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Field Evaluation of *Beauveria bassiana* and Novaluron Alone and Combined, versus Conventional Insecticides to Control *Lygus lineolaris* in Cotton

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Abstract

This study examined the efficacy of a native strain of *Beauveria bassiana* (*Bb*) (Balsamo) Vuillemin alone and in combination with the insect growth regulator (IGR) novaluron, as well as a set of insecticides, also tested individually and in combination for suppressing population of tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) and evaluating the effect on cotton. The suppression of TPB was significantly different among treatments for plots sprayed with tank-mixed of bifenthrim + novaluron + acephate for both years. No significant differences were observed between single or two tank-mixed synthetic insecticides (imidacloprid or sulfoxaflor or befenbrin + novaluron) compared with novaluron alone or novaluron + *Bb*, except for imidacloprid when the second spray was 7D after the first spray. Control and *Bb* alone had the lowest population suppression at all evaluation times regardless of sprays and years. This was reflected in the cotton lint yield per harvested plot, where *Bb* (411 ± 230 and 533 ± 58-SD kg/ha) showed no significant differences among control (487 ± 152 and 446 ± 42-SD kg/ha) with the lowest production between treatments for the years of 2018 and 2019, respectively. No significant differences in lint yield were observed between the plots spray with the set of synthetic insecticides, (919 ± 250 and 1178 ± 38-SD Kg/ha), novaluron alone (919 ± 265 and 882 ± 29-SD Kg/ha), and novaluron + *Bb* (899 ± 105 and 1061 ± 46-SD Kg/ha) for 2018 and 2019, respectively. These results indicated that IGR novaluron, alone and in combination with *Bb*, could suppress TPB population as effectively as synthetic insecticides. *Bb* alone could suppress populations of nymphs compared to the control; however, its performance was not enough to prevent cotton damage and affect lint cotton yields.

Keywords

Insecticides, IGR, *B. bassiana*, *Lygus*

1. Introduction

The tarnished plant bug (TPB), *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) continues to devastate cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae) in cotton belt states such as Mississippi, Arkansas, Tennessee, Louisiana, and Missouri, and is also emerging as a major pest in Georgia and Alabama [1]. Huoni *et al.* [2] mentioned that in Mississippi alone, TPB caused nearly $270/hectare in losses, accounting for approximately 70,000 lost bales per annum during 2021-2022. Since the early 1990s, TPB control has relied primarily on synthetic insecticides, which has contributed to insecticidal resistance [1] [3]-[6]. Currently, especially in the Mississippi Delta, control and resistance management of the TPB involves insecticide rotations, tank-mixing of multiple insecticides with different modes of actions, or two sequential insecticide applications at 4 - 7-day intervals [7] [8].

Cotton farmers worldwide saturate their cotton crops with pesticides classified as hazardous by the World Health Organization [1]. Regardless of resistance level, organophosphates and pyrethroids are the most commonly used contact insecticides to control this pest. During the last decade newer insecticides with a different mode of action that act on the central nervous system of insects have been introduced, including neonicotinoids, sufolxamines [9]. However, their frequent use can lead to insecticide resistance, and their impact extends beyond the target pests, raising concerns about environmental and public health effects [6]-[10]. A safer benzoylphenyl urea insecticide, the insect growth regulator (IGR) Novaluron, was introduced and approved for use on cotton in the early 2000s [1] [9]. It primarily affects immature stages of various insects by disrupting their ability to synthesize chitin, a key component of their exoskeletons [5]. Novaluron is considered to have low toxicity to mammals, birds, and beneficial insects like bees; however, it can be toxic to certain aquatic organisms (https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-124002_24-Sep-01.pdf).

Efforts have been undertaken to identify indigenous microbial pathogens as potential alternative control methods of TPB in integrated pest management (IPM) [11] [12]. Experimental work with the deutoromycete fungus *Beauveria bassiana* (Balsamo) Vuillemin alone and in combination with insecticides has been conducted frequently for management of various insect pests [13]-[19]. Little *et al.* [8] and Portilla *et al.* [20] conducted a two-year field experiment to study economic comparisons of a native entomopathogenic strain of *B. bassiana*, NI8, and conventional insecticides for TPB control in cotton. They found no significant differences between treatments in yield for non Bt and Bt cottons for the first year. However, NI8 was ineffective for the second year. These two studies are the only research that reported the effect of *B. bassiana* on TPB mortality and cotton yield; however, neither of the studies followed TPB populations under field conditions. The current study was conducted over a two-years period to assess the potential for using the IGR Novaluron alone and in combination with the NI8 strain, and the NI8 strain alone, vs. conventional insecticides alone and combined in tank mixes with different modes of actions. Cotton lint yield and insect density were used to evaluate the potential of the treatments.

