Topics in

Agricultural Entomology XIII

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Edimar Peterlini
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Sandy Sousa Fonsêca
Vinicius Ferraz Nascimento
Ricardo Antonio Polanczyk

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www.atenaeditora.com.br
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PREFACE

The Graduate Program in Agronomy (Agricultural Entomology) at the UNESP Faculty of Agricultural and Veterinary Sciences in Jaboticabal has always been characterized by its focus on Integrated Pest Management (IPM). Since its foundation, the program has graduated 287 students with a master's degree and 148 Ph.D. students. They are now active in various areas of the public or private sector and contribute to agriculture's economic and environmental sustainability.

This e-book entitled "Topics in Agricultural Entomology - XIII" was made possible through the immense effort of the Organizing Committee, formed by MSc and Ph.D. students from all research areas of our Graduate Program. In its 14 chapters, readers will find information on the most diverse areas of IPM, with a richness of information on both the fundamental and applied aspects of IPM.

As coordinator of the 2022 edition of the Winter Workshop on Agricultural Entomology, it is my pleasure to provide event attendees with an e-book of excellent content, demonstrating the importance of our research to society.

Prof. Ricardo Antônio Polanczyk

FCAV/UNESP

PPG Entomologia Agrícola Coordinator

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CAPÍTULO 13

ENDOPHYTIC ENTOMOPATHOGENIC MICROORGANISMS IN PEST MANAGEMENT

Lana Leticia Barbosa de Carvalho

Fabiana Santana Machado

Ricardo Antônio Polanczyk

1 I INTRODUCTION

Endophytic microorganisms were first mentioned in the early 19th century, but only at the end of the 20th century they became more relevant in scientific research. Microorganisms were believed to be neutral, that is, cause no benefits or harm to plants. However, over the years the benefits from plant-microorganism interaction, have been discovered and, in many cases, playing an important role in plant protection and development (Azevedo et al., 2000; Santos & Varavallo, 2011).

Currently, endophytic microorganisms are widespread in academia and have been used to improve agriculture, such as: phosphate solubilization, plant nutrition, plant growth promotion, among other advantages that are being discovered every day. Among the advantages of plant-microorganism interaction, pest control with endophytic entomopathogenic microorganisms is a technology in increasing development.

Two entomopathogenic microorganisms most used endophytically in research are fungi and bacteria. Both can penetrate plant tissues and establish a symbiotic relationship. The activation of plant mechanisms when in contact with these microorganisms can increase tolerance to different biotic and abiotic stresses.

Bacteria and plants have beneficial relationships. Through endophytic interaction, bacteria help plant growth and development, in addition to acting as pest and disease control agents (Kloepper et al. 1992; Barka et al. 2002; Mcgee, 2002). Biological control by endophytic bacteria has been widely studied and appears as an alternative to excessive use of chemicals in cropping areas (Pozzebon & Santos, 2016).

Fungi are the most studied entomopathogenic microorganisms used endophytically. Their implications and uses, ranging from growth promotion to plant protection against pathogens, stem from their promising results in insect control studies. Entomopathogenic species such as *Beauveria bassiana*, *Metarhizium* spp., and *Isaria fumosorosea* have been reported to have high pathogenic potential in insects when inoculated into plants (Mantzoukas & Lagogiannis, 2019).

Most studies on entomopathogenic endophytic fungi have focused on chewing pests. However, research on sucking insect pests has gained notoriety, mainly with fungus of the species

B. bassiana. Tests with endophytic entomopathogenic fungi have already been carried out for aphids, mealybugs, leafhoppers, whiteflies, and bed bugs of the Pentatomidae family (Sword; Tessnow & Ek-Ramos, 2017; Jaber; Araj & Qasem, 2018; Rondot & Reineke, 2018; Gonzalez-Mas et al., 2019).

In this chapter, some topics related to endophytic entomopathogenic microorganisms used for pest control will be addressed. We will begin by discussing the two most widespread microorganisms in agriculture, entomopathogenic bacteria and fungi, and their endophytic applications. We will also argue about sucking insect control by endophytic entomopathogenic fungi. Finally, we will provide a brief overview of perspectives on the use of plant colonization by entomopathogenic fungi for insect pest control.

