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Management strategy with bioinputs for *Dalbulus maidis* (Hemiptera: Cicadellidae) on a non-preferential host plant

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Abstract

Dalbulus maidis (DeLong & Wolcott) (Hemiptera: Cicadellidae) causes significant yield losses in various types of maize produced in Brazil through pathogen transmission. Its occurrence in fields lacking its preferential hosts has increased, including Solanaceae and Fabaceae crops. Chemical methods provide up to 90% control, but with a residual period of only seven days via seed treatment plus foliar applications. This short temporal efficacy window may be useful for integrating chemical and microbiological methods, a premise of Integrated Pest Management (IPM). Therefore, we evaluated the entomopathogenic fungi *Beauveria bassiana*

(Bb), *Metarhizium anisopliae* (Ma), and *Isaria fumosorosea* (Isa) against *D. maidis* on pea plants (var. Gallant), a candidate off-season host, under field conditions. We also assessed these fungi tank-mixed with the resistance inducer potassium silicate (SilK), including effects on crop yield (kg ha−1). Treatments were arranged in a (3×2)+2 factorial design, with three levels of the Fungi factor (Bb, Ma, or Isa), two levels of the SilK factor (with and without potassium silicate), plus two controls: an absolute control (water only) and a positive control (profenofos+lufenuron). Leafhoppers were sampled using yellow sticky traps at 0, 3, 6, 9, and 12 days after application (DAA). The mixture of SilK and entomopathogenic fungi increased the infection capacity of these entomopathogens on *D. maidis* adults. However, 85.63% of the sampled corn leafhoppers showed no fungal infection. A greater number of infected leafhoppers were sampled in plots treated with *I. fumosorosea* than with Bb or Ma. The addition of SilK also increased pea yield.

Keywords: entomopathogenic fungi, corn leafhopper, potassium silicate, non-host plant, biological control, crop yield

1. Introduction

Dalbulus maidis (DeLong & Wolcott) (Hemiptera: Cicadellidae) has been causing production losses of 70%, 50%, and 20% due to the transmission of phytoplasma, phytoplasma+spiroplasma, and spiroplasma, respectively, in various types of maize produced in Brazil (Massola Júnior, Bedendo, Amorim & Lopes, 1999). In the case of sweet corn (*Zea mays* var. *saccharata*), these losses are even greater due to its high susceptibility (Viana, Cruz & Waquil, 2002). This cicadellid is difficult to control, persisting through several off-season survival mechanisms (sensu Oliveira, Lopes & Nault, 2013) in agricultural environments in the absence of its three preferred hosts (maize, teosintes, and *Tripsacum* spp., all Poaceae) (Waquil, Viana, Cruz & Santos, 1999). These strategies include feeding on local spontaneous plants,

whether monocotyledons (Pitre, Combs & Douglas, 1966) or dicotyledons (Markham, Townsend, Plaskitt & Saglio, 1977), metabolic reduction under limiting abiotic conditions as observed in Mexico (Larsen, Nault & Moya-Raygoza, 1992), or even short-, medium-, and long-distance migration (Taylor, Nault & Styer, 1993) to areas with abundant food resources.

The number of corn leafhoppers sampled in agricultural fields planted with non-preferential host plants has drawn attention in recent years in the eastern, southeastern, and southern regions of the state of Goiás, Brazil, where a large portion of Brazilian agroindustries are established (Vilela, Melo, Boiteux & Clemente, 2012; CODEVASF, 2021). These agroindustries produce crops such as potato (*Solanum tuberosum* L., Solanaceae), tomato (*Solanum lycopersicum* L., Solanaceae), sweet corn (*Z. mays* var. *saccharata*), and pea (*Pisum sativum* L., Fabaceae) (Nascimento, Silva, Freitas & Boiteux, 2016; Rosa, Moribe, Yamamoto & Sperandio, 2018).

In industrial tomato plants (cv. Heinz 9553) in the state of Goiás, *D. maidis* populations were sampled with mean counts of 200 individuals per yellow sticky trap (Silva et al., 2018). These areas had successive crops of tomato and sweet corn, with two to three months between plantings at the same local scale. Populations of *D. maidis* have also been reported on common bean (*Phaseolus vulgaris* L. var. Huetar) captured with yellow sticky traps in the Central Valley region, Costa Rica (Castro, Rivera, Isard, Gamez & Jacqueline, 1992). More recently, populations of this insect have been observed in commercial pea plantings in the state of Goiás. Overall, quantitative reports of *D. maidis* on non-preferred hosts remain scarce and highly variable, which limits generalisation and motivates the direct field quantification undertaken here.

The high nitrogen fertilisation recommended for pea, tomato, potato, and sweet corn production may be related to the maintenance of high corn leafhopper populations in the field, as suggested by Power (1989). The accumulation of nitrogen and free amino acids, inhibiting

protein synthesis, is a recognised factor that supports high populations of sap-sucking and pathogen-transmitting insects in the field (Chen, Olson & Ruberson, 2010). Despite the absence of visual symptoms of mollicutes causing corn stunt spiroplasma and maize bushy stunt phytoplasma (Oliveira, Fernandes, Souza, Oliveira & Cruz, 2003; Pérez-López et al., 2018), as well as maize rayado fino virus (Oliveira et al., 2003) in these uncommon hosts, the maintenance of *D. maidis* populations, even in smaller numbers than in sweet corn, may justify the rapid and early re-infestation in the first vegetative stages of maize. This is relevant because the pathogens transmitted by this vector are of a persistent propagative nature (Nault, 1980), and *D. maidis* can maintain high quantities of phytopathogen inocula incubated in its salivary glands, even after months of fallow (Oliveira, Molina, Albres & Lopes, 2002). We stress, however, that a reservoir role for these non-preferred hosts during the maize off-season remains to be demonstrated and is treated here as a working hypothesis rather than an established fact.

The control percentage of this insect in different maize types by chemical methods currently reaches approximately 90% with thiamethoxam (neonicotinoid) via seed treatment and thiamethoxam + lambda-cyhalothrin (pyrethroid) in additional foliar applications, but with a residual period of only seven days (Albuquerque, Borges, Iacono, Crubelati & Singer, 2006). This short temporal efficiency window may be useful for integrating chemical and microbiological methods, which is a premise of Integrated Pest Management (IPM), promoting environmental, economic, and social sustainability in sweet corn agribusiness in Brazil and reducing high infestations in uncommon host plants. Moreover, field control failures and reduced susceptibility of *D. maidis* to pyrethroid and neonicotinoid insecticides have recently been reported in Brazil (Machado et al., 2024; Dias et al., 2025), reinforcing the need to integrate non-chemical tactics.

Therefore, we evaluated control methods for *D. maidis* based on the entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metschn.) Sorokin, and

Isaria fumosorosea Wize on pea plants (var. Gallant) under field conditions. Additionally, we assessed the performance of these fungi when tank-mixed prior to application with the resistance inducer potassium silicate (SilK), to test the hypothesis of a possible synergistic interaction between entomopathogenic fungi and silicate compounds, as previously reported in the literature (Akbar, Lord, Nechols & Howard, 2004; Gatarayiha, Laing & Miller, 2010; Anjum, Shakir & Ahmed, 2014; Shakir, Saeed, Anjum, Farid, Khan, Liaquat & Badshah, 2015).

