



## Monitoring and study of bio-ecological aspects of the citrus borer, *Diploschema rotundicolle* (Coleoptera:Cerambycidae), in southern-Uruguay lemon orchards

Monitoreo y estudio de aspectos bio-ecológicos del taladro de los cítricos, *Diploschema rotundicolle* (Coleoptera:Cerambycidae), en quintas de limón del sur de Uruguay

Monitoramento e estudo de aspectos bioecológicos da broca dos citros, *Diploschema rotundicolle* (Coleoptera:Cerambycidae), em pomares de limões do sul do Uruguai

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### Abstract

The citrus borer, *Diploschema rotundicolle*, is a South American cerambycid beetle considered a primary citrus pest in Uruguay. Serious focalized population explosions have been registered in recent years, particularly in lemon orchards. The larvae feed on the woody tissue, causing tree deterioration and reducing grove productivity and value. Current management relies on cultural control, which is expensive and ineffective partially because of imprecise timing of interventions. Monitoring tools for adults or information of their flight periods in Uruguay are not available thus far. Working in lemon groves in southern Uruguay throughout four flight seasons (2015-2020), we used cross-vane traps coupled with white light devices to capture night-flying adults, and mesh covers around infested trees to study adult emergence. Visual observation of larval activity and oviposition damage were also performed throughout the year. Finally, *Eucalyptus globulus* windbreaks were checked underneath loosen barks for hidden adults. Throughout all four seasons, adult flight occurred between late January and early April, with a maximum around mid-February. Emergence from mesh-covered trees was observed in the same period. In general, oviposition damage was observed closely after the peak of adult catches. Mostly females were both trapped in light traps and emerged from infested trees. Larval activity was registered all year long. Adults were found sheltered under *E. globulus* barks; in this case, mostly individual males or couples were found. This study provides a monitoring tool for *D. rotundicolle* adult flight and information on bio-ecological aspects of this pest in southern Uruguay.

**Keywords:** integrated pest management, light traps, Cerambycid beetles, Neotropical Cerambycinae

### Resumen

El taladro de los cítricos, *Diploschema rotundicolle*, es un cerambícido sudamericano considerado plaga primaria de los cítricos en Uruguay. Se observan explosiones poblacionales focalizadas, particularmente en cultivo de limón. Las larvas se alimentan de la madera, causando un deterioro en los árboles que impacta en la productividad y el valor de las quintas. El manejo recae en el control cultural, que es costoso e ineficiente parcialmente por la realización fuera de tiempo de las intervenciones. No existen herramientas de monitoreo de adultos, que tienen hábitos nocturnos, ni información sobre su período de vuelo en Uruguay. Trabajando en quintas de limón en el sur de Uruguay durante cuatro temporadas (2015-



2020), relevamos el vuelo de adultos en trampas de panel cruzado acopladas a luz blanca, y registramos emergencia de adultos mediante enmallado de árboles. Asimismo, se registró la actividad larval a lo largo del año y el daño de oviposición. Finalmente, se revisaron cortezas de cortinas de *Eucalyptus globulus* como refugio de adultos. En todas las temporadas, el vuelo y la emergencia de adultos sucedieron entre enero y abril, con un máximo a mediados de febrero. En general, el daño de oviposición se observó inmediatamente después del pico de mayores capturas. Se capturaron mayormente hembras en las trampas de luz y mallas. Se registró actividad larval durante todo el año. Se encontraron adultos refugiados bajo las cortezas de *E. globulus*; en este caso, mayormente machos aislados o parejas hembra-macho. Este trabajo provee una herramienta de monitoreo para el vuelo de adultos y aporta conocimiento sobre aspectos bio-ecológicos de *D. rotundicolle* en Uruguay.