2. Materials and Methods

2.1. Field Experiment

This experiment was conducted at the Southern Insect Management Research Unit's (SIMRU) research farm (33.3456, −90.9169), Leland, MS. It was conducted using Bollgard II™ cotton (DP1321B2RF®, Delta and Pine Land Company™, Scott, MS). Twenty cotton plots were planted in late May 2018 and replicated in 2019 arranged in a randomized complete block design with four blocks, each with five plots per block. Each plot (80.7 m long) consisted of 12 rows flanked by 4 rows of corn, *Zea mays* L. (VT2Pro® corn, DKC66-97®, DeKalb Genetics Corp., DeKalb, IL). The corn was planted in early March of each year as a barrier to prevent cross-contamination among plots and treatments. During the study, herbicides and plant growth regulators were applied to cotton plots as needed. Each treatment was randomly assigned to plots within each block of the experiment, and each treatment was used three times during each year as follows: 1) untreated check, 2) conventional insecticides alone and in tank mixed, 3) novaluron alone, 4) novaluron + *B. bassiana* NI8, 5) *B. bassiana* NI8 alone. The sprayable NI8 formulation was obtained by mixing 250 g of spore powder with 60 mL of Tween-80 (TWEEN 80; Sigma-Aldrich P8074, Darmstadt, Germany) (0.04%)/gallon of water (2.5×10^{11} spores/ha), as described in Portilla *et al.* [20]. The selected insecticides were those commonly used to control TPB population in Mississippi [6] [8] [21], which include novaluron-IGR (Diamond®, Adama Ltd., Raleigh, NC, USA), imidacloprid-neonicotinoid (AdmirePro™, BayerCropScience LP, Research Triangle Park, USA), sulfoxaflor-sulfoxamine (Transform WG™, Corteva Agriscience, Indianapolis, IN, USA), acephate-organophosphate (Bracket 90 WSP™, AgriSolutions, St. Paul, MN, USA), and bifenthrin-pyrethroid (Brigade® 2EC FMC Agricultural Sciences Company, Philadelphia, PA, USA). The commercial name, active ingredients, rates, and application dates for TPB control are listed in **Table 1**. Tarnished plant bug sampling began in mid- to late June for 2018 and 2019, respectively. It was used to determine the timing of the treatment applications. Each plot was sampled and TPB populations were recorded before each spray. The timing of applications was determined based on the threshold established by the MS Insect Control Guide for Agronomic Crops (first two weeks of squaring: 8 TPB/100 sweep nets, third week of squaring through bloom: 15 TPB/100 sweeps) [21]. The numbers of TPB collected in five sets of 25 sweep nets/row/plot determined application timing, and sampling five sets of 25 sweep nets/row/plot 2, 4 or 7 days after spray determined treatment effectiveness. Therefore, the experimental unit was considered a set of five 25 sweeps/plot/application. Cotton plots were sprayed with a tractor equipped with a multi-boom sprayer (TecjetTM-Conejet® TXVS12 nozzle) calibrated to apply 93.54 L of water/ha. The efficacy of each treatment in cotton lint yield was determined as described in Little *et al.* [8]. Briefly, the center 2 rows of each plot were harvested using a mechanical harvester (Jonh Deere 699 cotton picker modified for small plot research) and the weight of seed cotton was recorded. Lint yield was assessed as 37% of seed cotton weight [22]. The insecticide prices used in the control cost analysis were the four-year average (2022-2025) cost of each active ingredient obtained from the Mississippi cotton planning budgets (2021-2024). We used the price of a commercially available *B. bassiana* (BotaniGard 22WP) formulation. We adjusted the number of spores applied per hectare to obtain the application cost of the NI8 strain of *B. bassiana* which was $2.25 per hectare. The expenditure for each insecticide spray was the four-year average of the aerial application cost per hectare of applying 9.35 liters/ha which was $16.28 per hectare [23]-[26]. The price for cotton lint was set at $1.65/kg and the prices for each insecticide were calculated as follow: acephate (kg) $17.82, diamond (L) $59.34, imidacloprid (L) $26.29, bifenthrin (L) $25.61, transform (kg) $296.97, and botanigard/ha $2.25.

Table 1. Application dates, treatment, and rates for the efficacy of *Beauveria bassiana* strain NI8 alone and in combination with novaluron and conventional insecticides.

Application date	Sprays	Treatment	Rates AI*
06-21-2018	1	Control	354 ml MC-PGR**/ha
		*B. bassiana****	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Admire Pro)	354 ml MC-PGR + 118 ml Imidacloprid /ha
06-27-2018	2	Control	354 ml MC-PGR/ha
		B. bassiana	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Admire Pro)	354 ml MC-PGR + 118 ml Imidacloprid /ha
07-03-2018	3	Control	354 ml MC-PGR/ha
		B. bassiana	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Brigade + Diamond + Bracket)	354 ml MC-PGR + 189 ml Bifenthrin + 266 ml novaluron + 454 g acephate /ha

Continued

07-01-2019	1	Control	354 ml MC-PGR/ha
		B. bassiana	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Brigade + Diamond + Bracket)	354 ml MC-PGR + 189 ml Bifenthrin + 266 ml novaluron + 454 g acephate /ha
07-23-2019	2	Control	354 ml MC-PGR/ha
		B. bassiana	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Transform)	354 ml MC-PGR + 64 g Sulfoxaflor /ha
07-30-2019	3	Control	354 ml MC-PGR/ha
		B. bassiana	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR /ha
		B. bassiana + Diamond	2.5×10^{11} spores + 60 ml Tween-80 + 354 ml MC-PGR + 354 ml novaluron /ha
		Diamond	354 ml MC-PGR + 354 ml novaluron /ha
		Conventional (Brigade + Diamond)	354 ml MC-PGR + 238 ml Bifenthrin + 266 ml novaluron

*Active Ingredient. **Mepiquat chloride-Plant growth regulator. ****Beauveria bassiana* (*B.b.*) Stock conidia (Delta Native Strain NI8) were obtained from the USDA-ARS Southern Insect Management Research Unit (SIMRU), Stoneville, MS. Eight plastic bags came labeled as follows: Spore powder 250 g mix in 60 mL of Tween-80 (0.04%)/gallon of water, giving a concentration of 2.5×10^{11} spores/ha^{-1}. All treatment were applied in the morning when the weather conditions permitted.

2.2. Statistical Analysis

A randomized complete block design with factorial arrangements of 5 treatments, 5 sets of sweep net samples, and 4 replicates per application was used, with 0 to 2-D before and 2, 4, 7-D after spray, to assess population densities (nymphs, adults, and total TPB population). Five treatments replicated 4 times were used for cotton lint yield and net returns above tarnished plant bug control. The TPB population density, the efficacy of the treatments on cotton lint yield (Kg/ha), and net return above TPB control cost ($/ha) were analyzed using SAS 9.4 [27]. Variables were analyzed by using a t PROC GLM (ANOVA) followed by Tukey's HSD.

3. Results

3.1. Tarnished Plant Bug Population Density

During 2018, there were no statistically significant differences in TPB density for the initial population 1-D before spray for nymphs (F = 0.95; df = 4, 3; P = 0.4396), adults (F = 1.45; df = 4, 3; P = 0.2241), and total population (F = 1.25; df = 4, 3; P = 0.2976) ranging from 2 to 3 TPB per 25 sweeps/plot (Jun-21-2018) (**Figure 1(A)**). Significant differences in population densities were found 2-D after the first spray except for nymphs with <1 nymph/25 sweeps/plot : adults: (F = 3.82; df = 4, 3; P= 0.0006), and total population: (F = 5.59; df = 4, 3; P= 0.0005) (Figure 1(B)),

