Trends in Entomology
Vol. 14, 2018

Original Article

Push-pull strategy for handling the coffee berry borer

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ABSTRACT

Push-pull strategies for controlling pests have been developed for use in several different agroecosystems. The push-pull strategy involves the use of both repellent plants within crops to ward off pests and attraction plants at the edge of crops. Studies have demonstrated the repellent action of both *Nicotiana tabacum* L. (Solanaceae) and *Lantana camara* L. (Verbenaceae) against the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) and the attraction of *Emilia sonchifolia* L. (Asteraceae) for CBB. To manipulate the distribution and abundance of CBB, a push-pull strategy was applied to coffee plantations, and the field performance of this strategy was examined. The results showed that the push-pull strategy was effective, because the action of the repellent plants combined with the effects of the alcohol traps reduced the CBB infestation to 5.2%. This is the first time a push-pull system in coffee crops has been evaluated worldwide. The results indicate that the push-pull strategy for use in the agroecological management of CBB is promising.

KEYWORDS: *Lantana camara*, *Nicotiana tabacum*, *Emilia sonchifolia*, agroecology insect management.

INTRODUCTION

Several studies have investigated the involvement and incorporation of plant functional diversity within coffee plantations worldwide as a strategy to protect the soil, mitigate the effects of climate change, and increase the profitability of farms [1-7]. Few studies have related the functional advantages of the establishment of canopy or cover crops within coffee plantations infested with pests, particularly the coffee berry borer (CBB) *Hypothenemus hampei* [8-10].

One of the agroecological strategies used for pest management involves the introduction of functional diversity or optimization aimed at establishing regulatory relations [11]. When this introduction of diversity occurs between different plant families, it disrupts the monoculture and can provide adaptive benefits in the regulation of pests for both plant families. Among these beneficial relationships is a decrease in the concentration of resources, making the main crop less apparent to the pests, either because the introduced species creates a physical barrier or because it produces allelopathic signals that disrupt the mechanism governing the attraction of the resources for the pests [12]. The introduced species can also provide food and shelter for natural enemies [13]. Although various strategies exist for incorporating diversity into a main crop, the most appropriate strategy will depend on the functionality of the plants, the type of pest, and the conditions of the production system.

To take advantage of the insect repellency and attraction functions exerted by certain plants, a push-pull strategy has been used in some crops by manipulating the distribution and abundance of pests and natural enemies *via* a crop association design [14-20]. In this strategy, push plants are planted among the main crop plants to ward off

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pests, and pull plants are planted at the edge of the crop to attract the pests. In addition, both plants serve as hosts for beneficial insects that help reduce pest populations. This strategy has been used in different integrated pest management (IPM) programs in rice [21], sorghum, and millet [22] crops to maximize pest control and minimize environmental impact.

Knowledge of the biology of the insect pest and the chemical ecology of the interaction between a host and its natural enemies is required to apply a push-pull strategy. Previous olfactory studies on the interactions between coffee plants and the CBB identified *Nicotiana tabacum* (Solanaceae) and *Lantana camara* (Verbenaceae) as insect repellents, whereas *Emilia sonchifolia* (Compositae) was identified as an attractant. Controlled field trials corroborated the repellency of *N. tabacum* and *L. camara* to the CBB; these plant species released volatile sesquiterpenes, particularly β-caryophyllene and (E)-β-farnesene, into the environment, which were effective against CBB [23]. In addition, bubble caps of (E,E)-α-farnesene installed on coffee branches were shown to be potential repellents for CBB [24]. *Emilia sonchifolia* was selected as an attractant because it emits alcohols that attract insects, and this species was also shown to act as an insect attractant under laboratory conditions.

The objective of this research is to determine the repellent effects of *N. tabacum* and *L. camara* plants and the attraction effects of *E. sonchifolia* plants as part of a push-pull strategy in an open field.

MATERIALS AND METHODS

The experiment was carried out on a one-hectare lot during the third year of production of a sun-exposed monoculture system of *Coffea arabica* var. Castillo; the plants were spaced a distance of 2.0 m x 1.0 m and were treated under conventional management.

This coffee crop was located at the Naranjal Experimental Station of Cenicafé (Chinchiná - Caldas, Colombia) at an elevation of 1,381 m; the mean temperature was 21.4 °C, and the mean relative humidity was 68%.

As an experimental unit, each plot comprised 150 coffee plants that were distributed in a rectangle that consisted of 15 x 10 plants. Each plot was divided into two parts: the edge and the interior. The edge consisted of 48 coffee plants; a rectangle comprising 13 x 8 plants was left in the inside of the plot to separate the edge plants from the interior ones. The interior comprised 66 coffee plants.

