POTENTIAL INSECT DETERRENCE IN TRI-SPECIES PROGENY

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Abstract

Plant-incorporated *Bt* toxins and chemical applications are the primary means of managing insect pests in cotton. However, resistance to these management practices have led to renewed interest in host plant resistance as a more sustainable approach to crop management. β-caryophyllene derivatives (12-hydroxy-β-caryophyllene and hydroxy-β-caryophyllene acetate) from progeny of tri-species cotton hybrids consisting of either *Gossypium hirsutum* L., *G. arboreum*, and *G. armouranium* (line 23), or *G. hirsutum*, *G. arboreum*, and *G. turneri* (line 13) have demonstrated resistance to nematodes, drought, and heat, but their impacts on cotton pests are unknown. Field and laboratory experiments were conducted to examine the influence of these caryophyllene derivatives on cotton aphid, *Aphis goysspii* Glover (Hemiptera: Aphididae) colonization. The objective of this study was to evaluate cotton aphid population density on tri-species cotton progeny expressing caryophyllene derivatives in field and laboratory experiment. Field and laboratory results confirmed that the tri-species cotton hybrid does have an adverse effect on cotton aphid population development in both the field and in the laboratory. In the field, cotton aphid population densities were lower on the tri-species hybrid than on *G. hirsutum*. In the laboratory, cotton aphid population intrinsic rate of increase, doubling time, and finite rate of increase were lower on the tri-species cotton hybrid relative to *G. hirsutum*. However, this effect did not appear to be due to the expression of the β-caryophyllene derivatives but is due to an unknown factor associated with the tri-species hybrid.

Introduction

The cotton aphid, *Aphis gossypii* Glover, is a global pest of numerous crops. The aphid is an occasional pest of cotton (*G. hirusutum* L.) in the southern United States. Species attack and transmit plant viruses into cotton which delays plant development and productivity. Insecticides and natural enemies have been utilized as preventive tools to control aphid populations. However, insect resistance to chemicals and reduction of natural enemies as a consequence of applications is a concern in the mid-south (Gore et al., 2013). An additional integrated pest management strategy, host plant resistance (HPR) has become a more effective approach in response to control failures (Kennedy, 2008) by offering natural defense to crops to maintain sustainability.

β-caryophyllene is a defensive secondary plant metabolite in upland cotton. β-caryophyllene derivatives, caryophyllene acetate & alcohol and caryophyllene alcohol are natural defensive traits in wild cotton species *G. armouranium* and *G. turneri*. These wild cottons emit the most profuse volatiles from caryophyllene derivatives (William et al., 1997) to attract natural enemies and to deter insect feeding. Tri-species cotton hybrid breeding lines thirteen and twenty-three (*G. hirsutum*, *G. arboreum*, and *G. armouranium* (line 23) and *G. hirsutum*, *G. arboreum*, *G. turneri* (line 13)) were developed by USDA-ARS to not only tolerate harsh field conditions but to also improve the efficacy of pest control.

Materials and Methods

Field Experiment

Line 23 tri-species cotton progeny (*G. armouranium*, *G. arboreum*, and *G. hirsutum*) and commercial cotton varieties Tamcot 73 (*G. hirusutum*) (untreated) and Tamcot 73 (*G. hirusutum*) (imidacloprid insecticide seed treatment) containing fungicide was used to evaluate insect performance. A field experiment was held on Texas A&M University Farm in Snook, Texas and was designed as a randomized complete block design with four replications of individual treatments. On May 14, cotton was hand planted 6 in. apart on 40 ft. rows and each row had ~80 plants. Manual cultivation and irrigation were provided as necessary; however no insecticides were applied.

Line 23 Chemical Analysis

Because we did not know what each individual line 23 plant expressed, each plant was tagged. At the 5 true leaf stage the upper most expanded leaf from each line 23 plant was sampled for chemical analysis to determine which β-caryophyllene derivative that plant expressed. Leaves were collected in 2 ml microcentrifuge tubes and stored in -80℃ freezer. Dichloromethane (900 µl) was pipetted into crushed frozen samples, and then vortexed (20 sec.), sonicated (30 min.), and centrifuged (10 min.). The samples were allowed to concentrate to dryness for 1-2 hours then reconstituted to 5 ml using dichloromethane. The samples were transferred into labeled GC vials with volume inserts and sealed with caps in preparation for gas chromatography mass spectrometry (GC-MS). The results were used to categorize plants as either caryophyllene, caryophyllene acetate & alcohol, or caryophyllene alcohol in field experiment.