Mechanistically, silicon-based inducers can reinforce physical barriers and prime plant defences against sap-sucking, virus-transmitting insects (Reynolds, Keeping & Meyer, 2009; Ulhoa, Boiça Júnior, Davis & Stout, 2026; Leroy et al., 2019), while, at the leaf surface, silicate deposition may improve conidial adhesion, moisture retention and thermotolerance (Bell & Hamalle, 1974; Kim et al., 2014). Under the sub-optimal humidity and temperature typical of the maize off-season, tank-mixing potassium silicate with entomopathogenic fungi could therefore partially offset abiotic constraints on conidial performance and increase infection of *D. maidis* adults. Accordingly, we tested two hypotheses: (H1) the entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *I. fumosorosea* infect *D. maidis* adults occurring on pea under field conditions, with infection differing among fungal species; and (H2) tank-mixing potassium silicate (SilK) with these fungi increases the proportion of infected adults relative to fungi applied alone. We further assessed whether these treatments affected pea yield. Our aim was to quantify field infection of *D. maidis* on pea treated as a candidate off-season host, not to establish pea as a confirmed reservoir of the pathogens this insect transmits. In a companion field study on sweet corn, binary mixtures of these fungi with potassium silicate improved the control efficiency of *D. maidis* relative to fungi applied alone, with *M. anisopliae* performing similarly to the chemical standard (Ribeiro et al., 2026); the present work extends that approach to pea, an alternative host relevant during the maize off-season.

2. Materials and Methods

2.1 Experimental site and pea variety

The trial was conducted at Fazenda Morro do Peão (17° 17′ 59″ S, 48° 16′ 46″ W, 758 m a.s.l.) in Pires do Rio, southeastern Goiás, Brazil. The mean temperature during the experimental period was 19 ± 4°C, with relative humidity of 55 ± 10%. The pea variety Gallant (Gallatin Valley Seed Company, Montana, USA) has a medium cycle (69 days), short stature (65 cm mean height), and smooth, green seeds with a sieve size of 3.5 mm. It produces eight to nine grains per inflated green pod, with axillary white flowers and 14 to 15 nodes per plant at first flowering. Plants of the Gallant variety are afila-type, i.e. with absent leaves and terminal leaflets converted into tendrils (Pires, 1997). Stipules are abundant, occurring in pairs at stem axils, protecting buds. The tendrils coil around each other, helping plants remain erect and preventing lodging. This variety is resistant to *Fusarium* spp. and *Erysiphe* sp. (powdery mildew) but has no resistance to viruses or insect pests.

2.2 Soil preparation, fertilisation, seed treatment, and planting

Subsoiling followed by a levelling disc harrow provided adequate soil aeration and drainage. A rotary hoe was used to break soil clods. Dolomitic limestone was incorporated into the soil at 5000 kg ha−1. Planting fertilisation consisted of 103 kg ha−1 N, 100 kg ha−1 P2O5, and 108 kg ha−1 K2O. Top-dressing fertilisation during the vegetative phase was performed twice per cycle (from the emission of the 4th and 8th nodes), consisting of 270 kg ha−1 of NPK (5-37-00), 180 kg ha−1 of KCl, and 200 kg ha−1 of urea. The irrigation depth throughout the pea cycle was 220 mm.

Seed treatment (ST) included inoculation with *Rhizobium leguminosarum* bv. *viciae*, cobalt (15.60 g L−1) and molybdenum (273 g L−1) at 200 mL per 100 kg of seeds, along with

the fungicides metalaxyl-M (acylalaninate) and fludioxonil (phenylpyrrole) and the insecticide thiamethoxam (neonicotinoid) at 150 mL and 300 mL per 100 kg of seeds, respectively. Planting was carried out with a wheat planter adapted for pea seeds, at a speed of 5–6 km h−1, seed depth of 4 cm, and approximately 20 seeds per linear metre. The estimated plant population was 100,000 plants ha−1.

2.3 Experimental design and treatments

The experiment followed a randomised complete block design (RCBD) with four blocks (replicates) and eight treatments, the eight treatments being randomly and independently allocated to plots within each block. Here, a replicate refers to a complete block, whereas the samplings taken over time on each plot (see Section 2.4) constitute repeated measures rather than additional replicates. Each experimental plot contained 2000 plants and measured 6 m × 5 m (30 m2), with 5 cm × 30 cm spacing in single rows. The distance between experimental plots and between blocks was 10 m, intended to limit conidial drift and adult movement between plots; nonetheless, because the corn leafhopper is highly mobile and sampling relied on sticky traps, complete independence among plots cannot be fully guaranteed (see Discussion). A representative (schematic) diagram of the experimental layout is provided as Supporting Information (Figure S1), and the trial coordinates (Section 2.1) are reported to support reproducibility.

Treatments were arranged in a (3×2)+2 factorial design, with three levels of the Fungi factor (*B. bassiana*, Bb; *M. anisopliae*, Ma; and *I. fumosorosea*, Isa), two levels of the SilK factor (with and without potassium silicate), plus two additional treatments: an absolute control (water only) and a positive control (farm standard, FS), the latter consisting of one application of profenofos+lufenuron following the farm's conventional management programme. The

175 treatments were: (T1) Bb, (T2) Ma, (T3) Isa, (T4) Bb+SilK, (T5) Ma+SilK, (T6) Isa+SilK,
176 (T7) water (absolute control), and (T8) profenofos+lufenuron (positive control).
177 Boveril® Evo (Koppert do Brasil Holding Ltda, Piracicaba, SP, Brazil) (MAPA
178 registration no. 17520) with the isolate *B. bassiana* PL 63 (2 × 109 viable conidia g−1) was the
179 Bb source, applied at 1.2 kg product ha−1 in 200 L ha−1 spray volume. Metarril® WP E9
180 (Koppert do Brasil Holding Ltda) (MAPA registration no. 06605) with the isolate *M. anisopliae*
181 strain E9 (1.39 × 108 viable conidia g−1) was the Ma source, applied at 750 g ha−1 in 250 L
182 ha−1. Octane® (Koppert do Brasil Holding Ltda) (MAPA registration no. 30917) with the
183 isolate *I. fumosorosea* strain ESALQ-1296 (2.5 × 109 viable conidia mL−1) was the Isa source,
184 applied at 0.8 L product ha−1 in 250 L ha−1.
185 The SilK product was Silício Foliar (Solo Fértil SP Comercial Agrícola Ltda, São José
186 do Rio Preto, SP, Brazil) (MAPA registration no. 0944610000-9), containing 12% silicon (Si)
187 and 15% potassium (K2O), applied at 0.4 L K2SiO3 per 100 L−1 of water. The positive control
188 consisted of a factory-mix of profenofos (500 g L−1, organophosphate) and lufenuron (419.5
189 g L−1, benzoylurea) applied at 800 mL ha−1 in 150 L ha−1 spray volume.
190 All treatments were applied as foliar sprays using a pressurised CO2 sprayer (2 L) with a 3-m
191 lateral boom equipped with six conical spray nozzles (M 054) at 30 psi working pressure.
192 Sprays directed at the upper third of pea plants were performed once (at 0 DAA), after 17:00
193 h, at the beginning of the reproductive phase (40 days after germination).
194
195 **Regulatory compliance and product declaration.**
196 All commercial products used were registered with the Brazilian Ministry of Agriculture and
197 Livestock (MAPA); their registration numbers are given above, and all were applied at label-
198 recommended rates. The seed-treatment insecticide thiamethoxam and the
199 profenofos+lufenuron farm-standard treatment were transported, handled and applied