Three treatments were established in the plots. Treatment 1 was the absolute control, which consisted of 150 coffee plants without any accompanying repellent or attraction plants. In treatment 2, a line of *E. sonchifolia* attraction plants was planted on the outside of the edge coffee plants and repellent plants (30 *L. camara* and 30 N. *tabacum* plants) accompanied the 66 coffee plants that comprised the inner rectangle. Treatment 3 was the attraction control, in which the *E. sonchifolia* plants were substituted with bags that contained 25 ml of a mixture of ethanol and methanol alcohol (3:1); these bags were attached to the trunk of every other coffee plant at a height of 1.3 m, and the interior was identical to that in treatment 2. Figure 1 shows a schematic of the treatment design, and Figure 2 shows the arrangement of the plants within the coffee plantation.

L. camara was propagated by stakes, whereas *N. tabacum* was planted from seed. The plants remained in nursery for 3 to 4 months, after which they were transplanted 2 months prior to infestation evaluations in the field. *E. sonchifolia* was propagated by seed, which was sown directly in the experimental plot 2 months before the evaluations. The alcohol bags were replaced each month to ensure the proper release levels of the volatiles.

Each treatment consisted of five replicates in a randomized complete block design. The different levels of infestation by CBB represented the block factor.

The variable of interest was the mean percentage of CBB infestation. To calculate this variable, 40 trees from the edge and 40 trees from the interior were selected, and the most productive branch of each tree was marked for counting the total number of coffee fruits and the number of those infested by CBB. The preliminary field test results showed that the sample size would provide more than 75% confidence.

On the first day of the experiment, the mature fruits from each experimental unit were harvested;

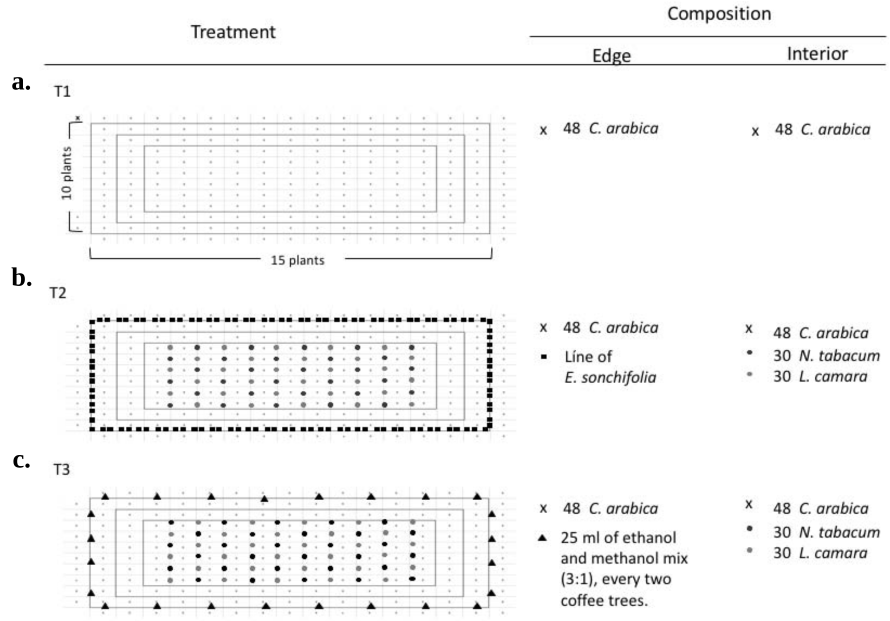

Figure 1. Push-pull plot design and treatments. **a.** T1, absolute control; **b.** T2, attraction plants at the edge and repellent plants in the interior; **c.** T3, alcohol mixture (ethanol:methanol at a 3:1 ratio) at the edge and repellent plants in the interior.

