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Optimizing torula bait for *Anastrepha suspensa* (Diptera: Tephritidae) trapping in the Dominican Republic

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Abstract

Torula yeast is the most common bait used by growers and agriculture professionals for trapping of tephritid flies in the Dominican Republic. However, the efficiency of the bait is influenced by weather conditions, aging, and contamination with undesirable microorganisms. Thus, additives such as benzalkonium chloride, a quaternary ammonium compound, have been used together with torula yeast as a bait stabilizer. This study evaluated the effect of the addition of benzalkonium chloride to torula yeast bait, and time of renewal in guava orchards for trapping of Caribbean fruit flies (*Anastrepha suspensa* Loew; Diptera: Tephritidae). A field study was conducted in 2 consecutive 8-wk periods between Oct 2019 and Feb 2020. Six treatments were evaluated based on the type of bait (torula yeast or torula yeast + benzalkonium chloride) and renewal frequency (weekly, biweekly, or without renewal). Treatments were arranged in a randomized complete block design with 4 replications. Data indicated that torula yeast was attractive to 85.2% and 80.2% more males and females of Caribbean fruit flies compared to torula yeast + benzalkonium chloride, respectively. Similarly, traps without renewal attracted an average of 49.8% more females than traps renewed weekly or biweekly, regardless of the bait type. Analysis of both baits showed a rapid decrease in pH of the torula yeast. The addition of benzalkonium chloride may have affected the microbial activity in the solution, leading to reduced decomposition of torula yeast + benzalkonium chloride and, therefore, reduced captures.

Key Words: Caribbean fruit fly; benzalkonium chloride; guava; torula yeast

Resumen

La levadura Torula es el cebo más comúnmente utilizado por los agricultores y los profesionales de la agricultura para atrapar moscas de la fruta en la República Dominicana. Sin embargo, la eficacia del cebo está influenciada por las condiciones climáticas, el envejecimiento y la contaminación con microorganismos. Dado esto, compuestos de amonio cuaternario, como el cloruro de benzalconio, son utilizados en mezcla con torula como estabilizador de cebo. Este estudio evaluó el efecto de la adición de cloruro de benzalconio al cebo de levadura torula y el tiempo de renovación en huertos de guayaba para atrapar moscas de la fruta del Caribe (*Anastrepha suspensa* Loew; Diptera: Tephritidae). Se realizó un estudio de campo en 2 períodos consecutivos de 8 semanas entre octubre de 2019 y febrero de 2020. Se evaluaron seis tratamientos de tipo de cebo (levadura torula o levadura torula + cloruro de benzalconio) y la frecuencia de renovación (semanal, quincenal o sin renovación). Los tratamientos se organizaron en un diseño de bloques completos al azar con 4 repeticiones. Los datos indicaron que la levadura torula fue atractiva para un 85,2% y un 80,2% más de machos y hembras de moscas de la fruta del Caribe en comparación con la levadura torula + cloruro de benzalconio, respectivamente. Del mismo modo, las trampas sin renovación atrajeron un promedio de 49,8% más de hembras que las trampas renovadas semanalmente o quincenalmente, independientemente del tipo de cebo. El análisis de ambos cebos mostró una rápida disminución del pH de la levadura torula. La adición de cloruro de benzalconio puede haber afectado la actividad microbiana en la solución, provocando una descomposición reducida de la levadura torula + cloruro de benzalconio y, por lo tanto, capturas reducidas.

Palabras Clave: mosca de la fruta del caribe; clorudo de benzalconio; guayaba; levadura torula

The Caribbean fruit fly (*Anastrepha suspensa* Loew; Diptera: Tephritidae) is one of the most damaging pests of guava orchards (*Psidium guajava* L.; Myrtaceae) in Central and South America (Bueno et al. 2004). Although the fly species is extremely polyphagous (over 100 hosts) (Burk 1983), it has been reported to show preference for guava, citrus (*Citrus* spp.; Rutaceae), and tropical almond (*Terminalia catappa* L.; Combetraceae) in the Dominican Republic (Serra & Ogando 2015). Traditionally, guava is produced for domestic consumption in the Dominican Republic, with an estimated planted area of 195 ha across the country and an average yield of 81,117 fruits per ha in 2019 (Dominican Department of Agriculture 2019). In recent yr, several governmental efforts have been aimed to promote the establishment of new guava orchards for export to Asian markets. However, given the direct impact of the Caribbean fruit fly on yield and quality of multiple fruit crops, it is imperative to determine their density and distribution on the growing fields (Toledo et al. 2009; Shelly et al. 2014).

Food-based baits have been one of the predominant attractants used in tephritid trapping, mainly due to their low cost (Cornelius et al.

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2000; Toledo et al. 2009; Leblanc et al. 2010). Adult fruit flies depend on sugar and protein for survival. The host fruit is used for both feeding and oviposition. Fruit volatiles can be detected by the adult flies several m away and serve as an olfactory stimulus to orient them upwind toward fruit host trees (Cornelius et al. 2000; Toledo et al. 2009). Volatile protein cues such as ammonia and putrescine are more attractive to tephritid flies (particularly females that need protein for ovarian maturation), and have provided the foundation for development of synthetic food-based lures (Kendra et al. 2005, 2008). Putrescine and cadaverine volatile cues are generated through bacterial decomposition of protein and underlie the attraction of tephritid flies to liquid protein baits. Liquid baits such as torula yeast undergo decomposition when exposed to hot and humid field conditions; the resulting volatiles can cause synergistic interactions with ammonium-based compounds that increase field captures of fly pest species such as *A. suspensa* (Kendra et al. 2008).

