

University of São Paulo
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Semiochemicals involved in the sugarcane-*Diatraea saccharalis*-opportunistic
fungi interaction

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Thesis presented to obtain the degree of Doctor in
Science. Area: Entomology

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RESUMO

Semioquímicos envolvidos na interação cana-de-açúcar-*Diatraea saccharalis*-fungos oportunistas

Interações entre planta, inseto e microrganismos no ambiente revela uma miríade de simbioses estratégicas. Por exemplo, semioquímicos (i.e. feromônios e voláteis de plantas) têm mostrado um papel decisivo nessas interações associadas a metabólitos voláteis e não voláteis. As alterações dos semioquímicos induzidas por microrganismos resultam em uma maior atração de insetos vetores para a planta, ou mudanças no comportamento sexual dos insetos e, portanto, aumento na dispersão de patógenos, comumente relatado em interações envolvendo bactérias, vírus e arqueas. Diferentemente dos patógenos transmitidos por vetores, os patógenos oportunistas presumivelmente não têm uma associação íntima com o inseto. *Fusarium verticillioides* e *Colletotrichum falcatum* são a muito tempo reportados como fungos oportunistas, utilizando das aberturas deixadas pela herbivoria de lagartas de *Diatraea saccharalis* para infectar a planta. No entanto, os semioquímicos envolvidos nesta interação ainda são desconhecidos. Diante do exposto, a presente pesquisa de doutorado aborda investigações que visam melhor compreender os semioquímicos envolvidos na interação cana-de-açúcar-*D. saccharalis*-fungos oportunistas. Aqui estabelecemos um novo papel para a associação inseto-fungo-cana-de-açúcar. Inicialmente, abordamos a identificação química da infecção por fungos nas defesas das plantas e a escolha da preferência do hospedeiro e, conseqüentemente, os benefícios do hospedeiro para a prole. Avaliamos o papel de *D. saccharalis* como vetor de disseminação do fungo *F. verticillioides*, e os possíveis efeitos da alimentação do fungo na biologia e no sucesso reprodutivo de *D. saccharalis*. Abordamos especificamente se o fungo modifica o comportamento de *D. saccharalis* de forma a aumentar a probabilidade do fungo ser disseminado por insetos contaminados. Enquanto *C. falcatum* mostrou um efeito neutro na associação com *D. saccharalis*, curiosamente, nossos resultados mostraram que os voláteis de plantas infectadas com *F. verticillioides* atraem fêmeas de *D. saccharalis* para oviposição. Além disso, o fungo *F. verticillioides* é transmitido verticalmente à sua descendência, que continua o ciclo inoculando o fungo em plantas saudáveis. As fêmeas contaminadas pelo fungo preferem colocar seus ovos em plantas mock quando comparadas a plantas infectadas pelo fungo *F. verticillioides*. Em seguida, nos concentramos na interação envolvendo apenas *D. saccharalis* e *F. verticillioides*. Nossos dados demonstram que *F. verticillioides* manipula tanto a planta hospedeira quanto o inseto herbívoro ao longo do ciclo de vida, para promover sua infecção e disseminação, formando associações mutualísticas, mas inerentemente egoístas, com a planta e inseto.

Palavras-chave: Interação inseto-planta-microrganismo, *Fusarium verticillioides*, *Colletotrichum falcatum*, Voláteis de plantas, Feromônio

ABSTRACT

Semiochemicals involved in the sugarcane-*Diatraea saccharalis*-opportunistic fungi interaction

