Attraction of Pissodes castaneus (Coleoptera, Curculionidae) to Pinus taeda: Laboratory and Field Evaluation

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Abstract

Coniferous trees of the genus *Pinus* (Pinaceae) are under continuous threats by numerous herbivorous insect species and pathogens attacking nearly all parts and tissues of the plants. To defend themselves, pine trees produce large amounts of oleoresin that is accumulated in a highly developed network of specialized resin ducts, which are distributed in the wood, bark, and needles. Such defense reactions in pines can be induced by the attack of herbivores. The banded pine weevil, *Pissodes castaneus* (De Geer, 1775) (Coleoptera, Curculionidae), is an important pest of *Pinus* in Brazil, where it has been an invasive species since 2001. The female lays its eggs under the tree bark of trees and the larvae feed in the phloem of the trunk and branches, interrupting the sap circulation and eventually causing its death. In the present study, we conducted detailed GC–MS analyses of volatiles emitted by twigs of *Pinus taeda L*. We analyzed how the attack by *P. castaneus* males and females affects the volatile pattern emitted by the twigs. When comparing volatiles produced by healthy plants and by female- and male-attacked *P. taeda*, qualitative and quantitative differences were detected, as the decreased production of limonene, germacrene D and (*E*)-caryophyllene and the increase of α-pinene. Laboratory bioassays showed that plants attacked by male and female *P. castaneus* were more attractive to the insects. Understanding about what compounds may attract or repel the insects may help in the development of more effective traps, as well as preventing stress to avoid infestation.

1. Introduction

The banded pine weevil, *Pissodes castaneus* (De Geer, 1775) (Coleoptera, Curculionidae), is an important pest of *Pinus* (Pinaceae) in South America (Iede et al. 2004). It was detected in Brazil in 2001 in the county of São José dos Ausentes, Rio Grande do Sul, on *Pinus taeda* L and later it was detected in the states of Santa Catarina and Paraná (Iede et al. 2004). In 2002, 7.6% of the trees of a plantation of *P. taeda* grown in Cambará do Sul, Rio Grande do Sul, were attacked by *P. castaneus* while in São Joaquim, Santa Catarina, 16.5% of the trees were attacked (Iede et al. 2004).

This insect represents a threat to Brazilian forest production, as it has the potential to cause economic losses, such as those that occurred in Uruguay where there was a mortality rate higher than 10% (Grez et al. 2000). More than 50% of trees in a stand can be attacked in one year (Cadahia et al. 1992).

Conifers are long lived gymnosperms, which includes many species that successfully inhabit large areas of our planet. As raw material for many products (wood, paper, plastic, fuel, and many chemicals), their economic impact on our society is of great importance. In European countries such as Sweden, 83% of the forests consist of conifers, mainly Norway spruce (*Picea abies* L.) and Scots pine (*Pinus sylvestris* L.) (Alin and Sundberg 2003). In South America, specifically in Brazil, pine trees, mainly *P. taeda* and *Pinus elliottii* Engelm., have been planted on commercial scale for over 30 years (Ahrens 2000). Currently, there are about 2 million hectares of reforested pine plantations, in large continuous areas and generally in narrow genetic base stands, mainly in the South and Southeast regions (Iede et al. 2004). The product of these plantations is destined mostly to the timber and cellulose industries (Cardoso and Lázzari 2003).

Insects use volatiles as their major method to find food, mates or an appropriate site to lay their eggs (El-Shafie and Faleiro 2017). For plants, which cannot escape threats, chemical constituents play an important role in their defense system. Their response to feeding or oviposition of herbivorous insects can be direct, by feeding deterrents, impairing digestion or producing toxins (Kessler and Baldwin 2002), or indirect, by the release of volatiles which attract natural enemies of the herbivorous insects (Dicke and van Loon 2000; Arimura and Pearse 2017).

However, there are cases that the induced compounds may act as kairomones (Nordlund and Lewis 1976; Keeling Christopher et al. 2006; Lusebrink et al. 2016; McCormick et al. 2016), i.e. chemical compounds emitted by an organism that induce a benefic response to the receiving organism from another species, or have a synergistic effect with insect pheromones – compounds used in intraspecific communication –, as happens with *Pinus ponderosa* when damaged by *Dendroctonus brevicomis* Lec. (Bedard et al. 1969).

