Neotropical Entomology

Trapping of Retrachydes thoracicus thoracicus (Olivier) and other Neotropical cerambycid beetles in pheromone and kairomone baited traps

--Manuscript Draft--

Manuscript Number:	NENT-D-21-00342R1	
Full Title:	Trapping of Retrachydes thoracicus thoracicus (Olivier) and other Neotropical cerambycid beetles in pheromone and kairomone baited traps	
Article Type:	Original Article	
Section/Category:	Ecology, Behavior and Bionomics	
Keywords:	Longhorn beetles; Cerambycinae; kairomone-pheromone synergism; 3-hydroxy-2-hexanone; ethanol	
Corresponding Author:	María Eugenia Amorós, MSc. Universidad de la Republica Facultad de Quimica Montevideo, Montevideo URUGUAY	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Universidad de la Republica Facultad de Quimica	
Corresponding Author's Secondary Institution:		
First Author:	María Eugenia Amorós, MSc.	
First Author Secondary Information:		
Order of Authors:	María Eugenia Amorós, MSc.	
	Lautaro Lagarde	
	Hugo Do Carmo	
	Viviana Heguaburu, PhD	
	Marcela Monné, PhD	
	José Buenahora, MSc.	
	Andrés González	
Order of Authors Secondary Information:		
Funding Information:	Comisión Sectorial de Investigación Científica (Beca de doctorado)	Mrs María Eugenia Amorós
	Programa para el Desarrollo de las Ciencias Básicas, UruguayPrograma para el desarrollo de las Ciencias Básicas, Uruguay	Mr Andrés González
	Comisión Sectorial de Investigación Científica (CSIC-Grupos)	Mr Andrés González
	Instituto Nacional de Investigacion Agropecuaria, Uruguay (Proyecto Sanidad Vegetal)	Mr José Buenahora
	Comisión Sectorial de Investigación Científica (Proyecto CSIC-Vusp)	Mr Andrés González

Abstract:

The subfamily Cerambycinae, one of the most diverse in longhorn beetles, is well-known for its remarkable chemical parsimony in male-emitted pheromones. Conserved shared structural motifs have been reported in numerous species, sometimes working in combination with plant volatile kairomones. Among other compounds, the most ubiquitous male pheromone in cerambycine species is 3-hydroxyhexan-2-one. We

	conducted field trials using intercept traps baited with 3-hydroxyhexan-2-one and observed abundant captures of several Neotropical cerambycine species. These were Retrachydes thoracicus thoracicus (Olivier), Megacyllene acuta (Germar), Compsocerus violaceus (White) and Cotyclytus curvatus (Germar) in high numbers; as well as Chydarteres striatus striatus (Fabricius) and Odontocroton flavicauda (Bates) in smaller numbers. When ethanol was added to the traps, a remarkable synergistic effect was observed for the attractiveness of 3-hydroxy-2-hexanone particularly for R. thoracicus thoracicus and M. acuta . Adding ethanol also resulted in the capture of Chrysoprasis aurigena (Germar). Incidental catches in pheromone lured traps of Trachelissa maculicollis (Audinet-Serville), Neoclytus pusillus (Laporte & Gory), Achryson unicolor (Bruch, 1908) and Achryson surinamum (Linnaeus), Megacyllene mellyi (Chevrolat) and Thelgetra adustus (Burmeister) were also observed. Pheromone chemistry has been reported for C. curvatus , M. acuta and N. pusillus , all three producing 3-hydroxy-2-hexanone; and for C. aurigena and A. surinamum , which produce other compounds. Our findings suggest that the captured species could either produce 3-hydroxyhexan-2-one for their pheromone communication system, or could be "eavesdropping" on the pheromones of other intra-guild species. The synergistic effect of ethanol is likely explained by its kairomonal role as a volatile cue for plant stress or ripeness.
Response to Reviewers:	Dear editors, here I attach the corrected files. I apologize for the format errors and missing sections, and hope everything has been corrected properly. Please contact me if there is anything else I can do. Sincerely, María Eugenia Amorós

Trapping of *Retrachydes thoracicus thoracicus* (Olivier) and other Neotropical cerambycid beetles in pheromone and kairomone baited traps

María Eugenia AMORÓS[1*], Lautaro LAGARDE[1], Hugo DO CARMO[2], Vivivana[2] HEGUABURU[2], Marcela MONNÉ[3], José BUENAHORA[4], Andrés GONZÁLEZ[1*]

[1]Facultad de Química, Udelar, Montevideo, URUGUAY

[2] Centro Universitario de Paysandú, Universidad de la República, Paysandú, Uruguay

[3]Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil

[4]Estación Experimental INIA Salto Grande, Instituto Nacional de Investigación Agropecuaria, Uruguay

Short title: **Neotropical cerambycid beetles in pheromone and kairomone traps**

* Correspondence: María Eugenia Amorós, Andrés González. Laboratorio de Ecología Química, Facultad de Química, Universidad de la República, Avda. Gral. Flores 2124, Montevideo, CP 11800, Uruguay. E-mail: eamoros@fq.edu.uy, agonzal@fq.edu.uy. Telephone: +598 99502638. ORCID 0000-0003-0937-4845

Abstract

The subfamily Cerambycinae, one of the most diverse in longhorn beetles, is well-known for its remarkable chemical parsimony in male-emitted pheromones. Conserved shared structural motifs have been reported in numerous species, sometimes working in combination with plant volatile kairomones. Among other compounds, the most ubiquitous male pheromone in cerambycine species is 3-hydroxyhexan-2-one. We conducted field trials using intercept traps baited with 3-hydroxyhexan-2-one and observed abundant captures of several Neotropical cerambycine species. These were *Retrachydes thoracicus thoracicus* (Olivier), *Megacyllene acuta* (Germar), *Compsocerus violaceus* (White) and *Cotyclytus curvatus* (Germar) in high numbers; as well as *Chydarteres striatus striatus* (Fabricius) and *Odontocroton flavicauda* (Bates) in smaller numbers. When ethanol was added to the traps, a remarkable synergistic effect was observed for the attractiveness of 3-hydroxy-2-hexanone particularly for *R. thoracicus thoracicus* and *M. acuta*. Adding ethanol also resulted in the capture of *Chrysoprasis aurigena* (Germar). Incidental catches in pheromone lured traps of *Trachelissa maculicollis* (Audinet-Serville), *Neoclytus pusillus* (Laporte & Gory), *Achryson unicolor* (Bruch, 1908) and *Achryson surinamum* (Linnaeus), *Megacyllene mellyi* (Chevrolat) and *Thelgetra adustus* (Burmeister) were also observed. Pheromone chemistry has been reported for *C. curvatus*, *M. acuta* and *N. pusillus*, all three producing 3-hydroxy-2-hexanone; and for *C. aurigena* and *A. surinamum*, which produce other compounds. Our findings suggest that the captured species could either produce 3-hydroxyhexan-2-one for their pheromone communication system, or could be “eavesdropping” on the pheromones of other intra-guild species. The synergistic effect of ethanol is likely explained by its kairomonal role as a volatile cue for plant stress or ripeness.

