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Aggregation Pheromone of *Cyrtogenius Luteus*, an Invasive Bark Beetle in Southern South America

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Research Article

Keywords: Coleoptera, Curculionidae, Scolytinae, Forest pest, Invasive pest, Aggregation pheromones, Pinus taeda

Posted Date: June 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9875783/v1

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Additional Declarations: Competing interest reported. AG is part of the Editorial Board of the Journal of Chemical Ecology CR is Associate Editor of the Journal of Chemical Ecology

Version of Record: A version of this preprint was published at Journal of Chemical Ecology on August 29th, 2026. See the published version at https://doi.org/10.1007/s10886-026-01752-0.

Abstract

Bark beetles are among the most important invasive pests affecting pine forestry worldwide, yet the chemical ecology of many introduced species remains largely unknown. *Cyrtogenius luteus* is an invasive scolytine established in pine plantations in South America, for which no pheromone had previously been identified. Here, we report the identification and biological evaluation of a male-produced aggregation pheromone in *C. luteus*. Volatiles from male-infested, female-infested, and unattacked *Pinus taeda* wood were collected and analyzed by thermal desorption GC–MS. A compound consistently detected only in male-infested pine wood was identified as p-mentha-1,8-dien-4-ol through comparison with a synthetic standard. In Y-olfactometer assays, both males and females were attracted to volatiles from unattacked pine relative to clean air and showed a significant preference for male-attacked pine over unattacked pine, as indicated by both first choice and residence time. Field trials with synthetic p-mentha-1,8-dien-4-ol confirmed its biological activity: cross-vane traps baited a combination of synthetic pheromone and host attractants increased adult captures more than 20-fold, in comparison with the conventional host-attractant baited traps. Males and females were captured in similar proportions. Together, the male-specific occurrence of p-mentha-1,8-dien-4-ol, the attraction of both sexes to male-attacked host material, and the response of both sexes to the synthetic compound in the field, identify this oxygenated monoterpene as a male-aggregation pheromone of *C. luteus*. This is the first pheromone reported for the species and provides new insight into the chemical ecology of an invasive bark beetle established in South American pine plantations.

INTRODUCTION

One of the consequences of globalization is the transport and introduction of pest insects into new regions beyond their natural range of distribution (Brockerhoff and Liebhold 2017). This issue is further exacerbated by climate change, which may pose additional challenges to host plants and may disrupt ecological processes (Ramsfield et al. 2016). Among these insects, bark beetles (Curculionidae: Scolytinae) are considered one of the most significant invasive pests threatening pine forestry worldwide (Biedermann et al. 2019; Brockerhoff et al. 2006; Raffa et al. 2015). Bark beetle populations can be significantly influenced by stochastic perturbations, which can trigger a shift from a stable phase—where they persist at low densities—to an epidemic phase (Lantschner and Corley 2023). This transition often leads to widespread damage to host trees, as the beetles proliferate and overcome the trees' defenses (Gómez et al. 2023). Since the 1980s, several species of Eurasian bark beetles have been introduced to the southern hemisphere, and this trend is likely to continue in the coming years (Brockerhoff et al. 2006; Faccoli et al. 2020; Lantschner and Corley 2023).

Cyrtogenius luteus (Blandford) (Coleoptera: Curculionidae: Scolytinae) was first reported in Uruguay in December 2009, coinciding with a significant bark beetle infestation affecting pine species in the country (Gómez et al. 2012). Although this was the first record of the species in South America, it was later identified in monitoring traps in Brazil as early as 2006, suggesting that its initial introduction into South America likely occurred in Brazil, from where it spread southward (Flechtmann and Atkinson 2018).

Since its initial detection, the species has rapidly spread nationwide and is now established on the two primary commercially planted pine species, *Pinus taeda* L. and *P. elliottii* Engelm., as well as on *P. pinaster* Aiton (Gómez 2016). Outside Uruguay, *C. luteus* has been documented infesting other Pinaceae, such as *Larix* and *Picea* (Beaver and Liu 2010), and there is also evidence suggesting its presence on other botanical families, including Araucariaceae and Cornaceae (Flechtmann and Atkinson 2018).

A monitoring scheme for *C. luteus* was established in 2009 in Uruguay by deploying interception window traps across six monitoring stations covering the major pine-growing areas in Uruguay (Gómez et al. 2012). The species is polygynous with at least two generations per year in South America (Lantschner and Corley 2023) and displays a distinct seasonal emergence pattern, with peak trap captures consistently observed during the summer months (Gómez et al. 2017). Its population levels are strongly influenced by precipitation, showing higher abundance during periods of increased rainfall, but no significant correlation with temperature (Gómez et al. 2020). As a result, the number of *C. luteus* captured in traps can be significantly predicted by total precipitation and seasonal factors. Interception traps were later replaced with either Lindgren funnel traps or cross-vane panel traps baited with ethanol and turpentine (Gómez 2016), as there is evidence that *C. luteus* is attracted to a mixture of ethanol and alpha-pinene, with or without the addition of known bark and ambrosia beetle pheromones such as ipsdienol or sulcatol (Fan et al. 2010; Flechtmann and Atkinson 2018). However, to date, no specific pheromone has been identified for *C. luteus*.

Here we report the chemical identification and biological evaluation of the male aggregation pheromone of *C. luteus*. Specifically, we report the chemical analysis of volatiles from male and female galleries, the racemic synthesis of the candidate male pheromone, laboratory olfactometer bioassays with natural volatiles and the synthetic compound, and field trials to evaluate the pheromone attraction activity in comparison with generic host tress attractants.

