EMERGENCE OF OVERWINTERING BOLL WEEVILS ASSOCIATED WITH MICROMETEOROLOGICAL FACTORS

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Abstract

A field study was conducted in the Brazos Valley of Texas to determine the effects of temperature, humidity, and other meteorological factors on the temporal emergence pattern of boll weevil (*Anthonomus grandis* Boheman), and to compare the physiological and morphological conditions of emerged and trapped boll weevils. Emergence cages were placed over leaf litter in a wooded area and infested with diapausing boll weevils in the fall. Daily emergence and microclimatic conditions of the leaf litter and ambient air were monitored until the subsequent summer. Temperature, relative humidity, incident solar radiation, and precipitation were significantly greater on emergence dates than on dates with no emergence, and barometric pressure was significantly lower on emergence dates than on dates with no emergence. Emerged and trapped boll weevils were dissected to assess their morphology. Emerged boll weevils tended to exhibit greater fat body development, greater atrophy of testes, and less ovary development than trapped boll weevils. None of the emerged boll weevils were rated extra lean and no trapped boll weevils were rated fat. These results are consistent with previously-reported effects of climatic factors on weevil emergence in other areas of the Cotton Belt, and add new information about the physiological and morphological characteristics of emerged weevils. This information on the dynamics and mechanisms of overwintering provides insight that will be helpful in formulating improved predictive models, risk assessments, and pest management strategies for boll weevils.

Introduction

The adult boll weevil, *Anthonomus grandis* Boheman, enters a state of dormancy (diapause) which contributes to its ability to survive winters in temperate regions (Brazzel and Newsom 1959). Adult boll weevils emerge from overwintering habitats in the spring and subsequently infest squaring cotton. The temporal pattern of emergence of boll weevils from overwintering is influenced by their physiological condition and the climatic conditions of their overwintering habitats (Rummel and Summy 1997).

Overwintering boll weevils utilize a variety of habitats which moderate adverse climatic conditions for several months before emergence. Although deciduous leaf litter has been reported as their primary overwintering habitat, boll weevils are known to utilize a variety of overwintering quarters. Beckham (1957) found overwintering boll weevils in pine straw from wooded areas near cotton fields in Georgia. Bondy and Rainwater (1942) reported that boll weevils successfully overwintered beneath ground cover comprised of Spanish moss or corn stalks. Boll weevils may also overwinter within cotton bolls on the ground (Cowan et al. 1963), weeping lovegrass (Brown and Phillips 1989), and in Conservation Reserve Program grasses (Carroll et al. 1993). Graham et al. (1978) found hackberry, granjeno, and open grass to be the three primary overwintering habitats in the subtropical Lower Rio Grande Valley of Texas. Winter habitats modify the microclimate experienced by overwintering boll weevils. For example, Parajulee et al. (1997) reported that average daily minimum temperatures were 3 to 6°C greater and average daily maximum temperatures were 4 to 6°C less than the respective air temperatures in shinnery oak, mesquite-grass pasture, pecan, and shelterbelt habitats.

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Several studies related overwinter survival and timing to temperature and precipitation patterns. Parencia et al. (1964) reported higher infestations of early-season fruiting cotton when maximum temperature exceeded 21.1°C and during months with appreciable precipitation. The influence of moisture on emergence appears to increase as temperatures increase (Mitchell et al. 1972). Shade afforded by leafy canopies reduces insolation and heating of overwintering habitats, and increases the variability of the microclimate and emergence patterns of boll weevils (Slosser et al., 1984a; 1984b, Slosser and Fuchs 1991).

Captures of boll weevils in pheromone traps have been used to estimate boll weevil emergence patterns. White and Rummel (1978) compared patterns of captures in pheromone traps with counts of boll weevils in cotton fields and determined that emergence from overwintering began several weeks before cotton was colonized. Carroll and Rummel (1985) found a strong correlation between the period of peak emergence from overwintering and peak response to pheromone traps. However, these studies did not examine the physiological and morphological characteristics of the emerged and trapped boll weevils.

Our objectives were to determine the effects of temperature, humidity, and other meteorological factors on the temporal patterns of boll weevil emergence, and to compare the physiological and morphological conditions of emerged and trapped boll weevils.