Keywords

Longhorn beetles; Cerambycinae; kairomone-pheromone synergism; 3-hydroxy-2-hexanone; ethanol

Acknowledgments

The authors wish to thank financial support from CSIC (Comisión Sectorial de Investigación Científica) Universidad de la República, and from PEDECIBA (Programa para el Desarrollo de las Ciencias Básicas, Uruguay) and INIA (Instituto Nacional de Investigación Agropecuaria). Technical advice and field assistance was kindly provided by Yamandú Pochintesta, Martín Lanfranco, Ramiro Vacca, Gabriel Bueno, Alejandro Borges, José Berreta, Johana Dalla Valle, Gustavo Rodríguez, Roy Mazondo, Anna Paula Burgueño, Diana Valle and Federico Rodrigo.

Introduction

There has been a remarkable progress in the identification of cerambycid beetle pheromones in the past fifteen years. As new pheromones were discovered, it became evident that cerambycids show a high degree of biosynthetic parsimony of male-emitted sex-aggregation pheromones. The same or similar pheromone components are shared by several species across genera, tribes, and even subfamilies [reviewed by (Hanks and Millar 2016)]. α-Hydroxyketones and 2,3-alkanediols are the most conserved chemical motifs within the subfamily Cerambycinae, with (*R*)-3-hydroxyhexan-2-one as the most widespread major or sole pheromone component (Hanks and Millar 2016). Indeed, this compound has been fully identified or suggested as a pheromone component for cerambycine species belonging to at least 25 genera across 10 tribes (Millar et al. 2019), including several sympatric and synchronic species (Mitchell et al. 2013). Research on chemical communication of Neotropical cerambycids also indicate that this compound is also used by many species native to the region (Aguirre Gil et al. 2021; Amorós et al. 2020; Silva et al. 2020; Silva et al. 2016; Silva et al. 2018; Silva et al. 2017).

Cerambycids are also know for using plant volatiles as kairomonal cues for resources such as food or refuge. Moreover, since many cerambycid species mate on the same host plants on which the adults and larvae feed, these plant chemical cues may also serve to bring the sexes together (Hanks and Millar 2016; Wang 2017). Many cerambycid species are attracted to stressed hosts, so research on host plant attractants has naturally focused on volatiles associated with plant stress. This is the case of ethanol, which is produced by stressed or diseased plants and has been reported to attract many species of wood-boring insects. Indeed, ethanol is commonly used as a general attractant for monitoring and quarantine surveillance programs in forests (Brockerhoff et al. 2006; Fan et al. 2018; Hanks and Millar 2013; Hanks et al. 2012; Miller et al. 2017). Plant volatile kairomones have also been shown to synergize with pheromones in the attraction of cerambycids, possibly adding specificity to the unspecific pheromone communication systems of cerambycids (reviewed by cerambycidae of the world (Hanks and Millar 2016; Wang 2017)).

In this study, we have conducted field trapping experiments specifically targeted to the citrus borer *Diploschema rotundicolle* (Audinet-Serville) (Cerambycidae: Cerambycinae), which also produces *R*-

3-hydroxyhexan-2-one (Amorós et al. 2020). As a side experiment of this work, we have systematically recorded captures of other native cerambycines in traps baited with generic pheromone components, and more recently we have added plant kairomone compounds to study potential pheromone-kairomone synergistic effects. Here we report these results, which represent a contribution to the knowledge of chemical communication systems of native cerambycines in southern South America.

Material and Methods

Field experiments

Field trials were performed in peach and citrus groves located in southern Uruguay. Homemade cross-vane traps (74 x 40 cm, black corrugated plastic) were used as trapping devices. Trap panels were coated with Fluon® (Insect-A-Slip, PTFE DISP30, BioQuip Products, Inc) (Graham et al. 2010). Trap basins (adapted Mc Phail traps, 19 cm height, 13 cm diameter) were partially filled with soapy water and salt to kill and preserve captured beetles.

Different trapping setups were laid out during summer and early fall (December to April) in 2015-2016 (season I), 2017-2018 (season II), 2018-2019 (season III), 2019-2020 (season IV). Two trapping experiments were also set up in the 2020-2021 (season V, Experiment 1: December-March; Experiment 2: March-May). Variables such as trap height, pheromone dispenser and combinations of attractant volatile stimuli were evaluated throughout the seasons (Table 1). To control for trap position bias, the treatments were assigned randomly to each trap on the day of trap set up, then rotated within the blocks when traps were serviced. Combined lures were hung as separate dispensers within a single trap. Captured beetles were removed from the traps once a week.

Chemicals

Racemic 3-hydroxy-2-hexanone (hereafter ketol) was purchased from ChemTica International, S.A. and Bedoukian Inc. (*R*)-3-Hydroxy-2-hexanone and 2,3-hexanediol (hereafter diol) were synthesized according to (Heguaburu et al. 2017). Lemon essential oil (hereafter LEO) was kindly provided by Novacore S.A (Paysandú, Uruguay).

Insect identification

Identification of the captured beetles was carried out in collaboration with a specialist on Neotropical Cerambycidae (MM, Museu Nacional, Universidade Federal do Rio de Janeiro, Brasil). Comparison with reference specimens from the entomological collection of Facultad de Agronomía (Universidad de la República, Uruguay), as well as online databases and specialized checklists were also used (Barriga-Tuñón 2009; Bezark and Monné 2019; Monné 2021; Monné and Bezark 2009)

Statistical analysis

Data was analyzed using R statistical software (0.99.892 version – © 2009-2016 RStudio, Inc.) (RStudioTeam 2015). Beetle trap captures (*i.e.* total catches per block/replicate throughout the seasons) were subjected to a generalized linear mixed model (GLM) with Poisson distribution. Treatment means were compared using Tukey's HSD test ($\alpha = 0.05$) (multcomp package (Hothorn et al. 2008). Treatments with zero catches were not considered in the analysis.