METHODS AND MATERIALS

Insects and Plant Material Unmated male and female adults of *Cyrtogenius luteus* were obtained from a seasonal rearing based on infested *Pinus taeda* logs stored at the National Institute of Agricultural Research (INIA) station in Tacuarembó, Uruguay, following a reported methodology (Gómez et al. 2017). In essence, during the flight season (November to March), cut logs (1 m height, 20 cm diam.) were left in the field for two weeks for natural infestation, checked every other day until presence of sawdust, then transferred to the laboratory, and placed in cylindrical white plastic bins (1 m height, 60 m diam.). The bins were covered with voile mesh to contain emerging insects while allowing ventilation. Adult beetle emergence was checked daily, and freshly emerged adults were immediately separated by sex and kept in refrigerated boxes until they were shipped on a weekly basis to the Chemical Ecology Laboratory (Faculty of Chemistry, Montevideo, Uruguay) for chemical and olfactometer studies. Freshly cut, non-attacked *P. taeda* logs were cut into 5-cm slices and shipped along with the insects.

To evaluate any potential effect of the refrigerated conditions on the insects, we conducted an experiment to measure adult survival under cold conditions. For this purpose, newly emerged adults were separated by sex and placed into vials containing up to 10 individuals. The vials were stored in a refrigerated chamber at 4°C ± 1°C and 55% relative humidity. The vials were inspected daily, and the number of surviving individuals was recorded until the death of the last specimen. In total, we monitored 100 individuals of each sex (Fig. S1).

Infestation and Collection of Gallery Volatiles Pine slices (5 cm height, 30 cm diam.) were infested with either 10 unmated males or females, and kept separated in-mesh covered plastic containers at 25°C. Upon releasing the insects onto the pine slices, they immediately moved towards the cambium area and buried a gallery (*ca*. 1 mm diam). One day after infestation, volatile organic compounds were collected from the entrance of the galleries using glass tubes containing Tenax® (200 mg). Air was drawn with a battery-operated air sampling pump (Apex 2) at 0.2 L/min. Collections were performed for 72 h with change of the adsorbent filter every 24 h. As a control, 1 mm holes were drilled in undamaged pine slices and volatiles were collected as described. Seven replicates were conducted to ensure consistent results.

Volatile Chemical Analysis Trapped volatiles were desorbed using a TD-30 thermal desorption system (Shimadzu Corp.) and analyzed on a QP 2010 Shimadzu GC-MS equipped with a Stabilwax-MS capillary column (30 m, 0.25 mm ID, 0.25 µm; Restek, USA). The thermal desorption system was operated by heating the tubes to 250°C for 5 min under a He flow of 20 mL/min, then focusing the volatiles at -20°C prior to injection. The GC-MS was operated with a column temperature program of 40°C (1 min) – 5°C/min – 250°C (5 min), split injection (1/20 split ratio), column flow of 1.5 mL/min (He) and interphase temperature of 230°C. The MS was operated in scan mode (*m/z* 30–350), ion source temperature of 200°C and 0.5 min cut-off time. Compounds were identified from fragmentation patterns and mass spectra database matches (NIST 17), as well as by retention indices and comparison with a synthetic standard of the identified pheromone.

Laboratory Bioassays Olfactometer bioassays were conducted to evaluate the attraction of males and females to volatiles from undamaged and male-attacked *P. taeda* slices. A Y-shaped acrylic olfactometer (branch width: 1.7 cm; central branch length: 6 cm; stimulus branch length: 7 cm; height: 1 cm) with bottom and top glass covers was placed horizontally within a box for visual isolation and lit from below with diffused white light. Charcoal-filtered humidified air was supplied by a compressor with a flow of 2 L/min and pushed into polyester bags containing pine slices similar to those described for the volatile collection. To further ensure air flow into the olfactometer, air was drawn from the entrance using an air sampling pump (Apex 2) at 1 L/min.

Bioassays were conducted with freshly emerged unmated adults, individually tested for 10 min after their release at the base of the central branch. First arm choice and total time spent in each stimulus arm were visually recorded. Insects that did not respond after 5 min were not included in the analysis, but percent responses are nonetheless reported. The volatile stimuli were tested in dual choice comparing

undamaged pine slice vs clean air and undamaged pine slice vs male-attacked pine slice. Male-attacked slices were prepared as described in the volatile collection section and used one day after infestation.

Synthesis The candidate pheromone compound, tentatively identified as 1,8-menthadien-4-ol, was synthesized as a racemic mixture for confirmation and field trials. Limonene was oxidized with SeO_2 as described elsewhere (Thomas and Bucher 1970): 1 g (7.3 mmol) of (*R*)-(+)-limonene (Sigma-Aldrich) was warmed to 50 ºC and added dropwise to a hot solution of 0.5 g (4.5 mmol) of selenium dioxide (Sigma-Aldrich) in 5 ml of ethanol. The mixture was stirred for 6 h at 95 ºC, then allowed to cool and filtered to remove the precipitated selenium. The solution was then concentrated and purified by column chromatography using silica gel 60M (230–400 mesh) and 9:1 hexane:ethyl acetate.

Field trials The synthetic pheromone candidate was tested in the field in comparison with turpentine and ethanol, generic bark beetle attractants used as reference trapping methodology (Lantschner et al. 2024). The field trial took place in a commercial 19-year-old *P. taeda* forested area located in Tacuarembó, Uruguay (Fig. S2). This area is located in Northern Uruguay, the main pine-forested region, and has recent records of *C. luteus* infestations. The trial was conducted for 8 weeks during April-June 2023 (see Fig. S2 for weather conditions). Twenty cross-vane traps (1 m height, 30 cm wide) with liquid collection cups (propylene glycol) were arranged in a 4 x 5 experimental design with 4 treatments and 5 blocks (Fig. S2). The traps within a block were spaced 50 m apart and included all four treatments in randomized positions. The 5 blocks were spaced 50 m.

