OKRA LEAF TRAIT EFFECT ON COTTON CANOPY MICROCLIMATE AND *HELICOVERPA ZEA* CONTROL

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Abstract

Microclimates within cotton (*Gossypium sp.)* canopies can vary due to many factors, including crop variety and plant architecture. Changes in microclimate conditions can also impact insect populations. Standard upland cotton (*G. hirsutum)* typically has large leaves that provide shade, potentially lowering the temperature and increasing the relative humidity within the canopy. Such conditions could allow pests, like the cotton bollworm (*Helicoverpa zea),* to thrive in desert environments. A field trial was conducted in eastern New Mexico to evaluate how plant architectural traits could affect bollworm populations. To determine the potential influences of microclimate, *H. zea* egg hatch was recorded in two upland cotton varieties with distinct architectures, an open canopy okra-leaf variety (UA107), and a closed canopy *Bacillus thuringiensis* (DP1845B3XF) variety with standard leaves. Clusters of approximately 30-60 bollworm eggs, oviposited on fabric, were placed on leaf surfaces at mid-canopy. After 48 hours egg clusters were retrieved and brought to the laboratory to record predation in the field and larval hatch at 48, 72, and 96 hours. Air temperatures and relative humidities in cotton canopies were recorded with HOBO sensors. Preliminary results showed higher egg hatch in the standard leaf cotton. These increases in egg hatch coincided with higher temperatures, suggesting that the warmer temperatures allow a higher proportion of eggs to successfully hatch. Okra-leaf cotton varieties might be able to play an important role in pest management especially in the arid Southwest.

Introduction

Upland cotton includes varieties differentiated by leaf shape and canopy structure which can alter microclimates and insect habitats in the cotton canopy—especially in the hot, arid growing environment of the Southwestern United States. The presence of lepidopteran pests in the Noctuidae family, cost growers millions of dollars in chemical control measures and yield losses from larval feeding on cotton fruiting bodies, squares and bolls (NCC, 2009; Anderson and Yeargan, 1998). The polyphagous feeding nature of *H. zea* makes it one of the most economically important pests as they feed on a wide variety of food, fiber, oil, and fodder crops. (Luttrell and Jackson, 2012; Sansone and Smith, 2001). The wide host range paired with the ability for wide dispersal, make *H. zea* one of the most damaging lepidopteran pests in cotton (Olmstead, 2015). Damage has been reduced by the common use of Bt cotton varieties although there is a growing concern with increasing resistance to Bt (Anilkumar et al 2008).

Adult bollworms are 1.9 cm long and appear yellow-white to brown (UCIPM, 2013). While adults feed on nectar, the most destructive behavior comes from larval feeding on fruiting forms of the cotton plant—the squares and bolls (Bohmfalk et al, 1914). Newly hatched larvae feed on plant terminals and young squares and have a development period that ranges from 14-16 days with six instars (Gianessi and Carpenter, 1999; Capinera, 2000). Larvae are much more difficult to control once they reach the third-instar when they are less susceptible to insecticidal treatments (UCIPM, 2013). The fifth instar damages bolls before burrowing in the ground to pupate (Gianessi and Carpenter, 1999; UCIPM, 2013). The pupal stage lasts approximately 13 days before adult moths emerge (Capinera, 2000). Because it is difficult to control later instars, it is advantageous to control eggs or young larvae. As an integrated pest management tool, choosing a resistant cotton variety can not only protect against problematic lepidopteran pests like *H. zea* but also provide suitable habitat for predatory insects.

Okra-leaf varieties have palmately-lobed leaves that grow to roughly 60-70% the size of the broad, standard leaf cotton variety (Andres et al, 2016). The smaller leaf size allows okra-leaf varieties to develop a relatively open canopy, permitting greater air circulation and light penetration (Andres et al, 2016). Okra-leaf cotton varieties are associated with higher mortality in square-feeding pests like the cotton boll weevil (*Anthonomous grandis*)—due to the desiccating effects from high temperatures under the open canopy (Jones et al 1976; Sorenson, 1995). However, studies with other lepidopteran pests like the pink bollworm, *Pectinophora gossypiella*, noted no significant difference in egg hatch between okra- and standard leaf cotton varieties after *P. gossypiella* eggs were introduced to cotton bolls

(Wilson et al 1986). Fye and Surber (1971) observed that reducing egg hatch in *P. gossypiella*, required temperatures above 35°C for over 4 days in a laboratory environment.

