

**FACTORS INFLUENCING SURVIVAL OF
OVERWINTERING BOLL WEEVILS
(*ANTHONOMUS GRANDIS*)
IN NORTHEAST ARKANSAS**

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Abstract

We examined different parameters that influence the survival of overwintering populations of the boll weevil, *Anthonomus grandis grandis* Boheman. Spring and fall surveys of boll weevil densities were conducted using Grandlure pheromone traps in 1994, 1995, and 1996 in four counties in Arkansas. Various habitat types were sampled in each county, and the average number of weevils caught in each habitat was calculated. Traps around wooded areas consistently contained the highest average of trapped boll weevils in all counties sampled. In Fall 1996, trap-caught boll weevils were dissected to determine their fat content and the condition of their reproductive organs in order to determine the diapause status of the boll weevil populations over time. Laboratory experiments were performed in Fall/Winter 1996 in which diapause-conditioned boll weevils were placed in containers and were submerged in a cold circulation bath at freezing or sub-freezing temperatures for varying hours per day for one to several days. Containers were either empty, or filled with dried grass or leaf material that either remained dry or was moistened prior to introducing the weevils. Laboratory results showed that very low temperatures and wet substrate are the least suitable conditions for boll weevil survival.

Introduction

Eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, continues to be of great importance to the Southern US cotton states. Implementation of large scale programs is very costly and risky. The formation of new strategies for boll weevil control can supplement the cultural, mechanical, and chemical practices which are already being conducted (El-Lissy and Myers, 1996).

Boll weevils spend the winter as diapausing adults in natural or man-made habitats near cotton fields (Brazzel and Newsom, 1959). Investigators realized that cold, harsh

winters increased the mortality rate of the overwintering boll weevils, and laboratory bioassays demonstrated that low temperatures and moisture reduced the survival rate of boll weevils in diapause condition (Parajulee *et al.* 1996; Slosser *et al.* 1996; Sorenson and George 1996; Sorenson and Layton 1996). Favorable overwintering habitats would, therefore, provide shelter against temperature and moisture. Surveys of boll weevil densities during spring emergence in various habitat types near cotton fields could offer information about how many boll weevils survive in those areas. Identification of the most suitable habitats would designate areas where control efforts should be focused. Treatment of only the areas around habitat which are most likely to harbor the largest numbers of emerging boll weevils would reduce costs and effort. In this study, habitat types were identified, and the number of boll weevils caught in pheromone traps were used to estimate the most favorable habitats for overwintering boll weevils.

In the fall when the cotton is defoliated, harvested, and then cleared from the fields, boll weevils begin to enter overwintering habitats. Before doing so, however, they need to enter a state of diapause so that they are able to tolerate the lack of food and the coldness of winter. A boll weevil is considered as being in diapause condition when metabolism rate is reduced, reproductive organs are atrophied, and fat content increases while water content decreases (Brazzel and Newsom, 1959; Earle and Newsom, 1964; Carter and Phillips, 1973; Grodowitz *et al.*, 1987). In an effort to reduce the number of diapause boll weevils entering overwintering habitats as part of the eradication program, insecticides are applied prior to and during cotton harvest. However, not all cotton is harvested at the same time, and not all boll weevils enter diapause at the same time. A second part of our project, therefore, was to determine when in late summer do the boll weevils show physical signs of being in diapause condition. Eliminating the food sources needed for the boll weevils to build-up their fat stores early enough in the season would help to reduce the number of insects successfully entering a diapause condition suitable for winter survival.

Materials and Methods

Density Survey: A survey of overwintering boll weevils was conducted in 1994, 1995, and 1996 in Lonoke, Crittenden, Craighead, and Mississippi counties in Arkansas. Boll weevil pheromone traps were placed in various habitats near cotton fields. Each trap location was determined using longitude and latitude measurements obtained with a Garmin global positioning instrument. Habitat types were described, and each trap was classified as representing one of these habitats. Habitat types included the following descriptions.