Tested treatments were: *i)* traps with no attractants (hereafter control); *ii)* turpentine/ethanol (T + E); *iii)* turpentine/ethanol/pheromone (T + E+P) and *iv)* ethanol/pheromone (E + P). Turpentine and ethanol (30–50 mL) were placed in separate 100 mL plastic flasks with holes in the lids to allow evaporation (Gómez 2016; Lantschner et al. 2024). The pheromone-loaded septa were hung from a wire in the center of the trap, shielded from rain and direct sunshine. All attractants were replaced every two weeks. Captured *C. luteus* were counted weekly and sexed under a stereoscopic microscope. Other scolytids captured in the traps were also identified and counted (data not shown).

Prior to the field trials, the emission rates of natural rubber septa dispensers were assessed. To do this, 1,8-menthadien-4-ol (50 mg) was loaded in 200 µL of CH_2Cl_2 in pre-weighed white rubber septa (int. diam. 2.2 mm; ext. diam. 4.5 mm; length 17 mm). The same solvent volume was added to control septa to assess the solvent evaporation rates. Septum weight loss was recorded daily for 10 days and every three days thereafter (n = 3). This preliminary experiment showed that, in laboratory conditions, the septa emitted approximately 50% of the pheromone load during the 2-week replacement period (Fig. S3). During this period, daily emission rates varied from an initial emission of 4.1 mg/day to a final rate of 0.85 mg/day, with an average emission of 2.3 mg/day.

Statistical analyses Survival rates for insects under storing/shipping conditions (4°C) were plotted over time and statistically analyzed using Kaplan-Meier survival analysis (*survfit* function, survival package, version 4.4.3). Sex-specific survival curves were compared using the log-rank test (*Mantel-Cox test*). For

olfactometer bioassays, first arm choice was analyzed using *GLM* with binomial error distribution and logit link function, with models fitted separately by sex. Arm residence times were analyzed by the *Wilcoxon test*. For the field test, total beetle trap captures per treatment (i.e. total catches per block throughout the season), total females and males per treatment (i.e. total catches of each sex per block throughout the season) and weekly catches (a separate analysis per week) were subjected to generalized linear models (*GLM*) with quasipoisson error (*GLM-qp*). Pairwise comparisons among treatments were performed using estimated marginal means (*EMMs*) with Tukey-adjusted p-values using the R package *emmeans* (Lenth and Piaskowski 2025) ($p < 0.05$). Generalized linear mixed models (*GLMMs*) were previously tested, however, since random effects contributed negligibly to model performance and simpler models provided an adequate fit, final analyses were conducted using *GLMs*. Due to limitations of *GLM* models, any treatment with zero catches was not included in the analysis. Data sets were evaluated for overdispersion using half-normal plots from the *hnp* package 1.2–6 (Moral et al. 2017). All analyses were performed in R (R Core Team 2024).

RESULTS

Volatile Chemical Analysis Volatile organic compounds trapped in Tenax tubes were analyzed by GC-MS using thermal desorption. All volatile blends, either from insect-infested or control samples, were dominated by typical pine volatiles such as estragole (Fig. 1A, major peak) and the monoterpene hydrocarbons α-pinene, β-pinene, β-myrcene, limonene and β-phellandrene (not shown). Since characterizing these volatiles was not the scope of this study, they were not further studied. However, a detailed comparison of volatiles from male-infested, female-infested and control samples, consistently showed a differential peak in the volatiles from male-infested pine wood, in comparison with female-infested and control samples (Fig. 1A, arrow), suggesting a male-emitted compound which was therefore targeted for identification.

The mass spectrum of the target compound (Fig. 1B) did not match spectra in available mass spectra databases. Moreover, no mass spectra from known scolytid pheromones (available at The Pherobase (El-Sayed 2026)) matched the fragmentation pattern of the target compound. The mass spectrum showed a tentative molecular ion of m/z 152, suggesting an oxygenated monoterpene with three rings or double bonds ($C_{10}H_{16}O$). Other significant ions were the base peak at *m/z* 69, most likely an isoprenyl ion $C_5H_9^+$, and *m/z* 84, possibly $C_5H_8O^+$ or, less likely, $C_6H_{12}^+$. The retention index of the compound was calculated 1686 for the polar column, as interpolated from the adjacent pine volatiles estragole (RI 1670) and α-terpineol (RI 1697). With all this information available, a thorough revision of mass spectra of reported scolytid semiochemicals was done and a candidate compound was hypothesized based on two findings. First, similarities were found between the mass spectrum of the target compound and that of p-menth-1-en-4-ol (4-terpineol), with two mass unit differences in key fragment ions. This suggested a structure with the tertiary alcohol in position 4 and an additional double bond. Second, an attractant with such structure, p-mentha-1,8-dien-4-ol (MW 152) was serendipitously found for the bark beetle *Taenoglyptes fulvus* (Scolytinae;Cryphalini) in Japan, in exploratory field trapping assays (Sumimoto et al.

1975). The mass spectrum of this compound, unavailable commercially, was only found in CAS SciFinder database (CAS # 3419-02-1), and showed clear similarities with the mass spectrum of our target compound, further strengthening our hypothesis for the male pheromone of *C. luteus*.

