Host-Free Survival of Boll Weevils (Coleoptera: Curculionidae) Previously Fed Vegetative-Stage Regrowth Cotton[1]

Charles P.-C. Suh[2] and Dale W. Spurgeon

USDA-ARS, Areawide Pest Management Research Unit, College Station, Texas 77845 USA

J. Entomol. Sci. 41(4): 277-284 (October 2006)

Abstract The need to minimize populations of overwintering boll weevils (*Anthonomus grandis* Boheman) in eradication programs is widely recognized, but the potential contribution of nonfruiting regrowth cotton (*Gossypium hirsutum* L.) to weevil survival has not been directly examined. We conducted experiments in 2002 and 2003 to examine the host-free survival of weevils previously supplied vegetative-stage regrowth cotton. Weevils, 1-3 d after eclosion, were caged with vegetative regrowth cotton under ambient environmental conditions (2002), or in a controlled environment (23.9 ± 2°C, 13:11 [L:D] h photoperiod, 2003). Four cohorts of each weevil sex were examined each year. Of the 60 weevils dissected in 2002, 12% possessed the hypertrophied fat bodies associated with diapause and extended host-free survival. In both years, the majority of weevils (>75%) died during the initial 3 wks of the host-free period. No differences in host-free survival were detected between weevil sexes, but differences were observed among replicates of the experiment established on different dates. Survival patterns among replicates, however, did not strictly follow a seasonal pattern. Overall, approximately 5% of the weevils survived $\geq$12 wks beyond the feeding period. Maximum observed host-free longevities were 21 wks in 2002 and 19 wks in 2003. Our findings suggest vegetative-stage regrowth cotton should not be disregarded in eradication zones, particularly in those experiencing unsatisfactory progress or resurgence of weevil populations.

Key Words boll weevil, *Anthonomus grandis*, regrowth cotton, *Gossypium hirsutum*, host-free survival

---

The need to minimize populations of overwintering boll weevils, *Anthonomus grandis* Boheman, in eradication programs is widely recognized (El-Lissy 1997, Carter et al. 2001). In fact, this is the basis for the diapause control tactic in such programs. An additional tactic to reduce boll weevil overwintering survival is timely post-harvest crop destruction. Even where cotton (*Gossypium hirsutum* L.) plants are destroyed after harvest, regrowth from stalks or growth of volunteer plants from unharvested seed can occur when environmental conditions permit. Occurrence of regrowth or volunteer cotton is particularly common in southern production regions of Texas. Long-standing regulations by the Texas Department of Agriculture have mandated the complete destruction of cotton plants by calendar dates specific to individual production regions. Crop destruction regulations were amended in 2002 to permit the occurrence of regrowth plants so long as those plants are not fruiting (Anonymous 2002). The regulatory change provided greater flexibility in the termination of crops in

---

[1] Received 15 November 2005; accepted 03 February 2006.

[2] Address inquiries (email: suh@usda-apmru.tamu.edu).

no- and reduced-tillage systems, and was deemed to minimally impact boll weevil eradication efforts because of the inability of weevils to reproduce on non-fruiting plants (Anonymous 2002). However, questions remain regarding the potential contribution of vegetative regrowth to weevil host-free survival.

Reports of weevil longevity on vegetative cotton plants, or plant parts, are contradictory. Field studies have indicated that survival of overwintered weevils on prefruiting plants is limited (Fenton and Dunnam 1927, Rummel and Carroll 1985). In contrast, Vanderzant and Davich (1958) reported that longevity of weevils fed excised cotyledons was similar to that of weevils fed squares (flower buds). In fact, Sterling et al. (1965) maintained a laboratory culture of weevils on Parafilm-wrapped cotton seedlings. Finally, in a study under controlled conditions, Esquivel et al. (2004) observed extended longevity of overwintered weevils on vegetative stage plants, and suggested nonfruiting plants provided weevils with greater nutrition than previously recognized. Because no information is available regarding the host-free survival of late-season weevils previously fed vegetative stage regrowth cotton, we conducted experiments to gain insights into this aspect of weevil ecology.

Materials and Methods

Weevil source. Adult weevils were reared from oviposition-punctured squares collected from commercial fields in Brazos (21 and 26 August, 2 September, and 28 October 2002), Hill (11 and 18 August 2003), Limestone (25 August 2003), and Navarro (1 September 2003) counties, TX. Infested squares were placed in screened cages, and were held at 29.4 ± 1°C and a photoperiod of 13:11 (L:D) h. Beginning 5 or 6 d after square collection and daily thereafter, pupae were harvested and placed on moistened vermiculite contained in Petri plates. Plates of pupae were held under the same environmental conditions as the squares and were inspected at least twice daily for adults. Newly-eclosed adults were sexed using the method of Sappington and Spurgeon (2000) and were held as single-sex groups at 23.9 ± 1°C and a photoperiod of 13:11 (L:D) h. Weevils were maintained without food for $\leq$ 2 d under these conditions until used in the experiment.

