SURVIVAL OF BOLL WEEVILS EXPOSED TO FREEZING TEMPERATURES WITH AND WITHOUT ICE NUCLEATORS

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Abstract

We assessed the impact of the presence of ice on the survival of over-wintering boll weevils (*Anthonomus grandis grandis* Bohman) through two sets of experiments. In the first, groups of weevils were exposed to three sub-freezing temperatures (-3.9, -7.0, and -9.4°C) for six hours either with or without free water present. Water was provided by sandwiching the weevils between two layers of wet cotton cloth. Whenever freezing of the water in the cloth took place, boll weevil survival was very low, regardless of tempera-ture. In the second set of experiments, the freezing points of individual weevils were determined with or without free water present. Dry weevils froze at temperatures between -4° and -18°C (mean =14.49°C); wet weevils froze essentially simultaneously with the free water present with them. Weevils that froze expired. No difference between the freezing points of trap-caught and square raised weevils were detected. These observations may help explain the distribution of boll weevils in the Missouri Bootheel.

Introduction

Winter kill is an extremely important factor in the annual population dynamics of the boll weevil (*Anthonomus grandis grandis* Bohman). While the effects of low temperatures on boll weevil winter survival have received much research attention (Pierce, 1916; Hixson and Sooter, 1937; Slosser et al, 1994; Sorenson and House, 1995), relatively little attention has been directed to the impact of the presence or absence of water on winter survival. Pfrimmer and Merkl (1981) examined overwintering survival in Mississippi and attempted to correlate survival with several weather parameters. They concluded that cold, dry weather was most detrimental to boll weevil populations. Taft and Hopkins (1966) found that survival was lowest under excessively moist conditions in a cage study in South Carolina. In southeast Missouri, two years of observation of the distribution of spring-emerging boll weevils suggest that over-winter survival is very poor in areas where suitable habitat is poorly drained (Sorenson unpublished data).

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We conducted these experiments to determine what impact the presence of free water in the form of ice had on the survival of over-wintering boll weevils exposed to sub-freezing temperatures.

Materials and Methods

We conducted two studies to assess viability of freeze exposed weevils and to determine the freezing characteristics of weevils.

Cold Bath Viability Tests: Weevils used in this study were collected as adults in late fall from pheromone traps. These insects were fed for at least two weeks on fresh squares and small bolls under cool, short day conditions (11:14h light: dark; 18.3°:15.6°C light: dark). After this feeding period, the weevils were held at 5°C in complete darkness until used in experiments. Examination of the fat body development of dissected weevils indicated that approximately 90 % had fat bodies consistent with diapause condition.

We used a Forma Scientific 2161 Circulating Bath and a modification of the technique developed by Slosser, et al. (1994) to evaluate the survival of groups of weevils exposed to freezing temperatures with and without free water present.

The standard test protocol was to place groups of 25 weevils in 9.5 cm diameter by 13 cm deep steel containers along with an electronic temperature logger (Stowaway™, Onset Computer Corp.) and attached thermistor. The weevils and the logger were isolated from each other by a plastic partition to prevent the insects using the logger as a refuge from the cold. Hook and loop tape was used to secure the steel containers to the inside of the cold bath lid. The temperature loggers recorded the temperature in the container every 30 seconds for the duration of the test.

The steel containers with weevils were submersed to a depth of about 10 cm for a 6 hour period. The weevils were then placed in a 5cm X 10cm plastic container and held at room temperature for 24 hours. Mortality was then recorded.

Three approximate temperatures were tested: -3.9, -7.0, and -9.4°C with two moisture regimens: “wet” and “dry”. The wet treatment was established by sandwiching the test group (along with the thermistor) between two saturated 12cm X 12cm pieces of cotton cloth. Each treatment was replicated 4 times in a randomized complete block design with time as the blocking factor.

Data were analyzed through ANOVA with means separation through LSD (a = 0.05) (SAS Institute 1988). Data were arcsine transformed prior to analysis; however, raw means are presented in the tables. Output from the temperature loggers was examined to determine if and when freezing of the water in wet treatments occurred and to assess variation from target temperatures.

Boll Weevil Freezing Points With and Without Water: Weevils used in this study came from two populations. The first population was the same one used in the previous experiment.

The second population was one obtained from squares. Squares were collected from cotton fields around Portageville, MO during September and October, and placed in cages in a walk-in environmental chamber with a temperature of 24°C and a photoperiod of 11 h light: 13 h dark. Adult weevils emerging from these squares were collected every other day and handled as described above for the trap-captured weevils.