following the manufacturers' safety instructions and Brazilian pesticide legislation (Federal Law No. 7,802/1989 and Decree No. 4,074/2002). The trial was carried out on a commercial farm with the owner's authorisation, and no protected or endangered species were involved.

2.4 Leafhopper sampling

For comparative purposes, leafhopper populations were also estimated in an adjacent sweet corn field (50,000 plants ha^{-1}) located at the same farm, using the same sampling methodology described below. Yellow sticky traps (15 cm × 10 cm) (BioControle – Métodos de Controle de Pragas Ltda, Indaiatuba, SP, Brazil), recommended for monitoring *D. maidis* (Castro et al., 1992), were used for sampling. Each experimental unit had one sticky trap placed immediately above the upper third of pea plants, with height periodically adjusted. Traps were replaced every three days. A total of 160 sticky trap samples were collected during the trial (0, 3, 6, 9, and 12 DAA). Traps installed at 0 DAA had been placed three days before treatment application.

2.5 Parameters quantified and statistical analysis

After collection, traps were maintained individually in sealed plastic containers (30 × 20 × 14 cm) with moistened paper towels for 72 h to allow previously contaminated leafhoppers to continue the infection process, enabling visual identification of fungal infection. Leafhoppers were counted under a stereomicroscope at 100× magnification and classified as infected or non-infected. Because adults were captured alive on the sticky traps and only then incubated, the 'infected' category reflects post-capture fungal sporulation on exposed adults; it is an indirect proxy for field infection and does not, by itself, establish fungal-induced mortality of the vector in the field. To confirm fungal species identity and verify no cross-contamination

between plots, samples of infected leafhoppers were sent to the Phytopathology Laboratory of IF Goiano (Urutaí, Goiás, Brazil)

Data for leafhopper counts (infected and non-infected) were assessed using box plots for outlier identification. Normality was verified using the Lilliefors test and complementarily by histogram (Ribeiro Junior & Melo, 2009). Data for leafhopper counts in the two controls did not follow a normal distribution and were transformed ($\sqrt{x+1}$). Various polynomial regression models were compared visually through plotting of observed and estimated data, and cross-validated using the holdout method to check for overfitting (Hawkins, 2004).

The significance of the isolated factors Fungi and SilK and their interaction was assessed through factorial ANOVA with additional treatments (3×2+2), comparing them with the controls, both for non-infected (Table 1) and infected leafhoppers (Table 2). Significant effects were explored through Tukey's mean comparison test at 5% probability.

Pea yield (kg ha−1) was estimated for each plot by harvesting all plants within a 1 m2 quadrat placed at the centre of each plot. Fresh pea seeds were counted and weighed. Yield comparisons among treatments were performed using ANOVA followed by Tukey's test at 5% probability. Because leafhoppers were sampled repeatedly on the same plots over time (0–12 DAA), temporal dynamics were described by regression over DAA (Figures 1–2), whereas treatment effects were tested on per-plot values summarised across the sampling period using the factorial ANOVA described above; a formal repeated-measures model was not fitted, which we acknowledge as a limitation. All analyses and figures were produced in SigmaPlot®, version 12.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

For contextual comparison, the estimated mean number of leafhoppers per sweet corn plant in the adjacent field (3.78 ± 0.60) was approximately two-fold higher than those estimated on pea

plants (1.89 ± 0.30) (F = 45.89, *P* = 0.02) (Figure 1A). The distribution of estimated leafhopper numbers over time followed a normal distribution for both sweet corn and pea, with a population peak at 6 DAA and lower values before (0 and 3 DAA) and after the peak (9 and 12 DAA) (Figure 1B).

Quadratic polynomial regression models best fitted the distribution of leafhopper numbers sampled on pea plants over time (Figure 2A), with distinct models for non-infected (y = 17,182.06 + 444.43x – 26.06x2, R2 = 0.83, F = 10.84, *P* = 0.04) and infected leafhoppers (y = 800.00 + 1,821.70x – 159.94x2, R2 = 0.90, F = 14.77, *P* = 0.03). A population peak at 6 DAA was observed for both models. Non-infected leafhoppers were, on average, 5.96 times more abundant than infected individuals (Figure 2A). Across all sampling intervals, treatments, and blocks, a total of 108,320 leafhoppers were collected, of which 85.63% were non-infected and 14.28% were infected by fungi (Figure 2B). It should be noted that this overall percentage includes leafhoppers from all treatments, including the two controls where no fungi were applied.

The isolated factors Fungi and SilK were significant for the number of non-infected leafhoppers (Table 1). The interaction between the factorial arrangement and the controls was also significant, while the Fungi × SilK interaction was not (Table 1). Treatments Bb and Ma had the highest number of non-infected leafhoppers, compared with Isa (F = 53.68, *P* = 0.04) (Figure 3A). For the SilK factor alone, the highest number of non-infected leafhoppers was sampled in the absence of SilK (F = 78.66, *P* = 0.04) (Figure 3B). Regarding the factorial vs. controls interaction, the lowest non-infected leafhopper counts were from Bb+SilK, Ma+SilK, Isa alone, and Isa+SilK treatments (Figure 4A). Treatments Bb and Ma without SilK resulted in leafhopper counts similar to the farm standard control, while the absolute control (water) had the highest counts (F = 112.31, *P* = 0.02) (Figure 4A).

For infected leafhoppers, the isolated factors Fungi and SilK were also significant (Table 2). The number of infected leafhoppers was higher when SilK was included in the spray mixture compared with its absence (Figure 5A). The Fungi × SilK interaction was also significant, unlike the comparison between controls (Table 2). The factorial vs. controls interaction was significant. The treatments with the highest number of infected leafhoppers were Bb+SilK, Ma+SilK, and Isa+SilK (Figure 4B). Intermediate values were observed for the isolated fungi (Bb, Ma, and Isa), with the lowest capture values in both controls, which did not differ from each other (Figure 5B).

Pea yield differed significantly among treatments ($F = 82.31$, $P = 0.03$) (Figure 6). The highest yields, above 7000 kg ha−1, were from Bb+SilK, Ma+SilK, Isa+SilK, and the farm standard treatments. Intermediate yields were obtained from the three isolated fungi treatments. The lowest yield (5780 kg ha−1) was from the absolute control (water only).