Materials and Methods

Boll weevil-infested squares were hand picked from fruiting cotton fields. The infested squares were divided among several 20 x 20 x 20-cm screened plexiglass cages with approximately 300 infested squares per cage. The cages were held in environmental chambers at 29.4°C and a photoperiod of 11:13 (L:D) h. The cages were inspected daily and newly emerged adults were removed. At the onset of adult emergence, infested squares were misted daily with distilled water to soften the squares and facilitate weevil emergence. Approximately 300 boll weevils were held in each of several cages identical to those used for rearing. Plexiglass cages were held in the laboratory at room temperature (~24°C). Adult boll weevils were provided a source of distilled water and fed at a rate of one small debracted boll (17 to 25 mm diameter) per ten boll weevils for three weeks after the first adults emerged. Bolls were replaced three times weekly (Mondays, Wednesdays, and Fridays). All boll weevils used in the study emerged within a 5-d period (6-10 October 1999). Dead boll weevils were removed when the bolls were replaced.

After the feeding period, boll weevils were sexed following the methods of Sappington and Spurgeon (2000) and marked on the elytra with a paint pen to identify the gender of the cohort group. An aliquot of 25 weevils was dissected to determine the proportion of boll weevils expressing diapause characters. Fat body, testis, and ovary condition were assessed in dissections according to the methods of Spurgeon and Raulston (1997) which, in turn, were based substantially on descriptions provided by Brazzel and Newsom (1959). Boll weevils were classified as fat if the fat body obscured most of the internal organs, intermediate if the fat body was well developed but portions of the digestive tract or reproductive organs were generally visible, and lean if internal organs were generally visible, regardless of presence of fat. Ovaries were rated according to the presence or absence of previtellogenic oocytes, oocytes containing yolk, and mature (chorionated) eggs. Testis rating was classified as pre-reproductive if the testes were translucent, early-reproductive if the testes had opaque centers and translucent lobes, late-reproductive if the testes had opaque centers and cloudy lobes, or atrophied if the testes were opaque due to dense deposits of yellow fat.

Pyramidal field emergence cages were constructed of a galvanized metal frame and steel window screening, and had a height of 0.5 m and basal area

of 1 m^2. Twenty cages were placed over relatively undisturbed deciduous leaf litter in a wooded area near the bank of the Brazos River, Burleson Co., TX, in fall 1999. Fifty randomly-selected marked boll weevils (25 males and 25 females) were placed in each sealed emergence cage on 30 October 1999 to induce boll weevils to overwinter within the leaf litter. After 13 January 2000, cages were inspected daily for the presence of emerged boll weevils.

A Campbell Scientific (Campbell Scientific, Inc., Logan, UT) CR21XL datalogger, AM416 multiplexer, and CR10 datalogger were used to record hourly meteorological data at the emergence site. Thermocouples (24 AWG; Omega Engineering, Inc., Stamford, CT) were placed within each cage on 29 October 1999 to measure air and leaf litter temperature. A thermocouple was placed at the interface between the soil and leaf litter within each cage. A second thermocouple was shielded by an inverted white plastic cup with a black-painted interior at a height of 0.3 m within each cage. Hourly measurements of ambient air temperature, soil temperature, ground surface temperature, relative humidity, barometric pressure, solar radiation, and precipitation were also recorded at a central weather station. Hourly meteorological data were summarized over 24-hour periods beginning at 1200 h CST to coincide with daily examination of the emergence cages.