Innoculative freezing temperatures of the boll weevils, the temperature at which they begin freezing in the presence of ice, were determined using a differential thermal analysis system consisting of an aluminum block with six independent chambers. Each chamber contained a thermoelectric heat pump module (Materials Electronic Products Corporation); attached to each module was a 0.013 cm thin-film copper-constantan thermocouple that monitored sample (insect) temperature. Output from the heat module and thermocouples were captured by a ADALAB-PC data acquisition system that also provided test chamber set-point temperatures. The aluminum block was housed in a temperature programmable Tenney JRTJR16 test chamber (Tenney Engineer-ing, Inc.)

Individual boll weevils were placed in the six cells in the aluminum block. Three of these insects were first wrapped in cheesecloth moistened with distilled water and then wrapped in aluminumk foil; the other three were wrapped in dry cheesecloth and then aluminum foil. The chamber was cooled at a rate of -5°C/hour from 0°C. Differential temperatures of each insect and the reference temperature were recorded every thirty seconds and outputted to a graphing program. Freezing of the insect was signaled by a heat spike in the temperature curve. This procedure was repeated a total of four times (n=12 insects per treatment) for each population.

The Mann-Whitney rank sum test (Mann and Whitney, 1947) was used to determine if freezing point means were different for square raised versus trapped weevils.

Boll Weevil Distribution In Missouri: A grid of boll weevil traps was established across the Missouri cotton growing region during the summers of 1994 and 1995. The grid was 6 X 6 miles in 1994 and 4 X 4 miles in 1995. In each year, the procedure was to seek the cotton field nearest to each intersection point and place three traps in the edge of the field at 100 to 200 meter intervals. Traps were deployed in late May and early June of both years. Locations of trapped fields were fixed with a hand-held Global Positioning System receiver (Magellan GPS®). The traps were monitored for five weeks in 1994 and for four weeks in 1995.

Trap capture data were used to generate relative density point estimate distri-bution maps through a computer mapping program (Atlas Pro 1.5 for Macintosh).

Distribution of forest cover in southeastern Missouri was obtained from the Geographic Resources Center, University of Missouri, Columbia.

Results

Cold Bath Viability Tests: Mortality in the wet -7.0 and -9.4°C treatments was significantly greater than in the remaining treatments; indeed, mortality in these treatments was essentially 100 percent (Table 1). While mortality in the wet -3.9°C was not significantly different from the three dry treatments, it should be noted that in the reps at this temperature where freezing of the cloth occurred relatively soon after submersion in the cold bath (as indicated by the temperature logger), mortality was greater than 40 percent.

Boll Weevil Freezing Points With and Without Water: Dry boll weevils from squares did not have freezing points significantly different from those of trap-captured weevils by the Mann-Whitney rank sum test ($p = 0.729$). Likewise, there was no difference between wet square-raised and wet trap-captured weevils ($p = 0.133$).

Mean freezing point for dry weevils was -14.49°C and -2.44°C for wet weev-ils (Table 2). In 6 out of 24 wet cases, no discernible peak could be detected.

Typical freezing curves for wet and dry weevils are presented in figures 1 and 2, respectively.

Boll Weevil Distribution In Missouri: Boll weevil relative density point estimate distribution maps are presented in Figures 3 and 4. Forest cover in southeast Missouri is depicted in Figure 5.

Discussion

Boll weevils appear to have very little tolerance for the formation of ice crystals in their immediate vicinity. In the viability test, all groups of weevils exposed to ice suffered heavy mortality. The only exception was one group at -3.9°C; in this instance the wet cloth did not freeze until approximately four hours into the course of the test. In one instance at this temperature, freezing did not occur over the entire course of the test; in this instance, mortality was only about 10 percent.

In the freezing point study, boll weevils essentially froze when the water sur-rounding them froze. When a heat spike could be detected for wet treatment weevils, it generally occurred soon after the water in the cheesecloth started freezing (Figure 4). In six cases the heat release from the weevil was indistin-guishable from that of the wet cheesecloth, suggesting that the weevils were freezing coincident with the water in the cotton cloth.

The distribution of boll weevils in both 1994 and 1995 was heavily skewed to the western parts of the Bootheel (Figures 5 and 6). The regions with highest boll weevil numbers correspond to Crowley's Ridge (A relatively high, dissected, and heavily wooded ridge of loessal origin oriented roughly North to South), the Malden Ridge (a former Mississippi River natural bank levee with relatively low relief and very sandy soils), and the southern portion of the Little River Drainage District's Floodway Drainage Ditches (the southern portion of these ditches have high, relatively steep, and heavily wooded spoil banks). The wooded areas in the eastern part of the Missouri cotton production region are typically bottomland hardwoods or cypress-tupelo associations on very flat ground. The relatively large wooded areas associated with the Mississippi River are for the most part not protected by levees and are therefore subject to flooding in late winter and spring.