Results

Overall, 13 diurnal species of Cerambycinae belonging to seven tribes were captured (Figure 1) (it was not possible to photograph the *Thelgetra adustus* (Burmeister) individual). All but one species (*Achryson surinamum* (Linnaeus)) are endemic to the Neotropics (Monné 2021) (Table 2).

In all cases, catches obtained in pheromone-baited traps were higher that control traps (Table 3). *Retrachydes thoracicus thoracicus* (Olivier) was the most common species attracted to pheromone traps, with more than three hundred beetles captured. Adults were clearly attracted to the ketol in comparison with control traps (2 catches throughout the whole study); a result that was consistently observed over all seasons and different study sites (Table 3). During seasons II and III, the high numbers of *R. t. thoracicus* captured allowed for a comparison of captures using the pure enantiomer (3*R*) or adding the diol as a minor component. In both cases, no significant differences were observed with respect to the traps lured with racemic ketol (GLM $P = 0.424$ y $P = 0.578$). Almost all catches were females, with 357 females and only 2 males obtained throughout the whole study.

Megacyllene acuta (Germar) (71 individuals), *Compsocerus violaceus* (White) (42 individuals) and *Cotyclytus curvatus* (Germar) (40 individuals), were the next most abundant trapped species. Almost all catches were obtained in ketol lured traps (Table 3). A few specimens of *Odontocroton flavicauda* (Bates) (4 individuals) and *Chydarteres striatus striatus* (Fabricius) (5 individuals) were also caught exclusively in ketol lured traps (Table 3).

The addition of ethanol to the ketol traps in season V showed a dramatic increase in the numbers of *R. t. thoracicus* and *M. acuta* captures (Figure 2) (GLM, Tukey's HSD, $P < 0.00$). In addition, *Chrysoprasis aurigena* (Germar) was captured for the first time in season V, only trapped in Ketol:EtOH traps (7 individuals) (Table 3).

Experiment 2 allowed us to confirm the synergistic effect of ethanol in the attraction of ketol to *R. thoracicus* when compared with the attraction of ethanol-lured traps (GLM, Tukey's HSD: $P= 0.00013$). However, in the case of *M. acuta* the attractivity of the combined ketol:EtOH lures was not significantly different from ethanol alone (GLM, Tukey's HSD: $P= 0.08239$) (Figure 3).

Single catches of *Trachelissa maculicollis* (Audinet-Serville), *Neoclytus pusillus* (Laporte & Gory), *Megacyllene mellyi* (Chevrolat) and *Thelgetra adustus* (Burmeister) were obtained in ketol taps. One *Achryson unicolor* (Bruch) and one *Achryson surinamum* (Linnaeus) in Ketol:LEO and Ketol:EtOH traps, respectively. These were only found in pheromone-baited traps, but they may be regarded as anecdotic due to their low numbers.

Discussion

In this study, 13 species of cerambycines were trapped (Table 2) in cross-vane traps lured with different pheromone-plant volatile combinations. All these species but one are native to the Neotropics (Table 2). Pheromone records are available for five of these species: *M. acuta*, *C. curvatus*, *C. aurigena*, *N. pusillus* and *A. surinamum* (Hanks and Millar 2016; Silva et al. 2017).

Retrachydes thoracicus thoracicus was the species with highest trapping numbers. This is a very polyphagous Neotropical cerambycine that belongs to the Trachyderini tribe, with yet unknown pheromone chemistry. It has been recorded in various host plants (Bentancourt and Scatoni 2010; Monné 2021), including fruit and forestry crops such eucalyptus, where it can potentially cause economic damage (Bentancourt and Scatoni 2010; Lindemberg Martins Mesquita et al. 2017; Monné et al. 2002). It is considered a pest of plants of the families Fabaceae (Costa et al. 2019), Moraceae, Ulmaceae (Di Lorio 1997) and Salicaceae (Machado et al. 2012). Regarding the knowledge of the chemical communication system in species of the Trachyderini tribe, reports are available for four species. 3-Hydroxyhexan-2-one, the ketol in our study, has been identified in *Batyle suturalis* (Say) and *Tragidion armatum* (LeConte) (Hanks and Millar 2016). Silva et al. reported field trapping of *Chydarteres dimidiatus dimidiatus* (Fabricius) and *Trachyderes succinctus duponti* (Aurivillius) in traps with racemic ketol and racemic 2-methylbutan-1-ol (Silva et al. 2018). These results suggest that the ketol may be an important compound in the pheromone communication of species within this tribe. We also observed catches of another trachyderini species, *C. striatus striatus*, all of them in traps lured with ketol (Table 3). Even though pheromone collection and analysis have not been yet reported for *R. t. thoracicus*, the consistent attraction of adults to the ketol strongly suggests this compound as an important component of the chemical communication system in this species. Millar et al. (2017, 2018) discussed the value of field screening bioassays as tools for initiating research on the chemical ecology of cerambycid beetles. Specifically, chemicals or blends of chemicals that attract cerambycid species in the field are likely pheromone components of these species (Millar et al. 2018; Millar et al. 2017). This seems logical and highlights the value of screening studies such as ours, given the difficulty of collecting or raising live cerambycid adults in enough numbers for volatile collections in the laboratory.

When ethanol was added as a potential plant kairomone to the traps, we observed a strong synergistic effect of ethanol and the ketol in the attraction of *R. t.thoracicus*. Ethanol is produced by stressed or diseased plants, or by woody plants that are long dead and decaying, so it is not surprising that ethanol attracts many species of wood-boring insects. Consistent with their attraction to ethanol, some cerambycid species whose larvae develop in deciduous woody plants are attracted to fermenting molasses or sugar solutions. It also has been suggested that fermenting baits might attract cerambycids because they mimic the volatiles from fermenting sap on which adult beetles feed, rather than volatiles from larval hosts (reviewed by (Wang 2017). As it has been mentioned, *R. t. thoracicus* is highly polyphagous and has been reported both on healthy as well as decaying woody plants. The adults also visit fruits, and attraction to fermenting lures has been reported (Bentancourt and Scatoni 2010; Bruhn and Beltrame 1980; Holdefer Woldan 2007; Lindemberg Martins Mesquita et al. 2017). In addition to a potential role as a cue for adult or larval feeding resources, the ketol-ethanol synergic effect may provide the insect with a mechanism to avoid cross-attraction with other sympatric and synchronic species in the context of shared pheromone compounds, as it has been reported for several other cerambycines (reviewed by (Wang 2017).