4. Discussion

Our results indicate that the pre-application mixture of SilK with entomopathogenic fungi increased the infection capacity of these entomopathogens on *D. maidis* adults in a pea crop under field conditions, even at low temperatures and relative humidity that are unfavourable for entomopathogens (Bouamama, Vidal & Fargues, 2010; Hallsworth & Magan, 1999). These inappropriate abiotic factors remain a major bottleneck for the use of entomopathogenic fungi under field conditions (Vega, 2018). We emphasise that conidia sporulation (a clear visual sign of fungal infection) on this host insect was low, with only 14.28% of the 108,320 individuals sampled during the entire experimental period showing infection. Given that chemical control of this insect has only a seven-day residual period (Albuquerque et al., 2006), the integration of chemical and microbiological methods within an IPM framework could promote sustainability in sweet corn agribusiness and help reduce this insect in uncommon hosts such

298 as pea. It is important to emphasise that entomopathogenic fungi, given their modest infection
299 rates under the conditions tested, should be considered as a complementary tool within an IPM
300 programme rather than as a standalone replacement for chemical control.

301 The main question of this study relates to the presence, in large numbers, of the corn
302 leafhopper on pea plants (Fabaceae), which is not its preferential host. The high nitrogen
303 fertilisation recommended for pea production may have contributed to maintaining high
304 leafhopper populations, as suggested by Power (1989). The accumulation of nitrogen and free
305 amino acids, inhibiting protein synthesis, is a recognised factor supporting high populations of
306 sap-sucking, pathogen-transmitting insects in the field (Chen et al., 2010).

307 The corn leafhopper employs various survival strategies in the absence of its preferred
308 hosts, including feeding on alternative plants and migration (Waquil et al., 1999). The large
309 number of leafhoppers found on pea in our study suggests that this insect is capable of at least
310 partially sustaining its biological cycle on this legume. Similarly, *D. maidis* populations have
311 been sampled on industrial tomato (cv. Heinz 9553) in areas with successive tomato–sweet
312 corn cropping (Silva et al., 2018) and on common bean in Costa Rica (Castro et al., 1992).

313 Our seed treatment in pea used thiamethoxam, which is recommended for corn
314 leafhopper control. However, this ST has efficacy of up to 80% with a residual period of one
315 week (Albuquerque et al., 2006). Since our treatment applications were made near the
316 reproductive stage (40 days after germination), the ST used may have been irrelevant for *D.*
317 *maidis* control. We note that no symptoms of phytoplasma or spiroplasma, or any visually
318 observable anomaly, were found in pea plants during the study.

319 The population peak at 6 DAA, with a clear normal distribution, resulted from both
320 non-infected and infected leafhoppers. For non-infected leafhoppers, we believe that natural
321 re-infestations occurred, which is common for this insect under favourable climate conditions,
322 generating normal distributions with population peaks as shown in other studies (Ávila & Arce,

2008; Oliveira, Lopes & Nault, 2013). For infected leafhoppers, the increase at 6 DAA likely reflected the time required for fungal infectivity and sporulation, which ranges from five to 10 days post-application (Ibarra-Aparicio, Moya-Raygoza & Berlanga-Padilla, 2005; Toledo, Lenicov & Lastra, 2007).

The high proportion of non-infected leafhoppers (85.63%) implies that factors inherent to pea plant architecture and climatic conditions (unfavourable to fungi) may have contributed to the low infections, regardless of the entomopathogenic species evaluated. Pea plants have modified leaves with terminal leaflets converted into tendrils, which have much smaller width and thus reduced leaf area for soil coverage. This may have facilitated greater UV radiation penetration, increased evaporation, and consequently reduced moisture retention throughout the lower, middle, and upper thirds of the plant. Fungal conidiogenesis is dependent on relative humidity and declines with greater UV exposure (Vega, 2018), although it also varies with fungal isolate, species, host phase, and time (Sosa-Gómez & Alves, 2000). Despite this complexity, the climatic conditions of our trial (19 ± 4°C and 55 ± 10% RH) were likely more favourable for pea cultivation (Nascimento et al., 2016) than for the entomopathogens, particularly those applied without SilK. We also note that the number of infected leafhoppers, identified by full mycelial growth on cadavers, may have been underestimated, as not all individuals killed by fungi display visible external mycelial growth (Toledo et al., 2007). The insect cuticle is the first barrier to fungal infection (Ortiz-Urquiza & Keyhani, 2013), and in *D. maidis* this barrier is reinforced by a protein–lipid brochosome coat and self-cleaning behaviour that reduce conidial adhesion and germination, keeping adult mortality low—below 35% within seven days—even under direct conidial application (Lopes, Faria & Oliveira, 2024). This vector-side defence offers a mechanistic explanation for the high proportion of non-infected adults recorded here and reframes the modest infection rates as an intrinsic property of the *D. maidis*–fungus interaction rather than a methodological shortcoming.

348 The significance of the isolated Fungi factor confirmed that the evaluated species differed in
349 their field efficacy. A greater number of non-infected leafhoppers in Bb and Ma treatments
350 compared with Isa indicated the higher infectivity capacity of *I. fumosorosea*, which is the only
351 one registered by MAPA (Brazilian Ministry of Agriculture) for use against this target insect.
352 However, several studies suggest control prospects for *D. maidis* using Bb (Toledo et
353 al., 2007) and Ma (Moya-Raygoza, Chacón-Torres, Palomera-Avalos, Becerra-Chiron &
354 Bernal, 2008). This comparison among fungi is complex, as other factors that may modify
355 fungal efficacy, such as the protective capacity of the insect cuticle in terms of antagonistic
356 bacteria, were not adequately evaluated. Toledo, Alippi and Lenicov (2011) reported that 91 of
357 155 bacteria present on the cuticle of *D. maidis* exhibited inhibitory effects against *B. bassiana*.
358 We also demonstrated that SilK has relevance in the fungus–host relationship
359 investigated here. When considering the significance of the isolated SilK factor, its absence
360 was determinant for higher numbers of non-infected leafhoppers. Studies have confirmed the
361 importance of silicate products as inducers of physical plant resistance, predominantly against
362 sap-sucking and virus-transmitting insects (Leroy, Tombeur, Walgra, Cornélis & Verheggen,
363 2019).
364 The significant interaction between the factorial arrangement and the controls indicated
365 that plots treated with entomopathogenic fungi under field conditions against *D. maidis* on pea
366 were associated with fewer non-infected adults and higher infection scores than the
367 profenofos+lufenuron farm-standard treatment; because sticky-trap captures reflect adult
368 activity rather than absolute mortality, this is a relative, trap-based comparison rather than a
369 direct ranking of control efficacy. This broadens the opportunities for adopting an IPM
370 programme in both sweet corn and pea. The significant Fungi × SilK interaction for infected
371 leafhoppers (Table 2), although not significant for non-infected leafhoppers (Table 1), suggests
372 that SilK may enhance the infectivity of certain fungal species under field conditions. This

373 indicates that SilK may serve as an abiotic resistance inducer for conidia, given that our
374 environmental conditions were unfavourable to the entomopathogens. The use of silicate
375 compounds associated with entomopathogenic fungi for insect and mite control has been
376 reported as effective (Akbar et al., 2004; Gatarayiha et al., 2010; Anjum et al., 2014; Shakir et
377 al., 2015). Furthermore, silicate compounds may confer greater thermotolerance to fungal
378 conidia. Bell and Hamalle (1974) reported that spores of *B. bassiana*, *M. anisopliae*, and
379 *Spicaria rileyi* maintained their virulence after three years stored at −20°C in tubes containing
380 silica gel crystals. Kim, Lee and Lee (2014) also confirmed greater silicate-mediated
381 thermotolerance through moisture retention for *I. fumosorosea*.

