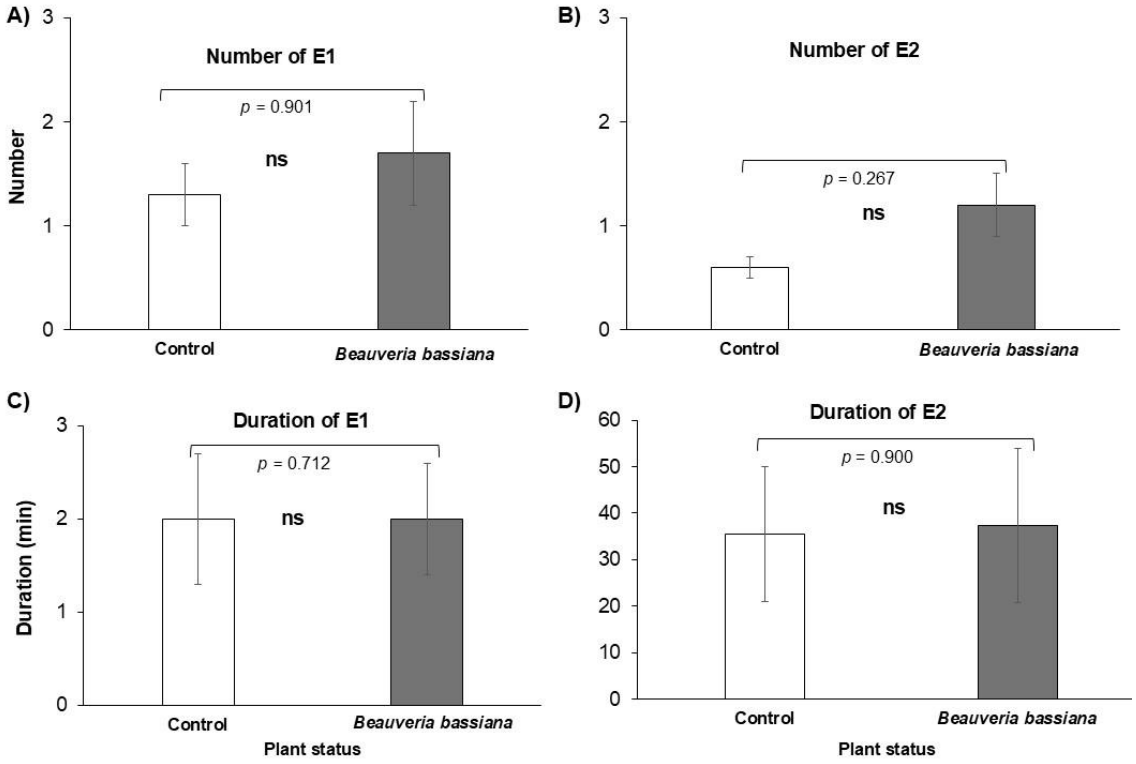


Figure 7. Effects of endophytic *Beauveria bassiana* strain ESALQ PL63 on phloem activities of *Bemisia tabaci* MEAM1 on soybean plants during 8-h recordings. Bars represent (mean ± SE). Asterisks (*) indicate significant differences between control and *B. bassiana*-colonized plants ($P < 0.05$), **ns**: non-significant.

Discussion

The present study demonstrates that endophytic colonization by entomopathogenic fungi can alter the probing behavior of phloem-feeding insects, but these effects are strongly dependent on the specific fungus–plant–insect interaction. Both *B. bassiana* strain ESALQ-PL63 and *C. javanica* strain ESALQ-1296 successfully colonized maize plants and disrupted *D. maidis* probing behavior, increasing interruptions and probing attempts while reducing phloem-associated activities. In contrast, although *B. bassiana* successfully established endophytically in soybean, its effects on *B. tabaci* MEAM1 were limited to a reduction in xylem ingestion, with no detectable changes in phloem activities. Moreover, *C. javanica* was not recovered from soybean plants under the tested conditions. These contrasting responses support our hypothesis that the effects of endophytic entomopathogenic fungi are not determined solely by successful fungal establishment but instead emerge from the interaction among fungal species, host plant, and insect.

Both fungal species were recovered from maize tissues within 24 h after foliar application, and colonization remained high during the first five days. However, fungal recovery subsequently declined and was absent or nearly absent 15 days after foliar application. A comparable pattern was observed for *B. bassiana* in soybean. The decline in fungal detection over time indicates that colonization resulting from foliar application was predominantly transient under the experimental conditions. Transient endophytic establishment following foliar inoculation has been reported for several entomopathogenic fungi and plant species, and colonization efficiency and persistence can vary according to fungal isolate, inoculation method, plant genotype, tissue sampled, developmental stage, inoculum concentration, and environmental conditions (Garrido-Jurado et al. 2017; Jaber and Ownley 2018; Vega 2018).