**Palabras clave:** manejo integrado de plagas, trampas de luz, cerambícidos, cerambicinos neotropicales

## Resumo

A broca dos citros, *Diploschema rotundicolle*, é um cerambicídeo sul-americano, considerado a principal praga dos citros no Uruguai. São observadas explosões populacionais, particularmente no cultivo de limão. Os adultos são noturnos e as larvas se alimentam da madeira, causando uma deterioração nas árvores que impacta a produtividade e o valor patrimonial das quintas. A gestão depende do controle cultural, que é caro e ineficiente, em parte devido aos tempos de intervenção inadequados. No Uruguai, não existem ferramentas de monitoramento dos insetos adultos, nem informações sobre seu período de voo. Durante 4 temporadas (2015-2020), usamos armadilhas de painel cruzado acopladas a luz branca e malhas envolvendo árvores infestadas, para estudar a emergência de adultos em limoeiros no sul do Uruguai. A atividade larval ao longo do ano e os danos à oviposição foram registrados. A casca dos arvores de *Eucalyptus globulus* que rodeiam os quadros foi revisada como refúgio para adultos. Em todas as estações, o voo e a emergência nas malhas ocorreram entre janeiro e abril, com máximo em meados de fevereiro. Em geral, o dano de oviposição foi observado imediatamente após o pico das maiores capturas. As fêmeas foram capturadas principalmente em armadilhas de luz e malha. A atividade larval foi registrada ao longo do ano. Adultos refugiados foram encontrados sob a casca de *E. globulus*; neste caso, principalmente machos isolados ou em pares fêmea-macho. Este trabalho fornece uma ferramenta de monitoramento do voo de adultos e fornece conhecimento sobre aspectos bioecológicos de *D. rotundicolle*.

**Palavras-chave:** controle integrado de pragas, armadilhas luminosas, Cerambicidos, Cerambicinos Neotropicais

## 1. Introduction

*Diploschema rotundicolle* (Audinet-Serville, 1834) (Coleoptera: Cerambycidae) is a South American citrus pest distributed throughout the center-south regions of Brazil, Argentina and Uruguay<sup>(1)</sup>. The adults are elongated (25-40×8-10 mm) and characterized by a light-brown elytra with a continuous dark-brown border, dark-brown head, pronotum, antennae and legs<sup>(2)</sup>. The adults are nocturnal, females oviposit in the apex of branches upon young flush and leaf axils<sup>(3)</sup>. After egg eclosion, the larvae perforate the epidermis and once in woody tissue they dig longitudinal galleries heading to thicker branches, usually reaching the principal trunk<sup>(4)</sup>. By the end of the larval stage, the larvae prepare a pupal chamber with an exit opening for the adult to exit<sup>(2)</sup>.

In subtropical areas —Sao Paulo, Brazil— the larval stage was reported to be uninterrupted from 8-10 months, and the adults were observed from November to April<sup>(2)</sup>. In temperate regions —Santa Catarina, RS, Brazil— larval activity was recorded from January to October of the second year, reaching up

to 20-22 months, with no activity in winter periods<sup>(5)</sup>. In these conditions, adults were reported active from November to January. In Pelotas, RS, Brazil, closer to Uruguay, adults were reported in February and March<sup>(6)</sup>. There are no records of biological aspects of this pest in Uruguayan climatological conditions.

Currently *D. rotundicolle* is considered a primary pest for citrus in Uruguay<sup>(7)</sup>. Over the last years, inefficient management of this pest has led to serious focalized population explosions, particularly in lemon orchards in the south (unpublished data). The deterioration caused by serious infestation levels is currently one of the limitations in an orchard's service life and has a direct impact on its productivity and value. Larval feeding results in poor yields, tree weakening and indirect damages due to invaders of empty galleries<sup>(1)</sup>.

Woodborer control is extremely complicated since the larvae are protected inside the wood<sup>(8)</sup>. Insecticides are also much restricted in citrus crops destined to fresh fruit consumption. Therefore, the cur-

rent management strategy for this insect relies almost exclusively on cultural control, which consists in the pruning of twigs with evidence of oviposition damage. This strategy has proven expensive and ineffective, partially because of imprecise timing of the interventions. No monitoring tools for adults are so far available.

The objective of this study was to determine the flight period of adults in the conditions prevalent in southern Uruguay and to evaluate a monitoring device for adults.