Traditionally, torula yeast (*Candida utilis* Henneber) has been the most commonly used bait by growers and agriculture professionals for tephritid trapping in citrus, papaya, mango, and guava in the Dominican Republic. Yeasts are eukaryotic microorganisms that produce enzymes and CO_2, commonly used in the fermentation process of many foods (USDA 2014). Torula yeast is a by-product of the paper mill industry, generally used in its autolyzed form, to obtain peptides and amino acids (Epsky et al. 2014). To establish the field traps, torula yeast pellets are hydrolyzed (diluted in water) to destroy the peptide bonds and create smaller chains of amino acids (Epsky et al. 2014). The aqueous solution usually is placed in yellow plastic McPhail traps and moved to the field for trapping. However, the efficiency of the bait is influenced by weather conditions (Thomas et al. 2001), bait aging, and contamination with undesirable microorganisms (e.g., mold). The addition of sodium borate (Borax) has been reported to reduce the decomposition of protein baits and generally is recommended to accompany torula yeast formulations to increase the solution stability and attraction properties (Epsky et al. 1993). A formulation of 3% hydrolyzed torula yeast + 4% borax was reported to attract the greatest numbers of Caribbean fruit fly in Florida (Lopez et al. 1971). In terms of bait aging, Malo (1992) evaluated the number of *Anastrepha* fruit flies captured in McPhail traps as a function of the decomposition time of torula yeast (2 to 10 d) and borax (4:5; 21 g; 500 mL of water). Data showed no significant difference among treatments, suggesting that the common approach of renewing the bait every 7 d did not allowed for proper decomposition time and served as a poor attractant (Malo 1992). In addition, new compounds have been evaluated in hydrolyzed protein baits to improve stability. Benzalkonium chloride is a quaternary ammonium compound, known for its broad spectrum anti-microbial activity, low human toxicity, and nonvolatile nature (Lasa & Williams 2017). Benzalkonium chloride was evaluated with a Captor + borax mix and replaced either weekly or not replaced for trapping of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae). Data showed a higher number of flies per trap per d for Captor + borax replaced weekly and Captor + borax + benzalkonium chloride not replaced, as compared to Captor + borax, not replaced (Lasa & Williams 2017).

Although tephritid trapping techniques are improving constantly around the world, the Department of Agriculture in the Dominican Republic endorses torula yeast bait with weekly renewal to maximize the efficiency of data collection. This approach is labor intensive and expensive at a local and regional level. There is little locally generated information regarding bait renewal frequency and potential bait stabilizers in the Dominican Republic. Hence, this study evaluated the effect of torula yeast with and without benzalkonium chloride in combination with 3 bait renewal frequencies on Caribbean fruit fly trapping in guava orchards in the Dominican Republic.

Materials and Methods

FIELD SITE AND CROPPING SYSTEM

A field study was conducted in 2 consecutive 8-wk periods, one between 23 Oct and 18 Dec 2019, and the second between 1 Jan 2019 and 24 Feb 2020. The experiments were established at a commercial guava farm in San Cristobal, Dominican Republic (Goya Santo Domingo farm, 18.7000°N, 70.1666°E). A guava orchard planted in 2001 with a reported history of *A. suspensa* pressure was used for evaluation. Guava trees of the cultivar 'Ruby' established 3 m apart between plants and 6 m apart between rows (555 trees per ha) were used for the experiments.

Crop practices at the Goya Santo Domingo farm during the study included commercial harvest commonly starting in Feb of each yr. Additionally, during the last wk of Nov, trees were top pruned to promote horizontal growth. Two drip irrigation lines supplied water throughout the yr. Drip emitters were located 0.5 m apart, with an average flow of 3.2 L per h. Irrigation was provided 3 times per wk for periods ranging between 1 and 1.5 h per irrigation event. Trees received an average flow of 4.7 m^3 per yr of water, assuming a full water film coverage under the canopy with 8 emitters per tree. Guava trees received approximately 300 kg per ha of nitrogen, 135 kg per ha of phosphate (P_2O_5) and 570 kg per ha of potassium (K_2O) per yr. During the experiments, insecticide applications were unchanged at the grower's request. On 9 Dec 2019, the orchard was sprayed with Aval 20SP® (Acetamiprid) at a rate of 2.5 L per ha, and Amistar® (Azoxystrobin) at a rate of 1.5 L per ha. Additionally, on 14 Jan 2020, plants were fumigated with Exalt 6SC® (Spinetoram) and Mastercop® (Copper sulfate pentahydrate) at rates of 2 and 4 L per ha, respectively.

BAIT COMPOSITION AND TREATMENT COMBINATION

Treatments were established in a randomized complete block design with 4 replications. Experimental units consisted of 2 traps placed parallel to each other and separated by 12 m (2 rows). Experimental units were placed about 21 m apart (7 trees) within the block, whereas blocks were spaced at 30 m (5 rows). The experimental site was approximately 1.5 ha. Plastic multibait McPhail traps (Soluciones Agrícolas, SRL, Santo Domingo, Dominican Republic) were composed of a yellow base covered by a transparent top, capable of holding 450 mL of bait solution. Six treatment combinations were established based on the type of bait and frequency of renewal: torula yeast renewed weekly, biweekly, or without renewal, and torula yeast + benzalkonium chloride renewed weekly, biweekly, or without renewal.

