<u>Abstract</u>

Populations of *Helicovepa zea* (Boddie) and *Heliothis virescens* (Fabricies) were commonly found on hophornbeam copperleaf, *Acalpha ostryaefolia*, in Arkansas and Mississippi. From 2002-2009, more than 25 colonies of *H. zea* were established from collections made on the wild host plant. One colony of *H. virescens* was made from hophornbeam copperleaf in Arkansas. More recent collections from hophornbeam copperleaf in Mississippi include both *H. zea* and *H. virescens*, but *H. virescens* appears to be more common in the recent Mississippi collections. The plant is commonly infested. Densities vary but can be as great as one larva per sweep. Larvae of both heliothine species were heavily parasitized but more than 50% of collected larvae survived to the adult stage. Hophornbeam copperleaf appears to be a preferred host. Based on our general observations, patches of hophornbeam copperleaf appears to be abundant and widely distributed in the agricultural landscape. More quantification is needed, but if this is correct and densities are as great as those we observed, hophornbeam copperleaf may be an important host in sustaining refuge populations outside of cultivated crops.

Introduction

Hophornbeam copperleaf, *Acalpha ostryaefolia* (Riddell), is a monoecious native summer annual in the family Euphorbiaceae. Horak et al. (1998) described biology and control of hophornbeam copperleaf, and most cooperative extension service programs in the South and Midwest publish contemporary information on its increased importance as a weed in agricultural crops. Interestingly, hophornbeam copperleaf does not produce the milky sap characteristic of most Euphorbiaceae. Seed require warm temperature (68-86°F) to germinate, and the plant is widely distributed across cotton growing regions of the southern U.S. It is commonly found on field borders, ditches and margins of glyphosate-treated areas.

Sudbrink and Grant (1995) recorded hophornbeam copperleaf as a host of *H. zea* and *H. virescens*. Cottrell and Yeargan (1998) and Griffin et al. (2002) studied relationships between the lady beetle, *Coleomegilla maculata* and *H. zea* infestations on the plant. Abundance of *C. maculata* and predation of *H. zea* eggs were greater in sweet corn plots near hophornbeam copperleaf. Hophornbeam copperleaf and velvetleaf (*Abutilon theophrasti*) were also found to serve as refuges for egg clusters of *C. maculata* (less predation than that observed on other weed hosts studied). Both of these weeds posses numerous glandular trichomes which may reduce foraging of predators on *C. maculata* eggs.

Since 2002, we have collected *H. zea* and *H. virescens* from cultivated and wild hosts throughout the Midsouth and cotton growing regions of the U.S. to measure susceptibility of subsequent generations to Bt insecticidal toxins expressed in transgenic cotton. Ali et al. (2004, 2005, 2006, 2007, 2008) and Luttrell and Ali (2009) describe the assay methods and report the range of susceptibilities measured. Hophornbeam copperleaf was a common source of collections from weeds, especially in late summer. Ali et al. (2004, 2005, 2006, 2007, 2008) grouped these collections with those from other weeds to characterize Bt susceptibility.

Collectively, these data indicated that *H. zea* and *H. virescens* from weed hosts were as susceptible or more susceptible to Bt toxins than those collected from crop hosts. In this summary, we report ecological information from the collections made from 2002-2009 and add additional information from later collections made from hophornbeam copperleaf in the Mississippi Delta.

Methods

Patches of hophornbeam copperleaf with seed heads (pistillate flowers on a long, terminal spike) located in pecan orchards, field borders, ditches, and occasionally within crop fields were sampled with a 15 inch diameter sweep net. The number of sweeps and sample time varied but most sample events included hundreds of sweeps. Contents of

multiple sweep samples (usually 25 sweeps) were emptied into plastic trays and examined for insects. Heliothine larvae were individually placed in 1 oz plastic cups containing a meridic diet (Luttrell and Ali 2009) with soft-touch forceps. The instar and density of larvae collected (number per sweep) were recorded. Individual cups containing a field-collected larva were taken to the laboratory and reared to adults following the procedures of Ali et al. (2008). Data were recorded on survival to pupation, survival to adult, mortality due to parasites, mortality due to disease, and unexplained mortality. Surviving adults were mass mated in 1 gallon cardboard cartoons. Resulting progeny were assayed for susceptibility to Bt toxins following the procedures of Luttrell and Ali (2009).

In 2010-2012, heliothine larvae were observed on hophornbeam copperleaf in Washington County, Mississippi, but detailed ecological and Bt susceptibility information were not collected. A few larval collections were made and both *H. zea* and *H. virescens* emerged as adults from the collections. The larvae were heavily parasitized and only a small fraction of collected larvae survived to adult. In 2013, collections were made from hophornbeam copperleaf near Jonestown, Tchula and Holly Ridge, Mississippi. The Jonestown, Mississippi population included 100 larvae. Twenty pupae were obtained from the 100 larvae. The Tchula, Mississippi population included 66 larvae. Twenty-five pupae were obtained. All were *H. virecens*. More detailed ecological information was collected from the Holly Ridge, Mississippi collection and is reported below.

