Enhancing *Dalbulus maidis* (Hemiptera: Cicadellidae) control: an integrated approach combining *Cordyceps javanica* (Ascomycota: Hypocreales) and insecticides

Thaís Berganton Poletto

Dissertation presented to obtain the degree of Master in Science. Area: Entomology

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Thaís Berganton Poletto
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DEDICATION

For my late grandparents, Sueli and Illari Berganton, Angelina and Pedro Poletto, and great grandparents, Urania and Quintilio Berganton, with all my heart.

"You will be everywhere my eyes land. Wherever my heart is, yours will keep beating."

Valerie Perrin
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"You are capable of more than you know. Choose a goal that feels right for you and strive to be the best, no matter how challenging the path. Aim high. Behave honorably. Be prepared to stand alone at times and endure failure. Persist! The world needs everything you can give."
Edward O. Wilson
APPENDICES................................................................................................................... 83
RESUMO

Aprimorando o controle de *Dalbulus maidis* (Hemiptera: Cicadellidae): uma abordagem integrada combinando *Cordyceps javanica* (Ascomycota: Hypocreales) e inseticidas

A cigarrinha-do-milho, *Dalbulus maidis*, destaca-se como uma praga importante no cultivo de milho, afetando a planta pela extração de seiva, injeção de saliva tóxica e, sobretudo, na transmissão de agentes patogênicos como enfezamento pálido (CSS), enfezamento vermelho (MBSP), vírus rayado fino (MRFV). O controle dessa praga tem sido realizado pela aplicação conjunta de agentes químicos e microbiológicos mas há pouca informação sobre as vantagens desta estratégia em comparação com tratamentos isolados. Nesse contexto, objetivou-se com esse trabalho avaliar a virulência de conídios aéreos, blastosporos e conídios submersos de *Cordyceps javanica* (ESALQ 1296) contra *D. maidis*, analisar a compatibilidade biológica desse fungo com os principais inseticidas para a cigarrinha-do-milho, investigar a eficácia da combinação de conídios aéreos com inseticidas e propor uma abordagem estratégica para o manejo de *D. maidis*. Para alcançar tais objetivos, realizaram-se ensaios de virulência com adultos da cigarrinha-do-milho, além de testes in vitro para avaliar a germinação e crescimento após a exposição de *C. javanica* a inseticidas. E por fim, foram conduzidos testes de compatibilidade por meio da aplicação das misturas nos insetos. Os blastosporos foram os mais eficazes no controle da cigarrinha-do-milho, exibindo taxas de mortalidade mais elevadas e um LT50 mais curto em comparação com outros propágulos. O isolado de *C. javanica* apresentou resistência aos inseticidas testados em misturas de calda. Adicionalmente, a inclusão de Silwet L-77 potencializou a eficácia de alguns inseticidas, revelando ser esse um componente útil em uma estratégia de Manejo Integrado de Pragas (MIP), juntamente com etiprole. Além disso, a combinação de *C. javanica* com etiprole e metomil exibiu um efeito sinérgico e aditivo, aumentando significativamente a mortalidade de *D. maidis*. Essas descobertas irão auxiliar no desenvolvimento de estratégias integradas no manejo de *D. maidis*, levando em consideração a eficácia de agentes microbiológicos e sua interação com inseticidas.

Palavras-chave: Fungo entomopatogênico, Compatibilidade químico e biológico, Manejo integrado de pragas do milho, Mistura de calda
ABSTRACT

Enhancing Dalbulus maidis (Hemiptera: Cicadellidae) control: an integrated approach combining Cordyceps javanica (Ascomycota: Hypocreales) and insecticides

The corn leafhopper, Dalbulus maidis, stands out as a critical maize pest, damaging the plant by sap extraction, toxic saliva injection, and, above all, transmission of pathogenic agents such as corn stunt spiroplasma (CSS), maize bushy stunt phytoplasma (MBSP), and maize rayado fino virus (MRFV). Current strategies for controlling this pest involve the application of chemical and microbial agents but there is a lack of information on the advantages of this strategy compared to each treatment alone. In this context, this project aimed to assess the virulence of aerial conidia, blastospores, and submerged conidia of Cordyceps javanica (ESALQ 1296) against D. maidis, analyze the biological compatibility of this fungus with key insecticides for the corn leafhopper, investigate the effectiveness of combining aerial conidia with insecticides, and propose a strategic approach for D. maidis management. To achieve these objectives, virulence assays were conducted with adult corn leafhoppers, along with in vitro tests to evaluate the germination and growth after the exposure of C. javanica to insecticides. Finally, compatibility tests were performed through insect application. Blastospores proved to be the most effective in controlling the corn leafhopper, exhibiting higher mortality rates and a shorter LT_{50} than other propagules. The C. javanica isolate demonstrated resistance to tested insecticides in spray mixtures. The inclusion of Silwet L-77 enhanced the effectiveness of some insecticides, making it a useful component along with ethiprole. Furthermore, the combination of C. javanica with ethiprole and methomyl exhibited a synergistic and additive effect, significantly increasing D. maidis mortality. These findings provide valuable insights for developing integrated strategies in D. maidis management, considering the effectiveness of microbiological agents and their interaction with insecticides.

Keywords: Entomopathogenic fungi, Chemical and biological compatibility, Maize integrated pest management, Tank mixture
LIST OF FIGURES

Figure 1. The life cycle of *Dalbulus maidis*, from mating behavior to adulthood Source: Waquil, 2004 and Oliveira; Frizzas, 2021 Edited by the author at BioRender, 2023

Figure 2. Visual symptoms resulting from diseases transmitted by *Dalbulus maidis* A) Corn stunt spiroplasma (CSS) B) Maize bushy stunt phytoplasma (MBS) C) Maize rayado fino virus (MRFV) D) Maize striated mosaic virus (MSMV) Source: Sabato et al., 2018; Vilanova et al., 2022. Edited by the author, 2023

Figure 3. A) Maize used for rearing *Dalbulus maidis* inside the cage B) Adults of *D. maidis* on maize leaves

Figure 4. A) *Cordyceps javanica* ESALQ 1296 sporulated Potato Dextrose Agar (PDA) plate B) *Beauveria bassiana* ESALQ 3366 sporulated PDA plate

Figure 5. Obtaining pure aerial conidial suspensions

Figure 6. A) Cages developed with a maize plant used for the bioassays B) Adults of *Dalbulus maidis* inside the cages after being transferred one day before the experiment of virulence

Figure 7. Procedure employed in the colony-forming units (CFU) assay

Figure 8. Procedure used in the viability assay

Figure 9. Adapted Potter tower to a constant-pressure compressor for spraying *Dalbulus maidis*

Figure 10. Submerged conidia yield grows in different modified culture media after five days of fermentation in flasks at 150 rpm at 10^6. Means (±SE) followed by different letters indicate significant differences according to the Tukey test (P < 0.05) Legend: M1 - Thomas et al. (1987), M2 Esther et al. (2013) with NS3, M3 - Esther et al. (2013) with NS2, M4 - Esther et al. (2013) modified with NS1

Figure 11. A-B) Submerged conidia observed at the beginning of conidiogenesis, 24 hours after inoculation C) Blastospores of *Cordyceps javanica* produced in modified Adamek medium (400x increase) D) Submerged conidia of *C. javanica* produced in modified Esther et al. (2013) medium (400x increase)
**Figure 12.** A) Proportion of surviving D. maidis during ten days after exposure to Octane (T2), pure aerial conidia (T3), blastospore (T4) and submerged conidia (T5) of Cordyceps javanica, and Beauveria bassiana isolate (T6) at the concentration of 1 x 10^7 spores mL^{-1}. B) Sporulated D. maidis along the images of the sporulated insects. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test (P < 0.05)………………….51

**Figure 13.** Propagules of *Cordyceps javanica* germinations (%) after exposure to 0.05% Silwet L-77 (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5) and imidacloprid + bifenthrin (T7), during 30 minutes A) Octane B) Pure aerial conidia C) Blastospores D) Submerged conidia. Means (±SE) followed by different letters indicate significant differences according to the Sidak test (P < 0.05)……….52

**Figure 14.** A) Formulated aerial conidia of *Cordyceps javanica* (Octane) germination (%) subjected to contact with 0.05% Silwet L-77 (T1), ethiprole (T2), methomyl (T3) and imidacloprid + bifenthrin (T4) over time B) Pure aerial conidia of *Cordyceps javanica* germination (%) subjected to contact with ethiprole, imidacloprid + bifenthrin and methomyl insecticides over time. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test (P < 0.05)……………………………………53

**Figure 15.** Number of colony-forming units (CFU) of propagules of *Cordyceps javanica* after exposure to 0.05% Silwet (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5), acetamiprid + bifenthrin (T6) and imidacloprid + bifenthrin (T7) A) Octane B) Pure aerial conidia C) Blastospores D) Submerged conidia. Means (±SE) followed by different letters indicate significant differences according to Tukey’s HSD test (P < 0.05)…………………………………………………………54

**Figure 16.** A) formulated conidia of *Cordyceps javanica* (Octane) number of colony-forming units (CFU) subjected to contact with 0.05% Silwet (T1) and insecticides ethiprole (T2), methomyl (T3) and imidacloprid + bifenthrin (T4) over time B) Pure aerial conidia of *Cordyceps javanica* number of colony-forming units (CFU) subjected to contact with ethiprole, methomyl, and imidacloprid + bifenthrin insecticides over time. Means (±SE) followed by different letters indicate significant differences according to Tukey test (P < 0.05)………………….55
**Figure 17.** A) Corrected mortality of *Dalbulus maidis*, after exposure to 0.05% Silwet (T1), Octane (T2), ½ dose ethiprole (T3), full dose ethiprole (T4), ½ dose ethiprole + Octane (T5), full dose ethiprole + Octane (T6), ½ dose methomyl (T7), full dose methomyl (T8), ½ dose methomyl + Octane (T9), full dose methomyl + Octane (T10), ½ dose imidacloprid + bifenthrin (T11), full dose imidacloprid + bifenthrin (T12), ½ dose imidacloprid + bifenthrin + Octane (T13), full dose imidacloprid + bifenthrin + Octane (T14) B) Sporulated *Dalbulus maidis* treated with the commercial product Octane, insecticides and combinations. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test of Sidak test (P < 0.05) C) Photos taken from sporulated *Dalbulus maidis*........................56

**Figure 18.** A) Corrected mortality of *Dalbulus maidis* after exposure to Octane (T2), ½ dose ethiprole (T3), full dose ethiprole (T4), ½ dose ethiprole + Octane (T5), full dose ethiprole + Octane (T6) with the solution prepared using distilled water (T1) B) Treatments with the solution prepared using 0.05% Silwet L-77 C) Corrected mortality of *Dalbulus maidis* after exposure to Octane (T2), ½ dose methomyl (T3), full dose methomyl (T4), ½ dose methomyl + Octane (T5), Full dose methomyl + Octane (T6) with the solution prepared using distilled water D) Treatments with the solution prepared using 0.05% Silwet L-77 Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test of Sidak test (P < 0.05)..............................................................57

**Figure 19.** A) Corrected mortality of non-methomyl-resistant (SUS) *Dalbulus maidis* after exposure to distilled water (T1), Octane (T2), ½ dose methomyl (T3), full dose methomyl (T4), ½ dose methomyl + Octane (T5) and full dose methomyl + Octane (T6) B) Methomyl resistant *Dalbulus maidis* after exposure to the same treatments..........................................................58
LIST OF ABBREVIATIONS AND ACRONYMS

Action threshold – AT
Corn stunt spiroplasma – CSS
Economic injury level – EIL
Entomopathogenic fungi – EF
Integrated pest management – IPM
Maize bushy stunt phytoplasma – MBSP
Maize rayado fino virus – MRFV
Maize striated mosaic virus – MSMV
Ministry of Agriculture, Livestock, and Supply – MAPA
Nitrogen source - NS
Potato Dextrose Agar – PDA
1 INTRODUCTION

Maize is the most widely cultivated and productive cereal worldwide, holding significant socioeconomic importance. At present, Brazil is the third-largest maize producer globally and boasts an extensive land area of 21,975.4 thousand hectares dedicated to maize cultivation, providing ample resources for diverse insect populations and diseases. Unfortunately, many of these insects are pests that cause substantial damage to this important crop. Corn leafhopper, *Dalbulus maidis* Delong & Wolcott (Hemiptera: Cicadellidae) has garnered significant attention in the last decade. This surge in interest is attributed to shifts in production systems, the expansion of cultivated areas, a prevalence of late plantings, the overlapping of plant cycles, and the discovery of a new virus transmitted by *D. maidis*. With a widespread presence across all Brazilian states, the corn leafhopper is responsible for inflicting considerable economic losses on the agricultural sector (Galvão et al., 2021; Oliveira; Frizzas, 2021; Vilanova et al., 2021; Conab, 2023).

