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Correlation between numbers captured and infestation levels of the Coffee Berry-borer, *Hypothenemus hampei*: A preliminary basis for an action threshold using baited traps

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Correlation between numbers captured and infestation levels of the Coffee Berry-borer, *Hypothenemus hampei*: A preliminary basis for an action threshold using baited traps

Adriano E. Pereira^{a*}, Evaldo F. Vilela^b, Ricardo S. Tinoco^b, José Oscar G. de Lima^{b,c}, Andreza K. Fantine^b, Elisângela G.F. Morais^b and Christiane F.M. França^b

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Sampling techniques currently used to determine control measures for the Coffee Berry-borer (CBB) are time-consuming and allow the grower only a small window of opportunity to select other options. Experiments were conducted in four coffee fields between 2005 and 2007 using IAPAR[®] traps that were baited with ethanol and methanol (1 : 3 ratio) and benzaldehyde at 1% volume, to test for a correlation between the number of captured adults and infestation levels of CBB, and to determine the action threshold level. For this study, a density 20 traps/ha was used in each experimental area. The number of CBBs captured and infestation level on coffee berries were recorded every 2 weeks. Significant correlation was observed between the trap capture and the infestation level of the CBB in the field. This correlation can enable us to determine action thresholds using traps as sampling methods. Trap catch was very low in all four fields during fruit maturation between March and July, and it increased sharply in August when the CBB emerged from the dry berries that remained on the plants or on the ground.

Keywords: attractant; benzaldehyde; Coffee Berry Borer; ethanol; methanol; semiochemical; trapping; Scolytidae

1. Introduction

The Coffee Berry-borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), is considered to be the most important pest in all coffee-growing regions (Baker 1999). The adult female beetles bore holes into coffee berries and make galleries inside the endosperm, where they lay eggs. The larvae feed on the endosperm and damage the seeds. Quantitative losses or direct damage occur as a consequence of the damaged, unripened berries, which fall to the ground. The gallery-damaged seeds can break up and crack during processing, and loss of berry weight can exceed to 20% (Souza and Reis 1997). On the other hand, qualitative losses or indirect damage can occur due to the low quality of the coffee seeds, as the galleries can allow pathogens to enter, leading to fermentation and tainting of coffee flavour (Batista 1986; Baker 1999; Wegbe et al. 2003).

A thorough harvest or “re-passing,” which involves the removal of dry berries from both the plant and the ground, is considered to be the best CBB management tactic, although it is not always feasible due to the labor costs involved in collecting the remaining berries in the field (Souza and Reis 1997; Baker 1999). These berries are a food source/refuge for the CBB. When the temperature increases to approximately 25°C in the spring and the relative humidity is between 90% and

100% after the first spring rains, colonizing females abandon the old berries that remain on the ground or on the plants, to search for new berries (Baker et al. 1992b; Mathieu et al. 1997a). In Brazil, spring occurs between September and December.

Sampling techniques which are used to estimate the adult density of CBBs are labor-intensive. Conventional sampling methods recommend the inspection of 100 berries per plot (Souza and Reis 1997), whereas sequential sampling recommendations involve inspection of 5 branches per coffee plant and 10 berries per branch to arrive at a decision (Bianco 2000). Chemical control is recommended when the level of infestation reaches a threshold of 3% or 5% of damaged berries, depending on whether the market price is high or low, respectively (Souza and Reis 1997). Endosulfan and chlorpyrifos are the most efficient and frequently used insecticides in Brazil. However, the use of insecticides has contributed greatly to environmental imbalance and pollution: it has adversely affected the CBB's natural insect enemies, and increased the risk that insect resistance might develop; thus it has threatened communities that live around the coffee fields (Brun et al. 1989; Souza and Reis 1997), and increased production costs (Baker 1999).