Testing this hypothesis required comparison with a synthetic standard, so we synthesized p-mentha-1,8-dien-4-ol (synthesis reported herein), and ordered a custom synthesis sample from a chemical company (BLD Pharmatech, USA). Mass spectra comparison of the natural and synthetic compounds precisely matched (Fig. 1C), confirming the identification of p-mentha-1,8-dien-4-ol as the male-specific compound obtained from *C. luteus* male infested pinewood.

Synthesis Oxidation of limonene with SeO_2 yielded 90 mg of p-mentha-1,8-dien-4-ol (8% yield). One- and two-dimensional NMR spectra (in $CDCl_3$, see Fig. S5a-d) confirmed the structure of the synthetic compound, showing the expected ^{1}H NMR and ^{13}C NMR signals and correlations (see Fig S5a for carbon labels): ^{1}H NMR (δ): 1.65 (s, 1H, OH), 1.69 (s, 3H, Me g), 1.74 (m, 1H, He), 1.80 (s, 3H, Me i), 1.83 (m, 1H, He'), 1.95 (m, 1H, Hf), 2.03 (broad d, J = 18 Hz, 1H, Hc), 2.20 (m, 1H, Hf'), 2.36 (broad d, J = 18 Hz, 1H, Hc'), 4.84 (broad s, 1H, Hj), 5.03 (s, 1H, Hj'), 5.33 (broad s, 1H, Hb). ^{13}C NMR (δ): 18.9 (CiH_3), 23.4 (CgH_3), 27.4 (CfH_2), 32.2 (CeH_2), 37.2 (CcH_2), 72.3 (Cd-O), 109.8 (CjH_2), 118.4 (CbH), 133.8 (Ca), 150.5 (Ch).

Laboratory bioassays Olfactometer bioassays showed that, as expected, both males and females were attracted to volatiles from their host three, in this case *P. taeda*, when contrasted to clean air (Fig. 2A, C). In these bioassays, the response rate was 92% for females and 96% for males, suggesting strong attraction to host volatiles. Considering first arm choice, both females and males showed a significant preference for pine slices over clean air (Fig. 2A; $z = 3.832$, $p < 0.001$ for females; $z = 2.532$, $p = 0.011$ for males). This preference was also observed in residence times within the olfactometer arms; both females and males remained significantly longer in the arm carrying volatiles from pine slices (Fig. 2C; $W = 1344.5$, $p < 0.0001$ for females; $W = 1861.0$, $p = 0.0006$ for males).

More important to our study, olfactometer bioassays contrasting male-attacked and unattacked pine slices showed that both males and females showed a significant preference for male-attacked pine slices (Fig. 2B; $z = 3.494$, $p < 0.001$ for females; $z = 2.794$, $p = 0.005$ for males), suggesting a male-emitted aggregation pheromone. Residence times showed a similar pattern, with significantly longer residence times the arms with volatiles from male-attacked pine slices, compared to undamaged pine slices (Fig. 2D; $W = 1536.5$, $p < 0.0001$ for females; $W = 1764,5$, $p < 0.0001$ for males). Here again, high response rates of 82% and 96% were observed for females and males, respectively.

Field trial To further study the presence of an aggregation pheromone in *C. luteus*, a field test was carried out to assess attraction to the synthetic pheromone. The main comparison of interest was the adult catches in traps loaded with the host volatiles turpentine and ethanol, usually employed for scolytid trapping, and catches when the synthetic pheromone was added to these volatiles. The results showed an increase of more than 20-fold in catches in turpentine/ethanol/pheromone traps, compared to traps with the host volatiles alone (Fig. 3A). The cumulative mean captures were 93.4 ± 11.6 and 4.4 ± 2.5

adults per trap in an 8-week period for turpentine/ethanol/pheromone traps and turpentine/ethanol traps, respectively (mean ± SEM). The unbaited traps showed similar captures (2.6 ± 1.9) than the traps with host volatiles, and traps with pheromone but no turpentine (ethanol/pheromone traps) showed similar captures (108.2 ± 12.5) as the whole traps (Fig. 3A). Multiple comparisons of adjusted means indicated that turpentine/ethanol/pheromone and ethanol/pheromone were significantly higher than both control and turpentine/ethanol ($p < 0.0001$ in all cases). No significant differences were detected between turpentine/ethanol/pheromone and ethanol/pheromone ($p = 0.8220$), nor between control and turpentine/ethanol ($p = 0.9439$).

Captured adults were separately counted by sex, showing equal attraction of males and females, concordant with an aggregation pheromone. Sex as a factor showed no significant effect, nor did the interaction sex-treatment ($p > 0.05$) (Fig. 3B). Weekly captures (Fig. S4) showed a similar pattern as cumulative captures regarding treatment effects. Variations in weekly captures may be attributed to fluctuations in environmental conditions (Fig. S2) and to the population dynamics of the insects.

DISCUSSION

Our study provides the first evidence of a male-produced aggregation pheromone in *Cyrtogenius luteus*, an invasive bark beetle established in pine plantations in South America. A compound detected specifically in volatiles from male-infested *Pinus taeda* wood was identified as *p*-mentha-1,8-dien-4-ol through GC–MS analysis and comparison with a synthetic standard. Behavioral assays showed that both females and males were attracted to volatiles from male-attacked pine slices over unattacked pine slices, while field trapping demonstrated a marked increase in captures when synthetic *p*-mentha-1,8-dien-4-ol was combined with host attractants. The attraction of both sexes in laboratory and field assays supports the classification of this compound as a male aggregation pheromone. Beyond providing new insight into the chemical ecology of this species, these results offer a semiochemical-based tool with direct potential for improving monitoring programs in pine-growing regions invaded by *C. luteus* (Gómez 2016; Gómez et al. 2012).