Corn leafhopper is an insect from the Hemiptera order that pierces the plant to extract sap, injects toxic spittle, and, most importantly, transmits disease-causing agents. This insect has a wide distribution and high migration potential, being found in tropical regions such as Brazil, Mexico, Argentina, the United States, and other countries in Latin America. This insect vector transmits three main pathogens: the corn stunt spiroplasma (CSS), maize bushy stunt phytoplasma (MBSP), and maize rayado fino virus (MRFV). Currently, chemical control represents the primary tactic for managing this pest in Brazil (Triplehorn; Nault, 1985; Taylor et al., 1993; Oliveira et al., 2007; Virla et al., 2021; Neves et al., 2022). In addition to these conventional products, biological control using microbial agents like entomopathogenic fungi presents a sustainable alternative (Polanczyk; Alves, 2006).

The control of corn leafhopper using entomopathogenic fungi can be achieved through *Beauveria bassiana* (Hypocreales: Cordycipitaceae), *Metarhizium robertsii* (Hypocreales: Clavicipitaceae), and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), all of which have demonstrated proven efficacy in the literature (Ibarra Aparicio et al., 2005; Toledo et al., 2007; Silva, 2009; Iwanicki et al., 2021). Presently, approximately 68 microbial products are commercialized and registered at the Ministry of Agriculture, Livestock, and Supply (MAPA) for *D. maidis*. These products are based on aerial conidia and composed of *B. bassiana* (IBCB 66) and *Cordyceps fumosorosea*, and recently, the ESALQ 1296 isolate was reclassified as *Cordyceps javanica* (Agrofit, 2023).
The production of aerial conidia through solid fermentation, the primary method used in the industry, is a time-consuming process with high production costs and labor demands. Due to these challenges, there has been a growing interest in submerged propagules offering shorter fermentation times and high infectivity. In this context, blastospores are the most economical and rapid way to produce microbiological control agents. On the other hand, submerged conidia remain relatively unexplored despite demonstrating proven efficacy in controlling insects (Jaronski; Jackson, 2012; Mascarin et al., 2015; Corrêa et al., 2020; Iwanicki et al., 2023).

Microbial insect control has proven to be effective in managing several agricultural pests, and there has been a significant increase in interest in mycoinsecticides. This interest arises from the limitations of certain chemical products against certain insects, the development of resistance, and the societal demand for more sustainable food production. However, the exclusive use of microbiological products may not result in satisfactory control of D. maidis, as their action takes longer, allowing the transmission of diseases to persist for an extended period. Combinations of strategies must be necessary to address these challenges and to improve control efficacy (Mascarin; Jaronski, 2016; Bolzan et al., 2019; Mateus et al., 2020; Lima et al., 2020).

One practical approach to managing the corn leafhopper involves applying chemicals and microbiological agents simultaneously. This strategy aims to increase mortality and reduce the time of death compared to using either agent alone, resulting in more effective control. Companies in the agricultural sector have recommended and implemented this combined approach. They advise using a chemical product, but at lower doses than recommended, in conjunction with the microbial agent. The rationale behind this recommendation is the potential synergistic effect between the products and the belief that insects killed by insecticides sporulate and serve as an inoculum for other insects. However, scientific studies do not conclusively prove these effects (Quintela et al., 2013; Oliveira; Frizzas, 2021).

The action of chemical products on pathogens can vary depending on factors such as the species and isolate of the pathogen, the chemical nature of the products, and the dosages used. Such interactions may have a synergistic, neutral, or antagonistic effect (Alves, 1998; Sain et al., 2019). Therefore, research investigating the effectiveness of different entomopathogenic fungi (EF) propagules against D. maidis and their biological compatibility with insecticides in vitro and in vivo is essential for effective Integrated Pest Management (IPM). This is
particularly crucial as no literature data is available regarding the compatibility of blastospores and submerged conidia with insecticides.
2 LITERATURE REVIEW

2.1 *Dalbulus maidis*: bioecology and economic impact

Presently, the *Dalbulus* genus encompasses 13 species grouped into three monophyletic categories. Among these, five species inhabit maize fields, and solely *Dalbulus maidis* is deemed a pest, primarily due to the capacity to maintain sizable populations on maize. Commonly referred to as the corn leafhopper, *D. maidis* is a member of the Cicadellidae family and was first described in 1923 by DeLong based on specimens collected from Puerto Rico. This insect boasts an expansive distribution, spanning the tropics across North, Central, and South America, with its center of origin in Mexico. Within maize plants, these insects create colonies encompassing both adults and nymphs, thriving within the maize cartridge and leaves (Nault, 1990; Todd et al., 1991; Waquil, 1998; Zurita, 2000; Oliveira; Frizzas, 2021; Cabi, 2021).

The life cycle of the corn leafhopper (Figure 1), from egg to adult, spans an average of 15 to 27 days, directly influenced by temperature and humidity. The optimal temperature range for corn leafhopper falls between 25 and 30 °C. Females are generally larger and heavier than males and lay approximately 400-600 eggs endophytically using their ovipositor, on the midrib or veins of maize leaves, preferably in healthy seedlings, causing more damage than males. The eggs are small, with a whitish color and a transparent chorion during the initial stages, measuring around 1.3 mm in length. As they continue to develop, within 7-10 days, they become white with red eye spots. The eggs typically hatch 9-12 days after being laid (Larsen; Nault, 1994; Waquil, 1998; Nault, 1998; Coll-Aráoz et al., 2020; Virla et al., 2021).
The nymphal stage of the corn leafhopper is characterized by five developmental instars. The first instars measure less than 1 mm long. In comparison, the last instar reaches just under 4 mm. Remarkably, irregular black spots appear on the anterior margin of the last two abdominal tergites in the second through fifth instars. In the fifth instar, the mesothoracic and metathoracic wing buds extend posteriorly over the abdomen. Under optimal developmental conditions, with a temperature of 23.4 ºC and 83% relative humidity, the nymphs hatch within a period of up to eight days and are usually found on the abaxial side of the leaves (Tsai, 1988; Nault, 1998; Waquil, 1999; Zurita et al., 2000; Oliveira; Frizzas, 2021).

The adults of *D. maidis* measure a few millimeters in length, ranging from 3.7 to 4.3 mm, and can be found on both the abaxial and adaxial sides of the leaves. They typically have a light color, varying from yellow to white. On their abdomen, there are variable black spots, and they also have two large spots always present on the head above the ocelli. Another distinguishing characteristic is the presence of two rows of spines on their posterior tibiae. Adult females mate and begin laying eggs within 1-2 days after emerging from the nymphal stage. The mean survival of adults is 7-8 weeks at 26 ºC (Triplehorn; Nault, 1985; Nault, 1998; Moreira; Aragão, 2009; Rafael et al., 2012).

Corn leafhopper pierces the plant and extracts its phloem sap, injecting toxic spittle and primarily inoculating disease-causing agents. It serves as a vector for three major pathogens:
corn stunt spiroplasma (CSS), maize bushy stunt phytoplasma (MBS), and maize rayado fino virus (MRFV), which cause red stunt, pale stunt, and stripe virus, respectively. Additionally, the transmission of maize striated mosaic virus (MSMV) was recently reported in Brazil (Vilanova et al., 2022). Considering this, plants attacked by the leafhopper commonly present multiple diseases simultaneously (Triplehorn; Nault, 1985; Waquil et al., 1999; Massola Junior, 2001; Oliveira et al., 2015).

_**Spiroplasma kunkelii**_ causes CSS, and its symptoms encompass the emergence of chlorotic stripes on the leaves, accompanied by stunting and a distinct reddening of the foliage. Meanwhile, MBSP is attributed to phytoplasma, which manifests symptoms such as leaf reddening, shortening of internodes, plant stunting, diminished grain yield, and the inhibition of lateral shoot production. Furthermore, MRFV induces the development of small chlorotic spots along the leaf veins; over time, these spots elongate and multiply. Lastly, MSMV is characterized by mild chlorotic streaks and mottling, in addition to a reduction in plant height (Figure 2) (Nault, 1980; Orlovskis et al., 2017; Sabato et al., 2018a; Vilanova et al., 2022; Cunha et al., 2023).

*Figure 2.* Visual symptoms resulting from diseases transmitted by *Dalbulus maidis* A) Corn stunt spiroplasma (CSS) B) Maize bushy stunt phytoplasma (MBS) C) Maize rayado fino virus (MRFV) D) Maize striated mosaic virus (MSMV) _Source_: Sabato et al., 2018; Vilanova et al., 2022 Edited by the author, 2023

Insects acquire pathogens from infected maize plants, undergo an incubation period where they multiply within their bodies, and then inoculate these pathogens into healthy plants. This disease transmission within the insect system happens in multiple stages: acquisition, where the vector obtains the pathogen while feeding on an infected maize plant; incubation or latency, during which the pathogen colonizes tissues and reaches the insect's salivary glands;
inoculation, when the vector must feed on a healthy plant to transmit the pathogen; and retention, the period during which the insect maintains the ability to transmit the pathogen (Alivizatos; Markham, 1986; Nault, 1997; Massola Júnior et al., 2001).

Besides its role as a pathogen vector, high incidences of *D. maidis* can lead to the death of young plants due to intense sap absorption. Furthermore, the excretion of honeydew creates a favorable environment for fungi that cause sooty mold on the leaf blades, thus hampering photosynthesis. The cumulative impact of corn leafhopper feeding and the transmission of phytopathogens results in significant losses in both productivity and quality within maize crops, and ten insects are sufficient to cause damage in 10-day-old seedlings. For instance, in Minas Gerais, the incidence of CSS ranged from 15.7% to 77.5%, and associated production damage was estimated at 60.7% to 84.1% (Massola Júnior, 1999; Oliveira et al., 2003; Moreira; Aragão, 2009; Sabato et al. 2013; Virla et al., 2021).

Until the late 1980s, *D. maidis* was considered a secondary pest. However, in recent times, it has gained notoriety due to recurring damage, particularly in the second maize season. Several factors have contributed to the significant increase in damage caused by these insects, including changes in the production system, the expansion of cultivated areas, the prevalence of late plantings, the overlapping of plant cycles, and the potential development of insecticide resistance. In Brazil, maize stands as the exclusive known host plant for corn leafhopper, with the insects enduring on tiguera maize post-harvest, facilitating sustained and heightened infestation rates (Oliveira; Frizzas, 2021; Oliveira et al., 2002).

Additionally, during the off-season, the insects can endure temporarily by utilizing sorghum seedlings, *Brachiaria ruziziensis*, and millet as both refuge and sustenance. A pivotal factor contributing to the widespread infestation in maize crops is the corn leafhopper’s high biotic potential, coupled with its expressive capacity to migrate long distances between fields (Taylor et al., 1993; Summers et al., 2004; Sabato et al., 2018b). Nowadays, this insect has become a significant challenge in maize crops, and relying solely on a single control method is insufficient to maintain its population at a low density and prevent the spread of diseases transmitted by it (Cota et al., 2021; Oliveira et al., 2023).

Moreover, the rapid transmission of pathogens poses a complex management issue. In this context, adopting Integrated Pest Management (IPM) has emerged as a viable alternative,
implementing multiple control measures simultaneously, aiming to mitigate the economic losses caused by this pest.

2.2 Integrated Pest Management (IMP) of *Dalbulus maidis*

IPM combines various strategies and techniques to minimize the impact of pests while promoting ecological balance and reducing the reliance on chemical interventions. The decision-making process relies on taxonomy, bioecology, monitoring, and economic thresholds, focusing on prioritizing conditions that foster plant equilibrium and natural pest control mechanisms. As a result, it is crucial to initiate control measures as soon as pest density crosses the action threshold (AT) to prevent these organisms from inflicting harm to crops and reaching the economic injury level (EIL). In the case of *D. maidis*, although an established EIL is absent, its low AT is of utmost significance due to its role as an insect vector (Kogan, 1998; Gallo et al., 2001; Gullan; Cranston, 2017).