The interaction between plant, insect and microorganisms in environment reveal myriad strategies of symbioses. For instance, semiochemicals (i.e., pheromone and plant volatiles) have played a decisive role in these interactions associated with volatile and non-volatile metabolites. The changes in semiochemicals induced by microorganisms result in the increased attraction of insect vectors to the plant, or changes in sexual behavior of insects and, hence, to increased pathogen dispersal, commonly reported to bacteria, viruses, and archaea interactions. Different from vector-borne pathogens, opportunistic pathogens presumably do not have an intimate association with the insect. It has long been assumed that *Fusarium verticillioides* and *Colletotrichum falcatum* are opportunistic fungi, where it takes advantage of the openings left by *Diatraea saccharalis* attack to infect the plant. However, the semiochemicals involved in this interaction are still largely unknown. Given the above, the present doctoral research approaches investigations to better understand the semiochemicals involved in sugarcane-*D. saccharalis*-opportunistic fungi interaction. Here, we establish a new paradigm for the insect-fungi-sugarcane association. Initially, we addressed the chemical identification of fungi infection in plant defenses, host preference choice, and offspring benefits. We also evaluated the role of *D. saccharalis* as a dissemination vector for *F. verticillioides* and the possible effects of *F. verticillioides* feeding on *D. saccharalis* biology and reproductive success. Finally, we specifically tackled whether the fungus modifies *D. saccharalis* behavior in ways that increase the likelihood of the fungus being disseminated by contaminated insects. Interestingly, our results showed that volatile emissions from *F. verticillioides*-infected plants attract *D. saccharalis* adults to lay eggs while *C. falcatum* has a neutral effect against *D. saccharalis*. The *F. verticillioides* is transmitted vertically to their offspring, which continues the cycle by inoculating the fungus into healthy plants. Then females carrying the fungus prefer to lay their eggs on mock plants than fungus-infected plants. Lather, we focus on *D. saccharalis*-*F. verticillioides* interaction. Our data demonstrate that *F. verticillioides* manipulate both the host plant and insect herbivore across the life cycle to promote its infection and dissemination, forming mutualistic but inherently selfish alliances with plants and insects.

Keywords: Insect-plant-microorganism interaction, *Fusarium verticillioides*, *Colletotrichum falcatum*, Plant volatile, Pheromone

1. GENERAL INTRODUCTION

What drives insects for mating partners, food sources, host preference for oviposition sites, and avoid dangers in their environment have fascinated scientists for centuries. Semiochemicals¹ cues play a decisive role in these ecological contexts mediate insect perception of different types of odors, the volatile organic compounds (VOCs), presents in nature by other species or by conspecifics, presumably the driving force behind the evolution of a complex olfactory system.

The interactions between insects and semiochemicals in the environment range from the specificity of sexual pheromones starting with the first VOC chemically recognized, bombykol, the *Bombyx mori* sex pheromone (Butenandt et al. 1959). However, the modulation of insect landscape results from dynamic interactions throughout life, including environment and multitrophic interactions. In fact, it is essential to point out that, within the evolutionary process, the plant factor plays an essential role in insect multitrophic interactions. The constitutive plant VOCs have been driving herbivores to avoid or chose the host to feed or oviposition site (Hanson and Dethier 1973; Visser 1986; Pare and Tumlinson 1999; Dicke 2000). Researchers later found that the plants emit constitutively and *de novo* blends of volatiles in responses to herbivory, identified as herbivore-induced plant volatiles (HIPVs), constitute important cues for parasitoids and predators to find prey or hosts and plant-plant communication (Baldwin and Schultz 1983; Turlings et al. 1990; Dicke and Baldwin 2010; Dicke 2016; Naranjo-Guevara et al. 2021; Silva et al. 2021). Recently ‘microbe-induced plant volatiles (MIPVs) have been considered in insect associations.

In general, relationships among microorganisms and insects are widespread, and this interaction's dynamics depend on their costs and benefits to hosts. If the benefits of interaction are consistently sturdy and mutualistic with beneficial effects on the hosts, selection should favor hosts and lead to fixation of the symbiosis; however, the antagonistic interactions, with adverse or no apparent effects on the hosts, can reduce the success of interaction and be lost (Batra 2016; Biedermann and Vega 2020; Hosokawa and Fukatsu 2020).

Since insects generally lack the capacity to synthesize sterols, which are the precursors for different hormones, they acquire either sterols or sterol precursors associated with microorganisms, include the cases of fungal cultivation by insects as ambrosia beetles (Morales-Ramos et al. 2000; Cardoza et al. 2003). Thus, the attraction of insects to infected-plant can be beneficial for insects and microorganisms, which that mothers choose the best oviposition sites for their offspring by superior nutritional quality for insect development through pathogens infection according to preference–performance hypothesis (“The mother know best”)(Jaenike 1978, Thompson 1988).

On the other hand, phytopathogens take advantage of insect hosts to increased pathogen dispersal. This symbiotic mode of transmission can be horizontal, in which hosts contagiously acquire symbionts through an environmental source or, vertically, through parents inheritance to offspring; however, as there is a great diversity of organisms, this transmission also it can be mixed, that is, horizontally and vertically (Bright and Bulgheresi 2010).