2 I ENDOPHYTIC ENTOMOPATHOGENIC BACTERIA

Bacteria are inhabitants of both internal and external tissues of most plants (Xie et al., 2020). To be endophytic, when colonizing internal plant tissues, they cannot have any negative effect on plants (Joo et al., 2021). An endophytic colonization provides a protective environment for microorganisms against ultraviolet radiation, scarcity of nutrients, excess rainfall, and extreme temperature fluctuations (Silva et al., 2006; Francis et al., 2010).

Likewise, several natural plant processes are improved by interactions with endophytic bacteria, inducing production of secondary metabolites, especially defense-related phytohormones such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Lazebnik et al., 2014), which are responsible for activation of acquired (ASR) and induced (ISR) systemic resistance pathways (Filho et al., 2010).

Bacteria of the *Enterobacter*, *Bacillus*, *Methylobacterium*, *Agrobacterium*, *Serratia*, *Acinetobacter*, *Arthrobacter*, and *Pseudomonas* genera are the most commonly microorganisms endophytically associated with plants. Baseline studies demonstrated *Bacillus thuringiensis* isolates colonizing cotton plant tissues (Monnerat et al., 2003). Praça et al. (2012) proved that *B. thuringiensis* isolates can act as endophytic organisms in cabbage plants, with a toxic effect against *Plutella xylostella* (Lepidoptera: Plutellidae). Macedo et al. (2012) selected and characterized native isolates of *B. thuringiensis* and observed that three of them caused mortality of above 75% in *Diatraea saccharalis* (Lepidoptera: Pyralidae).

In this sense, endophytic bacteria may act as biological control agents, protecting the host through synthesis of antimicrobial molecules and induction of ISR (Compant et al., 2010). These microorganisms can also produce toxins that play a role in insect control, becoming a great alternative for producers while reducing the use of agrochemicals, and hence environmental contamination (Pozzebon & Santos, 2016).

3 I ENDOPHYTIC ENTOMOPATHOGENIC FUNGI

Entomopathogenic fungi have been widely used as bioinsecticides, and a new line of research on endophytic species has been intensifying. These fungi can naturally colonize different host-plant tissues, including roots, stems, leaves, reproductive systems, and fruits (Baron & Rigobelo, 2022). Still, successful attempts to artificially introduce entomopathogenic fungi into plants have already been reported in several studies (Vega et al., 2008; Vega, 2018).

Application of entomopathogenic fungi via aerial conidia is advantageous compared to other methods. Among the benefits are the infection via insect integument, low risks of environmental imbalance and toxicity to humans and invertebrates, besides high capacity/reach of colonization in plants. Such advantages can be intensified when the fungi are endophytic. Examples of that include pest control by inducing systemic resistance and production of secondary metabolites, as well as promotion of plant growth by improving nutrition, producing siderophores and phytohormones, increasing photosynthetic efficiency, and relieving abiotic stresses (e.g., salinity).

4 I ENDOPHYTIC ENTOMOPATHOGENIC FUNGI FOR BIOLOGICAL CONTROL OF INSECT PESTS

Endophytic colonization of entomopathogenic fungi (Ascomycota, Hypocreales) can be beneficial to plants as it reduces pest infestation in economically important crops (Klieber & Reineke, 2016). Plant defense mechanism against insect and mite pests is not fully known yet. However, a few studies have reported systemic induction of resistance and production of secondary metabolites as causes of pest control by endophytic fungi.

Studies have shown that entomopathogenic fungi secrete chemical molecules into host plants soon after colonization. An initial plant response is to produce secondary metabolites, including alkaloids, flavonoids, and phenolic compounds to combat potential pathogenic threats (Zaynab et al., 2018).