Since its emergence in 1972, the concept of IPM has resulted in a diversity of definitions. These definitions encompass the prudent choice of pest control methods, individually or in tandem, all while accounting for the economic benefits to producers, society, and the environment. Additionally, they encompass decision-making frameworks that steer the selection of control measures. In the context of *D. maidis*, simultaneous strategies must be adopted to manage the vector insect and the diseases it transmits within maize (Council on Environmental Quality, 1972; Kogan, 1998; Oliveira et al., 2023).

Monitoring insect populations is a vital element of IPM, as it facilitates the efficient and economically sustainable control of pests in field crops. *D. maidis* densities can vary due to biome, crop location, air temperature, and rainfall. Consequently, it is essential to perform regular sampling within the crop. Monitoring corn leafhopper can be accomplished through direct insect counting within the maize whorl and by employing yellow pan traps (Arnaudov et al., 2012; Meneses et al., 2016; Santana Jr. et al., 2019; Foresti et al., 2022).

Cultural methods for controlling *D. maidis* include crop rotation to reduce infestation pressure and adjust the planting season, avoiding the period of highest leafhopper incidence, thus decreasing infestation during the early stages of the crop. Fallowing, on the other hand, aims to reduce the presence of the insect by eliminating available hosts in the field.
Additionally, critical cultural techniques involve managing volunteer plants by destroying residual maize plants after harvest to eliminate potential infestation sources. Finally, the use of stunting-resistant genetic varieties, which has proven effective in reducing associated damage, the maize cultivars currently available in the market still lack satisfactory resistance to this insect and the associated diseases (Summers et al., 2004; Cota et al., 2018; Faria, 2020; Cunha et al., 2023).

Currently, chemical control remains Brazil’s primary method for managing corn leafhopper, boasting 49 registered products intended for control via seed treatment and foliar applications. However, the current literature on chemical efficacy presents inconsistent results. This is because the effectiveness of insecticides can vary based on specific environmental conditions and the selection pressure exerted on the insect population. Nevertheless, it is recommended that insecticides be employed during the initial stages of maize growth. When utilized under these conditions, they offer a solid alternative for pest management. Combining seed treatments during the early crop development stages with early-stage insecticide applications ensures an elevated level of control reliability (Massola Junior et al., 1999; Oliveira et al., 2007; Agrofit, 2023).

While chemical control methods are more effective, concerns about environmental residues have raised alarms over time. Consequently, biological control has emerged as a promising alternative, managing insect populations through the intervention of agents of biotic mortality. Predators and parasitoids play pivotal roles in the inherent regulation of *D. maidis* populations. This includes egg parasitoids like *Anagrus* spp. (Hymenoptera, Mymaridae) and *Oligosita* sp. (Hymenoptera, Trichogrammatidae), nymph and adult parasitoids such as *Gonatopus* spp. (Hymenoptera: Dryinidae) and *Eudorylas* spp. (Diptera: Pipunculidae). Also, some predators feed different development stages of corn leafhoppers, including spiders, earwig, assassin bug, ladybugs and wasps (Oliveira; Lopes, 2000; Parra, 2002; Albarracin et al., 2006; Moya-Raygoza et al., 2012; Virla et al., 2015; Martins; Krinski, 2016; Querino et al. 2018; Hill et al., 2019).

Biological control strategies employing microorganisms, particularly entomopathogenic fungi, have garnered substantial attention for the management of corn leafhoppers. In Brazil, among the 45 products registered with MAPA, the majority are centered around *B. bassiana*, acknowledged as a potential agent for *D. maidis* control. Additionally, two other microorganisms are commercially available in Brazil. One is a blend of *Pseudomonas*
chlororaphis with *P. fluorescens*, and the other is a product based on *C. fumosorosea* (formerly *Paecilomyces fumosoroseus*). However, it's worth noting that both formulations lack scientifically proven efficacy, as documented in the literature (Ibarra-Aparicio et al., 2005; Silva et al., 2009; Agrofit, 2023).

In the literature, the fungus *M. robertsii* has shown remarkable virulence against adult leafhoppers, resulting in mortality rates exceeding 80%. Another species within the same genus, *M. anisopliae*, has also effectively infected it. Furthermore, the natural occurrence of *Batkoa* sp. and *Metarhizium brasiliense* has been observed in maize fields throughout Brazil's central-western and northeastern regions. As a result, entomopathogenic fungi offer an effective means of pest management, yielding efficiency levels comparable to those of synthetic insecticides (Ibarra-Aparicio et al., 2005; Toledo et al., 2007; Moya-Raygoza et al., 2008; Iwanicki et al., 2021; Souza et al., 2021; Agrofit, 2023).

### 2.3 Insecticides controlling *Dalbulus maidis*

Insecticides are chemical or natural compounds that, when applied directly or indirectly to insects, generally lead to their demise. From a toxicological standpoint, they can be categorized as either more or less toxic and exert their effects on living organisms through physiological or biochemical processes. These insecticides represent one of the strategies employed to mitigate the influence of early pests and prevent economic damage. Over the years, they have demonstrated their significance as an indispensable tool for enhancing productivity, primarily due to their ability to swiftly and effectively reduce insect populations (Cruz et al., 1999; Gallo et al., 2001).

In addition to its pivotal role in enhancing agricultural production, the intensive, incorrect, and indiscriminate use of insecticides can give rise to a host of pressing issues. These include environmental contamination, high residue levels in food items, disruptions in ecological balances arising from the elimination of natural enemies and non-target insects, and the emergence of insect populations that have developed resistance to these chemicals. Therefore, IPM aims to ensure the responsible use of insecticides, balancing crop protection with environmental and health preservation (Chen et al., 2014; Mendes et al., 2016; Chiarello et al., 2017; Amaral et al., 2023; Moreira et al., 2023).
However, certain significant pests in economically valuable crops rely solely on chemical control methods, offering a swift and essential solution for insect mortality, exemplified by the corn leafhopper. Within maize cultivation, initial pests affecting seeds, roots, and seedlings are commonly addressed through seed treatment. This strategy is cost-effective, easily applicable, and considered safe for human health and the environment. Moreover, seed treatment's effectiveness can often exceed that of spray applications when combating *D. maidis*, and presently, the primary registered products in Brazil are neonicotinoid-based (Cruz et al., 1999; Albuquerque et al., 2006; Martins et al., 2008; Viana et al., 2012; Neves et al., 2022).

To effectively address other pests that impact plants' vegetative and reproductive parts, insecticides are routinely administered via aerial spraying techniques. Currently, 65 products are registered with MAPA, specifically formulated for controlling *D. maidis*. These products predominantly consist of pyrethroids, organophosphates, neonicotinoids, and carbamates, all targeting the insect's nervous system and musculature. Moreover, many of these commercial formulations incorporate a blend of active ingredients, aiming to increase the effectiveness of pest management. These insecticides can be classified based on their mode of action and chemical structures (Table 1) (Viana et al., 2012; Agrofit, 2023).

<table>
<thead>
<tr>
<th>Chemical groups</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethroids</td>
<td>Sodium channel modulators</td>
</tr>
<tr>
<td>Neonicotinoids</td>
<td>Acetylcholine receptor modulators</td>
</tr>
<tr>
<td>Organophosphates and carbamates</td>
<td>Acetylcholinesterase inhibitors</td>
</tr>
<tr>
<td>Phenylpyrazoles</td>
<td>GABA-mediated chlorine channel blockers</td>
</tr>
<tr>
<td>Benzoylureas</td>
<td>Chitin biosynthesis inhibitors (0 type)</td>
</tr>
<tr>
<td>Diamides</td>
<td>Ryanodine receptor modulators</td>
</tr>
</tbody>
</table>

*Table 1. Chemical groups registered for Dalbulus maidis in the Ministry of Agriculture (MAPA) for spraying applications and their mode of action ordered by most representative and least representative.*

*Source: Irac, 2023*

Insecticide application is typically initiated upon the detection of the presence of *D. maidis* within maize fields, aiming to control this vector insect and minimize disease transmission. The synthetic insecticides acephate, methomyl, thiamethoxam, and imidacloprid + β-cyfluthrin have been widely employed to manage corn leafhoppers through spraying. Regarding seed treatment, neonicotinoids like imidacloprid, clothianidin, and thiamethoxam
have exhibited superior results. Additionally, it is essential to consider factors such as application timing, dosage, and the potential impacts on non-target organisms to develop a comprehensive pest management strategy (Tsai et al., 1990; Oliveira et al., 2008; Rangel Júnior, 2018; Ruegger, 2019; Silveira, 2019; Neves et al., 2022).

Few studies evaluate the impact of chemical management of *D. maidis* on other maize pests and non-target organisms. Nevertheless, Albuquerque et al. (2006) conducted a study assessing the application of insecticides on green-belly stink bugs, leafhoppers, thrips, and fall armyworms during the initial stages of the crop. Their findings pointed to thiamethoxam, when used as a seed treatment in combination with thiamethoxam + lambda-cyhalothrin in spray applications, as a viable chemical control option for this cluster of pests. Furthermore, some insecticides registered for corn leafhoppers can also be applied for other maize pests, such as ethiprole for *Diceraeus melacanthus* and methomyl for *Spodoptera frugiperda* (Agrofit, 2023).

On the other hand, insecticides can cause devastating consequences for non-target insects, particularly vital pollinators such as bees. The indiscriminate use of pesticides leads to increased mortality, colony losses and sublethal effects. For instance, neonicotinoids have demonstrated high toxicity to honeybees, resulting in reduced body weight and overwintering success. In stingless bees, these chemicals have been found to impact appetitive responses, learning, and memory. They also hinder wasp mobility. When insecticides are combined, as in tank mixtures, the risk to these organisms can be even more severe due to synergistic effects (Wang et al., 2020; Aguiar et al., 2023; Crispim, 2023; Lv et al., 2023). Therefore, it is crucial to test different combinations of insecticides or biological agents and provide this data for the safe implementation of IPM, aiming to preserve natural enemies and achieve effective control, thereby reducing the use of insecticides.

### 2.4 Microbial control of insects: the role of *Cordyceps javanica*

Microbial control entails the utilization of pathogens, such as viruses, fungi, bacteria, protozoa, or nematodes, to manage selective insect populations effectively, ensuring they remain below the economic threshold. This approach is highly valued for its potential to enhance environmental conservation and reduce reliance on insecticide applications. Nowadays, it has assumed growing significance, particularly within integrated pest management. In 2020, Brazil emerged as a global leader in the adoption and commercialization
of biopesticides, displaying growth rates that outpaced the global average (Alves, 1998; Lacey; Shapiro-Ilan, 2018; Croplife, 2021).

Entomopathogenic fungi (EF) have evolved to exploit insects, capable of colonizing different orders and causing epizootics under natural conditions. They encompass a wide range of morphologically, phylogenetically, and ecologically diverse fungal species that have independently evolved. They can be divided into Oomycetes, Microsporidia, Chytridiomycota, Entomophthoromycota, Basidiomycota, and finally, Ascomycota, the group that infects a vast number of arthropods and is explored for the development of bioinsecticides (Humber, 2008; Vega; Kaya, 2012; Araújo; Hughes, 2016).

Commercial products based on aerial conidia of EF in Brazil have been predominantly limited to species within the Hypocreales order, including B. bassiana, M. anisopliae, and C. fumosorosea (formerly known as Isaria fumosorosea and P. fumosoroseus). These fungi can actively penetrate an insect's exoskeleton through both mechanical and enzymatic means, thereby infecting the host insect at various stages of its development. Moreover, they demonstrate a remarkable capacity for replication and environmental dispersion through the release of spores (Faria; Wraight, 2007; Ali et al., 2010; Khan et al., 2012; Boomsma et al., 2014).

The genus Cordyceps (Hypocreales: Cordycipitaceae) was previously classified as Paecilomyces and, later, Isaria. These fungi have a global distribution and are commonly found in soil, infecting many hosts. Beyond their entomopathogenic capabilities, certain species also exhibit endophytic behavior, promoting plant growth. In Brazil, two species, C. fumosorosea, and C. javanica have economic interest and are commercially exploited for controlling insect pests in crops (Faria; Wraight, 2007; Zimmermann, 2008; Kepler et al., 2017; Sun et al., 2020; Gbif, 2023).

Two formulated products registered with MAPA to control D. maidis are based on these two fungal species. Some strains previously collected from Bemisia tabaci were initially identified as C. fumosorosea, 'I. fumosorosea,' and 'P. fumosoroseus'. However, more recently, they were accurately identified as C. javanica, as with the ESALQ 1296 isolate. C. javanica is well-known for its effectiveness in controlling whiteflies and is one of the most common entomopathogens that target nymphs and adults of B. tabaci (Hemiptera: Aleyrodidae). Furthermore, these fungi exhibit virulence against various other insects, including psyllid, as
Diaphorina citri (Hemiptera: Liviidae), aphid and lepidopterans (Ausique et al., 2017; Baja et al., 2020; Faria et al., 2022; Lopes et al., 2023; Wang et al., 2023; Domingues et al., 2024).