The orchestration of these phytopathogens to be transmitted by insects is fascinating and involves myriad strategies to manipulate plant and insect vectors to their own benefit, including alterations of volatile and non-volatile host metabolites. This change in MIPVs release after host–plant infection leads to a strategy to attract insects, leading to increased pathogen dispersal by insect vectors according to the “host manipulation hypothesis” (Heil 2016; Mauck et al. 2018). The manipulation of insect behavior by microorganisms can change the insect host preference, making

¹ Semio= Greek σημεῖον (semeion), signal. Chemical signals (categorized as either pheromones or allelochemicals) that are recognized and modify the behavior of the recipient organism.

the vector move to healthy plants to pinpointing the location of pathogen dissemination, very well reported in viruses and bacteria interactions (Ferreles and Moreno 2009; Ingwell et al. 2012; Mann et al. 2012; Keesey et al. 2017). For example, the *Barley yellow dwarf luteovirus* (BYDV) increase the quantity of volatiles, especially (Z)-3-hexenyl acetate in wheat plants, attracting virus-free aphids, in contrasting, virus-containing aphids prefer healthy plants, thereby resulting in higher virus uptake and spreading (Ingwell et al. 2012). Also, the *Cucumber mosaic virus* (CMV), a nonpersistent virus, elevates the overall emission of volatiles in Cucurbita pepo attracting *Myzus persicae* and *Aphis gossypii* virus vector. However, these infected plants are less palatable to virus vectors, and the vector dashes between plants spreading the virus (Mauck et al. 2010).

Different from vector-borne pathogens, opportunistic pathogens presumably do not have an intimate association with the insect. In sugarcane, infection by the fungi phytopathogens *Fusarium verticillioides* (pokkah boeng causal agent) and *Colletotrichum falcatum* (red rot causal agent) are widespread in areas of sugarcane. Even the disease can occur in the absence of borer-feeding insects (usually associated with crop damages or in endophytic association, entering it through a lesion in its structure), usually occurs associated with *Diatraea saccharalis*, the sugarcane-*D. saccharalis*-opportunistic fungi interaction (Ogunwolu et al. 1991; Franco et al. 2017). Colonization by these fungi promotes the inversion of sucrose into glucose and the disruption of water and nutrient transport, which are essential to produce sugars. Hitherto, it was assumed that the fungi take advantage of the openings made by the borer to penetrate the sugarcane stalk and infect the plant (Ogunwolu et al. 1991; Franco et al. 2017).

In previous studies, the sugarcane-*D. saccharalis*-opportunistic fungi interaction has been elucidated. Medeiros et al. (2012) and Franco and Santiago (2014) show that after the attack of *D. saccharalis*, herbivory strongly induced the genes encoding the plant expresses antifungal defense proteins (Sugarwin1 and Sugarwin2). Interestingly enough, contrary to the initial hypothesis, these proteins do not interfere in insects' development; however, they cause both fungal *C. falcatum* and *F. verticillioides*, death, but cannot affect *Aspergillus nidulans*, a non-sugarcane pathogenic fungus. Thus, the plant seems to predict pathogen infection as early as the first signs of insect herbivory protect the plant against opportunistic pathogens. Also, *F. verticillioides*-sugarcane infected release less attractive herbivore-induced plant VOCs to *Cotesia flavipes*, a parasitoid of *D. saccharalis* larvae (Peñaflor and Bento 2019), indicating an indirect benefit to the herbivore of co-occurring with the fungus as a strategy of feeding in a safe environment.

This close association between opportunistic fungi and the sugarcane borer led us to investigate the mechanisms of these interactions. In fact, in this dissertation, Franco (2017) shows that *C. falcatum* and *F. verticillioides* VOCs emission attract *D. saccharalis* larvae. Also, the fungi positively influencing the diet of *D. saccharalis* and larvae weight gain in diets supplemented with fungi. Chapter 2 focuses on *D. saccharalis* adult interaction among plants infected by opportunistic fungi, *C. falcatum*-infected plants, *F. verticillioides*-infected plants, and *C. falcatum*+*F. verticillioides*-infected plants. First, using chromatography tools, we addressed the chemical identification of plant defenses induced by fungi infection. Also, based on the theory of preference–performance hypothesis, we presented insights of insect behavior into oviposition preferences and consequently offspring benefits in infected plants. In concomitance, Gallan (2019), studying the possible role of *D. saccharalis* as a vector of *C. falcatum* and *F. verticillioides*, showed that, in fact, at least *F. verticillioides* was vertically transmitted to *D. saccharalis* offspring. Then, still in Chapter 2, using a mutant *F. verticillioides* (*Fr:DsRed*) tracked by laser confocal microscopy, we also explicitly evaluated Koch's postulates, *in vivo*, to confirm the vertical transmission and role of *D. saccharalis* on *F. verticillioides* infection in the sugarcane plant. Also, we evaluated the possible effects of *F. verticillioides* feeding on the biology and reproductive success of *D. saccharalis*. Finally, using electroantennography tools and olfactory behavior assays, we specifically tackled whether the fungus modifies *D. saccharalis* behavior to increase the likelihood of the fungus being disseminated by the contaminated insect,