Aphid Population Estimation

Aphid populations were evaluated within each plot by estimating the number of cotton aphids present on a single first expanded leaf in the upper plant canopy. Thirty plants total were sampled from middle rows (2, 3) of Tamcot 73 treated and untreated and every plant within line 23. Four field trials were conducted five days apart for the cotton aphids. Cumulative aphid days (CAD) was calculated using formula, $\Sigma[(x_i + x_{i-1})/2] \times (t_i - t_{i-1})$, where $(x_i + x_{i-1})/2$ is the aphid density x between progressive sampling periods *i*, and $(t_i - t_{i-1})$ is the number of days *t* between sampling periods (Brewer et al., 2017).

Laboratory Experiment

A single apterous adult of *A. gossypii* was collected from a chemical free cotton in greenhouse (USDA-ARS) to establish laboratory colony. Several generations were reared before initiating life table experiment.

Upland cotton *G. hirsutum* (untreated) and line 13 tri-species cotton progeny (*G. turneri*, *G. arboreum*, and *G. hirsutum*) referred as caryophyllene, caryophyllene acetate & alcohol, and caryophyllene alcohol was used to generate aphid life table statistics. The life table study was conducted in a growth chamber maintained at 26 ± 1° C and a photoperiod of 13:11 (L:D). Individual treatments (*G. hirsutum*, caryophyllene, c. acetate & alcohol, and c. alcohol) were assigned eight plant replicates in a completely randomized design. Plastic containers were filled with standard potting soil and watered when necessary. Two seeds were planted into plastic containers; however, one plant was removed at 1-2 true leaf stage. Clip cages were made using Neupane et al. methods (2019) to confine cotton aphids on individual plants.

At the 4th true leaf stage, 2-3 apterous adults were randomly selected from laboratory colony and carefully positioned on single expanded leaf of individual plants using a fine paint brush. Upon reproduction, all aphids but one caged newborn nymph was removed from cotton. After nymphs reached adulthood, their offspring were counted and removed daily. The monitoring and recording of aphid pre-reproductive period, and fecundity continued until death of adult.

Aphid life table parameters (pre-reproductive period, intrinsic rate of increase, doubling time, finite rate of increase, and longevity) were calculated. Pre-reproductive period is the time, in days, required to reach reproductive maturity. Intrinsic rate of increase is described as the rate of increase per individual under specified physical conditions (Neupane et al., 2019). Finite rate of increase is the rate of increase for each individual per unit of time, and doubling time is the amount of time necessary for a population to double in size (Neupane et al., 2019). Longevity is the time from birth until death. The intrinsic rate (r_m) for aphids on individual treatments was calculated using

formula (Birch, 1948) r_m = (log (R_0))/d. R_0 is the total number of nymphs produced by each female adult within its lifetime and d is defined as the pre-reproductive period (Neupane et al., 2019). Finite rate of increase (λF) and doubling time (DT) were calculated using formulas (Birch, 1948): $\lambda F = e^{rm}$ and DT = ln (2)/r_m.

Statistical Analysis

Cumulative aphid days was calculated using Brewer et al. (2017) formula, $\Sigma[(x_i + x_{i-1})/2]$ x $(t_i - t_{i-1})$, where $(x_i + x_{i-1})/2$ is the aphid density x between progressive sampling periods *i*, and $(t_i - t_{i-1})$ is the number of days *t* between sampling periods. A fit mixed model was used to analyze data in JMP (JMP Pro14 software, version 14.1.0 SAS Institute Inc., Cary, NC). Treatment (*G. hirsutum* (untreated/negative control), *G. hirsutum* (treated/positive control), caryophyllene (line 23 control), c. alcohol (positive line 23), and c. acetate and alcohol (positive line 23)), date, and treatment*date were set as fixed effects in a randomized complete block design of four replications. Caryophyllene and its derivatives data was pooled and then compared to *G. hirsutum*(untreated/negative control), *G. hirsutum* (treated/positive control) because there were no significant differences between the various caryophyllene treatments. Treatments were separated by date using Tukey-Kramer HSD, $P \leq 0.05$.

