Efficacy of *Cotesia flavipes* (Hymenoptera: Braconidae) in reducing *Diatraea tabernella* (Lepidoptera: Crambidae) injury in sugar cane

Belliney Arboleda[1], *and Germán Vargas*[2,*]

Abstract

Sugar cane stem borers, *Diatraea* spp. (Lepidoptera: Crambidae), are the most important pests affecting sugar cane in Colombia. To date, the use of egg parasitoids such as *Trichogramma exiguum* Pinto & Platner (Hymenoptera: Trichogrammatidae), and larval parasitoids such as *Billaea claripalpis* Wulp and *Lydella minense* Townsend (Diptera: Tachinidae) have been the principal biological control approaches for pest management. However, a pest outbreak of *Diatraea tabernella* Dyar in the northern Cauca River Valley demonstrated that conventional control measures are insufficient, and that new pest control methods must be sought. Field evaluations were made using 2 sources of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae): a commercial colony maintained in the laboratory, and a colony recovered from previous field releases (field-refreshed). Three releases of the parasitoid were made, each release consisting of 4 g of *C. flavipes* cocoons (about 4,000 wasps) per ha. The results from both sources of *C. flavipes* were compared with check plots where no releases were made. Larvae of *D. tabernella* were collected 2 different times (45 d and 75 d after the first release) and observed in laboratory. The proportion of larval parasitism ranged between 0.32 and 0.55, with no significant differences between sources of *C. flavipes*. Parasitism differed significantly from the check plots, where the proportion of larval parasitism was less than 0.1. Our results indicate that wasps from the check plots experienced an increase in the number of cocoons and wasps per parasitized larva between the first and the second larval collection. The high levels of parasitism in fields where *C. flavipes* was released resulted in a reduction of up to 65% in the percentage of bored internodes, demonstrating the potential of this natural enemy to effectively control *D. tabernella*. Changes in the number of progeny per parasitized larvae (cocoons and wasps) in the check plots can be explained as the increase of parasitoids in an area under the influence of nearby releases, and the subsequent effects of multiple parasitism. In addition, comparisons between the 2 parasitoid sources indicate higher biological efficiency in the field-refreshed plots expressed in an increase in adult longevity between the first and second collection times.

Key Words: biological control; stem borers; *Lydella minense*

Resumen

Los barrenadores del tallo, *Diatraea* spp., constituyen la plaga de mayor importancia en el cultivo de la caña de azúcar en Colombia. Hasta la fecha, el uso de parasitoides de huevo, como *Trichogramma exiguum* Pinto & Platner (Hymenoptera: Trichogrammatidae), y de larvas, como *Billaea claripalpis* Wulp y *Lydella minense* Townsend (Diptera: Tachinidae), ha sido el principal enfoque de control biológico en el manejo de esta plaga. Sin embargo, un brote de *Diatraea tabernella* Dyar (Lepidoptera: Crambidae) en el norte del valle del río Cauca ha evidenciado que las medidas tradicionales de control no son suficientes y que es necesario buscar nuevas alternativas de manejo. Se realizaron, por lo tanto, evaluaciones de campo utilizando dos fuentes de la avispa *Cotesia flavipes* Cameron (Hymenoptera: Braconidae): una colonia comercial criada en el laboratorio y otra colonia proveniente de material recuperado en el campo. Ambas se compararon con lotes de caña donde no se realizaron liberaciones (testigo). Se realizaron tres liberaciones del parasitoide, cada liberación consistente en 4 g de cocones de *C. flavipes* (acerca de 4,000 avispas) por hectárea. Se hicieron recolecciones de larvas en campo, a los 45 y 75 d luego de la primera liberación, que fueron observadas en condiciones de laboratorio. La proporción de larvas parasitadas varió entre 0.32 y 0.55, sin diferencias significativas entre fuentes de *C. flavipes*, pero sí se presentaron diferencias con las parcelas testigo donde la proporción del parasitismo no superó el 0.1. Los resultados indican que las avispas de las parcelas testigo presentaron un incremento en el número cocones y de avispas por larva entre la primera y segunda recolección. Los altos niveles de parasitismo en campos donde *C. flavipes* fue liberada estuvieron en concordancia con una reducción hasta del 65% del porcentaje de entrenudos barrenados y constituyen evidencia del potencial de este enemigo natural para controlar eficazmente *D. tabernella* en caña de azúcar. Los cambios en el número de cocones y de avispas por larva pueden ser explicados desde el incremento de parasitoides en un área bajo la influencia de liberaciones cercanas y el subsecuente efecto de parasitismo múltiple en la misma larva. Adicionalmente las comparaciones entre las fuentes del parasitoide indican una mayor eficiencia biológica de la fuente recuperada de campo debido al incremento en la longevidad de los adultos obtenidos entre la primera y la segunda recolección.