Collective data from 2002-2009 were summarized by host plant for *H. zea* and *H. virescens* colonies and studied by descriptive statistics and a one-way ANOVA in JMP 8.0. Numbers of samples varied widely, and information for instar (larval size) at collection and estimated population density were not available from all collections. For those with information on field density, number of larvae per acre was estimated using the following assumptions: velvetleaf densities were 1 plant per square foot, all agricultural crops were grown on 40 inch rows, corn and sorghum plants were at densities of 30,000 plants per acre, cotton plants were at densities of 40,000 plants per acre, garbanzo plants in sentinel plots were at a density of 1 plant per row foot, and one sweep of a 15 inch diameter sweep net sampled 3.1667 square feet.

Results and General Observations

Of the 323 collections characterized as *H. zea* (Table 1), a total of 23,269 larvae from 14 different host species were collected and studied. Twenty-five colonies were established from hophornbeam copperleaf. The number of larvae collected varied by host plant. Hophornbeam copperleaf collections averaged 90 larvae per collection. Those from sorghum averaged 157 larvae which was greater than the average of 25 larvae for cotton collections. Average instar was similar across all host plants sampled. Estimated density on hophornbeam copperleaf and ranged from 56 to 89% across all host plants (Table 2). Larval mortality due to parasitism was greatest on hophornbeam copperleaf (21.1%), and significantly lower for collections made from Bt corn, Bt cotton, corn, crimson clover, and sweet corn (0.0 to 3.2%). Larval mortality from disease was highest for collections from Bt corn (6.7%). Unexplained mortality was high and ranged from 1.8% on sweet corn to 35.2% on sorghum. Unexplained mortality for larvae collected on hophornbeam copperleaf was 15.5%.

Table	e 1. Number	of <i>H. z</i> .	<i>ea</i> larvae	collected	from	hophornbeam	copperleaf	and	other	host	plants	in	Arkansas	and
surrounding states during 2002-2006.														

Host Plant	No. Collections	No. Larvae Collected (SEM)	Avg. Instar (SEM)	No. Collections with Estimated Field Density	Estimated Density Per Acre (SEM)
alfalfa	1	30 () ab	3.1 ()	0	
black light	1	52 () ab		0	
Bt corn	41	76 (9.3) ab	3.3 (0.14)	7	31,971 (9354)
Bt cotton	19	68 (13.7) ab	3.3 (0.65)	1	1376 ()
corn	80	59 (6.7) ab	3.5 (0.10)	13	30,323 (6497)
cotton	32	25 (10.6) b	3.3 (0.27)	4	12,044 (9391)
crimson clover	51	75 (8.4) ab	2.9 (0.09)	23	2523 (559)
garbanzo	29	85 (11.1) ab	3.1 (0.12)	10	19,946 (6004)
geranium	13	55 (16.6) ab	3.1 (0.18)	4	2555 (1901)
hophornbeam copperleaf	25	90 (11.9) ab	3.2 (0.14)	12	4642 (1417)
peanut	3	54 (34.5) ab	3.7 (0.38)	1	825 ()
sorghum	15	157 (15.4) a	3.2 (0.21)	8	18,825 (4243)
soybean	9	123 (19.9) ab	3.5 (0.27)	3	1513 (781)
sweet corn	1	56 () ab	3.4 (0.65)	0	
velvetleaf	3	121 (34.5) ab	3.7 (0.46)	1	4356 ()

Host Plant	% Survival to Pupation (SEM)	% Survival to Adult Emergence (SEM)	% Parasitism (SEM)	% Diseased (SEM)	% Unknown Mortality (SEM)
alfalfa	76 ()	76 ()	6.7 () ab	0.0 () b	16.7 () ab
black light	75 ()	75 ()	0.0 () b	0.0 () b	15.4 () b
Bt corn	59 (3.0)	56 (3.2)	0.5 (1.7) b	6.7 (1.1) a	27.4 (2.8) ab
Bt cotton	69 (4.4)	67 (4.8)	2.4 (2.8) b	4.3 (1.8) ab	24.1 (4.2) ab
corn	59 (2.1)	58 (2.3)	1.7 (1.3) b	3.4 (0.8) ab	32.5 (2.0) a
cotton	64 (3.4)	62 (3.7)	3.8 (2.1) ab	0.4 (1.4) b	27.7 (3.2) ab
crimson clover	69 (2.7)	66 (2.9)	3.5 (1.5) b	3.5 (1.0) ab	20.9 (2.5) ab
garbanzo	68 (3.6)	59 (3.9)	5.3 (2.0) ab	0.7 (1.3) b	28.5 (3.4) ab
geranium	59 (5.3)	53 (6.0)	11.7 (3.0) ab	2.2 (1.9) ab	29.7 (5.1) ab
hophornbeam copperleaf	56 (3.8)	53 (4.3)	21.1 (2.2) a	1.9 (1.4) ab	15.5 (3.6) b
peanut	64 (11.0)	59 (12.0)	8.8 (6.4) ab	0.1 (4.1) b	29.2 (10.5) ab
sorghum	60 (4.9)	63 (5.4)	3.2 (3.5) b	1.4 (2.3) ab	35.2 (4.7) a
soybean	65 (6.4)	59 (6.9)	7.0 (3.6) ab	0.1 (2.4) b	28.9 (6.1) ab
sweet corn	89 (89 ()	0.0 () b	5.4 (7.2) ab	1.8 () b
velvetleaf	81 (11.0)	76 (12.0)	3.6 (6.4) ab	0.0 (4.1) b	17.1 (10.5) ab