The commercial product based on C. javanica (ESALQ 1296), before being named I. fumosorosea, is owned by Koppert do Brasil Holding S.A. and received its registration approval in 2018. Over time, it has expanded its target insect indications. Initially, it included only D. citri, but it nowadays also encompasses Helicoverpa armigera, D. maidis, Frankliniella schultzei (Thysanoptera: Thripidae), Tetranychus urticae (Acari: Tetranychidae), Planococcus minor (Hemiptera: Pseudococcidae), and Glycaspis brimblecombei (Hemiptera: Aphalaridae) (Agrofit, 2023).

And while the literature confirms an efficacy rate of 77.8% in mortality against D. citri, 86-93% against T. urticae, and 100% against G. brimblecombei caused by C. javanica strains, there is currently a lack of published data regarding its effectiveness against other pests. Additionally, despite being an isolate collected from B. tabaci with proven efficacy in the literature, causing an 83% mortality rate due to blastospores, there is no registration for such a pest (Ausique et al., 2017; Corrêa et al., 2020; Queirós et al., 2022).

Different fungal propagules can be produced through solid-state, liquid, and biphasic fermentation processes. Typically, these structures exhibit varying effectiveness in insect control, directly influencing the time to death or mean lethal time. While in Brazil, all bioinsecticides are based on aerial conidia, which require time for sporulation and labor and entail high production costs, liquid cultures have a significant potential for large-scale commercial fermentation in a short period, with automation. Therefore, it is considered the most economical and rapid method for producing microbial control agents (Jackson, 1997; Mascarin, 2010; Jaronski; Jackson, 2012).

Blastospores, the yeast-like propagules, may be considered even more effective than aerial conidia controlling whiteflies, leafhoppers, aphids, and beetles. On the other hand, submerged conidia are a relatively unexplored structure and have not yet been commercially developed as a biological agent for arthropod control. Characteristics including hydrophobicity, temperature tolerance, desiccation tolerance, virulence against insects, and germination rate differ among aerial conidia, submerged conidia, and blastospores (Bidochka et al., 1995; Poprawski; Jackson, 1999; Behle et al., 2006; Shapiro-Ilan et al., 2008; Esther et al., 2013; Iwanicki et al., 2023).
Exploring novel propagules of EF, particularly *C. javanica*, holds the promise of broadening the array of commercially viable options in the market while bolstering integrated management approaches for corn leafhopper control. Moreover, to guarantee the integration of new products with other pest management methods, including insecticides, conducting essential in vitro compatibility studies is necessary.

### 2.5 Interactions between insecticides and entomopathogenic fungi

Mixing insecticides in tanks is common and regulated by law in Brazil. Due to the challenges posed by corn leafhopper control, mixtures containing both chemical and microbiological products based on EF have become increasingly prevalent. This approach offers several advantages for agricultural sprays, including time and resource savings by reducing the need for multiple applications and mitigating plant damage. Additionally, it contributes to cost reduction and can help manage insect resistance. However, tank mixing requires care and attention to ensure the safety of non-target insects and to guarantee the compatibility of components with each other, following the manufacturer's recommendations (Ahmad et al., 2009; Gazziero, 2015; Gandini et al., 2020).

The interaction between chemical insecticides and microorganisms can yield positive outcomes when they exhibit synergistic action, resulting in improved pest control efficacy compared to the products used individually. Conversely, it can have adverse or antagonistic effects when the active ingredient of the chemical or its formulation inhibits the vegetative growth, conidiogenesis, and sporulation of the microorganisms, thereby reducing control effectiveness. Lastly, there can be neutral compatibility or additive, where no discernible effect occurs when the agents are mixed and, whether applied individually or combined, produces the same result (Alves et al., 1998).

This relation between insecticides and EF is a complex process influenced by various factors. These factors include the chemical nature of the products, their concentration, contact time, and the specific microbial agent species involved. These parameters directly impact the sporulation of the microorganisms. Additionally, this interaction may have far-reaching consequences, potentially leading to alterations in the microorganisms' genetic composition and infectivity capacity (Batista Filho et al., 2001; Loureiro et al., 2002; Alves; Lopes, 2008).
In vitro biological compatibility experiments employ various techniques, including product integration into the culture medium, pre-fungal inoculation product application, post-fungal inoculation product application, or the incorporation of the fungus into the product mixture. Nonetheless, it is crucial to consistently evaluate parameters such as germination (viability) and colony forming units (CFU) measurements in compatibility tests involving phytosanitary products. These parameters serve as essential criteria for assessing potential impacts on EF, as they simulate critical stages of insect body colonization. These in vitro experiments provide an advantage as they expose the pathogen to the full impact of insecticides, a scenario impractical to replicate in field conditions (Alves et al., 1998; Neves et al., 2001; Silva; Neves, 2005).

One of the key strategies involves using lower doses of insecticides in combination with EF. Since chemicals can induce stress, compromise an insect's immune system, and alter its behavior, they can potentially enhance the effectiveness of fungal pathogens. Furthermore, combining these agents may increase pest mortality and expedite the time needed for their demise compared to using either agent alone. This approach is a vital strategy for managing insecticide-resistant pests, rendering them more susceptible to microbial infection (Furlong; Groden, 2001; Ambethgar, 2009; Quintela et al., 2013; Rice; Furlong, 2023).

Imidacloprid has previously shown synergistic interactions when co-applied with entomopathogenic fungi in insects. It is compatible with various fungal species, including *B. bassiana*, *M. anisopliae*, *M. brunneum*, *M. flavoviride*, and *Verticillium lecanii*. Additionally, the insecticides acetamiprid and thiamethoxam do not negatively impact the germination, vegetative growth, and conidia production of *B. bassiana*, *M. anisopliae*, and *Paecilomyces* sp. (Neves et al., 2001; Loureiro et al., 2002; Russell et al., 2010; Ge et al., 2020).

On the other hand, imidacloprid and thiamethoxam exhibited moderate toxicity to *B. bassiana* when tested through product integration into the culture medium. However, in greenhouse experiments, these products were compatible (Pinto et al., 2012). This disparity could be linked to the varying doses tested and the methods employed to evaluate biological compatibility. For example, integrating insecticides into the culture medium represents a worst-case scenario for the entomopathogenic fungus, often resulting in incompatibility.

A recent study involving *C. javanica*, ESALQ 1296 isolate, identified several synthetic insecticides widely used for corn leafhopper control, demonstrating compatibility. These
insecticides include imidacloprid + β-cyfluthrin, ethiprole, and acephate. Combinations of imidacloprid + β-cyfluthrin and imidacloprid + bifenthrin were classified as highly compatible with the fungi based on their performance (Ribeiro et al., 2023). Furthermore, a previous study by Lopes et al. (2019) had confirmed the compatibility of acetamiprid and thiamethoxam with the ESALQ 1296. By aligning this current research to prior findings that confirm the compatibility of ESALQ 1296 isolate with the insecticides cited, we will establish a robust scientific foundation for their combined use, ultimately enhancing IPM.
3 HYPOTHESIS AND OBJECTIVES

We hypothesized that combining the commercial product based on *C. javanica* with insecticides would lead to a synergistic effect, resulting in increased mortality of *D. maidis*. Concerning compatibility, we hypothesized that blastospores and submerged conidia would be more sensitive to insecticides compared to aerial conidia. This assumption stemmed from the structural resilience of aerial conidia, which exhibit greater resistance to UV light and environmental conditions. We also anticipated that insecticides might reduce the viability and growth measured as colony-forming units (CFU) of *C. javanica* (ESALQ 1296), potentially inhibiting sporulation in the culture media or on the bodies of *D. maidis*. We also expect blastospores to demonstrate quicker lethality against adults of corn leafhopper compared to aerial conidia and submerged conidia. This expectation was based on the observed faster germination of blastospores on the integument surface of the host, as widely documented in the literature.

Thus, the main objective of this study was to assess the compatibility of *C. javanica* with the insecticides mostly used in combating *D. maidis*, aiming to recommend a control strategy for this insect.

The specific objectives were:

1. To compare the virulence of aerial conidia, blastospores, and submerged conidia from the *C. javanica* (ESALQ 1296) against *D. maidis*.

2. To determine the biological compatibility of aerial conidia, blastospores, and submerged conidia of *C. javanica* (ESALQ 1296) with the main insecticides used for *D. maidis* control.

3. To evaluate the efficacy of aerial conidia of *C. javanica* (ESALQ 1296) in controlling *D. maidis* when combined with the primary insecticides used for it.

4. To propose a strategic approach for managing *D. maidis*. 
4 MATERIALS AND METHODS

The experiments were carried out at the Department of Entomology and Acarology (LEA) of the 'Luiz de Queiroz' College of Agriculture (ESALQ), specifically in the Integrated Pest Management Laboratory, Laboratory of Pathology and Microbial Control of Insects (LPCM) and Arthropod Resistance Laboratory.

4.1 Rearing Dalbulus maidis and nurturing maize seedlings

The insects utilized in the experiments have been reared at the Laboratory of Insect Vectors of Plant Pathogens, located within the Department of Entomology and Acarology (LEA) at "Luiz de Queiroz" College of Agriculture (ESALQ) in Piracicaba, São Paulo. They were maintained on maize plants within specially designed cages measuring 45 cm (height) × 35 cm (length) × 35 cm (width). These cages were constructed with aluminum and acrylic materials and were fitted with an anti-aphid screen. The reared colony was situated within a greenhouse equipped with a "pad-fan" temperature regulation system and heaters, maintaining a controlled temperature of 25 ± 5 ºC. This greenhouse is also located within LEA.

Simultaneously, another population exhibiting resistance characteristics was selected to assess the synergistic effect of Octane with insecticides. The insects were sourced from Bahia and bred in the Arthropod Resistance Laboratory at ESALQ. They are reared in plastic cages fitted with anti-aphid screens, all within a controlled environment with regulated temperature. This population was collected from the field, and we are currently conducting ongoing research in the Arthropod Resistance Laboratory to study their resistance to the active ingredient methomyl. Furthermore, the experiments conducted during this project have yielded compelling evidence in this regard.

Maize seeds were planted weekly in vases measuring 60 cm in height and 13 cm in diameter, filled with commercial substrate. The seeds were fertilized with NPK 10-10-10 as needed until they reached the phenological stages V3 and V4. At these stages, the maize plants were used to maintain the rearing of D. maidis. Maintenance occurred once a week, involving replacing compromised plants with new ones, cleaning the cages, and transferring adults using a suction cup (Figure 3). The plants used in the experiment were cultivated in a greenhouse.
4.2 *Cordyceps javanica* (ESALQ 1296) and *Beauveria bassiana* (ESALQ 3366) maintenance

The *C. javanica* ESALQ 1296 and the *B. bassiana* ESALQ 3366 are maintained within the Collection of Entomopathogenic Microorganisms "Prof. Sérgio Batista Alves" at the Luiz de Queiroz College of Agriculture, specifically in the LPCM. These isolates were preserved in 2 mL cryogenic vials, each containing 1 mL of a sterile 10% glycerol solution, and were securely stored at an ultra-low temperature of -80 °C. Moreover, these isolates were routinely cultivated on Petri dishes filled with Potato Dextrose Agar (PDA, Difco®). The cultivation process took place in a controlled environment within a BOD chamber, maintaining a temperature of 25 °C for 7-15 days in a 12-hour photoperiod.

ESALQ 1296 isolate was originally collected from adult whiteflies of the *B. tabaci* biotype B in Jaboticabal, São Paulo, in 2001. The ESALQ 3366 isolate was collected using insect bait in *Tenebrio molitor* (Coleoptera: Tenebrionidae) in Inconfidentes, Minas Gerais, 2013 and was used as a positive control in the virulence experiments. The sporulated plates (Figure 4) were used to prepare conidial suspensions for the initial inoculum in liquid fermentation and bioassays requiring pure aerial conidia.
4.2.1 Pure aerial conidia production

Pure aerial conidial suspensions were prepared by transferring spores with a sterile spatula into 10 mL of a sterile aqueous solution containing 0.05% Tween® 80 or 0.05% Silwet L-77. The mixture was then agitated using a vortex. The spore concentration was determined using a Neubauer chamber and adjusted to the desired concentration of $5 \times 10^6$ conidia mL$^{-1}$ when used as an inoculum for the liquid culture medium and $1 \times 10^7$ conidia mL$^{-1}$ in virulence assays and in vitro experiments (Figure 5).