corroborating with the “host manipulation hypothesis”. The data of the three dissertations were combined and partially redrafted in Franco et al. (2021).

To make a four-year research story short, we showed the interaction in the following way (Figure 1): *D. saccharalis* moths orient towards volatiles emitted by infected plants, which is the preferred host for oviposition (1). When the larvae hatch, they penetrate the stems and are contaminated by the fungus (2). When they enter the pupal stage and develop into adult moths, they are already carriers of the fungus. Remarkably, the host preference rather than plastic changes whether insects themselves are carriers of the fungus. Instrumentalized by the VOCs, females not carrying *F. verticillioides* prefer to lay their eggs on fungus-infected plants, while females carrying the *F. verticillioides* prefer to lay their eggs on mock plants (3). The *F. verticillioides* spread to healthy plants by *D. saccharalis* vertical transmission through its offspring (4). In this scenario, our study indicates that *F. verticillioides* is not just an opportunistic fungus but also more intimately associated with an insect herbivore, manipulating the host plant and insect herbivore across the life cycle to promote its infection and dissemination.

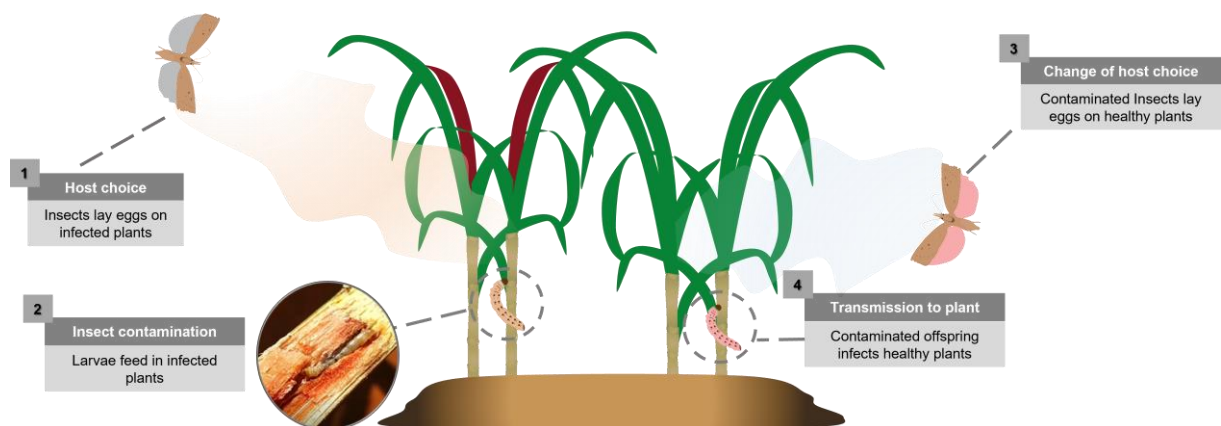


Figure 1. Semiochemicals involved in sugarcane-*Diatraea saccharalis*-opportunistic fungus interaction. Plants infected by *Fusarium verticillioides* emit VOCs (pink shadow) attractive to *D. saccharalis* moths to lay eggs (1). When the larvae hatch, they penetrate the stems, feed the contaminated plant, and are themselves contaminated by *F. verticillioides* (2). Females carrying the *F. verticillioides* (pink females) are attracted by VOCs emitted by healthy plants (light green shadow) to lay their eggs (3). The offspring from genitors fed the fungus (pink larvae) transmit the *F. verticillioides* to a new healthy plant (4).