Palabras Claves: control biológico; barrenadores del tallo; *Lydella minense*

[1]Laboratorios Biocol S.A.S., Cra 8 #5-49, La Victoria, Valle del Cauca, Colombia; E-mail: b.arboleda@laboratoriosbiocol.com (B. A.)
[2]Colombian Sugarcane Research Center (Cenicaña), Varietal Program, Entomology, Calle 58 N #3BN-110, Cali, Valle del Cauca, Colombia;
E-mail: gavargas@cenicana.org (G. V.)
*Corresponding author; E-mail: gavargas@cenicana.org

Colombia offers perhaps one of the best examples of effective biological control of the sugarcane stem borers, *Diatraea* spp. (Lepidoptera: Crambidae), major pests of sugar cane in the country (Vélez 1997). The newly hatched larvae of this pest feed on leaf parenchyma and migrate toward the cane stem, which they penetrate after the second larval instar, feeding on plant conducting tissues, building tunnels, and finally leaving the stalk as moths (Vargas et al. 2015a). Sugarcane stem borers can reduce crop performance significantly by decreasing stalk weight, and in some cases causing plant death. Losses due to stem borer damage have been estimated at around 0.83% of reduced cane weight, and an additional 0.26% yield at milling per ha for each percentage unit of internodes bored (Vargas et al. 2015a). To control this pest, biological control using egg or larval parasitoids has proved effective not only in Colombia, but elsewhere in the Americas (Vargas et al. 2015a).

In 2012, *Diatraea tabernella* Dyar was detected attacking sugar cane crops in the Cauca River Valley, between Viterbo (Department of Caldas) and La Unión (Department of Valle del Cauca). It was also the predominant and most widely distributed species in cane fields of the Risaralda Sugarcane Mill (Vargas et al. 2013), surpassing in number and aggressiveness *Diatraea saccharalis* F. and *Diatraea indigenella* Dyar & Heinrich, species traditionally found in the region. As early as 1914, this insect had been reported in Colombia in the Department of Chocó (Box 1931), but it was only in 2012 that its presence was recorded in commercial sugar cane fields in the Cauca River Valley (Vargas et al. 2013).

Diatraea tabernella also is considered the most important and widely distributed pest of sugar cane in Panama and Costa Rica, and has been observed to attack from germination through harvest in Costa Rica (Valverde et al. 1991). Based on the observed impact of this pest in Central American countries, its potential to limit sugar cane production, not only in the Cauca River Valley but elsewhere in Colombia, should not be overlooked.

According to Guagliumi (1962), an alternative to effectively reduce sugarcane borer populations could be the use of the wasp *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). The biological characteristics of this natural enemy, such as its short life cycle and high population growth rate, are fundamental elements of effective biological control (Bellows & van Driesche 1999). *Cotesia flavipes* is a larval endoparasitoid native to Southeast Asia, first found parasitizing larvae of the genus *Chilo* (Lepidoptera: Crambidae) (Mohyuddin 1971). A large number of host species of the families Pyralidae and Noctuidae have been reported for this species (Li et al. 2005). The life cycle of *C. flavipes* consists of 4 development stages: egg, 3 larval instars, pupa (cocoon), and free-living winged adult (Hernández 2010). These parasitoids take 13 to 14 d to complete larval development within the host, after which they exit the *Diatraea* larvae to build a silk cocoon in which they pupate.

Cotesia flavipes has been introduced as a biocontrol agent of the sugarcane borer in several countries, including Brazil, Peru, Madagascar, Indonesia, and the US (David & Easwaramoorthy 1991; Fernández et al. 2006). However, in Colombia, and especially in the Cauca River Valley, this parasitoid has not been used because of its history of low adaptation in this region (Aya et al. 2017), explaining its absence from *Diatraea* hosts in the region thus far (Vargas et al. 2013). The objectives of this study were to evaluate the effect of releasing *C. flavipes* in areas highly infested by *D. tabernella* in the northern Cauca River Valley where they were absent at the time of this study, and then to analyze its adaptation to both the host and the study area. We also sought to determine whether continuous rearing of *C. flavipes* under laboratory conditions could decrease the adaptation potential of the released parasitoid as compared to its offspring obtained from material recovered in the field (field-refreshed).

Materials and Methods

The study was carried out in 9 commercial sugar cane fields located in the municipality of Toro, Department of Valle del Cauca (4.6028°N, 76.0355°W), planted with the sugar cane variety CC 85-92, and having similar agronomic practices. To verify the absence of *C. flavipes* in the test area, on 9 Sep 2014 an initial larval collection was made of 22 larvae, which produced 21 *D. tabernella* moths and only 1 *D. saccharalis* moth, demonstrating, as expected, that the parasitoid was rare or absent in the study area. In each commercial field, a plot measuring 100 m × 100 m (1 ha) was established, and separated from all other plots by at least 100 m.

The commercial insectary Laboratorios Biocol S.A.S. supplied the biological material used in the study. The *C. flavipes* specimens were from 2 different sources: a commercial source permanently maintained in the laboratory, and a field refreshed source. The latter consisted of individuals from a colony obtained from recovered parasitized individuals from fields in different sugar cane farms where previous releases were made (Vargas et al. 2015b).

Treatments used were as follows: release of individuals from the commercial source, release of individuals from a field refreshed source, and plots with no releases of the parasitoid. Each treatment had 3 replicates (plots) randomly assigned to each treatment. Three releases of *C. flavipes*, at 15 d intervals, were made in a 2 mo-old crop on 9 Oct, 23 Oct, and 6 Nov 2014. Each release consisted of 4 g parasitoid cocoons per ha, totaling 12 g per ha in the 3 releases, which is equivalent to approximately 12,000 wasps per ha, with a 1.5:1.0 female:male ratio.