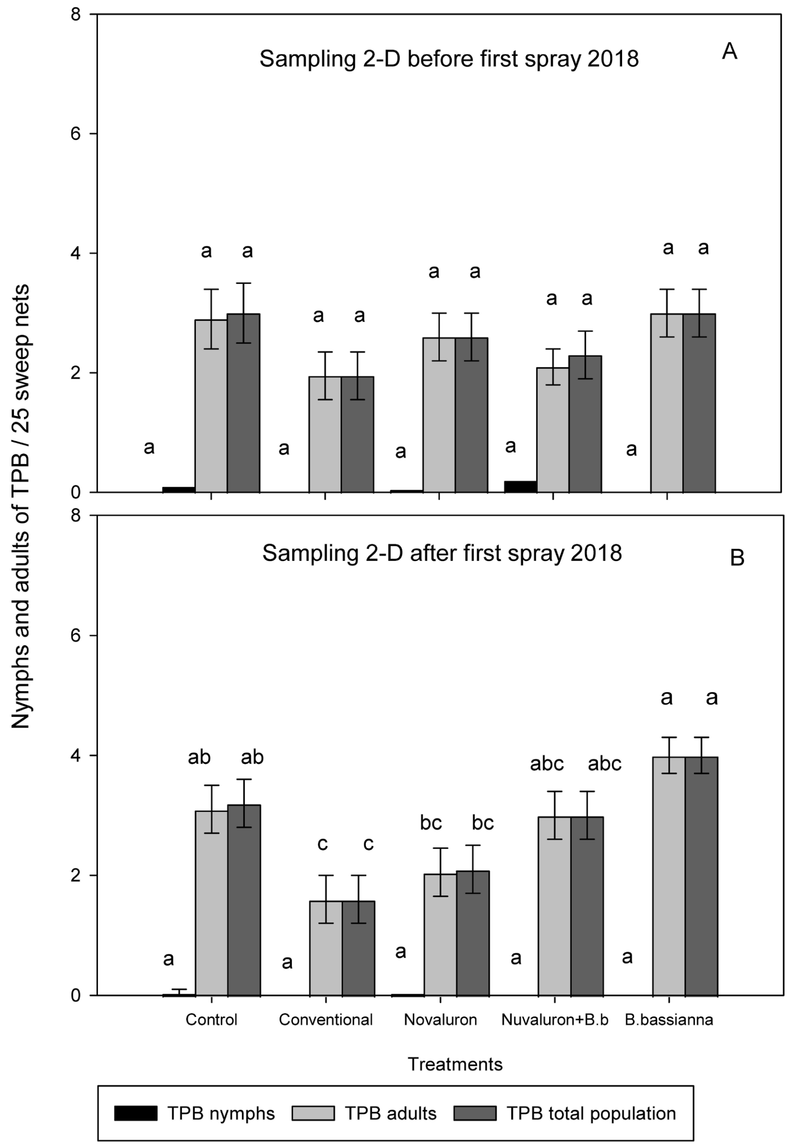

Figure 1. Mean ± SD number of tarnished plant bug nymphs, adults, and total population/25 sweeps/plot before (A) and after (B) application in 2018 (first spray-conventional: imidacloprid). Means separated by a common letter are not significant different according to Tukey Test LSD (p = 0.05).

with the lowest TPB population in plots sprayed with the conventional insecticide imidacloprid and the highest density in plots that were sprayed with *B. bassiana*. No significant differences in populations were observed between novaluron alone, novaluron + *B. bassiana*, and control. Figure 1(B) shows that none of the treatments were effective in suppressing TPB population below threshold 2-D after spray, with the population densities increasing 5-D after the first spray (Figure 2(A)) ranging from 2 to 5 TPB/25 sweeps/plot. The initial population 1-D before the second spray (Jun-27-2018) showed no highly significant differences in adults ($F = 0.95$; df = 4, 3; $P = 0.4396$) and total population density ($F = 0.95$; df = 4, 3; $P = 0.4396$) among treatments, where the control treatment had the highest population density (**Figure 2(A)**). No significant differences in nymph populations

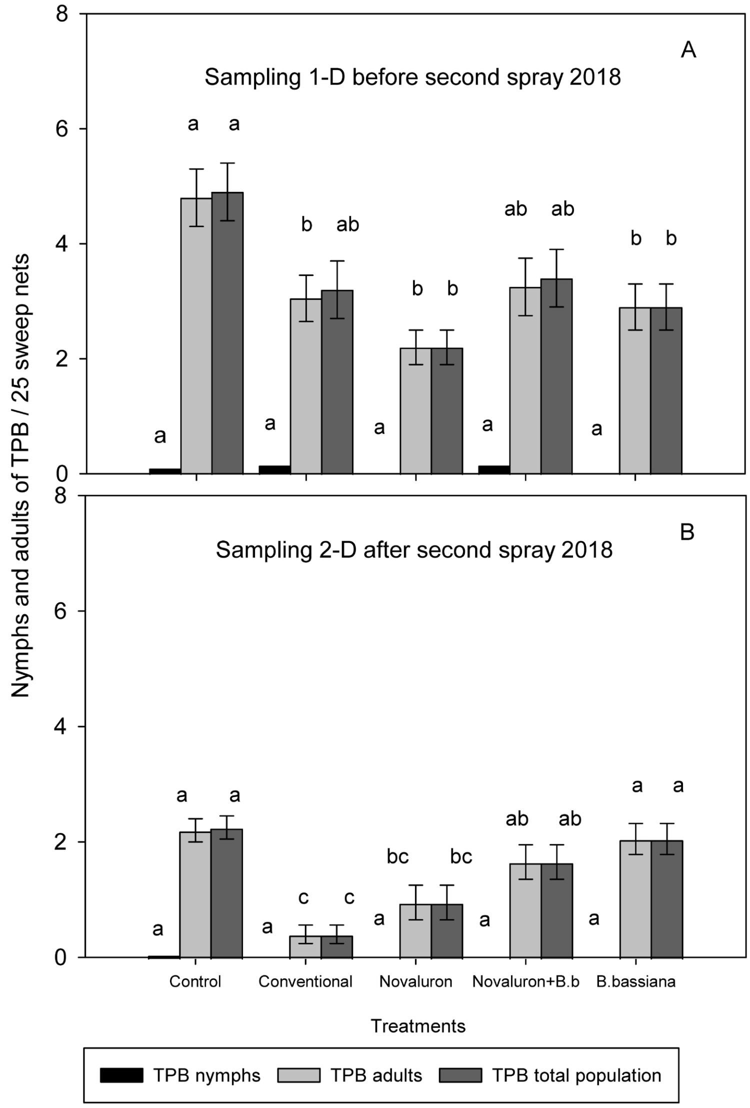

Figure 2. Mean ± SD number of tarnished plant bug nymphs, adults, and total population/25 sweeps/plot before (A) and after (B) application in 2018 (Second spray-conventional: imidacloprid). Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

were observed among treatments ($F = 0.95$; df = 4, 3; $P = 0.4396$). The population density dropped below the threshold 2-D after the second spray for the conventional plots (imidacloprid) and novaluron alone with a total population of <1 and 1 TPB/25 sweeps, respectively (**Figure 2(B)**). No significant differences were found between novaluron +*B. bassiana*, *B. bassiana* alone and control with 1.5, 2, and 2.5 TPB/25 sweeps/plot, respectively. Nymphs were not observed for