382 The variation in pea yields reported for Brazil ranges from 3000 to 13,000 kg ha−1
383 depending on type and use (Nascimento et al., 2016). The mean Brazilian yield for green peas
384 was recently estimated at 3590 kg ha−1 (IBGE, 2021), which confirms that the Gallant variety
385 responds with more than double the national average when appropriate investments in
386 biological nitrogen fixation, seed treatment, phytosanitary products, and fertilisation
387 (particularly nitrogen) are made.

388 The tank mixtures of fungi with SilK resulted in the highest pea yields compared with
389 isolated fungi treatments. *Beauveria bassiana* through endophytic association has been shown
390 to increase plant height, leaf number, and dry mass in cowpea (*Vigna unguiculata* L., Fabaceae)
391 (Pachoute, Nascimento & Souza, 2021). *Metarhizium anisopliae* (Clifton, Jaronski, Coates,
392 Hodgson & Gassman, 2018) and *I. fumosorosea* (Dash et al., 2018) have also been
393 endophytically associated with Fabaceae, enhancing growth and yield parameters while
394 regulating pest insect attack. Although in our study the fungi were applied as foliar sprays
395 rather than as endophytic inoculants, foliar applications can also lead to endophytic
396 colonisation (Vega, 2018), which may partially explain the observed yield increases. Plant
397 growth hormone regulation (auxins, gibberellins, cytokinins, and ethylene) or improved soil

nutrient uptake may also contribute to this yield increase (Berg, 2009). Consistent with this, silicon supply has been shown to improve legume growth and development under field conditions (Ulhoa, Boiça Júnior, Davis & Stout, 2025). Additionally, SilK may have had positive effects, as Ismail, Soliman, El-Aziz and Abdel-Aziz (2022) reported that SiO2 application significantly increased vegetative growth, relative water content, and antioxidant defence in pea plants. We hypothesise that the combined effect of fungi (potentially through plant hormone regulation) and SilK (potentially through improved water relations) may have contributed to the higher yields observed in binary mixture treatments. However, the specific mechanisms underlying this yield increase were not directly evaluated in the present study and warrant further investigation.

Several methodological limitations of this study should be acknowledged. First, our assessment of fungal infection was based on visible mycelial growth on leafhoppers captured by sticky traps, which is an indirect measure of efficacy. Individuals that died from fungal infection before reaching the traps were not accounted for, potentially underestimating the true infection rate. Second, although fungal species identity was confirmed by laboratory analysis and the 10-m distance between plots was designed to minimise cross-contamination, we cannot entirely rule out the possibility that some infections resulted from naturally occurring entomopathogens rather than from the applied treatments. Third, the comparison of leafhopper densities between pea and sweet corn was based on an adjacent field without the same experimental design, and should therefore be interpreted as descriptive context rather than a formal statistical comparison. Fourth, the overall infection rate of 14.28% is modest compared with chemical control efficacy, reinforcing that entomopathogenic fungi should be viewed as a complementary tool within an IPM framework rather than as a standalone alternative to insecticides. Finally, the mechanisms underlying the observed yield increases in fungus+SilK treatments were not directly investigated; whether they resulted from reduced pest pressure,

endophytic colonisation, silicon-mediated plant defence, or a combination of these factors remains to be elucidated in future studies.

5. Conclusions

The pre-application mixture of potassium silicate (SilK) with entomopathogenic fungi increased the infection capacity of these entomopathogens on *D. maidis* adults in pea under field conditions. Of the sampled leafhoppers, 85.63% showed no fungal infection, suggesting that pea plant architecture and unfavourable climatic conditions contributed to low infection rates. Among the evaluated fungi, *I. fumosorosea* showed higher infectivity than *B. bassiana* or *M. anisopliae*. The resistance inducer SilK played a significant role in the fungus–host relationship, as its absence was determinant for higher numbers of non-infected leafhoppers.

This management approach using binary mixtures of fungi and SilK also increased pea yield. Because infection was scored on trap-captured adults rather than by direct mortality assays, these findings identify pea as a candidate off-season host on which *D. maidis* can be infected by entomopathogenic fungi; confirming their contribution to vector management will require maize-focused trials that measure vector mortality and pathogen transmission directly.

Author Contributions

Dalvan dos Santos: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. João Batista Coelho Sobrinho: Investigation. Yamile Salvatierra Nuñez: Investigation. Luciana Barboza Silva: Supervision, Writing – review & editing. Lucas Adjuto Ulhoa: Methodology, Investigation, Visualization, Writing – review & editing. Jardel Lopes Pereira: Conceptualization, Supervision, Funding acquisition. Alexandre Igor Azevedo Pereira: Conceptualization, Methodology, Supervision, Funding acquisition, Project administration, Writing – review & editing.

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Competing Interests

The authors declare no competing interests.

Ethics approval

This study did not involve human participants or vertebrate animals; therefore, no ethics approval was required. All field procedures complied with Brazilian legislation on the registration and use of agricultural inputs.

Clinical trial number: not applicable

Not applicable.

Data Availability

The data supporting the findings of this study are available in Zenodo at https://doi.org/10.5281/zenodo.19618316

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Tables

Table 1. Factorial ANOVA (3×2+2) with three levels of the Fungi factor (*Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*), two levels of the SilK factor (with and without potassium silicate), plus two controls: absolute control (water) and positive control (farm standard, FS, with profenofos+lufenuron) for the number of non-infected leafhoppers on pea.