Conifers can use a large array of structurally diverse mono-, sesqui- and diterpenoids as a chemical defense against herbivores (Lewinsohn et al. 1991; Phillips and Croteau 1999; Trapp and Croteau 2001; Martin et al. 2002, 2003). Induction of volatiles terpenoids may result in a quantitative or qualitative difference in the chemical profile produced by a plant (Fitzgerald 2003). The herbivore-induced change in the plant volatiles can be specific for the plant and herbivore species, the plant age and the herbivore stage (Takabayashi et al. 1994; De Moraes et al. 1998).

Defense responses of induced terpenoid have been intensively studied using spruce (*Picea* spp.) as model plant. Anatomical defenses like traumatic resin duct formation or polyphenolic parenchyma cell activation are induced by wounding or by attacking insects and pathogens (Tomlin et al. 1998; Nagy et al. 2000; Franceschi et al. 2002; McKay et al. 2003). Besides, induction of terpenoids have been observed in spruce after mechanical wounding or treatment with methyl jasmonate, a plant hormone known to upregulate plant defenses (Nault and Alfaro 2001; Martin et al. 2002; Faldt et al. 2003).

These terpenoids can act as repellents or toxins against those herbivores, defending conifers directly, or mimic juvenile hormones (Lewinsohn et al. 1991; Langenheim 2003; Mumm et al. 2004). Likewise, conifer volatiles can also attract predatory and parasitic insects, consequently defending conifers indirectly against herbivores (Nadir and Raffa 2001; Mumm et al. 2003; Sullivan and Berisford 2004; Hilker et al. 2005).

In previously studies it was described that *P. castaneus* adults were attracted to the volatiles emitted by *P. taeda* (Marques et al. 2012) and previously stressed plants showed a higher infestation (Iede et al. 2004). In the present study, we conducted detailed GC–MS analyses of volatiles emitted by twigs of *P. taeda*, both healthy and attacked. We analyzed how the attack by *P. castaneus* males and females affects the volatile pattern emitted by *P. taeda* twigs. We also carried out laboratory and field assays to evaluate the attraction of *P. castaneus* to healthy and stressed host plants.

2. Materials And Methods

2.1. Field evaluation of the attraction of *P. castaneus* by *P. taeda*

2.1.1. Characterization of the experimental area

The attractiveness experiments were performed in two plantations with density of 1,666 1 year old plants/ha, located in Três Barras (TB), Santa Catarina, Brazil (26°07'41"S, 50°19'30"W, 802m altitude) and Cambará do Sul (CS), Rio Grande do Sul, Brazil (29°02'52"S, 50°08'41"W, 1031m altitude). In each area, the experiment was repeated on three plots, 200m apart from each other. For the experiment's purpose, plants found in CS were considered stressed due to poor planting sites in flooded areas with poor drainage, whereas plants found in TB were considered healthy.

2.1.2. Evaluation of the attractiveness of *P. castaneus* to *P. taeda* log traps

To evaluate the attractiveness of *P. castaneus* to *P. taeda*, two variables were analyzed: the effect of stressed (CS) x non-stressed (TB) environments, and the effect of seasonal variations through a 12-month experiment.

In each plot of each area, log traps composed of 20 freshly cut 1m long and 8–10 cm diameter *P. taeda* logs were installed every month between July 2012 and June 2013 for adults of *P. castaneus* to lay eggs in. After 30 days, the logs were removed from the field, new log traps were installed in the same plots, and the removed ones were transported to the laboratory. For each batch of 20 logs, 17 were stored for 1 year in screened cages in a room at 20^{o}C, humidity on 70 ± 10% and photoperiod of 12h. The logs were monitored weekly to assess the emergence of viable adults. Any emerged adult was removed from cages.

The average number of adults emerged in each area per month was obtained by averaging the total number of adults emerged in the three plots in each area using BioEstat 5.0 (Ayres et al. 2007). To access the effect of seasonality and location in the attractiveness of the insects, a factorial analysis of variance (ANOVA) was performed using Statistica software (version 8.0; StatSoft Inc, Tulsa, OK, USA). The homogeneity of data was evaluated through a Levene's test, and a Duncan post-hoc test was performed to evaluate which means were statistically different at 5%, both using Statistica 8.0 software.