Volatile collections from attacked and unattacked pine material consistently showed a differential peak only in male-infested material, indicating that the compound was associated with male colonization rather than constitutive pine emissions or unspecific damage responses. The compound was subsequently identified as p-mentha-1,8-dien-4-ol supported by the agreement between the natural compound and the synthesized standard in GC–MS analyses.

Olfactometer bioassays provided behavioral evidence that further supported the presence of a male-emitted pheromone. First, host volatiles themselves proved to be attractive by themselves to *C. luteus*; both males and females were attracted to unattacked *P. taeda* slices relative to clean air, in agreement with previous field observations showing attraction of this species to host-related compounds such as ethanol and α-pinene (Fan et al. 2010; Flechtmann and Atkinson 2018). This response likely reflects the importance of volatile signals associated with suitable colonization material during dispersal.

Nevertheless, the stronger behavioral response observed when insects were offered male-attacked *vis-a-vis* unattacked pine slices indicates that male colonization substantially increases the attractiveness of host material. Because male-infested pine was the only treatment associated with the detected pheromone candidate, the most parsimonious explanation is that *p*-mentha-1,8-dien-4-ol acts as an aggregation pheromone signal released in association with pioneer male colonization of the host.

The evidence for a male-emitted aggregation pheromone in analytical and behavioral studies was further supported by a field trial in which synthetic *p*-mentha-1,8-dien-4-ol, tested along with the host volatiles turpentine and ethanol, resulted in more than a 20-fold increase in adult captures compared with the host volatiles alone. Interestingly, captures in traps baited with the synthetic pheromone in combination with ethanol suggest that a simplified host attractant may suffice to synergize with the pheromone. Further experimentation with different pheromone/host attractant blends as well as with the pheromone by itself will provide more information on the most attractive lure combination.

Even though turpentine and ethanol are regarded as adequate attractants for *C. luteus* (Flechtmann and Atkinson 2018; Gómez 2016), in the absence of pheromone they did not increase captures relative to unbaited traps. Taking into account that the field test was carried from April through June, later in the season than the main flight period of *C. luteus* (Gómez et al. 2017), the results suggest that host attractants may be useful generic lures during peak flight season, but the synthetic pheromone is a key attractive component under low population levels, which is particularly relevant for delimitation surveys, early detection in uninvaded areas, and surveillance of the continuing expansion of *C. luteus* in the Southern Hemisphere.

The similar capture rates of females and males in pheromone-baited traps further support the assignment of *p*-mentha-1,8-dien-4-ol as an aggregation pheromone rather than a sex pheromone. This pattern is also consistent with the laboratory results, in which both sexes preferred male-attacked host material. For monitoring purposes, attraction of both sexes is advantageous because it increases trap sensitivity and may provide a more representative estimate of adult flight activity than a lure attracting only one sex.

Interestingly, *p*-mentha-1,8-dien-4-ol had previously been reported as an attractant for *Taenoglyptes fulvus*, a scolytine beetle of the tribe Cryphalini. However, the ecological function in that species was identified through exploratory field assays rather, than through direct association with male emissions (Sumimoto et al. 1975). The present study therefore extends the relevance of this oxygenated monoterpene within Scolytinae. Clearly, *p*-mentha-1,8-dien-4-ol is structurally related to host monoterpenes, so its production could be linked to the transformation of host-derived precursors during male gallery establishment. Such a mechanism would be consistent with the integration of host and conspecific information during host colonization by bark beetles.

Within the Dryocoetini, male aggregation pheromones have been reported for the most part in the genus *Dryocetes*, namely *D. confusus*, *D. affaber* and *D. autographus*, and for *Xylocleptes bispinus*.(Borden et al. 1987; Camacho et al. 1994; Klimetzek et al. 1989; Kohnle and Vité 1984; Stock et al. 1990) In *Dryocetes*

species, pheromone chemistry is based on the fatty-acid-derived bicyclic acetals exo- and endo-brevicomins (Vanderwel et al. 1992), while in *X. bispinu*s, which is not associated with conifers, the main male-specific pheromone is the acyclic monoterpene ipsenol, structurally more related to the male-aggregation pheromone herein reported for *C. luteus*. To our knowledge, p-mentha-1,8-dien-4-ol has not yet been reported as a pheromone in scolytines, therefore extending the known chemical diversity of aggregation pheromones in bark beetles.

Attraction of both sexes to male-attacked pine volatiles is consistent with the reproductive biology of *C. luteus*. The species is described as polygynous (Lantschner and Corley 2023), and the emission of an aggregation pheromone by males would facilitate the recruitment of females to occupied breeding material, while also attracting additional males to a suitable host resource. In bark beetles, aggregation can enhance the exploitation of host material and, in systems involving living or recently stressed trees, may contribute to overcoming host defenses through coordinated colonization. Although the present assays do not establish the consequences of aggregation for successful attack or reproduction in *C. luteus*, the strong attraction to male-associated odors suggests that chemical communication is likely to play a central role in its colonization dynamics. This may be especially important for an introduced species exploiting commercially planted hosts across extensive areas of South America.

The discovery of an aggregation pheromone may also improve the interpretation of monitoring data for this invasive species. Current knowledge of *C. luteus* seasonal flight activity and its association with rainfall in Uruguay has been obtained using interception traps or traps baited with generic host volatiles (Gómez et al. 2017; Gómez et al. 2012; Gómez et al. 2020). A more attractive pheromone-based lure could provide greater temporal resolution of flight peaks and improve the power to associate captures with environmental drivers, population increases, or forest management practices.