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2. PHYTOPATHOGENIC FUNGUS INDUCED CHANGES IN SUGARCANE MODULATES BEHAVIOR IN THE MOTH *Diatraea saccharalis*

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ARTICLE



Fungal phytopathogen modulates plant and insect responses to promote its dissemination

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Abstract

Vector-borne plant pathogens often change host traits to manipulate vector behavior in a way that favors their spread. By contrast, infection by opportunistic fungi does not depend on vectors, although damage caused by an herbivore may facilitate infection. Manipulation of hosts and vectors, such as insect herbivores, has not been demonstrated in interactions with fungal pathogens. Herein, we establish a new paradigm for the plant-insect-fungus association in sugarcane. It has long been assumed that *Fusarium verticillioides* is an opportunistic fungus, where it takes advantage of the openings left by *Diatraea saccharalis* caterpillar attack to infect the plant. In this work, we show that volatile emissions from *F. verticillioides* attract *D. saccharalis* caterpillars. Once they become adults, the fungus is transmitted vertically to their offspring, which continues the cycle by inoculating the fungus into healthy plants. Females not carrying the fungus prefer to lay their eggs on fungus-infected plants than mock plants, while females carrying the fungus prefer to lay their eggs on mock plants than fungus-infected plants. Even though the fungus impacts *D. saccharalis* sex behavior, larval weight and reproduction rate, most individuals complete their development. Our data demonstrate that the fungus manipulates both the host plant and insect herbivore across life cycle to promote its infection and dissemination.

Introduction

Plant-insect-fungus interactions involve plant defense responses that eventually influence insect behavior or pathogen infection [1–3]. Changes in plant metabolites and insect behavior can be explained by the “host manipulation hypothesis”, which states that the pathogen can manipulate the insect vector and/or final host response to guarantee infection and dissemination [4–7]. Phytopathogens can change plant phenotypes, nutritional profiles, and the emission of volatile organic compounds (VOCs) [3], as a strategy to attract vectors and disseminate them [6]. An example is when the phytopathogen infection increases pre-existing host-location cues that are attractive to the vector, but also reduces host nutritional quality, making the vector move to a healthy plants [6]. Microorganisms can also alter insect behavior, for example, when they induce an increase in aggregation pheromone production resulting in the attraction of more individuals and, consequently, infecting more insects [8], when they affect insects’ feeding behavior [9], perception of odors [10, 11] or tastes [12]. An extraordinary example of changes of insect behavior is “zombie-ants”

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Abstract

Vector-borne plant pathogens often change host traits to manipulate vector behavior in a way that favors their spread. By contrast, infection by opportunistic fungi does not depend on vectors, although damage caused by an herbivore may facilitate infection. Manipulation of hosts and vectors, such as insect herbivores, has not been demonstrated in interactions with fungal pathogens. Herein we establish a new role for the insect-fungi-sugarcane association. It has long been assumed that *Fusarium verticillioides* and *Colletotrichum falcatum* are opportunistic fungi, where it takes advantage of the openings left by *Diatraea saccharalis* attack to infect the plant. In this work, we show that sugarcane infection attracts *D. saccharalis* adults to lay their eggs. However, only *F. verticillioides* seems to modulate the behavior of the *D. saccharalis* moth. More specifically, infected plants dramatically increased amounts of volatile organic compounds (VOCs) emission, attracting female moths for oviposition. Once they become adults, the fungus is transmitted vertically to their offspring, which continues the cycle by inoculating the fungus into healthy plants. Females not carrying the fungus prefer to lay their eggs on fungus-infected plants than mock plants, while females carrying the fungus prefer to lay their eggs on mock plants than fungus-infected plants. Most individuals complete their development even though the fungus impacts *D. saccharalis* sex behavior, larval weight, and reproduction rate. Our data demonstrate that the fungus manipulates both the host plant and insect herbivore across the life cycle to promote its infection and dissemination.