Regrowth plants. Regrowth plants were obtained by cutting heavily-fruiting, greenhouse-grown plants (DP 436 RR, Deltapine and Land Co., Scott, MS) between the 4th and 6th mainstem nodes, and removing all remaining branches and foliage. Each pot contained 2 or 3 plants which had been maintained for about 6 mos to provide fruit for other studies. During that time each pot was fertilized with 10-15 g of Osmocote Slow Release Plant Food (19-6-12, N-P-K, Scott-Sierra Horticultural Products, Marysville, OH) every 1-2 mos to maintain a high level of fruit production. Cut plants were maintained in a greenhouse (24-38°C) and were provided only water for 10-14 d to allow development of regrowth. At the end of this period each plant had 1-3 terminals growing from each node and quantities of foliage were similar to those associated with 10- to 12-leaf stage plants.

Experimental procedures. Two single-sex cohorts of weevils (one of each sex) constituted an experimental replication. Experimental replications were established on 2, 6, and 12 September and 5 November 2002. Because of the limited availability of infested squares, the number of weevils eclosing on a single day was often smaller than desired for a replication of the experiment. Therefore, adults eclosed over 2 or 3 consecutive days were pooled to increase sample sizes. The first two replications each consisted of 40 weevils of each sex. The third replication consisted of 20 weevils of each sex, and the fourth consisted of 14 males and 21 females. Each single-sex cohort was held in a separate cage (100 × 70 × 60-cm) screened with nylon organdy and provisioned with nonfruiting regrowth at a rate of 3 plants per 10-15 weevils. Before weevils were introduced to the cages, regrowth plants were inspected for the presence of squares. Less than 30% of plants had one or more pin-head squares at that time, and those squares and associated fruiting branches were removed. Additionally, plants were watered and the soil surface of each pot was covered with a layer of Perlite™ to assist in the recovery of weevils. Petri plates containing weevils were placed on the Perlite near the base of plants, and the lid was removed to allow weevils to crawl onto the plants. The cages were not opened during the feeding period to minimize the potential for weevil escape, and feeding was restricted to 14 d to minimize square development. Plants were watered periodically through the top of the cage. Cages were held in a screened insectary so the weevils would be exposed to natural environmental conditions during the feeding period. A HOBO datalogger (Onset Corp., Pocasett, MA) in an exposed position on top of one cage recorded the temperature hourly during each feeding period.

Immediately following the feeding period, weevils were recovered from each cage and the plants were examined for the presence of squares. Ten weevils of each sex from each of the first two replications, and 5 of each sex from each of the third and fourth replications were randomly selected and dissected to assess fat body condition. Fat bodies were classified as either fat, intermediate, or lean using the criteria of Spurgeon et al. (2003). Ratings of either fat or intermediate indicated a hypertrophied condition characteristic of diapause and extended host-free survival (Brazzel and Newsom 1959, Rummel et al. 1999).

The remainder of each single-sex cohort was placed in a separate screened-Plexiglass™ cage (20 × 20 × 20-cm). Each cage contained a crumpled piece of craft paper as a refuge and a source of water in the form of saturated cotton wick extending from the lid of a 29.5-ml diet cup filled with deionized water. Cages were held in an environmental chamber at 23.9 ± 1°C and a photoperiod of 13:11 (L:D) h, and survival was recorded weekly until all weevils were dead.

The study was repeated in 2003 using replications established on 21 and 28 August and 4 and 11 September. Procedures were similar to those in 2002 except each single-sex cohort consisted of 30 weevils, cohorts were held under controlled conditions (23.9 ± 2°C and a photoperiod of 13:11 [L:D] h) during the feeding period, and no weevils were dissected. The purpose of controlling environmental conditions during the feeding periods was to minimize confounding effects of changing ambient conditions on different replications of the experiment. No weevils were dissected in 2003 to maximize the number of weevils available for assessments of host-free survival.

Statistical analysis. Because experimental procedures differed somewhat between years, data from each year were analyzed separately. Homogeneity of survival curves between sexes, and among replications, was examined with the log-rank test using the STRATA statement of PROC LIFETEST (SAS Institute 2002). In these analyses, replicates of each sex were pooled to examine differences between sexes, and sexes were combined to examine differences among replications. Also, cohorts within each year were pooled to estimate an overall survival curve for each year. Estimates of respective survival distribution functions and associated 95% confidence intervals were generated using the OUTSURV option of the SURVIVAL statement in the LIFETEST procedure (SAS Institute 2002). Confidence intervals were based on the LOG-LOG transformation of the survival distribution functions.

