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Studies on the chemical ecology of the wood-boring beetles *Euplatypus parallelus* (Coleoptera: Curculionidae) and *Dinoderus minutus* (Coleoptera: Bostrichidae)

Hugo Leoncini Rainho

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

Piracicaba
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Hugo Leoncini Rainho
Agronomist Engineer

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Advisor:
Prof. Dr. **JOSÉ MAURÍCIO SIMÕES BENTO**

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DEDICATION

This indefeasible work of my life is dedicated to my family, Carlos Alberto Rainho, Suzete Coelho Leoncini Rainho, Heitor Leoncini Rainho, and to my lovely wife, Aline Gastardeli de Oliveira.

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EPIGRAPH

“It is always darkest just before the day dawneth.”

Thomas Fuller *– A Pisgah-Sight of Palestine and the Confines Thereof (1650)*

RESUMO

Estudo sobre a ecologia química das coleobrocas *Euplatypus parallelus* (Coleoptera: Curculionidae) e *Dinoderus minutus* (Coleoptera: Bostrichidae)

O besouro-da-ambrosia, *Euplatypus parallelus* (Fabricius) (Coleoptera: Curculionidae: Platypodinae) e a broca-do-bambu, *Dinoderus minutus* (Fabricius) (Coleoptera: Bostrichidae: Dinoderinae), estão entre as pragas mais cosmopolitas e economicamente importantes das suas respectivas famílias. Apesar da importância econômica destes besouros, poucas estratégias eficientes e ecologicamente seguras para o seu controle estão disponíveis. Semioquímicos, incluindo compostos atraentes e repelentes, têm sido utilizados para o Manejo Integrado de Pragas (MIP) das famílias Curculionidae e Bostrichidae em todo o mundo. Atraentes feromonais ou voláteis de plantas (cairomônios) não têm sido investigados para *E. parallelus* e *D. minutus*. Na presente tese, um feromônio sexual putativo e atraentes voláteis de plantas para *E. parallelus* e *D. minutus* foram identificados e validados em experimentos de campo. Essa tese foi dividida em dois capítulos. O **Capítulo 1** apresenta a identificação e avaliação de uma mistura de compostos voláteis sexo-específicos, produzidos por machos de *E. parallelus*, combinada ou não ao etanol, um composto presumidamente produzido por plantas hospedeiras desse besouro. O **Capítulo 2** apresenta a identificação e avaliação de compostos orgânicos voláteis produzidos por plantas de bambu que desempenham uma função na localização hospedeira para machos e fêmeas de *D. minutus*. No **Capítulo 1**, os dados mostraram que: (i) machos de *E. parallelus* produzem cinco compostos sexo-específicos (três álcoois, um monoterpeno álcool e um acetato éster), que constituem um sinal químico (i.e., feromônio sexual putativo) que atua na atração de fêmeas conspecíficas somente quando combinado ao etanol supostamente produzido por plantas hospedeiras em condição ideal para a reprodução dessa coleobroca; (ii) machos de *E. parallelus* são atraídos somente por etanol, que serve como um sinal químico determinante para localização de plantas hospedeiras por machos dessa espécie; (iii) potenciais inimigos naturais de *E. parallelus*, pertencentes ao gênero *Sosylus* (Coleoptera: Bothrideridae), são atraídos pela combinação da mistura dos compostos produzidos por machos de *E. parallelus* com etanol, revelando que estes inimigos naturais utilizam os canais químicos de comunicação da sua presa para localizá-la. No **Capítulo 2**, foi observado que: (i) colmos de plantas de bambu se tornam atrativos e começam a ser broqueados por adultos de *D. minutus* após o corte das plantas, o que está relacionado com os compostos voláteis liberados pelos colmos; (ii) adultos de *D. minutus*, tanto machos quanto fêmeas, são atraídos por uma mistura de compostos liberados pelos colmos cortados de bambu, a qual desempenha uma função na localização de plantas hospedeiras em condição ideal para reprodução dessa espécie. Os semioquímicos identificados são promissores para o monitoramento e controle das coleobrocas *E. parallelus* e *D. minutus*.