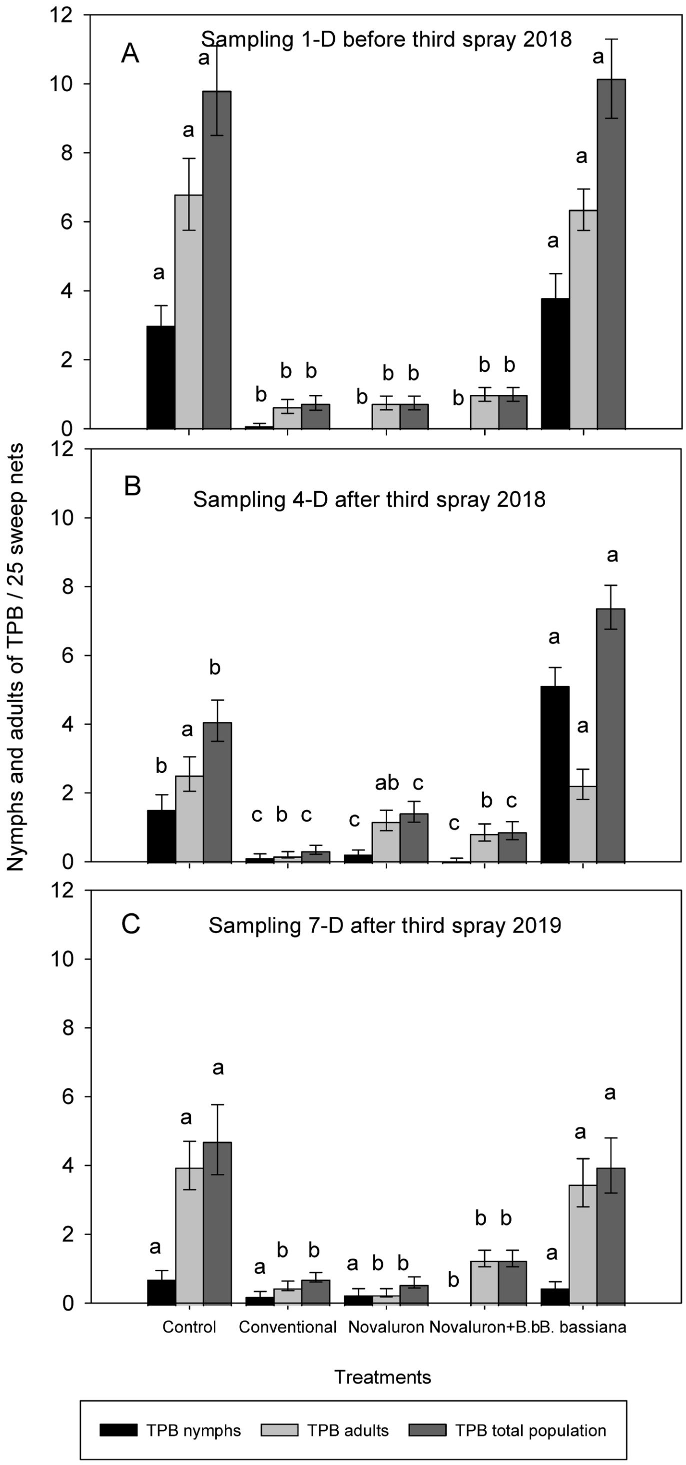

Figure 3. Mean ± SD number of tarnished plant bug nymphs, adults, and total population/25 sweeps/plot before (A) and after (B) application in 2018 (third spray-conventional: acephate + bifenthrin + novaluron). Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

any treatment except for the control 2-D after the second spray. Figure 3(A) shows that although the population was below threshold for conventional, novaluron alone, and novaluron + *B. bassiana*, a third spray was applied on July-03-2018 due to densities of TPB nymphs and adults in the control and *B. bassiana* treatment (10 TPB/25 sweeps/plot (**Figure 3(A)**)). The conventional treatment for this spray was a tank mix of bifenthrin + novaluron + acephate (**Table 1**), which had the lowest population (0.3 TPB/25 sweep nets/plot) 4-D after spray with no significant differences among novaluron + *B. bassiana* (0.7 TPB/25 sweep nets/plot) (**Figure 3(B)**). The population pattern continued 7-D after spray with the highest densities on the control and *B. bassiana* treatments; however, nymph population densities drastically decreased with *B. bassiana* alone, although no significant differences were observed with the control treatment for nymphs, adults, and total population (**Figure 3(C)**).