An initial collection of *Diatraea* larvae was conducted 15 d after the last release of the parasitoid (i.e., 45 d after the first release, on 24 Oct 2014, from this point forward referred to as the first collection), and the level of parasitism by *C. flavipes* in the borers was evaluated. A second collection was carried out 1 mo after the first collection (i.e., 75 d after the first release, 19 Nov 2014, and from this point forward referred to as the second collection) to determine the establishment potential of the parasitoid in the field. Both collections were made with a sampling effort of 2 man-h per plot.

Collected larvae were tagged and placed individually in plastic Petri dishes for identification of the stem borer species at Laboratorios Biocol S.A.S., located in La Victoria, also in the department of Valle del Cauca. Larvae were fed slices of fresh corn cobs, according to the methodology of Rodríguez et al. (2004), and were maintained at temperatures of 24 ± 2 °C and 65% RH, with a photoperiod of 12:12 h (L:D). The following variables were recorded: total number of captured larvae, proportion of borers parasitized, number of *C. flavipes* cocoons formed per parasitized larva, number of wasps emerged per parasitized larva, female:male ratio of parasitoids obtained, time from cocoon formation to adult parasitoid emergence, and adult longevity.

To assess the effect of the releases of natural enemies on the reduction of pest injury, at 6 mo of crop age the percent of bored internodes was estimated. A randomly distributed sample of 20 stalks was observed within each plot to determine the level of boring.

A 3 × 2 completely randomized factorial design was used for the field experiment, with 3 treatments and 2 collection times. A binomial-type distribution was assumed for proportion variables. Data were analyzed using the Glimmix procedure, and multiple comparisons between treatments were performed based on the Tukey-Kramer test ($\alpha = 0.05$) using SAS 9.3 (SAS Institute 2011). The GLM procedure was used to analyze the percentage of bored internodes, whereas multiple tests were performed using the Tukey test ($\alpha = 0.05$), and using SAS 9.3 (SAS Institute 2011).

Results

Despite the decrease in the average number of *D. tabernella* larvae captured between the first (38 larvae per plot in 2 man-h) and second collection times (23 larvae per plot in 2 man-h), no effect could be attributed to the treatments. The average proportion of parasitism by *C. flavipes* on *D. tabernella* ranged from 0.32 to 0.55 among the 2 *C. flavipes* sources and the 2 collection times. Although no difference in parasitism was observed between the 2 sources of *C. flavipes* (Fig. 1), statistical differences were found between the commercial source and the check for both collection times (F = 12.21; df = 2, 12; P = 0.001). Parasitism tended to increase between the first and second collection, but this was not a statistically significant difference (F = 3.23; df = 1, 12; P = 0.097). Also, there was no interaction between treatments and collection times (F = 0.06; df = 2, 12; P = 0.941). The proportion of parasitism observed in the check plots ranged from 0.04 and 0.09 for the 2 collection times, indicating that check plots were probably colonized by parasitoids released in nearby plots, assuming there were no previously parasitized larvae in the field. On the other hand, a natural proportion of parasitism by *L. minense* was observed in larvae collected in the different plots, and ranged from 0.07 to 0.16 between treatments and collection times.

Statistical differences found in the duration of the cocoon stage of *C. flavipes* were attributable to the treatment (F = 5.21; df = 2, 6; P = 0.048) and collection times (F = 14.57; df = 1, 116; P < 0.001). A significant interaction was observed between treatment and collection times (F = 11.9; df = 1, 116; P < 0.001), where *C. flavipes* wasps in the check treatments displayed a shorter development time (up to 1 d) in the first collection time (Fig. 2).

With respect to the number of cocoons formed per parasitized larva (Fig. 3A), the maximum average number was 62.2 cocoons per larva from the refreshed source of *C. flavipes*, and the minimum average was 26.5 cocoons per larva from check plots. However, no statistically significant differences were found among treatments (F = 1.43; df = 2, 5; P = 0.320). The number of cocoons per larva increased between the first and the second collection times, especially in the check and the refreshed source of *C. flavipes* treatments (F = 14.36; df = 1, 146; P < 0.001). In addition, a significant interaction was found between treatments and collection times (F = 7.59; df = 2, 143; P < 0.001). The same pattern was observed when considering the number of wasps emerging per larva, with a tendency for fewer wasps per larva in check plots, but there was not a statistical difference among treatments (F = 4.71; df = 2, 5; P = 0.059). However, statistically significant differences were found between the 2 collection times (F = 26.11; df = 1, 145; P < 0.001). Fewer wasps were found per larva from check plots on the first collection time, and a significant interaction was found between treatments and collection times (F = 14.54; df = 1, 145; P < 0.001) (Fig. 3B). As in the case of the number of cocoons per larva, the number of wasps per larva tended to increase between the first and second collection times for the refreshed source of *C. flavipes* and colonizing wasps from the check plots. However, there was a statistically significant difference only in the case of the check treatment, where the number of wasps produced per larva increased up to 3 times from the first to second collection times (Fig. 3).