## 2. Materials and methods

Field surveys were performed in four citrus groves located in San José (Kiyú, 2 groves: 34°42'1"S 56°43'37"W; 34°25'59.8"S 57°40'56.3"W); Montevideo (El Espinillo, 34°48'58.6"S 56°22'53.6"W), and Canelones (Las Brujas, 34°37'13.1"S 56°21'18.5"W), all of them in southern Uruguay. Assays were performed from December to April, in four seasons (2015-2016, 2017-2018, 2018-2019 and 2019-2020). In Kiyú we worked in one grove, "Quinta 20", in 2015-2016; the others seasons we worked in another orchard nearby (4,7 km apart), "Quinta 7".

Homemade cross-vane traps with standard design (e. g., Alpha Scents Inc., West Linn, Oregon, USA; ChemTica Internacional SA, San Jose, Costa Rica) were used as trapping devices (black corrugated plastic or sheet iron; panel measures: 74×40 cm) and coupled in the bottom to plastic trap basins. These were partially filled with soapy water and salt to kill and preserve captured beetles. White fluorescent tubes (60 cm; 380-750 nm – Cold white, 6500 K) or LED light devices (50 cm per vane; LEDs 5730, 120 LEDs/m- cold white, 6000-6500 K) were coupled to the traps.

Populations were monitored with 1 to 3 traps/grove depending on the location and year. The traps were deployed within 1-ha citrus plots, 6 m apart from the plot border and with a separation of at least 20 m between traps. They were hung either from tree branches or a holder, with a height of 60 cm from the ground to the basin.

Mesh covers were set up around highly infested trees chosen from visual observation of abundant sawdust at their base. The trees were partially pruned and the mesh covered the whole tree structure, tied around the trunk at ground level. Throughout the seasons, a total of 25 trees were mesh-covered covering the four studied groves.

Traps and wrapped trees were checked weekly from early summer (mid-December) to early fall (April).

When present, *Eucalyptus globulus* windbreaks surrounding the citrus plots were checked for hidden *D. rotundicolle* adults. This was performed by extracting loosen barks and searching underneath for adults. Trees surrounding at least four citrus blocks were checked in each monitoring date.

Monthly visual observation of larval activity, recorded as presence of fresh frass in the base of the trees, was carried out throughout the whole year. Also, visual observation of oviposition damage was recorded. The damage is observed as characteristic wilting of the apex of branches in their first ca. 20 cm.

Weather recordings were obtained from Estación Experimental INIA Las Brujas, Canelones, Uruguay.

*Statistical analysis:* Male and female comparisons of caches in light traps, emergence on mesh-covered trees and extraction of windbreaks barks were all done with Chi-square tests.

Data included in the article were processed for clarity. Raw data are available upon request.

## 3. Results

Light traps showed good performance for monitoring *D. rotundicolle* adult flight. In all seasons and groves, adult catches were observed between late January and April. Peak of higher catches was observed around mid-February, except for the 2015-2016 season, in which higher catches were recorded later in February (Figures 1 and 2). Oviposition damage was observed shortly after the peak of higher catches: 2015-2016: February 16; 2017-2018: February 19; 2018-2019: February 20, and 2019-2020: March 27.

Results of the 2015-2016 season could have slight timing inaccuracies caused by monitoring dates.

In general, 2019-2020 season showed lower catches in comparison with previous seasons (Figure 1), which may be explained by a dry summer season with low precipitations (Figure 1 in Supplementary material). In 2018-2019, three groves were monitored and the results show slight differences in the onset of adult flights in the different groves (Figure 2).

Figure 1. *Diploschema rotundicolle* flight period in Kiyú groves, throughout the four surveyed seasons (2015-2020). Arrows indicate oviposition damage observation dates. Total catches are not comparable since devices (meshes and light traps) evaluated per grove per season were not equivalent. Cumulative data is shown for better observation of the peak