The initial density for 2019 started with significant differences among treatments 2-D before spray for both adults (F = 3.85; df = 4, 3; $P = 0.0014$) and the total population (F = 5.45; df = 4, 3; $P = 0.0006$). Nymph populations were similarly to 2018 with low densities that did not differ among treatments (F = 1.63; df = 4, 3; $P = 0.1737$) (**Figure 4(A)**). Highly significant differences were found in 2-D after the first spray on July-01-2019 for adults and total population, with significantly lower populations of TPB (0.05 TPB/25 sweeps/plot) in plots sprayed with a tank mix of conventional insecticides (bifenthrin + novaluron + acephate) (**Figure 4(B)**). No significant differences were observed in numbers of TPBs between novaluron and novaluron + *b. bassiana* plots. Although *B. bassiana* alone lowered the population, it was not significantly different compared to the control; however, it differed from the control 7-D after spray, with the total TPB population below threshold (1.5 TPB/25 sweeps/plot), which was lower than control (2.5 TPB) but higher than conventional, novaluron, and novaluron + *B. bassiana* with 0.5, 0.9, and 0.4 TPB/25 sweeps/plot, respectively (**Figure 4(C)**). Population densities remained low for the next 3 weeks after the first spray. High densities suddenly appeared after three weeks, with the total population ranging from 7 (conventional plots) to 18 (untreated plots) TPB/25 sweeps/plot (**Figure 5(A)**). Highly significant differences of both nymphs (7.20; df = 4, 3; $P = 0.0001$) and adults were observed 2-D before applying the second spray on July-23-2019 (**Figure 5(A)**). Although, after the application, the population decreased with highly significant differences in densities among treatments, none of the treatments lowered the population below threshold (**Figure 5(B)**). No significant differences were observed between conventional (sulfoxaflor), novaluron, and novaluron + *B. bassiana* with 3.3, 4.6, and 4.0 TPB/25 sweeps/plot, respectively; but they differed among the control (9.3) and *B. bassiana* alone treatments (10.4) (**Figure 5(B)**). **Figure 6(A)** shows that 6-D after the second application the population density drastically increased, ranging from 6.3 (conventional plots) to 19.0 (control plots) TPB/25 sweeps/plot, with the highest significant differences among treatments for both nymphs (12.34; df = 4, 3; $P = 0.0001$) and adults (19.20; df = 4, 3; $P = 0.0001$). **Figure 6(B)** shows the densities 3-D after spray, where none of the treatments decreased populations. Similar to the first and second application, no significant differences in population density were observed in the third spray 3-D after application between conventional, novaluron, and novaluron + *B. bassiana*. *Beauveria bassiana* alone had a lower population than the control with significant differences among them. The population density continued to decline 7-D after spray for all treatments, including the control (**Figure 6(C)**).

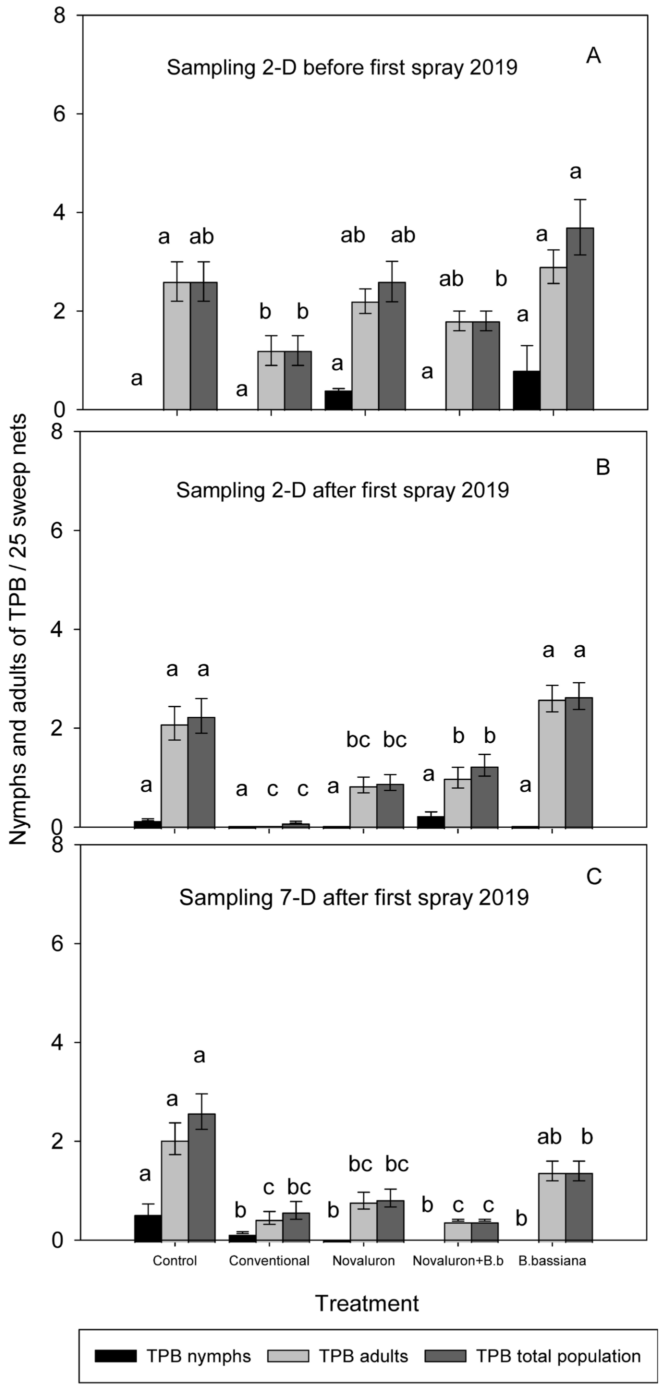

Figure 4. Mean ± SD number of tarnished plant bug nymphs, adults, and total population/25 sweeps/plot before (A) and after (B) application in 2019 (First spray-conventional: acephate + bifenthrin + novaluron). Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

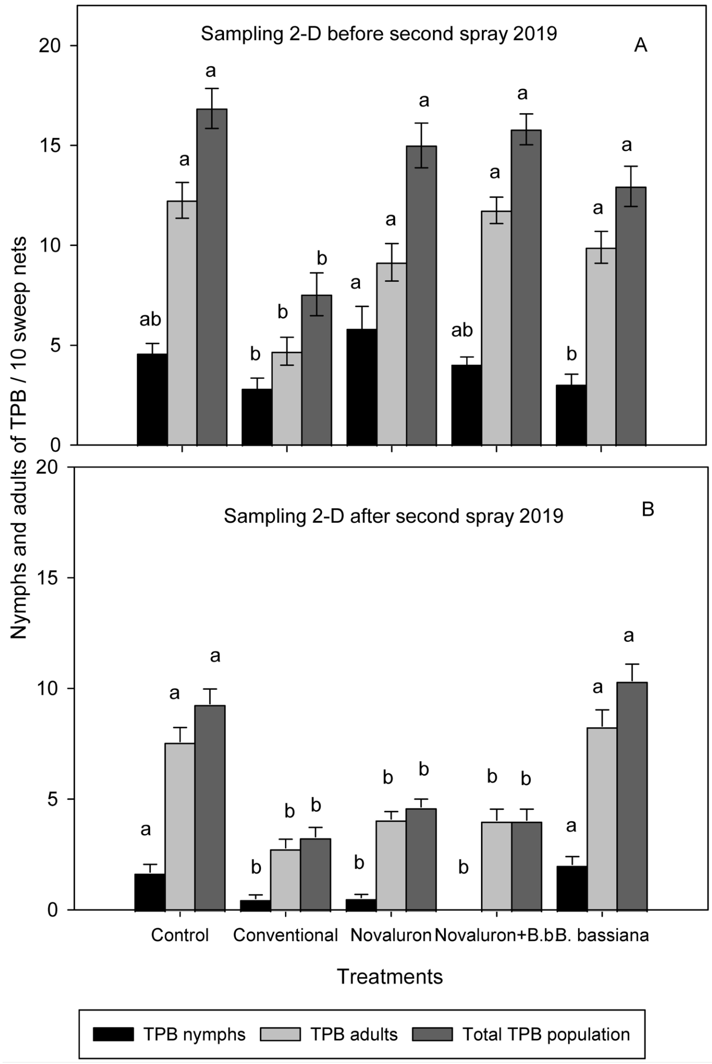

Figure 5. Mean ± SD number of tarnished plant bug nymphs, adults and total population/25 sweeps/plot before (A) and after (B) application in 2019 (Second spray-conventional: Sulfoaflor). Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