2.2. Chemical analysis of the attraction of *P. castaneus* by *P. taeda*

2.2.1. Insects

For chemical assays, the insect colony was started with specimens collected from log traps installed in Três Barras plantation (26°07'41"S, 50°19'30"W, 802m altitude). To obtain the insects for the bioassays, *P. taeda* freshly cut logs were placed in the field. Log traps were installed from July 2012 to June 2013. Each log trap was composed of 20 freshly cut 1m long and 8–10 cm diameter pine logs for adults of *P.*

castaneus to lay eggs in. After 30 days, the logs were removed from the field and placed in screened cages measuring 30 cm height by 30 cm diameter until adult emergence. After their emergence, the adults were separated by sex and each group were kept for 5–10 days in an incubator at 22 ± 2°C, 70 ± 10% relative humidity and 12L:12D photoperiod. Females and males were selected for the trials and placed alone in plastic screened cages with ventilation at the sides under the same conditions as described above. Adults were fed a natural diet consisting of pieces of fresh *P. taeda* branches. The cages were cleaned, and the food supply replaced at regular intervals of five days.

2.2.2. Plants

All trees of *P. taeda* used in this work were 1–2 years old and were obtained from greenhouses near Curitiba, Paraná, Brazil.

2.2.3. Y-tube bioassays

Bioassays were conducted with a vertical glass Y tube (2.5 cm ID, bottom arm 20 cm, top arms 20 cm) with ground glass female joints at the end of each arm, and matching male joints terminating in hose nipples. In the system, the speed of humidified, charcoal filtered air was adjusted to 2.0 L/min. All connections were made of Tygon tubing (0.6 cm ID). After each trial, all materials were washed with neutral soap, dipped in 70% ethanol, and placed in the oven at 50 ^{o}C for 30 min to avoid any residual volatile compounds. Test insects were introduced individually into the bottom of the Y tube and, after 5 min of acclimation, allowed 20 min to respond. When the insect moved through the arm containing the stimulus (male or female-attacked *P. taeda* branches), the result was considered positive while the result was negative when it moved through the control arm (healthy *P. taeda* branches). However, when the insect did not move towards any of the arms within 20 min, it was considered non-responsive. The location of stimulus and control were rotated after each test. The insects used in the tests were given no food 12 h before the bioassays. The branches of *P. taeda* used as stimuli were collected one day before conducting the bioassays, placed in the thermal box, and transported to the laboratory where they were kept in the refrigerator at 10 ± 2°C until tests. Bioassays were conducted in a temperature- (23 ± 2 ^{o}C) and humidity-controlled room (70 ± 10% relative humidity). Each stimulus was tested with 30 sexually mature virgin adult insects of each sex.

The data obtained with insects that reached the corresponding odor sources in the Y tube assay were compared by the χ^2 test using BioEstat 5.0 (Ayres et al. 2007). Results showing $p \leq 0.05$ were considered statistically significant.

2.3. Tentative Identification of healthy and attack-induced compounds produced by *P. taeda*

2.3.1. Healthy trees

Four branches of healthy trees of *P. taeda* (200g) were aerated in a 1 L glass chamber. Aerations were carried out under controlled conditions at 23 ± 2°C, relative humidity of 70 ± 10% and a photoperiod of

12L:12D. Volatiles were trapped on a 0.4 cm long bed of Super Q resin (Alltech, Deerfield, Illinois, USA) held in place by glass wool plugs in a glass tube (4 mm ID). Volatiles were collected for two days (flow 1.0 L/min), then eluted with hexane (3 x 0.5 mL). Extracts were concentrated as required (c.a. 100 µL) under Argon. All procedures were carried out in triplicate.

2.3.2. *P. castaneus* attacked trees

Saplings of *P. taeda* (1–2 years old) were placed in plastic screened cages with ventilation at the sides, at 22 ± 2°C, 70 ± 10% relative humidity and 12L:12D photoperiod, with 10 males or 10 females of *P. castaneus*. After 72 hours of attack, branches of these trees were cut and aerated the same way healthy trees were.

