EFFECT OF TEMPERATURE AND HABITAT ON SURVIVAL OF OVERWINTERING BOLL WEEVILS (*ANTHONOMUS GRANDIS*) IN ARKANSAS

D.R. Johnson, M.P. Maret, D.W. Atwood, T.L. Singer, L.D. Page, H.B. Myers
Cooperative Extension Service, University of Arkansas
Little Rock, AR
R.W. McNew
University of Arkansas
Fayetteville, AR

Abstract

Laboratory experiments were performed in fall/winter 1997 in which diapause-conditioned boll weevils, *Anthonomus grandis grandis* Boheman, were subjected to freezing temperatures within containers submerged in a cold circulation bath and held for one to eight hours. Containers were either empty, or filled with dry or moist leaf litter. Results show that temperature, duration of exposure, and litter, as well as all interactions were significant factors of boll weevil mortality. Mortality increased with temperature reduction and exposure time. The presence of dry litter significantly improved weevil survival over those in empty containers at -10.0 and -12.5°C, and over those in moist litter at -5.0 to -12.5°C. Over 70% of weevils were able to survive temperatures of -2.5°C for eight hours, in either moist or dry litter, while high (>75%) mortality occurred at -10°C or colder temperatures in moist litter, even for short (1 hour) exposures.

Introduction

The need for improved control and the boll weevil eradication program has created a need to increase the ability to predict boll weevil, *Anthonamus grandis grandis* Boheman, winter survival patterns. Overwinter survival is important to understand because it largely determines the magnitude of early cotton field populations (Parajulee et al. 1996, Rummel and Carrol 1993, Fuchs and England 1989) especially in areas of the cotton production region where winter mortality of the boll weevil is significant. These predictions could help focus strategic planning efforts for boll weevil control.

Climatic factors, such as the severity of winter freezes, are important indicators of boll weevil winter survival and thus spring infestations (Pfrimmer and Merkl 1981, Gaines 1943, Bondy and Rainwater 1942). Many investigators have also found relationships between weevil survival and exposure to sub-freezing temperatures in laboratory tests (Sorenson and House 1995, Slosser et al. 1996, Sorenson et al. 1996, Sorenson and George 1996). However, these laboratory tests were conducted without a litter substrate for the weevils and instead exposed directly to inorganic substrates. The insulating effect is known to have an effect on overwintering survival of boll weevils in the field. Results from preliminary tests (Singer et al. 1997) suggest that such use of leaf litter substrate has a great influence on the survival of the boll weevil.

The presence of moisture also influences boll weevil winter survival in freezing temperatures. In the relatively arid climate of the Texas rolling plains, greater winter rainfall is associated with increased survivorship (Price et al. 1985, Stone et al. 1990, Parajulee et al. 1996) apparently due to reduced freeze-drying affects. Dry, cold winter weather has also been highly lethal to Mississippi weevil populations (Pfrimmer and Merkl 1981). On the other hand, Taft and Hopkins (1966) reported that weevil mortality in South Carolina was highest under excessively moist conditions, and in southeast Missouri, over-winter survival was low in wet, poorly drained areas (Sorenson and George 1996). In addition, we know that weevils cannot survive once ice crystals form in their tissues and freezing occurs at warmer temperatures in dry than in moist environments (Sorenson and George 1996).

The main objective of this study was to determine the relationship between sub-freezing temperatures, exposure time, and presence of moisture in leaf litter substrate and boll weevil survival. A second objective was to assess the effectiveness of using empty versus litter-filled containers in laboratory cold bath studies for estimating temperatures for winter kill.

Methods

Adult boll weevils were collected from pheromone traps near cotton fields or were allowed to emerge from cotton squares placed in plastic, ventilated cages in the laboratory. All collections were made in September and early October 1997, in Lonoke County, Arkansas. Collected and newly emerged weevils were induced into a diapause state using techniques described by Slosser et al. (1996).