After fungal infection, plant-secreted secondary metabolites are at first a barrier to colonization by endophytic entomopathogenic fungi. To overcome this obstacle, these fungi produce detoxification and degradation enzymes such as -1,3-glucanases, chitinases, laccases, cellulases, and amylases. Endophytes produce a variety of species-specific secondary metabolites of bioactive structure (e.g., phenolic acids, benzopyrones, quinones, and steroids) (Tan & Zou, 2001), which are widely used as agrochemicals, antibiotics, antiparasitic, and antioxidants.

Fungal secondary metabolites are also used for signaling, defense, or interacting with the host plant. They may also influence the host plant secondary metabolite profile and directly influence pathogen attack (Baron & Rigobelo, 2022). In this way, these fungi can improve the self-defense system of plants by activating systemic resistance pathways that protect them against pathogens and pests (Chadha et al., 2015). Endophytic colonization may have a priming effect, preparing plants for new infections by entomopathogenic microorganisms (Latz et al., 2018).

5 I ROLE OF ENDOPHYTIC ENTOMOPATHOGENIC FUNGI IN PLANT GROWTH PROMOTION

Endophytic entomopathogenic fungi have been used to improve plant growth (Akutse et al., 2013). According to the literature, plant growth is promoted due to the activation of mechanisms by endophytic entomopathogenic fungi, such as: improvement in plant nutrition, production of phytohormones, increase in photosynthetic efficiency, etc.

Endophytic entomopathogenic fungi can improve macronutrient or micronutrient uptakes from organic matter and increase their supply to host plants (Rana et al., 2020). For instance, Behie & Bidochka (2014) found that five *Metarhizium* species and *B. bassiana* can kill insect larvae in endophytically colonized plants, and transfer nitrogen from insects to plants. Numerous reports in the literature have highlighted an improvement in phosphorus uptake by fungal inoculation due to endophytic interactions (Ortega-García et al., 2015; Baron et al., 2018).

Although little explored, endophytic entomopathogenic fungi can also promote plant growth by stimulating phytohormone production. Rivas-Franco et al. (2020) verified corn colonised endophytically by *M. anisopliae* show increased levels of certain phytohormones. Among the most produced phytohormones are auxins (Aux), gibberellins (GAs), and cytokinins (CKs).

The main auxin produced by endophytic fungi is indole-3-acetic acid (IAA). It is a plant growth regulator and has several positive effects on shoot and root developments (Jaroszuk-Ściseł; Kurek & Trytek, 2014). Gibberellins are other hormones secreted by endophytic fungi that are essential for various plant processes, including seed germination, stem elongation, sexual expression, flowering, fruit formation, and senescence (Bömke & Tudzynski, 2009).

Endophytic entomopathogenic fungi have also been reported to influence plant photosynthetic rate. Moustaka, Meyling & Hauser (2021) noted that *Metarhizium brunneum* and *Beauveria bassiana*-inoculated plants tend to have higher photosynthetic rates than those non-inoculated, mainly after an insect pest attack. Photosynthetic rate is known to directly affect crop yields and determine plant growth and development.

6 I CONTROL OF SUCKING INSECTS BY ENDOPHYTIC ENTOMOPATHOGENIC FUNGI

Studies in the literature on endophytic entomopathogenic fungi, such as *Beauveria bassiana* and different *Metarhizium* species, are regularly used to control lepidopteran or coleopteran pests. Yet, new researches have been using them to control sucking insects, mainly due to a lack of biological products registered against these pests.

Aphids (Hemiptera, Aphididae) are the most studied sucking pests using endophytic entomopathogenic fungi. These insects directly affect plants by sap suction, damaging the final product. Indirect damages such as injection of toxins or acting as disease vectors are also observed; therefore, more punctual control methods are required, such as the use of pathogenic plants.

Aphis gossypii is an efficient vector of cucumber mosaic and cucurbit aphid-borne yellow viruses. Endophytic colonization of melon plants with *B. bassiana* promoted decreases in *A. gossypii* feeding and virus inoculation rates *(Gonzalez-Mas et al., 2019)*. Thus, in addition to controlling *A. gossypii* (Lopes et al., 2014) *B. bassiana* may reduce virus transmission by aphids and be used in integrated pest management (IPM) programs.