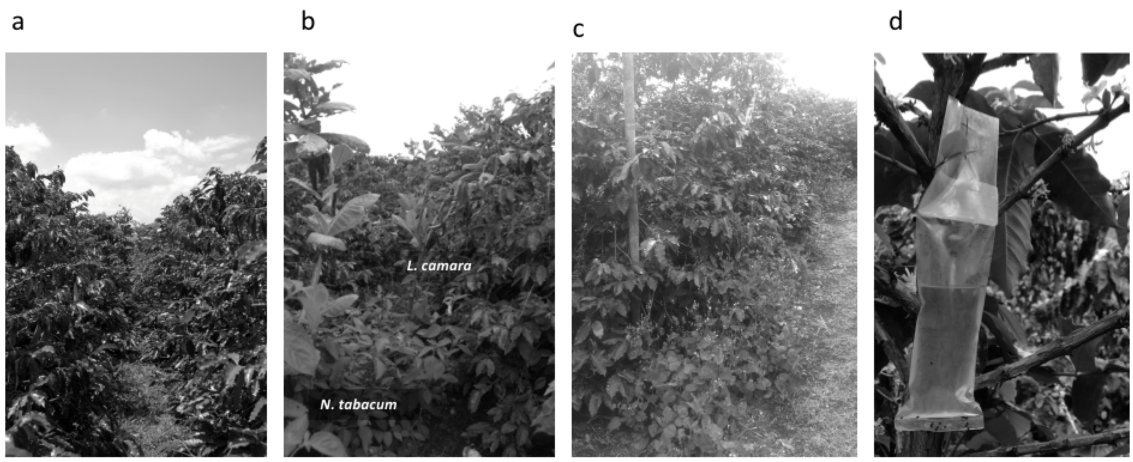

Figure 2. Establishment of push-pull plants in the experimental plot. **a.** Coffee plants in the absolute control treatment 1; **b.** repellent *N. tabacum* and *L. camara* push plants in the interior of the plots of both treatment 2 and treatment 3; **c.** attractant *E. sonchifolia* pull plants at the edge of the coffee plantation in treatment 2; **d.** bag of attractant (pull) alcohols attached to a coffee plant at the edge of treatment 3.

therefore, the CBB infestation levels were obtained from green coffee fruits at the edge and in the interior of each experimental unit. In block 5, all CBB-infested fruits (both mature and green) were removed to start the test at a 0% infestation level.

Every 18-20 days, the mature fruits in each experimental unit were manually harvested, after which the CBB infestation of the green fruits of the productive branch marked on each tree was evaluated. These evaluations occurred for 6 months (from April to September 2016), guaranteeing that fruits from flowering in February 2016) until maturity (for harvest, which began in September 2016) were evaluated. In total, ten evaluations were performed.

During the experiment, no insecticides or herbicides were sprayed, and weeds were manually removed. During each evaluation, the companion plants were examined, and the *L. camara* and *N. tabacum* plants were pruned such that they were not taller than the coffee plants.

Two statistical analyses were performed to evaluate the treatment effects. First, we compared the means of the infestation percentage within the plots, between the different treatments and compared the percentages of the infestations at the edge of the plots, between the different treatments *via* analysis of variance (ANOVA) in conjunction with the Tukey honest significant difference(HSD) multiple comparison test at $P < 0.05$. Second, we evaluated differences in the percentage of infestation between the edge and the interior of the plots for each treatment, using ANOVA and the least significant difference (LSD) test ($P < 0.05$). Additionally, block 5 was analyzed separately, with the statistical analyses being performed using the Duncan test ($P < 0.05$).

RESULTS

The initial mean infestation of the plots was 54%, and for all treatments, this infestation decreased with time because of the dynamics of the CBB and harvesting of the ripe fruits every 18-20 days. The results of the comparisons of the percentages of the CBB infestations at the edges of each treatment over time are presented in Figure 3A. The Tukey HSD test showed significant differences