Torula yeast pellets consisting of 34.2% torula yeast + 57.2% borax (Susbin, San Juan, Argentina) and 80% purity benzalkonium chloride were used for the experiments (Reactivos Analíticos DS S.R.L., Santo Domingo, Dominican Republic). For treatments of weekly renewal, a new bait solution was prepared at the beginning of each wk. Similarly, for treatments with biweekly renewal a new bait solution was prepared every 14 d. Each bait consisted of an aqueous solution of 4 torula yeast pellets (4 g per pellet) diluted in 400 mL of water per trap with or without the addition of 32 mL of benzalkonium chloride (8% v/v). For treatments with biweekly renewal, an additional 200 mL of solution was mixed per trap. Additional bait solution was added until reaching 400 mL per trap, 7 d after setting the traps to compensate for evaporation losses. For the treatments without renewal, 2 stock solutions were made by diluting 250 g of torula yeast pellets (4 g per pellet) in 25 L of water. Two L of benzalkonium chloride (8% v/v) were added to 1 solution for the treatments including benzalkonium chloride. Stock solutions were stored at a constant temperature of 28 ± 1 °C and 60% relative humidity. The same bait volume (400 mL) was used at the beginning of the study for traps without renewal. Additional bait from the stock solutions was added to compensate for evaporative losses. All trap volumes were maintained at 400 mL, with weekly additions of bait as required.

SAMPLING

Traps were hung in trees branches about 1.5 m aboveground, avoiding direct sunlight, on 23 Oct 2019 and 1 Jan 2020. All treatments remained in the field for a period of 8 wk. Traps were checked every 7 d. The insects collected were separated from the solution using a sieve and moved into 25 mL plastic containers with 70% ethanol. The insects were transported to the Entomology Laboratory at the Loyola Specialized Institute of Superior Studies (San Cristóbal, Dominican Republic) for further counting, identification to the species level, and gender classification.

One data logger (HOBO U23 Pro V2, Onset, Bourne, Massachusetts, USA) was placed near 1 of the traps, avoiding direct sunlight, to measure air temperature and relative humidity. Data points were collected every h from 23 Oct 2019 to 23 Feb 2020. Additionally, 2 separated 200 mL samples of torula yeast and torula yeast + benzalkonium chloride solutions (initial pH = 7) were mixed under laboratory conditions on 10 Mar 2020. Solution concentrations were as described for the field experiment. Samples were placed in 500 mL beakers, replicated 5 times, and maintained at 28 ± 1 °C. The pH of the solutions was measured with a pH meter (Ohaus ST300; Parsippany, New Jersey, USA) every d throughout a 14 d period to identify potential changes in pH due to gradual degradation of the torula yeast with and without addition of benzalkonium chloride.

DATA ANALYSIS

The number of adult males and females of Caribbean fruit fly were analyzed separately using an analysis of variance. An initial analysis was conducted to identify the effect of the 2 evaluation periods. Data were compared considering the fixed factors of treatment (bait type × renewal frequency), 8 wk evaluation period (periods 1 and 2), and their interaction. A second analysis of variance was used to identify treatment main effects and interactions. This analysis considered the fixed effect factors of bait type (torula yeast, torula yeast + benzalkonium chloride) and renewal frequency (weekly, biweekly, without renewal). In addition, the random effect of block was considered. In case of significant effect, means were separated by LSD test. To comply with the analysis of variance assumptions, all insect counts were log-transformed (Y = log [X + 1]). Linear regression analysis was used to describe the daily pH changes of the types of baits (torula yeast, torula yeast + benzalkonium chloride). All analyses were conducted using Statistics software (Statistix version 9, Tallahassee, Florida, USA) and *p*-values less than 0.05 were considered significant.

Results

BAIT TYPE AND RENEWAL EFFECT ON CARIBBEAN FRUIT FLY

There was an interaction between evaluation period (2019 and 2020) and treatment combination (bait type + renewal frequency) for male ($F_{5,47}$ = 2.53; P = 0.048) and female Caribbean fruit fly ($F_{5,47}$ = 2.99; P = 0.024). Thus, data from each of the 8 wk periods was analyzed and presented separately. Caribbean fruit fly populations varied from Oct 2019 to Feb 2020. Captures in 2020 were 26.2% higher than captures in 2019 for Caribbean fruit fly females. Similarly, male captures increased 29.5% in 2020 compared to 2019 (Table 1). Differences in Caribbean fruit fly captures between evaluation periods could be related to the higher availability of fruits in Jan and Feb, which seemed to promote an increment in Caribbean fruit fly populations, compared to Oct and Dec.

There was no interaction between bait type and bait renewal time in both 2019 and 2020. There was a significant effect of bait type on male ($F_{1,23}$ = 65.55; P < 0.0001 in 2019, and $F_{1,23}$ = 205.45; P < 0.0001 in 2020) and female numbers of Caribbean fruit fly ($F_{1,23}$ = 66.29; P < 0.0001 in 2019, and $F_{1,23}$ = 131.79; P < 0.0001) during both yr of evaluation, with no significant effect of bait renewal time, with the exception of female counts in 2019 (Table 1). Torula yeast attracted an average of 85.2% and 80.2% more males and females of Caribbean fruit fly compared to torula yeast + benzalkonium chloride, respectively. Similarly, torula yeast attracted 94.3% and 91.4% more males and females than torula yeast + benzalkonium chloride in 2020 (Table 1).

Bait renewal time did not affect male counts in both yr. Average male captures per wk were 4.17 and 4.10 flies per trap in 2019 and 2020, respectively (Table 1). Similarly, average weekly female captures in 2020 were 20.2 per trap, with no effect of the bait renewal time. In 2019, bait with no renewal attracted 49.8% more females than treatments of weekly and biweekly renewal, whereas there was no difference

Table 1. Effect of bait type and time of bait renewal on average male and female of Caribbean fruit fly (*Anasthrepha suspensa*) captures in guava production in San Cristobal, Dominican Republic, between Oct 2019 and Feb 2020.

