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Extraction of coffee berry borer adults and larvae from fruits

Abstract – The objective of this work was to develop a new method for extracting *Hypothenemus hampei* adults and larvae from coffee (*Coffea arabica*) fruits. The extractor consists of a set of two plastic containers, with one fit on top of the other: the one on top is used to place the fruits inside and the one on the bottom, as a base to capture adults and larvae. The efficiency of the extractor was compared with that of the dissection method (control). The number of live adults, dead adults, and larvae does not differ significantly between the two evaluated methods. The developed extractor reduces hand labor, is affordable, and is effective in capturing larvae and adults of *H. hampei*.

Index terms: *Coffea arabica*, *Hypothenemus hampei*, extractor, dissection, rearing insects.

Extração de adultos e larvas da broca-do-café de frutos

Resumo – O objetivo deste trabalho foi desenvolver um novo método para extração de adultos e larvas de *Hypothenemus hampei* de frutos de café (*Coffea arabica*). O extrator é composto por um conjunto de dois recipientes plásticos, um encaixado sobre o outro: o de cima serve para colocar os frutos dentro e o de baixo, como base para a captura de adultos e larvas. A eficiência do extrator foi comparada com a do método de dissecação (controle). O número de adultos vivos, adultos mortos e larvas não difere significativamente entre os dois métodos avaliados. O extrator desenvolvido reduz o trabalho manual, é de custo acessível e é eficaz para a captura de adultos e larvas de *H. hampei*.

Termos para indexação: *Coffea arabica*, *Hypothenemus hampei*, extrator, dissecação, criação de insetos.

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The search for alternatives to control *H. hampei* depends on the development of laboratory research, which is only possible through improved techniques of insect rearing and maintenance (Parra, 2014). Currently, dissection is the most used method for obtaining eggs, larvae, and adults from the borer; however, it is an expensive process

Hypothenemus hampei (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae), commonly known as coffee berry borer, is the most important insect pest of coffee (*Coffea* spp.) worldwide (Vega et al., 2015). However, the control of *H. hampei* is difficult because of its cryptic habits (Vega et al., 2017). The borer can reduce the yield of coffee plantations by up to 80%, as well as the quality of the bean and beverage due to the weight loss of infested coffee (Vega et al., 2015). In addition, the borer is not evenly distributed throughout the crop, which makes monitoring and managing this pest difficult (Mariño et al., 2017).

that demands time and labor because it is performed manually (Infante, 2001).

The objective of this work was to develop a new method for extracting *H. hampei* adults and larvae from coffee (*Coffea arabica* L.) fruits.

In March 2021, fruits from the Mundo Novo IAC 376 - 4 coffee cultivar were collected at the agricultural experimental station of Syngenta Proteção de Cultivos Ltda, located in the municipality of Holambra, in the state of São Paulo, Brazil (22°38'57"S, 47°5'29"W, at an altitude of 600 m). The field experimental area, with six-year-old plants spaced at 1.70×2.70 m, totaled 1.0 ha. Each plot consisted of a row with ten plants, and the eight central plants were considered the working area.

A total of 10 L of infested coffee fruits (unripe, half-ripe, and ripe berries) were collected and separated into volumes of 0.5 L, totaling 20 plots, of which 10 were used for the dissection method (control) and the other 10 for the developed extractor. The fruits were placed in properly identified paper bags and transported to the Laboratory for Insecticide Resistance Monitoring, located at the experimental station, where they were subjected to both evaluated methods.

The dissection method consisted of counting the fruits and opening them in the crown region using a scalpel and then counting the number of adults and larvae in each fruit. In the extractor method, the fruits were immersed in a 5% sodium hypochlorite solution for 1 min, washed in running water, and then dried at room temperature (25±2°C) for about 1 hour; the fruits were also counted. The developed extractor consisted of a set of two plastic containers of same dimensions (length × width × height of 25×17×8 cm), with one fit on top of the other (Figure 1 A and B).