- woods - deciduous trees, moderate undergrowth, sometimes standing water

- ditch bank - few trees, heavy undergrowth, sometimes standing water
- buildings - homes, farm buildings, gins
- roadside - cleared, grassy embankments along roads
- bayou (tree line) - continuous row of deciduous trees, following a bayou, moderate undergrowth
- well head - area around well usually located in a grassy clearing, serving as a pivot point in the center of fields to be irrigated

Every two weeks during the survey period, the number of boll weevils caught in each trap was counted, and the data was recorded using PSION data collectors. Descriptions of overwintering habitats were updated as necessary. The average number of weevils per trap were calculated by date and by habitat using SAS 6.11.

Dissections: Pheromone-trapped boll weevils were freezer killed and then stored in isopropyl alcohol at room temperature. Boll weevils were dissected on each collection date in the fall of 1996. Dissections determined sex, fat content, and reproductive condition. Fat content for both male and female weevils was described as 1) no fat; 2) moderate amounts of fat in the form of oily and/or mealy material; or 3) well-formed fat globules. Reproductive conditions for females were described as 1) empty: small, transparent ovarioles; 2) undifferentiated: small, opaque ovarioles in which follicles are not formed; 3) differentiated: well-formed follicles, but no eggs; 4) well-developed: eggs and follicles developed and in good condition; and 5) reabsorbing: ovarioles containing eggs that are detached from the ovariole walls and are being reabsorbed into the surrounding tissue. Reproductive conditions for males were described as 1) poor: testes small, dense (often yellowed), and indistinctly lobed; and 2) well-developed: testes opaque at center and transparent along edges, with distinct lobes.

Cold Bath Experiments: Boll weevils were collected from pheromone traps or allowed to emerge from cotton squares in plastic, ventilated cages (25cm X 38cm X 18cm) in the laboratory at room temperature (approx. 26°C). On the day that adult boll weevils emerged, they were placed into cages with fresh squares, small bolls, or artificial diet for food. The cages were kept inside a Percival Scientific I-36VL Biological Incubator with a Series 982 Microprocessor Controller that was set for temperatures and photoperiods which are known to induce diapause in boll weevils: 18.3:15.6°C for a light:dark photoperiod of 11:13hr (Earle and Newsom, 1964; Slosser et al., 1996). Additional food was provided every other day, and after 2-3 weeks, the weevils were moved to second, cooler (10°C) biological incubator and left there in total darkness until they were used in an experiment. Cold bath experiments were conducted from October through December 1996. Tin cans (diameter = 10cm, height = 13.5 cm) were filled up to a 6 cm mark with 1) dry leaf substrate; 2) dry grass substrate; 3,4) leaf or grass substrate that had been

moistened with 3 full sprays of water (one squirt at each of 3 different levels in the substrate) from a plant and garden sprayer bottle; or 5) the cans were empty. Fifteen boll weevils were placed inside a tin can for each treatment. The weevils were restricted from crawling to the top of the can by a screen barrier which sat inside the can at the 6 cm mark. Each can was closed with a plastic lid and then attached to the metal covering of the cold bath with Velcro hook and latch strips that were adhered to the lid of the can and the bath cover. The cans were then submerged into a Forma Scientific Model 2067 CH/P countertop bath filled with a 50% water:50% antifreeze solution. The inherent buoyancy of the cans in the water solution was overcome with weights placed onto the top of the bath cover. The cans were left in the cold bath for one of 50 different exposure periods (Tables 3-6). After testing, living and dead weevils were sorted and stored in a freezer in vials of isopropyl alcohol. The weevils were then dissected to determine sex and fat content.