The progressive decline in fungal recovery observed in the present study is consistent with Posada et al. (2007), who reported that although *B. bassiana* successfully established endophytically in coffee plants, its recovery decreased with increasing time after inoculation, highlighting the temporal dynamics and limited persistence of endophytic colonization

Importantly, the relatively short persistence observed here does not necessarily limit the biological relevance of endophytic colonization. The high recovery rates recorded during the first days after application coincided with the period in which the EPG assays were conducted, indicating that the behavioral effects observed in *D. maidis* occurred while the fungi were actively detectable within maize tissues.

In maize, endophytic colonization by both fungi increased the number of probing attempts and activities associated with non-phloem tissues. Insects feeding on colonized plants also spent more time with their stylets withdrawn from the plant and less time probing, although the number of non-probing events differed significantly only in the *C. javanica* treatment. This pattern suggests that colonized plants were less readily accepted or that insects encountered factors that repeatedly interrupted stylet progression through epidermal and parenchymal tissues. The increased number of Dm1 events, without a corresponding increase in their total duration, further indicates that *D. maidis* initiated the stylet pathway more frequently but did not necessarily spend longer in each pathway event. Thus, the effect appears to involve repeated interruption and reinitiation of probing rather than simply a prolonged pathway phase. Such responses may arise from changes in physical or chemical plant traits perceived during probing, including alterations in tissue composition, defense-related metabolites, or other plant-mediated cues induced by fungal colonization.

462 The effects were especially relevant during the phloem phase. On maize plants colonized by *B.*
463 *bassiana*, *D. maidis* produced fewer Dm5 events, and the proportion of individuals that reached phloem
464 ingestion decreased from 100% in control plants to 72.2% on colonized plants. Although Dm4 and Dm5
465 durations did not differ significantly, total phloem duration was numerically reduced by approximately 60
466 min. These results indicate that *B. bassiana* did not consistently prevent salivation into the sieve elements
467 but reduced the likelihood that phloem contact progressed to sustained sap ingestion. The absence of
468 significant differences in Dm4 duration also suggests that the principal effect occurred after or near the
469 transition from phloem conditioning to ingestion. This distinction is biologically important because it
470 indicates that endophytic colonization affected the ability of *D. maidis* to successfully exploit the phloem
471 rather than producing only a generalized reduction in probing.

472 A broader range of EPG variables differed from the control in maize colonized by *C. javanica* than
473 in maize colonized by *B. bassiana*. In addition to increasing non-probing, probing attempts, and Dm1
474 events, *C. javanica* reduced the number of both Dm4 and Dm5 events and shortened the total duration of
475 Dm4. The proportion of insects exhibiting Dm5 also declined from 100% in control to 68.8% on colonized
476 plants. Collectively, these changes indicate interference with both phloem conditioning and the subsequent
477 ingestion of phloem sap. Although Dm5 duration was numerically lower but not statistically different, the
478 marked reductions in the number of Dm5 events and in the proportion of insects producing this waveform
479 provide clear evidence that successful phloem ingestion became less frequent. Differences between the
480 effects of *B. bassiana* and *C. javanica* may reflect fungus-specific interactions with maize physiology,
481 including differences in tissue colonization, spatial distribution within the plant, elicitation of defense
482 responses, or production and induction of biologically active metabolites. These mechanisms were not
483 directly measured here and should therefore be investigated through fungal quantification, microscopy,
484 transcriptomics, metabolomics, and analyses of plant defensive pathways.

485 The disruption observed following endophytic colonization differs mechanistically from the
486 previously reported effects of direct fungal exposure on *D. maidis*. Maluta, Castro and Lopes (2023) showed
487 that topical exposure to *C. javanica* markedly disrupted both non-phloem and phloem activities before
488 insect death. In that case, behavioral changes could have resulted from the developing infection and its
489 effects on insect physiology. In the present study, the fungus acted through the maize plant, demonstrating
490 that disruption of *D. maidis* probing can also occur without prior direct treatment of the insect. Despite
491 these different routes of exposure, both studies revealed reduced phloem exploitation and increased

492 interruption of probing. This convergence suggests that entomopathogenic fungi may affect vector behavior
493 through complementary direct and plant-mediated pathways. Under field conditions, foliar applications
494 may therefore provide effects beyond direct infection, provided that the fungus successfully establishes
495 within plant tissues.