4.2.2 Blastospore production

To produce blastospores from the ESALQ 1296 isolate, a modified Adamek medium (Iwanicki et al., 2018) was employed. The medium’s composition per liter included 40 g of...
glucose, 80 g of yeast extract, 40 g of corn steep liquor, 20 mL of Tween 80, 2.5 g KH$_2$PO$_4$, 1.0 g CaCl$_2$.2H$_2$O, 0.83 g MgSO$_4$.7H$_2$O, 0.3 g FeSO$_4$.7H$_2$O, 29.6 mg CoCl$_2$.6H$_2$O, 12.8 mg MnSO$_4$.H$_2$O, 11.2 mg ZnSO$_4$.7H$_2$O, as well as thiamine, riboflavin, pantothenate, niacin, pyridoxamine, and lipoic acid (each at 0.2 mg). The pH was adjusted to 6.8 before autoclaving. The fungal isolate was cultivated in 45 mL of medium in 250 mL Erlenmeyer flasks, each containing 5 mL of inoculum, over 3 days at 28°C and 350 rpm using a rotary incubator shaker (Solab®, Piracicaba, Brazil).

4.2.3 Submerged conidia production

Due to the absence of literature on the production of submerged conidia in *C. javanica*, insights from studies involving *C. fumosorosea* and *B. bassiana* were utilized as a foundation. Consequently, culture media were modified and tested to attain a sufficiently concentrated yield of these propagules, enabling the execution of experiments. Nevertheless, in the medium proposed by Esther et al. (2013), NH$_4$NO$_3$ is as the nitrogen source (NS) employed, subject to restrictions and controlled by the Brazilian Army. Therefore, alternative sources were investigated as possible substitutes, namely NS1, NS2, and NS3 (Table 2).
Table 2. The composition of selected or tested culture media for the submerged conidia production of *Cordyceps javanica* ESALQ 1296

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Esther original</th>
<th>Thomas original</th>
<th>Esther modified M1</th>
<th>Esther modified M2</th>
<th>Esther modified M3</th>
<th>Esther modified M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
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<td>50 g</td>
<td>50 g</td>
<td>50 g</td>
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<td>50 g</td>
</tr>
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<td>NS3</td>
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<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>NS2</td>
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<td>-</td>
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<tr>
<td>FeCl₃·6H₂O</td>
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<td>12 mg</td>
<td>20 mg</td>
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<tr>
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<tr>
<td>CuSO₄·5H₂O</td>
<td>15 mg</td>
<td>0.5 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>0.5 g</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>pH</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All submerged conidial productions were conducted using a rotary shaker (Solab®, Piracicaba, Brazil) with a fermentation period of 5 days at 28 °C and 150 rpm, during which 5 mL of the inoculum with a concentration of 5 x 10⁶ conidia mL⁻¹ was cultivated in 45 mL fermentation flasks (Bellco®), as described by Iwanicki et al. (2023), repeated three times at different times, totaling six samples per treatment. Distinctions between blastospores and submerged conidia were made based on morphological characteristics and were quantified through direct counting to assess the proportion of these propagules in the final yield obtained (Vidal et al., 1998).

Furthermore, to confirm that the fungal propagules were indeed submerged conidia, daily samples were collected from a flask production to observe fungal growth and visualize the conidiophore responsible for producing these submerged conidia. The observations were conducted using a Leica Microsystems® optical microscope (DM 4000 B) with a camera...
attached to the Leica Microsystems® microscope (DFC295), enabling the capture of images using LAS V4.1 software. The M4 medium (Esther et al., 2013 with NS1) was identified as the most suitable and was subsequently utilized in all further experiments.

4.3 Virulence of different *Cordyceps javanica* propagules against *Dalbulus maidis*

The virulence of blastospores, submerged conidia, and pure aerial conidia from *C. javanica* (ESALQ 1296) was assessed and compared with the formulated biological product Octane (Koppert®) against adult corn leafhoppers. In this experiment, an isolate of *B. bassiana*, already known for its high virulence to corn leafhoppers, was used as a positive control. Blastospores and submerged conidia obtained through liquid fermentation, following the parameters described above, were subjected to centrifugation to remove the culture medium with a triple wash, using the potassium buffer saline solution as previously mentioned.

On the day before the bioassay, 12 unsexed adults of *D. maidis*, aged 7-10 days post-emergence, were transferred and confined in cylindrical acetate cages measuring 60 cm in height and 13 cm in diameter. These cages contained a maize plant in the vegetative stage (V3 or V4), cultivated in a plastic pot with 10.5 cm in diameter and 10 cm in height. The top of the cage was covered with voile fabric to provide better ventilation. To prevent dead insects from contacting the soil, pieces of black, non-woven fabric were placed at the bottom of the cage to line the exposed substrate (Figure 6). The insects were kept in a bioassay room within the LPCM at ESALQ/USP, where conditions were maintained at around 26 ± 2 °C, under a 12-hour photoperiod with an uncontrolled relative humidity level of approximately 60-70%.
The bioassays comprised six treatments, with five replicates (cages) each, totaling approximately 360 insects. The treatments included: T1 - Control (0.05% Silwet L-77), T2. Octane, T3. Aerial conidia, T4. Blastospore, T5. Submerged conidia and T6. Positive control (*B. bassiana*). All insect-spraying suspensions were prepared at the standard concentration of $1 \times 10^7$ spores mL$^{-1}$, considering viability. They were applied to adult corn leafhoppers using an airbrush. A total of 2 mL of each treatment was evenly sprayed across the entire plant base and leaves. The equipment was cleaned with 70% alcohol and distilled water twice between treatments.

Evaluations were conducted at 2-day intervals for ten days after application, during which the count of live and deceased insects was recorded. Dead adults were transferred to Elisa™ plates to confirm mortality by observing the sporulation (mycosis). The sporulated leafhoppers were separated, and photos were taken using a Leica M250C Stereo Microscope equipped with a Leica DFC450 camera in the Laboratory of Chemical Ecology and Insect Behavior, ESALQ/USP. This experiment was repeated three times using different cohorts of insects and fungal cultures.
4.4 In vitro screening of insecticides

The in vitro compatibility assessment between insecticides used for *D. maidis* control and the propagules of ESALQ 1296 isolate was conducted in a sterile laboratory environment, considering factors such as viability and colony-forming units (CFU). The insecticides used in these experiments included acetamiprid + fenpropathrin, β-cyfluthrin + imidacloprid, ethiprole, bifenthrin + imidacloprid, methomyl, and bifenthrin + carbosulfan, all of which are registered with the MAPA for controlling corn leafhopper in maize crops (Agrofit, 2023) (Table 3).

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Commercial name</th>
<th>Concentration tested*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamiprid + fenpropathrin</td>
<td>Bold</td>
<td>37 g and 56.2 g in 0.5 L</td>
</tr>
<tr>
<td>β-cyfluthrin + imidacloprid</td>
<td>Connect</td>
<td>10 kg and 1.25 kg in 100 L</td>
</tr>
<tr>
<td>Ethiprole</td>
<td>Curbix</td>
<td>20 kg in 100 L</td>
</tr>
<tr>
<td>Bifenthrin + imidacloprid</td>
<td>Galil</td>
<td>50 kg and 10 kg in 200 L</td>
</tr>
<tr>
<td>Methomyl</td>
<td>Lannate</td>
<td>21.5 kg in 100 L</td>
</tr>
<tr>
<td>Acetamiprid + bifenthrin</td>
<td>Sperto</td>
<td>75 g in 1 L</td>
</tr>
<tr>
<td>Bifenthrin + carbosulfan</td>
<td>Talisman</td>
<td>0.5 kg and 15 kg in 10 L</td>
</tr>
</tbody>
</table>

*Concentration of the active ingredient in the respective order Source: Agrofit, 2023

The insecticide dilution was prepared following the manufacturer's recommendations as provided on the product label. In all suspension prepared in vitro experiments, 0.05% Silwet L-77 was used. This choice was based on the study of Arnosti et al. (2018), which demonstrated that Silwet L-77 is a superior wetting agent compared to others and is more effective when used with *C. javanica* ESALQ 1296 isolate, enhancing the conditions for germination.

When dealing with blastospores and submerged conidia, it was necessary to centrifuge the final production (10 minutes; 5000 rpm; 27 °C) to obtain pure propagules, eliminating the culture medium through a triple washing process. To accomplish this, a potassium buffer saline solution was used, with the following composition per liter: NaCl 8.0 g, KCl 0.2 g, Na₂HPO₄ 1.44 g, KH₂PO₄ 0.24 g, with the pH adjusted to 6.0 (phosphate-saline buffer) for the washing process.

In CFU assay, a stock solution with a concentration of $2 \times 10^6$ spores mL$^{-1}$ was prepared for each of the *C. javanica* propagules based on Neubauer chamber counts. Additionally, a 20
mL suspension of each treatment with insecticide was prepared in a sterile 45 mL polypropylene centrifuge tube (Falcon). Thus, 10 μL from the stock solutions were added to the treatment suspensions, initiating the contact time across insecticides and the *C. javanica* propagules. The tubes were then placed on a shaker (Marconi®, SP, Brazil) at 150 rpm for 30 minutes to simulate the mixing in a spray tank.

After agitation, 100 μL with a final concentration of $2 \times 10^2$ spores mL$^{-1}$ was spread on Petri dishes containing PDA (Kasvi®) using a sterile Drigaski loop. Subsequently, all the plates were incubated at $(27 \pm 2 \, ^\circ C)$ for 48 hours. After this period, the number of colonies was directly counted using a manual counter (Figure 7). Each treatment had two replicates per plate, and this process was repeated three times over time.

![Figure 7. Procedure employed in the colony-forming units (CFU) assay](image)

To assess viability, the propagules were suspended in the 20 mL insecticide solution at the concentration of $5 \times 10^6$ conidia mL$^{-1}$, based on Neubauer chamber counts and as required by this protocol. Subsequently, the solutions were placed on a shaker (Marconi®, SP, Brazil) at 150 rpm for 30 minutes to simulate the mixing in a spray tank. After the agitation period, 100 μL of the suspensions were plated onto Rodac™ plates containing PDA (Kasvi®) and then incubated in a BOD for 16 hours in the case of aerial conidia and submerged conidia and 8 hours for blastopore (at 25 °C with a 12-hour photoperiod). Viable propagules were directly counted, with a minimum of 100 propagules per sample, while cases of excessive hyphal growth or clumping were excluded from the analysis (Figure 8).
Observations were made using an optical microscope Leica Microsystems® (DM 4000 B) at 400x magnification. The camera attached to the Leica Microsystems® microscope (DFC295) facilitated image capture using LAS V4.1 software.

Subsequently, three insecticides - ethiprole, imidacloprid + bifenthrin, and methomyl - were selected to assess their compatibility with the tank mix after 90 and 150 minutes. The goal was to verify if there would be a significant reduction in germination or growth measured as CFU over the contact time, following the same protocol. The selection of ethiprole and methomyl was based on their viability and/or CFU being equal to or higher than that of the control, suggesting a potential stimulatory effect of the insecticide on the growth of *C. javanica*. In contrast, imidacloprid + bifenthrin was chosen because it was the least effective among the tested treatments. The compatibility testing was carried out using aerial conidia produced on laboratory plates, as described in previous sections, and aerial conidia formulated in the commercial product Octane.

### 4.5 Compatibility of *Cordyceps javanica* with insecticides on *Dalbulus maidis*

A compatibility study of the commercial product Octane (Koppert®, ESALQ 1296) with the selected insecticides from the in vitro experiments was assessed through bioassays involving adults of *D. maidis*. The cage setup and transfer of corn leafhopper adults were conducted as previously described. The insecticides selected were ethiprole, methomyl, and imidacloprid + bifenthrin based on their performance, as previously explained. The suspensions were prepared following the same procedure as experiments in vitro. The insecticides were first
added to the 20 mL suspension with 0.05% Silwet L-77, and subsequently, the commercial product Octane was added following the label recommendations.