Palavras-chave: Besouro da ambrosia; Broca do bambu; Feromônio sexual; Cairomônios; Semioquímicos; Voláteis de planta; Atraentes; Interação inseto-planta; Interação tritrófica; Seleção hospedeira; Ecologia Química

ABSTRACT

Studies on the chemical ecology of the wood-boring beetles *Euplatypus parallelus* (Coleoptera: Curculionidae) and *Dinoderus minutus* (Coleoptera: Bostrichidae)

The ambrosia pinhole borer, *Euplatypus parallelus* (Fabricius) (Coleoptera: Curculionidae: Platypodinae), and the bamboo borer, *Dinoderus minutus* (Fabricius) (Coleoptera: Bostrichidae: Dinoderinae) are among the most invasive and economically important pests of their respective families. Despite these beetles' economic importance, a few efficient and ecologically-safe strategies for their management are available. Semiochemicals, including attractant and repellent compounds, have been applied for the Integrated Pest Management (IPM) of beetles within the families Curculionidae and Bostrichidae worldwide. Attractant pheromones or plant volatiles (kairomones) for *E. parallelus* and *D. minutus* have not been addressed. In this thesis, a putative sex pheromone and attractant volatiles of plants for *E. parallelus* and *D. minutus* were identified and validated by field bioassays. This thesis was divided into two chapters. **Chapter 1** presents the identification and evaluation of a blend of male-specific volatile compounds of *E. parallelus*, combined or not to the ethanol, a compound presumably produced by host plants of this beetle. **Chapter 2** presents the identification and evaluation of volatile organic compounds (VOC's) produced by bamboo plants, which play a role in the host location by adult males and females of *D. minutus*. In **Chapter 1**, the results showed that: (i) males of *E. parallelus* produce five sex-specific compounds (three alcohols, one monoterpene alcohol, and one acetate ester), which consisting of a chemical cue (i.e., a putative sex pheromone) on the attraction of conspecific females only when combined with the ethanol supposedly produced by suitable host trees for the breeding of this wood-boring beetle; (ii) males of *E. parallelus* are attracted only to the ethanol, which serves as a determinative chemical cue for the host-plant location by males of this species; (iii) potential natural enemies of *E. parallelus* belonging to the genus *Sosylus* (Coleoptera: Bothrideridae) are attracted to the combination of the *E. parallelus* male-specific compounds with ethanol, revealing that these natural enemies use the chemical channels of communication of its prey to locate it. In **Chapter 2**, it was observed that: (i) culms of bamboo plants become attractive and start to be drilled by adults of *D. minutus* after cutting the plants, which is related to the volatile compounds released by the culms; (ii) adult males and females of *D. minutus* are attracted to a blend of compounds released by the cut bamboo culms, which plays a role in the location of suitable host plants for the breeding of this species. The identified semiochemicals are promising for the monitoring and control of the wood-boring beetles *E. parallelus* and *D. minutus*.

Keywords: Ambrosia beetle; Bamboo borer; Sex pheromone; Kairomones; Semiochemicals; Plant volatiles; Attractants; Insect-plant interaction; Tritrophic interaction; Host selection; Chemical Ecology

1. SEMIOCHEMICAL-BASED ATTRACTANT FOR THE AMBROSIA PINHOLE BORER *Euplatypus parallelus*

Hugo L. Rainho*, Weliton D. Silva and José Maurício S. Bento

Department of Entomology and Acarology, University of São Paulo, Piracicaba, SP, Brazil

*Correspondence: Avenida Pádua Dias, 11, University of São Paulo, Piracicaba, SP 13418900, Brazil. E-mail: hugo.lleoncini@gmail.com

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Abstract

A semiochemical-based attractant for *Euplatypus parallelus* was identified and field-tested. Analyses of headspace volatile extracts of conspecific males revealed the presence of 1-hexanol along with lesser amounts of 3-methyl-1-butanol, hexyl acetate, 1-octanol and trans-geraniol, which were not found in equivalent extracts from females. Emission of 1-hexanol coincided with the emergence of adults of both sexes during afternoon hours. A synthetic blend of these compounds, with and without ethanol, was tested in the field. The blend alone attracted a small number of females and no males. Ethanol alone attracted a small number of females (not significantly different from the blend alone) but significantly more males than the blend alone. More females were caught with the blend combined with ethanol than the combined catch of either attractant alone, suggesting a synergistic interaction. Attraction of males appeared to be a response to ethanol alone. During the trials, two potential natural enemies of *E. parallelus* were caught, indicating that they might be eavesdropping on the semiochemical channels of their prey. Traps containing the male-specific volatile compounds combined with ethanol could be applied as an effective attractant for detection and monitoring of *E. parallelus* as well as for recruitment of its natural enemies.