Under laboratory conditions, Pierce and Monk (2010) determined that *H. zea* eggs exposed to high temperature and low humidity had lower egg hatch. Very low egg hatch—down to 4%—was observed under the most extreme conditions of high temperature (35°C) and low humidity (17%) (Pierce and Monk, 2010). Under field conditions with daily maximum temperature exceeding 40°C in plant canopies, *H. zea* egg hatch rates were still at least 37% after 48 hours of exposure (Pierce and Monk, 2010). To manipulate temperature and humidity in the crop canopy, Pierce and Monk (2010) altered row spacing and orientation and noted that the location of *H. zea* eggs on leaves was influenced by microclimate conditions altered by leaf shading and plant respiration within the canopy. Although row spacing and orientation have been studied as control measures for *H. zea* egg hatch, there is little information how *H. zea* egg hatch responds to microclimate conditions with okra-leaf vs standard leaf cotton varieties.

The impact of different canopies on predation has not been often explored in cotton agroecosystems and reports of predator abundance in soybeans may provide some insights applicable to this study. Anderson and Yeargan (1998) showed the most common *H. zea* egg predators in soybeans systems were damsel bugs (F: Nabidae), big-eyed bugs (F: Geocoridae), minute pirate bugs (F: Anthocoridae), and miscellaneous spiders (O: Araneae). There were no differences in predator abundance or predation rates between open and closed soybean canopies (Anderson and Yeargan, 1998). Using traditional weekly sweep net sampling, Booze et al (2005) sampled predatory insect populations in okra-leaf and standard leaf cotton varieties and found no differences in predatory insect abundance between varieties. However, using sentinel eggs placed n the field with and subsequent examination of eggs and remains could provide insight into the degree of predation on *H. zea* eggs.

A cotton variety with a canopy that suppresses *H. zea* hatch and provides suitable habitat for predatory insects can be part of integrated management programs for problematic Lepidopteran pest like *H. zea*. The objectives of this study were: 1) compare microclimates and *H. zea* egg hatch rates between two varieties of cotton, a palmately-lobed okra-leaf variety and a broad-leafed standard variety, 2) Quantify the impact of predators on *H. zea* eggs in a okra-leaf variety and a broad-leafed standard variety.

Materials and Methods

Helicoverpa zea **Colony**

Helicoverpa zea eggs were from a colony maintained in chambers set to 25°C and 50% relative humidity at the New Mexico State University Artesia Science Center insectary. Colony eggs originated from Frontier Agrosciences rearing facility in Newark, DE (Corn earworm eggs, E9394). Larvae were maintained on a wheat germ protein diet made up of a dry pre-mix for Lepidoptera larvae (Beet Army Worm Diet without aureomycin #F9221B, Frontier Agricultural Sciences, Newark, DE).

Egg Hatch and Predation in Field Trials

Three egg hatch and six predation field trials were conducted at the NMSU-ASC-Artesia from mid-July to mid-August of 2020 using two cotton varieties. The two varieties were planted on April 22, 2020—the palmately-lobed okra-leaf cotton (Cotton Cultivar UA107, University of Arkansas) and standard broad leaf (Bollgard® 3- DP1845B3XF, Monsanto Corporation) cotton. Experimental plots consisted of six, 15.2 m-long rows. Okra-leaf plots were replicated five times and the standard leaf plots were replicated four times on a randomized block design. Border rows comprised of standard leaf cotton separated blocks. The entire study was encased by additional border rows of standard leaf cotton that separated the study from other cotton cultivar trails at the study site.

For egg hatch trials, groups of 30-60 *H. zea* eggs on cheese cloth were stapled to bright 4x2 cm index cards before being placed in field (Figure 1). Damaged eggs were removed from cloth with forceps before cloth was stapled to index cards. Index cards with eggs were stapled to leaves at mid-canopy and left in the field for 48 hours. Index cards with eggs were then collected from field and were observed in laboratory under a stereo microscope (Zeiss Stemi 2000-C Stereo Microscope 6.5x - 50x) to assess egg hatch and predation. Undisturbed eggs (not fed upon by predators) were placed in 100x15 mm polystyrene petri dishes with a 2x1x1 cm cube of diet and sealed with parafilm (parafilm M, laboratory film) and left under observation for an additional 48 hours. Larval egg hatch was assessed at 48, 72, and 96 hrs after eggs were introduced to field and placed at mid-canopy. One assay was conducted under cooler conditions (September 8th, 2020) and had a delayed egg hatch which was observed up to 144 hours.

