RESEARCH

Molecular markers as a method to evaluate the movement of *Hypothenemus hampei* (Ferrari)

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ABSTRACT. The objective of this research was to develop a methodology to describe the movement of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae) in the field through: (i) the evaluation of allele variation of a microsatellite marker on polymorphic Colombian *H. hampei* populations; (ii) the invention of a device for releasing *H. hampei* adults; (iii) the standardization of a release-recapture technique for *H. hampei* populations; (iv) the estimation of the flight distance of the insect; and (v) the calculation of a mathematical expression that describes the movement of *H. hampei* in space over time. The results indicated that: (i) the microsatellite molecular marker HHK.1.6 was exclusively present in a population from Guapotá-Santander, was dominant and allows the evaluation of *H. hampei* movement for several generations; (ii) a device that released 88.8% of *H. hampei* adults in 2 s was designed; (iii) this device was used as *H. hampei* populations containing HHK.1.6 marker release strategy, and coffee seeds as recapture strategy; (iv) it was estimated that *H. hampei* adults flew as far as 65 m, however, 90% were recovered in a radius of $<$40 m. Finally, (v) the mathematical expression that described the movement of *H. hampei* in space over time was $\hat{Y} = \alpha\beta^{X_i}$, being $\hat{Y}$ the average number of borer beetles recaptured per tree, and *x* the distance in meters. This method will allow to determine the movement of *H. hampei* from different environmental and ecological scenarios.

Key Words: coffee, dispersal, releasing device, microsatellites, *Coffea arabica*

Coffee production is the economic basis of several countries in the tropics. Currently, this industry is threatened by its major insect pest, the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae), as well as climatic change and an increase in the global average temperature (Jaramillo et al. 2009). In agriculture, an increase in temperature affects the conditions for the development of pests and diseases, which influences the effects that these organisms have on the vegetation on which they feed. In a number of insect pests, these phenomena can directly influence the geographical distribution of the species, through the tendency to move to other latitudes or higher altitudes, which alter their life history, the number of generations per year, longevity and dispersal patterns (Jaramillo et al. 2009). Therefore, it is important to measure the movement of insects in both their natural habitat and invaded areas, in order to understand their biology, demography and ethology (Hagler and Jackson 2001). Besides, this type of studies would allow to better design sampling programs, to estimate properly population sizes (Sevacherian and Stern 1972) and plan ahead control strategies in invaded areas.

The ability of coffee berry borer to disperse has been inferred. According to Alonzo (1984), the mobility of the coffee berry borer beetles to infest new areas is reduced and the mode of transportation is hitchhiking on clothing and tools through workers from infested plantations; however, Castaño et al. (2005) demonstrated that the removal of one hectare of coffee allows the flight of more than three millions coffee berry borer adults over a period of 170 d, indicating that pruning infested coffee trees is the agronomic practice that most beetles disperse in field conditions. Similarly, Castro et al. (1998) and Moreno et al. (2001) demonstrated that around 25,000 borer beetles can fly from the picking, collection and wet mill of 300 kg of coffee fruits.

Other authors disclose that the movement of the coffee berry borer is probably not restricted to the local dispersion, but to a larger scale wind-aided. Baker (1984, 1999) showed under laboratory conditions, that coffee berry borer has the ability to fly for up to 3 h, which allows massive migration and causing rapid infestation in adjacent coffee crops. Experimentally, the greatest distance that a coffee berry borer individual was able to reach was recorded in 348 m (Leefmans 1923), constituting evidence that the borer beetles would migrate and cause infestation in the surrounding areas (Ruiz et al. 2003).

To assess the dynamics and movement of insect populations under natural conditions, a wide variety of markers, such as paints, inks, powders, and molecular markers, have been used (Acevedo et al. 2009). According to Robledo and González (2009) molecular markers are useful tools for the study of the movement at different ecological levels; they are frequently used as individual identification tags or "barcode" that allow establishing relationships among individuals. They are also useful to determine the origin of migrant populations and characterize dispersal patterns of organisms. In addition, molecular markers provide historical information on migration rates among populations and are used in evolutionary ecology to study the spatial dynamics of genes and their interaction. Molecular markers are useful in mark-release-recapture studies because they provide a measure of dispersion as well as gene flow without affect the external appearance or behavior of individuals (Huettel et al. 1976).

Although the flight ability of the coffee berry borer is partially known, there is not information about its dispersal in space and time. Therefore, the objective of this research was to develop a methodology to describe the movement of *H. hampei* in field conditions, as a tool to study its behavior in different ecological and agronomic coffee growing areas.