Although *B. bassiana* is the most tested entomopathogenic fungus for endophytic use, other species have been widely described in the literature. Mantzoukas & Lagogiannis (2019) reported mortality rates of green peach aphid (*Myzus persicae*) above 80% in sweet pepper (*Capsicum annum*) inoculated with *B. bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea*.

Rondot & Reineke (2018) evaluated inoculation of grapevines with *B. bassiana* and had promising results against two piercing-sucking insects; they observed infestation reductions for vine mealybug (*Planococcus ficus*) in semi-field tests and grape spittlebug (*Empoasca vitis*) in a field vineyard.

The use of endophytic entomopathogenic fungi has also shown to be efficient in controlling whitefly (*Bemisia tabaci*), especially when combined with other strategies. The combined application of these microorganisms and plant extracts, such as *Calotropis procera*, has an additive effect on mortality at almost all developmental stages of that insect (Jaber; Araj & Qasem, 2018).

Despite the agriculture damages caused by bed bugs of the Pentatomidae family, few are the reports in the literature concerning the use of endophytic entomopathogenic fungi to control such pests. However, this endophytic technology might be extremely efficient, as already observed by Sword, Tessnow & Ek-Ramos (2017). These authors noted the feeding preference of bed bugs for cotton plants not inoculated with *B. bassiana*. Behavioral responses indicated that insects were repelled before contacting with plant tissues of colonized-plants, highlighting the crucial role of volatile compounds in mediating negative responses (Sword; Tessnow & Ek-Ramos, 2017).

7 I PERSPECTIVES ON THE USE OF PLANTS COLONIZED BY ENDOPHYTIC ENTOMOPATHOGENIC MICROORGANISMS IN PEST CONTROL

Studies on the application of endophytic entomopathogenic agents in pest control have intensified in recent years. This increase in interest is mainly due to the positive results of research on the plant-host relationship for pathogen control. Besides the interest in the pathogenic potential of these endophytic microorganisms, several potential implications, such as plant growth promotion, have also raised lots of interest by researchers.

As discoveries advance, endophytic fungi and bacteria have become new biological resources. These microorganisms have great research value and broad development prospects, opening doors not only for pest control, but also for plant development and crop productivity improvement (Xie et al., 2020).

Throughout the text, some cases of use of this technique for pest control were reported; however, it is not yet used commercially on a large scale directly in agriculture. Despite the numerous findings about mechanisms of this interaction and reaction of plants to pathogen attacks, there is still much to be studied and revealed about this symbiotic relationship.

Modern procedures, such as genetic, physiological, and semiochemical techniques, among others, have allowed to better understand how such microorganisms can benefit plants, especially when attacked by pathogens. Accordingly, research on the use of these microorganisms in pest control tends to intensify increasingly. Furthermore, the search for answers on how the technique works and can be applied will create possibilities for the use of endophytic microorganisms on a large scale in agriculture, mainly due to current demands for more sustainability and reduction of agricultural inputs.