Groups of four cages were clustered into four greenhouse treatment groups and one untreated group. Treatments consisted of greenhouse enclosures without artificial heating (GR4-1, GR4-2, GR4-3, and GR4-4), and with heating to a constant air temperature of 15°C (GR3-1, GR3-2, GR3-3, and GR3-4), 20°C (GR2-1, GR2-2, GR2-3, and GR2-4), and 25°C (GR1-1, GR1-2, GR1-3, and GR1-4). An unheated and uncovered set of ambient cages (i.e., AMB-1, AMB-2, AMB-3, and AMB-4) was also maintained. Greenhouses were constructed of clear plastic sheets draped over an arched framework of PVC pipes, with a maximum height of about 1.2 m. Plastic sheets were sealed at the base of the framework with 2.44-m lengths of 0.05 m x 0.10 m lumber. Plastic sheets were opened only to inspect the cages and heaters within each greenhouse. Greenhouse heating systems consisted of a Vornado EH1-0005 (Air Circulation Systems, Inc., Wichita, KS) vortex electric heater, a Duracraft CZ-320 (Duracraft Corp., Southburrough, MA) oscillating electric heater, a Lasko 2009 (Lasko Metal Products, West Chester, PA) oscillating fan, and a bimetallic thermostat which were operated to maintain desired air temperatures. A Miller Bobcat 225 (8500-W peak power; Miller Electric Mfg., Appleton, WI) gasoline-powered generator supplied power to the greenhouse heating system. Greenhouses were used continuously from 24 January 2000 to 14 February 2000, when daily minimum air temperatures were forecast to be < 10°C.

A single boll weevil pheromone trap was deployed on the margin of the wooded area and about 200 meters north of the emergence cages. Emergence cages and the pheromone trap were inspected daily at about 1300 h CST from 11 January to 10 July. When emergence cages were examined, boll weevils visible on the sides of the cages were removed in addition to those in the collection jars. Collected boll weevils were stored in a refrigerator and dissected on the day of collection, except for a total of four boll weevils on 28 March, 8 April, and 21 April that were dissected the following day, and one crushed boll weevil on 29 March that was not dissected.

Dissection data for emerged and trapped boll weevils were merged with climate data by date, greenhouse, and cage. Mean differences of sex ratio among greenhouses were tested for significance using ANOVA (PROC GLM; SAS Institute 1988). Mean differences of air temperature, leaf litter temperature, and leaf litter relative humidity among emergence cages were tested for significance using ANOVA and separated using the Waller-Duncan K-ratio t-test (PROC GLM). Mean differences of daily climatic factors for emergence dates and dates with no emergence were tested using the t-test statistic (PROC TTEST; SAS Institute 1988). Because the infrequent occurrence of some physiological and morphological conditions, physiological and morphological factors were each pooled into two classes for contingency tables that could be tested using the Fisher Exact Test (PROC FREQ; SAS Institute 1988). Fat body ratings were re-classified as hypertrophic (intermediate or fat) and normal (extra lean or lean). Testis rating was reclassified as atrophied and not atrophied. Statistical differences were interpreted using individual cell contributions to the χ^2 value (CELLCHI2 option of PROC FREQ).

Results

Emergence Patterns

Boll weevil emergence occurred over a period of 128 d (on 87 days) from 24 February to 1 July (Figure 1). Significantly early (80. 3 d) and late (143.8 d) mean dates of emergence occurred in the uncovered, unheated cages, AMB-1 and AMB-4, respectively. Boll weevils emerged from a single-day maximum of 19 cages on 29 March. Maximum daily emergence (53 boll weevils) occurred on 3 May. The greatest number of boll weevils emerged from GR1 and the least from AMB (Table 1). Six-hundred nineteen marked boll weevils emerged, and the remains of 159 boll weevils were recovered when the leaf litter was excavated on 10 July. The number of emerged females (N=316) and emerged males (N=303) were not significantly different. The within-treatment range of total emergence among cages was greatest (10 to 45 weevils) for GR4 and least (30 to 41 weevils) for GR2. During the emergence period, 183 boll weevils were captured in the single pheromone trap over 49 days. A daily maximum of 15 boll weevils were captured in the pheromone trap on 4 May. Daily capture of boll weevils in the pheromone trap were significantly related ($F = 66.510$, $df = 1, 168$, $P = 0.0001$) to daily emergence of boll weevils with $R^2 = 0.28$ using linear regression (PROC REG; SAS Institute 1988).

Significant differences in daily mean leaf litter temperature and ambient air temperature were detected among cages between 1 November 1999 and 1 July 2000 (Table 2). However, mean daily minimum air temperatures were not significantly different among cages for 1 November 1999 to 1 July 2000.