The use of traps that are baited with semiochemicals is one of the recognized tools for pest detection

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and mass-trapping. The major advantage of using traps is rapid detection of the CBB, even when the insect density is low during either adult emergence or the movement of adults into the field from neighboring farms. The initial CBB captures can be used as an early-warning tool for farmers, to initiate the monitoring before the number of pests reaches the action threshold (Wall 1989). The usefulness of semiochemicals to attract CBB was demonstrated during the late 1960s when Prates (1969) observed the attraction of CBBs to ripe and green coffee berry extracts. Since then, other researchers have demonstrated that volatiles obtained from coffee berries are attractive to the CBB (Giordanengo et al. 1993; Mathieu et al. 1997b, 1998, 2001; Ortiz et al. 2004; Mendesil et al. 2009), and Lima et al. (2004) successfully demonstrated the CBB's attraction to benzaldehyde and methyl salicylate, which are present in the coffee berries.

Using Lindgren traps (Lindgren 1983), Mendoza-Mora (1991) was the first to test and demonstrate the olfactory attraction of the CBB to blended ethanol-methanol at 1 : 1 and 1 : 3 ratios; the two chemicals had a synergistic effect. Trapping also allows the identification of the "transition phase" of CBB; this occurs when the colonizing females leave the old berries to search for new fruits, as described by Mathieu et al. (1997a). In Brazil, the transition phase is considered to be the post-harvest period between August and February. During this period, chemical control is more efficient at targeting the pest (Gutiérrez-Martínez et al. 1993; Souza and Reis 1997; Cárdenas 2000; Gonzáles and Dufour 2000; Villacorta et al. 2001; Dufour 2002a; Barrera et al. 2004, 2005; Dufour and Frérot 2008), which results in a lower cost of production for the farmer (Mathieu et al. 1999; Barrera et al. 2004).

A system for adult detection using pheromone or semiochemical-baited traps can be an alternative method for identifying the action threshold. Very few studies have been directed at this technique. Bento et al. (2001) demonstrated the efficiency of traps that were baited with pheromones in determining the action threshold to initiate chemical control of the citrus fruit-borer.

The positive correlation between pest trap catches and the field population density is a feature that can be used to determine when pest control is necessary, as the number of insect pests present in the traps could reflect the real pest population in the field (Mathieu et al. 1999; Asaro and Berisford 2001; Faccoli and Stergule 2004; Bacca 2006).

The aims of this research were to test for a correlation between trap catch and infestation level of the CBB using IAPAR[®] traps baited with semiochemicals, and to test the hypothesis that the data obtained from the correlations can generate an action threshold level for CBB control, in the southern region of Minas Gerais State, Brazil.

2. Materials and methods

2.1. Experimental fields

The experiments were conducted in the Viçosa municipality (20°45'14"S, 42°52'5"W; 670 m a.s.l.), Minas Gerais state, southeast region of Brazil, from March 2005 to February 2007 in four coffee fields cultivated in the full sunlight system, with high CBB infestation levels: three commercial fields ("Catuaí" variety – *Coffea arabica* L.) and one experimental field ("Conilon" variety – *Coffea canephora* Pierre ex A. Froehner) owned by the Federal University of Viçosa. Field 1 comprised the "Catuaí" variety; it contained 20 traps in an area of 1.0 ha set up on four coffee rows (not consecutively), was planted in 1990 at 737 m a.s.l. and had a spacing of 2.70 m × 0.70 m between row and plant, respectively, and was located at latitude (S) 20°48'24" and longitude (W) 42°52'56". Field 2 comprised the "Catuaí" variety; it contained 20 traps in an area of 1.0 ha set on four coffee rows, was planted in 1992 at 669 m a.s.l. and had a spacing of 3.50 m × 0.70 m between row and plant, respectively, and was located at latitude (S) 20°43'34" and longitude (W) 42°51'3". Field 3 comprised the "Conilon" variety; it contained only 6 traps in one row due to its small size (0.33 ha), was planted in 1997 at 678 m a.s.l. and a spacing of 3.00 m × 0.90 m between row and plant, respectively, and was located at latitude (S) 20°44'55" and longitude (W) 42°50'36". Field 4 comprised the "Catuaí" variety; it contained 15 traps in 0.75 ha set in three coffee rows, was planted in 2000 at 704 m a.s.l. and a spacing of 2.70 m × 0.90 m between row and plant, respectively; it was located at latitude (S) 20°46'36" and longitude (W) 42°49'55". Viçosa county and region have an annual average precipitation of 1.227 mm. The experimental areas were different regarding the location, age, size, variety, and spacing. During the years of experiment, fields 1, 2 and 3 were not sprayed with insecticide against the CBB; only field 4 had the surrounding fields sprayed with endosulfan.