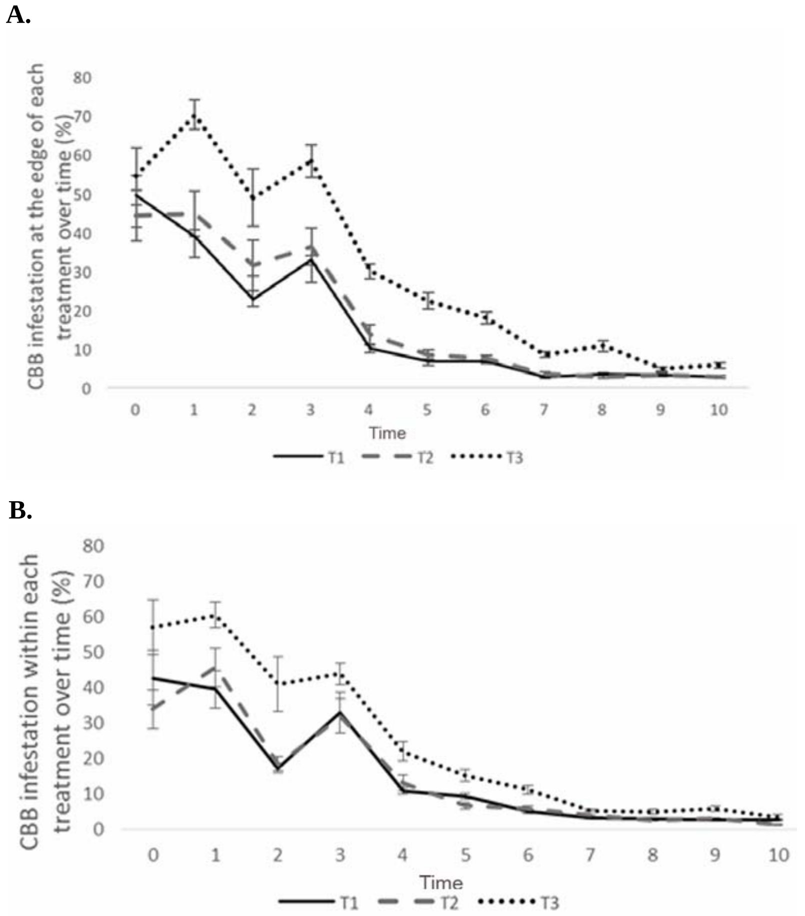

Figure 3. Percentage of CBB infestation **A.** at the edge and **B.** in the interior of each treatment over time. Each evaluation was performed every 15-18 days. T1, control; T2, *E. sonchifolia* at the edge and *N. tabacum* and *L. camara* in the interior; T3, alcohol mixture at the edge and both *N. tabacum* and *L. camara* in the interior. The lines represent the means and SDs of the data.

between the CBB infestations at the edge of treatment 3, which contained a mixture of attractant alcohols, and the edges of the other two treatments throughout the experiment. The results on each evaluation date were statistically analyzed and compared with those on the first evaluation date. Differences were observed 18 days after the bags of the alcohol mixture were placed on the coffee plants at the edge of treatment 3 (F = 7.77, *P* = 0.134); the CBB infestation increased from 54 to 70%. However, in the other two treatments, the infestation was maintained between 40 and 50%. Over time, the infestation decreased in all treatments, but the decrease was much lower on the edge of treatment 3; the infestation in treatment 3 significantly differed from that in treatments 1 (the control) and 2, both of which included *E. sonchifolia*. In evaluations 3 (F = 6.85, *P* = 0.0185), 4 (F = 9.43, *P* = 0.0079), 5 (F = 13.29, *P* = 0.0029), 6 (F = 15.21, *P* = 0.0019), 7 (F = 25.91, *P* = 0.0003), and 8 (F = 6.59, *P* = 0.0204), no significant differences were found between treatment 1 (the control) and treatment 2 with respect to the edges, throughout the experiment.

A comparison of the mean percentage of CBB infestation within each treatment is shown in Figure 3B. Differences between the interior of treatment 3 and that of treatments 1 and 2 were due to the significant increase in infestation in treatment 3 at each evaluation, except for the first one. This increase is attributed to the influence of the alcohol mixture at the edge, in that treatment. No differences were observed between the interior of treatment 2 and that of the control.

The edge and interior within each treatment were compared; the results are shown in Figure 4. In this comparison, a block effect (F = 74.16, *P* = 0.0005) occurred: blocks 5, 3, and 1 differed, and blocks 4 and 2 were grouped together according to their mean percentage of infestation. As shown in Figure 4A, no significant differences were observed between the edge and the interior of treatment 1 (the control), except in evaluation 5 (F = 16.89, *P* = 0.009), in which the interior of the plot had greater infestation values than did the edge, and evaluation 6 (F = 11.82, *P* = 0.0263), in which the edge of the plot had greater infestation values than did the interior.

According to the Tukey HSD analysis of all the blocks, the CBB infestations on the edge (with *E. sonchifolia*) and inside (with *N. tabacum* and *L. camara)* were similar throughout the experiment. Significant differences were observed between the edge and the interior of treatment 2 only in evaluation 2 (F = 73.06, *P* = 0.0005) (Figure 4B).

In treatment 3, significant differences were observed between the edge and the interior in evaluations 3 (F = 8.80, *P* = 0.0413), 4 (F = 45.01, *P* = 0.0026), 5 (F = 16.68, *P* = 0.015), 6 (F = 20.78, *P* = 0.0103), 7 (F = 12.81, *P* = 0.0232), and 8 (F = 8.07, *P* = 0.0468) (Figure 4C). The infestation increased at the edge, which contained the alcohol mixture, between the third and eighth evaluations.

In block 5, at the beginning of the experiment, infested fruits more than 90 days old were collected such that the initial infestation in the lot was 0%. Analysis of the comparisons between the three treatments revealed a block effect throughout the experiment: block 5 differed from the other blocks, both in the comparisons between the edges of the treatments (F = 8.61, *P* = 0.0054) and in the comparisons between the interior regions of the treatments (F = 6.46, *P* = 0.0127). Therefore, this block was statistically analyzed separately.

When comparing the mean percentages of CBB infestation between the edges of the three treatments in block 5, we observed significant differences between treatment 3, which contained the alcohol mixture, and the other two treatments, from the first evaluation (F = 24.61, *P* = 0.0001) until the 8th evaluation, with the exception of the second evaluation (Figure 5A) (Evaluations 3: F = 6.98, *P* = 0.0014; 4: F = 10.27, *P* = 0.0001; 5: F = 7.15, *P* = 0.0012; 6: F = 7.88, *P* = 0.0006; 7: F = 23.61, *P* = 0.0001; and 8: F = 13.94, *P* = 0.0001). These findings corroborate the attraction effect of the alcohol mixture. No significant differences were observed between the control and treatment 2 at any of the evaluation times. As a result, no evidence of *E. sonchifolia* attraction was observed in treatment 2 compared with the control treatment.