Significant differences were observed between daily climatic factors associated with emergence dates and dates of no emergence, respectively (Table 3). Within emergence cages, daily leaf litter temperatures were 4.4 to 4.9°C higher and air temperatures were 1.5 to 3.5°C higher on emergence dates than on dates of no emergence. Outside of the emergence cages, mean temperatures of the soil, ground surface, and ambient air were significantly higher on emergence dates than on dates of no emergence. Daily mean relative humidity, total precipitation, and total solar radiation were significantly higher, and daily mean barometric pressure was significantly lower, on emergence dates than on dates of no emergence.

Morphological Characteristics

The initial aliquot of boll weevils to determine the proportion of diapause following the feeding period included 16 males and nine females. All of the males and eight of the females (96% total) exhibited diapause characters.

Significant differences in morphological characteristics were evident between the emerged and trapped boll weevils. Emerged boll weevils tended to have more substantial fat bodies (Fisher Exact Test, $P < 0.0001$) (Figure 2). A greater proportion (85.6%) of emerged boll weevils had hypertrophic fat body than did trapped boll weevils (2.3%). Trapped boll weevils contributed 78.0% of the χ^2 value. The proportion of emerged boll weevils rated as intermediate or fat decreased throughout the emergence period (Figure 3). The last boll weevil rated fat emerged on 29 May and the last boll weevil rated intermediate emerged on 19 June. None of the emerged boll weevils were rated extra lean and none of the trapped boll weevils were rated fat. The single unmarked boll weevil that emerged on

3 May had an intermediate fat body rating, as did 80.7% of the marked emerged boll weevils. Emerged boll weevils (50.5%) were more likely to have atrophied testes than were trapped boll weevils (4.6%) (Fisher Exact Test, $P < 0.0001$). Trapped boll weevils with atrophied testes accounted for 46.4% of the χ^2 value. Trapped boll weevils (20.6%) exhibited a greater propensity of having previtellogenic oocytes than did emerged boll weevils (0.6%) (Fisher Exact Test, $P < 0.0001$). Trapped boll weevils with previtellogenic oocytes accounted for 72.4% of the χ^2 value. A greater proportion of trapped boll weevils (8.3%) had oocytes with yolk than did emerged boll weevils (0.0%) (Fisher Exact Test, $P < 0.0001$). Trapped boll weevils which had oocytes with yolk accounted for 75.0% of the χ^2 value. No mature eggs were present in either trapped or emerged boll weevils.

Discussion

Daily capture of boll weevils in the pheromone trap was significantly related with emergence of boll weevils in emergence cages, but peak values of these variables were often asynchronous. The divergence between emergence and trap capture patterns was most evident in the comparative pattern of peak values. Six of ten dates with either a major trap peak or emergence peak were not characterized by synchronous peaks, as in the case of prominent peaks in emergence (50 weevils on 29 March) and trap capture (12 weevils on 9-10 March). A hailstorm occurred on 1 May substantially reduced canopy shading of the emergence study area and increased the penetration of solar radiation to heat the ground surface. In turn, the open canopy created by the hailstorm contributed to marked increases in surface heating, and may have been responsible for the subsequent major emergence events. Seven major emergence peaks were associated with rain events and warm temperatures. The observed decrease in the amplitude of the emergence peaks after peak emergence was likely an artifact of population sampling without replacement.

The results indicate that boll weevils removed from the emergence cages tended to possess more high developed fat bodies than did boll weevils captured in traps. This difference may be the result of two distinct scenarios. First, it is possible (and probably likely) that the boll weevils established in the emergence cages were generally better suited for overwintering survival than the bulk of boll weevils that enter overwintering quarters naturally. On the other hand, many of the boll weevils entering naturally do not survive to the following year. More information is need to determine if the differences in fat body ratings due to differences between the fat body ratings of boll weevils that enter overwintering quarters naturally or artificially. Second, boll weevils captured in traps may have been similar to those obtained from emergence cages, and their fat body ratings reflect their high activity (flight) since emergence. Further, the preponderance of boll weevils with fat body ratings of intermediate or fat suggests that these boll weevils may not necessarily be part of a "suicidal emergence" before the availability of squaring cotton. Additional experimentation on the rate of fat body consumption will be required to assess this explanation.