A density of 20 traps/ha was used, based on previous work that showed this density to be optimal for capturing and monitoring the CBB (Villacorta et al. 2001; Dufour 2002b; Barrera et al. 2004, 2005; Lima et al. 2004; Dufour and Frérot 2008). In all the fields, the traps were separated from each other by c. 22 m both within and between coffee plant rows. The experimental fields were separated from each other by c. 5–15 km.

2.2. Trap, dispenser and semiochemicals

The traps used for the experiments were the IAPAR[®] model, developed by researchers from Paraná Agriculture Research Institute (IAPAR), because they are cheap and easy to assemble, and due to their efficiency in attracting the CBB (Villacorta et al. 2001). They

were constructed using clear plastic 2-L capacity soda bottle with a window opened on the side (15.0 cm × 9.5 cm). All of the traps were spray-painted red (Renner Colorjet, Code # 1735) to maximize attractiveness to the CBB (Mendoza-Mora 1991; Mathieu et al. 1997a; González and Dufour 2000; Dufour and Frérot 2008). A dispenser containing the semiochemicals was placed inside the trap to attract the CBBs. Water with detergent (5%) was added at the bottom of the bottle to help drown the CBB adults. The semiochemicals were placed in a 10-ml glass vial with a rubber cap in which two very small, hollow stainless steel pipes (10 mm in length, 1.2 mm in diameter) were introduced to allow the semiochemicals to evaporate from the vial. The volatiles used as semiochemicals to attract CBB were ethyl alcohol (99%) (ethanol), methyl alcohol (100%) (methanol) at a 1 : 3 ratio, and benzaldehyde (Merck 99.5%, redistilled) at 1% volume, because of the synergistic effect of this volatile, based on the results of Lima et al. (2004) showing a high level of CBB attraction. Under laboratory conditions of $24 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ R.H., the release rate can reach 98 mg/day according to Lima et al. (2004), who also showed that this rate is optimal for attracting CBB females to the semiochemical blend used. The traps were fixed to the coffee plants at 1.5 m above ground level (Barrera et al. 2005; Dufour and Frérot 2008).

2.3. Data compilation

2.3.1. Evaluation of CBB infestation levels in the fields

Seven branches (numbered 1–7) between 1.0 m and 1.5 m above ground level were selected, marked, and number-labeled on seven individual plants (one branch per plant) that were located symmetrically around each trap to represent the infestation level. Branch 1 was located on the plant on which the trap was placed. Branches 2 and 3 were 5 m to the right and 5 m to the left of the plant with the trap, respectively. Branches 4 and 5 were on the first and second rows next the row with the trap, and branches 6 and 7 were on the first and second rows next the row with the trap, on the opposite side of branches 4 and 5. The branches and plants were identified with “Yellow and Black” tape, and the same branches and berries were evaluated visually during every sampling. The sampling was performed every 2 weeks until the berries reached an infestation level of 100% or had fallen (dry). All of the fields around the experimental plots had a period of harvest and post-harvest during the 2005 and 2006 seasons. Field 1 was harvested between 18 July and 11 October in 2005 and between 19 June and 15 September in 2006. Field 2 was harvested between 1 August and 25 October in 2005 and between 3 July and 18 September in 2006. Field 3 was harvested between the 14 and 18 July in 2005 and between the 10 and 15 June in 2006. Finally, field 4 was harvested between 14

July and 20 August in 2005 and between 25 July and 20 August in 2006 (Figure 1). The experimental area (plot) of all four fields, containing the traps, was not harvested. The sampled berries ripened from green to red and then dried as the experiment continued. During the sampling, 20 berries of the Catuaí variety (fields 1, 2 and 4) and 25 berries of the Conilon variety (field 3) were sampled. The berries were not removed from the trees during each sampling, and the number of infested berries was counted. The percentage of infested berries was calculated, even when few berries were left over. At the beginning of the sampling of the first season, in April 2005, or the second season in January–February 2006, most of the berries were green and red (ripened). By April to May, all of the berries had ripened or dried.