In block 5, when comparing treatments in the interior of the plots, we observed significant differences between treatment 2 and treatment 1 (the control) (Figure 5B) (evaluations 3: F = 6.26, *P* = 0.0029; 5: F = 1.77, *P* = 0.1759; 6: F = 2.39,

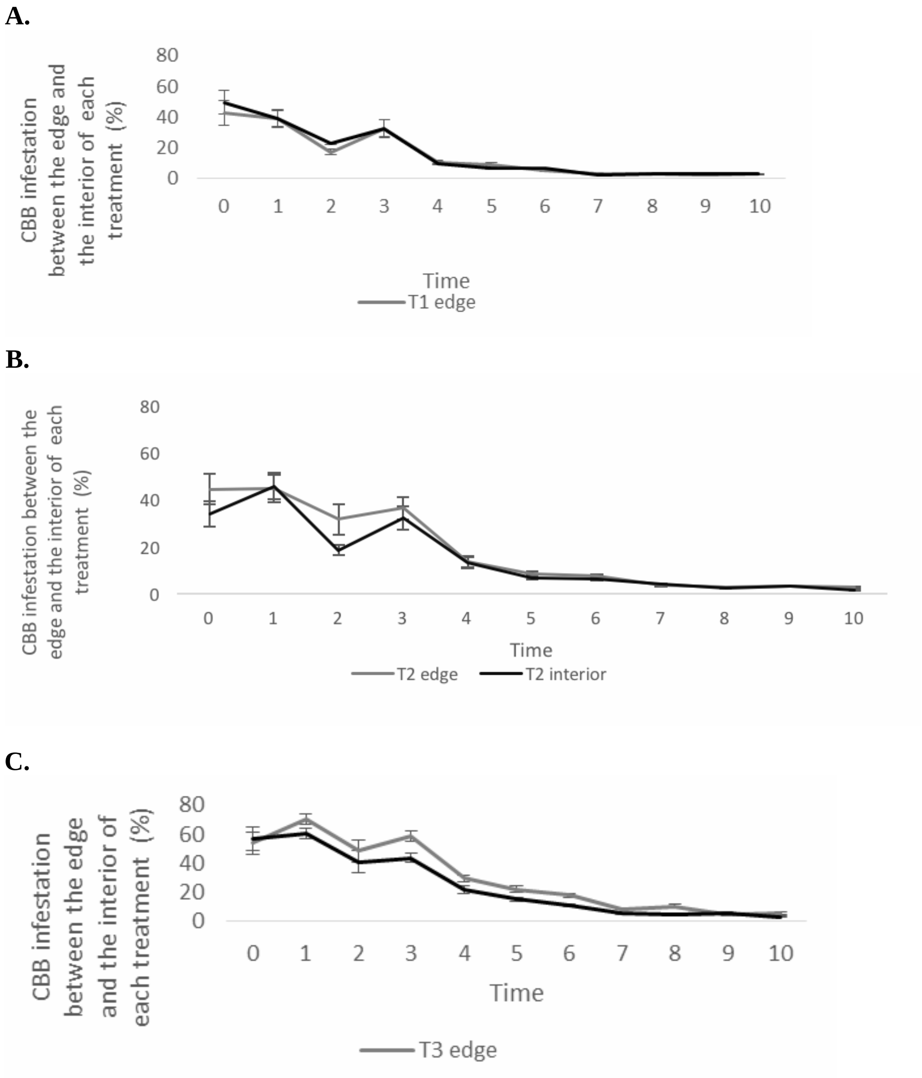

Figure 4. Percentage of CBB infestation at the edge and in the interior of each treatment. **A.** T1, control; **B.** T2, *E. sonchifolia* at the edge and both *N. tabacum* and *L. camara* in the interior; **C.** T3, alcohol mixture at the edge and both *N. tabacum* and *L. camara* in the interior.

P = 0.0975; and 8: F = 7.16, *P* = 0.0013). However, during evaluation 1, a decrease in the percentage of CBB infestation was observed in treatment 2 compared to the control and treatment 3. Compared with that in treatment 1 (the control), the mean reduction in CBB infestation in treatment 2 was 4.9% (the reductions were 4.92, 3.94, 12.33, 3.59, 4.01, and 1.09 for evaluations 1 to 6, respectively), and treatment 2 presented the least infestation throughout the experiment (see the arrow in Figure 5B).