For the life table statistic data, a one-way analysis of variance (ANOVA) with cultivar (*G. hirsutum* (untreated/negative control), caryophyllene (line 13 control), c. acetate & alcohol (positive line 13), and c. alcohol (positive line 13)) were the treatments in a completely randomized design with eight replications per treatment. Cotton aphid longevity, fecundity, pre-reproductive period, and life table statistics was analyzed using JMP. There was no difference between caryophyllene and its derivatives, therefore data was pooled to compare *G. hirsutum* (untreated/negative control) and tri-species cotton. Means were separated using Tukey-Kramer HSD, $P \leq 0.05$.

Results and Discussion

Field Experiment

Based on cumulative aphid days, the untreated *G. hirsutum* had significantly more aphids than imidacloprid-treated *G. hirsutum*, caryophyllene, and caryophyllene derivatives on the first collection date (Figure 1). Over time, the cumulative aphid days increased across all individual treatments for 15 days, however caryophyllene, caryophyllene acetate & alcohol, and caryophyllene alcohol cumulative days had significantly fewer aphids than the untreated *G. hirsutum* on 11 and 16 June, but did not differ from the imidacloprid-treated *G. hirsutum*. On the last sample date, 21 June, only the c. alcohol treatment differed from the untreated *G. hirsutum*. These results suggest that the line 23 tri-species hybrid negatively impacted cotton aphid population development, and was similar to *G. hirsutum* treated with an insecticide seed treatment.

Laboratory

The average intrinsic rate of increase of cotton aphid was significantly lower when exposed to caryophyllene and c. alcohol tri-species treatments (Figure 3). C. alcohol had an adverse effect on the average aphid population doubling time by taking much longer to double than on *G. hirsutum* (Figure 5). The average finite rate of increase of cotton aphid was significantly lower on caryophyllene and caryophyllene alcohol than on *G. hirsutum* (Figure 7). Because the individual caryophyllene treatmetns were not sigficantly different from each other, those treatments were pooled as tri-species cotton for the aphid life table statistics and then compared to *G. hirsutum*. The tri-species cotton negatively affected aphid intrinsic rate of increase, finite rate of increase, and doubling time (Figures 4, 6 and 8). The cotton aphid life span and generation time was not significantly influenced by caryophllyene and its derivatives (Figure 8 and 9).

In field study, tri-species cotton had less cotton aphids than *G. hirsutum* and the life table statisics experiment conducted in laboratory supports field data. The tri-species cotton seems to be influencing aphid population development. However, it does not appear that it is the caryophyllene derivatives that's affecting population development, but is due to an unknown antibiotic factor related to the trispecies hybrid.