Significant microclimatic gradients in overwintering habitats and their effects on overwintering emergence of boll weevils have been presented. This bioclimatic relationship would better explain variability of estimated emergence patterns compared to that estimated by a single climatological station. This information on the dynamics and mechanisms of overwintering provides insight that should be helpful in formulating improved predictive models, risk assessments, and pest management strategies for boll weevils.

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Disclaimer

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Table 1. Total emergence of overwintering adult boll weevils from leaf litter at the Brazos River site, Burleson Co., TX, 24 February to 1 July 2000.

Greenhouse	Heater Setting	Total	Percent of Total Released
GR1	25°C	146	73.0
GR2	20°C	142	71.0
GR3	15°C	115	57.5
GR4	-----	125	62.5
AMB	-----	91	45.5
Total	-----	619	61.9

One unmarked female emerged on 3 May from GR3-4, but was excluded from the emergence count.
Sex ratios among the greenhouses were not significantly different (α = 0.05) using ANOVA (PROC GLM; SAS Institute 1988).
Remains of 70 males and 89 females were recovered from leaf litter at the conclusion of the study on 10 July, but were excluded from the total counts.

Table 2. Daily mean climate statistics and mean emergence dates for 20 emergence cages in the boll weevil overwintering emergence study, Brazos River site, Burleson Co., TX, 1 November 1999 to 1 July 2000.

Green-house	Cage	Litter Temperature (°C)		Air Temperature (°C)		Emergence Date (Day-of-year)	
AMB	1	19.1	ef	15.9	b	80.3	g
AMB	2	21.6	b	15.9	b	111.1	e
AMB	3	17.1	gh	15.9	b	97.9	f
AMB	4	15.9	i	15.9	b	143.8	a
GR1	1	22.6	a	17.3	ab	128.0	bcd
GR1	2	19.9	cde	17.5	a	129.5	bcd
GR1	3	19.3	ef	17.2	ab	135.1	abc
GR1	4	20.4	cd	17.2	ab	129.7	bcd
GR2	1	17.2	g	16.8	ab	134.7	abc
GR2	2	17.3	g	17.0	ab	124.9	cd
GR2	3	17.3	g	17.0	ab	126.4	cd
GR2	4	17.2	g	16.9	ab	126.2	cd
GR3	1	18.9	f	16.6	ab	128.7	bcd
GR3	2	16.1	i	16.7	ab	126.9	cd
GR3	3	16.2	hi	16.7	ab	122.7	d
GR3	4	16.0	i	16.7	ab	121.5	de
GR4	1	20.7	bc	16.1	ab	121.6	de
GR4	2	15.8	i	16.1	ab	91.4	fg
GR4	3	18.7	f	16.3	ab	130.9	bc
GR4	4	19.6	def	16.1	ab	138.4	ab

Greenhouses were deployed from 24 January through 14 February 2000, and electric heaters were operated in GR1 (25°C), GR2 (20°C), and GR3 (15°C). GR4 was enclosed in a greenhouse without heaters, and AMB was not enclosed in a greenhouse. Column means with the same letter are not significantly different using the Waller-Duncan K-ratio t-test (α = 0.05).

Table 3. Daily climate statistics for dates with and without boll weevil emergence at the Brazos River site, Burleson Co., TX, 14 January to 1 July 2000.

Variable	Emergence dates	Dates of no emergence
Inside of Emergence Cages		
Minimum Leaf Litter Temperature (°C)	23.4	18.5
Maximum Leaf Litter Temperature (°C)	26.6	22.2
Mean Leaf Litter Temperature (°C)	24.8	20.1
Minimum Air Temperature (°C)	17.5	14.0
Maximum Air Temperature (°C)	26.2	24.7
Mean Air Temperature (°C)	20.9	18.4
Outside of Emergence Cages		
Minimum Air Temperature (°C)	19.5	15.0
Maximum Air Temperature (°C)	27.8	24.9
Mean Air Temperature (°C)	22.9	19.4
Mean 4" Soil Temperature (°C)	23.0	19.6
Mean Ground Surface Temperature (°C)	23.0	19.5
Total Solar Radiation (kW m^{-2})	1.27	1.22
Mean Barometric Pressure (kPa)	100.7	101.1
Mean Relative Humidity (%)	90.1	80.4
Total Precipitation (mm)	3.7	1.7

All row means are significantly different (α = 0.05) using the t-test statistic (PROC TTEST; SAS Institute 1988). The equal variance assumption was validated for each test.