3.2. Efficacy of the Treatments on Cotton Lint Yield and Net Return

High variation among replications was observed for cotton lint yield among treatments in 2018 (Figure 7(A)). During 2019 distributions of the minimum and maximum values of plots were much smaller than that in 2018 for all treatments except for *B. bassiana* alone (Figure 7(B)). Highly significant differences were observed between treatments in 2018 (11.09; df = 4, 3; $P = 0.0005$), but no significant differences were obtained between conventional (919.23 ± 125 Kg of lint/ha), diamond alone (919.23 ± 265 Kg of lint/ha), and diamond + *B. bassiana* (898.91 ± 105 Kg of lint/ha) for 2019. The cotton lint yields for diamond + *B. bassiana* (898.91 ± 105 Kg of lint/ha) did not differ statistically from the control (487.55 ± 152 Kg of lint/ha). *B. bassiana* alone had the lowest cotton lint production (411.37 ± 115 Kg of lint/ha). In 2019 significant differences in cotton lint yields among

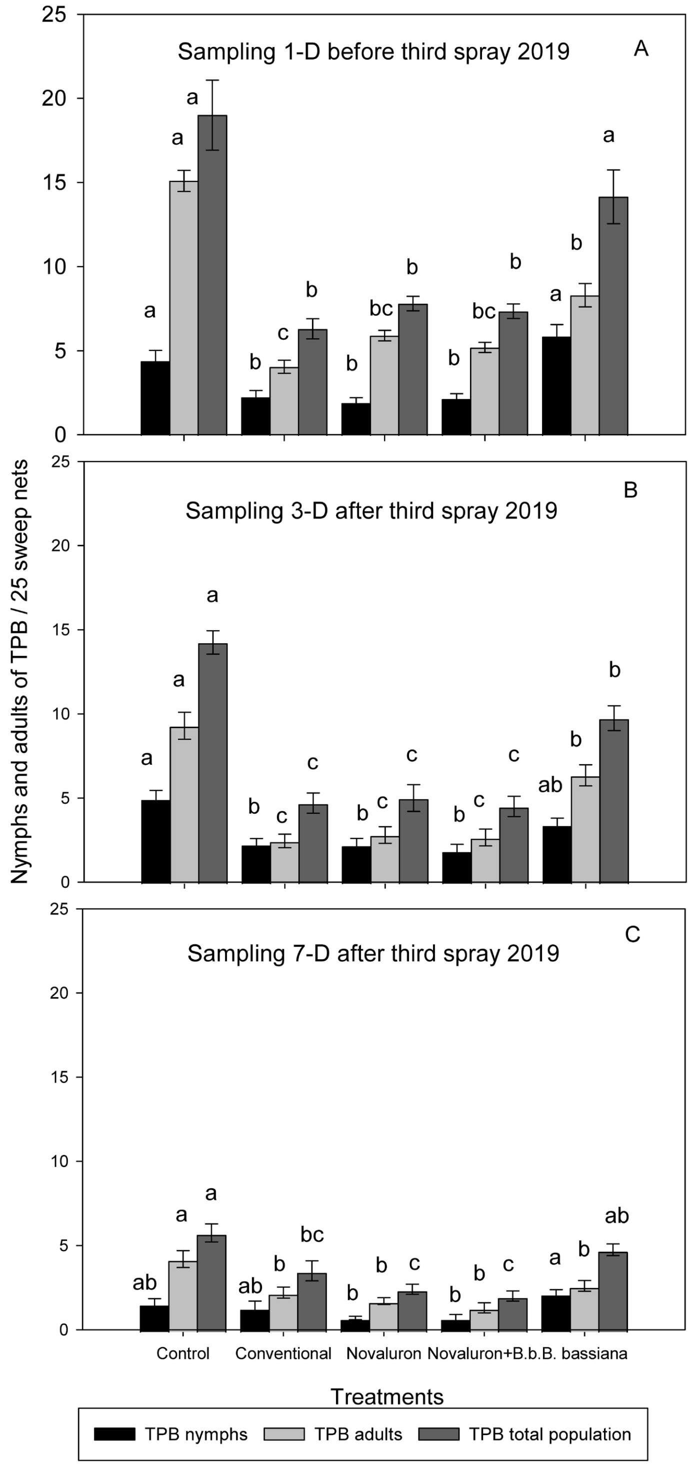

Figure 6. Mean ± SD number of tarnished plant bug nymphs, adults and total population/25 sweeps/plot before (A) and after (B) application in 2019 (Third spray-conventional: bifenthrin + novaluron). Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

treatments were observed (42.04; df = 4, 3; $P = 0.0001$) with the highest amount of lint (1178.19 ± 38 Kg of lint/ha) for the conventional treatment and the lowest amount of lint (446.95 ± 42 Kg of lint/ ha) for the control. The cotton lint production for diamond + *B. bassiana* (1061.46 ± 45.70 Kg of lint/ha) was higher than diamond alone (883.72 ± 29 Kg of lint/ha). The combined production of both years also resulted in highly significant differences between treatments; however, no significant differences were found between conventional (2097.42 ± 111 Kg of lint/ha), diamond alone (1802.96 ± 247 Kg of lint/ha), and diamond + *B. bassiana* (1960.37 ± 72 Kg of lint/ha). Yet, *B. bassiana* alone (944.74 ± 155) and the control (934.50 ± 175) had the lowest production (Kg of lint/ha) with no significant differences (**Figure 7(C)**). The values of cotton lint were reflected in the calculation of the net returns above TPB control for 2018, 2019, and both years combined (**Figures 8(A)-(C)**).