2.3.3. Chemical analysis

Extracts and *n*-alkane standards were analyzed by coupled GC/MS with a Shimadzu CGMS-QP2010 Plus system equipped with a quadrupole detector using a Rtx-5MS (Crossbond 5% diphenyl / 95% dimethyl polysiloxane) low bleeding column (30 m x 0.25 mm x 0.25 µm), using Helium as carrier gas, at flow of 1.02 mL/min. Oven temperature was initially held at 60°C for 1 min and increased at a rate of 3°C/min to 250°C. The mass spectrometer operated in electron impact mode (70 eV) with a mass range set from 40 to 350 m/z. The interface and source temperatures were set at 250°C. Kovats retention index was calculated for the compounds and their mass spectra were compared with the literature (Adams 2007). The C_8H_{18}-$C_{18}H_{38}$ and $C_{20}H_{42}$ *n*-alkanes standards were purchased from Aldrich (Deisenhofen, Germany). Compounds detected in at least 2 of 3 samples of the attack-induced pine branches were considered.

3. Results

3.1. Number of *P. castaneus* that emerged from log traps of *P. taeda*

The average number of adults of *P. castaneus* that emerged from the log traps differed between the two study sites. In CS, higher average number of insects (196.778 ± 27.552) were collected when compared to TB, where in average 110.556 ± 22.775 insects were collected (Table 1).

Table 1

Mean number (± SE) of adults of Pissodes castaneus that emerged from the logs trap installed in periods (months) compared within in a Pinus taeda forest in Três Barras (TB), Santa Catarina, Brazil, and Cambará do Sul (CS), RS, Brazil, July/2012 up to June/2013.

Period (month)	Site			
	CS		TB	
	Average nº of insects (n = 3)	Significantly distinct months within area (Duncan)	Average nº of insects (n = 3)	Significantly distinct months within area (Duncan)
Jul.12	283 ± 40.427 *	ABC	46.333 ± 31.424 *	ABC
Ago.12	149.667 ± 15.431	DEF	61.333 ± 12.548	DE
Set.12	116.333 ± 38.942	AGHI	82.333 ± 35.366	F
Out.12	230 ± 79.775	J	240.333 ± 20.497	ADFGHI
Nov.12	308.667 ± 17.023	DGKLM	198 ± 68.000	BJKL
Dez.12	185 ± 65.957	N	118 ± 40.633	
Jan.13	288 ± 33.307	HOP	212 ± 30.238	CEMNO
Fev.13	233 ± 83.720	Q	180.333 ± 22.821	PQ
Mar.13	304 ± 55.426 *	EIRS	106.333 ± 66.520 *	
Abr.13	0 ± 0	BFJKNOQRT	44 ± 7.234	GJM
Mai.13	100 ± 37.005	CLPS	27.333 ± 14.769	HKNP
Jun.13	163.667 ± 41.378 *	MT	10.333 ± 4.702 *	ILOQ
Mean	196.778 ± 27.552		110.556 ± 22.775	

* at “Average nº of insects” rows indicate mean values that were statistically different at 5% significance level among different areas within the same month. Similar letters at “Duncan” rows indicates that the mean values were statistically different at 5% significance level among different months within the same area

Although ANOVA results revealed a correlation between seasonality treatment and mean number of insects obtained ($F = 6.618$, $p < 0.001$; Table 2), no such correlation was observed when both sites and

seasonality were analyzed together (F = 1.763, p = 0.0878; Table 2). This can be explained by the differences found in the average number of insects collected per month for each area (Table 1). For CS, the lowest number of insects was found during the months of April (0 ± 0) and May (100 ± 37.005), whereas the highest number was during the months of November (308.667 ± 17.023) and March (304 ± 55.426). On the other hand, for TB the lowest values were those of June (10.333 ± 4.702) and May (27.333 ± 14.769), whereas the highest ones were those of October (240.333 ± 20.497) and January (212 ± 30.238).

Table 2
ANOVA results for the two treatments (period and site), as well as for the two factors combined.

	Degr. of freedom	F	p
Period (Month)	11	6.618	< 0.001
Site	1	24.381	< 0.001
Month*Site	11	1.763	0.088

Since more insects were collected from the traps coming from a plantation in which the trees were visually more stressed, caused by abiotic factor such as floods, we decided to evaluate in laboratory if the stress caused by *P. castaneus* males and females attack could also result in a greater insect attraction.

3.2. Y-tube bioassays

In Y-tube bioassays, test insects were separated by sex and responded to two different stimuli: healthy and *P. castaneus* attacked branches of *P. taeda* (Table 3). It was observed that males and females were more attracted to attacked branches than to healthy ones.