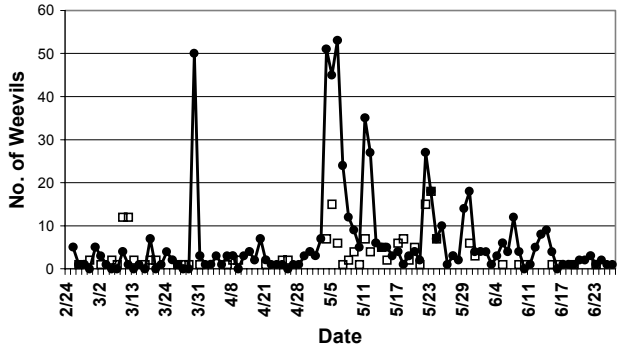

Figure 1. Daily capture of boll weevils in phermone traps (squares) and overwintering emergence cages (solid circles) at the Brazos River site, Burleson Co., TX, 1999-2000.

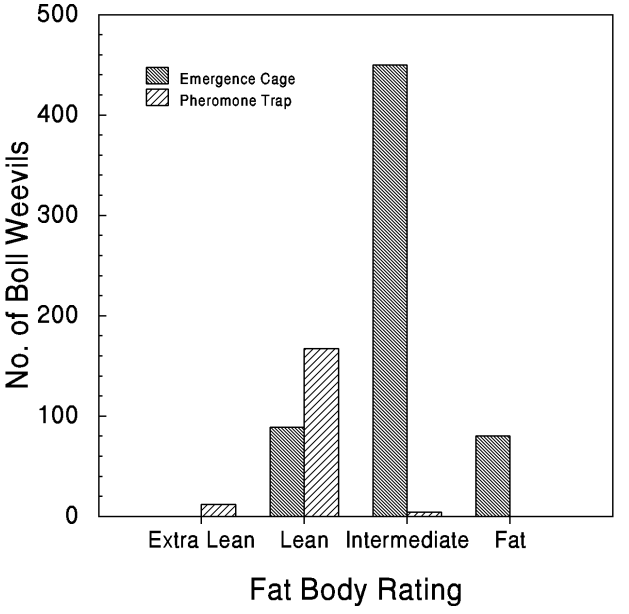

Figure 2. Overall fat body ratings of boll weevils captured from overwintering emergence cages and pheromone traps at the Brazos River site, Burleson Co., TX, 1999-2000.

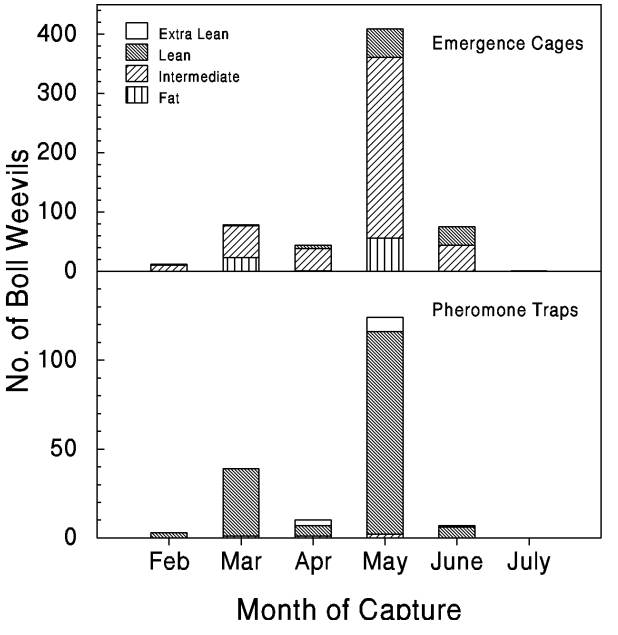

Figure 3. Monthly fat body ratings of boll weevils captured from overwintering emergence cages and pheromone traps at the Brazos River site, Burleson Co., TX, 1999-2000.