The observation that boll weevils apparently cannot tolerate ice formation in their immediate vicinity may help to explain the distribution of overwintering weevils in southeastern Missouri. All the areas where high overwintering numbers combine wooded areas with substantial land form relief and/or very well drained soils; most of the wooded areas in the East are very level (or even depressions) and have poor surface drainage. Heavy winter precipitation may keep leaf litter in these areas saturated most of the winter, while that on Crowley's Ridge and the other areas described above drains fairly rapidly after precipitation events. If this is the case, then weevils in the flat wooded areas may proportionally higher mortality due to freezing of the water in the leaf litter they inhabit. This might also help explain the observations of Taft and Hopkins (1966). Further investigation of the relationships between boll weevil survival and moisture conditions are warranted.

The fact that no difference in freezing point could be detected between trapped weevils and weevils raised from squares under conditions known to induce diapause suggests that fall-caught adults, fed under cool, short day conditions for two weeks, assume diapause condition.

Acknowledgments

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Table 1. Mortality of Missouri boll weevils exposed to freezing temperatures with or without water present.

Treatment and	Temperature, °C	% Dead
-9.4 wet	100.0a	-9.4 dry
	27.0 b	-6.7 wet
	99.0a	-6.7 dry
	21.0 b	-3.9 wet
	37.0 b	-3.9 dry
	28.0 b	

Means with same letter not significantly different at p=0.05, LSD

Table 2. Freezing points (°C) of boll weevils raised from squares ("Square") or captured in traps ("Trap"), with ("Wet") or without ("Dry") ice nucleators present.

	Trap, Dry	Trap, Wet	Square, Dry	Square, Wet
	-17.14	*	-15.46	*
	-16.63	-2.30	-16.80	*
	-16.73	*	-17.00	-6.72
	-17.95	-1.68	-16.92	-1.66
	-8.67	-2.49	-15.09	-2.61
	-10.82	*	-13.72	-2.32
	-14.38	-1.86	-17.14	-2.93
	-13.09	-1.76	-8.72	-1.81
	-17.19	-1.81	-17.70	-2.12
	-16.65	-1.42	-15.58	*
	-10.23	-1.37	-12.23	-3.32
	-17.75	-3.79	-3.98	-1.86
mean:	-14.21	-2.05	-14.77	-2.82

*No peak was observed for this insect; freezing apparently occurred simultaneously with freezing of water in cheese cloth.

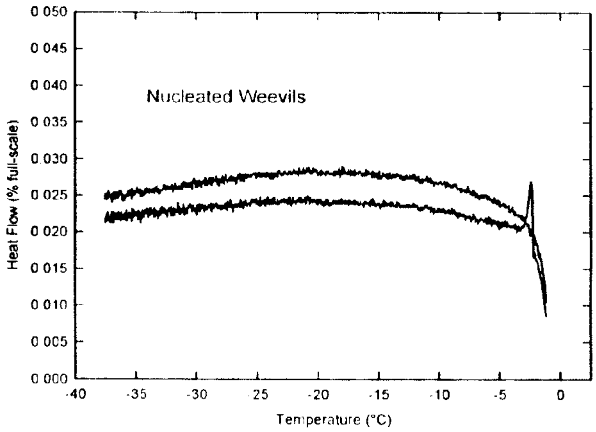

Figure 1. Typical freezing curves for two ice-nucleated (wet cheesecloth-wrapped) boll weevils.

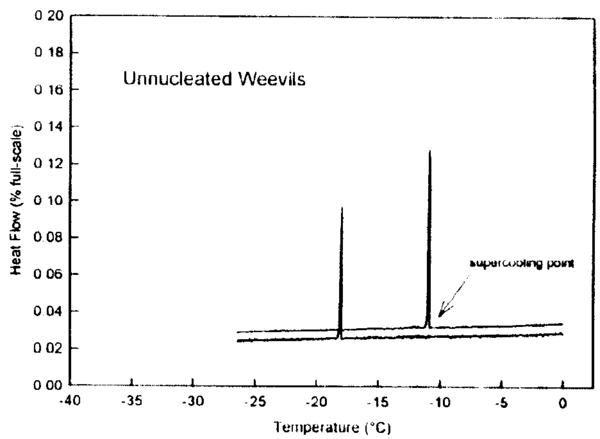

Figure 2. Typical freezing curves for two unnucleated (dry cheesecloth-wrapped) boll weevils. The arrow indicates the supercooling point for one of the insects.