2.3.2. Trap contents and other scolytids

The trap contents were collected every 2 weeks. The water containing the insects was poured into a 500 ml glass vial and transferred to the laboratory to count and identify the specimens. Scolytids other than CBB were attracted and caught in the traps and the differentiation between CBB and other scolytids was based on morphological characters examined using a stereomicroscope. The total numbers of CBB and other scolytids were recorded separately. The correlation between other trapped scolytids and the CBB infestation level was calculated to determine whether the scolytids could interfere with decisions regarding CBB control measures.

2.4. Statistical analysis

Means and standard errors for the number of CBB adults captured and the percentage of infested berries were calculated. A population oscillation curve for the infestation level and the CBBs that were captured in traps baited with the semiochemicals was generated (Figure 1).

Three correlations were performed using Pearson's correlation (r); one was the comparison between CBBs captured in the traps and the CBB infestation level in the field, to verify whether the number of CBBs that were captured in the traps reflected the infestation level in the field; the other was the comparison between the number of other scolytids captured and the number of CBBs captured in the traps; instead of, and the number of other scolytids captured and CBB infestation level, to evaluate whether the capture of other scolytids in the traps interfered with the decision-making process involved in controlling the CBB.

Normality and homogeneity of variances were evaluated prior to statistical analysis. The data for infestation level were normally distributed. In contrast, the data for trap catches were not normally distributed, and so were transformed. Linear regression was