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Materials and Methods

This research was based on the mark-release-recapture technique. Thus, in order to identify genetically distinct coffee berry borer beetles in Colombia, we evaluated the microsatellite molecular marker HHK. 1.6, designed by Gauthier and Rasplus (2004) from Ethiopian populations of *H. hampei*, on Colombian populations composed of individuals from more than 80 municipalities. Subsequently, we designed, developed and tested a device for releasing coffee berry borer adults. A release-recapture technique was evaluated under field conditions at the experimental station of Cenicafé "La Catalina", located in the municipality of Pereira-Risaralda, using the coffee berry borer polymorphic population and the releasing device. In this exploratory research, we established as a conjecture that it was possible to differentiate genetically distinct populations in Colombia to develop a mark-release-recapture method to measure the movement of the coffee berry borer in field conditions. To confirm this, we proceeded with the following methodology and analysis:

Allele Variation of the Microsatellite HHK.1.6 in Colombian Coffee Berry Borer Populations

The microsatellite molecular marker HHK.1.6 was synthesized (Gauthier and Rasplus 2004), and used for the amplification of over 100 DNA samples from coffee berry borer adults from more than 80 municipalities in Colombia, in the Entomology laboratory at the National Center for Coffee Research (Centro Nacional de Investigaciones de Café, Cenicafé). Subsequently, 100 coffee berry borer beetles were field collected at "La Catalina" Experimental Station, in order to determine the genotype of the borer beetles there present, so the absence of the marked populations could allow the monitoring of the genetically different individuals released in a mark-release-recapture strategy.

The amplification of the microsatellite HHK. 1.6 loci was performed in a final volume of 20 µl; each reaction contained 4 μl of DNA ($\sim$20 ηg), 0.2 μl of the "forward + reverse" (CGGCACGAATAATCCCTAC + CCTGAATTATCGACGTCGG, respectively) primers (0.5 μM each), 1.6 μl of 0.2 mM dNTP, 4 μl of 1 X buffer, 1.2 μl of 1.5 mM $MgCl_2$, 0.1 μl of Taq DNA polymerase (0.5 U) (Promega), and 8.9 μl of autoclaved MilliQ water. The amplification conditions were as follows: an initial denaturation for 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 30 s at 52°C, and 45 s at 72°C, followed by 5 min at 72°C for the final extension. After the amplification, the DNA banding pattern for each individual was visualized using 4% polyacrylamide gel electrophoresis.

Design, Development and Evaluation of a Device for Releasing Coffee Berry Borer Adults

We developed a device to release coffee berry borer adults and to ensure that the individuals flew simultaneously. The main components of the device were temperature to activate the coffee berry borer adults and wind to blowing out the coffee berry borer adults (Fig. 1). The device efficiency was evaluated by measuring the average percentage number of borer beetles that flew at different times (8:00 h, 11:00 h, 14:00 h and 17:00 h) after releasing 10 groups containing 100 individuals.

Releasing and Recapturing Marked Coffee Berry Borer Populations

Once identified the genetic differences among coffee berry borer beetles in Colombia and those present at "La Catalina" Cenicafé Experimental Station, the movement of *H. hampei* was evaluated at different times and distances, after releasing the same time and in single point 5,000 adults of a polymorphic population, at 14 h, in an epicenter of a coffee crop. This bioassay was carried out between October 2012 and February 2013, the experimental area contained 2,353 coffee trees (*Coffea arabica* var. Colombia) distributed over an area of $\sim$6,644 m^2 (Fig. 2). All coffee trees of the experimental plot were harvested to remove borer beetle infested fruits and reduce their initial population to a minimum. Besides, a synthetic mesh fence to a height of 8 m was erected in order to avoid at most the entry of coffee berry borer insects to the experimental plot from surrounding coffee plantations.

The polymorphic borer beetles used in this study were reared on artificial diet following the methodology described by Portilla (1999). We selected 5,000 most active females from a bulk of 10,000, recognized as those that actively walked toward an artificial light source.

The recapture of the released marked borer beetle population, 24 h after the release, in each quadrants of the experimental plot (north, south, east and west), at 16 distances within a radius of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, and 80 m. We counted the number of coffee berries bored by the coffee berry borer beetles in each tree. The infested berries were then marked using white indelible ink. We repeated the same procedure at 5, 15, 30, 45, 60, 75, and 90 d after the release (sampling times), marking with a different indelible ink color at each time. In order to confirm that the recaptured populations were the marked released, 95 d after, in each quadrant, we collected 100 coffee berry borer infested coffee fruits (first and second generation) and 20 *H. hampei* individuals from "La Catalina" Cenicafé Experimental Station as control population. A coffee berry borer adult was extracted from each infested coffee berry collected and genomic DNA was isolated. The allele of interest, using the microsatellite molecular marker HHK.1.6, was amplified following the methodology described earlier to confirm the marked population.