Some aspects of the pheromone system remain to be resolved. First, the compound evaluated here was synthesized and tested as a racemic mixture. Thus, although the synthetic lure was strongly attractive in the field, the absolute configuration of the naturally produced pheromone and the relative behavioral activity of its enantiomers remain unknown. Identification of the natural stereoisomer may allow further improvement of lure efficacy and may provide information on the specificity of chemical communication in *C. luteus*. Second, while the present results demonstrate attraction to p-mentha-1,8-dien-4-ol, additional minor pheromone components may occur below the detection threshold of the analytical approach or may enhance attraction in particular ecological contexts. Third, further field trials should evaluate lure dose, release rate, trap design, seasonal performance, and possible interactions between the pheromone and host-related volatiles. Such information will be required before implementing an optimized operational monitoring protocol.

In conclusion, this study identifies *p*-mentha-1,8-dien-4-ol as a male-associated aggregation pheromone of *C. luteus* and demonstrates that a synthetic formulation containing this compound and host volatiles is highly attractive under field conditions. The compound mediates attraction of females and males and substantially outperforms the generic host-derived attractants previously used for trapping this invasive

beetle. These findings expand current knowledge of the chemical ecology of *C. luteus* and offer a promising tool for improving surveillance and management of this invasive scolytine in South American pine plantations.

DECLARATIONS

Competing Interests

AG is part of the Editorial Board of the Journal of Chemical EcologyCR is Associate Editor of the Journal of Chemical Ecology

Author Contribution

GZ conducted all laboratory and field experiments. MA performed the statistical analyses. CR contributed to data analysis and experimental planning. GS planned and advised on the organic synthesis work and NMR data analysis. GMC provided advice on all entomological aspects of the study and led insect rearing and identification. AG conceived, planned, and led the study and interpreted the GC–MS data. CR and AG acquired funding for the research. GZ, MA, GMC, and AG contributed to the preparation of the manuscript and figures. All authors reviewed and approved the final version of the manuscript.

Acknowledgement

The authors wish to thank Mariela Suárez and Analía García for insect rearing and field trial assistance, also to Dr. Luciana Ingaramo and the forest company Lumin S.A. for facilitating the field site. Fellowships to GZ from the National Agency for Research and Innovation (Uruguay) (POS_NAC_2022_1_174146 and POS_NAC_2022_1_183703) and a grant from the National Institute for Agricultural Research (Uruguay) (INIA-FPTA-232) are also acknowledged.