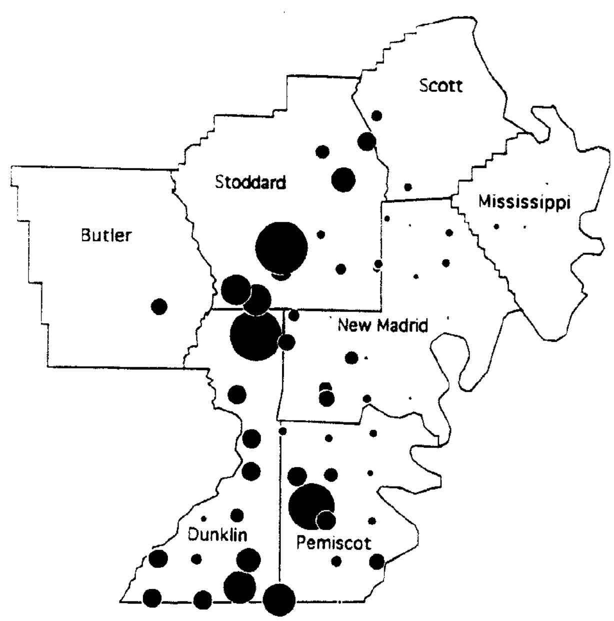

Figure 3. Distribution of over-wintered boll weevils in Southeast Missouri, May-June 1994. The size of the dot is proportional to capture at that site, range 0-2.4 boll weevils/ trap/ day. The distribution of the dots also describes the distribution of cotton in the region.

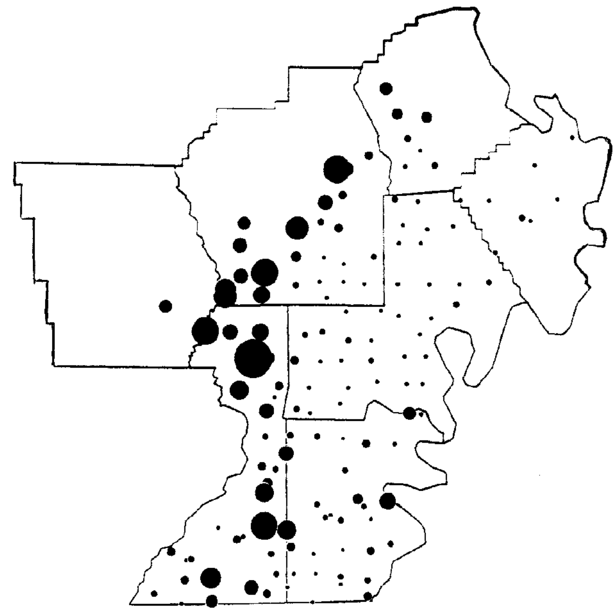

Figure 4. Distribution of over-wintered boll weevils in Southeast Missouri, May-June 1995. The size of the dot is proportional to capture at that site, range 0-12.5 boll weevils/ trap/ day. The distribution of the dots also describes the distribution of cotton in the region.

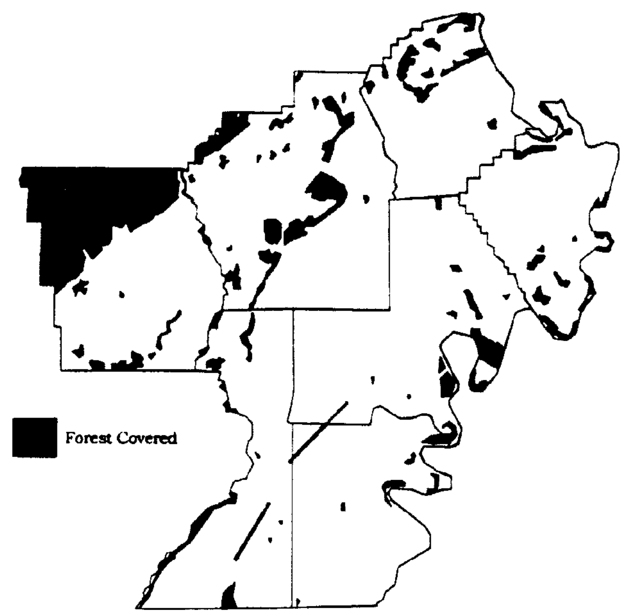

Figure 5. Distribution of forest cover in Southeast Missouri. This map does not include wooded hedgerows or woodlots smaller than about 4 acres.