Results

Density Survey: The mean number of boll weevils caught per trap per day for each collection date were calculated for 1994, 1995, and 1996 (Figs. 1a-d, 2a-d). The mean number of boll weevils caught in each trap (mean trap catch = MTC) was always higher in the fall than in the spring, showing the effects of winter kill. In 1996, the number of boll weevils trapped in the spring was the lowest for the three years sampled, reflecting the effect of the severity of the winter of 1996. The percent reduction in MTC was determined by calculating the proportion of the overall mean trap catch in the spring to the overall mean trap catch in the fall, and then multiplying by 100. Subtracting this percentile from 100% gave the percent reduction of boll weevils from the fall to the spring. The percent reduction in mean trap catch (MTC) shows approximately a 90% reduction in mean catch from the fall of 1994 to the spring of 1995 (Figs. 1a-d), while the reduction from the fall of 1995 to the spring of 1996 was 99% in 3 of the 4 counties surveyed (Figs. 2a-d). In the more southern Leno County, the percent MTC reduction was 97%. The mean trap catch was also calculated according to habitat type in each county for 1995 and 1996 (Tables 1,2). Again, 1996 winter apparently greatly reduced the number of boll weevils emerging in the spring (Table 2). In general, however, traps in which the highest number of boll weevils were caught during spring emergence periods were those traps located near wooded habitats or tree lines around bayous. In Mississippi County, wooded habitats are sparse, and the higher trap catches also occurred around larger ditch banks with several trees and undergrowth on the banks.

Dissections: Boll weevils were collected and dissected (number of weevils dissected = 12-30 weevils per date) until no more weevils were caught in pheromone traps when freezing temperatures occurred during the night. Results showed that approximately 50% of both male and female

boll weevils were in diapause condition by mid-September and approximately 90% were in diapause condition by late September (Figs. 3-6). The daylength in mid September is approximately 12:30 and in late September is approximately 12:00. Dissections revealed that the percent of weevils with well-developed fat bodies increased over time in both male and female boll weevils (Figs. 3,5). Just as expected, the percent of weevils with non-functioning reproductive organs also increased over time (Figs. 4,6).

Cold Bath Experiments: Results from the cold bath experiments showed that at 0°C, 80-100% of the boll weevils survived all exposure periods (Table 3). At -5°C, the number of weevils that survived the treatments declined especially when the leaf or grass substrates were moistened and as the number of days of exposure increased (Table 4). The most boll weevil mortality occurred at -10°C in all treatments (Table 5). Percent survival was highest when the boll weevils were placed in cans with dry leaf substrate and second highest in cans with dry grass substrate. An empty can in -10°C provided the least shelter from the cold for the boll weevils.

Preliminary results from dissections of boll weevils used in the cold bath experiment revealed that most of the weevils tested had medium amounts of fat (Tables 6,7). However, of the weevils that survived exposure to cold temperatures, the percentage of weevils with high fat content was greater than the percentage with low fat content (Table 6). Conversely, of the weevils that died during the experiments, the percentage of weevils with low fat content was higher than the percentage of weevils with high fat content (Table 7).

Discussion

The results of the boll weevil survey in four Arkansas counties reveal how the number of weevils caught in pheromone traps decline after cotton is harvested and removed from the fields each fall and as the cool winter temperatures set in. The boll weevils move into their overwintering habitats and enter a diapause state. In the spring, the boll weevils gradually emerge from their habitats and are at first attracted to the pheromone lures in the traps. As the cotton develops and squares become more readily available, the attractiveness of the artificial lures declines.

Each year, the number of boll weevils caught in traps in the fall is up to 2 orders of magnitude higher than the number of weevils trapped in the spring (Figs. 1a-d, 2a-d). The reduction in MTC is almost certainly due to the harshness of the winter on the survival rate of this boll weevil of tropical origin. Evidence for the negative effect of harsh weather on the survival of the boll weevil is most apparent in the very low numbers of weevils caught after the winter of 1995-96 (Figs. 2a-d). The winter of 1995-96 was very severe, with temperatures dropping to -13.0°C as late as February.

Since the location and habitat type for each boll weevil pheromone trap had been determined using global positioning devices and aerial photography, the number of boll weevils trapped in certain areas was used to determine which specific habitats were most suitable for the survival of overwintering boll weevils. Overall, wooded habitats appeared to be the most suitable areas for boll weevil survival (Tables 1,2). The centers of these habitats contain a layer of leaf litter into which the weevils can crawl. Wooded areas also have edges that are thick with bramble, sumac, and thick grass. In survey areas where traps near ditches and bayous caught high numbers of boll weevils, those habitats were well-drained and had heavy undergrowth. Areas around buildings often provide suitable overwintering habitats because of shelters, crevices, and the accumulation of trash, debris and old farm equipment. A new strategy for boll weevil control might be to use aerial photography and remote sensing of cotton-producing landscapes to determine habitat areas that have the greatest potential for protecting overwintering weevils. Then treatment for boll weevils can be concentrated in these areas.