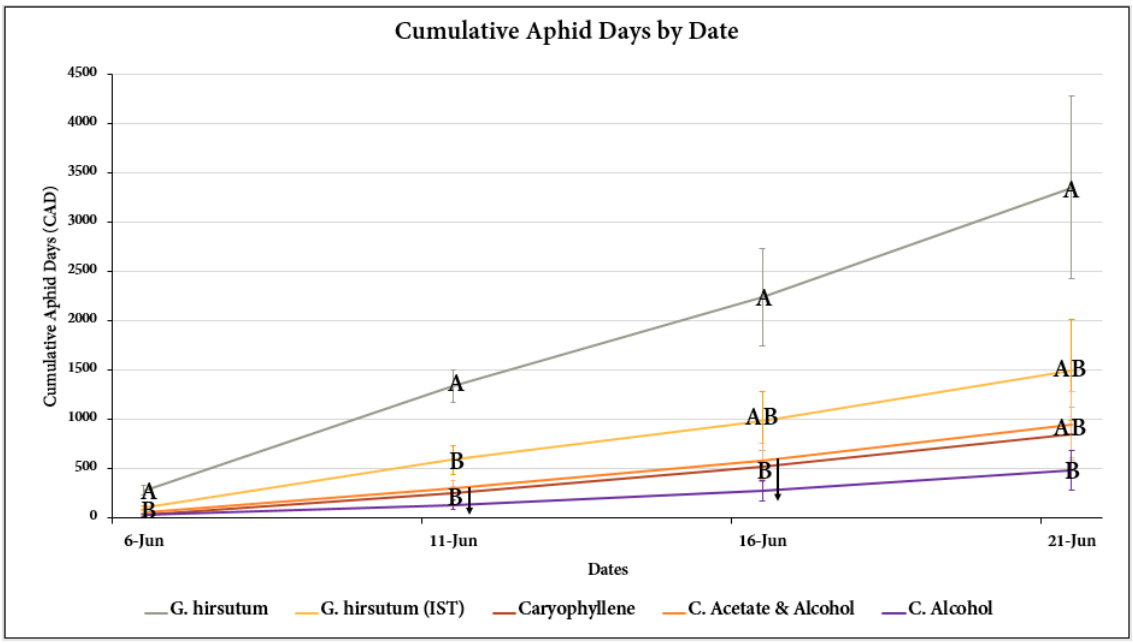

Figure 1. Average cumulative aphid days by date on individual treatments.
*IST - insecticide seed treatment

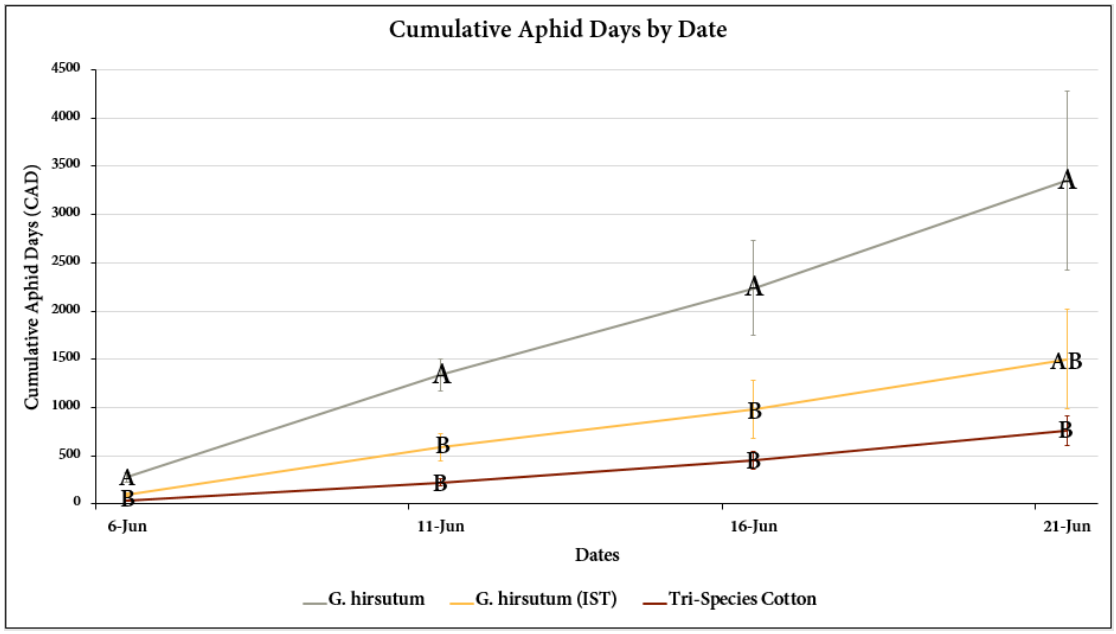

Figure 2. Average cumulative aphid days by date on individual treatments
after caryophyllene treatments are pooled.
*IST - insecticide seed treatment