496 Changes in Dm4 and Dm5 may also have epidemiological implications because these waveforms
497 are associated with salivation into sieve elements and phloem sap ingestion, respectively. The major
498 pathogens transmitted by *D. maidis*, including *S. kunkelii*, maize bushy stunt phytoplasma, and maize
499 rayado fino virus, are associated with phloem tissues and are acquired or inoculated during particular stylet
500 activities. Consequently, reductions in the occurrence and duration of phloem activities could decrease
501 opportunities for pathogen acquisition or inoculation. However, changes in EPG variables cannot by
502 themselves demonstrate reduced transmission. Depending on the pathogen and transmission mechanism,
503 repeated short probes or salivation events could have different epidemiological consequences, and
504 behavioral disruption does not invariably result in reduced transmission efficiency. Experiments directly
505 measuring pathogen acquisition and inoculation are therefore required before concluding that endophytic
506 colonization reduces disease spread. Nevertheless, the lower proportion of insects reaching Dm5,
507 particularly on *C. javanica*-colonized plants, provides a biologically relevant basis for testing this
508 hypothesis.

509 Studies combining confirmation of endophytic colonization with EPG analysis indicate that the
510 effects of entomopathogenic fungi on insect probing behavior are highly context dependent. Endophytic *B.*
511 *bassiana* strain EABb 01/33-Su altered the number of short probes of *Aphis gossypii* Glover, 1877
512 (Hemiptera: Aphididae) on melon in the first 10-minutes recording, although these behavioral changes did
513 not fully explain the reduction in virus transmission observed in colonized plants (González-Mas et al.
514 2019).

515 Host-dependent responses are further supported by studies involving *Metarhizium brunneum*
516 Petch, 1935 (Hypocreales: Clavicipitaceae) strain EAMa 01/58-Su and the meadow spittlebug *Philaenus*
517 *spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae), important vector of *Xylella*
518 *fastidiosa* (Xathomonadales: Xanthomonadaceae) in Europe. Endophytic colonization of *Sonchus*
519 *oleraceus* by *M. brunneum* produced few feeding alterations and insects feeding on colonized plants
520 reached xylem vessels faster than insects feeding on control. (Yousef-Yousef et al. 2023), whereas
521 colonization of olive plants increased unsuccessful probing and non-probing activities while delaying and

reducing xylem-related activities (Lago et al. 2026). Collectively, these contrasting outcomes indicate that the effects of endophytic entomopathogenic fungi on probing behavior arise from the specific fungus–plant–insect combination rather than from fungal colonization alone.

Unlike the response of *D. maidis* on maize, endophytic colonization of soybean by *B. bassiana* did not substantially alter the probing behavior of *B. tabaci* MEAM1. The absence of differences in stylet pathway and phloem variables, including E1, E2, total phloem duration, and the proportion of insects reaching these activities, indicates that fungal recovery from plant tissues does not necessarily imply sufficient changes in plant suitability to disrupt insect feeding.

More pronounced effects were reported for *B. tabaci* MED on tomato plants colonized by *B. bassiana* strain Bb252, including increased non-probing duration and reduced total probing, salivation and phloem ingestion (Wang et al. 2024). These findings contrast with the limited behavioral response of *B. tabaci* observed on *B. bassiana*-colonized soybean plants in the present study, suggesting that successful fungal establishment does not necessarily result in comparable effects across host plants. In that system, fungal colonization also altered plant-specialized metabolites and affected the physiological characteristics of *B. tabaci*. Tomato and soybean may respond differently to endophytic *B. bassiana*, and these responses may not generate the same chemical or nutritional changes in the phloem.

Endophytic entomopathogenic fungi may influence insect probing behavior indirectly through fungus-induced physiological and metabolic changes in the host plant. Recent metabolomic evidence showed that endophytic colonization of *Citrus aurantium* L. 1753 (Sapindales: Rutaceae) by *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones and Samson, 2011 (Hypocreales: Ophiocordycipitaceae) induced systemic reprogramming of host metabolism, including changes in primary metabolism and the activation of secondary defense pathways (Bouzembila et al. 2026). These findings support the hypothesis that the changes in probing behavior observed here for *D. maidis* on *B. bassiana*-colonized maize plants may result, at least in part, from plant-mediated responses induced by fungal colonization. However, because plant metabolic changes were not assessed in the present study, further metabolomic and biochemical analyses are required to determine the mechanisms underlying these behavioral effects.

The only significant response of *B. tabaci* was a reduction in the total duration of xylem ingestion. Xylem feeding in whiteflies is generally associated with water uptake and osmoregulatory balance, particularly because phloem sap has a high concentration of soluble sugars. A reduction in xylem activities

552 may therefore indicate a change in plant water relations, xylem accessibility, or the physiological need for
553 water uptake. However, because the number of G events and all phloem-associated variables remained
554 unchanged, the biological importance of this isolated response should be interpreted cautiously.