Estimation of the Coffee Berry Borer Flight Distance

Using the data obtained from the recapture experiments, we estimated the flight distance of coffee berry borers during the 30 d following the release.

Estimation of the Mathematical Expression that Describes the Movement of the Coffee Berry Borer in Space Over Time

The expression selection was based on the following criteria: the function that presents the lowest mean square error, in which the model and each of the coefficients would be significant ($P < 0.05$), and that the residuals followed a normal distribution. For the analyses, the data were separated as follows:

1. Average per tree of recaptured marked borer beetles, from day 1 to day 30, which represents the recapture of the initial released population.
2. Average per tree of recaptured marked borer beetles, from day 45 to day 90, which represents the recapture of the second and third generation of the released population.

Data Analysis. We used descriptive analysis to estimate:

1. The polymorphic population of coffee berry borer in Colombia.
2. The number of samples of coffee berry borers in "La Catalina" Cenicafé Experimental Station containing alternative alleles of polymorphic populations.
3. The means and confidence intervals for the percentage of *H. hampei* adults that flew using the releasing device at each assessed time.
4. The proportion and confidence intervals (95%) of fruits containing borer beetles with presence of a polymorphic allele in the released population.
5. The average number of borer beetles recaptured by sampling date and distance per tree and their standard error.
6. The adjustment of the mathematical expression that best describes the coffee berry borer movement in space over time.

Results

Allele Variation of the Microsatellite HHK.1.6 in Colombian Coffee Berry Borer Populations. The microsatellite molecular marker HHK. 1.6 amplified a band of 175 bp in samples of the coffee berry

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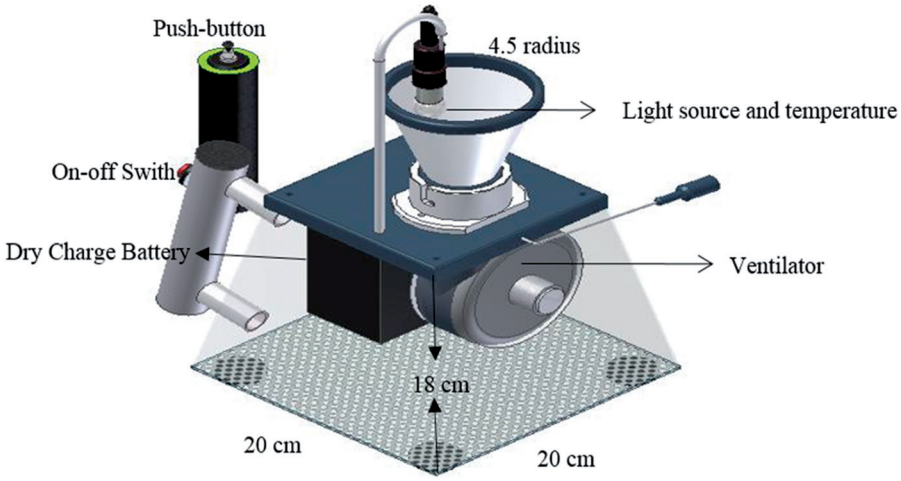

Fig. 1. Device to release coffee berry borer populations.