The treatments with the isolated agents and the combinations were: T1 - Control (0.05% Silwet L-77), T2 - C. javanica ESALQ 1296, T3 - ½ dose ethiprole, T4 - Full dose ethiprole, T5 - ½ dose ethiprole + C. javanica, T6 - Full dose ethiprole + C. javanica, T7 - ½ dose methomyl, T8 - Full dose methomyl, T9 - ½ dose methomyl + C. javanica, T10 - Full dose methomyl + C. javanica, T11 - ½ dose imidacloprid + bifenthrin, T12 - Full dose imidacloprid + bifenthrin, T13 - ½ dose imidacloprid + bifenthrin + C. javanica, T14 - Full dose imidacloprid + bifenthrin + C. javanica. The half dose was employed following the recommendations of companies in the agricultural sector, which advise using a chemical product, but at lower doses, in conjunction with the microbiological agent.

The treatments were applied to adult corn leafhoppers using an airbrush. A total of 2 mL of each treatment was evenly sprayed across the entire plant base and leaves. One equipment was used for the control and Octane, and the other for insecticides and combinations. The airbrush was cleaned with acetone, 70% alcohol, and distilled water twice between treatments. Evaluations were carried out daily for eight days after application, during which the count of live and dead insects was recorded. Dead adults were transferred to Elisa™ plates to confirm mortality by observing the sporulation (mycosis) when relevant. The sporulated leafhoppers were separated, and photos were taken using a Leica M250C Stereo Microscope equipped with a Leica DFC450 camera in the Laboratory of Chemical Ecology and Insect Behavior, ESALQ/USP. This experiment was repeated twice in time with different cohorts of insects.

Following this experiment, a publication on the enhancing effect of Silwet and other adjuvants was released, and new bioassays were added to the schedule (El-Bassoieny et al., 2023). The preparation of the spray solution using distilled water and its application to D. maidis aimed to evaluate with 0.05% Silwet L-77 enhanced the insecticides' effects. The methodology followed the previously described procedure, with the only modification being the replacement of the adjuvant with sterile distilled water, including the control with sterile distilled water. In these experiments, the insecticides ethiprole and methomyl were selected for application to corn leafhoppers based on their performance.
4.6 Bioassay with methomyl-resistant and non-resistant *Dalbulus maidis*

The experiments with methomyl-resistant insects were conducted in the Arthropod Resistance Laboratory. Due to this, the application method was modified to use an adapted Potter tower within a fume hood, β-cyfluthrin + imidacloprid to a constant-pressure compressor (Figure 9). For this experiment, methomyl was the selected insecticide, with a dose of 600 mL ha\(^{-1}\) and a spray volume of 160L ha\(^{-1}\). The solution was prepared with sterile distilled water, and 20 mL of the suspension was mixed in a falcon tube. The recommended dose of Octane was used, which is consistent with previous trials.

![Figure 9. Adapted Potter tower to a constant-pressure compressor for spraying *Dalbulus maidis*](image)

Insects were aspirated and transferred to glass tubes, each equipped with voile coverings to prevent the leafhoppers from escaping. Approximately 12 unsexed individuals, aged 7-10 days, were then exposed to CO\(_2\)-induced dormancy and placed in Petri dishes where they were treated with 1 mL of suspension corresponding to their respective treatments. Finally, the corn leafhoppers were introduced into cylindrical acetate cages (60 cm in height × 13 cm in diameter), each containing a maize plant in the vegetative stage (V3 or V4). These maize plants were grown in plastic pots measuring 10.5 cm in diameter and 10 cm in height. The Potter tower was thoroughly cleaned with acetone, 70% alcohol, and distilled water before each treatment change.
The insects were kept in the LPCM bioassay room at 26 ± 2 °C, with a 12-hour photoperiod and uncontrolled humidity. Evaluations were carried out daily for eight days after application, during which the count of live and dead insects was recorded. Dead adults were transferred to Elisa™ plates to confirm mortality by observing the sporulation (mycosis) when relevant.

4.7 Statistical analysis

In the statistical analysis of all data sets, preliminary assessments for residual normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test) were conducted before implementing an analysis of variance (ANOVA). Furthermore, an exploratory analysis was undertaken to identify treatment means, standard errors, and outliers in each case. The data about submerged conidia production from modified culture media were subjected to a linear model (LM), and mean comparisons among treatments were conducted using the Sidak test, with a confidence level of 95%.

The mortality rates were adjusted using the Abbott formula (Abbott, 1925) and then analyzed employing a generalized quasi-binomial model (GLM). A Scott-Knott test was also carried out to compare the treatments with a significance level of 0.05%. For sporulation analyses, a comparison of means by Scott-Knott was conducted, with a significance level of 0.05%. In cases of survival data, a Weibull distribution was applied, and pairwise comparisons between treatments were performed using the Log-Rank test.

Viability data obtained from in vitro experiments were analyzed separately for each fungal propagule. Subsequently, a combined analysis was conducted using a quasi-binomial (logit) generalized linear model (GLM) and the Scott-Knott test, with a significance level set at 0.05%. UFC data were analyzed using a quasi-poisson GLM. Finally, a Tukey HSD test was employed for multiple comparisons of means, with a 95% family-wise confidence level. All analyses and graphs presented were created using the software ‘R’ (R Core Team, 2016).

To assess the independence between two categorical variables and identify potential antagonistic, additive, or synergistic effects among treatments showing statistical differences, a chi-square test at a 5% significance level was conducted. Following the approach outlined by Alexandre et al. (2008), employing the formula $\chi^2 = \text{ME} \times \text{ME}$ where MO represents
observed mortality, and ME is expected mortality. The expression for ME is $\text{ME} = \text{M1} + \text{M2} \times (1 - \text{M1})$ where M1 denotes the mortality of the entomopathogen alone, and M2 is the mortality of the insecticide alone. When the calculated $\chi^2$ value exceeded the tabulated value (3.84 for 1 degree of freedom, $P < 0.05$), we interpreted the effect as non-additive, indicating synergy or antagonism. If the difference between $\text{M1,2}$ (mortality resulting from the combination of the entomopathogen and insecticide) and ME was positive, we considered the effect as synergistic; if negative, it was interpreted as antagonistic. Calculated $\chi^2$ values lower than the tabulated value indicated an additive effect.
5 RESULTS

5.1. Cordyceps javanica (ESALQ 1296) submerged conidia production

The production of submerged conidia from modified culture media was assessed after five days of fermentation. There was a significant difference among the tested modified culture media (P < 0.05) (Figure 10). The M4, Esther et al. (2013) modified with NS1 as the nitrogen source yielded a significantly higher final production of $2.61 \times 10^8$ submerged conidia mL$^{-1}$. Furthermore, the broth from the final production was aqueous, easy to handle, and purple, similar to what is observed in sole cultures. It was observed that the culture media M1 and M2, based on Thomas et al. (1987) and Esther et al. (2013) with NS3, respectively, resulted in excessive hyphal growth, leading to an extremely low production of submerged conidia. Additionally, M2 exhibited hyphal and propagule deformation. Although M3, based on Esther et al. (2013) with NS2, showed reasonable production, it generated a thick challenging-to-handle fermentation broth (Figure 10).

Figure 10. Submerged conidia yield grows in different modified culture media after five days of fermentation in flasks at 150 rpm at 10$^6$. Means (±SE) followed by different letters indicate significant differences according to the Tukey test (P < 0.05)

Legend: M1 - Thomas et al. (1987), M2 Esther et al. (2013) with NS3, M3 - Esther et al. (2013) with NS2, M4 - Esther et al. (2013) modified with NS1

The conidiogenesis of the submerged conidia was monitored to distinguish them from other fungal propagules produced in a liquid medium (Figure 11 A-B). The compositional
analysis also revealed that blastospores constituted less than 10% of the total propagule production in the submerged culture of *C. javanica*. The propagules were differentiated based on their morphology, with the larger and elongated structures classified as blastospores. In comparison, the smaller and round structures were identified as submerged conidia, following the criteria established by Vidal et al. (1998).

![Figure 11. A-B) Submerged conidia observed at the beginning of conidiogenesis, 24 hours after inoculation C) Blastospores of *Cordyceps javanica* produced in modified Adamek medium (400x increase) D) Submerged conidia of *C. javanica* produced in modified Esther et al. (2013) medium (400x increase)](image)

5.2 Virulence of different *Cordyceps javanica* propagules against *Dalbulus maidis*

The virulence of blastospores, submerged conidia, and pure aerial conidia from *C. javanica* (ESALQ 1296) was assessed and compared to the formulated biological product Octane. This experiment also included a positive control represented by an isolate of *B. bassiana*. Statistically significant differences in *D. maidis* mortality were observed among the treatments (*P* < 0.05). The positive control using *B. bassiana* resulted in higher total mortality, 64 ± 2.1%. However, among the various fungal propagules, the blastospores of *C. javanica* exhibited a statistically significant difference, resulting in a corrected mortality rate of 40 ± 3.0%. All of the other propagules were statistically different from the negative control, as Octane (25.47 ± 1.97%), pure aerial conidia (23.73 ± 2.44%), and submerged conidia (22.24 ± 2.42%).
Different survival times were observed between the treatments, and the *B. bassiana* and blastospores of *C. javanica* killed *D. maidis* adults faster than the other propagules (*P* < 0.05). And notably, blastospores exhibited the highest virulence, as indicated by LT<sub>50</sub> value of 10 days (Figure 12A). Other propagules of ESALQ 1296 had a LT<sub>50</sub> value higher than the duration of bioassay evaluation. Statistical differences in the percentage of sporulated insects, or confirmed mortality, were also observed (Figure 12B).

![Figure 12](image)

**Figure 12.** A) Proportion of surviving *D. maidis* during ten days after exposure to Octane (T2), pure aerial conidia (T3), blastospore (T4) and submerged conidia (T5) of *Cordyceps javanica*, and *Beauveria bassiana* isolate (T6) at the concentration of 1 x 10^7 spores mL⁻¹. B) Sporulated *D. maidis* along the images of the sporulated insects. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test (*P* < 0.05)

### 5.3 Insecticides screening in vitro conditions

The viability was assessed after a 30-minute exposure to a tank mixture containing six different insecticides. Despite statistically significant differences observed among the tested treatments and propagules (*P* < 0.05), none of the treatments substantially compromised *C. javanica* viability. All treatments exhibited a germination rate superior to 80%, allowing for a tank mixture with insecticides for 30 minutes without causing a negative effect. However, it was not possible to conduct a count of samples related to the acetamiprid + bifenthrin insecticide, and so, only the CFU was conducted.

Treatment T4 (ethiprole) had the least impact on the germination of all fungal structures, as no propagule exhibited germination below 93%. Moreover, it performed similarly to the
control. However, imidacloprid + bifenthrin affected germination, reducing the germination to 80% of *C. javanica* after 30 minutes of contact. Analyzing all fungal propagules, submerged conidia and aerial conidia showed the highest tolerance (P < 0.05), whereas the most affected by the insecticides was the formulated product Octane (Figure 13).

(T2) acetamiprid + fenpropathrin  (T3) β-cyfluthrin + imidacloprid  (T4) ethiprole  (T5) methomyl  (T7) imidacloprid + bifenthrin

![Graphs showing germination percentages for different treatments](image)

**Figure 13.** Propagules of *Cordyceps javanica* germinations (%) after exposure to 0.05% Silwet L-77 (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5) and imidacloprid + bifenthrin (T7), during 30 minutes **A)** Octane **B)** Pure aerial conidia **C)** Blastospores **D)** Submerged conidia. Means (±SE) followed by different letters indicate significant differences according to the Sidak test (P < 0.05)

Subsequently, aerial conidia, both in pure and formulated form (Octane), were subjected to 90 and 150 minutes of contact with three selected insecticides, ethiprole, methomyl, and imidacloprid + bifenthrin. There were statistically significant differences among the treatments and contact times (P < 0.05).

For *C. javanica* ESALQ 1296, at the 90 and 150-minute, treatments T2 (ethiprole) and T3 (methomyl) showed significant differences between each other and in comparison to the other treatments, ethiprole maintained the viability higher than 90% in all samples (P < 0.0001).
Additionally, in T3 (methomyl), a significant difference was observed between the 30 and 90-minute time points, reducing the viability to 77% at 150 minutes (P < 0.0001) (Figure 14A). Regarding aerial conidia, it was noted that at the 90 and 150-minute time points, treatments T2 (ethiprole) and T4 (imidacloprid + bifenthrin) differed significantly from each other, and from the other treatments, imidacloprid + bifenthrin affected more the viability, reducing for 80% (P < 0.01) (Figure 14B).