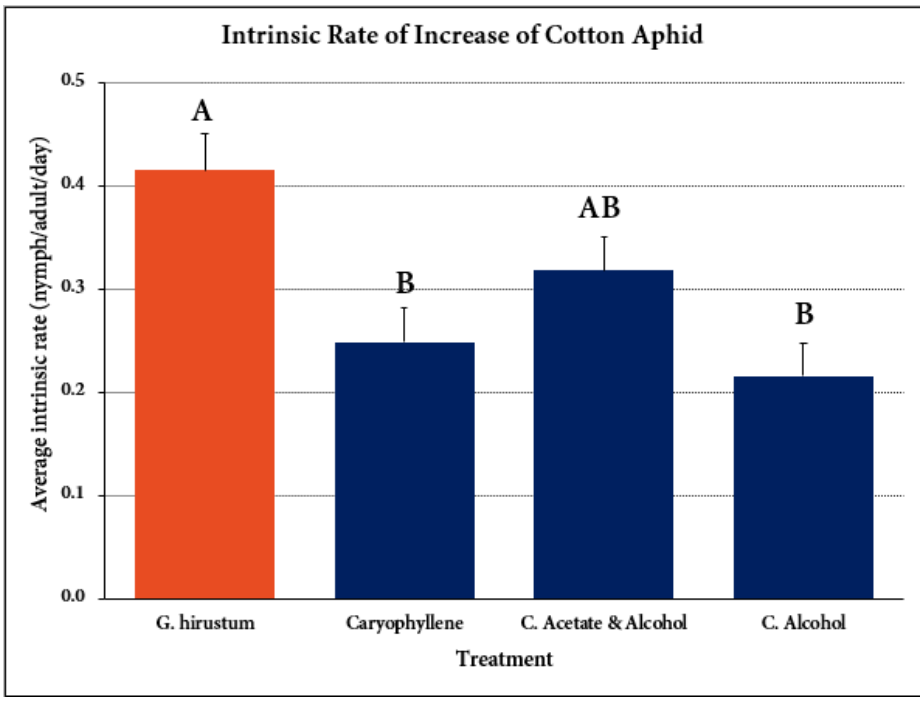

Figure 3. Average intrinsic rate of aphid expressed as nymph per adult per day on individual treatments.

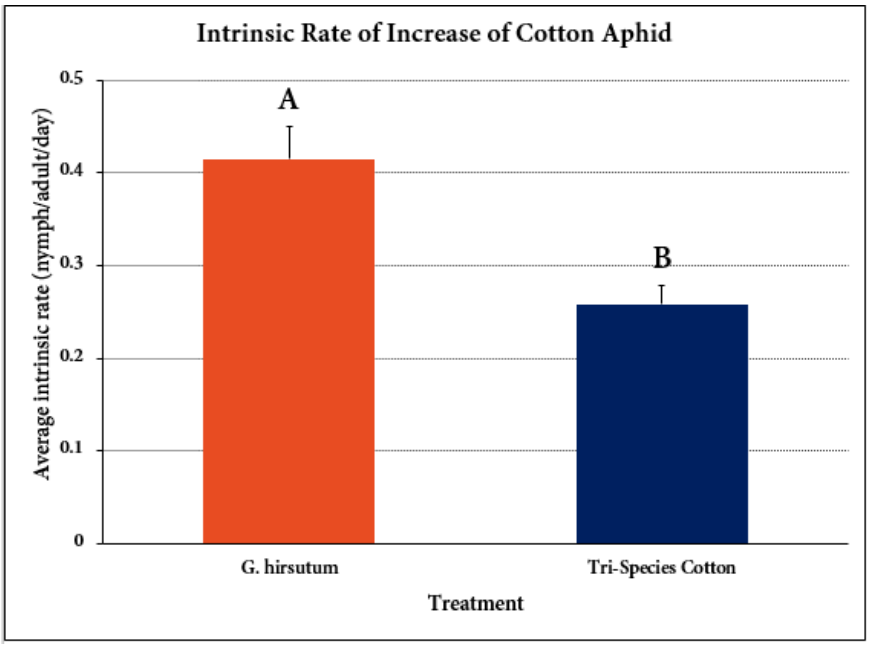

Figure 4. Average intrinsic rate of aphid expressed as nymph per adult per day on individual treatments after caryophyllene treatments are pooled.