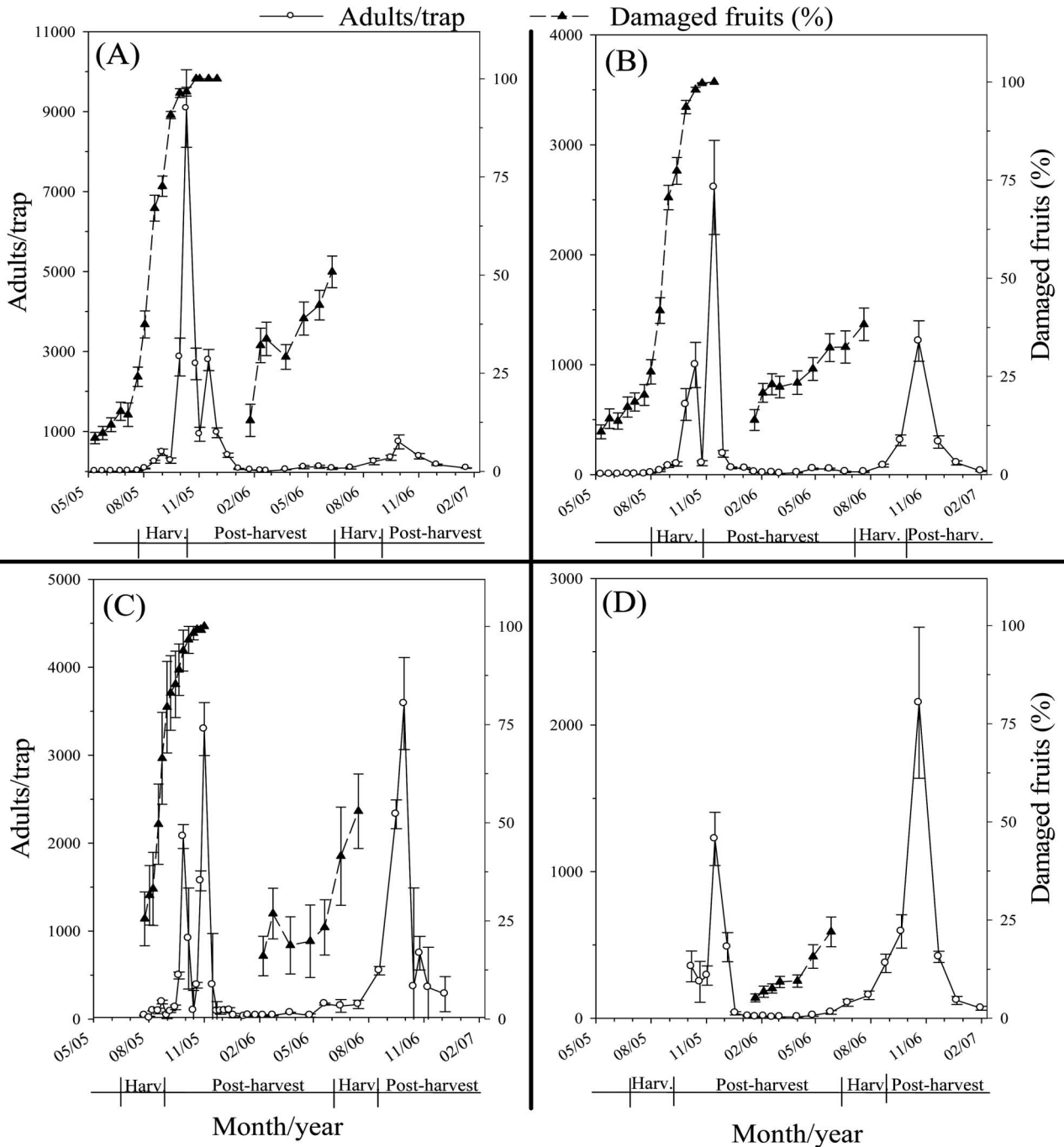


Figure 1. Population distribution of adult Coffee Berry-borers that were caught in semiochemical-containing traps every 15 days, and the percentage of damaged berries on the coffee plants in fields 1 (A), 2 (B), 3 (C) and 4 (D) during the harvest and post-harvest periods. Viçosa county, MG, 2005 and 2006 seasons.

performed on the data (infestation level and capture) to examine the relationship between trap catch and infestation level (Figure 2). Statistical analysis was performed using the statistical software SAEG- Statistical Analysis System (Ribeiro Júnior 2001).

2.5. Meteorological data

Regional data for daily precipitation (mm), relative humidity (%), and temperature (°C) were obtained

from the Meteorological Station at the University of Viçosa from the beginning of the experiment in March 2005 until February 2007.

3. Results

3.1. The correlation between numbers captured and CBB infestation level

All of the experimental fields were very similar with respect to increases and decreases in the trap captures

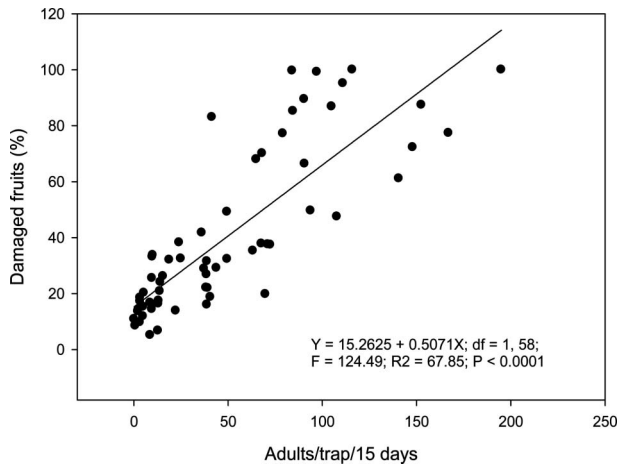


Figure 2. The regression of the mean of infestation level and trap captures until 250 Coffee Berry-borer/trap per 15 days. Each data point is the mean of the infestation level and trap captures for each sampling date of all four experimental fields.

throughout the year. At the beginning of the experiment, the fields demonstrated low CBB captures until July (except field 4 that started in October 2005) followed by a significant increase from August until the end of November. In December and thereafter, captures were reduced substantially in all four fields. Likewise, in 2006 captures were low until July and started to increase until November and decrease from December (Figure 1A–D).

During the experiment, field 1 had c. 466 000 CBBs captured over 21 months; field 2 had > 143 000 CBB captured over 21 months; field 3 had > 114 000 CBBs captured over 17 months, and field 4 had > 100 000 CBBs captured over 15 months. Field 3 had the greatest number of captures/trap, reaching 1117 CBB/trap per month ($114\,000/17 = 6706$ CBBs per month; $6706/6$ traps = 1117).

All four fields demonstrated a significant correlation between the infestation level and the number of CBBs captured. The capture peaks were registered in late October and November in all four fields, reaching the highest peak in the field 1, with more than 9000 CBB/trap in 15 days (Figure 1A–D).