No statistically significant differences were found in offspring sex ratio at the treatment level (F = 2.63; df = 2, 5; P = 0.165) or due to collection time (F = 0.01; df = 1, 135; P = 0.915), nor the interaction between treatment and collection time (F = 1.12; df = 1, 135; P = 0.327). However, the first collection time revealed a higher female:male ratio in check plots (2.6:1) and in the refreshed source of *C. flavipes* (1.8:1) compared to the commercial source of *C. flavipes* (1.3:1). On the other hand, in the second collection, all treatments presented a female:male ratio of 1.8:1, which corresponds to a proportion of 0.64 females.

The proportion of adults emerging fluctuated between 0.51 for check plots in the first collection time and 0.94 for the same treatment in the second collection; however, due to large variations in data, no differences were found among treatments (F = 0.52; df = 2, 5; P = 0.625). In the case of sources of *C. flavipes*, the proportion of adult emergence ranged from 0.83 (commercial source in the second collection time) to 0.87 (check in the first collection time). On the other

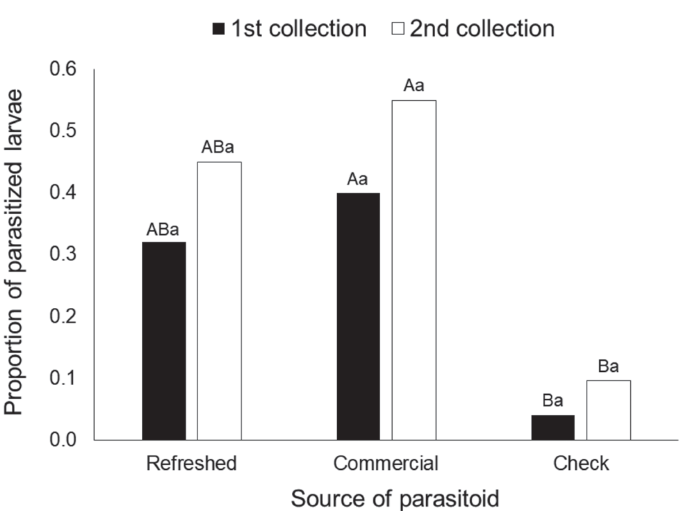

Fig. 1. Average proportion of larval parasitism of *Cotesia flavipes* on *Diatraea tabernella* after 3 releases of 4 g of cocoons per ha each, from 2 sources of *C. flavipes* (field refreshed and commercial), compared to a check with no releases. Observations were made at a first collection time (45 d after the initial release) and a second collection time (75 d after the initial release). Bars with the same capital letter do not differ between treatments within the same collection time. Bars with the same lowercase letter do not differ within each treatment between collection times (Tukey-Kramer, α < 0.05).

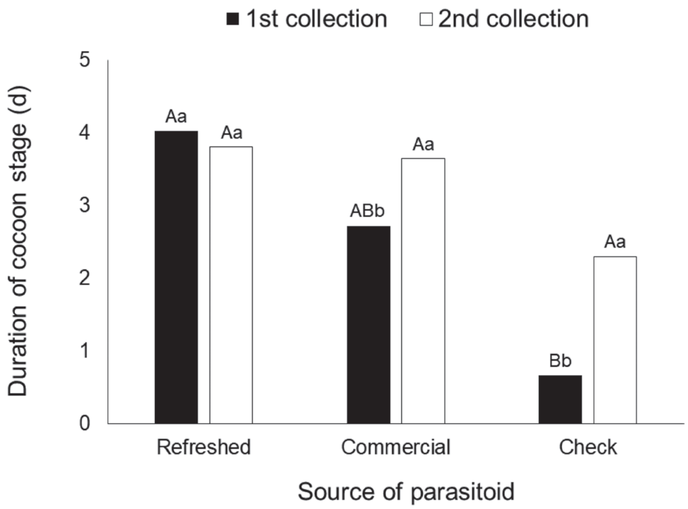

Fig. 2. Average number of d spent on the cocoon stage (d from cocoon to adult emergence) of *Cotesia flavipes* obtained on larvae of *Diatraea tabernella* after 3 releases of 4 g of cocoons per ha each, from 2 sources of *C. flavipes* (field refreshed and commercial), compared to a check with no releases. Observations were made at a first collection time (45 d after the initial release) and a second collection time (75 d after the initial release). Bars with the same capital letter do not differ between treatments within the same collection time. Bars with the same lowercase letter do not differ within each treatment between collection times (Tukey-Kramer, α < 0.05).