![Figure 14. A) Formulated aerial conidia of Cordyceps javanica (Octane) germination (%) subjected to contact with 0.05% Silwet L-77 (T1), ethiprole (T2), methomyl (T3) and imidacloprid + bifenthrin (T4) over time B) Pure aerial conidia of Cordyceps javanica germination (%) subjected to contact with ethiprole, imidacloprid + bifenthrin and methomyl insecticides over time. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test (P < 0.05)](image)

In some experiments, the viability of ethiprole was superior to the control, showing statistical differences. This led to the assumption that this active ingredient might have a stimulating or synergistic effect, prompting the selection of this insecticide for the compatibility stage with D. maidis. Similarly, the insecticide Imidacloprid + bifenthrin was chosen based on indications of its negative effects, aiming to clarify whether the same would be observed when applied to the insect.

The evaluation of colony-forming units (CFU) was initially performed after 30 minutes of contact. Significantly, none of the treatments completely compromised the propagules of C. javanica, and the number of colonies varied depending on the propagule. For Octane, this number was 49 ± 24; for pure aerial conidia 58 ± 20. For blastospores, 114 ± 47, and for submerged conidia, 54 ± 16. In this experiment, a statistically significant difference was observed only among the treatments involving aerial conidia, where T6 (acetamiprid +
bifenthrin) negatively affected the colony count, reducing the number of colonies to 18 ± 9 (P < 0.05). The remaining treatments did not affect the colony formation (Figure 15).

![Figure 15. Number of colony-forming units (CFU) of propagules of *Cordyceps javanica* after exposure to 0.05% Silwet (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5), acetamiprid + bifenthrin (T6) and imidacloprid + bifenthrin (T7) A) Octane B) Pure aerial conidia C) Blastospores D) Submerged conidia. Means (±SE) followed by different letters indicate significant differences according to Tukey’s HSD test (P < 0.05)](image)

Subsequently, pure aerial conidia, in pure and formulated form (Octane), were subjected to 90 and 150 minutes of contact with three selected insecticides, ethiprole, methomyl, and imidacloprid + bifenthrin. In the formulated product (Octane), no statistical differences were observed among the various treatments and contact times (Figure 16A). Conversely, for pure aerial conidia, a significant difference was noted only between the contact times of 30, 90, and 150 minutes (P < 0.05) (Figure 16B).
5.4 Compatibility of *Cordyceps javanica* with insecticides on *Dalbulus maidis*

The compatibility of Octane (ESALQ 1296 isolate) based on *C. javanica* was tested in vivo with *D. maidis* adults. A statistically significant difference was observed among the various treatments (P > 0.05) (Figure 17A). Notably, treatments T6 (full dose of ethiprole + Octane) and all combinations of methomyl (T7, T8, T9, and T10) and imidacloprid + bifenthrin (T11, T12, T13, and T14) resulted in mortality close to or exceeding 89%. An evident synergistic effect was noted in T5 ($\chi^2 = 5.4$), while an additive effect was observed in T6 ($\chi^2 = 3.1$). This is evidenced by the fact that individual products failed to induce mortality beyond 65%, whereas the combined application achieved an impressive 86%.

Moreover, survival analysis unveiled a statistically significant distinction in LT$_{50}$ values, as T6 demonstrated a duration of 3 days in contrast to T4 (full dose ethiprole), which required five days. The LT$_{50}$ of T2 (Octane) exceeded that of 8 days, while T3 (½ dose ethiprole) and T5 (½ ethiprole + Octane) showed a 7-day; the remaining treatments resulted in a 1-day.

Additionally, sporulated insects confirmed the compatibility observed in the in vitro insecticide screening. Insects killed in treatments involving a combination of insecticide and Octane, as well as the commercial microbiological product isolated, sporulated despite the insecticides' presence (Figure 17B-C).
Figure 17. A) Corrected mortality of *Dalbulus maidis*, after exposure to 0.05% Silwet (T1), Octane (T2), ½ dose ethiprole (T3), full dose ethiprole (T4), ½ dose ethiprole + Octane (T5), full dose ethiprole + Octane (T6), ½ dose methomyl (T7), full dose methomyl (T8), ½ dose methomyl + Octane (T9), full dose methomyl + Octane (T10), ½ dose imidacloprid + bifenthrin (T11), full dose imidacloprid + bifenthrin (T12), ½ dose imidacloprid + bifenthrin + Octane (T13), full dose imidacloprid + bifenthrin + Octane (T14) B) Sporulated *Dalbulus maidis* treated with the commercial product Octane, insecticides and combinations. Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test of Sidak test (P < 0.05) C) Photos taken from sporulated *Dalbulus maidis*

Based on these results, the formulation of the spray solution with distilled water and its subsequent application to *D. maidis* was designed to investigate the hypothesis that including 0.05% Silwet L-77 amplifies the efficacy of the employed insecticides. The results indicated a difference between the treatments with insecticides prepared with distilled water and 0.05% Silwet L-77. Notably, for the insecticide ethiprole, a positive interaction between the adjuvant and treatments T3 (½ dose ethiprole) and T4 (full dose ethiprole) was observed (Figure 18). Additionally, this experiment also revealed again a synergistic effect between the ethiprole treatments and the commercial product Octane (T5), compared to the isolated agents (P < 0.05%). And the treatment T6 prepared with destilled water had a synergistic effect ($\chi^2 = 10.4$), different that observed with Silwet L-77.
For the insecticide methomyl, 0.05% Silwet L-77 also enhanced the insecticidal effect, especially in the treatments T3 (½ dose methomyl) (P > 0.05). Furthermore, the combination observed in treatment T5 (½ dose methomyl + Octane) prepared with distilled water exhibited an additive effect ($\chi^2 = 0.20$), causing higher mortality among the insects than when applied individually (Figure 18).

![Figure 18](image_url)

**Figure 18.** A) Corrected mortality of *Dalbulus maidis* after exposure to Octane (T2), ½ dose ethiprole (T3), full dose ethiprole (T4), ½ dose ethiprole + Octane (T5), full dose ethiprole + Octane (T6) with the solution prepared using distilled water (T1) B) The same treatments above with the solution prepared using 0.05% Silwet L-77 instead of water C) Corrected mortality of *Dalbulus maidis* after exposure to Octane (T2), ½ dose methomyl (T3), full dose methomyl (T4), ½ dose methomyl + Octane (T5), Full dose methomyl + Octane (T6) with the solution prepared using distilled water D) Treatments with the solution prepared using 0.05% Silwet L-77 instead of water Means (±SE) followed by different letters indicate significant differences according to the Scott-Knott test of Sidak test (P < 0.05)

The survival time for ethiprole prepared with water and Silwet L-77 remained consistent, featuring a notable LT$_{50}$ of 1 day for T6, as opposed to the prolonged durations of 5 days for T4, six days for T5, and seven days for T3, in contrast to more than eight days for the remaining treatments. In the case of methomyl, the survival time for T4, T5, and T6 prepared with distilled water and Silwet L-77 was also one day. The LT$_{50}$ exhibited variation only for T3 and T2 with water, which were two days and more than eight days for T2, respectively.
5.5 Bioassay with methomyl-resistant and non-resistant *Dalbulus maidis*

Experiments with methomyl-resistant insects were conducted to confirm their resistance and assess the combination's efficacy in another population of *D. maidis*. The results indicate a significant difference between the insect population from the Laboratory of Insect Vectors of Plant Pathogens breeding and the field-collected population from the Arthropod Resistance Laboratory (P < 0.05) (Figure 19).

In the methomyl-resistant population, the combination of Octane and the insecticide methomyl, observed in treatments T5 (½ dose methomyl + Octane) increased the mortality of *D. maidis* compared to the isolated agents, demonstrating a synergistic effect ($\chi^2 = 8.6$), and and T6 (full dose methomyl + Octane) had an additive effect ($\chi^2 = 1.37$). Additionally, T6 was the only treatment that caused 44% of dead insects compared to 8% of the same dose of methomyl isolated. The survival analysis revealed a significant difference (P < 0.05), in which the full dose of methomyl + Octane killed the insects rapidly, with LT$_{50}$ of 1 day, compared to the full dose of methomyl alone, with an LT$_{50}$ of 5 days.

In the population susceptible to methomyl, the mortality observed was visually higher. A additive effect was observed between T3 (½ dose methomyl) and T5 (½ dose methomyl + Octane), as the cumulative mortality with both agents was 77% compared to 47% of methomyl isolated. The survival analysis also revealed a significant difference (P < 0.05), that T3, T4, T5, and T6 killed the insects rapidly, with LT$_{50}$ of 1 day only.

**Figure 19.** A) Corrected mortality of non-methomyl-resistant (SUS) *Dalbulus maidis* after exposure to distilled water (T1), Octane (T2), ½ dose methomyl (T3), full dose methomyl (T4), ½ dose methomyl + Octane (T5) and full dose methomyl + Octane (T6) B) Methomyl resistant *Dalbulus maidis* after exposure to the same treatments
6 DISCUSSION

The discoveries presented in this study revealed the efficacy of blastospores from C. javanica in managing D. maidis. Additionally, our research introduces the production of submerged conidia based on a modified culture medium, representing their first reported effectiveness against the corn leafhopper. Despite these advancements, our study reveals significant resistance exhibited by the ESALQ 1296 isolate against the tested insecticides in tank mixtures. Notably, synergistic effects were observed between C. javanica and ethiprole. Furthermore, our observations shed light on the impact of combinations involving methomyl and C. javanica in controlling methomyl-resistant D. maidis, and supporting for the existing evidence of insecticide resistance within field populations.

The production of submerged conidia of C. javanica was achieved through media modification, resulting in a yield of $2.61 \pm 6.55 \times 10^8$ submerged conidia mL$^{-1}$. This production aligns with observations by Asaff et al. (2009), who, using NH$_4$NO$_3$ as a nitrogen source, produced submerged conidia of C. fumosorosea, reaching a concentration of $2 \times 10^8$ cells mL$^{-1}$. For B. bassiana, Iwanicki et al. (2023) reported yields ranging from $1.88 \times 10^8$ to $3.92 \times 10^8$ mL$^{-1}$. Thus, the optimization performed in the culture medium based on Esther et al. (2013) was satisfactory and can be adopted for submerged conidia production of C. javanica.

The compositional analysis also revealed that blastospores constituted less than 10% of the total propagule production in the submerged culture of C. javanica. In a similar context, Esther et al. (2013) reported a blastospore proportion of 15.6% in the submerged culture of C. fumosorosea. These proportions might be linked to comparable concentrations of organic nitrogen (N), carbon (C), or the C:N ratio (Thomas et al., 1987; Jackson; Jaronski, 2009). Therefore, further in-depth studies on the production of this propagule with ESALQ 1296 isolate may better elucidate the nutritional requirements of this isolate, considering it can vary according to the strain.

The medium proposed by Thomas et al. (1987) and Esther et al. (2013), modified with NS3 and NS2, resulted in pronounced hyphal growth, potentially linked to variations in the C:N ratio or the presence of elevated sugar concentrations in the culture medium. On the other hand, the deformities observed in the medium with NS3, could be associated with osmotic pressure, as it plays a critical role in submerged fermentation, directly impacting the morphology of the propagules (Kobori et al., 2015; Mascarín et al., 2023).
Among *C. javanica* propagules, blastospores caused a corrected mortality of 40 ± 3.0% against *D. maidis*, more rapidly than aerial conidia. This has also been observed in *C. fumosorosea* and *B. bassiana* controlling *B. tabaci*, *S. frugiperda*, and *Chrysodeixis includens* (Jackson et al., 1997; Côrrea et al., 2020). In *M. anisopliae*, also demonstrated that blastospores are more virulent than aerial conidia (Kleespies; Zimmermann, 1994). This result supports the hypothesis that blastospores exhibit quicker lethality against adults of the corn leafhopper than other propagules. This expectation was based on the faster germination of blastospores on the integument surface of the host (Vega et al., 1999).

The performance of submerged conidia was similar to that of pure and formulated aerial conidia, causing a mortality of 22 ± 1.9%. The expectation was that this propagule would cause mortality equal to or greater than blastospores, as the virulence of submerged conidia of *B. bassiana* and *C. fumosorosea* has been demonstrated previously, causing mortality exceeding 75% against *Anthonomus grandis* and *Galleria mellonella* (Esther et al., 2013; Iwanicki et al., 2023). This result may indicate that the isolate is not truly promising in controlling corn leafhoppers or that the modification in the culture medium, such as some unmet nutritional need, influences the virulence of *C. javanica* (Ali et al., 2009).