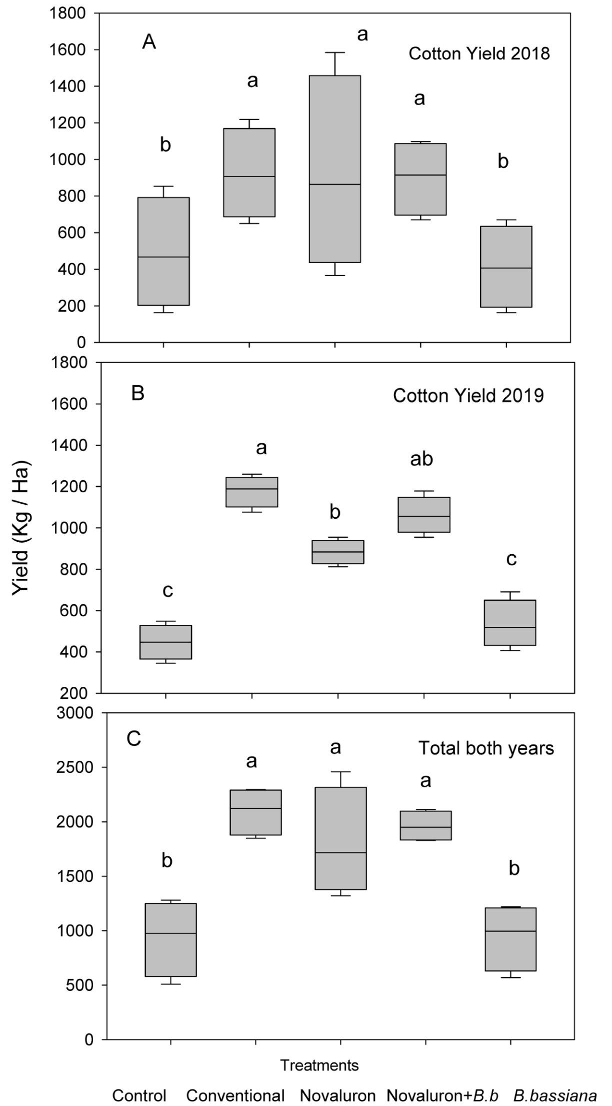

Figure 7. Mean total cotton yield (Kg of lint/ha) in field plots treated with insecticides treatments (conventional), IGR (novaluron alone), IGR + *B. bassiana*, and *B. bassiana* alone in 2018 (A), 2019 (B), and both years combined (C). Different letters among boxes indicate significant differences according to Tukey's HSD tests ($p = 0.05$).

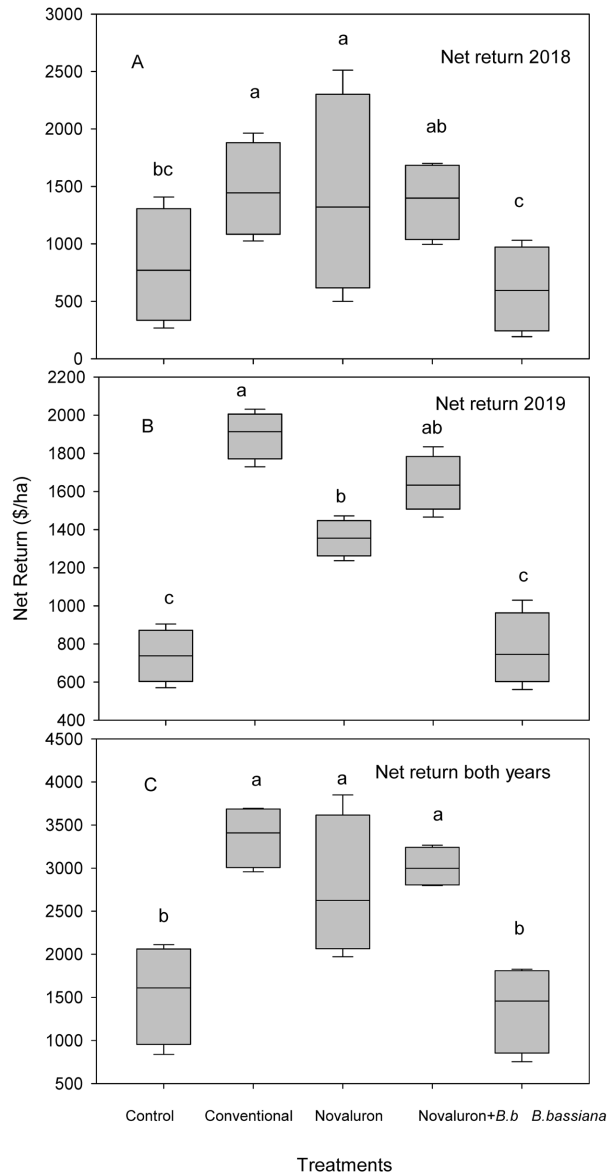

Figure 8. Net returns above TPB control ($/ha) in field plots treated with insecticide treatments (conventional), IGR (novaluron alone), IGR + *B. bassiana*, and *B. bassiana* alone in 2018 (A), 2019 (B), and both years combined (C). Different letters among boxes indicate significant differences according to Tukey's HSD tests ($p = 0.05$).

4. Discussion

In Mississippi, the action thresholds for cotton are generally 8 TPB/100 sweeps during the first two weeks of squaring and 15 TPB per 100 sweeps from the third week of squaring until bloom [28]. The present study indicated that after reaching the threshold, only the tank-mixed treatment of conventional insecticide that contained acephate (organophosphate) + bifenthrin (pyrethroid) + novaluron (IGR) decreased TPB population density to 1 or less than 1 TPB/25 sweeps 2-D after spray, as shown for the third spray of 2018 and the first spray of 2019. In contrast, the tank mix of bifenthrin + novaluron (third spray 2019) exhibited lower num-

bers of TPB as well as the individual insecticide applications, such as imidacloprid (first and second spray 2018) or sulfoxaflor (second spray 2019), including novaluron alone and in combination with *B. bassiana*. It is essential to note that regardless of the applications none of the conventional insecticide treatments can maintain suppression for more than 7 days. This may be due to the high resistance levels of TPBs to insecticides, which have been reported in Mississippi Delta since 1996 [3] [4]. Graham and Smith [7] noted that an alternate or a tank-mix of insecticides of different chemistries and modes of action is recommended for controlling TPB to help delay the development of insecticide resistance. However, there are some cases where even this strategy has resulted in resistance to multiple chemistries at once
https://www.epa.gov/pesticide-registration/slowing-and-combating-pest-resistance-pesticides#:~:text=The%20general%20recommendation%20when%20using,crop%20damage%20before%20applying%20pesticides.