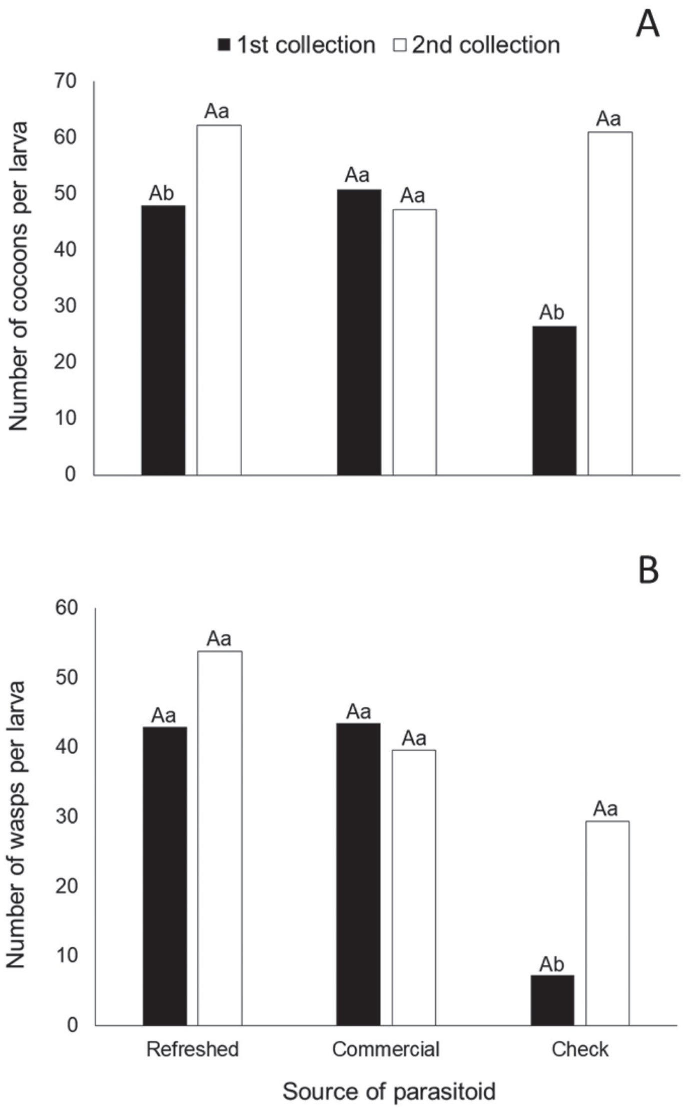

Fig. 3. Average number of *Cotesia flavipes* cocoons (A) and adults (B) formed per *Diatraea tabernell*a larva after 3 releases of 4 g of cocoons per ha each, from 2 sources of *C. flavipes* (field refreshed and commercial), compared to a check with no releases. Observations were made at a first collection time (45 d after the initial release) and a second collection time (75 d after the initial release). Bars with the same capital letter do not differ between treatments within the same collection time. Bars with the same lowercase letter do not differ within each treatment between collection times (Tukey-Kramer, α < 0.05).

hand, significant differences were observed due to collection time (F = 6.8; df = 1, 139; P = 0.010). There also was a significant interaction between treatment and collection time (F = 5.47; df = 1, 139; P = 0.005), specifically involving check plots, which recorded a 50% increase in adult emergence from the first to second collection times.

Adult longevity ranged from 2.0 d (check plots at first collection time) to 3.7 d (refreshed source at second collection time), but no statistically significant differences were found between treatments (F = 1.28; df = 2, 6; P = 0.345). On the other hand, longevity increased 1 d between the first and the second collection times in the refreshed source of *C. flavipes* (F = 4.24; df = 1, 145; P = 0.040). Likewise, a significant interaction was found between the treatment and collection time (F = 3.82; df = 1, 145; P = 0.024) (Fig. 4).

With respect to the effect of releases of *C. flavipes* on pest damage, a decrease up to 65% in the percentage of bored internodes was observed at 6 mo of crop age in plots where releases of *C. flavipes*

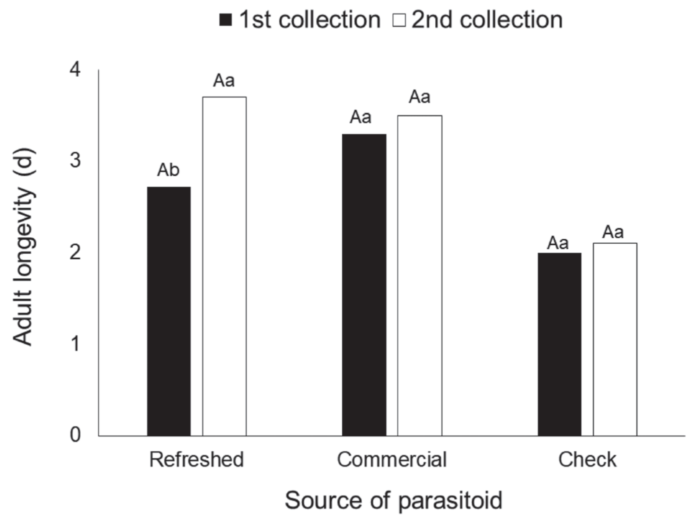

Fig. 4. Average adult longevity (d) of *Cotesia flavipes* wasps obtained on *Diatraea tabernella* larvae after 3 releases of 4 g of cocoons per ha each, from 2 sources of *C. flavipes* (field refreshed and commercial), compared to a check with no releases. Observations were made at a first collection time (45 d after the initial release) and a second collection time (75 d after the initial release). Bars with the same capital letter do not differ between treatments within the same collection time. Bars with the same lowercase letter do not differ within each treatment between collection times (Tukey-Kramer, α < 0.05).

were made (commercial and refreshed) as compared with check plots (F = 5.68; df = 2; P = 0.004). Post-hoc tests do not reveal differences between the 2 sources of the parasitoid (Tukey, α < 0.05; Fig. 5).