Boll weevil mortality patterns in sub-freezing temperatures (0, -2.5, -5.0, -7.5, -10.0 and –12.5°C) were examined for three experimental substrate types and four duration of exposure. Substrate types included moist leaves, dry leaves, and no leaves. Duration of exposure were 1, 2, 4, and 8 consecutive hours. A split plot experimental design was used with temperature as the whole plot and substrate × exposure as the subplot. All six temperatures could not be run simultaneously because only two cold baths were available, so order of temperatures were randomly assigned. Treatments at each temperature were replicated four times, blocked in three-day periods during late October and November 1997.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1011-1015 (1998)
National Cotton Council, Memphis TN

Weevils (n=20) were placed inside a 29.6 ml clear plastic cup with a paper lid. Approximately 20 small holes had been punctured into each cup to allow some airflow. Twelve cups (a complete 3×4 factorial of substrate and exposure) were arranged on the bottom of a metal bread pan (23×13×7cm), which was immersed into a circulating cold bath (Forma Scientific Model 2067 CH/P, Forma Scientific, Marietta, OH). A solution of equal parts ethylene glycol antifreeze and water was used as the cooling solution in the cold baths. Temperatures within the cups were verified using a thermocouple attached to an electronic data recorder (Stowaway^{TM} XTI, Onset Computer Corporation, Pocasset, MA). Target temperatures in cups took approximately 15 minutes to equilibrate.

Leaf fragments were used within the plastic cups for the moist and dry leaf treatments (substrate). Leaf fragments had been collected from partially decomposed leaves (2-5 cm^2 fragments) beneath a nearby oak stand. Following collection, leaves were oven-dried. Water was added for moist leaf treatments and mixed until leaves had moistened (55-75% moisture). Weevils were placed within the leaves by first filling cups halfway with leaves, adding weevils, and then quickly adding more leaves until cups were loosely filled. Weevils were added to empty cups in "no leaves" treatments.

After initial pan immersion and a fifteen-minute equilibration time, cups were removed from the cold baths at 1, 2, 4, and 8-hour intervals. Cup removal and pan re-immersion required approximately 20-30 seconds, and trials with a thermocouple and electronic data recorder showed no measurable temperature change within the re-immersed cups. Weevils remained in removed cups and held at room temperature until evaluated.

Weevil survival was evaluated 16 to 24 hours (overnight) after cups were removed from the cold bath. Only individuals which were able to stand and walk were considered as having survived exposure to freezing temperature. Non-walking weevils were monitored for several minutes to verify their condition.

The boll weevil mortality for 1 hour exposure did not reach 100% and as a result another temperature was tested. Immediately following completion of the first set of temperatures (mid-November), boll weevil survival was tested for an additional cold temperature, -15°C, to potentially determine a threshold temperature weevil survival in moist, dry and no litter. A second temperature (-10°C) was used as a control for any later test date effect. Weevils were placed in leaf-treated cups within pans, and pans immersed in cold bath, as described previously, for a duration of one hour (plus a 15-minute temperature equilibration time). Treatments at each temperature were replicated five times, blocked by hour.

Boll weevil mortality data were tested using a General Linear Regression Method (GLM) test (SAS Institute 1990). GLM was utilized to account for unequal number of replications among Temperature (whole plot) treatments. Additional GLM tests were performed on mortality data within each Temperature to determine Litter type, Duration of exposure, and interaction of main effects. Treatment means were separated using protected least significant difference (LSD) ($\propto = 0.05$).

Results and Discussion

The boll weevil mortality in this study was affected by temperature, substrate, and moisture level (Table 1). The trends were similar to those in previous studies but the significant difference in the results is attributed to the effects of substrate and moisture on boll weevil mortality.