Treatment	Caribbean fruit fly counts (average fruit flies per trap per week)			
	Average across 8 wk periods			
	Oct to Dec 2019[a]		Jan to Feb 2020	
Bait type	Male	Female	Male	Female
Torula yeast	9.47 ± 5.66	47.98 ± 18.78	13.45 ± 4.33	65.06 ± 20.29
Torula yeast + benzalkonium chloride[b]	1.40 ± 3.37	9.47 ± 19.23	0.77 ± 0.64	5.60 ± 5.50
Significance[c]	*	*	*	*
Bait renewal				
None	6.08 ± 5.26	34.48 ± 14.94 a	4.37 ± 7.97	21.90 ± 35.30
Weekly	3.17 ± 5.32	15.21 ± 14.09 b	4.49 ± 8.15	22.44 ± 39.15
Biweekly	3.27 ± 5.75	19.41 ± 27.11 b	3.46 ± 7.44	16.37 ± 32.24
Significance	NS	*	NS	NS
Bait type × bait renewal	NS	NS	NS	NS

[a]Field evaluations between 27 Oct and 21 Dec 2019; field evaluations between 1 Jan and 23 Feb 2020; [b]Torula yeast pellets (34.2% torula yeast + 57.2% borax) and benzalkonium chloride (80% purity); [c]Values followed by different letters indicate that the means are significantly different ($P \le 0.05$) according to Fisher's protected least significant difference test; NS, * = Nonsignificant or significant at $P \le 0.05$.

between the 2 latter (Table 1). The variations in female counts seemed to be related to the reduced number of fruits ready for harvest in late 2019 compared to the early 2020.

CUMULATIVE CAPTURES OVER TIME

Populations of Caribbean fruit fly did not seem to change substantially across evaluation wk; however, female captures were 80.6% higher than male captures across evaluations. Linear regression analysis showed high influence of time (wk) in the cumulative captures of males and females, without large fluctuations among weekly evaluations (Figs. 1, 2). Coefficients of determinations for treatment × wk regressions ranged between 0.87 to 0.98 for all treatments, across both yr. During the 2019 evaluation period, torula yeast captured a total of 451 females and 86 males, whereas torula yeast + benzalkonium chloride captured a total 85 females and 13 males (Fig. 1). In 2020, torula yeast captured 542 females and 115 males, whereas torula yeast + benzalkonium chloride captured 56 females and 7 males (Fig. 1).

Bait renewal time had little influence in the cumulative weekly captures. Changing the baits weekly and biweekly did not increase the captures after their renewal (Fig. 2). In 2019, female captures in baits without renewal were 2.3- and 1.7-times higher compared to captures with weekly and biweekly renewal, respectively. Similarly, males captured in baits without renewal were 1.8-times higher compared to baits with weekly or biweekly renewal (Fig. 2). A similar trend was observed for female and male captures from 2020 evaluations (Fig. 2).

NON-TARGETED INSECTS

Non-targeted insects captured also were recorded and identified to the family level. Lance flies (Lonchaeidae), ants (Formicidae), sawflies, wasps, and bees (Hymenoptera) and butterflies and moths (Lepidoptera) were more attracted by the torula yeast compared to the torula yeast + benzalkonium chloride in both yr (Table 2). Similar to the response of Caribbean fruit fly, none of the families and genera mentioned were influenced by bait renewal frequency with an average of 13.6 lance flies, 40.5 ants, 4.9 among sawflies, wasps, and bees, and 2.6 butterflies and moths (Table 2).

Hump-backed flies (Phoridae) were influenced by bait and time of bait renewal in 2019. Torula yeast attracted increased hump-backed fly captures by 89%, while treatment without renewal and with biweekly renewal increased the captures by 58.5% (Table 2). There was no influence of the renewal time in 2020 in hump-backed fly captures. Also, there was no interaction between factors for any of the mentioned species. Nevertheless, there was an interaction between bait and bait renewal for attraction of house flies (Muscidae) and small fruit flies (Drosophila) in both yr (Table 2).

Torula yeast with weekly, biweekly, or without renewal attracted similar numbers of house flies with an average of 370 flies per trap per wk (Table 3). Torula yeast + benzalkonium chloride with weekly and biweekly renewal increased the attraction of house flies compared to torula yeast + benzalkonium chloride without renewal. Overall, torula yeast attracted more house flies than torula yeast + benzalkonium chloride. Similarly, torula yeast without renewal attracted a 92% higher number of small fruit flies compared with weekly and biweekly renewal. Conversely, torula yeast + benzalkonium chloride attracted a higher number of small fruit flies when renewed weekly or biweekly (Table 3).

BAIT'S PH VARIATION OVER TIME

During the bait's laboratory analysis, the initial pH (1 d) of both solutions showed little variation. Torula yeast had an initial pH of approximately 8.9, whereas torula yeast + benzalkonium chloride had a pH of 8.8 (Fig. 3). Over time, torula yeast showed a quicker reduction in pH than torula yeast + benzalkonium chloride, although data showed a tendency to stabilize around 8.5. after 14 d of evaluation (Fig. 3).

ENVIRONMENTAL CONDITIONS

There were no major changes in the average temperature and relative humidity between evaluation periods. Average air temperature during the 2019 experiment was 27.4 °C, whereas maximum and minimum temperature was 33.2 °C and 21.6 °C, respectively (Fig. 4A). Similarly, during the 2020 experiment, the average air temperature was 27.1 °C, with a maximum air temperature of 33.4 °C, and a minimum air temperature of 20.7 °C (Fig. 4B). Average relative humidity ranged between 62.5% and 86.7% from Oct 2019 to Feb 2020 (Fig. 4A, B).

Discussion

After 4 mo of evaluation, 2 key findings clearly stand out: (1) the addition of benzalkonium chloride to torula yeast bait decreases the capture of Caribbean fruit fly, either due to a repelled effect or inhibition of microbial activity, therefore reducing attractive emissions, and (2) replacing the torula yeast solution at 1- or 2-wk intervals does not improve Caribbean fruit fly captures, compared to bait allowed to age in the field. Previous studies have reported that females of *A. suspensa* are more responsive to ammonia when sexually immature (Kendra et al. 2005), which is consistent with their necessity to feed to reach maturity and produce eggs. However, increasing concentrations of ammonium-based compounds may have caused torula yeast + benzalkonium chloride baits to be less attractive or have a repellent effect on females of *A. suspensa*.