Compsocerus violaceus (tribe Compsocerini) was another commonly trapped species which lacks reports on pheromone chemistry. Silva et al. had also reported field catches of *C. violaceus* with racemic ketol and racemic 2-methylbutan-1-ol (Silva et al. 2018), so our results are in line with previous observations.

Megacyllene acuta and *C. curvatus,* belong to the Clytine tribe, in which (3*R*)-ketol has been identified as an important or even sole component of male-emitted pheromones in Neotropical species (Silva et al. 2017). Pheromone chemistry reports are available for these two species: Silva et al. reported that *C. curvatus* males emit exclusively (*R*)-3-hydroxyhexan-2-one, whereas *M. acuta* produces the same compound along with lesser amounts of (2*S*,3*S*)-2,3-hexanediol and (*S*)-2-methylbutan-1-ol (Silva et al. 2018). This may explain that more catches were observed in ketol traps across all seasons for *C. curvatus* (20) than for *M. acuta* (6) (Table 3), which may need minor compounds to enhance attraction. Another Clytine species, *N. pusillus,* has been shown to emit exclusively the ketol (Silva et al. 2017). We obtained one capture of this species in ketol traps.

Megacyllene acuta was strongly attracted to Ketol:EtOH and ethanol alone, without significant differences between these treatments. This species is known to visit flowers and feed on rotting fruit (Martins and Galileo 2011), and poses a threat as an invasive species in other regions of the world because their larvae are easily transported overseas in wooden cratings (Duffy 1953). Along with *C.*

curvatus larvae, *M. acuta* can cause economic damage to fruit trees such as apple, pear, quince (Rosaceae), avocado (Lauraceae), and fig (Moraceae) (Martins and Galileo 2011), so finding attractants may have applied significance for surveillance programs. As mentioned for *R. t. thoracicus*, attraction to ethanol may be explained by the natural history of these species, and pheromone specificity may be in this case provided by minor compounds (Silva et al. 2018).

In our experiments, *C. aurigena* was only captured in Ketol:EtOH traps early in the summer of season V. Although no pheromone chemistry has been reported for *C. aurigena*, Silva et al. reported field catches in pheromone traps (blends of ketol plus 1-(1H-pyrrol-2-yl)-1,2-propanedione and ketol plus 1-(1H-pyrrol-2-yl)-1,2-propanedione plus 3-methylthiopropan-1-ol) (Silva et al. 2017). Their follow up experiments, however, revealed that the adults did not produce either of these compounds. While our results suggest that ethanol may be important in attracting this species, no catches were obtained in traps lured with ethanol alone, so further experiments possibly targeting different flight periods are needed.

Species trapped in lesser amounts in ketol-lured traps include *O. flavicauda*, which belongs to the Rhinotragini tribe n has no reports of pheromone composition. *Trachelissa maculicollis, M. mellyi, A. unicolor, A. surinamum* may be considered incidental catches, or they may be the result of low populations of these species in the agro-ecosystems in which we based our study. *Achryson surinamum* is a widely distributed species that has been reported to produce anti-2,3-octanediol and traces of 2-methylbutan-1-ol (Hanks and Millar 2016). In our study, one individual of *A. unicolor* and one of *A. surinamum* were caught in Ketol:LEO and Ketol:EtOH traps, with no other *Achryson* species caught in any of our experiments.

It is likely that the species captured in our study either use ketol in their chemical communication systems, or that they “eavesdrop” on the pheromone communication system of other guild members, which may serve as an efficient method of finding suitable hosts for mating and oviposition, as has been reported for other species (Hanks and Millar 2013). Our results open possibilities for new trapping and surveillance devices for these potentially damaging cerambycids. Furthermore, valuable information about the chemical ecology of Neotropical cerambycid beetles is provided, which keeps proving the remarkable parsimony in pheromone chemistry among cerambycines from all over the world.

Statements and Declarations

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

María Eugenia Amorós and Andrés González contributed to the study conception and design, and wrote the manuscript. Lautaro Lagarde and María Eugenia Amorós performed material preparation, data collection and analysis. Hugo Do Carmo, Vivivana Heguaburu and José Buenahora contributed with the synthetic compounds tested. Marcela Monné identified the insects.

Funding Information

The authors wish to thank financial support from CSIC (Comisión Sectorial de Investigación Científica) Universidad de la República, and from PEDECIBA (Programa para el Desarrollo de las Ciencias Básicas, Uruguay) and INIA (Instituto Nacional de Investigación Agropecuaria).

Bibliography

Aguirre Gil OJ et al. (2021) Screening known Cerambycidae pheromones for activity with the Peruvian fauna Agr Forest Entomol doi:https://doi.org/10.1111/afe.12454

Amorós ME, Lagarde L, Do Carmo H, Heguaburu V, González A (2020) Pheromone chemistry of the citrus borer, Diploschema rotundicolle (Coleoptera: Cerambycidae) Journal of Chemical Ecology 46:809-819 doi:DOI 10.1007/s10886-020-01203-4

Coleoptera Neotropical (2009) Barriga-Tuñón, J. E. http://www.coleoptera-neotropical.org/paginas/2_PAISES/Uruguay/Cerambycidae/cerambycinae-uru.html. Accessed June 2019

Bentancourt CM, Scatoni IB (2010) Guía de insectos y ácaros de importancia agrícola y forestal en el Uruguay. 3 edn. Facultad de Agronomía, Montevideo

Bezark LG, Monné MA (2019) Checklist of the Oxypeltidae, Vesperidae, Disteniidae and Cerambycidae, (Coleoptera) of the Western Hemisphere 2019 Edition (updated through 31 December 2018).