Field 1 showed a correlation in September 2005 ($r = 0.29$; $P = 0.002$) and in February ($r = 0.80$; $P = 0.01$), May ($r = 0.53$; $P = 0.02$), and June 2006 ($r = 0.46$; $P = 0.04$). Field 2 presented correlation in September 2005 ($r = 0.29$; $P = 0.03$) and in January–February ($r = 0.54$; $P = 0.02$), June ($r = 0.53$; $P = 0.02$), and July 2006 ($r = 0.66$; $P = 0.001$). Field 3 presented correlation in September 2005 ($r = 0.31$; $P = 0.02$) and March ($r = 0.91$; $P = 0.01$), April ($r = 0.94$; $P = 0.01$), and July 2006 ($r = 0.94$; $P = 0.01$). Field 4 presented correlation just in 2006 in January ($r = 0.63$; $P = 0.01$), March ($r = 0.63$; $P = 0.01$), April ($r = 0.54$; $P = 0.04$), and May ($r = 0.74$; $P = 0.002$).

3.2. Trapping other scolytids

Several scolytid beetles other than the CBB were caught in the semiochemical-baited traps in each field during the experiment (field 1 = 9048 scolytids; field 2 = 4924 scolytids; field 3 = 2850 scolytids; field 4 = 4817 scolytids). The majority of the scolytids belonged to the tribe Xyleborini (Tito Bacca, a Colombian Entomologist, pers. comm.). The “false” CBB, *Hypothenemus obscurus* (Fab.), was also captured in all four experimental fields and was distinguished from *H. hampei* based on morphological features (Souza and Reis 1997).

A positive correlation was recorded between the capture number of other scolytids and the number of CBBs captured in field 2 ($t = 17.33$; $r = 0.56$; $P = 0.0001$), field 3 ($t = 7.15$; $r = 0.43$; $P = 0.0001$) and field 4 ($t = 8.97$; $r = 0.44$; $P = 0.0001$). A positive correlation was also found between the percentage of infestation of CBBs and the number of other scolytids captured in fields 1 ($t = 13.92$; $r = 0.56$; $P = 0.0001$) and 2 ($t = 15.47$; $r = 0.59$; $P = 0.0001$).

4. Discussion

The correlation we found between trap captures and infestation levels can indicate the pest status in the field and is a useful tool for monitoring the level of CBB infestation. When an increase in the number of CBBs captured in traps is reported in consecutive samplings, attention should be paid to the CBB infestation level in the berries, which will certainly increase as well. A strong correlation between the trap captures and damaged berries was observed for the first samplings in all of the experimental fields until the capture rate started to decrease.

These results demonstrate that the use of bottle traps baited with semiochemicals can predict the infestation level of the CBB in the field, and so be a useful tool both for detecting the colonizing females (Mathieu et al. 1997a) and identifying the “transition phase” of the CBB (Souza and Reis, 1997). Mathieu et al. (1999) also showed a positive correlation between CBB captures and infestation level, using funnel traps. Asaro and Berisford (2001) and Faccoli and Stergule (2004) demonstrated a positive correlation between trap captures of the Nantucket pine tip moth and the European spruce bark beetle and the level of pest damage in the field. Using pheromone traps, Bacca (2006) found a positive correlation between the numbers of captured male coffee leaf-miners and the number of eggs laid on the leaf surface. However, it is important to note that most of the CBB females that infest the berries and were captured in the traps during the peak between July and December came from residual dry berries located on the ground or on the plants from the previous season (Mathieu et al. 1999; Dufour et al. 2007). As stated above, experimental

areas were not harvested during the experiment; this is likely to have contributed to increase in trap captures and infestation level, but there are neighboring producers who might not control CBB appropriately.

The use of baited traps to capture CBBs can generate a map of CBB infestation in the area that reveals the highly infested trees or hotspots near the traps. The map can facilitate CBB control at each site and potentially indicate the need for an early harvest of the coffee plants located around the traps (depending on the fruit maturation) to prevent the CBB population from reaching too high a level. This strategy would lead to a cost reduction of coffee farm management due to the decrease in insecticide use, labor, and sampling time, as suggested previously by Mathieu et al. (1999) and Cárdenas (2000). Concerning field 1, which demonstrated the highest level of infestation, >466 000 CBB females were captured during 21 months of trapping. Considering that each female lays an average of 50 eggs, the number of CBB females would be approximately 21 million assuming the sex ratio of CBB to be 10 females to each male (Bergamin 1943). However, this number would be lower considering the natural mortality factors in the environment (Baker et al. 1992a, 1994).