Table 3
Responses of individual male and female *P. castaneus* adults to treatments in Y-tube olfactometer

Bioassay	Sex Responding	Stimuli	Positive	Negative	χ^2	*P*
1	Female	Male attacked P. taeda	22	8	**6.533**	**0.0176**
2	Female	Female attacked P. taeda	22	8	**6.533**	**0.0176**
3	Male	Male attacked P. Taeda	23	7	**8.533**	**0.0062**
4	Male	Female attacked P. taeda	23	7	**8.533**	**0.0062**

3.3. Tentative Identification of *P. castaneus* Attack-induced compounds

The volatiles produced by branches of *P. castaneus* attacked trees were identified by GC-MS and the results are shown in Table 4. When comparing volatiles produced by non-attacked and male and female *P. castaneus* attacked plants, qualitative and relative amount differences were detected.

Table 4
Volatile compounds produced by healthy *P. taeda* and by female- and male *P. castaneus*-attacked plants

Relative mean area (%)					
	Terpene	**K.I.†**	**Healthy** P. taeda	Attacked by females	Attacked by males
1	**α-pinene**	939	22.73 ± 1.5	63.76 ± 15.8	68.93 ± 8.1
2	**camphene**	954	–	0.77 ± 0.2	0.73 ± 0.1
3	**β-pinene**	979	10.37 ± 1.6	11.59 ± 1.6	12.67 ± 2.2
4	**myrcene**	991	16.14 ± 3.0	13.72 ± 12.0	1.77 ± 1.8
5	**limonene**	1030	3.21 ± 0.3	–	1.10 ± 0.3
6	**β-phellandrene**	1032	17.08 ± 5.3	–	–
7	**(Z)-β-ocimene**	1040	–	4.16 ± 3.0	–
8	**Unknown 1**	1121	–	1.37 ± 0.4	1.67 ± 0.4
9	**methyl octanoate**	1125	5.26 ± 0.5	–	–
10	**(E)-caryophyllene**	1419	1.23 ± 0.3	–	–
11	**prezizaene**	1448	–	1.34 ± 0.7	1.21 ± 0.7
12	**α-humulene**	1455	0.69 ± 0.1	–	–
13	**Unknown 2**	1480	–	0,24 ± 0.1	–
14	**germacrene D**	1485	15.91 ± 3.8	–	–
15	**(E)-muurola-4(14),5-diene**	1491	–	2.59 ± 1.5	5.51 ± 2.9
16	**α-muurolene**	1501	1.11 ± 0.3	–	–
17	**zonarene**	1526	–	0.19 ± 0.1	0.19 ± 0.1
† Kovats Index					

The chromatograms (Fig. 1) showed significant differences in the compounds produced by healthy (A) and female- (B) and male- (C) *P. castaneus* attacked trees. Compounds as β-phellandrene (6), methyl octanoate (9), *E*-caryophyllene (10), α-humulene (12), germacrene D (14) and α-muurolene are produced

only by healthy trees and their production is suppressed when the plant is attacked by *P. castaneus*. Several compounds such as camphene (2), Z-β-ocimene (7), prezizaene (11), (*E*)-muurola-4(14),5-diene (15) and zonarene (17) were produced only by attacked plants. The production of α-pinene (1), β-pinene (3), myrcene (4) and limonene (5), differed in relative percentage in the extracts from healthy and attacked plants; α-pinene (1) and β-pinene (3) were present in higher amounts in attacked-plants extracts than in healthy plants; myrcene (4) and limonene (5) were present in lower percentage in plants attacked by *P. castaneus*.

4. Discussion

4.1. Attractiveness of *P. castaneus* in a plantation of *P. taeda*

The results obtained show that the log traps are effective to collect *P. castaneus* in *P. taeda* plantations, since they are good sites for oviposition. It was expected, once it was demonstrated in a previous work, that males and females of *P. castaneus*, were attracted by branches of the host plant, *P. taeda*, in Y-tube bioassays (Marques et al. 2011). The number of insects collected varied according to the plantation site. The number of insects collected in CS was higher than in TB, probably due to a stress caused by environmental conditions, such as planting sites in flooded areas with poor drainage. This apparently led to a higher infestation in CS, so it can be proposed that *P. castaneus* feed preferentially on damaged or stressed trees, which could favor the dissemination of the insect.