Table 2. Survival to pupation, adult emergence and incidence of parasitism and disease in *H. zea* larvae collected from hophornbeam copperleaf and other host plants in Arkansas and surrounding states during 2002-2006.

Of the 28 collections made from 2002-2009 and characterized as *H. virescens* (Table 3), only one was from hophornbeam copperleaf. More collections were made from velvetleaf (7) and sentinel plots of garbanzo bean (6). Estimated densities were much higher on velvetleaf (176,418 per acre) than on hophornbeam copperleaf (2476 per acre). Survival to pupation was similar for all weed hosts (67.6 to 75.3%), but lower for a few collections of *H. virescens* from corn (Table 4). These collections were all made in June when corn was silking and few or no broadleaf crop hosts were attractive as a host. Larval instar was not recorded, but notes indicated that all larvae were neonates. Only 38.7% of these *H. virescens* larvae collected on corn survived to pupation. Parasitism on hophornbeam copperleaf was 18.9%, the highest observed on any host plant. Unexplained mortality was least for the collection from hophornbeam copperleaf (4.4%) and greatest for the *H. virescens* collected as neonates from corn silks (51.4%).

Host Plant	No. Collections	No. Larvae Collected (SEM)	No. Collections wi Estimated Instar	Avg. Instar (SEM)	No. Collections with Estimated Field Density	Estimated Density Per Acre (SEM)
corn	5	32 (15.3)	0		0	
cotton	4	50 (17.2)	2	3.5 (0.71)	2	23,000 (86,543)
garbanzo	6	71 (14.0)	5	2.8 (0.48)	5	11,367 (70,662)
geranium	1	90 ()	0		0	
hophornbeam copperleaf	1	90 ()	1	3.9 ()	1	2476 ()
palowonia	4	24 (17.2)	1	2.8 ()	0	
velvetleaf	7	74 (12.9)	5	1.9 (0.45)	5	176,418 (86,543)

Table 3. Number of *H. virescens* larvae collected from hophornbeam copperleaf and other host plants in Arkansas and surrounding states during 2002-2006.

Table 4. Survival to pupation, adult emergence and incidence of parasitism and disease in *H. virescens* larvae collected from hophornbeam copperleaf and other host plants in Arkansas and surrounding states during 2002-2006.

Host Plant	% Survival to Pupation (SEM)	% Survival to Adult Emergence (SEM)	% Parasitism (SEM)	% Diseased (SEM)	% Unknown Mortality (SEM)
corn	38 (12.4) b	37 (6.7) b	2.9 (3.36)	1.0 (0.51)	51.4 (7.54) a
cotton	53 (20.6) ab	52 (7.8) ab	10.3 (5.33)	0.0 (0.80)	36.7 (8.44) ab
garbanzo	75 (14.6) a	71 (6.2) a	6.1 (3.06)	0.1 (0.46)	18.0 (6.89) b
geranium	82 () a	80 () a	0.0 ()	1.1 ()	18.8 () ab
hophornbeam copperleaf	74 () a	74 () a	18.9 ()	0.0 ()	4.4 () b
palowonia	67 (17.8) ab	61 (7.5) ab	3.4 (3.76)	0.0 (0.57)	35.4 (8.49) ab
velvetleaf	73 (14.2) a	66 (5.7) ab	6.0 (2.85)	0.3 (0.43)	17.4 (6.38) b

Hophornbeam copperleaf, a native annual plant that grows throughout the geographic range of *H. zea* and *H. virescens*, appears to be a suitable host for refuge populations of both insect species during late summer and fall. Populations of both insects collected from hophornbeam copperleaf were heavily parasitized but more than 50% of larvae collected from the plant survived to the adult stage. Densities of heliothine larvae varied but they were consistently found on the plant in late summer. At times densities greater than one larva per sweep were observed. Patches of hophornbeam copperleaf appear to be common across the southern landscape. Additional landscape-level estimates of host abundance and movement of these polyphagous pest species from host to host through the year are needed. If our general assessment of plant abundance and common infestations are applicable across the landscape, this may be an important refuge population not exposed to insecticidal proteins in transgenic crops. Maintaining a reservoir of susceptibility (i.e. refuge) is an important component of contemporary resistance management programs.

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