Figure 1: Cluster of *H. zea* eggs on cloth attached to pink index card before being placed in field.

All sentinel eggs were evaluated for predation after 48 hours in the field. In addition to the field to lab assay that evaluated the impact on egg hatch, additional trials were conducted to only evaluate predation. Eggs and their remains were evaluated in laboratory to attempt to determine the family of the predator which fed on the *H. zea* eggs. Classification of predatory damage was based on past work with sentinel eggs in cotton and sorghum (Pierce et al, 2017). Predators with sucking mouthparts like damsel bugs (F: Nabidiae) or big-eyed bugs (F: Geocoridae) left eggs deflated and collapsed. Predators with mandibles like ladybird beetles (F: Coccinellidae), collops beetles (F: Melyridae) or crab spiders (F:Thomisidae) left chewed eggs or only partial remains. Ladybird beetle or Lacewing larvae (F: Chrysopidae) left sucked out, transparent eggs that retained their form (Figure 2).

Figure 2: Lacewing larvae (F:Chrysopidae) feeding on *H. zea* egg. Egg is left nearly transparent after feeding.

Welch two-sample t-test was conducted using R software to determine significant differences in means and F-test for comparison of variances between cotton varieties. Data analyzed included those from each individual egg hatch and predation trials. Data was also pooled to determine overall impact on eggs hatch and predation.

<u>Temperature and Relative Humidity</u>

Temperature and relative humidity were recorded throughout trials by placing two HOBO sensors (HOBO Pro v2, U23-001, Bourne, MA) in each field plot (n=18) attached to a meter-long PVC pipe. Sensors remained at mid-canopy height and recorded temperature and relative humidity every 30 minutes for the duration of the trials. Two out of eighteen sensors failed to record relative humidity data after first trial, therefore data were not used from those sensors. Both temperature and relative humidity data were analyzed using Welch two-sample t-test to evaluate mean differences and F-tests were used to compare the variance between cotton varieties using R software.

<u>Leaf Area and Light Measurements</u>

Differences in plant leaf area were recorded by collecting all leaves from ten random plants from the inner rows of each block from both varieties of cotton (n=20). Petioles were removed and each leaf was spread and taped to sheet of paper for scanning. Scanned images were analyzed with Easy Leaf Area and Canopy Cover open-source software (Copyright (c) 1991 - 1995, Stichting Mathematisch Centrum Amsterdam, The Netherlands) to measure total leaf area in cm^2 for each plant.

Light measurements were conducted on ten randomized plants in each plot (n=90) with a digital light meter (Model LT300, Extech instruments, Nashua, New Hampshire). Light readings were taken at mid-canopy at mid-day were measured in klux (1000 lux, 1 lux = luman/m^2).

Leaf area and light measurements were analyzed using Welch two-sample t-test to check for differences in means and F-test for equality of variance between cotton varieties using R software.

Results

Egg Hatch and Predation in Field

For two of the three trials, *H. zea* hatch was significantly lower in the okra-leaf variety of cotton (Table 1). Egg hatch was significantly lower in okra-leaf plots on July 13th with only 19% egg hatch versus 51% in standard leaf cotton ($t = 3.05$, $df = 5.94$, $P<= 0.022$). Egg hatch was also significantly lower in okra-leaf on August 17th with only 27% egg hatch versus 52% in standard leaf plots ($t = 2.63$, $df = 4.89$, $P< = 0.04$). Mean percent egg hatch was not significant on July 29th trial with 40% and 60% hatch in okra-leaf and standard leaf plots respectively. There was a delay in egg hatch on the September 8th trial due to colder weather. There were no significant differences in mean percent egg hatch with 21.1% and 18.6% hatch in okra-leaf and standard leaf plots respectively.

Table 1. Percent egg hatch at 48, 72 and 96 hours after introduction to okra leaf and standard cotton plots.

	7/13/2020		7/29/2020		8/17/2020		9/8/2020	
Hours	Okra (%)	Standard (%)	Okra (%)	Standard (%)	Okra (%)	Standard (%)	Okra (%)	Standard (%)
48	0	0	0.4	0	0	0	0	0
72	17	48	38	52	4	16	0	0
96	2	3	2	8	23	36	0.3	0.3
120	-	-	-	-	-	-	19.7	17
144	-	-	-	-	-	-	1	1.1
Total	19	51*	40	60	27	52*	21.1	18.6

*Percent egg hatch statistically significant $P< = 0.05$.