The most common tank-mixed conventional insecticide treatments used particularly in cotton production in the Mid-South are listed in the Insect Control Guide [28] [29] as follows: novaluron + acephate, novaluron + bifenthrin, acephate + bifenthrin, and imidacloprid + bifenthrin. The combination of the three tank-mixed conventional insecticides used in this research was not found in any other study. Still, it was used because of the high population of TPB sampled before spray (over 10 TPB nymph and adults/25 sweeps/row/plot). It was based on general observations from recent studies where high efficacy of acephate + bifenthrin [30] was noted, while others demonstrated high TPB suppression using tank-mixes of acephate + novaluron [7] [31]. Yet, it is uncertain whether the combination of both pyrethroid + organophosphate or organophosphate + IGR provided the knockout of TPB adults or if it was the action of organophosphate (acephate) only. Portilla *et al.* [6] found that acephate caused more than 50% mortality 0-D after spray, reaching more than 90% mortality by the second day. They also reported TPB mortality of 20% or less 0-D after application for bifenthrin, imidacloprid, and thiamethoxam [6]. Although our results cannot be compared with other studies, we were able to confirm that this treatment (organophosphate + pyrethroid + IGR) reduced a large TPB population to almost 0 TPB individuals/25 sweeps 2-D after application. It was evident that the mixture of organophosphate (acephate) and pyrethroid (bifenthrin) provided a quick knockdown of adults as was cited by Smith *et al.*, [30] while novaluron targeted the immature stages [31] and more than likely controlled the new progeny of younger TPBs as reported by Catchot *et al.* [5]. In general, our results demonstrated and corroborated those of others [7] [31], showing that tank-mixes provided better control than individual insecticide applications.

Although there are several IGR (pyriproxyfen, diflubenzuron, methoxyfenozide, and buprofezin) approved for pest control in cotton, novaluron is the only IGR widely used on this crop both for TPB or bollworms [5]. It is well known that novaluron acts by disrupting the growth and development of immature stages of insects and prevents oogenesis and oviposition in young adults [5] [32]-[36]. Such act is a slow action behavior that will not be reflected in old TPB adult population. This behavior was clearly observed in our study where the suppression of TPB in plots treated with novaluron alone and novaluron + *B. bassiana* were much slower compared to the conventional treatments regardless of tank-mixes or individual insecticides either for nymphs or/and adults of TPB. Yet, no significant differences in cotton yield (kg of lint/ha) were found between novaluron alone, novaluron + *B. bassiana*, and the conventional treatment in 2018. In 2019, differences were found between novaluron alone which resulted in a significantly lower cotton lint yield compared to the conventional treatment. Interestingly, there were no significant differences in lint yields between novaluron + *B. bassiana* with the conventional treatment. These results may be explained by the findings of other studies where it was identified that *B. bassiana* could increase or reduce the efficiency of some insecticides [13] [19]. Hassan and Charnley [37] documented the original observations of IGRs and the synergisms with *B. bassiana*. They and some other authors [37]-[41] suggested that both *B. bassiana* and the IGR cause tissue disruption that could increase the efficacy of the IGR or increase the proliferation of *B. bassiana*'s spores in an insect's hemolymph. Although the mechanism by which IGRs could increase or decrease efficacy of a fungal pathogen or vice versa is still uncertain, our research demonstrated that novaluron + *B. bassiana* was more effective in controlling TPB than novaluron alone and *B. bassiana* alone. It could be comparable with the cotton lint production and TPB suppression in plots sprayed with conventional insecticides.

Unfortunately, as expected, *B. bassiana* alone did not differ from the control treatment, with no suppression of TPB population once the threshold was reached in 2018 and 2019. The high populations found in both control and *B. bassiana* treatments negatively impacted cotton lint yields which were significantly lower (Kg of lint/ha) compared with conventional, novaluron alone and novaluron + *B. bassiana* treatments. These results corroborate studies conducted by Little *et al.* [8] and Portilla *et al.* [20]. They found that the conventional treatment had significantly higher lint yield than the untreated control and *B. bassiana* treatments in both *Bt* and non-*Bt* cottons. Although, the application of the treatments in the previous studies and in our study was determined based on the sampled populations in the conventional treatment. Meaning that all treatments were applied once the threshold for TPB was reached in the conventional plots. Future studies should consider applications based on the threshold of the respective treatments. These should increase the number of applications for *B. bassiana*, which will not differ from the 5 - 7 application required for Mississippi Delta or up to 10, depending on the severity of the TPB infestation [29] [41].

5. Conclusions and Recommendations

In general, different insecticides and bio-pesticides vary in their mode of action targeting different tissues and organs at various growth stages of TPB. Their effects determine the lint yield, which is the standard for evaluating TPB damage to cotton [22]. For instance, the mortality rates of novaluron alone and novaluron + *B. bassiana* or any individual insecticide were not as impressive as the combination of organophosphate + pyrethroid + IGR. However, novaluron + *B. bassiana* showed no significant differences in cotton yield lint, hence, in net return. Based on EPA (http://www.epa.gov/) recommendations, the use of single insecticide may lead to resistance more quickly; therefore, using novaluron + *B. bassiana* only should be considered. On the other hand, this combination could be part of the conventional insecticide rotation system as is mentioned by Portilla *et al.* [20] and Houni *et al.* [2]. Consequently, when both TPB adults and nymphs are present in high numbers, new progenies will be proliferating, then the tank-mixed of acephate + bifenthrin + novaluron could suppress both old and young populations and their progenies at the same time. Cook *et al.* [42] noted that farmers often mixed insecticides and possibly fungicides together to minimize time and labor costs. In such circumstances, as indicated by the data generated in this study, the three insecticides tank-mixed and novaluron + *B. bassiana* can be recommended to be part of the rotational system to control TPB in the Mississippi Delta. Overall, to further improve the effectiveness of *B. bassiana* + insecticides applications within a functional IPM system for TPB, a variety of operational factors, such as the application time, rate, coverage, and methods must be considered carefully.

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Consent for Publication

All authors read and approved the manuscript for publication.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors do not have conflicts of interest to declare that are relevant to the content of this article.

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