Keywords: ambrosia beetle; chemical ecology; pheromone; traps; quarantine species; monitoring; Platypodinae; Bothrideridae

Introduction

The Neotropical ambrosia pinhole borer, *Euplatypus parallelus* (Fabricius) (Coleoptera: Curculionidae: Platypodinae), is one of the most important invasive forest pests worldwide, causing damage in natural and managed forests in over 50 countries [1–3]. This beetle is highly polyphagous, attacking conifers and broadleaf trees of over 80 species from ~25 botanical families [2–4]. Adult *E. parallelus* damage trees by boring deep galleries into the wood, inside of which they inoculate fungal symbionts that create a substrate for their larvae to feed [2,5]. In addition to the damage by the beetles, staining of the woody tissues by the associated fungi compromises the wood quality specially for furniture and veneer production [2,6].

Outbreaks of *E. parallelus* are commonly associated with massive attacks on trees stressed by biotic or abiotic factors, such as damage by other insects, phytopathogens, storms, drought, fire, and forestry management practices [2,7–14]. The attacks compromise the physiology of trees, making them vulnerable to infection by phytopathogens that may result in high mortality in tree stands [7–9,12]. In Brazil, outbreaks of *E. parallelus* have been reported in commercial plantations of *Pinus* [10], *Eucalyptus* [11], *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg. [15], and *Khaya senegalensis* (Desv.) A. Juss. (present study, see Supplementary Material).

Attraction of adult platypodine beetles to stressed trees is mediated by volatile organic compounds (VOCs) [16,17]. Ethanol is released in large amounts by stressed trees [18–20], and platypodine beetles use this VOC as a chemical cue to locate their host [21,22]. In this case, adult males appear to locate and initiate colonization of a suitable host tree. After initiating a gallery, males emit a pheromone to attract conspecific females for mating and reproduction [5].

To our knowledge, male-produced attractant pheromones in platypodine beetles have been identified for five species: *Megaplatypus mutatus* (Chapuis) [23,24], *Myoplatypus flavicornis* (Fabricius) [25], *Platypus cylindrus* (Fabricius) [26], *Platypus koryoensis* (Murayama) [27], and *Platypus quercivorus* (Murayama) [28]. The pheromone of *E. parallelus* has not hitherto been addressed. Despite evidence of attraction of adult *E. parallelus* to ethanol-baited traps [29], there is no further information, either on the efficacy of this alcohol compared to other attractants or controls or on the sex ratio of attracted beetles.

Traps containing semiochemical blends (*e.g.*, pheromones and kairomones) have been applied with success in surveillance programs for native and exotic forest pests [30]. Our objective was to identify a semiochemical-based attractant for *E. parallelus* that could be incorporated into traps for early detection or delineation of the geographical spread of this invasive species.

Conclusions

Adult male *E. parallelus* sex-specifically produced 1-hexanol (major), 3-methyl-1- butanol, hexyl acetate, 1-octanol, and *trans*-geraniol. In the field, a synthetic blend of these volatile compounds, in combination with ethanol, attracted conspecific females. Males were attracted equally to ethanol alone or to male-specific volatiles + ethanol. It is unclear if all the blend compounds are necessary and sufficient for the attraction that we observed. Two potential natural enemies of *E. parallelus* were attracted by the combination male-specific volatiles + ethanol blend. Traps containing this semiochemical-based attractant may be useful in surveillance and management programs for this important platypodine species.