Brockerhoff EG, Jones DC, Kimberley MO, Suckling DM, Donaldson T (2006) Nationwide survey for invasive wood-boring and bark beetles (Coleoptera) using traps baited with pheromones and kairomones Forest Ecology and Management 228:234-240

Bruhn JC, Beltrame JBI (1980) Los taladros Praxithea derourei Chabrilac, Trachyderes thoracicus Olivier y T. striatus Fabricius (Coleoptera: Cerambycidae) y su relación con los cultivos de manzanas en Uruguay Investigaciones Agronomicas 1:11-14

Costa MKC, Diodato MA, Fernandes JPP, Santos JPS (2019) Insetos nocivos a Prosopis sp. no Rio Grande do Norte (Brasil) e Piura (Peru). Agrop Cient Semiárido 15:158-116

Di Lorio ORR (1997) Plantas hospedadoras de Cerambycidae (Coleoptera) en el Espinal periestépico y en la provincia de Buenos Aires, Argentina. Revista de Biología Tropical 44(3)/45(1):159-165

Duffy EAJ (1953) A monograph of the immature stages of British and imported timber beetles (Cerambycidae). Brit. Mus. (Nat. Hist.), London

Fan J et al. (2018) Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests Journal of Pest Science doi:https://doi.org/10.1007/s10340-018-0997-6

Graham EE, Mitchell RF, Reagel PF, Barbour JD, Millar JG, Hanks LM (2010) Treating panel traps with a fluoropolymer enhances their efficiency in capturing cerambycid beetles Journal of Economic Entomology 103:641-647

Hanks LM, Millar JG (2013) Field bioassays of cerambycid pheromones reveal widespread parsimony of pheromone structures, enhancement by host plant volatiles, and antagonism by components from heterospecifics Chemoecology 23:21-44

Hanks LM, Millar JG (2016) Sex and aggregation-sex pheromones of cerambycid beetles: Basic science and practical applications Journal of Chemical Ecology 42:631-654 doi:10.1007/s10886-016-0733-8

Hanks LM, Millar JG, Mongold-Diers JA, Wong JCH, Meier LR, Reagel PF, Mitchell RF (2012) Using blends of cerambycid beetle pheromones and host plant volatiles to simultaneously attract a diversity of cerambycid species Canadian Journal of Forest Research 42:1050-1059

Holdefer Woldan DR (2007) Análise faunística de cerambycidae (Coleoptera) em duas situações florísticas no município de União da Vitória – Paraná. UNIVERSIDADE COMUNITÁRIA REGIONAL DE CHAPECÓ

Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models Biometrical Journal 50:346–363

Lindemberg Martins Mesquita A, Teles Portela Policarpo G, Emilson Cardoso J, Cavalcante de Souza Mota M (2017) Novas Ocorrências de Cerambycidae (Insecta:Coleoptera) em cajueiro no Brasil e recomendações de manejo vol Comunicado Técnico, 231. Embrapa Agroindústria Tropical,

Machado VS, Botero JP, Carelli A, Cupello M, Quintino HY, Simões MVP (2012) Host plants of Cerambycidae and Vesperidae (Coleoptera, Chrysomeloidea) from South America. Revista Brasileira de Entomolgia 56:186-198

Martins UR, Galileo MHM (2011) Tribo Clytini Mulsant, 1839. In: Martins UR GM (ed) Cerambycidae Sul-Americanos

(Coleoptera): taxonomia,, vol 12. Sociedade Brasileira de Entomologia, São Paulo, pp 8-64

Millar JG et al. (2018) Identifying possible pheromones of cerambycid beetles by field testing known pheromone components in four widely separated regions of the United States Journal of Economic Entomology 111:252-259 doi:doi: 10.1093/jee/tox31

Millar JG et al. (2017) Identifying possible pheromones of cerambycid beetles by field testing known pheromone components in four widely separated regions of the United States Journal of economic entomology

Millar JG, Richards AB, Halloran S, Zou Y, Boyd EA, Quigley KN, Hanks LM (2019) Pheromone identification by proxy: identification of aggregation-sex pheromones of North American cerambycid beetles as a strategy to identify pheromones of invasive Asian congeners Journal of Pest Science 92:213–220 doi:https://doi.org/10.1007/s10340-018-0962-4

Miller DR, Crowe CM, Mayo PD, Reid LS, Silk PJ, Sweeney JD (2017) Interactions between ethanol, syn-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one lures on trap catches of hardwood longhorn beetles in Southeastern United States Journal of Economic Entomology:1-10 doi:doi: 10.1093/jee/tox188

Mitchell RF, Millar JG, Hanks LM (2013) Blends of (*R*)-3-hydroxyhexan-2-one and alkan-2-ones identified as potential pheromones produced by three species of cerambycid beetles Chemoecology 23:121-127

Monné M (2021) Catalogue of the Cerambycidae (Coleoptera) of the Neotropical Region. Part I. Subfamily Cerambycinae. Available from cerambyxcat@com/Part 1 Cerambycinae. pdf. Accessed August 2021

Monné MA, Bezark L (2009) Checklist of the Cerambycidae, or longhorned beetles (Coleoptera) of the Western Hemisphere. https://www.cerambycoidea.com/titles/monnebezark2009.pdf.

Monné MM, Bianchi M, Sánchez A, Escudero R (2002) Cerambícidos (Coleoptera) que atacan Eucalyptus globulus y Eucalyptus grandis en Uruguay Agrociencia 6:63-68

RStudioTeam (2015) RStudio: Integrated Development for R. RStudio. Boston, MA Patent,

Silva WD, Hanks LM, Alvarez JCS, Madalon FZ, Bento JMS, Bello JE, Millar JG (2020) Variations on a theme: two structural motifs create species-specific pheromone channels for multiple species of South American cerambycid beetles Insects: Special Issue "Ecology and Management of Invasive Insects in Forest Ecosystems 11 doi:doi:10.3390/insects11040222

Silva WD, Millar JG, Hanks LM, Bento JMS (2016) 10-methyldodecanal, a novel attractant pheromone produced by males of the south american cerambycid beetle *Eburodacrys vittata* vol 11. doi:10.1371/journal.pone.0160727

Silva WD, Millar JG, Hanks LM, Costa CM, Leite MOG, Tonelli M, Bento JMS (2018) Interspecific cross-attraction between the south american cerambycid beetles *Cotyclytus curvatus* and *Megacyllene acuta* is averted by minor pheromone components Journal of Chemical Ecology 44:268-275 doi:https://doi.org/10.1007/s10886-018-0933-5

Silva WD, Zou Y, Bento JMS, Hanks LM, millar JG (2017) Aggregation-sex pheromones and likely pheromones of 11 South American Cerambycid beetles, and partitioning of pheromone channels Frontiers in Ecology and Evolution 5:1-9

Wang Q (2017) Cerambycidae of the world: biology and pest management. Cerambycidae of the world: biology and pest management. CRC Press, Boca Raton, Florida