Our results differed from Lasa and Williams (2017), because they found that Captor + borax containing 0.24 mg per mL of benzalkonium chloride and without renewal for 6 wk remained as effective as newly prepared Captor + borax. In this study, the benzalkonium chloride served as a stabilizer of the solution, buffering natural changes in pH. A common application of benzalkonium chloride is as an antimicrobial preservative. The slower change in pH of the torula yeast + benzalkonium chloride, compared to torula yeast alone, seemed to be an indicator of lower microbial activity in the solution, leading to a longer time of decomposition. Although, this preservation property was suitable when used in combination with Captor + borax, it was not useful when combined with the yeast-based bait. Volatiles of food baits, such as 3-methyl-1-butanol, largely are a byproduct of microbial metabolic pathways for protein breakdown (Drew et al. 1983; Davis et al. 2013; Biasazin et al. 2018). Ethyl hexanoate, 3-methylbutyl acetate, butyl acetate, and 3-methyl-1-butanol are volatiles commonly produced as a byproduct of different yeast fermentation process, including torula yeast (Biasazin et al. 2018). The production of these volatiles had been related to attraction of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), *Bactrocera zonata* (Saund) (Diptera: Tephritidae), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura) (Diptera: Tephritidae), *Anastrepha ludens* (Loew) (Diptera: Tephritidae), and *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) (Lee et al. 1995; Scheider et al. 2015; Biasazin et al. 2018). It is possible that the addition of benzalkonium chloride affected the microbial activity of both torula yeast and external microbes that could have been interacting with it, leading to a reduced production of volatiles and, hence, reduced attraction effects.

Additionally, the torula yeast solution seems to increase its attraction properties over time. Our treatments without renewal resulted in increased insects captures. Although the traps were not renewed completely, it is worth mentioning that they were still adjusted for evaporative losses. Our results resemble those of Malo (1992), who found no significant difference among treatments of torula yeast + borax with decomposition times of 2, 4, 6, 8, and 10 d for Caribbean fruit

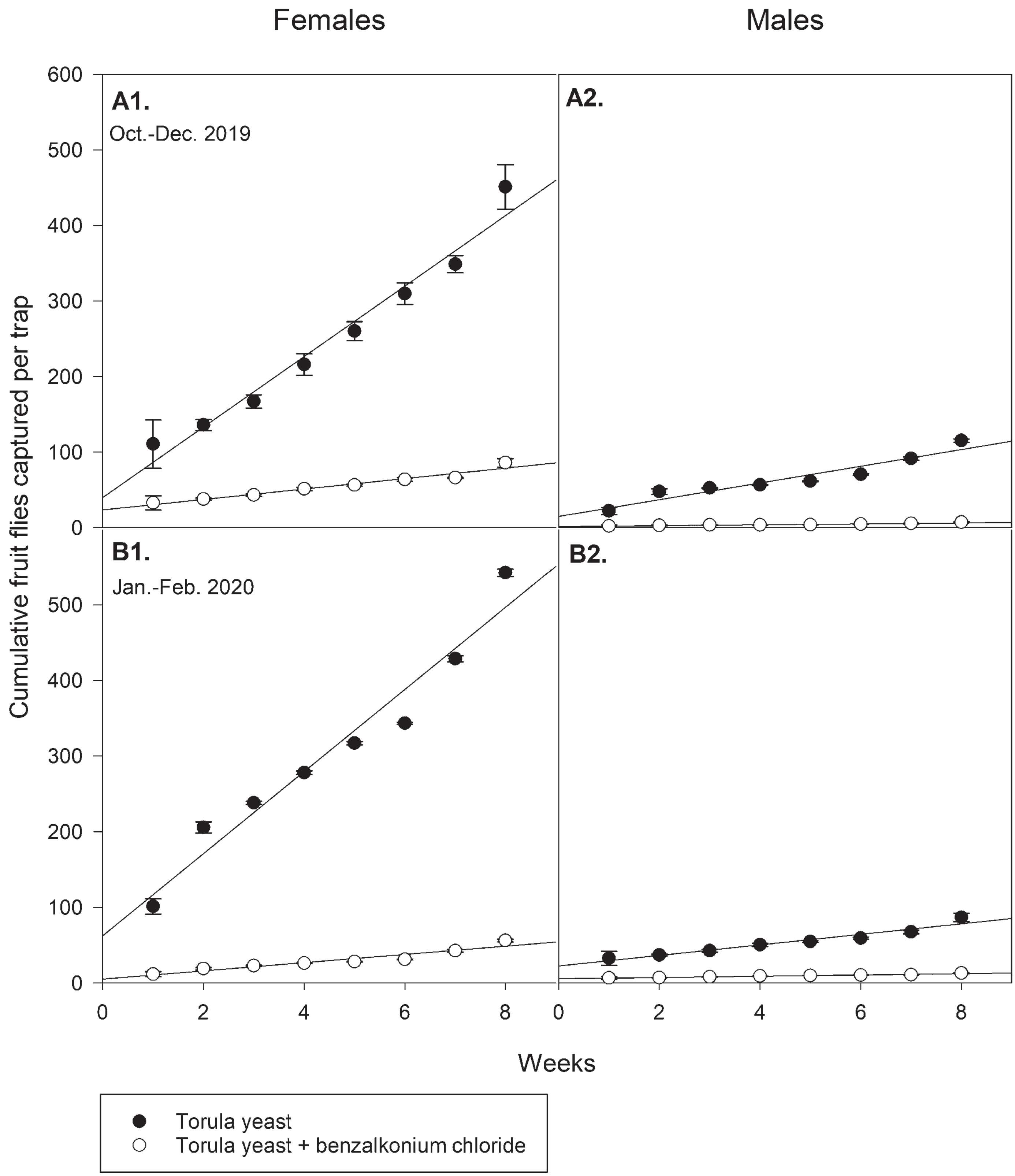

Fig. 1. Effect of torula yeast and benzalkonium chloride on the cumulative captures of females (A1) and males (A2) from Oct to Dec 2019, and females (B1) and males (B2) from Jan to Feb 2020 of Caribbean fruit fly (*Anastrepha suspensa*) per wk in guava production in San Cristobal, Dominican Republic.

fly captures. He suggested that traps renewed every 7 d served as poor attractants. This concurs with our results because weekly and biweekly renewal resulted in either lower or similar captures.