Keywords: Plant-fungi interaction, *Fusarium verticillioides*, *Colletotrichum falcatum*, vertical transmission, host manipulation hypothesis

2.1. Introduction

Plant-insect-fungus interactions involve plant defense responses that eventually influence insect behavior or pathogen infection (Ingwell et al. 2012; Franco et al. 2017; Sharifi and Ryu 2017). The “host manipulation hypothesis” can explain changes in plant defense pathways in primary and secondary metabolisms and insect behavior, which states that the pathogen can manipulate the insect vector and/or final host response to guarantee infection and dissemination (Ferreles and Moreno 2009; Mauck et al. 2010, 2018; Ingwell et al. 2012; Heil 2016). Phytopathogens can change plant phenotypes, nutritional profiles, and the emission of volatile organic compounds (VOCs) (Franco et al. 2017) as a strategy to attract vectors and disseminate them (Mauck et al. 2010; Ingwell et al. 2012). An example is when the phytopathogen infection increases pre-existing host-location cues that are attractive to the vector, but also reduces host nutritional quality, making the vector move to a healthy plant (Mauck et al. 2010). Microorganisms can also alter insect behavior, for example, when they induce an increase in aggregation pheromone production resulting in the attraction of more individuals and, consequently, infecting more insects (Keesey et al. 2017) when they affect insects feeding behavior (Mauck et al. 2010; Stafford et al. 2011), perception of odors or tastes (Becher et al. 2010a; Stensmyr et al. 2012a). An extraordinary example of changes in insect behavior is “zombie-ants” which fungus manipulates the ants to behave like “zombies,” to bite the underside of vegetation and spread the spores on nature (Hughes et al. 2011).

On the other hand, insects can participate in fungal dispersion by spreading spores over long distances (Phoku et al. 2016) and/or facilitating the entry of opportunistic fungi through feeding, as can occur in Ambrosia beetles (Zhao et al. 2019). As a vector, insects can transmit pathogens horizontally in which hosts contagiously acquire symbionts through an environmental source or, vertically, through parents inheritance to offspring; however, as there is a great diversity of organisms, this transmission also it can be mixed, that is, horizontally and vertically (Bright and Bulgheresi 2010).

Opportunistic pathogens presumably do not have an intimate association with the insect; moreover, the association between *Fusarium* spp. and borer-feeding insects have been reported, including the role of insects as fungal vectors, such as *Eldana saccharina* (Ako et al. 2003; McFarlane et al. 2009). In fact, “Bt crops” have been associated with lower mycotoxin levels because of the absence of moth larvae in the field, as *Ostrinia nubilalis*, the European corn borer (Bakan et al. 2002, Gatch and Munkvold 2002), *E. saccharina*, the African corn borer (Schulthess et al. 2002) and

Helicoverpa zea, the corn earworm (Gatch and Munkvold 2002; Bowers et al. 2013). Furthermore, the interaction between *F. verticillioides* and plants from the family Poaceae is a global problem for human and animal food due to the fumonisin production by the fungus of *Fusarium* spp. (Theumer et al. 2010).

The same fungi, *F. verticillioides*, usually occur in association with *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) and the fungus *Colletotrichum falcatum*, a prevalent pest in sugarcane fields (Ogunwolu et al. 1991). Hitherto, it was assumed that the fungus takes advantage of the openings made by the borer to penetrate the sugarcane stalk and infect the plant, resulting in additional crop damage due to pokkah boeng and red rot disease, respectively.

Nevertheless, recent research shows that *D. saccharalis* herbivory strongly induces genes encoding in the sugarcane plant to express defense proteins (Sugarwin1 and Sugarwin2). Interestingly, these proteins affect specific *C. falcatum* and *F. verticillioides* morphology, causing fungal death, but are not able to affect *Aspergillus nidulans*, a non-sugarcane pathogenic fungus, even the *D. saccharalis*, the insect that induces the plant to produce these proteins (Medeiros et al. 2012, 2016; Franco et al. 2014). Thus, the sugarcane plants seem to protect the plant against opportunistic pathogens associating the *D. saccharalis* herbivory to *C. falcatum* and *F. verticillioides* pathogenicity. Then, the sugarcane-*D. saccharalis*-opportunistic fungi interaction appears to be more elaborate than the pre-established concept of the insect mediating fungal infection. Therefore, the sugarcane-*D. saccharalis*-opportunistic fungi were considered. Based on the previous association between *Fusarium* spp. and borer-feeding insects and the strong interaction between *F. verticillioides* and *D. saccharalis*, we investigated the role of *D. saccharalis* as a dissemination vector *F. verticillioides* possibly corroborating the “host manipulation hypothesis”. We specifically addressed whether the fungus modifies *D. saccharalis* behavior in ways that increase the likelihood of the fungus being disseminated by the contaminated insect. Additionally, we evaluated whether this interaction benefits both organisms or only *F. verticillioides*.