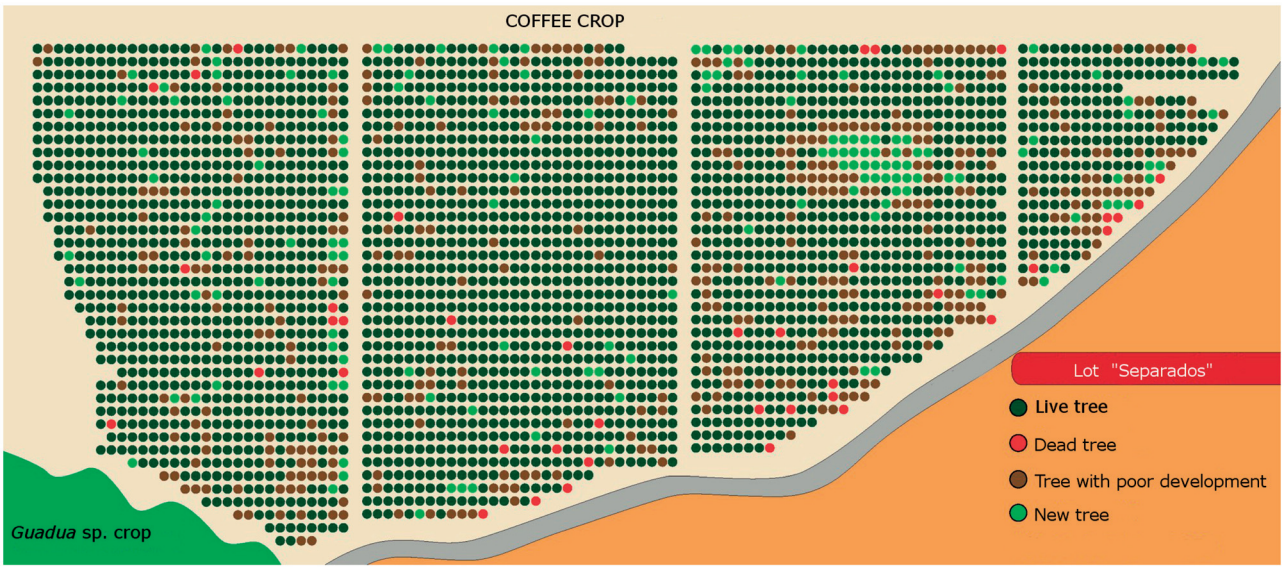

Fig. 2. Schematic representation of the experimental plot at "La Catalina", Cenicafé Experimental Station, used in the process of mark-release-recapture of borer beetles.

borer from Colombia, except those collected in the municipality of Guapotá (Santander), which showed genetic differences amplifying an alternative allele of smaller molecular size, with 173 pb (Fig. 3). With this evidence, the borer beetles of the Guapotá region would be useful as the marked population in a mark-release-recapture strategy as long as this allele was not identified in the existing population in the experimental plot at "La Catalina" Cenicafé Experimental Station. Thus, the genotype of the 100 borer beetles collected in "La Catalina" was the same and did not contain the allele of 173 bp. This indicates, that the alternative allele of the polymorphic population was not present in populations of the borer beetles at the release site. Polymorphism was easily distinguished (Fig. 3).

Design, Development and Evaluation of a Device for Releasing Coffee Berry Borer Adults. The efficiency of the release device was measured by estimating the average percentage of borer beetles that flew in 2 s during four releasing times. The values obtained were between 88.3 and 89.1%. According to Student's *t*-test ($P < 0.05$), the mean values of this variable at the different release times were statistically similar. Thus, the environmental conditions implicit at each time did not affect the insect flight. Therefore, we conclude that the device allows the fly of 88.8% of the insects, with a confidence interval of 2.4 and a coefficient of 95%. This device ensures a homogeneous release of coffee berry borer adults from a central point, as a basic condition to assess the movement in field conditions.

Releasing and Recapturing Marked Coffee Berry Borer Populations. The environmental conditions on the day of the release of the 5,000 polymorphic coffee berry borer adults from Guapotá were: average temperature 21.8°C, relative humidity 77%, rainfall 0.4 mm, radiation of 8.7 h/d and wind speed 1.67 m/sec.

To confirm that the released marked insects were in fact the target population, we amplified the allele fragment that recognizes the population from Guapotá. The percentage of individuals recaptured containing the 173 bp alternative allele on 100 borer beetles in each quadrant of the experimental plot was obtained. The proportion was between 68.1 and 100% across distances (Table 1); therefore, the foundation of this study, which was to recapture the released populations, was pursued. These results recommend the use of coffee fruits as traps, in studies involving parameters of movement and dispersal of the coffee berry borer, since they do not affect the behavior of the insect.

Estimation of the Coffee Berry Borer Flight Distance. The flight distance of the coffee berry borer was evaluated through the bored coffee berries after the release. It was noted that the coffee berry borer flew