Since at least half of the boll weevils caught in pheromone traps began to show signs of diapause condition in mid-September (Figs. 3-6), control measures would be better if taken prior to this time period. Boll weevils need to feed on cotton squares or bolls in order to achieve the fat levels required to survive the winter. If the cotton fields are treated with boll weevil insecticides and defoliated before the weevils cease reproduction and increase feeding activity, then the number of hardy boll weevils entering overwintering habitats could be reduced. Daylength and temperature obviously have an affect on the onset of diapause during early fall, but the exact effects of these factors cannot be determined from this study. It would be interesting to know if there are boll weevils which are genetically predestined to enter diapause and never reach a reproductive state. It is also conceivable that boll weevils already prepared to enter the winter habitats are not attracted to pheromone traps, and thus our samples might not exactly reflect the actual percent of boll weevils in diapause condition.

The survival of boll weevils in the laboratory cold bath treatments reflect the data that was collected in the field. Boll weevil survival was the highest in dry leaf substrate (Tables 3-5), which is the kind of ground cover found in well-drained wooded areas. The percent of boll weevils that survived in the dry grass substrate was also high, suggesting that thick, dry grassy or weedy habitats that are in areas protected from wind and rain (such as in ditch banks or near buildings) may serve as favorable overwintering habitats. The data confirms our assumptions for field data and the results of other laboratory studies (Sorenson and George, 1996) that the wet areas cause greater boll weevil mortality during the winter. Periods of exposure to -5°C and -10°C for more than one day also increased boll weevil mortality.

Although not all boll weevils have been dissected from the cold bath treatments, preliminary results confirm that weevils that were not fat were less likely to survive in the cold temperatures (Tables 6,7). This also shows that not all of the boll weevils maintained and fed in the growth chambers reached optimal diapause status. Therefore, interpretation of the laboratory data should consider the condition of the boll weevils when they were subjected to the cold bath. Further evaluation of this data will enhance our understanding of the ability of boll weevils to survive winters in Arkansas.

The results of all of our studies provide information that will be useful for predicting the severity and localities of boll weevil spring outbreaks in accordance with habitat availability, winter temperatures, and precipitation in the form of rain or snow. Remote sensing of cotton production areas can help in identification of habitat types which can be depicted on a map created with a Geographic Information System. Locating boll weevil "hot spots" and considering weather factors will help cotton producers and consultants to make better assessments about where to concentrate boll weevil control efforts.

Acknowledgements

We thank Cotton, Inc. and the University of Arkansas for financial support, the cotton producers in Lonoke, Craighead, Crittenden and Mississippi Counties for allowing us to place our pheromone traps on their land, Dr. Jake Phillips for showing us the what to look for in our boll weevil dissections, and CES technicians, Kimberly Hunt, Jarrod Pettis, and Mike Dillion, for their assistance in performing our studies.

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Table 1. Mean number of boll weevils caught per trap in various habitat type areas in four Arkansas counties in spring of 1995.

	County			
<u>Habitat</u>	<u>Lonoke</u>	<u>Craighead</u>	<u>Crittenden</u>	<u>Mississippi</u>
woods	42.8	57.6	16.1	27.7
ditch bank	4.3	29.0	13.1	27.3
building	23.5	11.3	3.5	n. a.
roadside	34.1	16.0	12.6	14.9
bayou	32.0	24.9	n. a.	15.1
wellhead	n. a.	1.6	7.2	8.2

Table 2. Mean number of boll weevils caught per trap in various habitat type areas in four Arkansas counties in spring of 1996.

	County			
<u>Habitat</u>	<u>Lonoke</u>	<u>Craighead</u>	<u>Crittenden</u>	<u>Mississippi</u>
woods	8.8	5.7	0.4	4.0
ditch bank	4.0	1.1	0.4	2.5
building	5.2	0.7	0.1	n. a.
roadside	3.0	0.8	0.3	0.3
bayou	9.5	0.6	n. a.	0.3
well head	n. a.	0.0	0.0	0.2

Table 3. The percent of surviving boll weevils inside containers with or without substrate after exposure to 0°C water/anti-freeze solution in a cold bath.