Discussion

In the study conducted in the Cauca River Valley, the proportion of parasitism was as high as 0.53 in plots where releases were made, clearly demonstrating the suitability of *C. flavipes* for parasitism of *D. tabernella* under field conditions. The occurrence of parasitism in check plots also confirmed that the parasitoid adapted well to the

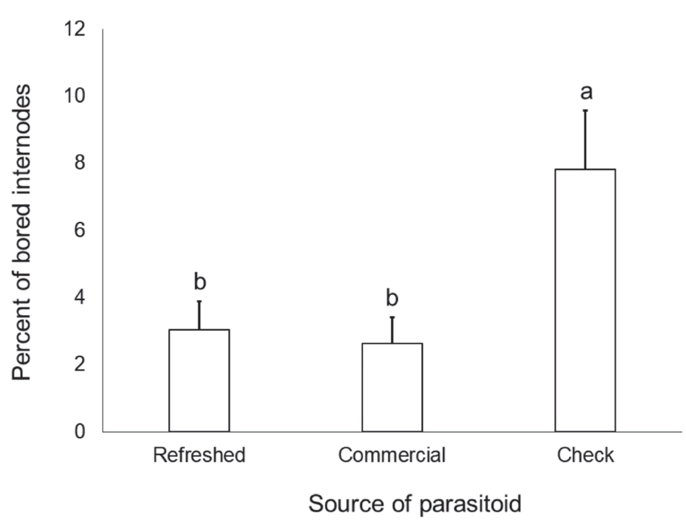

Fig. 5. Average percentage of bored internodes (± SE) evaluated in 6-mo-old sugar cane plots (20 stalks evaluated per plot) after 3 releases of 4 g of cocoons per ha each, from 2 sources of *C. flavipes* (field refreshed and commercial), compared to a check with no releases. Bars with the same letter do not differ statistically (Tukey, α < 0.05).

study area, considering the low level of natural parasitoid dispersal (Sallam et al. 2001). Badilla (2002) reported similar levels of parasitism (50%) in Costa Rica and concluded that the introduction of *C. flavipes* had proved successful in managing the pest, parasitizing the 3 borer species found in that country (*D. tabernella*, *D. saccharalis*, *D. guatemalella* Schaus). According to the same author, *C. flavipes* also was found to adapt well to the different ecological regions where sugar cane is planted in Costa Rica.

According to Sallam et al. (2001) a female *C. flavipes* can travel up to 64 m during her lifespan of 2 to 3 d, and although plots in this study were at least 100 m apart, the time between each of the 3 releases (15 d) and the 2 collection times (30 d) totaled 75 d between the first release of the parasitoid and the second collection time. The generational time of *C. flavipes* is around 20 d (Hernández 2010). Therefore, 2 generations could have passed when the first collection was made 15 d after the 3 releases had been completed, and at least 3 generations by the second collection, 45 d after the 3 releases had been made. During the multiple generations, individuals could have travelled between the plots where the releases were made and the check plots. This also could affect the ability to contrast the different sources of *C. flavipes.*

It is important to mention that *L. minense* has been widely released as a biocontrol agent of *Diatraea* spp. in the study area; however, there is no evidence that releases of *C. flavipes* triggered a decrease in the activity of these tachinid flies during the study period. Although the work carried out by Weir and Sagarzazu (1998) showed that flies were more effective than wasps in inoculations on *D. saccharalis* larvae in the laboratory, Rossi and Fowler (2004) observed a marked tendency for populations of the tachinid flies *L. minense* and *B. claripalpis* to decrease at sites in Brazil where *C. flavipes* had been released. However, these studies in the Cauca River Valley are, to the best of our knowledge, the first in Colombia, and additional studies should be conducted on future releases of *C. flavipes* in relation to population densities of *L. minense.*

An interaction was found between treatments and collection times with respect to the number of cocoons and adults formed by larvae, indicating that in check plots there was an increase in the number of cocoons and wasps per larva in relation to collection time. This might be explained by the fact that there were no parasitoid releases in these plots, therefore fewer ovipositing adults and perhaps less multiple parasitism, leading to fewer cocoons and wasps per parasitized larva in the first larvae collection. The increase in parasitism in the check plots between collection times can, therefore, be explained by the additional releases and time for dispersal from the refreshed and commercial plots, and as Potting et al. (1997) suggest, a lack of discrimination between already parasitized hosts and non-parasitized ones, mostly in non-experienced females.

Although wasp size was not measured in the present study, fewer parasitoids per larva resulted in a shorter cocoon stage in the case of the checks, which is consistent with a tradeoff between favorable conditions during development (e.g., number of individuals per larva) and reduced development time, and possibly, larger-sized adults. In fact, in the context of life history evolution, it is assumed that there is a tradeoff between the time an individual takes to develop and its size as a sexually matured adult (Stearns 1992). Larger adult size generally is understood to translate into higher fecundity in insects (Honěk 1993), and in parasitoid wasps in particular (Charnov et al. 1981).

In this regard Goubault et al. (2007) pointed out that the optimal oviposition per host would not only be affected by future reproduction expectancy, but also would be flexible and affected by intergenerational biological efficacy, where larger individuals (the result of less sibling competition) would translate into more competitive adults. Mayhew and Glaizot (2001) in turn pointed out that one of the greatest tradeoffs in the life history of parasitoid insects is between clutch size and resulting size of offspring; however, in the particular case of *C. flavipes* the equation also needs to consider fitness consequences of superparasitism under a very common low rate of host encounter (Potting et al. 1997).