Compared with treatment 1 (the control), treatments 2 and 3 presented less infestation during evaluations 4 (F = 2.38, *P* = 0.0985) and 8 (F = 7.16, *P* = 0.0013), and no differences between treatments 2 and 3 were observed. Compared with treatments 1 (the control) and 2, treatment 3 showed significantly greater infestation and significant differences at evaluations 1 (F = 4.22, *P* = 0.0191) and 7 (F = 5.94, *P* = 0.0038). At evaluation 2, no significant differences were observed between the treatments; however, at evaluation 9, treatments 2 and 3 were more infested than the control was (F = 3.46, *P* = 0.0357).

In general, treatment 3 presented the greatest infestation throughout the experiment. Because

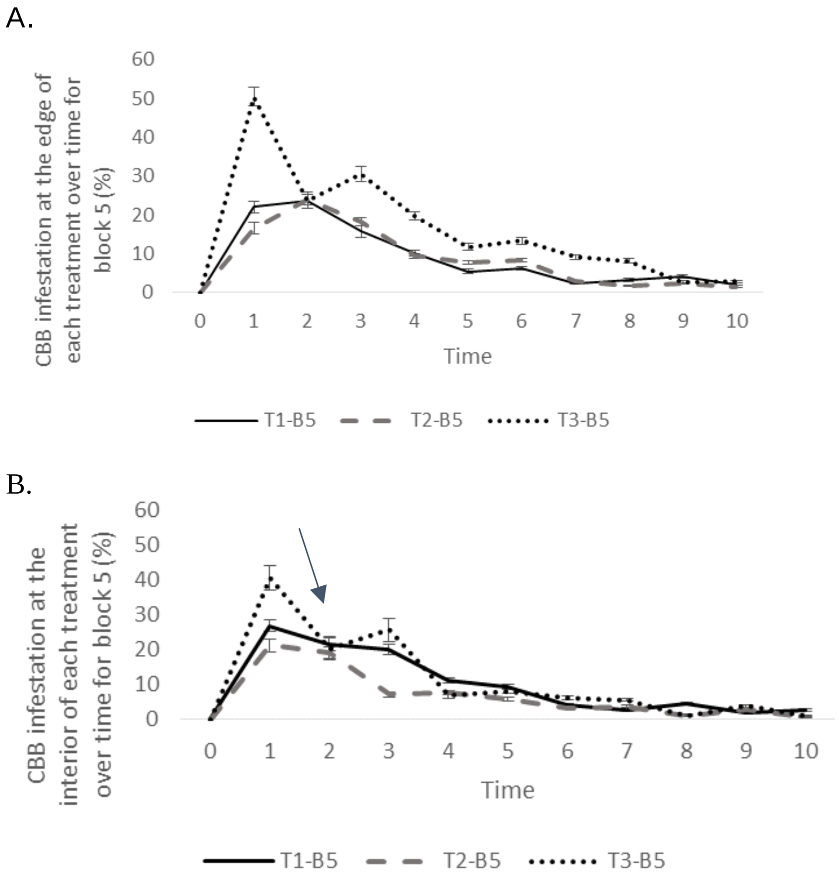

Figure 5. Percentage of CBB infestation in block 5. **A.** at the edge and **B.** within each treatment over time. T1, control; T2, *E. sonchifolia* at the edge and both *N. tabacum* and *L. camara* in the interior; T3, alcohol mixture at the edge and both *N. tabacum* and *L. camara* in the interior.

the accompanying plants within the plots of treatments 2 and 3 were the same, the variation is attributed to the emission of the alcohol mixtures by the traps located at the edge of treatment 3.

The results of the comparisons of the CBB infestations within each treatment at the edge versus the interior of the plots are shown in Figure 6. With respect to treatment 1 (the control), no significant differences were observed between the edge and the interior of the plots except at evaluation 5 (F = 6.01, P = 0.0164), in which the interior was more infested than was the edge, and at evaluation 9 (F = 6.59, P = 0.0121), in which the edge was more infested than was the interior. In general, the infestation was similar across the plot (Figure 6A).

With respect to treatment 2, the LSD test revealed significant differences in infestation reduction between the interior regions and edges of the plots at evaluations 3 (F = 17.15, P = 0.0001) and 6 (F = 15.61, P = 0.0002). The numerical values reflected reduced CBB infestation levels at evaluations 2, 3, 4, 5, and 6, whose differences were 6.22, 10.4, 1.64, 2.36, and 5.34%, respectively. A mean infestation reduction of 5.20% within the interior of the plots compared with the edge of the plots was observed (Figure 6B).