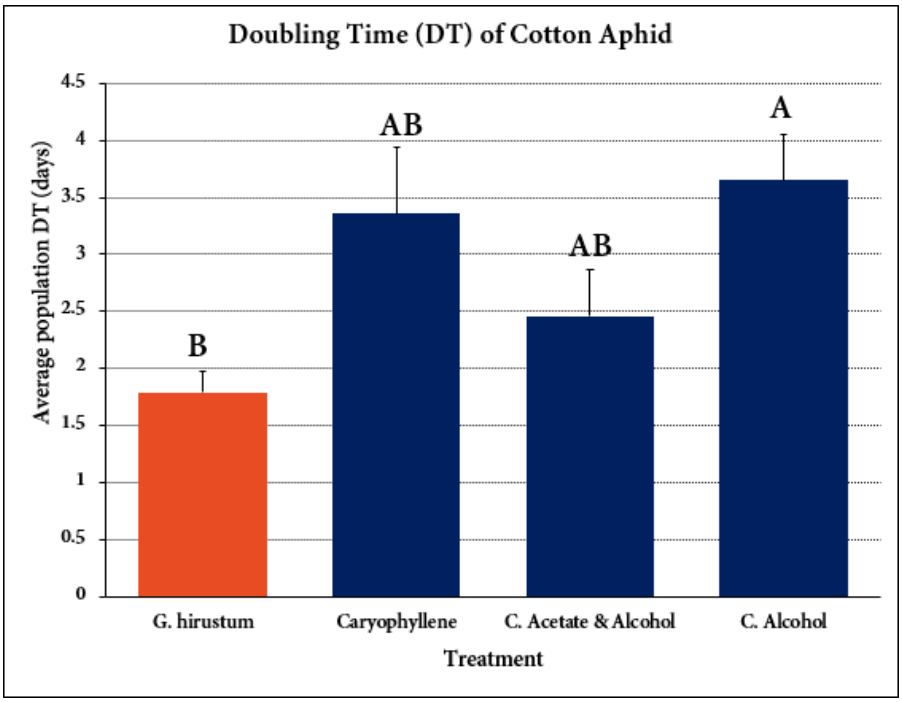

Figure 5. Average population doubling time of aphid on individual treatments.

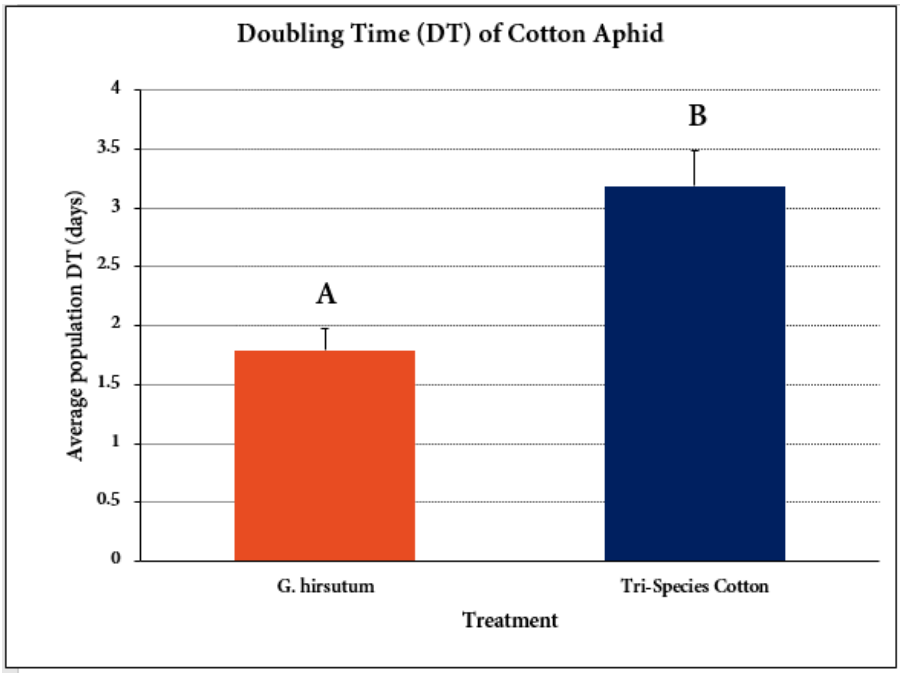

Figure 6. Average population doubling time of aphid on individual treatments after caryophyllene treatments are pooled.