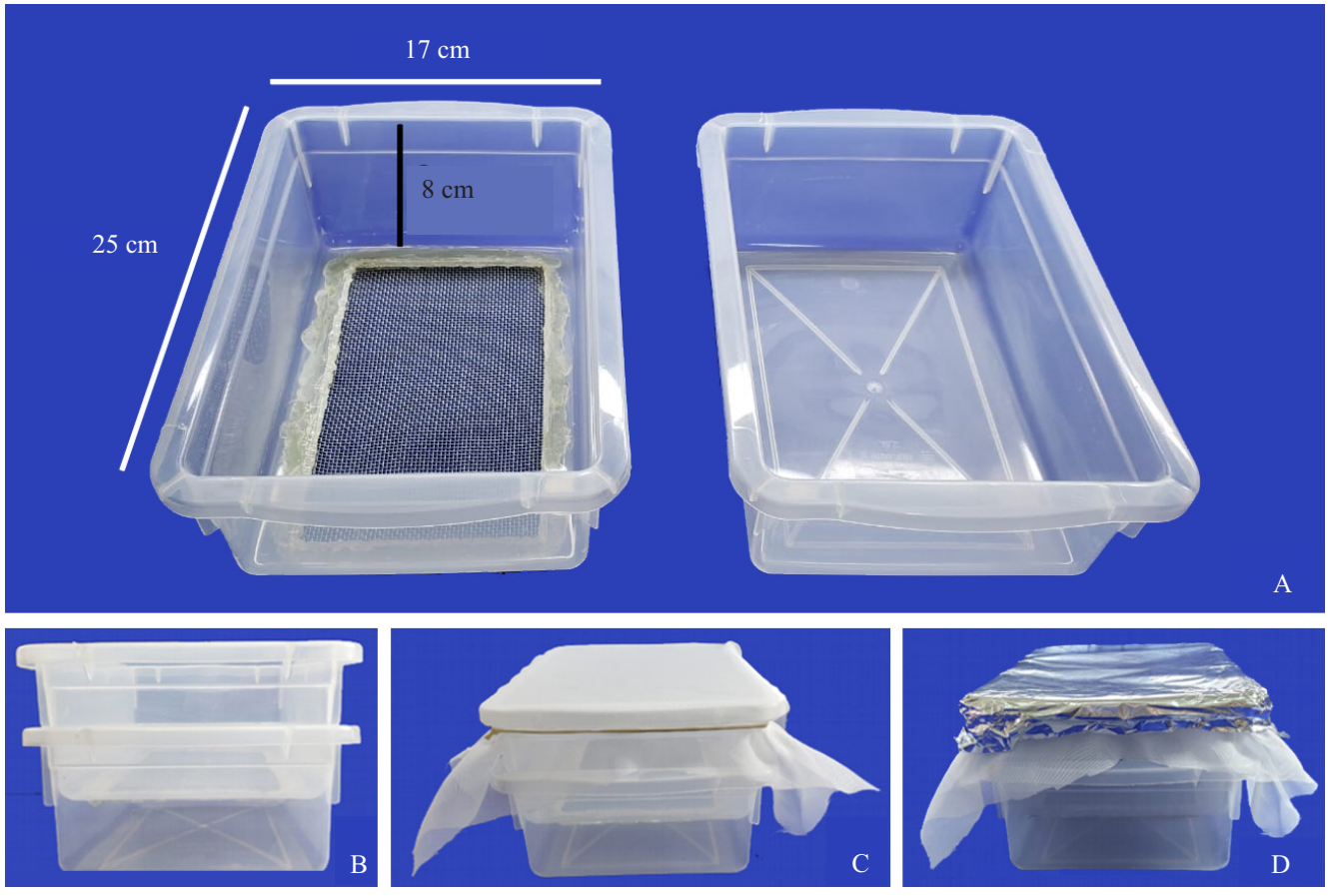

Figure 1. Overview of the developed extractor, consisting of a set of two plastic containers of same dimensions (A), with one fit on top of the other (B) and the top one being covered with voile fabric fixed with a rubber band (C) and with aluminum foil (D). Photos by Adriana Cristina Nardon.

The first container was used to place the fruits inside and had a screen (18×16 mm mesh, 30 thread) placed at its bottom, functioning as a sieve, to separate the insects from the fruits; the second container was used as a base to capture adults and larvae of the berry borer (Figure 1 A). The top container was covered with voile fabric fixed with a rubber band and also with aluminum foil (Figure 1 C and D) due to the endophytic habit of the insect. Ten container sets were kept in a climate-controlled room (25±2°C and 60±10% relative humidity) for five days. The *H. hampei* adults and larvae were kept in the bottom container after they left naturally the interior of the fruits and were then captured with entomological tweezers.

Shapiro-Wilk's test, at 5% probability, was used to assess the normality of the data. A possibly significant difference regarding the number of fruits, live adults, dead adults, and larvae obtained by the two methods was verified by the paired t-test, also at 5% probability (Table 1).

Although half of the 10 L of the collected coffee was used for each method, the average number of fruits differed significantly. However, the number of live adults, dead adults, and larvae did not differ significantly between the two evaluated methods ($p<0.05$) (Table 1). The average of 0.70 live adult per fruit obtained using the developed extractor was similar to that of 0.60 live adult per fruit using dissection. Souza et al. (2014) collected 1,380 adults from 2,400 dissected fruits, which is equivalent to 0.57 adults per fruit. In the present study, the average number of dead adults, regardless of the extraction method, was considered low, indicating that the extractor does not interfere with mortality.

Table 1. Number (mean±standard error) of fruits and of live adults, dead adults, and larvae of *Hypothenemus hampei* obtained by two extraction methods from 10 L of fruits from the Mundo Novo IAC 376 - 4 coffee (*Coffea arabica*) cultivar[(1)].

Method	Number of fruits	Live adults	Dead adults	Larvae
Dissection	259.3±4.8	155.9±8.7	0.8±0.3	19.6±1.7
Extraction	239.4±6.7	169.5±6.5	0.5±0.3	20.9±2.3
p-value	0.0076	0.2660	0.403	0.5263

[(1)]Significant difference verified by the t-test, at 5% probability.

Moreover, the developed extractor allowed obtaining a higher average of 20.9 larvae when compared with those reported in the literature. Hamilton et al. (2019), for example, found an average number of 14.42±1.11 larvae by dissection. According to Vega et al. (2015), one of the main obstacles to conducting studies at this stage of development is the difficulty in obtaining larvae using the latter method.

The number of live adults, dead adults, and larvae does not differ between the dissection and extraction methods. The developed extractor does not harm adults and larvae because it acts passively, allowing them to be transferred to rearing on an artificial diet. Furthermore, the method reduces hand labor, is affordable, and is effective for obtaining *H. hampei* adults and larvae from coffee fruits.

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