Figure captions

Fig. 1 Photographs of representative specimens of cerambycine beetles trapped in pheromone-baited traps. **a)** *Retrachydes thoracicus thoracicus* (Olivier); Trachyderini. **b)** *Megacyllene acuta* (Germar); Clytini. **c)** *Compsocerus violaceus* (White); Compsocerini. **d)** *Cotyclytus curvatus* (Germar); Clytini. **e)** *Odontocroton flavicauda* (Bates); Rhinotragini. **f)** *Chydarteres striatus striatus* (Fabricius); Trachyderini. **g)** *Chrysoprasis aurigena* (Germar); Dichophyiini. **h)** *Neoclytus pusillus* (Laporte & Gory); Clytini. **i)** *Megacyllene mellyi* (Chevrolat); Clytini. **j)** *Achryson unicolor* (Bruch); Achrysonini. **k)** *Trachelissa maculicollis* (Audinet-Serville); Trachyderini. **l)** *Achryson surinamum* (Linnaeus); Achrysonini

Fig. 2 Field results of Season V - Experiment 1. The bars show the mean catches ± sd (n = 3) for each evaluated attractant from December through March. Different letters indicate significant differences (GLM, Tukey’s HSD, $P < 0.001$)

Fig. 3 Field results of Season V - Experiment 2. Boxplots show the catches over the season for each evaluated attractant (n = 6) from March through May. Asterisks show significant differences (GLM, Tukey’s HSD, P<0.05), ns stands for not significant

Figures were created with R statistical software and Microsoft Power Point.

Table 1. Materials and methods details of all experiments performed throughout the seasons of study.

	Season	Treatments	Abbreviation	Loading	Dispenser	Hunged on	Replicates	Trap height[a]	Lure replace (weeks)
Multiple seasons	Isolated traps	Racemic 3-hydroxy-2-hexanone	Ketol	50 mg/1 mL of isopropanol	Double press seal bags 5 x 7 cm plus a 5 cm cottong wick	Citrus trees	4	60 cm	3
2015-2016	I	Racemic 3-hydroxy-2-hexanone	Ketol	50 mg/1 mL of isopropanol	Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick	Citrus trees	2	60 cm	2
		2,3-hexanediol (mixture of stereoisomers)	Diol	50 mg/1 mL of isopropanol					
		Isopropanol	Control	1 mL isopropanol					
2017-2018	II	Racemic 3-hydroxy-2-hexanone	Ketol	50 mg/1 mL of isopropanol	Double press seal bags 5 x 7 cm plus a 5 cm cottong wick	Citrus trees	10	60 cm	2
		(*R*)-3-hydroxy-2-hexanone	3*R*-Ketol	25 mg/1 mL of isopropanol					
		Racemic 3-hydroxy-2-hexanone plus lemon essential oil	Ketol : LEO	50 mg/1 mL of isopropanol : 10 mL					
		Isopropanol	Control	1 mL isopropanol					
2018-2019	III	Racemic 3-hydroxy-2-hexanone	Ketol	50 mg/1 mL of isopropanol	Double press seal bags 5 x 7 cm plus a 5 cm cottong wick - separate bags for each stimuli	Citrus, peach and eucalyptus trees	6	60 cm	2
		Racemic 3-hydroxy-2-hexanone plus 2,3-hexanediol (mixture of stereoisomers)	Ketol : diol	50 mg/1 mL of isopropanol : 25 mg/1 mL of isopropanol					
		Isopropanol	Control	1 mL isopropanol					
2019-2020	IV	Racemic 3-hydroxy-2-hexanone	Ketol	50 and 500 mg neat	Eppendorf tube (1 mL) with a perforated cap (1 mm) (low emission rate) - Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick (high emission rate)	Citrus trees : PVC Water pipes next to citrus trees	5	60 cm (low traps) - 180 cm (high traps)	1
		Dispenser materials	Control	x	x				
2020-2021	V - E1	Racemic 3-hydroxy-2-hexanone	Ketol	500 mg neat	Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick	PVC Water pipes next to citrus trees	3	180 cm (high traps)	2
		Racemic 3-hydroxy-2-hexanone plus ethanol 95%	Ketol:EtOH	500 mg neat: 100 mL	Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick : Simple press seal bags 10 x 15 cm				
		Racemic 3-hydroxy-2-hexanone plus lemon essential oil	Ketol:LEO	500 mg neat : 10 mL	Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick : two separate press seal bags 5 x 7 cm plus two 5 cm cottong wick				
		Dispenser materials	Control	x	x				
	V - E2	Racemic 3-hydroxy-2-hexanone	Ketol:EtOH	500 mg neat	Simple press seal bags 5 x 7 cm plus a 5 cm cottong wick : Simple press seal bags 10 x 15 cm		6		
		Ethanol 95%	EtOH	100 mL	Simple press seal bags 10 x 15 cm				Not needed