Data Availability

Environmental data was obtained from https://www.inia.uy/gras

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Figures

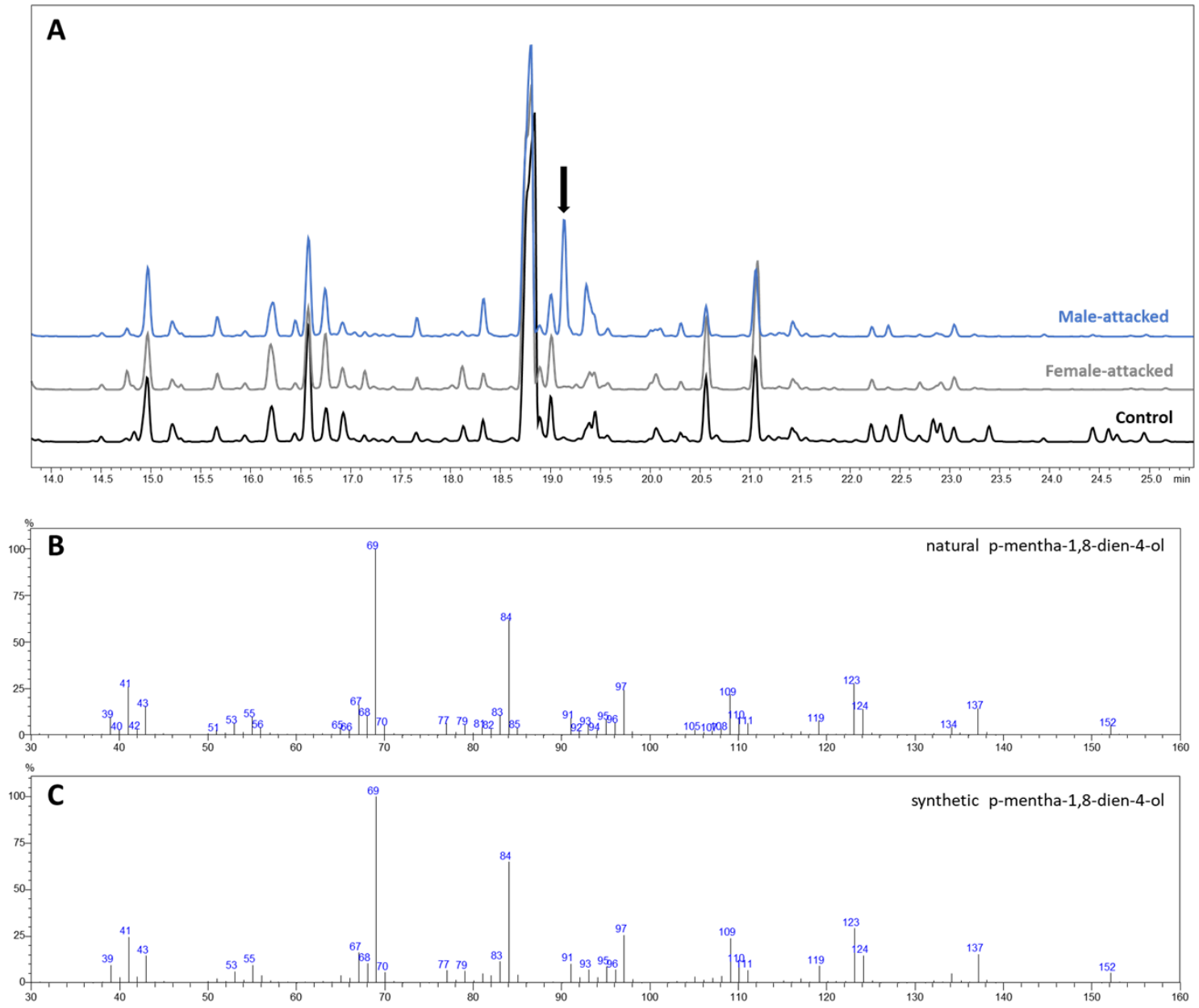


Figure 1

A: Comparison of total ion chromatograms of thermally desorbed volatiles from pinewood slices infested by males (upper, blue trace), females (middle, grey traces) and artificially drilled galleries (lower, black trace). The arrow indicates the male-specific compound identified as the male aggregation pheromone, p-mentha-1,8-dien-4-ol. **B**: Mass spectrum of the natural male-specific compound obtained from volatile entrainment of pine wood infested by *C. luteus* males: m/z (% rel. int.): 153 (0.6), 152 (5.3), 138 (1.4), 137 (14.1), 135 (1.3), 134 (6.7), 132 (2.3), 125 (12), 124 (14.1), 123 (26.8), 122 (0.5), 120 (14), 119 (10.3), 118 (0.5), 117 (3.8), 116 (0.6), 115 (2.2), 112 (0.5), 111 (6.4), 110 (9.5), 109 (22.3), 108 (3.3), 107 (2.2), 106 (15), 105 (3.5), 104 (0.5), 103 (0.9), 98 (1.7), 97 (24.1), 96 (6.8), 95 (8.5), 94 (2.7), 93 (6.5), 92 (3.4), 91 (12.4), 85 (4.0), 84 (63.5), 83 (10.9), 82 (3.9), 81 (48), 80 (1.2), 79 (6.6), 78 (17), 77 (74), 71 (0.7), 70 (5.5), 69 (100.0), 68 (10.8), 67 (16.8), 66 (2.9), 65 (47), 64 (0.6), 63 (11), 58 (0.5), 57 (15), 56 (46),

55 (11.0), 54 (1.7), 53 (7.5), 52 (14), 50 (0.9), 45 (0.7), 43 (18.0), 42 (41), 41 (31.2), 40 (3.6), 39 (12.6), 38 (0.6), 31 (0.9). **C**: Mass spectrum of synthetic p-mentha-1,8-dien-4-ol.