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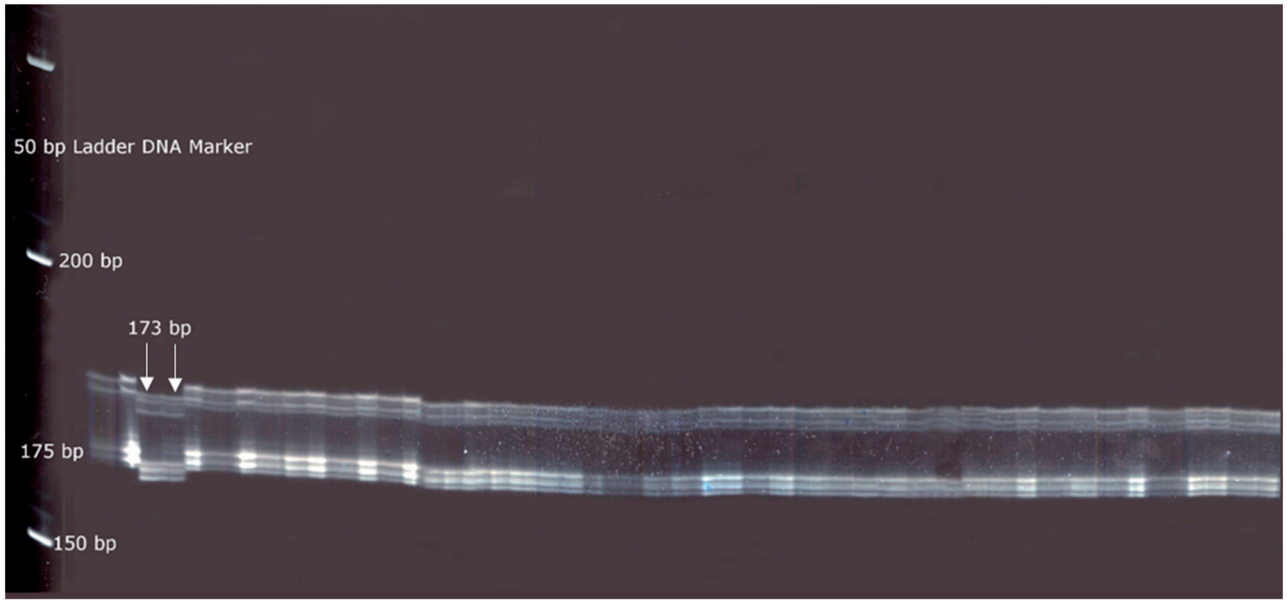

Fig. 3. Polyacrylamide gel fragment showing allelic variation of the microsatellite molecular marker HHK.1.6, amplified on 100 samples of *Hypothenemus hampei* from "La Catalina" Cenicafé Experimental Station. The amplification of the allele shows a 175 bp band in the samples La Catalina, compared with the control population, Guapotá, which amplified a smallest alternative allele of 173 bp (lanes 3 and 4).

Table 1. Percentage and confidence limits of coffee berry borer adults containing the 173 pb alternative allele of the Guapotá population in each evaluated distance, 95 d after the release, which contain first and second generation of berry borer

Distance (m)	Percentage	Lower limit*	Upper limit*
5	97.2	91.7	100
10	94.4	86.7	100
15	94.4	86.7	100
20	94.4	86.7	100
25	94.4	86.7	100
30	86.1	74.4	97.8
40	88.9	78.3	99.5
45	83.8	71.5	96.1
50	87.0	72.6	100
55	93.1	83.5	100
60	83.8	71.5	96.1
65	78.5	66.2	85.3

*Confidence coefficient of 95%.

Table 2. Number of borer beetles recaptured in north, south east and west quadrants, in 16 distances and up to 30 d after the release of 5,000 marked borer beetles

Distance (m)	Quadrant															
	North				South				East				West			
	Sampling times (days)															
	1	5	15	30	1	5	15	30	1	5	15	30	1	5	15	30
5	7	44	0	1	43	47	0	0	15	53	0	0	0	6	0	0
10	12	107	0	0	20	48	0	0	25	68	0	0	0	2	0	0
15	2	55	1	0	19	57	0	0	21	59	0	0	0	27	0	0
20	0	57	0	0	4	31	0	0	1	34	0	0	0	9	0	0
25	3	54	3	3	0	18	0	0	2	40	0	0	0	4	0	0
30	2	44	1	1	2	32	0	0	3	29	0	0	0	6	0	0
40	3	37	0	0	0	12	0	0	1	19	0	0	0	16	0	0
45	0	26	0	2	0	0	0	0	1	5	0	0	0	8	0	0
50	0	27	1	0	0	0	0	0	0	0	0	0	0	2	0	0
55	0	22	2	2	0	0	0	0	0	0	0	0	0	0	0	0
60	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

up to 65 m, recorded in the northern quadrant on the fifth day of the evaluation (Table 2). Interestingly, borer beetles were observed colonizing coffee fruits after day 5, only in the north quadrant, where the largest recaptures were recorded. The average of recaptured borer beetles per tree at 5 m from the releasing point was ~50% higher than to 10 m (Table 3).

Estimation of the Mathematical Expression that Describes the Movement of the Coffee Berry Borer in Space Over Time. For the data adjustment, the average number of recaptured borer beetles as a function of the distance at each of the times, the following models were evaluated: The function of the Taylor relation as $y = e^{(a+bX^c)}$, where c is the density change rate with distance, a is the sample size, and b is the scale factor of the measurement units for the distance (Taylor 1980). The density function of $V(X) = (\alpha + \beta x^{\phi})^{-1/\vartheta}$ was also assessed, where $x, \alpha, \beta, \phi, \vartheta > 0$ y ϕ, ϑ were parameters. Thus, the decreasing exponential function of $\hat{Y} = \alpha\beta^{X_i}$ was evaluated. This function consistently provided a better adjustment to the data in addition to providing reliable parameters to describe the coffee berry borer movement in space over time. However, the data from each study are unique, and a function cannot be generalized because the choice depends on the nature of the data and the researcher interest.