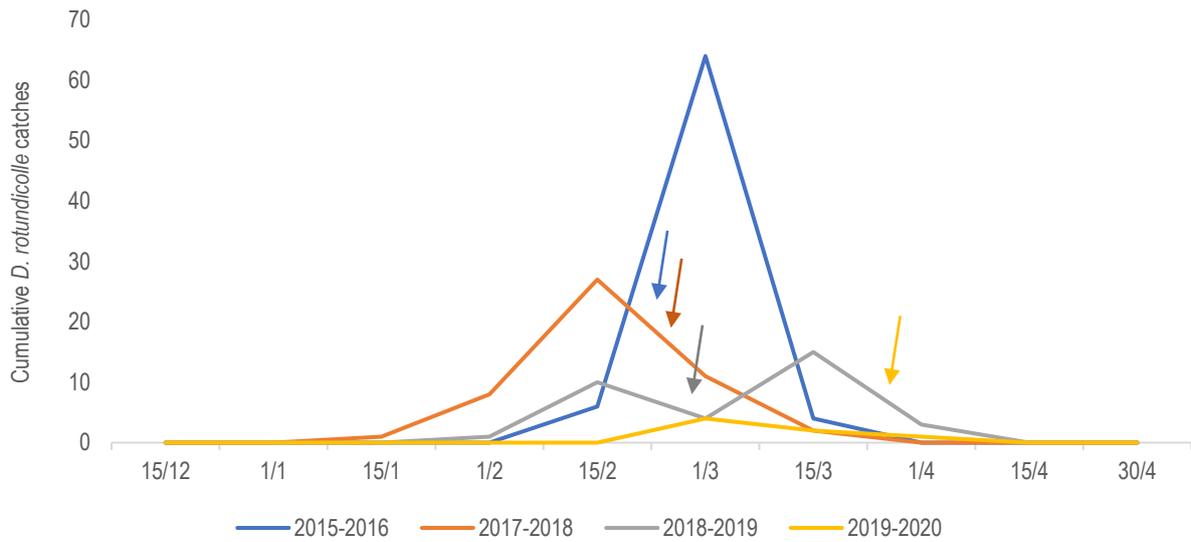
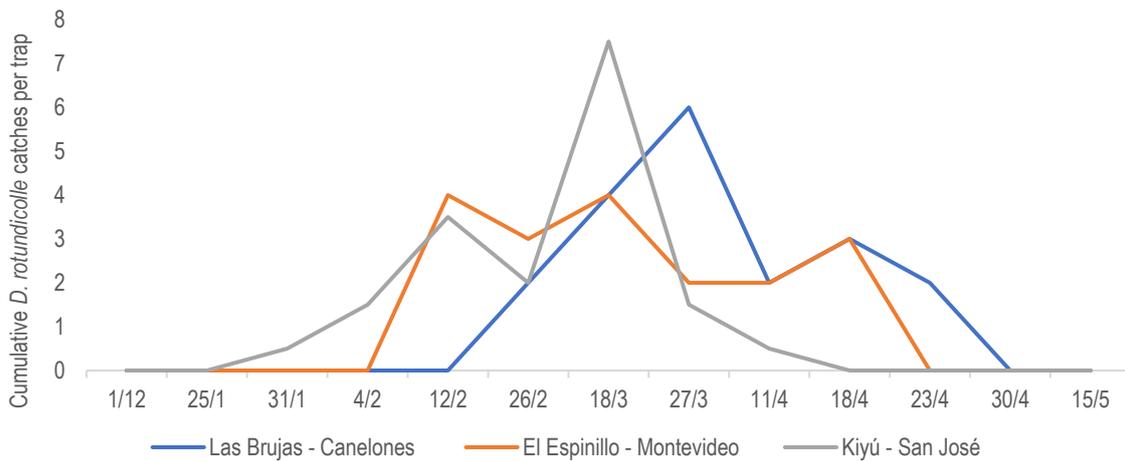


Figure 2. Cumulative *Diploschema rotundicolle* adult catches per trap in three groves in the 2018-2019 season



Significantly higher amounts of females were trapped in light traps in all seasons (5.2 females per male;  $P < 0,00001$ ) (Figure 3A). Further, emergence upon mesh-covered trees also showed higher female emergence (1.8 females per male;  $P < 0,0001$ ) (Figure 3B). A media of  $6.8 \pm 5.4$  adults emerged per tree.

During the flight season, adults were found underneath loosen barks of *Eucalyptus globulus* windbreaks surrounding the citrus plots, particularly in seasons and groves with high population levels. Adults where either found as individual males or as one single male-female couple, suggesting that daytime shelter occurs with no gregarious behavior. Overall, adults under *E. globulus* windbreaks barks were more males than females (Figure 4).