2.2. Conclusion

Our results showed at the list for *F. verticillioides*, strong interaction with *D. saccharalis* in sugarcane-infected plants and support a paradigm change in which the fungus is not merely an opportunistic player manipulates both the host plant and insect throughout its life cycle to promote infection and dissemination. The *D. saccharalis* moths orient towards volatiles emitted by infected plants, which is the preferred host for oviposition. Interestingly, the host preference rather than plastic changes whether insects themselves are carriers of the fungus. Females not carrying *F. verticillioides* prefer to lay their eggs on fungus-infected plants, while females carrying the *F. verticillioides* prefer to lay their eggs on mock plants. In addition, *D. saccharalis* vertically transmits the fungus *F. verticillioides* to healthy plants by its offspring. In this scenario, our study indicates that *F. verticillioides* is not just an opportunistic fungus but also more intimately associated with an insect herbivore, manipulating the plant and the insect to increase its potential for dissemination.

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3. PHYTOPATHOGENIC FUNGUS PROMOTES CHANGES IN SEXUAL BEHAVIOR OF MOTH

Diatraea saccharalis

Manuscript in preparation for submission

Abstract

A remarkably complex and fascinating network among *Diatraea saccharalis*-*Fusarium verticillioides*-sugarcane interaction elucidates the fungus manipulation to attract the moth *D. saccharalis* to lay eggs and feed on contaminated plants, promoting its infection and dissemination. The fact that these insects feed on these fungi without causing any consequences for their biology we argue whether the acquisition of the fungus *F. verticillioides* would alter the sexual behavior of *D. saccharalis*. Few examples of entomopathogenic microorganisms associated with insects resulting from pheromone modification and the attraction of more individuals than, consequently, infecting more insects or change the insect preference of mating. There is a paucity of information on microorganisms not associated with insects such as phytopathogens. Using chromatography and electroantennography tolls, we were able to evaluate the *F. verticillioides* effects in *D. saccharalis*.

Keywords Sugarcane-borer, Insect-microorganism interaction, *Fusarium verticillioides*, Pheromone

3.1. Introduction

Insect-microorganism interactions have been reported in several cases as oviposition stimulants and host location, and attraction to food resources (Becher et al. 2010; Stafford et al. 2011; Stensmyr et al. 2012; Soldano et al. 2016; Engl and Kaltenpoth 2018; Weisskopf et al. 2021). In addition, the most common microbial symbionts as *Wolbachia* and *Rickettsia* can also impact their host reproductive and sexual behavior to facilitate their spread since they can be sexually transmitted (Schneider et al. 2019; Flatau et al. 2021). Although, a shred of insufficient literature evidences the impacts of chemical communication between individuals by microorganisms, including symbiont and entomopathogenic microorganisms (Zurek et al. 2002; Keeseey et al. 2017; Ren et al. 2020), there is a paucity of information on microorganisms not associated with insects such as phytopathogens.

A recent study showed that infection in sugarcane modulates behavior in the moth *Diatraea saccharalis*, guiding the moths to acquire *Fusarium verticillioides* and further enhance pathogen dispersal vertically transmitted to the offspring. Furthermore, even though the fungus no impacts the insect performance, it was demonstrated that the presence of the fungus influences *D. saccharalis* sexual behavior, decreasing the latency period and unsettle the copulation success when the adults were contaminated by the fungus (Franco et al. 2021). We argue whether the acquisition of the fungus *F. verticillioides* would alter the sexual behavior of *D. saccharalis*. Such discovery would be beneficial since *D. saccharalis* is a significant pest in Poaceous crops and a potential vector of *F. verticillioides*, the causal agent of pokkah boeng disease in sugarcane crop, and also, a global problem for human and animal food due to the fumonisin production by the fungi of *Fusarium* spp. (Theumer et al. 2010; Blacutt et al. 2018).

3.2. Conclusion

The advancement of a holistic view of insect-microbe interaction can increase insect pest and disease management efficacy. For instance, the knowledge of ecological interaction among plants, invertebrates, and fungi contributes to reduces pathogenic problems through insect pest control before transmitting the inoculum. Thus, such

an approach would be ideal for controlling mycotoxins, a global human and animal health problem. However, the results were not conclusive.