[a] Ground level to collector bucket

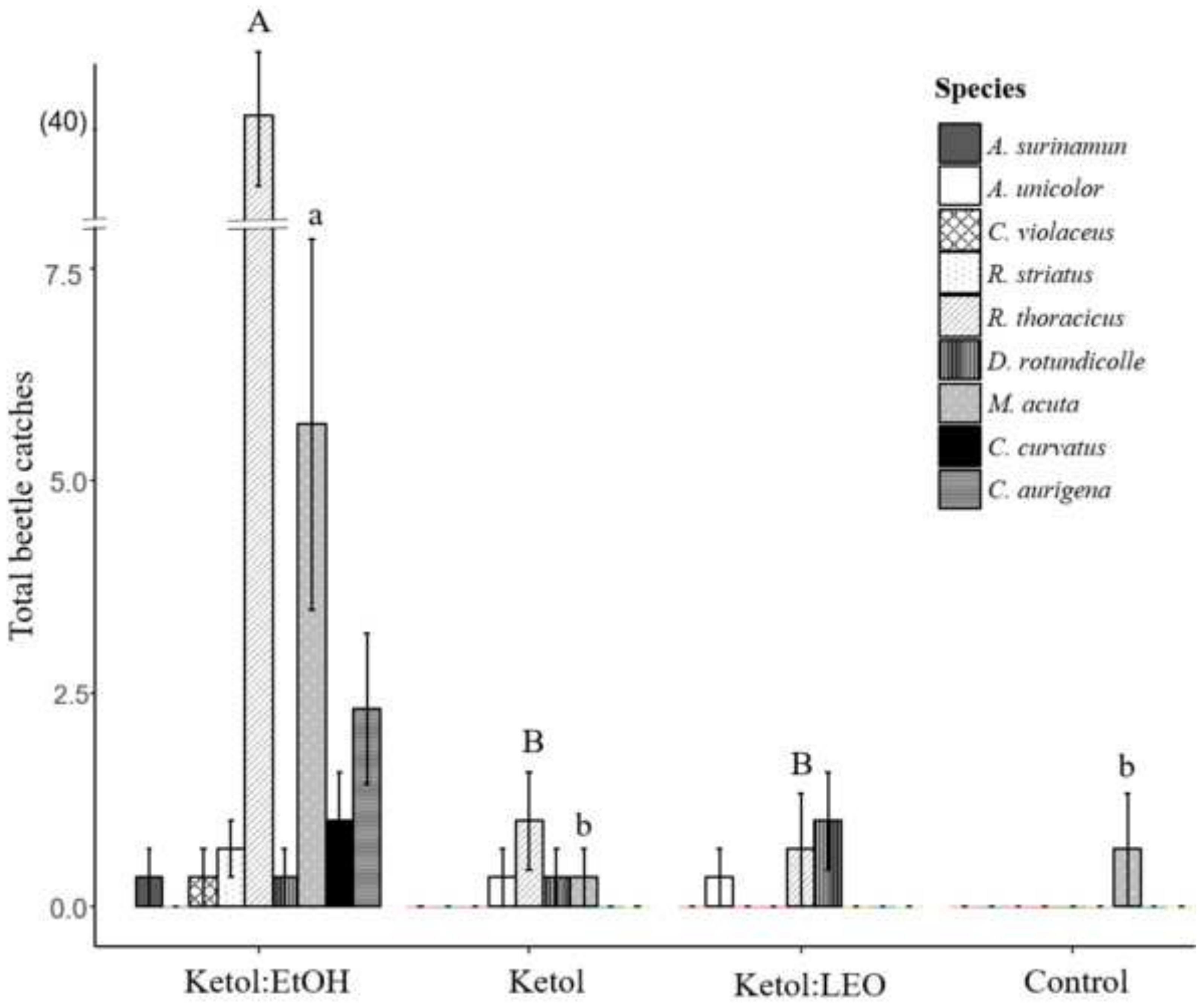
Table 3. Total sum of cerambycine beetles captured in pheromone-baited traps in different setups and seasons.

	Monitoring isolated traps[a]	**season I: 2015-2016[b]**			**season II: 2017-2018[c]**				**season III: 2018-2019[d]**		
	Ketol	Ketol	Diol	Control	Ketol	3*R*-Ketol	Ketol:LEO	Control	Ketol	Ketol:diol	Control
Retrachydes t. thoracicus	8	5	0	0	23	31	23	0	34	31	2
Megacyllene acuta	3	0	0	0	0	0	0	0	2	7	0
Compsocerus violaceus	5	0	4	0	1	0	6	0	17	3	3
Cotyclytus curvatus	17	5	0	0	0	3	3	0	3	1	0
Odontocroton flavicauda	0	0	0	0	0	0	0	0	4	0	0
Chydarteres s. striatus	0	0	0	0	0	0	2	0	0	0	0
Chrysoprasis aurigena	0	0	0	0	0	0	0	0	0	0	0

	season IV: 2019-2020[e]		**season V: 2020-2021 Experiment 1[f]**				**season V: 2020-2021 Experiment 2[g]**		**Totals**		
	Ketol	Control	Ketol	Ketol:EtOH	Ketol:LEO	Control	Ketol:EtOH	EtOH	All pheromone treatments	Controls	TOTAL
Retrachydes t. thoracicus	13	0	3	130	2	0	42	12	345	2	359
Megacyllene acuta	0	0	1	17	0	2	25	14	55	2	71
Compsocerus violaceus	0	0	0	1	0	0	2	0	39	3	42
Cotyclytus curvatus	0	0	0	3	0	0	3	2	38	0	40
Odontocroton flavicauda	0	0	0	0	0	0	0	0	4	0	4
Chydarteres s. striatus	0	0	1	2	0	0	0	0	5	0	5
Chrysoprasis aurigena	0	0	0	7	0	0	0	0	7	0	7

[a]- 4 monitoring isolated traps; [b]- citrus grove - 4 traps/treatment; [c]- citrus grove - 10 traps/treatment; [d]- citrus and peaches groves - 16 traps/treatment;

[e]- citrus grove - 5 traps/treatment; [f]- citrus grove - 3 traps/treatment; [g]- citrus grove - 6 traps/treatment.


A
a
B
b
B
b
(40)
7.5
5.0
2.5
0.0
Total beetle catches
Ketol:EtOH
Ketol
Ketol:LEO
Control
Species
A. surinamun
A. unicolor
C. violaceus
R. striatus
R. thoracicus
D. rotundicolle
M. acuta
C. curvatus
C. aurigena