The number of insects collected on each area also varied according to the time the trap was installed (seasonality treatment), which can be related with *P. castaneus* population variation, that is associated with abiotic factors of each region. For the areas studied, such variation was not simultaneously observed for both areas. Therefore, although for each area the parameter “month” has had a significative variation, those did not coincide among the evaluated areas.

4.2. Laboratory bioassays

4.2.1. Y-tube bioassays

In Y-tube bioassays, test insects walked rather than flew when they responded to stimuli. The attractiveness of males and females to volatiles emitted by the healthy versus attacked host plant was determined. The bioassays showed that females and males were significantly more attracted by attacked host plant when compared to the healthy host plant, as shown in Table 3.

4.2.2. Tentative identification of Attack-induced compounds

Volatiles of *P. castaneus* attacked *P. taeda* have significant differences from the ones produced by the healthy trees. The relative amount of α-pinene is about three times higher and the production of β-phellandrene and germacrene D is suppressed (Fig. 2). On the other hand, attacked trees produce

compounds not produced by the healthy ones, such as prezizaene, (*E*)-muurola-4(14),5-diene and (*Z*)-β-ocimene, the latter one interestingly produced only by female-attacked *P. taeda* (Fig. 3).

In a search for compounds that contribute to the perception of the host in *Pissodes castaneus* (= *P. notatus*), Bichão and co-workers (2003) identified olfactory receptor neurons that respond to α-pinene, β-pinene, limonene, β-phellandrene, (*E*)-caryophyllene, camphene and other compounds. So, any change in the amount of those compounds will have influence in the perception of the host by the insect. In a recent published work, Skrzecz and co-workers (2019) showed that feeding *P. castaneus* were baited in olfactory bioassays and field traps using α-pinene and ethanol as attractant.

Some of the volatiles produced only by the wounded trees, such as camphene, (*Z*)-β-Ocimene and (*E*)-muurola-4(14),5-diene were previously detected in unhealthy *Pinus sylvestris* trees, either by pollution (Judžentienė et al. 2006; Kupcinskiene et al. 2008) or by insect feeding (Mumm et al. 2003), but also in *P. sylvestris* healthy plants. However, this is the first report that detect prezizaene and zonarene being produced in the genus *Pinus*.

Volatiles of many species of *Pinus* induced when attacked by different insects (Barnola et al. 1994; Sadof and Grant 1997; Mumm et al. 2003, 2004; Miller et al. 2005) including other *Pissodes* (Tilles et al. 1986; Nordlander 1991) were studied. In most cases, there was a decrease of α-pinene and an increase of limonene production, differently from what was observed in this study. (*S*)-(-)-limonene is a known repellent and α-pinene is an attractant to some weevils (Nordlander 1990) and many others species of herbivores (Sadof and Grant 1997). (*S*)-(-)-limonene is also known to be more toxic to several Curculionidae compared to other monoterpenes, typically α-pinene, β-pinene, 3-carene and myrcene (Smith 1965; Werner 1995; Chiu et al. 2017). Likewise, another compound known to be a repellent to many insects, (*E*)-caryophyllene (Bedini et al. 2015; Bougherra et al. 2015; Alquézar et al. 2017), had its production interrupted when the tree was attacked by *P. castaneus*.

So, as compounds that act as repellent or are toxic to insects ((*S*)-(-)-limonene and (*E*)-caryophyllene) have its production decreased and attractants (α-pinene) production increase, this can explain why the *P. castaneus* prefer to previously wounded or stressed trees, either by abiotic factors or other insects species.

5. Conclusions

Our results indicate that it is possible to discriminate, using GC-MS analysis, between healthy plants of *P. taeda* and *P. castaneus* attacked ones based on the terpenes emitted. The preference of *P. castaneus* adults to attacked *P. taeda* makes it likely that this pest locates the host plants using the specific volatiles that it emits and that the presence for stressed trees can contribute to the attraction of *P. castaneus* to the site.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Gustavo Frensch, Scheila R. M. Zaleski, Francisco A. Marques, Beatriz H. L. N. S. Maia and Sonia M. N. Lazzari contributed to the study conception and design. Material preparation, data collection and analysis were performed by Gustavo Frensch, Scheila R. M. Zaleski and Marina Krasniak. Statistical analysis was perfomed by Gustavo Frensch and Liliane G. Dantas. The first draft of the manuscript was written by Gustavo Frensch and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