For this study, the expression that best described the average borer beetles recaptured per tree, depending on the distance to the times 30 and 90 d was:

$$\hat{Y} = \alpha\beta^{X_i}$$

Where:

$\hat{Y}$ = Average borer beetles recaptured per tree.
x = Recapture distance in meters.

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Table 3. Average per tree and standard error for the number of recaptured borer beetles per sampling date and distance and up to 30 d after the release of 5,000 marked borer beetles

Distance (m)	Sampling times (days)							
	1		5		15		30	
	Average	Stand. error	Average	Stand. error	Average	Stand. error	Average	Stand. error
5	2.7	0.9	7.4	1.4	7.4	1.4	7.2	1.3
10	1.4	0.1	4.1	0.4	4.1	0.4	4.1	0.4
15	1.4	0.1	2.7	0.2	2.7	0.2	2.7	0.2
20	1.0	0.0	1.8	0.1	1.8	0.1	1.8	0.1
25	1.7	0.3	2.1	0.3	2.1	0.3	2.2	0.3
30	1.4	0.2	1.7	0.2	1.7	0.2	1.7	0.2
40	1.3	0.3	1.7	0.2	1.7	0.2	1.7	0.2
45	1.0	0	1.5	0.2	1.5	0.2	1.6	0.2
50	0	0	1.4	0.2	1.4	0.2	1.4	0.2
55	0	0	1.3	0.1	1.3	0.1	1.4	0.1
60	0	0	1.6	0.4	1.6	0.4	1.6	0.4
65	0	0	1.7	0.3	1.7	0.3	1.7	0.3

Therefore, for the period from day 1 until day 30, the equation was:

$$\hat{Y} = 0{,}94889 * -0{,}04634^{X_i}$$
$$(0{,}31667)\ (0{,}00798)$$
$$R^2 = 0,75$$
$$F1;11 = 33{,}72 \quad P < 0{,}001$$

The values in parentheses represent the standard error of the coefficients.

$\hat{Y}$ = Average borer beetles recaptured per tree.
x = Recapture distance in meters.

The Fig. 4 is a descriptive representation of the average of recaptured borer beetles per tree in relation to the distance and time from day 1 to day 30 (first generation).

The function that describes the behavior of the coffee berry borer movement from day 45 to day 90 (second generation), including the time, can be expressed as:

$$Y_i = \beta_1 X_{2i}^{\beta_2} X_{3i}^{\beta_3} e^{u_i} \quad (1)$$

Where:

Y = Average number of recaptured borer beetles per tree.
X_2 = Recapture distance in meters.
X_3 = Times in days.
U = Stochastic disturbance term.
e = Natural logarithm base.

Transforming Equation (1) to logarithm function produced the following equation:

$$\ln Y_i = \ln \beta_1 + \beta_2 \ln X_{2i} + \beta_3 \ln X_{3i} + u_i$$

Therefore, the model was linearized in the parameters B_1, B_2, and B_3. In this model, B_2 is the rate (partial) of the average recapture of borer beetles with respect to the distance, i.e., it is a measure of the percentage change in the recapture of the borer beetles because of a 1% variation in the distance, with the time remaining constant. Likewise, B_3 is the percentage variation (partial) in the average recapture of borer beetles with respect to time, with the distance remaining constant.

Using the NLIN procedure from the SAS statistical software to solve the problem, the following was obtained:

$$\ln Y_i = -3{,}0996 - 0{,}669 \ln X_{2i} + 1{,}90 \ln X_{3i}$$
$$(0{,}00087)\ (0{,}0508) \quad (0{,}2501)$$
$$R^2 = 0{,}891$$
$$F3;61 = 166{,}21 \quad P < 0{,}001$$

The values in parentheses represent the standard error of the coefficients. In this case, by keeping the time constant, an increase of 1% in distance leads to an average decrease of 0.7% in the recapture of the borer beetles. Similarly, by keeping the distance constant, an increase of 1% in the time implies in an average increase of 1.9% in the recapture of the borer beetles (Fig. 5).