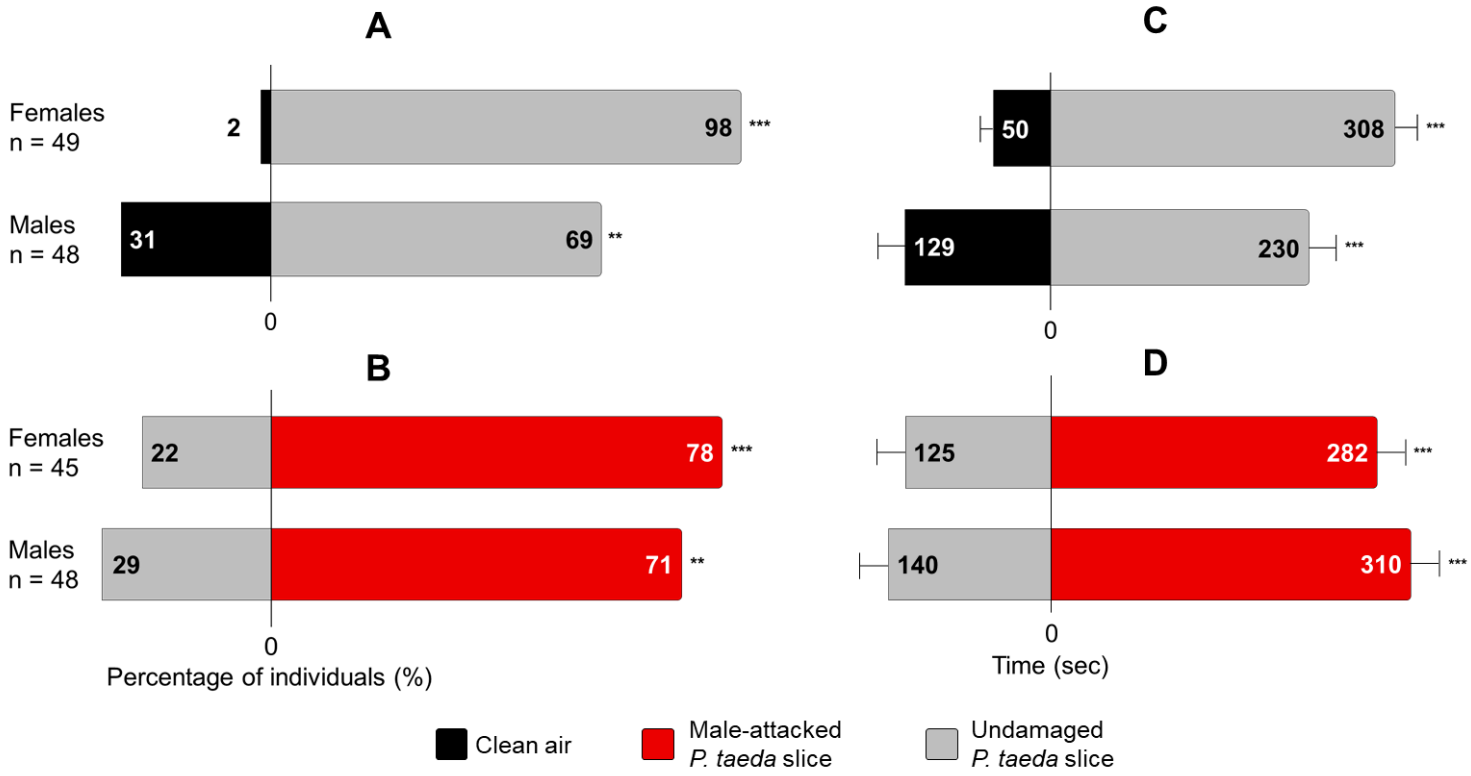


Figure 2

Olfactometer bioassay results showing the first choice of *C. luteus* adults (**A**, **B**) and the residence time in olfactometer arms (**C**, **D**). Contrasted volatile treatments were clean air (black bars) *vs* undamaged pine slices (grey bars), and male-attacked (red bars) *vs* undamaged pine slices. Significance was determined using GLM for first arm choice and Wilcoxon tests for residence time (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Numbers within bars indicate the percentage of individuals (**A**, **B**) or mean time (**C**, **D**). Error bars indicate SEM.

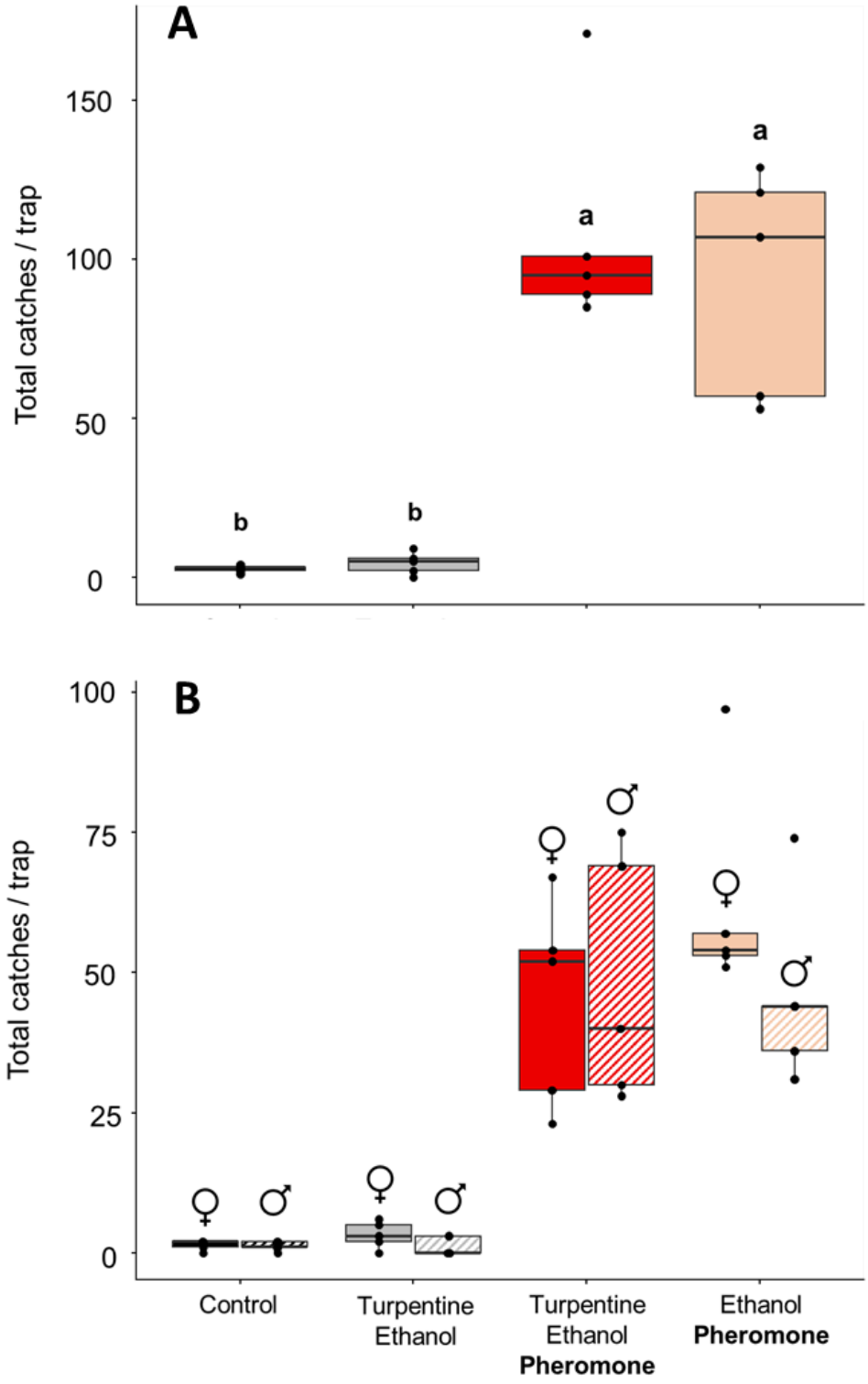


Figure 3

A: Cumulative (8 weeks) *C. luteus* adult captures per trap for unbaited control traps and traps baited with turpentine/ethanol, turpentine/ethanol/pheromone or ethanol/pheromone. **B**: Cumulative adult captures as in (A), separated for females (filled bars) and males (stripped bars). Data are shown as boxplots with black points indicating cumulative individual trap captures (n = 5 traps per treatment). Different letters above bars indicate significant differences as per GLM-qp analyses and pairwise comparisons (EMMs).

Supplementary Files

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