A similar attraction pattern was observed for captured non-targeted insects such as hymenopterans and lepidopterans. The latter were attracted mostly to torula yeast and were not influenced by the renewal

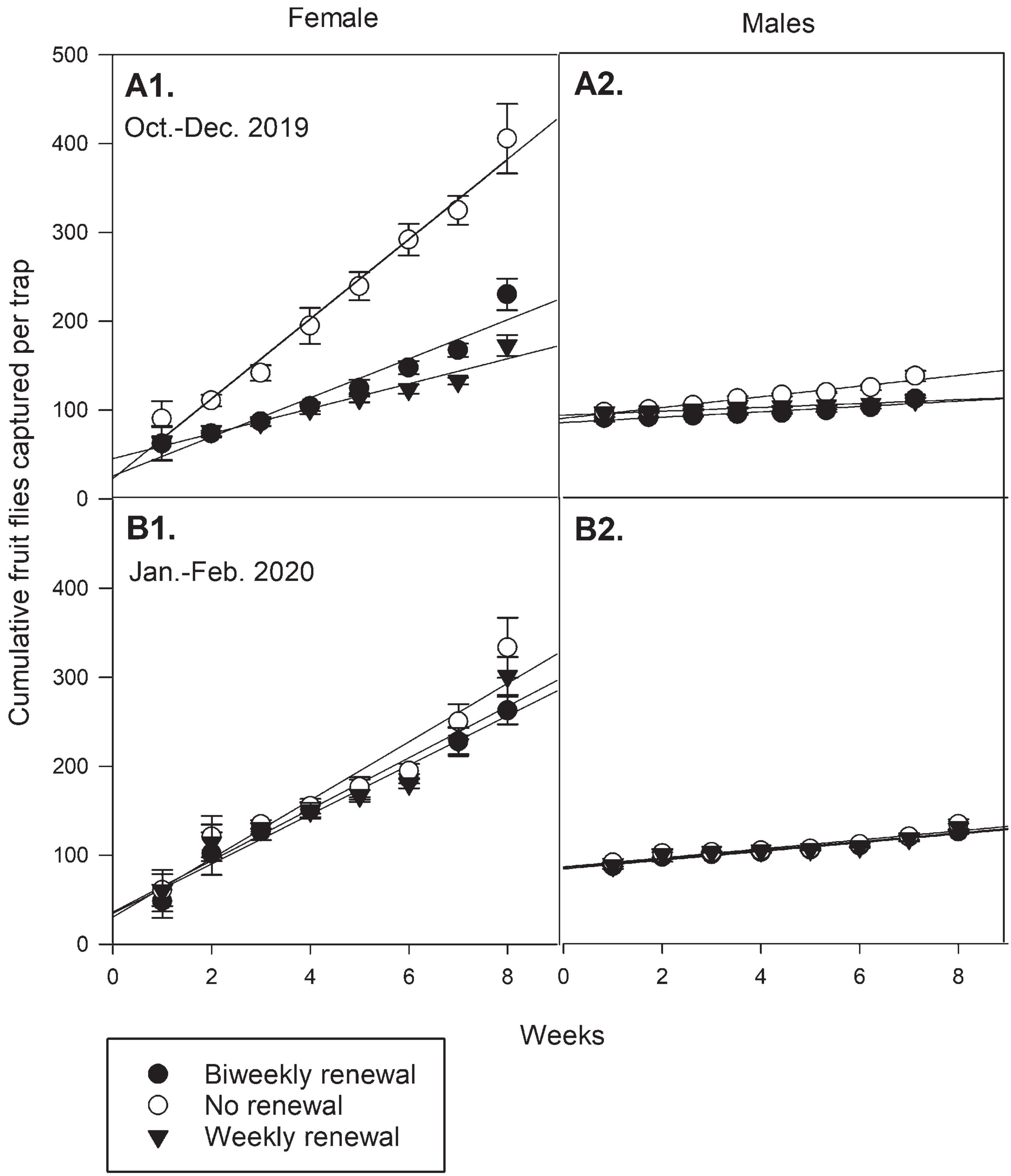

Fig. 2. Effect of weekly and biweekly renewal and no renewal of baits on the cumulative captures of females (A1) and males (A2) from Oct to Dec 2019, and females (B1) and males (B2) from Jan to Feb 2020 of Caribbean fruit fly (*Anastrepha suspensa*) per wk in guava production in San Cristobal, Dominican Republic.

frequency. On the other hand, other dipterans such as phorids, muscids, and drosophilids were influenced by the renewal frequency. This could be a response to a different volatile spectrum resulting from early fermentation processes and different protein requirements compared to Caribbean fruit fly. Nonetheless, none of the non-targeted fly species collected during the evaluations were identified as economically important.

Another interesting finding was the changes in population and ratio of male to female captures. Common male:female ratios are 1:2.4 for

Table 2. Effect of bait type and time of bait renewal on non-targeted insects in guava production in San Cristobal, Dominican Republic.