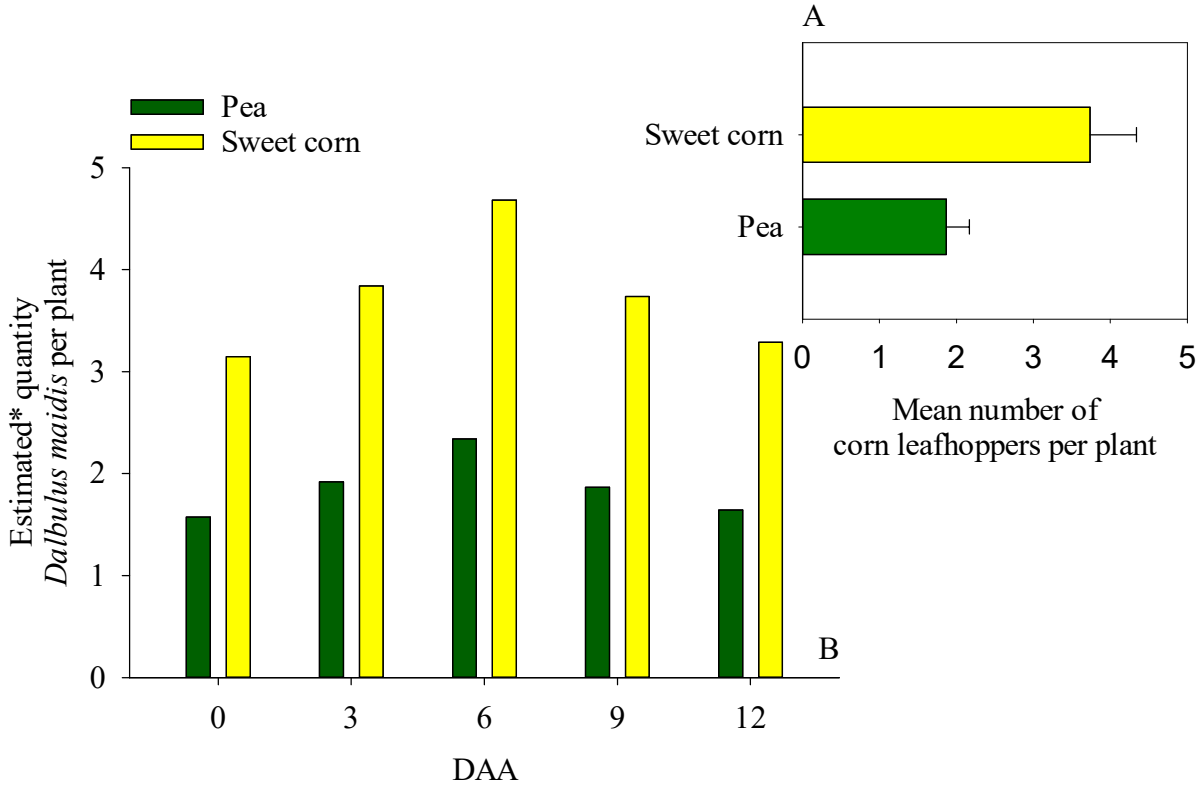
Source of Variation	df	SS	MS	*F*	F_{tab} (0.05)
Blocks	3	4203.99	1401.33	0.696	
Treatments	(7)	(326333.8)	46619.11	23.170	
Fungi	2	48585.38	24292.69	11.768	0.00054
SilK	1	90452.72	90452.72	43.816	0.00000
Fungi × SilK	2	10389.54	5194.77	2.516	0.10873
Controls	1	79640.41	79640.41	95.353	0.00007
Factorial vs. Controls	1	103674.60	103674.60	11.557	0.00193

Residual	21	42253.37	2012.07	
Total	31	278087.05		

Table 2. Factorial ANOVA (3×2+2) with three levels of the Fungi factor (*Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*), two levels of the SilK factor (with and without potassium silicate), plus two controls: absolute control (water) and positive control (farm standard, FS, with profenofos+lufenuron) for the number of infected leafhoppers on pea.

Source of Variation	df	SS	MS	*F*	F_{tab} (0.05)
Blocks	3	3797.12	1265.71	1.166	
Treatments	(7)	(52051.58)	7435.94	6.852	
Fungi	2	1912.95	956.47	8.652	0.02358
SilK	1	5474.10	5474.10	7.730	0.03936
Fungi × SilK	2	2963.21	1481.61	7.009	0.03415
Controls	1	426.32	426.32	2.060	0.20119
Factorial vs. Controls	1	39650.01	39650.01	30.509	0.00000
Residual	21	22789.69	1085.22		
Total	31	278087.05			

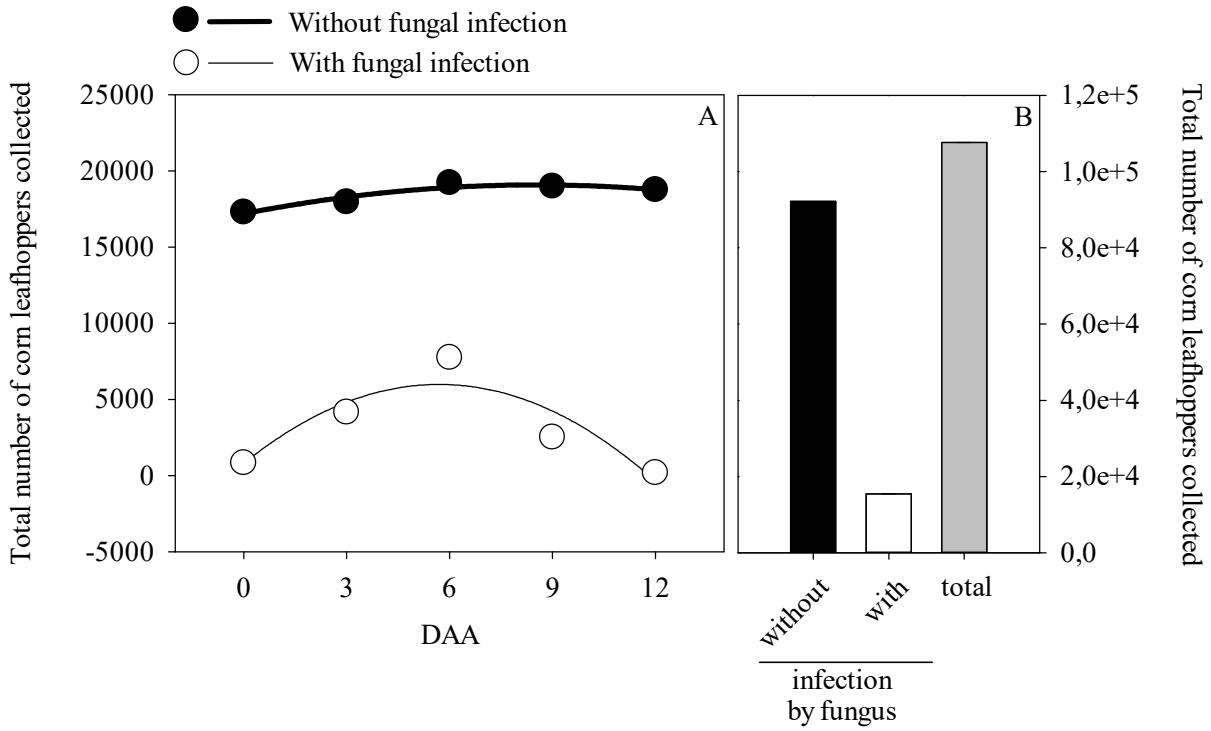
Figures



647

648 **Fig. 1** Estimated number (mean ± SE) of leafhoppers, *Dalbulus maidis* (Hemiptera:
649 Cicadellidae), per pea plant (100,000 plants ha−1) and sweet corn plant (50,000 plants ha−1),
650 considering all treatments and sampling intervals (0, 3, 6, 9, and 12 days after application,
651 DAA) (A), and total leafhoppers per plant considering all treatments over DAA (B).