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4. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The present thesis was conducted to obtain a comprehensive picture of the semiochemicals involved in sugarcane-*Diatraea saccharalis*-opportunistic fungi interaction, the fungi *Fusarium verticillioides*, and *Colletotrichum falcatum*. Briefly, the main goals of the work were to address the chemical identification of plant defenses induced by both fungi infection, separated, or concomitant in the plant. Consequently, we evaluated the effects of plant defenses on insect behavior and fitness. Also, we evaluated the role of *D. saccharalis* as a vector of *F. verticillioides* infection in the sugarcane plant. Finally, we combined behavioral, electrophysiological, microscopy, and chromatography approach to testing our hypotheses specifically.

In fact, the sugarcane infection by the fungus separated or associated with the plant induces volatiles and nonvolatile chemical defenses. Even though *C. falcatum* has a neutral effect against *D. saccharalis*; the *F. verticillioides*-infected plants release VOCs, attract *D. saccharalis* adults to lay their eggs. The *F. verticillioides* is transmitted vertically to their offspring, which continues the cycle by inoculating the fungus into healthy plants. However, in contrast to other interactions, in which pathogen infection provides superior nutritional quality for the vector's offspring, *F. verticillioides* infection in sugarcane does not directly benefit the insect. Then, females carrying the fungus prefer to lay their eggs on mock plants than fungus-infected plants. Altogether, our results support a paradigm change in which the fungus is not merely an opportunistic player but also manipulates both the host plant and the insect throughout its life cycle to promote infection and dissemination, forming a mutualistic but inherently selfish association with sugarcane and *D. saccharalis*.

Even the sugarcane-*D. saccharalis*-opportunistic fungi interaction is the prevalent pest in sugarcane fields; a shred of insufficient literature evidences the chemical identification of plant volatiles in this interaction. Essentially, this disease control has been associated with managing the *D. saccharalis* larvae using the biological-control agent *Cotesia flavipes* on a large scale (Parra 2014). However, the absence of injury of the insect vector can increase insect pest and disease management efficacy; mainly allied to the fact that plants infected with *F. verticillioides* release less attractive herbivore-induced plant VOCs to *C. flavipes* (Peñaflor and Bento 2019), which can reduce the efficiency of parasitism in the field of co-occurring of *D. saccharalis* and *F. verticillioides*.

Because of the close sugarcane-*D. saccharalis*-*F. verticillioides* association and the specificity of *D. saccharalis* feeding on plants of the family Poaceae, the background of plant volatiles could influence pheromone perception in *D. saccharalis* previously reported to Noctuidae moths (Kromann et al. 2015; Badeke et al. 2016; Hoffmann et al. 2020). Also, microbe-induced plant volatiles (MIPVs) have been considered in developing new emerging techniques for sustainable plant disease management and confer immunity to neighboring susceptible cultivars (Lee 2017).

The co-occurrence of the pathogen, herbivore, and plants implicates complex interactions between them and is a complex task for many researchers; because this has been overlooked. However, this study changes our previous knowledge regarding sugarcane-*D. saccharalis*-opportunistic fungi interaction and enhances our understanding of plant-insect-fungus interactions, which can be extended to other systems.

In fact, the association between *Fusarium* spp. and borer-feeding insects have been reported, including the role of insects as fungal vectors, such as *Eldana saccharina*, the African corn borer (Ako et al. 2003; McFarlane et al. 2009), and *Ostrinia nubilalis*, the European corn borer (Gatch and Munkvold 2002; Bowers et al. 2013). Furthermore, "Bt crops" have been associated with lower mycotoxin levels because of deterring kernel injury from *Ostrinia nubilalis*, the European corn borer (Bakan et al. 2002, Gatch and Munkvold 2002), *E. saccharina*, (Schulthess et al. 2002), and *Helicoverpa zea*, the corn earworm (Gatch and Munkvold 2002; Bowers et al. 2013). The interactions among *Fusarium*

spp. and these Lepidopteran pests are a global problem for human and animal food due to the fumonisin, which exposes a toxic effect on farm animals and humans (Theumer et al. 2010; Drakulic et al. 2017; Blacutt et al. 2018).

On top of that, social problems associated with this interaction, the knowledge of ecological interaction among plants, herbivores, and fungi might contribute to reducing pathogenic problems through insect pest control before transmitting the inoculum. Thus, such an approach would be ideal for controlling mycotoxins, a global human and animal health problem.

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