Treatment	Oct to Dec 2019[a] – Average non-targeted insects per trap per wk						
Bait type	Muscidae	Lonchaeidae	Phoridae	Formicidae	Drosophila	Hymenoptera[d]	Lepidoptera
Torula yeast	212.80 ± 111.73	40.69 ± 12.96	26.54 ± 19.33	168.82 ± 356.85	3.57 ± 3.65	15.22 ± 16.68	5.31 ± 4.22
Torula yeast + benzalkonium chloride[b]	39.74 ± 19.57	1.95 ± 1.55	7.91 ± 9.67	18.05 ± 9.81	5.31 ± 4.48	1.51 ± 2.45	1.75 ± 2.23
Significance[c]	*	*	*	*	NS	*	*
Bait renewal							
None	133.8 ± 171.40 a	10.75 ± 21.27	25.30 ± 24.56 a	76.62 ± 431.01	3.89 ± 2.50 ab	4.25 ± 13.95	3.79 ± 5.37
Weekly	73.1 ± 81.27 b	9.00 ± 24.61	8.33 ± 8.80 b	60.66 ± 221.85	3.07 ± 5.23 b	7.32 ± 19.75	3.17 ± 3.49
Biweekly	80.2 ± 105.75 b	10.75 ± 25.32	14.85 ± 10.81 ab	37.02 ± 80.51	6.76 ± 3.77 a	4.89 ± 14.09	2.72 ± 2.13
Significance	*	NS	*	NS	*	NS	NS
Bait type × bait renewal	NS	NS	NS	NS	NS	NS	NS
	Jan to Feb 2020[a] – Average non-targeted insects per trap per wk						
Bait type	Muscidae	Lonchaeidae	Phoridae	Formicidae	Drosophila	Hymenoptera	Lepidoptera
Torula yeast	330.13 ± 331.97	73.13 ± 64.56	127.82 ± 112.58	50.29 ± 78.61	203.17 ± 1188.45	11.30 ± 8.68	4.50 ± 3.05
Torula yeast + benzalkonium chloride	25.30 ± 94.68	2.89 ± 14.38	15.60 ± 6.96	9.72 ± 6.52	18.50 ± 19.34	1.14 ± 1.08	0.48 ± 0.88
Significance	*	*	*	*	*	*	*
Bait renewal							
None	94.50 ± 462.78	27.18 ± 94.43	60.66 ± 161.80	30.62 ± 99.42	94.50 ± 1439.61	2.80 ± 7.21	1.51 ± 2.96
Weekly	78.43 ± 148.91	11.30 ± 26.85	37.02 ± 40.99	23.55 ± 43.03	57.88 ± 60.27	6.59 ± 11.95	1.69 ± 2.55
Biweekly	108.65 ± 207.38	12.80 ± 27.08	40.69 ± 61.49	15.60 ± 28.61	42.65 ± 81.69	3.68 ± 5.09	2.98 ± 2.64
Significance	NS	NS	NS	NS	NS	NS	NS
Bait type × bait renewal	*	NS	NS	NS	*	NS	NS

[a]Field evaluations between 27 Oct and 21 Dec 2019, field evaluations between 1 Jan and 23 Feb 2020; [b]Torula yeast pellets (34.2% torula yeast + 57.2% borax) and benzalkonium chloride (80% purity); [c]Values followed by different letters indicate that the means are significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference test; NS, * = Nonsignificant or significant at $P \leq 0.05$; [d]Hymenoptera includes sawflies, wasps and bees, Lepidoptera includes butterflies and moths.

Table 3. Effect of bait type and time of bait renewal interaction on non-targeted insects in guava production between Jan and Feb 2020, in San Cristobal, Dominican Republic.

Treatment	Average non-targeted insects per trap per wk					
	Muscidae			Drosophila		
	Bait renewal					
Bait type	None	Weekly	Biweekly	None	Weekly	Biweekly
Torula yeast	615.59 ± 403.92 Aa[a]	280.83 ± 65.01 Aa	217.77 ± 251.89 Aa	1095.47 ± 1705.62 Aa	113.81 ± 51.90 Ab	66.60 ± 107.24 Ab
Torula yeast + benzalkonium chloride[b]	13.79 ± 2.94 Bb	21.38 ± 9.10 Bab	53.95 ± 148.50 Ba	7.51 ± 5.67 Bb	29.90 ± 25.48 Ba	27.84 ± 11.91 Aab

[a]Values followed by different uppercase letters (columns) indicate that the means of the bait are significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference test within time of bait renewal; values followed by different lowercase letters (rows) indicate that the means of the time of bait renewal are significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference test within bait type; [b]Torula yeast pellets (34.2% torula yeast + 57.2% borax) and benzalkonium chloride (80% purity).

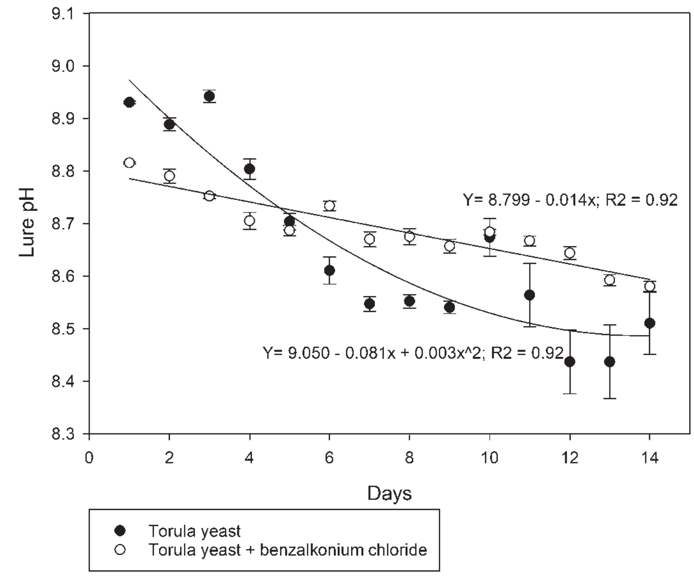

Fig. 3. Daily pH changes of torula yeast pellets (34.2% torula yeast + 57.2% borax) (two 4 g pellets in 200 mL of water) and torula yeast pellets + benzalkonium chloride (80% purity) (two 4 g pellets in 200 mL of water + 16 mL of benzalkonium chloride) under laboratory conditions in San Cristobal, Dominican Republic.