On average 64 eggs hatched per plot in the okra-leaf cotton versus 92 in the standard leaf cotton. There was a difference in the percentage of hatched eggs across all egg trials (Figure 3; $t = 3.06$, $df = 19.32$, $P< = 0.006$). Mean egg hatch in okra-leaf cotton across all field to lab assays was 16% compared to 23% in the standard leaf 72 hours after the eggs were introduced to the field. The difference was also significant after 96 hours with the mean egg hatch 24% in okra-leaf and 33% in standard leaf ($t = 2.929$, $df = 19.001$, $P< = 0.008$).

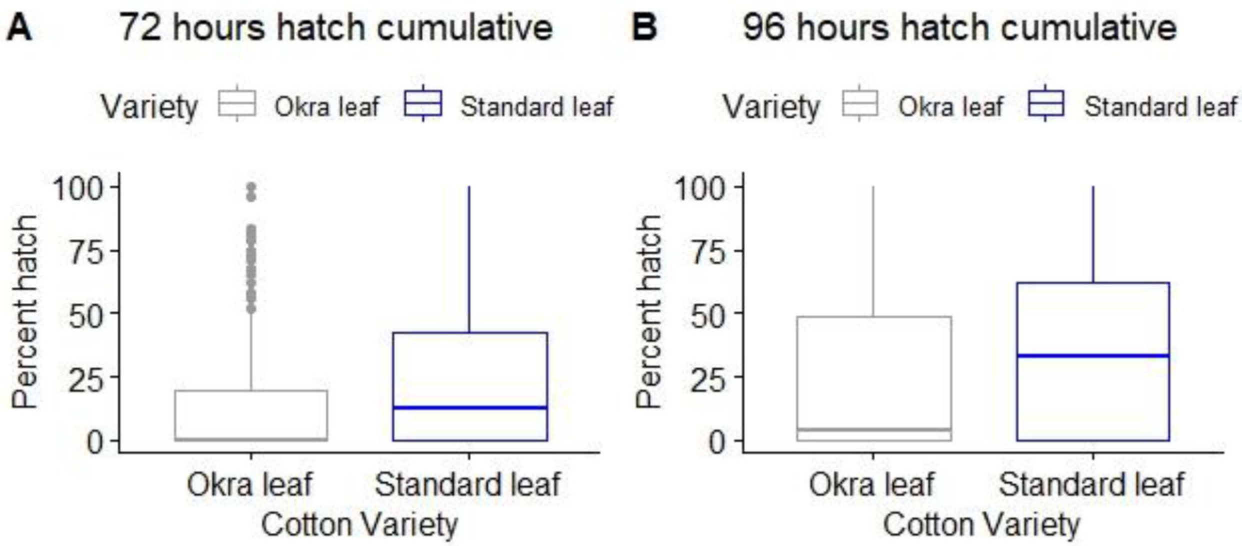

Figure 3: Percent egg hatch of *H. zea* 72 (A) and 96 hrs (B) after eggs were introduced to field and placed at mid-canopy.

Observations of *H. zea* egg remains showed a higher number of collapsed or 'sucked out' eggs—in okra-leaf cotton compared to standard leaf cotton (Table 2; t = 2.05, df = 1006, *P*< = 0.040). The majority of *H. zea* egg remains appeared to be partially consumed or 'chewed,' however there was no significant difference in the number of 'chewed' eggs between okra leaf and standard leaf cotton varieties. Overall predation was very similar across both cotton leaf varieties with 52.8% in okra leaf cotton compared to 52% in standard leaf cotton.

Table 2. Percent *H. zea* eggs collapsed ("sucked out"), chewed by predators, sucked out clear, and total predation after 48 hours in field plots

	Okra-leaf (%)	Standard leaf (%)
Sucked out*	9.0	7.2
Chewed	28.0	28.7
Sucked out (transparent)	14.8	14.7
Total Predation	52.8	52.0

*Egg predation statistically significant *P*≤ =0.05.