Percent of Living Weevils (number of weevils treated):					
Exposure Period*	Can = Empty	Can with: Dry Leaves	Can with: Wet Leaves	Can with: Dry Grass	Can with: Wet Grass
2h / 1d	90.0 (30)	96.5 (30)	100 (30)	100 (29)	100 (30)
2h / 2d	96.5 (30)	100 (30)	96.5 (29)	100 (30)	100 (30)
2h / 4d	93.5 (30)	96.5 (29)	93.5 (30)	100 (30)	96.5 (31)
4h / 1d	100 (30)	96.5 (30)	90.5 (30)	100 (30)	100 (30)
4h / 2d	100 (30)	90.0 (30)	100 (30)	87.0 (30)	93.5 (30)
4h / 4d	--	93.0 (15)	100 (15)	100 (15)	100 (15)
6h / 1d	100 (30)	100 (30)	100 (30)	100 (30)	100 (30)
6h / 2d	--	100 (15)	100 (15)	100 (15)	100 (15)
8h / 1d	100 (31)	100 (30)	100 (30)	100 (30)	100 (30)
8h / 2d	80.0 (25)	100 (10)	--	100 (25)	100 (10)

* h = hour; d = day; e.g. 2h/1d = exposure for 2 hours on only 1 day

Table 4. The percent of surviving boll weevils inside containers with or without substrate after exposure to -5°C water/anti-freeze solution in a cold bath.

Percent of Living Weevils (number of weevils treated):					
Exposure Period *	Can = Empty	Can with: Dry Leaves	Can with: Wet Leaves	Can with: Dry Grass	Can with: Wet Grass
2h / 1d	100 (30)	100 (30)	70.0 (30)	100 (30)	90.0 (30)
2h / 2d	96.5 (30)	80.0 (30)	53.5 (30)	100 (30)	41.5 (29)
2h / 4d	60.0 (15)	--	33.0 (15)	--	47.0 (15)
4h / 1d	100 (30)	100 (30)	80.0 (30)	100 (30)	93.5 (30)
4h / 2d	90.0 (30)	100 (30)	77.7 (45)	73.5 (30)	75.0 (31)
4h / 4d	93.0 (15)	--	--	--	40.0 (15)
6h / 1d	97.7 (45)	93.3 (45)	75.3 (45)	90 (30)	75.0 (31)
6h / 2d	--	--	--	--	--
8h / 1d	96.5 (25)	73.3 (35)	93.3 (40)	100 (25)	80.0 (30)
8h / 2d	--	--	--	--	--

* h = hour; d = day; e.g. 2h/1d = exposure for 2 hours on only 1 day

Table 5. The percent of surviving boll weevils inside containers with or without substrate after exposure to -10°C water/anti-freeze solution in a cold bath.

Percent of Living Weevils (number of weevils treated):					
Exposure Period *	Can = Empty	Can with: Dry Leaves	Can with: Wet Leaves	Can with: Dry Grass	Can with: Wet Grass
2h / 1d	3.5 (30)	53.5 (30)	19.7 (45)	47.0 (29)	30.0 (29)
2h / 2d	0.0 (30)	56.5 (30)	7.0 (29)	37.0 (30)	0.0 (30)
2h / 4d	--	--	--	--	--
4h / 1d	0.0 (30)	36.5 (30)	20.0 (30)	33.3 (30)	0.0 (30)
4h / 2d	3.5 (30)	36.5 (30)	17.0 (30)	13.5 (30)	0.0 (30)
4h / 4d	--	--	--	--	--
6h / 1d	0.0 (25)	40.5 (30)	27.0 (30)	43.0 (30)	40.0 (30)
6h / 2d	0.0 (10)	5.0 (25)	5.0 (20)	10.0 (25)	3.5 (25)
8h / 1d	0.0 (30)	30.0 (30)	13.5 (30)	27.0 (30)	0.0 (30)
8h / 2d	--	--	--	--	--