Figure 3. Comparison of females and males captured in light traps (A) and emergence from mesh-covered trees (B). Data are shown as boxplots and correspond to cumulative catches in all groves an

d seasons. Asterisks indicate significant differences (Chi-square test,  $P < 0,05$ )

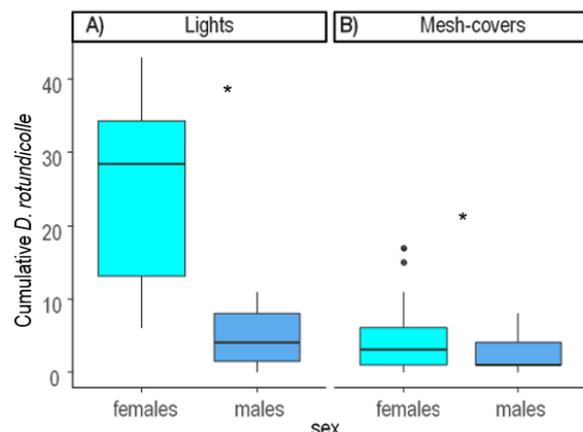
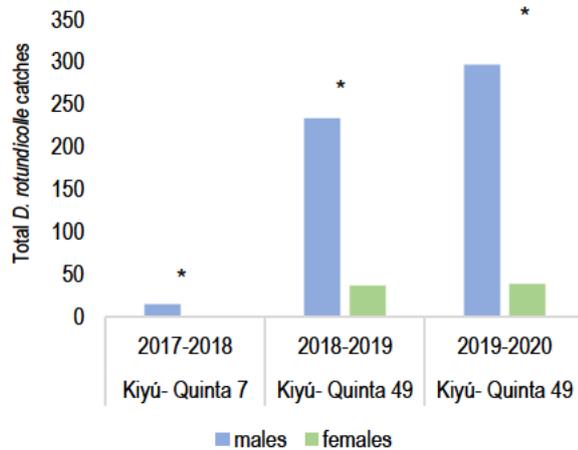


Figure 4. Extraction of *D. rotundicolle* adults from underneath loosen barks of *Eucalyptus globulus* windbreaks surrounding the citrus plots. Asterisks indicate significant differences (Chi-square test,  $P < 0,05$ )



Visual observation of larval activity was recorded throughout the whole year. Damage was not evenly distributed in the orchard but rather focused in certain parcels.

#### 4. Discussion

Within the framework of integrated pest management, monitoring of insect populations in space and time is remarkably important in order to make informed decisions on control measures<sup>(9)</sup>. The results of this study showed that light cross-vane traps proved an effective tool for monitoring *D. rotundicolle* adults. Flight was consistently observed between January and April in southern Uruguay conditions, with population peaks between mid and late February. Emergence from mesh-covered trees was observed in the same period as adult catches in light traps. This may be an alternative method to detect the adult emergence period, although it is a more disruptive and laborious methodology.

Oviposition damage was generally observed close or shortly after the peak of higher catches in light traps, between mid and late February. However, in the 2019-2020 season, oviposition damage was observed approximately one month after the usual recorded period. This season, also characterized by low trap catches, was particularly dry, which may have caused increased eggs desiccation or delayed oviposition. Slightly lower relative humidity and higher temperatures were also observed during this season compared to 2017-2018 and 2018-2019 summers (Supplementary material, Figures 1 and 2).

Some variability was observed across seasons and groves. Particularly remarkable are the results obtained in season 2019-2020 in the three surveyed groves within Kiyú and Las Brujas, less than 50 km apart. In this season, flight onset was observed with a gap of about a month between groves. This flight period gap may be extremely relevant for an appropriate timing of cultural control interventions, and suggests that each grove should be monitored independently to maximize trimming effectiveness. Indeed, our observations indicate that trimming may be significantly optimized if it is planned according to adult monitoring information rather by the visual observation of wilted twigs, when the larvae may have already migrated proximally within the branch.