555 Although *C. javanica* successfully colonized maize, it was not recovered from soybean under the
556 conditions tested, indicating that its endophytic establishment is host- and context-dependent. Nevertheless,
557 this fungus has been reported as an endophyte in other plant species. Following seed inoculation, *C.*
558 *javanica* was recovered from the leaves, stems, and roots of tomato plants for up to 60 days (Sani et al.
559 2023), whereas foliar application resulted in its establishment within pepper leaf tissues for up to five weeks
560 under semi-field conditions (Bocco et al. 2021). These contrasting results suggest that endophytic
561 colonization by *C. javanica* depends on several interacting factors, including the host plant, fungal isolate,
562 inoculum concentration, and inoculation method. Therefore, the lack of recovery from soybean in the
563 present study should not be interpreted as an inherent inability of this fungal species to colonize soybean,
564 as other isolates, application rates, or inoculation routes may produce different outcomes.

565 Overall, our findings show that the effects of endophytic entomopathogenic fungi on insect
566 probing behavior are strongly context-dependent and cannot be predicted solely from successful fungal
567 establishment within the plant. The pronounced disruption of *D. maidis* probing on maize, particularly the
568 reduced occurrence of phloem ingestion, demonstrates that *B. bassiana* strain ESALQ-PL63 and *C.*
569 *javanica* strain ESALQ-1296 can exert biologically relevant plant-mediated effects beyond their direct
570 insecticidal activity. Conversely, the limited response of *B. tabaci* on *B. bassiana*-colonized soybean
571 reinforces that the functional outcome of endophytic colonization emerges from the specific fungus–plant–
572 insect interaction.

573 From a practical perspective, our findings may contribute to the development of more preventive
574 and strategically timed biological control programs. The transient nature of fungal establishment observed
575 in this study suggests the existence of a temporal window during which endophytic colonization may
576 provide plant-mediated effects in addition to the direct insecticidal activity of the fungi. Defining this
577 functional window could help optimize the timing of biological applications, allowing entomopathogenic
578 fungi to be positioned at specific stages of crop development or periods of increased pest risk, before pest
579 populations reach damaging levels. Such an approach could shift the use of fungal biocontrol agents from
580 predominantly reactive interventions toward more preventive management strategies that integrate direct
581 insecticidal and plant-mediated effects. Future studies should therefore determine the physiological

582 mechanisms underlying these interactions and, particularly, establish the persistence and consistency of
583 their effects under field conditions and their consequences for pest population dynamics and pathogen
584 transmission. Ultimately, understanding when and for how long these complementary effects operate may
585 enable more effective integration of entomopathogenic fungi into preventive IPM programs.
586

587 **Acknowledgements**

588 We thank Koppert Biological Systems for providing the products Octane® and Boveril Evo ® used in the
589 assays. The authors would like to acknowledge the financial support from São Paulo State Research
590 Foundation (FAPESP Grant N°. 2018/02317-5 and 2024/17729-8) as part of the SPARCBIO (São Paulo
591 Advanced Research Center for Biological Control) located at ESALQ/USP with support of FAPESP,
592 Koppert, and University of São Paulo (USP). Finally, we thank the anonymous reviewers for many useful
593 comments and suggestions, which helped to improve the manuscript.
594

595 **Statements & Declarations**

596 **Funding**

597 This study was supported by the São Paulo Research Foundation (FAPESP) [Grant No. 2018/02317-5], as
598 part of the São Paulo Advanced Research Center for Biological Control (SPARCBIO). The first author
599 received a fellowship from FAPESP [Grant No. 2024/17729-8] to conduct this research.
600

601 **Competing interests**

602 The authors declare no competing interests.
603

604 **Authors Contributions**

605 All authors contributed to the development of experimental methodology. NM conducted all experiments
606 and was responsible for all electrical penetration graph (EPG) analyses, including waveform identification
607 and classification, as well as data processing, statistical analyses, and interpretation of the results. All
608 authors (NM, TC, CMM and JRSL) contributed to the writing, critical revision, and editing of the
609 manuscript. All authors read and approved the final version of the manuscript.
610

611 **Data availability**

Data sets used or analyzed during current study are available from the corresponding author on reasonable request.

Ethics approval

The experimental research and collection of plant and insect materials of this study comply with the relevant institutional, national, and international guidelines and legislation.

Consent to participate

Not applicable. This study did not involve human participants; therefore, informed consent to participate was not required.

Consent to publish

Not applicable. This study did not involve human participants or contain identifiable personal information, images, or data requiring consent for publication. The authors have permission to use all biological materials used in this study. Also, all methods were carried out in accordance with relevant guidelines and regulations.

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