652

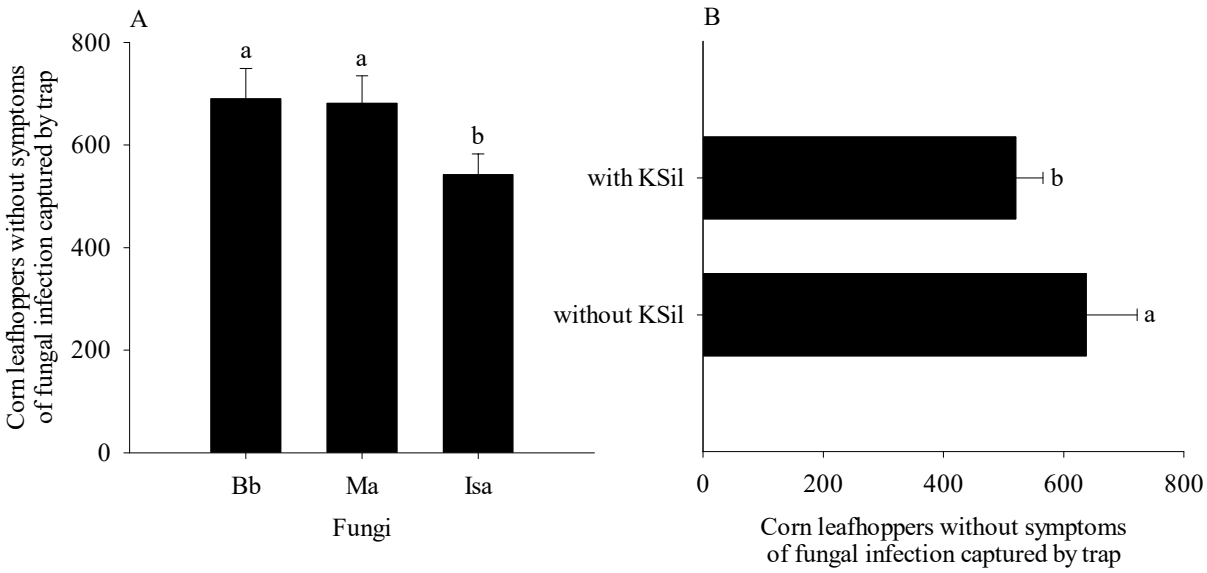


653

654 **Fig. 2** Regression analysis for population fluctuation of leafhoppers, *Dalbulus maidis*
655 (Hemiptera: Cicadellidae), without (or with) fungal infection symptoms, collected on pea by
656 yellow sticky traps during the experimental period (0, 3, 6, 9, and 12 DAA) (A). Total number
657 of leafhoppers (non-infected or infected) and overall total (non-infected + infected) throughout
658 the trial (B).

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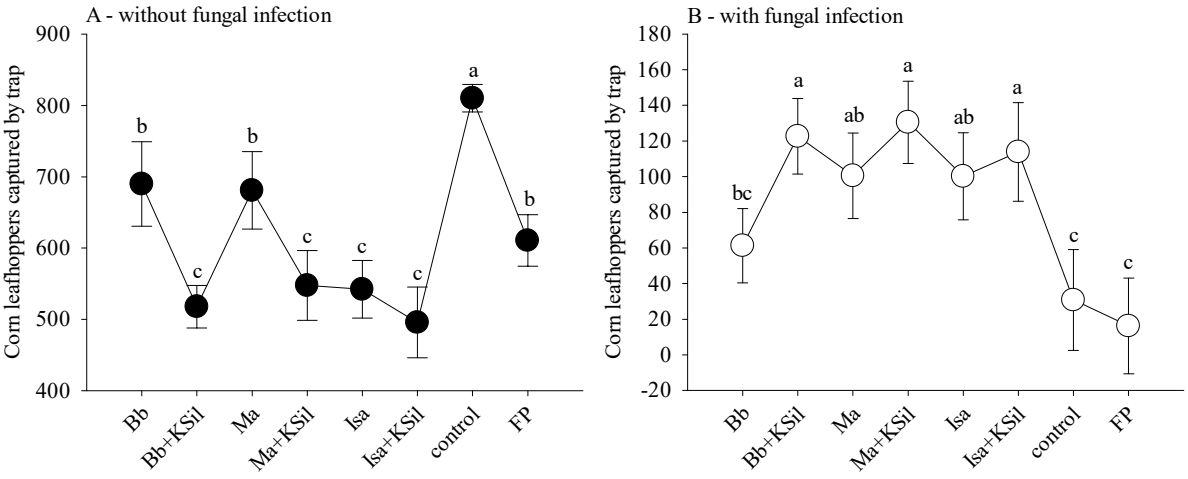
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661

662 **Fig. 3** Breakdown of the Fungi (A) and SilK (B) factors found to be significant in Table 1 for
663 the number (mean ± SE) of non-infected leafhoppers captured on pea as a function of fungal
664 species: Bb (*Beauveria bassiana*), Ma (*Metarhizium anisopliae*), and Isa (*Isaria fumosorosea*)
665 (A), and as a function of the presence or absence of SilK (potassium silicate) in the spray
666 mixture (B). Means followed by the same letter do not differ at 5% probability by Tukey's test.