* h = hour; d = day; e.g. 2h/1d = exposure for 2 hours on only 1 day

Table 6. Percent of boll weevils alive after cold bath experiments which had low, medium, or high body fat content. (Preliminary Data)

Temperature	Males			Females		
	low	medium	high	low	medium	high
0°C	5.5	61.1	33.0	10.0	60.0	30.0
-5°C	5.3	58.0	36.6	9.8	54.4	35.7
-10°C	0.0	45.0	55.0	4.6	67.0	32.5

Table 7. Percent of boll weevils dead after cold bath experiments which had low, medium, or high body fat content. (Preliminary Data)

Temperature	Males			Females		
	low	medium	high	low	medium	high
0°C	0.0	100	0.0	0.0	100	0.0
-5°C	37.8	55.6	6.7	32.5	65.0	2.5
-10°C	19.3	68.9	11.7	17.9	68.4	13.5

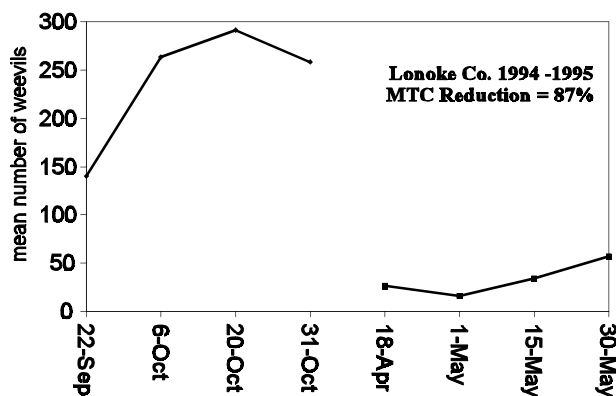


Figure 1a. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Lonoke Co., AR in fall 1994 and spring 1995.

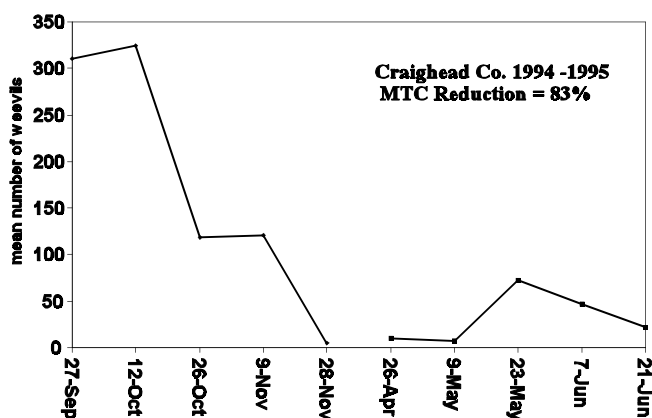


Figure 1b. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Craighead Co., AR in fall 1994 and spring 1995.

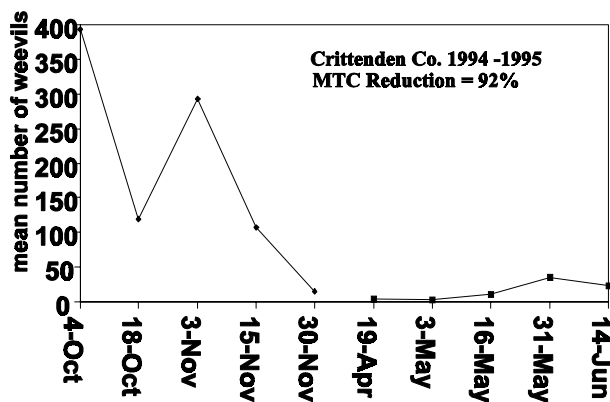


Figure 1c. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Crittenden Co., AR in fall 1994 and spring 1995.