Discussion

The advantages of having a molecular marked populations, which is easily visualized on polyacrylamide gels, is that they are present in the insect for the entire lifetime and are passed throughout several generations. On the contrary, using Day-Glo fluorescent pigments as a method of marking *H. hampei* adults are hard to visualize, are of short duration (up to 5 d) and do not allow assessments over time and for several generations (Acevedo et al. 2009). Monitoring field insect populations that have molecular markers are a rapid method for measuring their dispersal and movement (Steinberg and Jordan 1998), assertion that is confirmed in the present investigation where the field work was completed in a period of only 5 mo.

The recapture of the released marked borer beetle population was possible because the coffee beans were used as traps: *H. hampei* is specific to *Coffea* species, and once the colonizing female begins oviposition, it remains inside the fruit taking care of its offspring. Furthermore, coffee trees produce secondary metabolites that attract borer beetle adults (Bustillo 2006); the behavior of the species ensures the recovery of the released population through the coffee fruits. The method of using the host plant as a trap has also been used to measure the emergence and colonization of insects (Bucher and Cheng 1970). In the case of the Elaterid larvae, they are attracted to the carbon dioxide (CO_2) released during the process of seed germination; the investigators Williams et al. (1992) made use of this behavior to sample populations of this beetle, putting the seeds onto the soil to germinate and subsequently recovering elaterids. Besides, traps containing baits have been used successfully in mark-release-recapture techniques because they constitute a quick and easy method for

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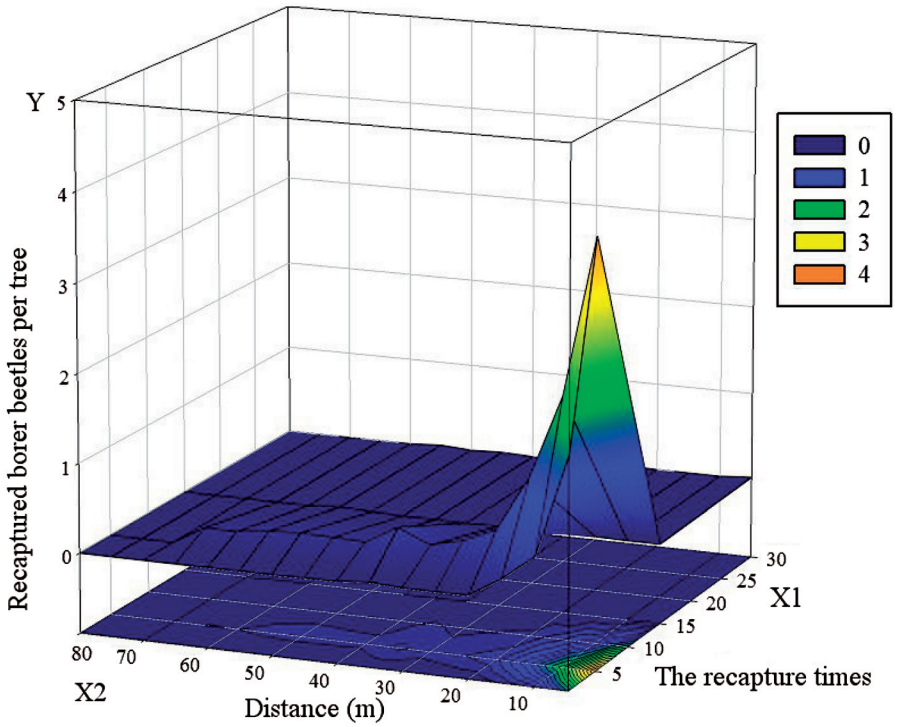

Fig. 4. Descriptive model for the coffee berry borer movement in space over time from day 1 to day 30 (first generation). X_1 represents the recapture times, X_2 represents the different distances of the recaptures from the central point of release, and Y is the average of recaptured borer beetles per tree at different times and distances.