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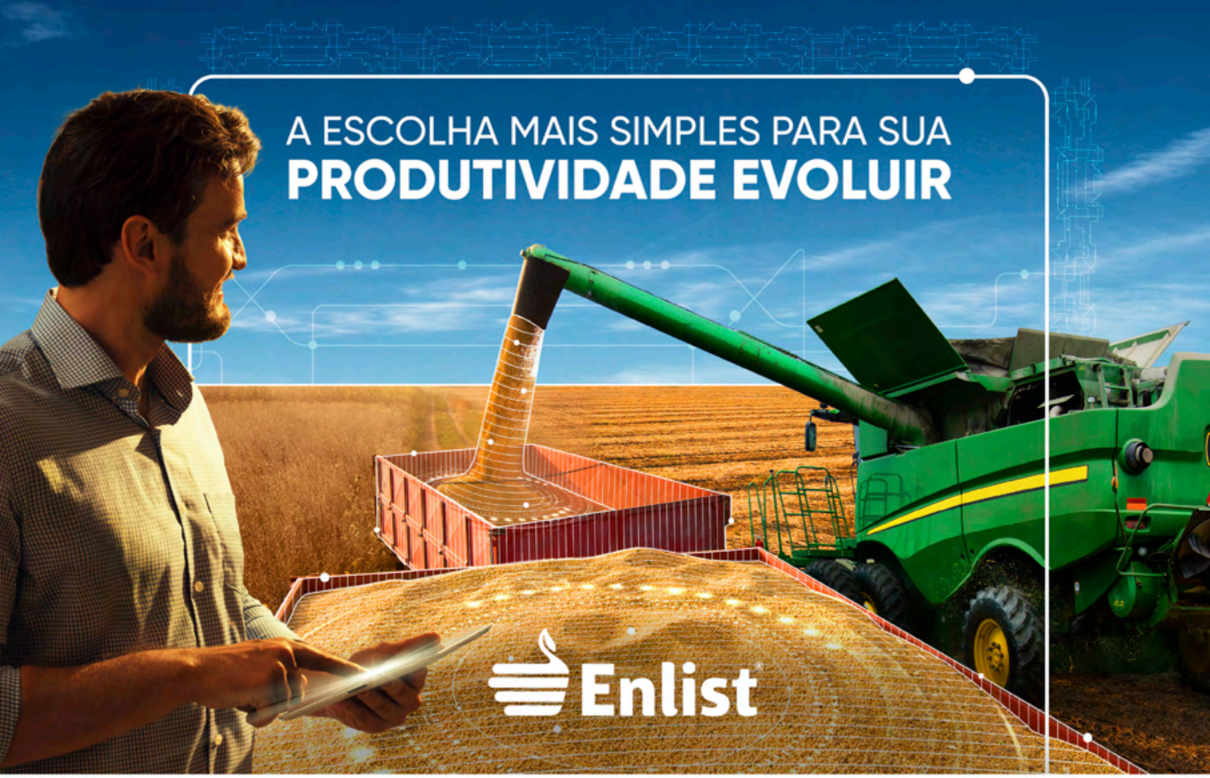

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GENÉTICA DE ALTA PERFORMANCE

Com variedades altamente produtivas dos principais parceiros

Enlist® Certo
Boas Práticas Agrícolas

MAIS FACILIDADE PARA ALTA PRODUTIVIDADE

MAIOR CONTROLE
Redução no potencial de deriva e ultrabaixa volatilidade na aplicação, além da proteção contra lagartas.

CONVENIÊNCIA
Redução de odor, proporcionando comodidade aos aplicadores e comunidades vizinhas.

DIVERSIDADE
Sementes tolerantes aos herbicidas Enlist® Colex-D® (novo 2,4-D sal colina), glifosato e glufosinato.

FLEXIBILIDADE
Compatível com diversos sais de glifosato, de acordo com a bula.

Os eventos de soja transgênica contidos nas variedades de sojas Enlist E3® e Conkesta E3® são desenvolvidos e pertencem conjuntamente à Corteva Agriscience e à M.S. Technologies L.L.C.
Enlist® Colex-D® deve ser usado em dessecação da soja, em pré-plantio (aplique/plante) e em pós-emergência das sojas Enlist E3® e Conkesta E3®.

ATENÇÃO PRODUTO PERIGOSO À SAÚDE HUMANA, ANIMAL E AO MEIO AMBIENTE; USO AGRÍCOLA; VENDA SOB RECEITUÁRIO AGRONÔMICO; CONSULTE SEMPRE UM AGRÔNOMO; INFORME-SE E REALIZE O MANEJO INTEGRADO DE PRAGAS; DESCARTE CORRETAMENTE AS EMBALAGENS E OS RESTOS DOS PRODUTOS; LEIA ATENTAMENTE E SIGA AS INSTRUÇÕES CONTIDAS NO RÓTULO, NA BULA E NA RECEITA; E UTILIZE OS EQUIPAMENTOS DE PROTEÇÃO INDIVIDUAL.

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