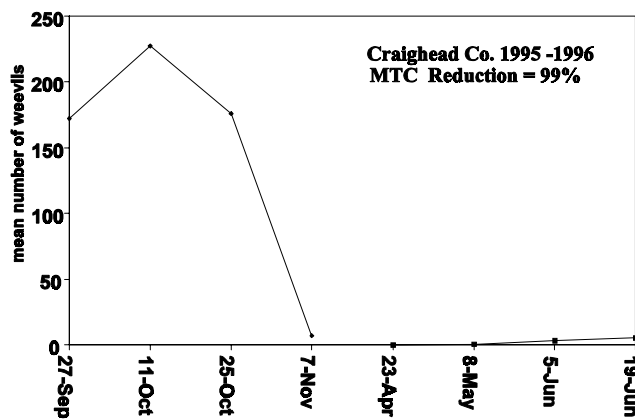


Figure 2b. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Craighead Co., AR in fall 1995 and spring 1996.

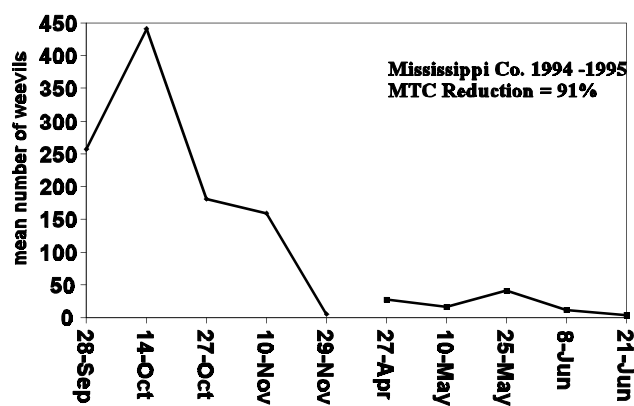


Figure 1d. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Mississippi Co., AR in fall 1994 and spring 1995.

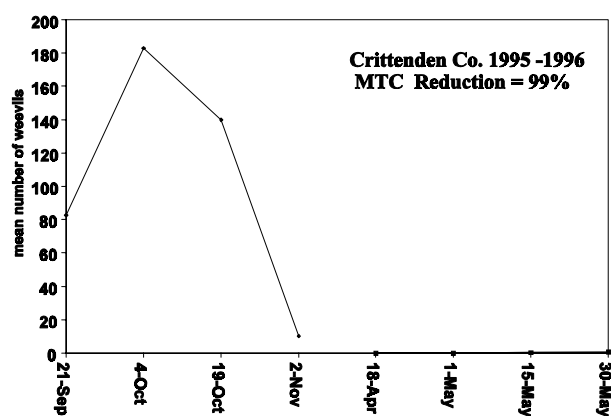


Figure 2c. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Crittenden Co., AR in fall 1995 and spring 1996.

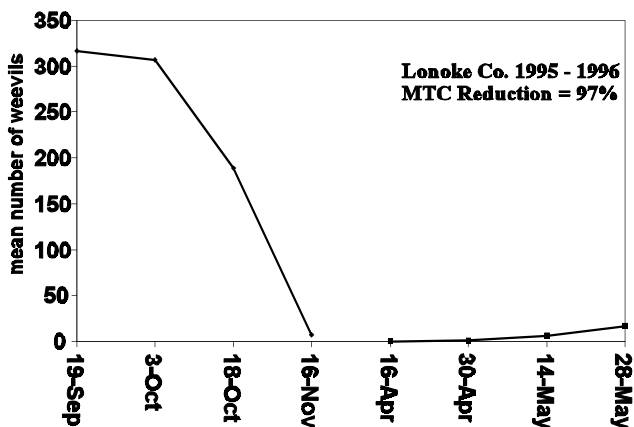


Figure 2a. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Lonoke Co., AR in fall 1995 and spring 1996.

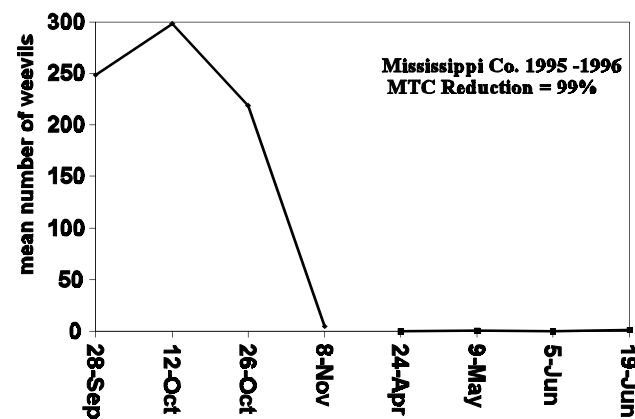


Figure 2d. The mean number of boll weevils caught per trap (mean trap catch = MTC) in Mississippi Co., AR in fall 1995 and spring 1996.