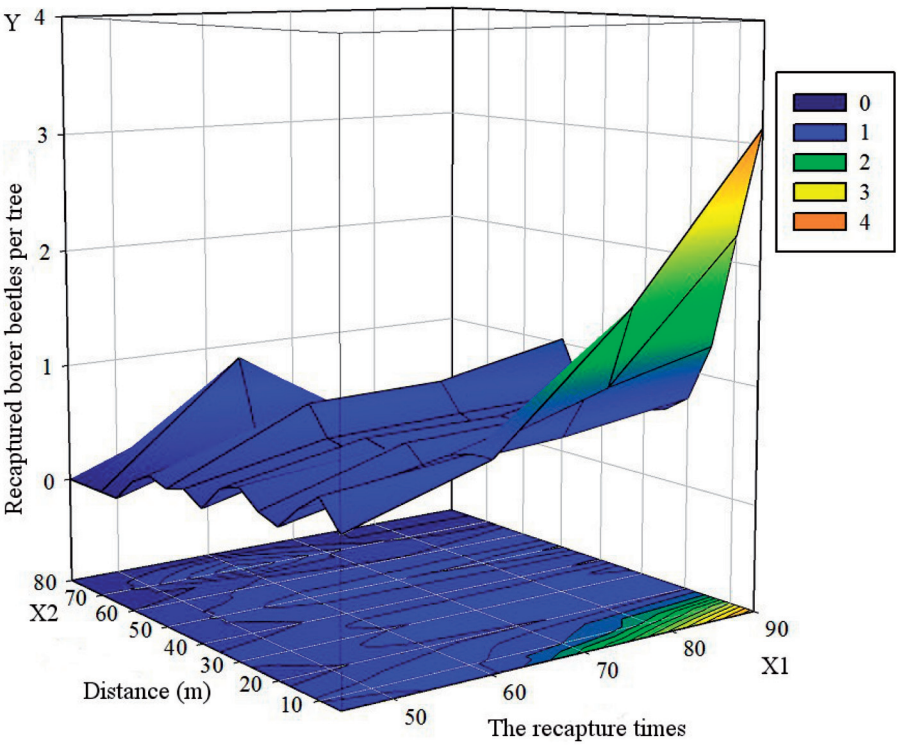

Fig. 5. Descriptive model of the coffee berry borer movement in space over time from day 45 until day 90 (second generation). X_1 represents the recapture times, X_2 represents the different distances of recaptures from the central point of release, and Y is the average number of recaptured borer beetles per tree at different times and distances.

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sampling insects (Reynolds et al. 1997). Nevertheless, using traps for estimating the movement and dispersal of organisms poses a dilemma because the traps intercept the flight of insects and stop their dispersal, which affects the actual measurement of the movement. Moreover, the effect of traps is short-term and can change the behavior of insects because the response is inherent to the traps and baits.

Although the coffee berry borer adults reached distances of up to 65 m, the data demonstrated that the insects tended to bore fruits located <40 m from the epicenter, and the largest number of recaptured borer beetles was within 5 and 10 m. This observation indicates that the coffee berry borer flies little in a productive coffee crop and its movement ability is restricted to the local area; perhaps because the food is guaranteed and volatile emissions from coffee plants and fruits quickly attract insects. These data are consistent with a report by Castaño et al. (2005) in which the pruning effect of neighboring coffee plants was evaluated. It was determined that the insect had a greater influence on the 15 m adjacent to the eliminated coffee plantation; however, the greatest influence on coffee berry borer infestation levels was noticeable in the first four rows. According to Alonzo (1984), the flight of coffee berry borer adult females (the only individuals with this capability) is reduced to a few meters unless they take advantage of air currents. Taking into consideration the time that a coffee berry borer adult can fly, the hilly geographic location of Colombian coffee growing areas and the number of adults flying from coffee crops pruned each year, we may assume a dispersion at large distances given speed conditions and wind direction (Benavides 2010).

To prevent the dispersal of the polymorphic population in the central Colombian coffee zone and ensure their use in subsequent investigations, at the end of the experimental phase of this study at "La Catalina" Cenicafe Experimental Station, we performed integrated pest management practices for the coffee berry borer on the released population.

This is the first work with coffee berry borer where mark-release-recapture strategy and molecular markers were combined; also, it is the first field experiment with this insect where the host (coffee beans) are used as a trap, in contrast to other studies using semiochemicals as traps (Zolubas and Byers 1995, Byrne et al. 1996, Byers 1999, Acevedo et al. 2009).

The results obtained here on the dispersion of the coffee berry borer *H. hampei* (Ferrari) (Coleoptera: Curculionidae) allow us to conclude:

1. The alternative allele 173 bp present in the populations of coffee berry borer from Guapotá (Santander—Colombia), and absent in the populations of the central Colombian coffee area, are useful for ecological studies of the coffee berry borer, over time and for several generations of the insect. However, the fertility and aggressiveness of this polymorphic coffee berry borer populations should not overpass that of the existing population.
2. It was confirmed that coffee berry borer does not disperse long distances in a productive coffee plantation since *H. hampei* individuals do not need to fly long distances in order to obtain food. Therefore, control strategies for this insect and monitoring with traps should take into account the distances and times for the coffee berry borer flights reported in this study.
3. The methodology presented here can be used in future research to determine; (i) the parameters of the distribution of populations of coffee berry borer in space and time, (ii) the movement of populations of *H. hampei* in different agronomical and ecological conditions of coffee crops, (iii) the dispersion of pesticide resistant genes, (iv) more accurate models to make real predictions of pest pressure on coffee crops, and (v) determine the effects of climate variability on insect populations.

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