Studies carried out on *C. flavipes* in Kenya corroborate that the parasitoid tends to produce a higher proportion of females, even when exposed to different hosts such as *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (0.55 females) and *Chilo partellus* (Swuinhoe) (Lepidoptera: Crambidae) (0.68 females) (Obonyo et al. 2008). Similarly, Wiedenmann et al. (1992) found high female:male ratios in the lab when studying *C. flavipes* parasitism on *D. saccharalis*. Studies carried out in sugar cane fields in Cuba by Barroso et al. (2003) also demonstrated a higher proportion of females (0.58 females) in *C. flavipes* during the acclimatization and colonization processes. The observed tendency of a higher proportion of females in the first evaluation of both the refreshed source and check suggests that the female:male encounter rate was high, and attributable to a balanced sex ratio of released insects. It also suggests good parasitoid adaptation (Fernandez & Sharkey 2006).

Both rate of host encounters and adult longevity notably affect the reproductive success of parasitoid insects in the field, indicating that these insects generally are affected by time constraints (Bezemer & Mills 2003). The significant interaction between treatment and collection time for wasp longevity indicates that the refreshed source was able to increase its longevity by 1 d between the first and second collection times, which translates into more opportunities for wasps to search for hosts, and in turn increases parasitic potential, as illustrated by the greater number of cocoons per parasitized larvae, especially during the second evaluation 45 d after release. Factors such as nutritional quality, host size, temperature, and relative humidity are known to affect the longevity and fecundity of *C. flavipes* females (Charnov et al. 1981; Jervis & Copland 1996; Emana 2007). In this case, we argue that factors associated with increased longevity in the refreshed source would be related to a higher biological efficiency mediated by selection in the field to tolerate greater environmental stress (van Lenteren 2003).

The reduction of pest injury observed in sugar cane plots where the wasp was released, as compared with check plots, is consistent with the parasitism observed, and confirms the parasitoid's potential to adapt to the environmental conditions of the northern Cauca River Valley, as well as to the host *D. tabernella*. The reduction in the level of pest injury is similar to that reported by Vargas et al. (2015a), when estimating the effect of releases of the tachinid *L. minense* on reducing the damage caused by *Diatraea* spp. in sugar cane fields.

This study presents useful biological observations on the parasitoid's functionality when introduced into a new environment. Comparisons between a source previously exposed to field conditions and those maintained in the lab suggest differences at the level of biological efficiency by an increase in adult longevity between the first and second collection times in the refreshed source, which translates into more opportunities for wasps to search for hosts, and in turn an increase in parasitic potential. More recent observations in the Cauca River Valley have proven its potential for adaptation, persistence, and effectiveness as a biological control agent under field conditions, as *C. flavipes* has been expanding its distribution range in the region (Aya et al. 2017; Vargas et al. 2018). Therefore, *C. flavipes* is currently included in the biological control programs, not only in the northern Cauca River Valley, but across the region. It should be highlighted that *Cotesia* also could prove useful in the management of other *Diatraea* species; however, additional studies are needed to explore the potential of this natural enemy to manage different species of the *Diatraea* complex.

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References Cited

Aya VM, Echeverri C, Barrera G, Vargas G. 2017. *Cotesia flavipes* (Hymenoptera: Braconidae) as a biological control agent of sugarcane stem borers in Colombia's Cauca River Valley. Florida Entomologist 100: 1–5.

Badilla F. 2002. Un programa exitoso de control biológico de insectos plaga de la caña de azúcar en Costa Rica. Manejo Integrado de Plagas y Agroecología 64: 77–87.

Barroso F, Aday O, Acosta S, Díaz FR, Barroso J. 2003. Aclimatación y colonización de *Cotesia flavipes* Cam. en áreas de producción en Villa Clara, Cuba. Centro Agricola 30: 55–59.

Bellows TS, van Driesche RG. 1999. Life table construction and analysis for evaluating biological control agents, pp. 199–223 *In* Bellows TS, Fisher TW [eds], Handbook of Biological Control: Principles and Applications of Biological Control. Academic Press, San Diego, California, USA.

Bezemer TM, Mills NJ. 2003. Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. Animal Behaviour 66: 1119–1128.

Box HE. 1931. The Crambine genera *Diatraea* and *Xanthopherne* (Lepidoptera: Pyralidae). Bulletin of Entomological Research 22: 1–50.

Charnov E, Los-Den Hartogh RL, Jones WT, van den Assem J. 1981. Sex ratio evolution in a variable environment. Nature 289: 27–33.

David H, Easwaramoorthy S. 1991. Biocontrol Technology for Sugarcane Pest Management. Sugarcane Breeding Institute, Coimbatore, India.

Emana GD. 2007. Comparative studies of the influence of relative humidity and temperature on the longevity and fecundity of the parasitoid, *Cotesia flavipes*. Journal of Insect Science 7: 1–7.

Fernández F, Sharkey M. 2006. Introducción a los Hymenoptera de la Región Neotropical. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia. Bogotá, Colombia.