Temperature and Relative Humidity in The Cotton Canopy

Temperature and relative humidity data from 16 HOBO data loggers showed significant differences in temperature (t = 3.28, df = 9198, *P*< = 0.001) and relative humidity (t = -3.0341, df = 9223.2, *P*< = 0.002) between okra and standard leaf cotton. There were statistically significant differences in temperature and relative humidity in three trials on July 20th, July 29th, and August 8th. On average temperature was 1.2°C warmer in standard leaf cotton vs okra-leaf cotton as well as 2% less humid (Table 3).

Table 3. Average daily temperatures and relative humidity in seven microclimate trials with okra-leaf and standard leaf cotton.

	Temperature ° C			Relative humidity (%)		
	Okra leaf	Standard leaf	Difference	Okra leaf	Standard leaf	Difference
7/13/2020[1,2]	34	35	1	40	38*	-2
7/20/2020[2]	31	32*	1	51	47*	-4
7/29/2020[1,2]	37	40*	3	38	34*	-4
8/2/2020[2]	37	39*	2	31	28*	-3
8/12/2020[2]	36	36	0	36	35	-1
8/17/2020[1,2]	33	33	0	44	45	1
9/8/2020[1,2]	18	18	0	58	59	1
Averages	32	33	1	43	41	-2

[1]Egg hatch assay.
[2]Predation assay.
*Temperature and relative humidity statistically significant P=≤ 0.05.

Light Penetration, Density, And Leaf Area

Light penetration measurements were taken at mid-canopy in both varieties of okra-leaf and standard leaf in September when canopies were fully formed. Illumination, measured in lux (1 lumans per meter squared), differed between okra-leaf and standard leaf varieties (t = 6.102, df = 88, p-value = 1.37E-8). On average okra-leaf had an illumination measure of 37.1 Klux, more than double the average measurement in standard leaf canopies at 17.1 Klux. There was no significant difference in average plant heights, number of nodes, or plant density between each cotton variety (n=90). The average okra-leaf leaf area was 441.1 cm^2, only 55% the average standard leaf area which was 798.3 cm^2.

Discussion

The original hypothesis was that egg hatch might be lower in okra-leaf cotton and that it might be due to an increase in temperature and lower relative humidity within the plant canopy of okra leaf cotton. Fewer eggs did hatch in the okra-leaf treatment than the standard leaf treatment. Forensic analysis of egg remains from all seven predatory trials showed that cotton variety did not influence the total number of predated eggs. However, there was a difference between varieties in incidences of *H. zea* eggs fed on by predators with sucking mouthparts, likely damsel, or big-eyed bugs.

The reduced *H. zea* egg hatch in okra-leaf canopies agrees with past work by Jones et al (1976) which indicated the open canopies of new okra-leaf varieties, 'Gumbo' and 'Pronto,' show higher mortality of late-season lepidopteran pest infestations. Jones et al. (1976) concluded that the canopy architecture of okra-leaf allowed cotton plants and soil surfaces to become warmer and dryer than standard leaf canopies. However, our data showed that temperature in standard leaf cotton canopies were, on average, 1.2° C higher than canopy temperature in okra-leaf cotton. These findings also are also inconsistent with work by Pierce and Monk (2010) which showed reduced egg hatch of *H. zea* in warmer cotton canopies. Other factors such as disturbances from wind and canopy aerodynamics or direct radiation need to be investigated to see if there is some relationship to *H. zea* egg hatch and okra leaves as suitable habitats.

There was no significant differences in total predations between both okra-leaf and standard cotton varieties. This is consistent with studies in soybean systems by Anderson and Yeargan (1998) that showed no differences in predator abundance or predation of *H. zea* eggs in open and closed crop canopy treatments. However, there was a significant difference in the amount of collapsed or 'sucked out' eggs. These differences may point to better host-seeking behavior or preferences by these predators or perhaps larger populations in the okra-leaf canopies. Weekly sweep net samples need to be tabulated and analyzed to see if this is a possible reason for this difference in 'sucked out' predation; perhaps due to some other factors such as seasonality or other difference in canopy microclimate. The presence of certain types of predatory insects in okra-leaf cotton provide biological control measures to *H. zea* and other lepidopteran pests in cotton systems and is useful information to growers to inform management interventions. Specifically, growers managing okra-leaf cotton should carefully consider insecticide applications because the use of insecticides would provide an adverse effect to predatory insect populations. While the exact mechanism of microclimates within the cotton canopy controlling *H. zea* egg is not known, the potential for controlling Lepidopteran pests make okra-leaf cotton an attractive variety to plant in arid cotton growing regions like New Mexico.

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