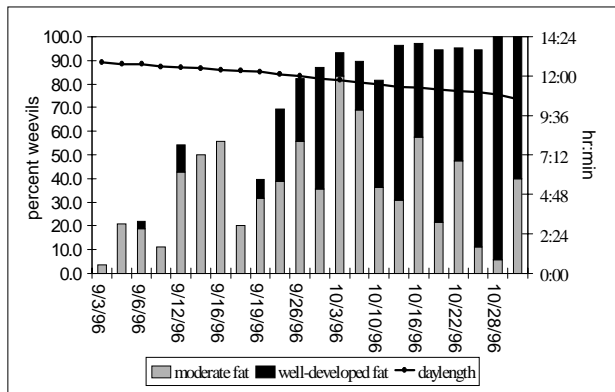


Figure 3. Percent of male boll weevils that contained moderate amounts of fat or had well-developed fat bodies which is indicative of being in a diapause state.

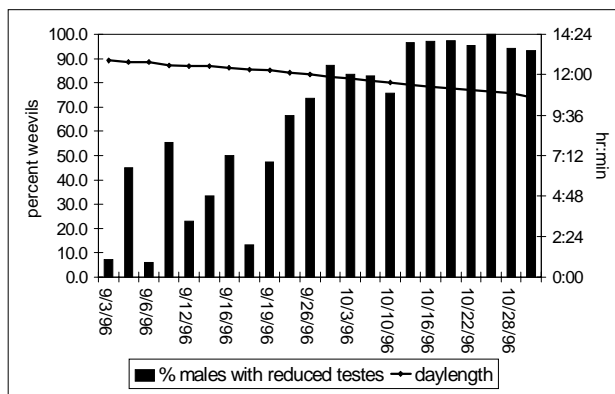


Figure 4. Percent of male boll weevils whose testes were reduced indicating that these reproductive organs were not functional.

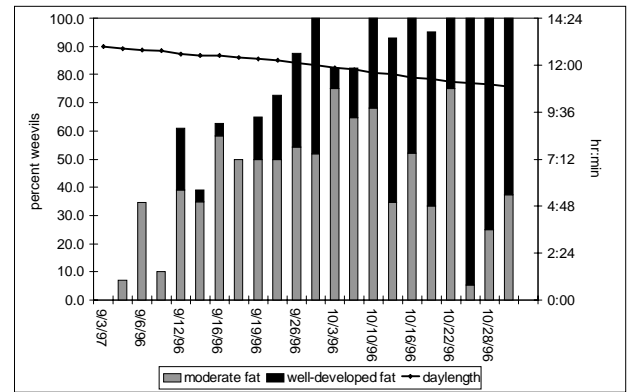


Figure 5. Percent of female boll weevils that contained moderate amounts of fat or had well-developed fat bodies which is indicative of being in a diapause state.

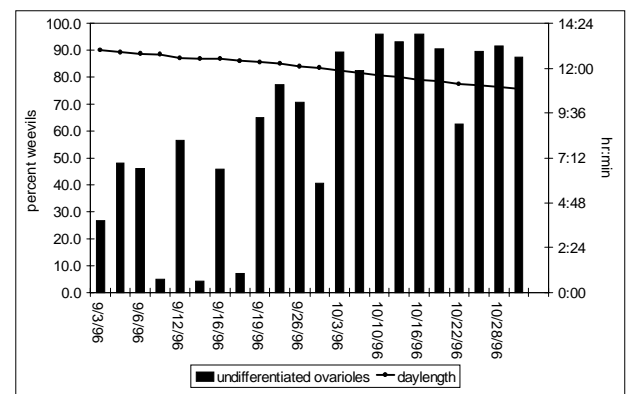


Figure 6. Percent of female boll weevils whose ovarioles were undifferentiated indicating that these reproductive organs were not functional.