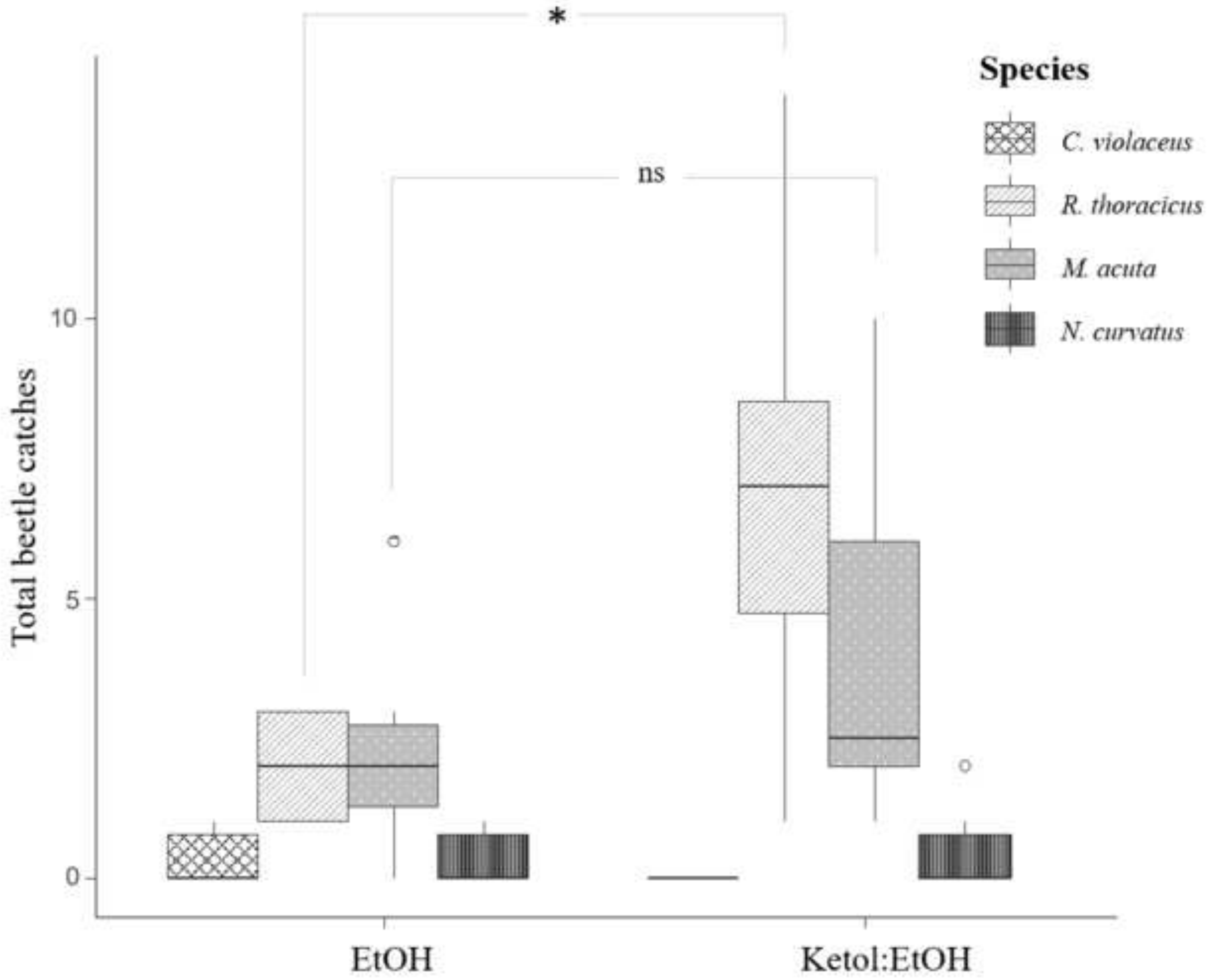
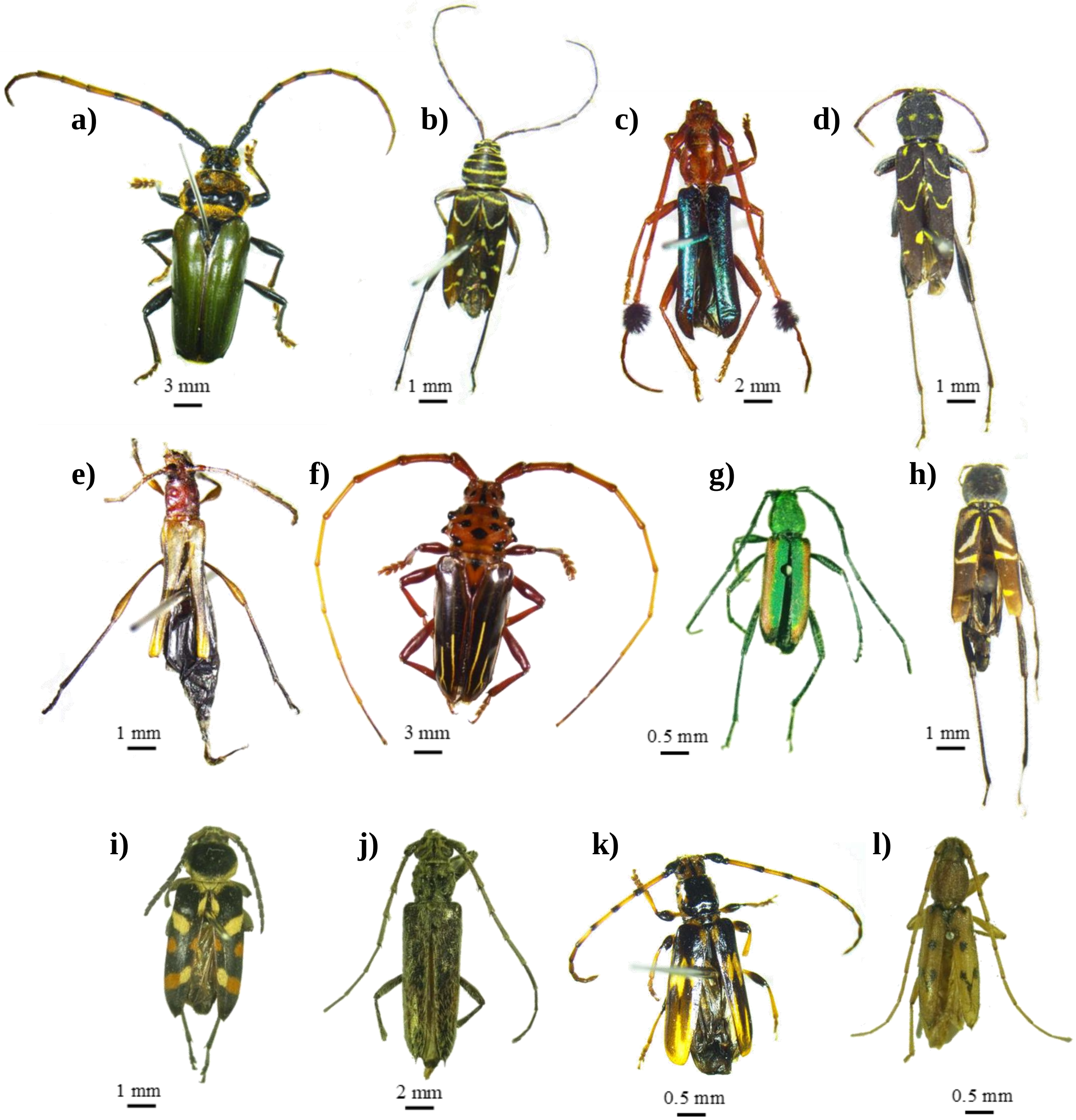

*
ns
Species
C. violaceus
R. thoracicus
M. acuta
N. curvatus
Total beetle catches
10
5
0
EtOH
Ketol:EtOH

fig 1


a)
3 mm
b)
1 mm
c)
2 mm
d)
1 mm
e)
1 mm
f)
3 mm
g)
0.5 mm
h)
1 mm
i)
1 mm
j)
2 mm
k)
0.5 mm
l)
0.5 mm