Boll weevil mortality levels in empty cups were not significantly different from those in dry substrate, for the temperature exposures ranging from 0 to -7.5°C. However, at colder temperatures (–10 and –12.5°C), weevils in dry substrate had significantly greater survival than those exposed in empty cups. No weevils survived the coldest temperature tested (-15°C for one hour), regardless of the presence of leaves within cups (Table 1). These comparisons indicate that the dry substrate increased boll weevil survival at intermediate sub-freezing temperatures, probably due to a conductivity effect. This difference is important because several authors have reported boll weevil mortality estimates based upon laboratory results from cold exposures within empty containers (Slosser et al. 1996, Sorenson et al. 1996, Sorenson and George 1996, Sorenson and House 1995). However, in the field, weevils overwinter under a cover of plant litter (Bondy and Rainwater 1942). These results indicate that laboratory techniques used to measure weevil survival under field conditions at sub-freezing temperatures may result in extra-conservative estimates of weevil survival.

Temperature, substrate type, and length of exposure were all significant factors in boll weevil survival. Interactions between substrate and exposure, as well as among temperature, substrate and exposure time were also significant elements in mortality. Weevil mortality increased with temperature reduction and increased exposure time, and was greater in moist substrate than in dry substrate (Figure 1).

Separate analysis was also conducted for each temperature. These analysis showed that at intermediate freezing temperatures (-5.0, -7.5, -10.0 and –12.5°C), litter type, exposure time, and an exposure × substrate interaction were all significant factors of weevil mortality. For these temperatures, moist substrate produced significantly higher mortality levels than did dry substrate, especially with increased exposure times (Table 1). For example, few (<25%) weevils survived greater than one hour exposures

at these temperatures in moist substrate. At -5°C, greater mortality with exposure time was only apparent in the moist litter treatments (Table 1).

At the warmest (0.0 and -2.5°C) temperatures tested, exposure time and substrate type were not significant factors of weevil mortality. Most (>70%) weevils were able to survive freezing temperatures of –2.5°C or higher for up to eight hours duration, even when exposed within moist substrate, and at -5.0°C in dry or no litter. Slosser et al. (1996) reported similar results, with over 90% of diapausing boll weevils surviving an eight hour exposure to temperatures of -5°C or warmer. Although our mortality rates were about 5-20% higher than those of Slosser et al. (1996) were, these differences were probably due to the temperature measurement since our test measured temperatures directly in the cups and not on the machine monitor. In these tests, temperature probes indicated a 1.5 to 2.5 degree difference between the temperatures inside the cups and the cooling solution. Most other research has relied on the temperature recordings in the external cooling solution rather than the internal test area (Slosser et al. 1996).

No weevils survived a one-hour exposure at the coldest temperature (-15.0°C) tested. Sorenson and House (1995) reported greater survival at this temperature in a similar study, with over 20% survival following a 1.5 hour exposure and three hours required for complete mortality.

These results indicate that moist substrate and length of freezing temperatures are important factors of overwinter weevil mortality in Arkansas. However, these factors are most important, when litter temperatures drop to -5.0°C or below when moist, or to -7.5°C in dry litter. Warmer temperatures result in high overall survival, while few weevils can survive a temperature drop to –15C for even a short period of time.

Acknowledgments

The authors are grateful for the support of the Arkansas Cotton Support Committee and Cotton Incorporated in the effort to improve technology to manage the boll weevil. We also wish to thank the cotton producers of Arkansas for their cooperation and support of our work.

References

Bondy, F.F. and C.F. Rainwater. 1942. Boll weevil hibernation, survival, and emergence under South Carolina conditions. J. Econ. Entomol. 35:495-498.

Fuchs, T.W. and A. England. 1989. Winter habitat sampling for overwintering boll weevils as a component of an integrated pest management program. Southwest. Entomol. 14:265-269.

Gaines, R.C. 1943. Relation between winter temperatures, boll weevil survival, summer rainfall, and cotton yields. J. Econ. Entomol. 36:82-84.

Parajulee, M.N., L.T. Wilson, D.R. Rummel, S.C. Carroll, and P.J. Trichilo. 1996. Climatic data-based analysis of boll weevil (Coleoptera: Curculionidae) overwintering survival and spring emergence. Environ. Entomol. 25:882-894.