Anastrepha ludens (Loew) and *Anastrepha obliqua* (Macquart) (both Diptera: Tephritidae) (Malo 1992). In our study, we found a 1:5.3 and 1:4.9 male:female ratio for *A. suspensa* in 2019 and 2020, respectively. Over all evaluations, higher numbers of females were captured, presumably because females required the protein for ovarian development and sexual maturation (Malo 1992). Furthermore, there was a small increase in the Caribbean fruit fly population in 2020 compared to 2019. Environmental conditions did not seem to have an effect on fruit fly population. As mentioned before, the increase in population from period to period could be related to the higher number of fruits being produced by the plants as we were approaching the beginning of the harvest, allowing the flies to have less competition for oviposition sites, and increasing their overall reproduction. Additionally, the main increases in population seem to be related to an increase in the number of males, as suggested by the changes in male:female ratio.

Maintenance and trap renewal for fruit fly scouting in large commercial fields is labor intensive and expensive. Similarly, government efforts to monitor different species of fruit flies across a country such as the Dominican Republic require intensive organization, a large body of labor, and resources. Any effort aiming to optimize the scouting process of Tephritidae species could represent a reduction in cost and better time management of the teams involved.

Our results suggest that *A. suspensa* trapping can be done efficiently with torula yeast (34.2% torula yeast + 57.2% borax) without the addition of a stabilizer such as benzalkonium chloride. Additionally, there is little requirement for the bait to be renewed, other than for compensation for evaporative losses. It is recommended to prepare and store a stock solution of torula yeast for later use to compensate for evaporative losses of the traps, while allowing a proper decomposition time to maximize their attraction properties.

There is a lack of basic information regarding fruit fly trapping methods, population changes over time, and seasonal changes among fruit crops in the Dominican Republic. Future research in the Dominican Republic should focus on evaluating extended periods of storage of torula yeast to identify the maximum and most efficient decomposition times. Additionally, changes in population dynamics of *A. suspensa* and other fruit fly species should be evaluated according to seasonal changes of fruit crops of economic importance.

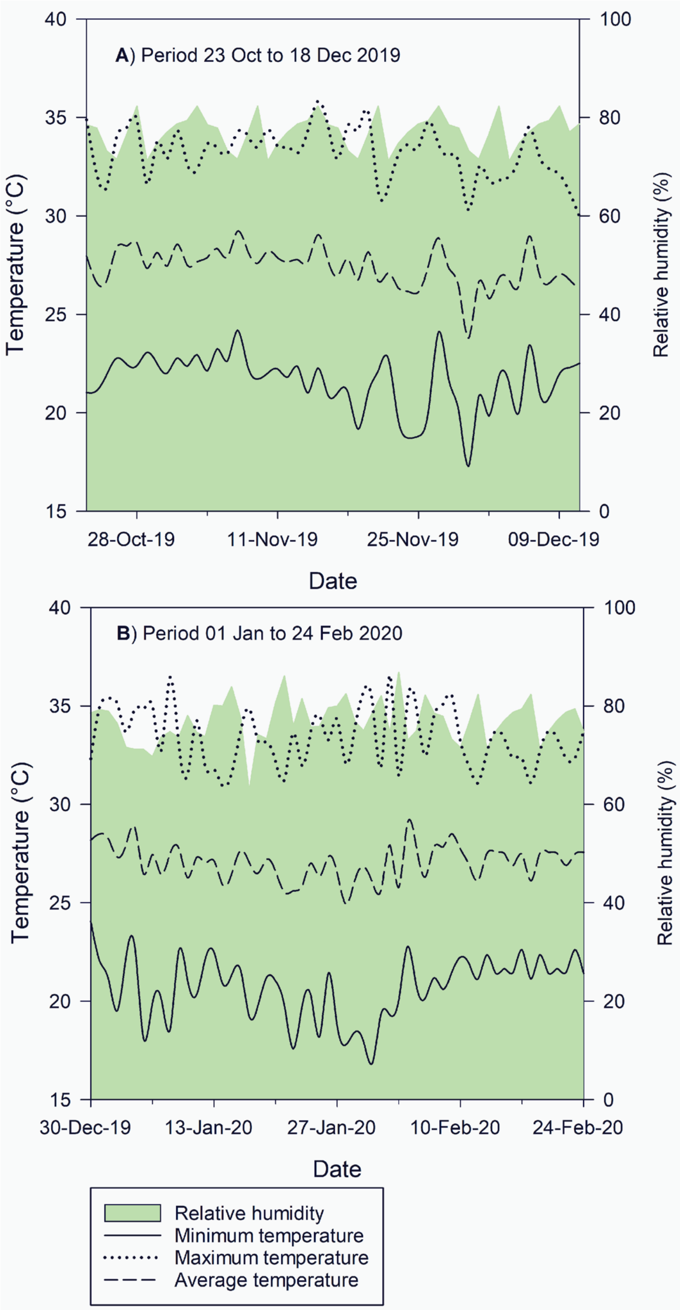

Fig. 4. Minimum, average, and maximum air temperature, and relative humidity at the experimental site between 23 Oct 2019 and 23 Feb 2020 in San Cristobal, Dominican Republic; (1.8 × °C) + 32 = °F.

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