Goubault M, Mack AFS, Hardy ICW. 2007. Encountering competitors reduces clutch size and increases offspring size in a parasitoid with female-female fighting. Proceedings of the Royal Society B 274: 2571–2577.

Guagliumi P. 1962. Las plagas de la caña de azúcar en Venezuela. Ministerio de Agricultura y Cría, CIA, Maracay, Venezuela.

Hernandez D. 2010. Estudio de algunos aspectos biológicos de *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) parasitoide de *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae). Entomotropica 25: 69–81.

Honěk A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66: 483–492.

Jervis MA, Copland MJW. 1996. The life cycle, pp. 63–160 *In* Jervis M, Kidd N [eds.], Insect Natural Enemies. Chapman & Hall, London, United Kingdom.

Li Z, You L, Ma J, Chen L, Xiao F. 2005. Analysis of the phylogenetic relationships among the *Cotesia flavipes* complex (Hymenoptera: Braconidae: Microgastrinae). Journal of Hunan Agricultural University 31: 24–28.

Mayhew J, Glaizot G. 2001. Integrating theory of clutch size and body size evolution for parasitoids. Oikos 92: 372–376.

Mohyuddin A. 1971. Comparative biology and ecology of *Apanteles flavipes* (Cam.) and *A. sesamiae* Cam. as parasites of graminaceous borers. Bulletin of Entomological Research 61: 33–40.

Obonyo M, Schulthess F, Gerald J, Wanyama O, Le Ru B, Calatayud P. 2008. Location, acceptance and suitability of lepidopteran stemborers feeding on a cultivated and wild host-plant to the endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). Biological Control 45: 36–47.

Potting RPJ, Snellen HM, Vet LEM. 1997. Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*. Entomologia Experimentalis et Applicata 82: 341–348.

Rodríguez LC, Gómez I, Peñaloza Y, Tejada M. 2004. Desarrollo del parasitoide *Cotesia flavipes* Cameron, 1891 (Hymenoptera: Braconidae) en *Diatraea tabernella* Dyar y *Diatraea saccharalis* Fabricius, 1794 (Lepidoptera: Pyralidae), y su efectividad en el control de *Diatraea tabernella*. Tecnociencia 6: 85–94.

Rossi MN, Fowler HG. 2004. Spatial and temporal population interactions between the parasitoids *Cotesia flavipes* and Tachinidae flies: considerations on the adverse effects of biological control practice. Journal of Applied Entomology 128: 112–119.

Sallam MN, Overholt WA, Kairu E. 2001. Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. Entomologia Experimentalis et Applicata 98: 210–217.

SAS Institute. 2011. SAS software, version 9.3. Cary, North Carolina, USA.

Stearns SC. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, United Kingdom.

Valverde L, Badilla F, Fuentes G. 1991. Pérdidas de azúcar a nivel de fábrica causadas por *Diatraea tabernella* en tres variedades de caña de azúcar (*Saccharum* spp.) en la zona alta de San Carlos, Costa Rica. Agronomía Costarricense 15: 7–12.

van Lenteren JC. 2003. Need for quality control of mass-produced biological control agents, pp 1-18 *In* van Lenteren JC [ed.], Quality Control and Production of Biological control Agents. CABI, Wallingford, United Kingdom.

Vargas G, Gómez LA, Michaud JP. 2015a. Sugarcane stem borers of the Colombian Cauca River Valley: pest status, biology and control. Florida Entomologist 98: 728–735.

Vargas G, Lastra LA, Ramírez GD, Solis MA. 2018. The *Diatraea* complex (Lepidoptera: Crambidae) in Colombia's Cauca River Valley: making a case for the geographically localized approach. Neotropical Entomology 47: 395?402.

Vargas G, Lastra LA, Solís MA. 2013. First record of *Diatraea tabernella* (Lepidoptera: Crambidae) in the Cauca River Valley of Colombia. Florida Entomologist 96: 1198–1201.

Vargas G, Villegas A, Ramírez D, Barco LE, Gutiérrez Y, Herrera D, Valencia IC. 2015b. Eficacia de las liberaciones de enemigos naturales para el manejo de *Diatraea tabernella* y *D. busckella* en la zona norte del valle del río Cauca, pp. 411–422 *In* Cruz JR [ed.], Memorias X Congreso de la Asociación Colombiana de Técnicos de la Caña de Azúcar, Tecnicaña. Cali, Colombia. 14–18 Sep 2015.

Vélez R. 1997. Plagas agrícolas de impacto económico en Colombia: bionomía y manejo integrado. Editorial Universidad de Antioquia, Medellín, Colombia.

Weir EH, Sagarzazu L. 1998. Interspecific competition between *Metagonystilum minense* (Diptera: Tachinidae) and *Cotesia flavipes* (Hymenotera: Braconidae) parasitoids of sugarcane borers (*Diatraea* spp., Lepidoptera: Pyralidae). Revista Biología Tropical 46: 1135–1139.

Wiedenmann RN, Smith JW, Darnell P. 1992. Laboratory rearing and biology of the parasite *Cotesia flavipes* (Lepidoptera: Crambidae) using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as a host. Environmental Entomology 21: 1160–1167.