Pfrimmer, T.R. and M.E. Merkl. 1981. Boll weevil: winter survival in surface woods trash in Mississippi. Environ. Entomol. 10:419-423.

Price, J.R., J.E. Slosser, and G.J. Puterka. 1985. Factors affecting survival of boll weevils in winter habitat in the Texas Rolling Plains. Southwest. Entomol. 10:1-6.

Rummel, D.R., and S.C. Carrol. 1993. Biological basis for a boll weevil winter survival and spring emergence model. Proceedings Beltwide Cotton Conferences. pp. 932-934.

SAS Institute. 1990. SAS Users Guide, Release 6.11 Edition. Cary, NC. SAS Institute Inc.

Singer, T.L., D.R. Johnson, L.M. Page, H.B. Meyers, C.D. Klein, and R. Bagwell. 1996. Factors influencing survival of overwintering boll weevils (*Anthonomus grandis*) in northeast Arkansas. Proceedings Beltwide Cotton Conferences. pp. 960-965.

Slosser, J.E., R. Montandon, D.R. Rummel, L.T. Wilson, and T.W. Fuchs. 1996. Survival of diapausing and nondiapausing boll weevils (Coleoptera: Curculionidae) subjected to freezing temperatures. Environ. Entomol. 25:407-415.

Sorenson, C.E. and J. House. 1995. Winter habitat microclimate and winter cold tolerance of boll weevils in Missouri; a preliminary report. Proceedings Beltwide Cotton Conferences. pp. 1025-1028.

Sorenson, C.E. and M. George. 1996. Survival of boll weevils exposed to freezing temperatures with and without ice nucleators. Proceedings Beltwide Cotton Conferences. pp. 969-972.

Sorenson, C.E., Parker, and M.B. Layton. 1996. Relative cold tolerance of boll weevils from Missouri, Mississippi, and coastal Texas. Proceedings Beltwide Cotton Conferences. pp. 715-717.

Stone, N.D., D.R. Rummel, S.C. Carroll, M.E. Makela and R.E. Frisbie. 1990. Simulation of boll weevil (Coleoptera: Curculionidae) spring emergence and overwintering survival in the Texas rolling plains. Environ. Entomol. 19: 91-98.

Taft, H.M. and A.R. Hopkins. 1966. Effects of different hibernation environments on survival and movement of the boll weevil. J. Econ. Entomol. 59:277-279.

Table 1. Mean boll weevil mortality (%) following exposure to sub-freezing temperatures in no substrate, dry leaf litter substrate and moist leaf litter substrate and four exposure times.

Exposure	Leaf Substrate Type			
Time (hours)	None	Dry	Moist	All Types**
	Temperature = 0.0ºC			
1	3.8	12.4	2.6	6.3a
2	7.2	6.4	2.6	5.4a
4	12.5	9.1	1.3	7.6a
8	11.8	1.3	3.8	5.6a
All Exposures*	8.8a	7.3ab	2.6b	
	Temperature = -2.5ºC			
1	18.5	24.5	5.0	16.0a
2	16.8	20.3	6.6	14.6a
4	23.9	7.4	7.5	12.9a
8	13.7	9.9	26.6	16.7a
All Exposures*	18.2a	15.5a	11.4a	
	Temperature = -5.0ºC			
1	15.2	16.8	27.2	19.8a
2	14.5	5.2	29.5	16.4a
4	15.0	9.7	70.5	31.7b
8	20.6	14.6	77.0	37.4b
All Exposures*	16.3a	11.6a	51.1b	
	Temperature = -7.5ºC			
1	15.8	21.5	23.5	20.2a
2	19.8	18.8	78.8	39.1b
4	18.2	15.8	93.7	42.5b
8	36.5	22.2	100.0	52.9c
All Exposures*	22.6a	19.6a	74.0b	
	Temperature = -10.0ºC			
1	30.2	25.8	57.1	37.7a
2	36.2	28.7	100.0	54.9b
4	95.6	73.9	100.0	89.8c
8	100.0	93.0	100.0	97.7c
All Exposures*	65.5a	55.4b	89.3c	
	Temperature = -12.5ºC			
1	70.0	37.9	97.6	68.5a
2	100.0	100.0	100.0	100.0b
4	100.0	100.0	100.0	100.0b
8	100.0	100.0	100.0	100.0b
All Exposures*	92.5a	84.5b	99.4c	
	Temperature = -15.0ºC			
1	100.0	100.0	100.0	