In treatment 3, significant differences in CBB infestation were observed from evaluations 4 to 8, with the exception of evaluation 5 (evaluations 4: F = 14.89, P = 0.0003; 6: F = 8.35, P = 0.0053; 7: F = 3.89, P = 0.0533; and 8: F = 17.01, P = 0.0001). The percentage values of reduction in CBB infestation from evaluations 4 to 8 were 12.0, 3.5, 6.5, 3.6, and 6.2% (Figure 6C). Although the reductions in CBB infestation were higher

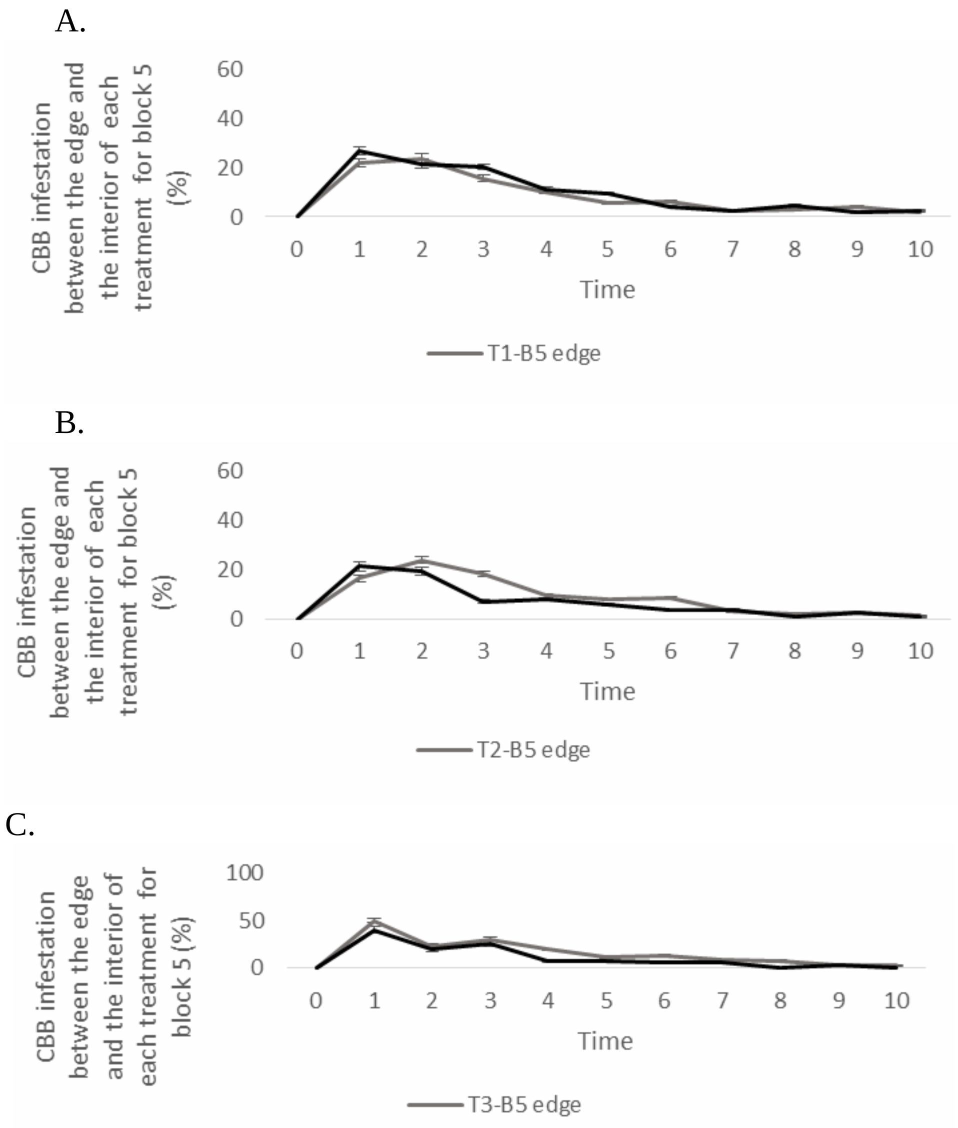

Figure 6. Percentage of CBB infestation in block 5 at the edge and interior of each treatment over time. **A.** T1, control; **B.** T2, *E. sonchifolia* at the edge and both *N. tabacum* and *L. camara* in the interior; **C.** T3, alcohol mixture at the edge and both *N. tabacum* and *L. camara* in the interior.

in this treatment than in the other treatments, both the edge and the interior of the plots presented greater infestation levels than did the other treatments.