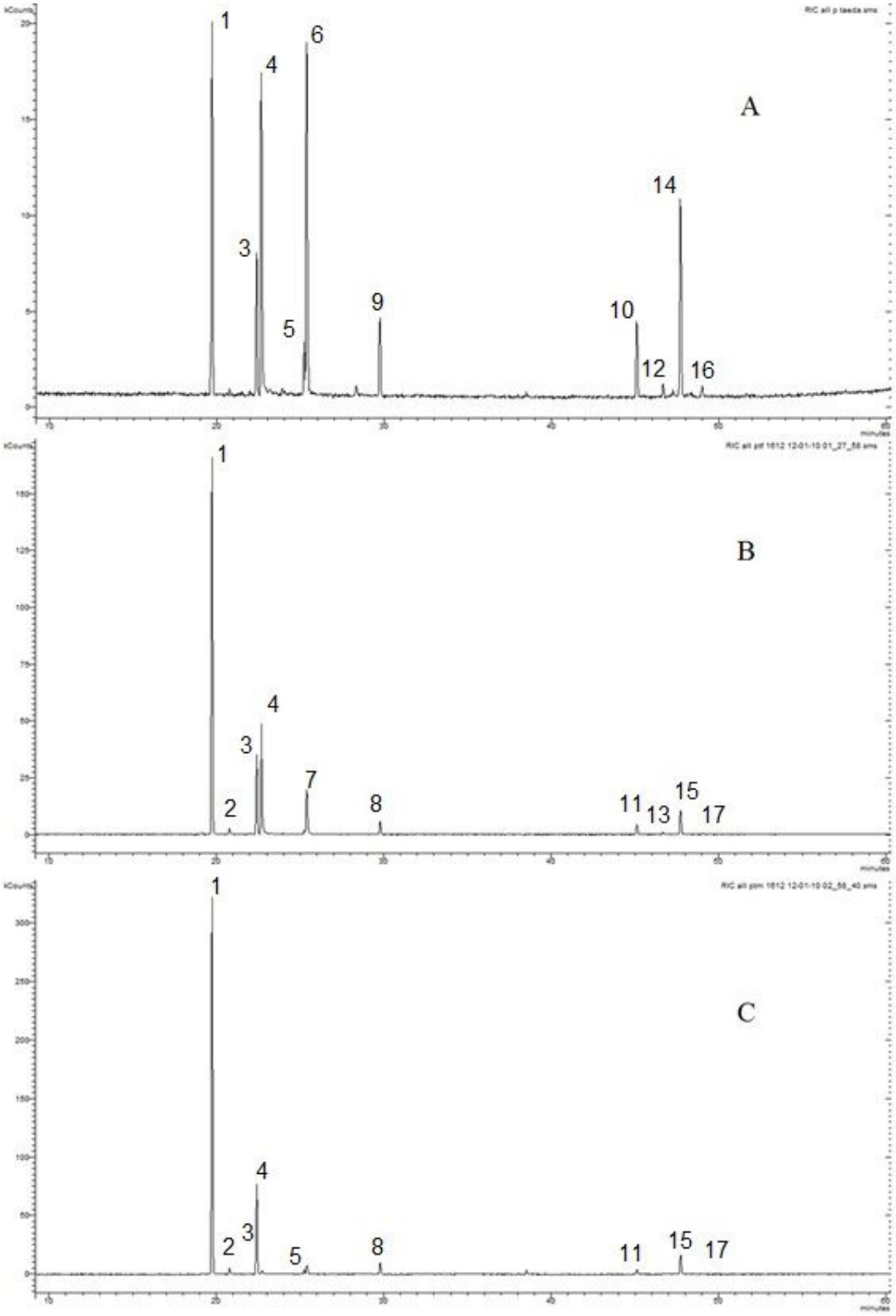

Figure 1

Total ion chromatogram of the aeration of (A) healthy, (B) female- and (C) male-attacked *P. taeda.* All identifications are tentative and made by comparation with Adams (2007). Compounds: 1) α-pinene; 2) camphene; 3) β-pinene; 4) myrcene; 5) limonene; 6) β-phellandrene; 7) (*Z*)-β-ocimene; 8) Unknown 1; 9) methyl octanoate; 10) (*E*)-caryophyllene; 11) prezizaene; 12) α-humulene; 13) Unknown 2; 14) germacrene D; 15) (*E*)-muurola-4(14),5-diene; 16) α-muurolene; 17) zonarene.