The differences among the experimental areas, especially as regards the trap capture numbers, might be attributable to plant age, size, and mechanization of the farming practice, and certainly due to the level of infestation of the CBB in the previous year in each field. Fields 1 and 2 were the oldest; field 3 comprised the Conilon variety and was not mechanically managed due to its small size; it had the highest capture rate per trap per month. Field 4 was only 5 years old at the time of study, and the farmer previously controlled CBBs by spraying enfosulfan, if infestation was high. "Conilon" coffee plants are taller and produce more coffee berries per cluster (this is why 25 coffee berries per branch were sampled to record infestation level) compared with Catuaí coffee plants, probably contributing to making field 3 have the highest capture rate/trap per month.

The peak of CBB capture was the same for all of the fields; it started in mid-October to early November (Figure 1A–D), immediately after the rains and when the relative humidity and temperature had increased. Subsequently, the number of trap captures in all of the fields decreased significantly. Beginning in December, the females started to locate new berries within which to lay their eggs and start a new lifecycle.

In fields 1 and 2, a decrease in the size of the peak of trap capture was observed in 2006 compared with 2005 (Figure 1A, B). This could be explained by the trapping of the colonizing females that occurred in 2005. On the other hand, fields 3 and 4 showed an increase in the size of the peak of trap capture in 2006 compared with 2005 (Figure 1C, D). Although the coffee fields surrounding field 4 were sprayed with insecticide, the neighboring

farmers might not have sprayed their fields, causing the CBB population for 2006 to be higher in field 4. This reasoning also applies to field 3, although there was no insecticide spraying in 2005.

Since other scolytid species were captured in the traps, we decided to identify and relate their numbers to both CBB trap counts and infestation level. The region of Viçosa county is surrounded by mountains and by a small section of the native Atlantic rainforest, and the other scolytids might have migrated from the trees, pines, and *Eucalyptus* comprising the forest, to the coffee areas. According to Souza and Reis (1997), the scolytid *H. obscurus* bores a hole only through the pericarp of the coffee berry without damaging the seed. The number of other scolytids that were captured in the traps was insufficient to allow interpretation of the CBB data for decision-making purposes, and thus, coffee-growers do not need to separate the number of other scolytids that are captured in the traps. However, growers must be aware that the interpretation of the data starts from July onwards, when the monitoring of the CBB begins in Brazil. From December until June, the number of captured CBB was very low and the number of other scolytids that were captured was sometimes higher.

We can infer from both the positive correlation between the capture of CBBs and other scolytids, and that between CBB infestation level and the capture of other scolytids, that optimal temperature and rainfall are the main reasons why scolytids emerge from the wood or trees at the same time as CBBs emerge from old or overpopulated berries to either colonize coffee berries or be captured in the traps.

From the experimental data, it was not possible to obtain the action threshold values for CBB at 3% and 5% of infestation using baited traps, because of the high infestation of the fields sampled. Based on the equation for the linear regression (Figure 2) ($Y = 15.2625 + 0.5071X$), when the captures were zero, the infestation level was c. 15% and this value is much higher than 3% or 5%, which are the action thresholds for the CBB, depending on the coffee price in the market, as discussed before. To obtain the action threshold for CBB using traps, other fields with low CBB infestation should be sampled and there was no time left to sample more fields during the Masters' program.

We have shown that the use of bottle traps baited with ethanol-methanol at a ratio of 1 : 3 with benzaldehyde added at 1% volume is efficient in attracting CBBs, and consequently enables infestation level in the coffee fields to be predicted. This prediction with traps can be a tool for monitoring CBB movements within the coffee-growing area, as CBB females start to colonize other coffee berries from July, in Brazil. From December onwards, captures decrease substantially when the females are already colonizing new coffee berries. In those areas in which native

forests or other trees surround the coffee fields, other scolytid beetles might eventually be attracted to the traps but will not interfere in the decision-making to control the CBB. Our findings suggest that the action threshold value estimated by using baited bottle traps can be obtained in areas with low CBB infestation. This technique can improve the CBB management in the field by making sampling easier and feasible.

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