667

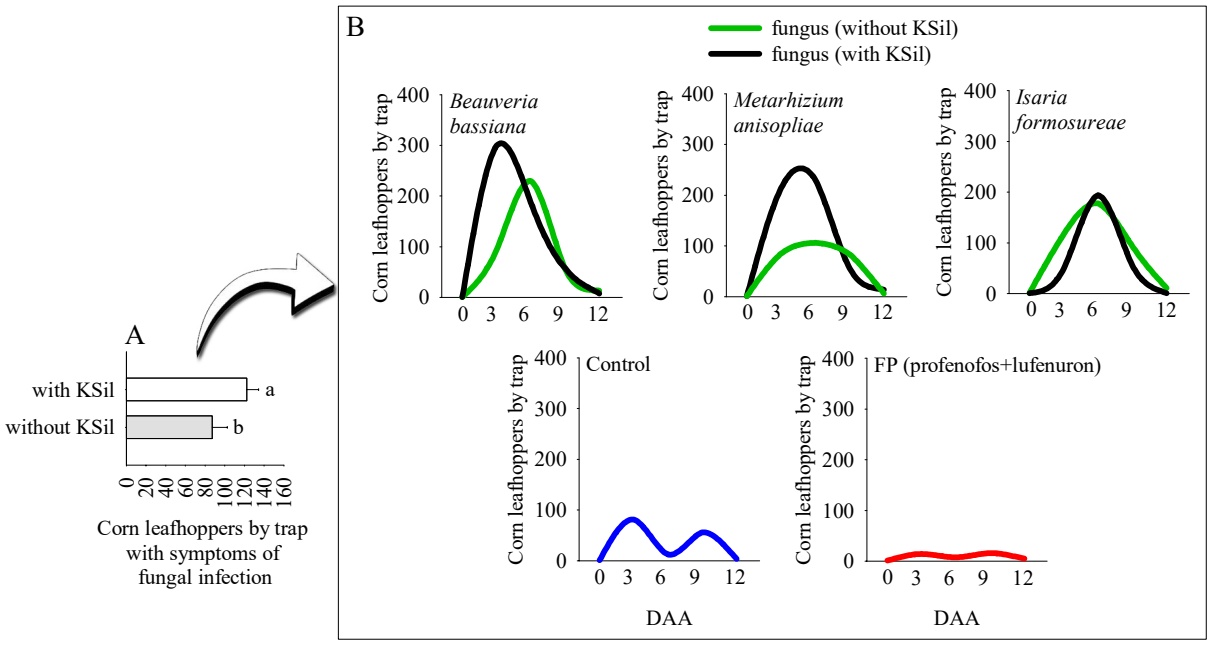


668

669 **Fig. 4** Breakdown of the Factorial vs. Controls source of variation found to be significant in
670 Tables 1 and 2 for the number (mean ± SE) of leafhoppers, *Dalbulus maidis* (Hemiptera:
671 Cicadellidae), without (A) and with (B) fungal infection symptoms captured on pea during the

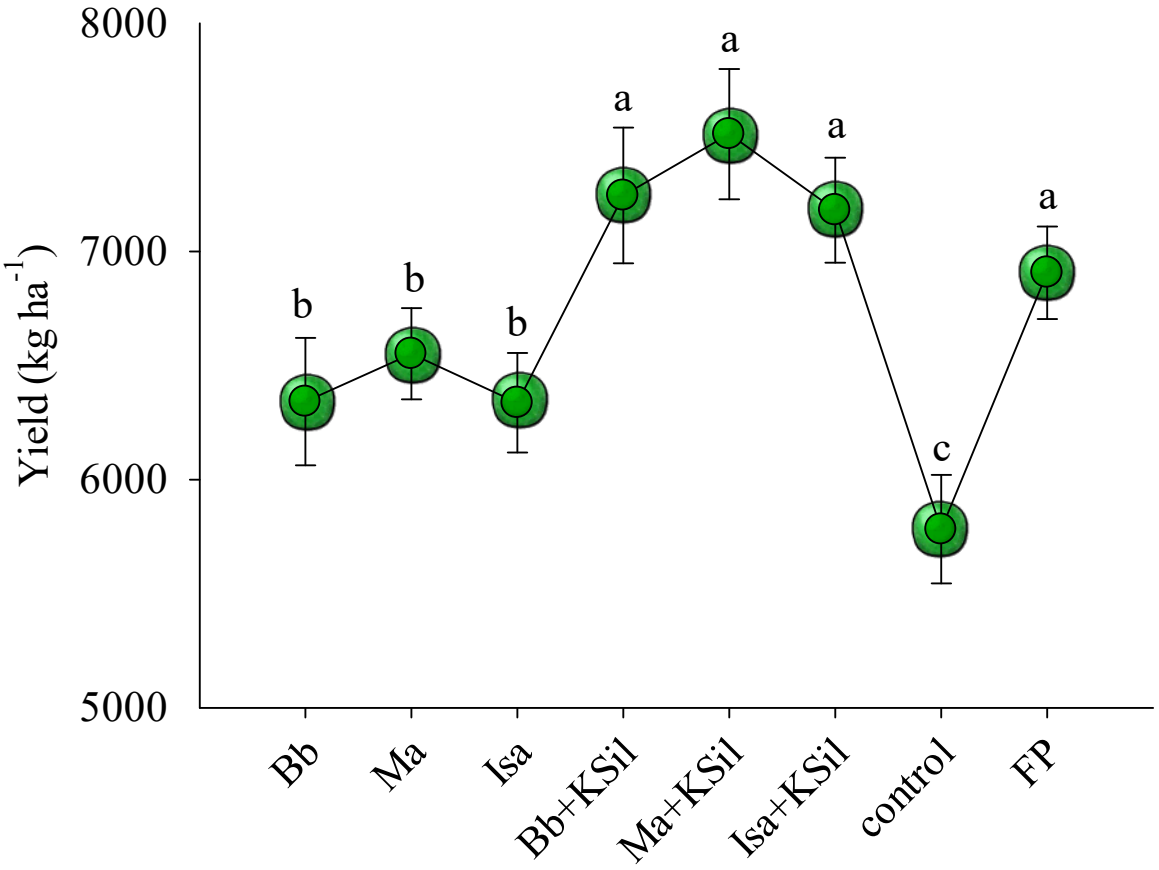
672 entire trial (mean of total sampled at 0, 3, 6, 9, and 12 DAA) as a function of treatments: Bb
673 (*Beauveria bassiana*), Bb+SilK, Ma (*Metarhizium anisopliae*), Ma+SilK, Isa (*Isaria*
674 *fumosorosea*), Isa+SilK, control (water only), and FS (farm standard, with
675 profenofos+lufenuron). Means followed by the same letter do not differ at 5% probability by
676 Tukey's test.

677



678

679 **Fig. 5** Breakdown of the SilK factor found to be significant in Table 2 for the number (mean ±
680 SE) of infected leafhoppers captured on pea with or without SilK (potassium silicate) in the
681 spray mixture (A). Number of leafhoppers captured (mean) as a function of treatments and
682 days after application (DAA) (B). Means followed by the same letter do not differ at 5%
683 probability by Tukey's test.



684

685 **Fig. 6** Yield (kg ha−1) (mean ± SE) of pea as a function of treatments: Bb (*Beauveria*

686 *bassiana*), Bb+SilK, Ma (*Metarhizium anisopliae*), Ma+SilK, Isa (*Isaria fumosorosea*),

687 Isa+SilK, control (water only), and FS (farm standard, with profenofos+lufenuron). Means

688 followed by the same letter do not differ at 5% probability by Tukey's test.

689

690 **Supplementary Material**

691 **Fig. S1** Representative (schematic) layout of the randomised complete block design (RCBD):

692 four blocks × eight treatments, each treatment randomised independently within each block.

693 Plots measured 6 m × 5 m (30 m²), with 10 m between plots and between blocks. Treatment

694 positions illustrate the randomisation scheme and do not represent the exact field allocation.

695 Treatments: T1, *Beauveria bassiana* (Bb); T2, *Metarhizium anisopliae* (Ma); T3, *Isaria*

696 *fumosorosea* (Isa); T4, Bb + potassium silicate (SilK); T5, Ma + SilK; T6, Isa + SilK; T7,

697 absolute control (water); T8, positive control (profenofos + lufenuron)

698

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1experimentaldesign.png