*Substrate type means (for all four exposures) sharing the same letter were statistically indistinguishable (α=0.05).

**Exposure time means (for all three substrate types) sharing the same letter were statistically indistinguishable (α=0.05).

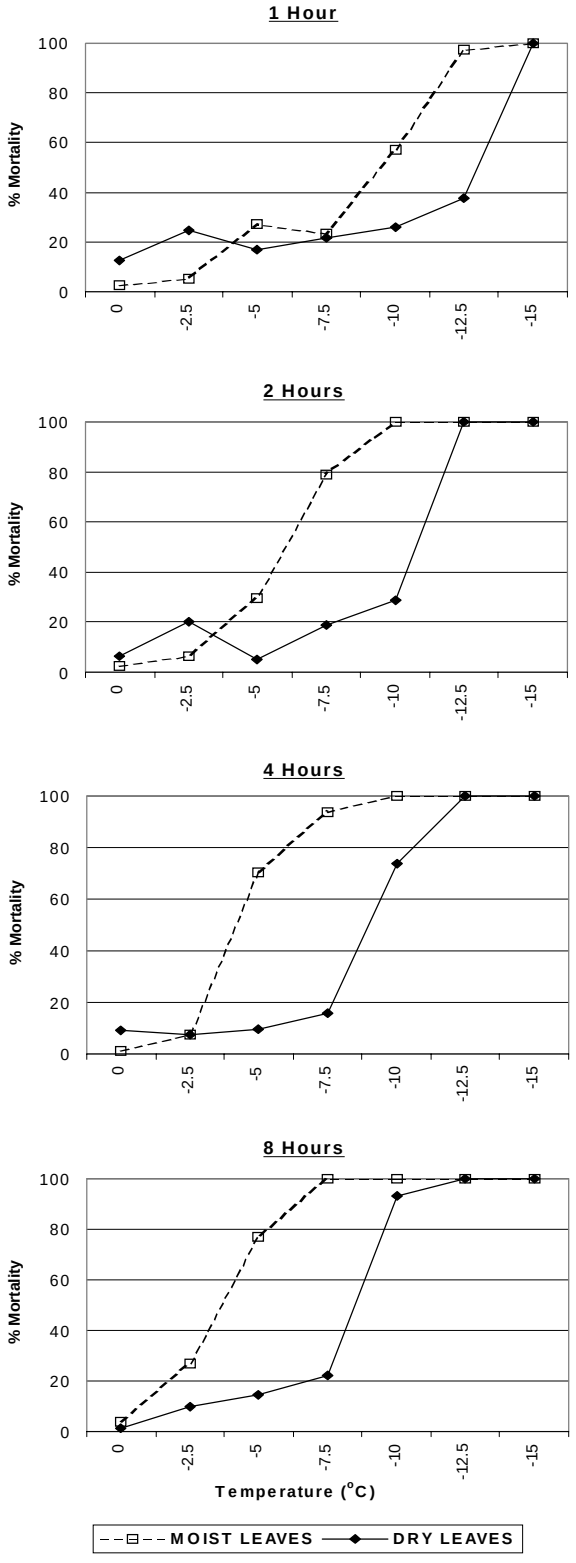

Figure 1. The relationship between temperature reduction and mortality levels for boll weevils exposed within dry or wet litter for periods of one, two, four and eight hours.