DISCUSSION

During the establishment of this experiment, the climatic conditions at Naranjal Station during January 2016 included a mean temperature of 23.4 °C, a minimum temperature of 16.2 °C, a maximum temperature of 31.0 °C, and a mean relative humidity of 74.0%, with a minimum humidity of 39.9% and a maximum humidity of 100% [25]. These conditions coincide with the El Niño phenomenon, which progressed from mid-2014 until the first quarter of 2016, facilitating high CBB populations and a high percentage of CBB infestation [26]. At the whole-plot level, the initial mean infestation was 54%; this infestation decreased over time because of the insect dynamics and the harvesting of the mature fruits every 18-20 days; however, no differences were observed among the treatments. Nevertheless, in block 5, the initial infestation was reduced to 0%, and the repellent effect of the *N. tabacum* and *L. camara* plants was evident in this block. For this type of push-pull strategy to be effective in coffee production, we suggest using a CBB-free coffee plantation when the repellent plants are introduced. Therefore, establishment of companion plants before the formation of the coffee berries and the second harvest is important so that the CBB can be repelled before arrival instead of being forced out of a patch in which it is already established; in other words, the push-pull management approach of the CBB should be preventative.

The inclusion of the repellent plants contributed to a reduction in CBB infestation during the critical period in coffee production, which occurred from the second evaluation until the sixth evaluation. The evaluated plants flowered at the end of January 2016, so the duration of fruit development was known; the first CBB infestation evaluation was performed in April 2016 when the fruits were 80 days old on average. At the second evaluation, which occurred at 120 days after flowering, the fruits had entered the critical insect attacking period during which the CBB could successfully infest and produce offspring inside the berries.

We expected that the mean percentages of CBB infestation in treatments 2 and 3, both of which contained *N. tabacum* and *L. camara* plants, would be similar but that the values would be lower than those of the control; however, in treatment 3, the effect of the alcohol mixture was so effective at increasing the infestation at the edge that it seemed to attract the CBB regardless of whether the repellent plants were present in the interior of the plots. Therefore, the infestation both at the edge and in the interior exceeded that in treatment 1 (the control) and treatment 2. The results suggest that, although the use of the alcohol mixture within a push-pull management program may be a promising approach to guide the attraction of CBB out of the interior of the coffee crops, particularly in the coffee-producing region of Colombia where the CBB is concentrated, understanding of the level of attraction of the alcohol bags will be needed, as this attraction information is pertinent if studies to determine the amount and distance necessary for alcohols to attract the CBB are to be carried out without affecting the interior of the coffee crop. Studies of this type have been proposed for the management of coleopteran pests. For example, [27] evaluated traps with alcohols for the mass capture of the exotic *Ambrosia* sp. (Coleoptera: Scolytinae) beetles in ornamental trees at the nursery production stage.

The *E. sonchifolia* plants at the edge of treatment 2 had no attraction effect. This result could be due to the amount of attraction plants relative to the number coffee plants; that is, the concentration of volatiles in a single row may have been insufficient to attract the CBB away from the coffee plants.

Olfactometry tests have revealed that *N. tabacum* and *L. camara* produce CBB repellents, and *L. camara* presents high contents of β-farnesene [23]. An isomer of β-farnesene is involved in the repellency of CBB [25]. Although statistically controlled field tests have revealed effects for only *N. tabacum* [23], the use of both plants might be important because of increased complementary or redundant functional diversity. Combinations of the volatile compounds emitted by these two plants mitigate resistance to pests in general because of their multigenic nature and avoid any selection pressure generated by each component separately, such as that which occurs when only one plant is used [28]. In fact, climatic conditions during this experiment affected the survival of *N. tabacum*; during drought, the plants of this species were attacked by lepidopterans, and during rain, they suffered from stem rot.

At the beginning of the experiment, the ratio between *L. camara* and *N. tabacum* was 50%. This relationship was not maintained throughout the experiment, and in the end, the high proportion of *N. tabacum* was replaced with a high proportion of *L. camara*. Therefore, the CBB infestation data from coffee plants near empty spaces were not included in the analyses.

The results of this study suggest that an agroecological strategy for the control of the CBB *via* the use of repellent plants that accompany crop plants is promising.

CONCLUSIONS

Here, we demonstrated the repellent effect of a set of plants (*N. tabacum* and *L. camara*) accompanying a crop that initially had zero percent infestation, whose ripe fruits were harvested every 18-20 days.

No attraction effect of *E. sonchifolia* was observed, but the attraction effect of an alcohol mixture commonly used in Colombian coffee cultivation for CBB infestation diagnosis was corroborated. This study was the first to evaluate the use of a push-pull system in coffee production. Taken together, the results suggest that the push-pull strategy is a promising alternative method for the agroecological management of the CBB.

ACKNOWLEDGEMENTS

The authors thank Dr. Ruben Medina for his advice on the statistics and thank the laboratory team and field workers from the Cenicafé and Naranjal Experimental Research Station for their support in the establishment and development of this experiment. This research was funded by Colciencias Grant 567 and the Cenicafé-FNC.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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