A difference in virulence has been identified among the various types of propagules of *C. javanica*. In this context, it is noteworthy to consider the emerging trend in the biopesticide market, indicating the necessity to conduct studies with blastospores during the screening of isolates for pest control. As previously demonstrated, this propagule type can induce faster mortality, more effectively meeting the requirements for vector insect control. Additionally, it underscores the importance of testing different isolates of the same fungal species, as virulence also varies according to the characteristics of each isolate. Taking the example of *B. bassiana* ESALQ 3366 yielded a corrected mortality rate of 64 ± 2.1%, with an LT$_{50}$ of 8 days. The isolate IBCB 66 is registered with MAPA for *D. maidis* control, and field data indicates a 37% mortality rate (Ribeiro Junior, 2020). In laboratory conditions, another *B. bassiana* isolate (CEP 147) caused 49.9% mortality after 14 days, with an LT$_{50}$ of 8.6 days (Toledo et al., 2007). Thus, different isolates can achieve different mortality rates. And comparing these results with *C. javanica* ESALQ 1296, the *B. bassiana* ESALQ 3366 demonstrated significant promise for controlling corn leafhopper.

Although the results for various propagules of *C. javanica* did not reach the same potential demonstrated by the *B. bassiana* isolate, an alternative would be their simultaneous
use with chemical insecticides to enhance the effectiveness of biological control. In this regard, compatibility studies were required to ensure that the insecticide components do not interfere with the development of the microorganism. Therefore, in vitro experiments demonstrated that the insecticide Ethiprole had the most negligible impact on the germination and growth of aerial conidia, blastospores, and submerged conidia of *C. javanica*. Ribeiro et al. (2023) classified it as one of the most compatible with the ESALQ 1296 isolate after assessing germination, CFU, conidia production, and colony area. Initially, they noted a significant impact on germination after 1.5 hours of contact, but in other evaluated parameters, there was no pronounced negative effect. However, despite the method being very similar, in this present study, germination remained above 90%, even after 150 minutes of contact, differing from the germination results they obtained.

Methomyl significantly reduced the viability of ESALQ 1296 after 150 minutes of contact but had no impact on the number of colonies (CFU). One possible explanation is that the direct germination count considers observations made after 16 hours of incubation. It is well-documented that certain active ingredients can initially delay the germination of EF (Silva et al., 2013; Muthabathula; Biruduganti, 2022). Therefore, while some insecticides may have affected germination in the early hours, there was no lasting effect. Von Nowakonski (2019) evaluated compatibility after seven days of incubation, and methomyl didn't notably affect germination, reducing it to 92.9%. Based on vegetative growth, viability, and conidiogenesis, the same insecticide was considered toxic to *B. bassiana* (Tamai et al., 2002; Duarte et al., 2016). These varying levels of compatibility can be explained by the different species tested (Alves, 1998).

Imidacloprid + bifenthrin significantly impacted the germination of propagules, reducing it to approximately 77% after 150 minutes of contact. However, similar to methomyl, it did not exert a lasting impact on the number of colonies (CFU). In the literature, bifenthrin has been acknowledged as toxic to some isolates of *C. javanica*, causing a pronounced effect on germination (Sain et al., 2022). Lastly, the insecticide acetamiprid + bifenthrin adversely affected the colony count in aerial conidia CFU, aligning with the result obtained by Ribeiro et al. (2023), who classified it as incompatible with the ESALQ 1296 isolate.

The insecticides selected in the in vitro screening followed specific criteria, such as minimal impact on different propagules of *C. javanica* and the fact that some of them exhibited a germination rate higher than the control in specific experiments, like the insecticide ethiprole.
This observation raised the hypothesis that there might be a stimulating effect of the insecticide favoring the fungus's germination, as reported by Quintela et al. (1996), who demonstrated that certain components of formulated imidacloprid stimulated the rate and amount of conidial germination on the insect body.

The fact that the insecticides showed low toxicity to the ESALQ 1296 isolate may be related to the ability of *C. javanica* to degrade compounds for development and reproduction (Alves, 1998). A study evaluated the expression pattern of EF genes in the presence of insecticides. In the presence of pyrethroid, deltamethrin, and cypermethrin insecticide, genes from the catalase family showed the highest induction, suggesting their involvement in the fungal antioxidant response and degrading insecticides potential (Forlani et al., 2014; Muthabathula; Biruduganti, 2022). The results revealed the potential of EF-degrading insecticides, which may occur with *C. javanica*, since the ESALQ 1296 isolate was not affected by the chemical molecules.

Overall, the most resilient propagules were the submerged conidia and aerial conidia. This fact can be attributed to these propagules composition and cellular structure, rendering them more resistant to factors such as UV radiation and temperature (Bidochka et al., 1995; Bernardo et al., 2020). The results challenge the initial hypothesis that blastospores and submerged conidia would be more susceptible; however, despite minimal effects, and none of the tested insecticides had a severe impact on these propagules.

*C. javanica* showed minimal susceptibility to the impact of the tested insecticides. One plausible assumption is that the formulation of the biological product, which is based on oil suspension, might have interacted unfavorably with the formulations of the insecticides, resulting in a relatively low level of compatibility between them (Jones; Burges, 1998). Notwithstanding, it is noteworthy that nearly all the insecticides tested demonstrated compatibility with *C. javanica* ESALQ 1296. This underscores its potential for integration into corn leafhopper control strategies.

Synergistic and additive effects were observed with the combination of methomyl and ethiprole with Octane, resulting in increased pest mortality and a shorter time for their demise compared to using each agent alone. The doses of these insecticides induced stress, compromising the immune system of *D. maidis* and rendering them more susceptible to *C. javanica* (Sharififard et al., 2011). This strategy, reported in the literature with different active ingredients, proves valuable IPM, reducing the quantity of chemicals used (Santos et al., 2018). Synergism between imidacloprid and *M. anisopliae, C. fumosorosea*, and *B. bassiana* has been observed in the control of *Diaprepes abbreviatus, B. tabaci*, and *Culex quinquefasciatus,*
respectively (Quintela; McCoy, 1998; Zou et al., 2014). Notably, Quintela and McCoy (1998) also observed an increase in mycosis in this association between insecticide and EF, which did not occur in the present study.

To further enhance the biological effect, in addition to the potential of the combined use of biological and chemical control, the use of adjuvants can also provide advantages. A significant positive effect of Silwet L-77, compared to distilled water, was observed, indicating a synergistic interaction between these adjuvants and insecticides, enhancing their overall effectiveness. Adjuvants are frequently utilized to optimize microbiological product formulations, stabilize the pathogen, aid in product application, protect the fungus from environmental factors, and increase pathogenic activity (Jones; Burges, 1998; Knowles, 2008). In addition, Silwet has been reported to enhance the efficacy of the isolate ESALQ 1296 controlling D. citri (Arnosti et al., 2018).

These adjuvants are effective on the hydrophobic insect integument, facilitating the distribution and germination of conidia on the insect surface and optimizing the fungal action (Peng; Xia, 2011). As Arnosti et al. (2019) observed, adjuvants have an effect on the insect cuticle, such as the reduction of lipids and other substances, making insects more susceptible to colonization by EF. Therefore, it occurred with the combination of Silwet and insecticides, promoting the spreading and wetting of the insect's body, increasing mortality. Silwet L-77 can be incorporated into IPM strategy to enhance the success of C. javanica in association with the insecticides ethiprole and methomyl controlling D. maidis.

Variability in susceptibility to insecticides has been documented in populations (Siqueira et al., 2000). Notably, a significant difference was observed between the laboratory-bred insect population and the field-collected population. It is widely acknowledged that laboratory populations tend to be more susceptible to insecticides than their counterparts in the field (Baliota et al., 2022). D. maidis population collected in Bahia showed resistance to methomyl in preliminary trials. Methomyl is an insecticide that acts on the nervous system and musculature, inhibiting acetylcholinesterase (Irac, 2023; PubChem, 2023). This insecticide prevents the enzyme acetylcholinesterase from binding to acetylcholine, resulting in an accumulation in the synapse. This effect causes hyperexcitability due to the continuous and uncontrolled transmission of nerve impulses, leading to muscle paralysis, hindering respiration, and ultimately causing death (Gepner et al., 1978).
The combination of Octane and methomyl significantly increased the mortality of methomyl-resistant *D. maidis* compared to the isolated agents, demonstrating a synergistic effect. This approach is a crucial strategy for managing insecticide-resistant pests, making them more susceptible to microbial infection (Rice; Furlong, 2023). The potential efficacy of blending blastospores with insecticides could enhance mortality, especially considering that this propagule exhibited the highest virulence. Further studies are imperative to confirm the use of blastospores with methomyl, validating and optimizing the effectiveness of this integrated approach, including field studies. This paves the way for a more sustainable pest control strategy.
7 CONCLUSION

In conclusion, the study highlights the remarkable efficacy of blastospores as the most effective propagule in controlling *D. maidis*, demonstrating superior mortality rates and a shorter LT$_{50}$ compared to other propagules. *C. javanica* (ESALQ 1296) resisted the tested insecticides in tank mixtures. The incorporation of Silwet L-77 further enhanced the impact of certain insecticides, presenting an opportunity for its inclusion in an IPM strategy to bolster the success of *C. javanica* in conjunction with insecticides for corn leafhopper control.

The strategic combination of *C. javanica* with ethiprole and methomyl showcased a synergistic and additive effects, leading to a remarkable escalation in *D. maidis* mortality. This synergistic interaction is particularly valuable in the context of managing insect populations that have developed resistance to traditional control methods. Importantly, even following exposure to the insecticides, the insects demonstrated sporulation. This resilience underscores the potential effectiveness of this integrated approach in addressing the challenges posed by resistant insect populations.
REFERENCES


ALVES, S. B. *Controle microbiano de insetos*. FEALQ, 1998. 1163p


CRUZ, I.; VIANA, P. A.; WAQUIL, J. M. Manejo de pragas iniciais de milho mediante o tratamento de sementes com inseticidas sistêmicos. Embrapa Milho e Sorgo, 1999. 41p (Circular Técnica 31)


DA SILVA, R. Z.; NEVES, P. M. O. J. Techniques and parameters used in compatibility tests between Beauveria bassiana (Bals) Vuill and in vitro phytosanitary products. Pest Management Science, v. 61, n. 7, p. 667-674, 2005. DOI: https://doi.org/10.1002/ps.1035


FARIA, R. D. Resistência de genótipos de milho Bt e não-Bt a *Dalbulus maidis* (Hemiptera: Cicadellidae) e molícutes. Faculdade de Ciências Agronômicas, 2020. 77p (Dissertações de Mestrado - Proteção de Plantas)


GEPNER, J.; HALL, L.; SATTELTE, D. Insect acetylcholine receptors as a site of insecticide action. *Nature*, v. 276, p. 188–190, 1978. DOI: https://doi.org/10.1038/276188a0


IRAC. Comitê de Ação à Resistência a Inseticidas: Brasil. 2023. <https://www.irac-br.org/modo-de-acao>


MALUTA, N.; CASTRO, T.; LOPES, J. R. S. Entomopathogenic fungus disrupts the phloem-probing behavior of *Diaphorina citri* and may be an important biological control tool in citrus. *Scientific Reports*, v. 12, n. 7959, 2022. DOI: https://doi.org/10.1038/s41598-022-11789-2


MUTHABATHULA, P.; BIRUDUGANTI, S. Analysis of biodegradation of the synthetic pyrethroid cypermethrin by *Beauveria bassiana*. *Current Microbiology*, v. 79, n. 46, 2022. DOI: 10.1007/s00284-021-02744-x


ZIMMERMANN, G. The entomopathogenic fungi Isaria farinosa (formerly Paecilomyces farinosus) and the Isaria fumosorosea species complex (formerly Paecilomyces fumosoroseus): biology, ecology and use in biological control. Biocontrol Science and Technology, v. 18, n. 9, p. 865-901, 2008. DOI: https://doi.org/10.1080/09583150802471812


### APPENDICES

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Appendix A. Representative images of the direct germination count (viability) of different propagules of *Cordyceps javanica* after exposure to 0.05% Silwet L-77 (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5) and imidacloprid + bifenthrin (T7) per 30 minutes.
**Appendix B.** Representative images of colony forming units (CFU) of different propagules of *Cordyceps javanica* after exposure to 0.05% Silwet L-77 (T1) and insecticides acetamiprid + fenpropathrin (T2), β-cyfluthrin + imidacloprid (T3), ethiprole (T4), methomyl (T5), acetamiprid + bifenthrin (T6) and imidacloprid + bifenthrin (T7) per 30 minutes.