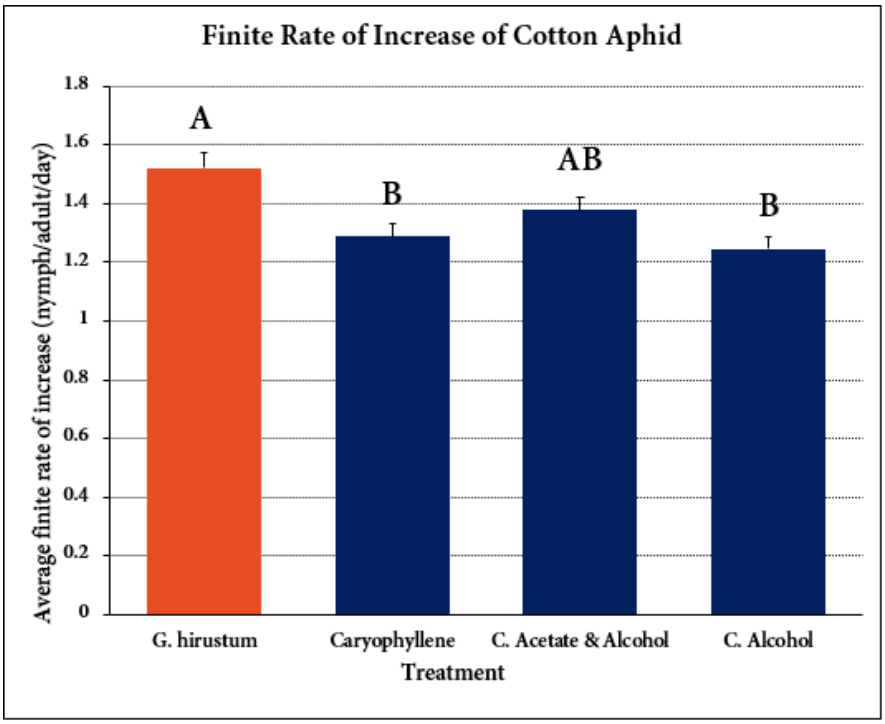

Figure 7. Average finite rate of increase of aphid expressed as nymph per adult per day on individual treatments.

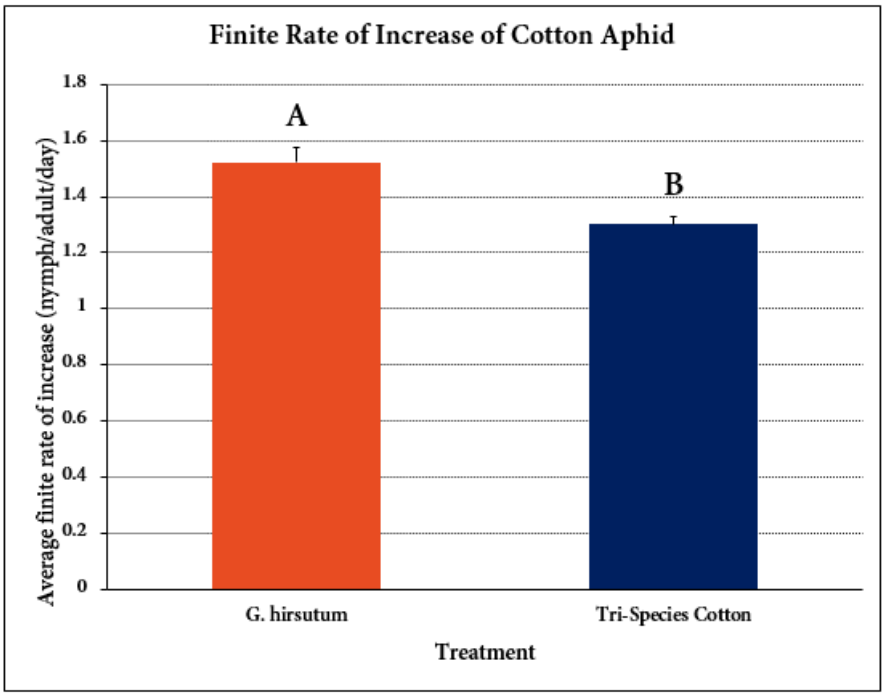

Figure 8. Average finite rate of increase of aphid expressed as nymph per adult per day on individual treatments after caryophyllene treatments are pooled.