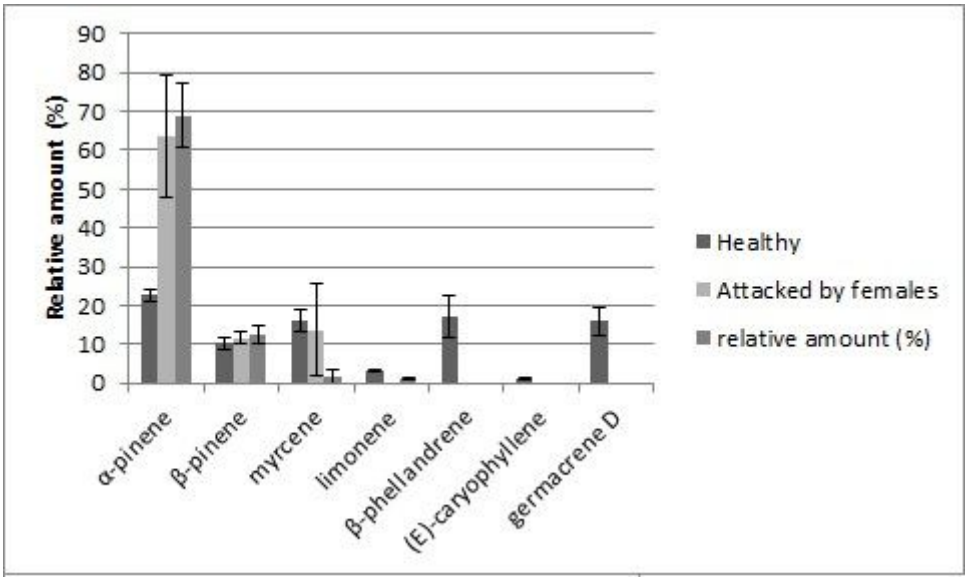

Figure 2

Relative amount of volatiles produced by healthy *P. taeda* trees and by *P. castaneus* female- and male-attacked plants. All identifications are tentative and made by comparation with Adams (2007).

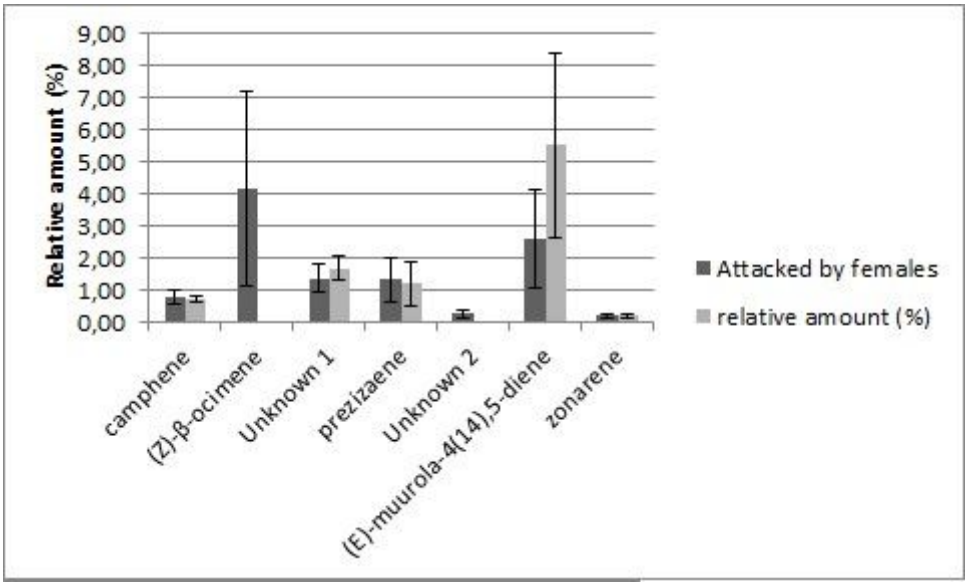

Figure 3

Relative amount of volatiles produced only by female- and male-attacked *P. taeda.* All identifications are tentative and made by comparation with Adams (2007).

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