In this study we worked with 1-3 traps per citrus plot, a number of traps that proved to be sufficient in years and plots of higher population levels; when problematic situations arise and there is a need for stronger interventions. It should be mentioned, however, that poorer results were obtained in seasons of low populations, in which more conclusive results might have been obtained with a higher number of traps per plot. However, 1-3 traps were sufficient to generate general knowledge of the flight period, enough for management decision making at the grove scale. Further studies may be needed to define the monitoring strategy in situations of lower populations densities.

The fact that more females are trapped in light traps may be due to an unbalanced sex ratio, as observed in the emergence from mesh-covered trees. Another hypothesis is that females are the more mobile sex, and are trapped in their search for oviposition sites. Indeed, the trapping of more males underneath the refuge of loosen barks of *E. globulus* windbreaks, very close to citrus trees, is in line with this last hypothesis. It is possible that courtship and mating may take place within refugee sites, after which females take off for oviposition on citrus trees.

Interestingly, our confirmation that *D. rotundicolle* adults hide in eucalyptus trees enables another strategy of cultural control, that is, the manual removal of barks and beetles. This strategy may also be defined based on monitoring adult flight. Despite the fact that this measure requires crew labor and it might be as expensive as twig pruning, it may be performed intensively in more affected areas and seasons. Further, when possible, it would be desirable to remove this species of eucalyptus as windbreakers in groves and to avoid them in new orchards.

Finally, non-interrupted larval activity was observed throughout the year, as reported by Faria and others<sup>(2)</sup>; further, attacked trees within the groves were observed in focalized spots. The later observation highlights the need for an effort to monitor adult flight in different areas of the grove, to maximize not only the temporal but also the special benefits of following the dynamics of adult populations.

## 5. Conclusions

This study provides evidence that cross-vane light traps are an effective tool for monitoring *D. rotundicolle* adult flight period. One trap per ha would be enough to define interventions, especially when populations are high. In southern Uruguay, the adult flight period spans between January and April, with a peak around mid-February. Oviposition damage is generally observed around mid-late February. *Eucalyptus globulus* windbreaks surrounding citrus groves should be avoided since they provide suitable shelter for *D. rotundicolle*.

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## Author contribution statement

María Eugenia Amorós and Andrés González contributed to the study conception and design, and wrote the manuscript. Lautaro Lagarde, Valentina Poloni and María Eugenia Amorós performed material preparation, data collection and analysis.

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**Supplementary material**

Figure 1. Effective precipitation period 2017-2020. Data extracted from Estación Experimental INIA Las Brujas, Canelones. The arrow shows a summer season of particular low precipitation records, which may explain unusual survey results in the 2019-2020 season.

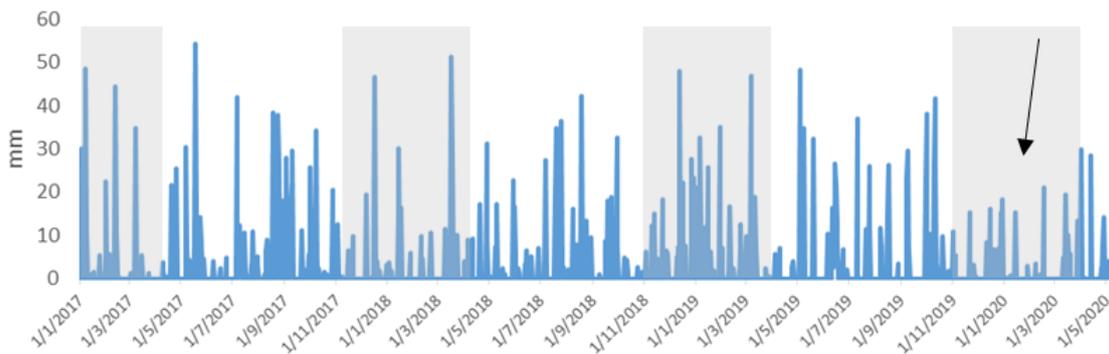


Figure 2. Boxplots of temperature (°C) and relative humidity (RH %) measurements from January to April in 2018, 2019 and 2020, in Kiyú, San José. Data extracted from a sensor in the grove.