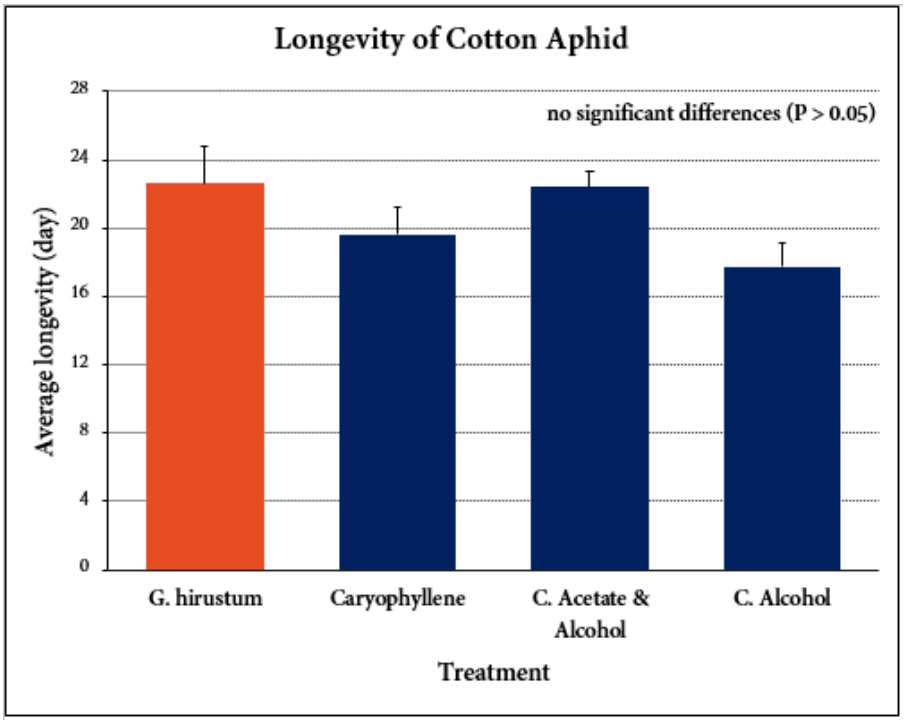

Figure 9. Average longevity of aphid on individual treatments.

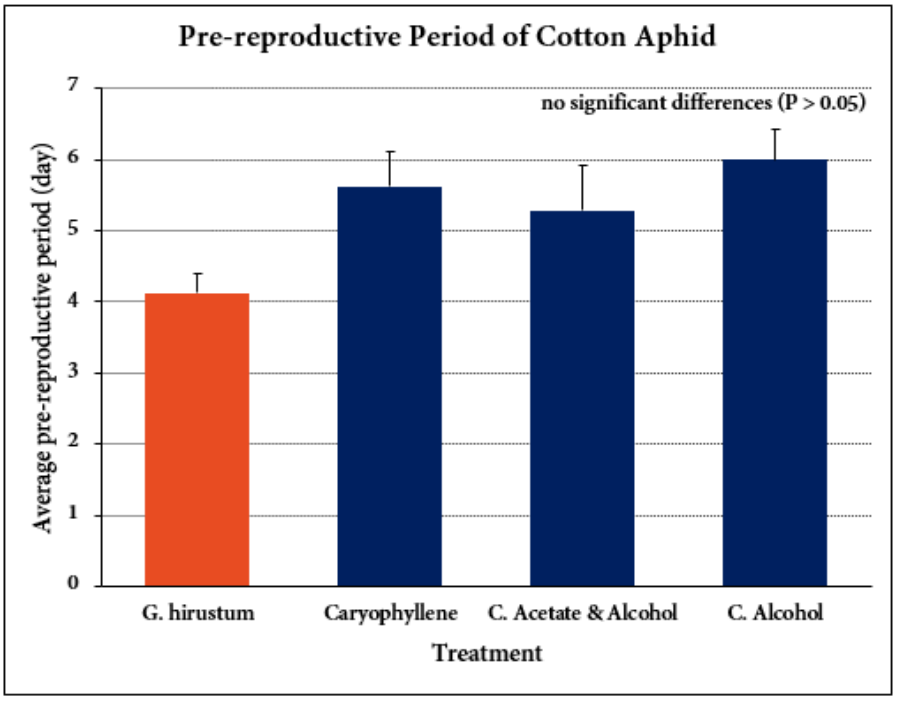

Figure 10. Average pre-reproductive period of aphid on individual treatments.

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