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2. THE BAMBOO BORER, *Dinoderus minutus* IS ATTRACTED TO A BLEND OF VOLATILE COMPOUNDS PRODUCED BY CULMS OF BAMBOO PLANTS

Hugo L. Rainho*, Weliton D. Silva, Marcoandre Savaris, Felipe G. Gonçalves and José Maurício S. Bento

Department of Entomology and Acarology, University of São Paulo, Piracicaba, SP, Brazil

*Correspondence: Avenida Pádua Dias, 11, University of São Paulo, Piracicaba, SP 13418900, Brazil. E-mail: hugo.lleoncini@gmail.com

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Abstract

The bamboo borer, *Dinoderus minutus* is the major pest of post-harvested bamboo and its products. This cosmopolitan species is a great concern as a quarantine pest in tropical and subtropical regions that produce bamboo and its timber products. Semiochemical-based attractants for monitoring and management of this invasive species have not been addressed. Thus, the objective of this study was to identify volatile organic compounds produced by the bamboo culms that comprise an attractant for *D. minutus*. Attraction and boring activity of *D. minutus* to bamboo culms was observed 24 h after cutting the plants. However, the peak of attack intensity by the beetles occurred 35 days after cutting. The release of substantial amounts of some volatile compounds by the cut culms of bamboo was observed at the peak of beetles' attacks. Significant electroantennographic responses of both adult males and females of *D. minutus* were observed to a single synthetic volatile compound. Synthetic candidate kairomones identified from bamboo culms were tested in field bioassays conducted in Brazil. Traps baited with different blends of the candidate compounds attracted more adult beetles compared to control. Adult males and females were significantly attracted to a bonafide blend of compounds. Thus, this blend is involved with the long-range attraction of adult *D. minutus* to the culms of decaying plants of bamboo.

Keywords: Plant volatiles; Kairomones; Insect-plant interaction; Host-plant location; Primary attraction; Powderpost beetle

Introduction

The bamboo borer, *Dinoderus minutus* (Fabricius) (Coleoptera: Bostrichidae: Dinoderinae), is the major pest of post-harvested bamboo and its finished products throughout the world [1]. This cosmopolitan species has been reported in 48 countries, belonging to almost all biogeographic regions [2]. The increasing international trade of bamboo-derived products and its timber has raised concerns about the bamboo borer introduction in localities with tropical and subtropical climate and suitable host species of bamboo [3].

Dinoderus minutus has a narrow range of host plants, particularly bamboo species (Poaceae: Bambusoideae) [1,4,5]. The high degree of host-plant specificity of the bamboo borer and the relatively high susceptibility of post-harvested and raw bamboo timber to the attack by the beetle give it overt economic importance [1,6]. Economic damage results from the boring activity and feeding of adults and larvae on the tissues of bamboo culms and their timber products, leading to the destruction of raw materials [1].

Evidence of plant volatile organic compounds (VOC's) mediating the attraction of adult beetles have been reported for the major pests of the stored grain of cereals within the family Bostrichidae, for example, the lesser grain borer *Rhyzopertha dominica* (Fabricius) [7,8] and *Dinoderus bifoveolatus* (Wollaston) [9]. Adult of *D. minutus* attacks only recently felled, broken, or cut bamboo plants' culms [1]. Attacks to live plants of bamboo have not been reported [6]. Thus, we hypothesized that the volatile emissions of decaying bamboo plants' culms play a role in the host-plant location by *D. minutus* adults.

To our knowledge, semiochemical-based attractants (i.e., kairomones and pheromones) for *D. minutus* have not been addressed. This study aimed to identify attractant compounds for adult *D. minutus* among the volatile organic compounds emitted by suitable culms of bamboo plants.

Conclusions

Adults of *Dinoderus minutus* were attracted to newly-cut culms of the bamboo, *Bambusa vulgaris*, and started to build gallery holes within 24 h after cutting the plants. The attack intensity on cut culms of bamboo by the adults increased over time. At the peak of the beetles' attacks, the cut culms of bamboo released substantial amounts of some volatile organic compounds. In the field, synthetic blends of these bamboo-produced volatile compounds attracted *D. minutus* adults. The bonafide blend of compounds attracted both adult males and females of *D. minutus*. This blend was identified as the chemical cue involved with the host-plant location by the adults of *D. minutus*. Traps baited with this semiochemical-based attractant have the potential to be exploited in the monitoring and control of *D. minutus*.

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