Results

Of the 235 weevils used in the 2002 study, 12 males and 10 females died during the feeding periods, 1 female was killed during recovery, and 30 weevils of each sex were dissected. Thus, 72 males and 80 females were available for assessments of host-free survival. In 2003, 21 males and 23 females died during the 14-d feeding periods, leaving 99 males and 97 females for assessments of host-free survival.

In 2002 temperatures during the feeding periods for the first three replications ranged from 12.9-46.9°C, and the average temperatures during the respective periods were 28.7, 27.8, and 26.9°C. In comparison, temperatures during the feeding period for the fourth replicate were considerably cooler, and ranged from 2.5-37.9°C with an average of 16.4°C. These temperatures, however, may not accurately reflect field conditions during the respective feeding periods because the datalogger was not shielded from sunlight. Nevertheless, they provide relative estimates of temperatures during each feeding period.

Weevils extensively damaged the terminals of branches and thereby prevented development of squares on most plants. However, several squares up to match-head size (about 3 mm diam) were found on the plants provided to the male weevils of the fourth replication, and there was evidence that some squares had been fed upon. No squares were found on plants provided to the other cohorts.

Of the 60 weevils (30 of each sex) dissected in 2002, fat bodies of 4 females and 3 males were rated as intermediate. The fat bodies of the remaining weevils (88%) were rated as lean.

No differences in host-free survival were detected between sexes in either year (2002, $\chi^2 = 0.83$, df = 1, $P = 0.363$; 2003, $\chi^2 = 2.46$, df = 1, $P = 0.117$). However, differences in survival were detected among replicates (2002, $\chi^2 = 65.86$, df = 3, $P < 0.001$; 2003, $\chi^2 = 106.37$, df = 3, $P < 0.001$) (Fig. 1). When cohorts were pooled within years, the median times of host-free survival were 2 wks in 2002 and 1 wk in 2003. Although the majority of weevils (>75%) died during the initial 3 wks of the host-free period, approximately 5% of the weevils in each year survived ≥12 wks (Fig. 2). The maximum host-free longevity was 21 wks in 2002 and 19 wks in 2003 (Fig. 2).

Discussion

Since Brazzel and Newsom (1959) formally characterized reproductive diapause in the boll weevil, numerous investigators have examined the factors or cues responsible for diapause induction. However, this aspect of boll weevil biology remains poorly understood (Rummel and Summy 1997; Spurgeon et al. 2003). In fact, many reports of diapause induction cues are contradictory. For example, Earle and Newsom (1964) and Lloyd et al. (1967) reported that both photoperiod and nighttime temperatures influenced the incidence of diapause, although their results were not consistent. However, Lloyd et al. (1967) found that when weevils were held on intact cotton plants the types of available food (squares or bolls) controlled the diapause response irrespective of photoperiod and temperature except when nighttime temperatures were 10°C. Wagner and Villavaso (1999) suggested that photoperiod influenced diapause only when daylength was decreasing. However, their experimental

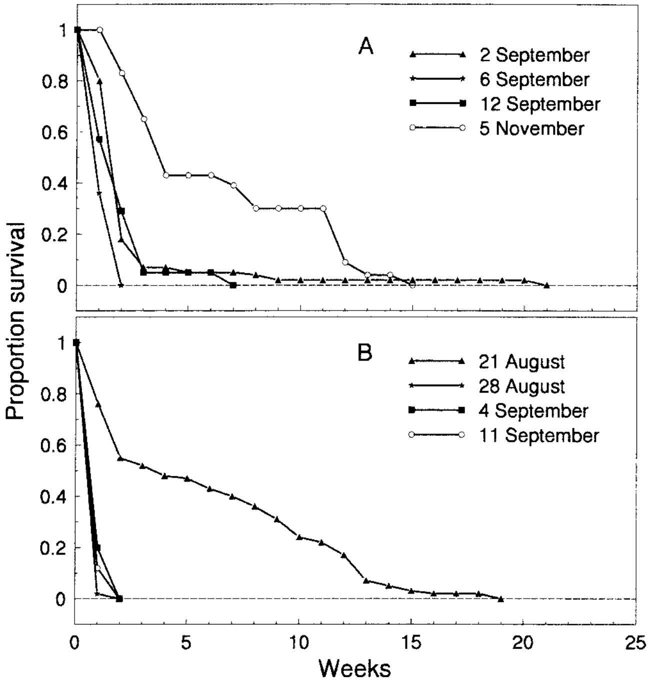

Fig. 1. Host-free survival of boll weevil cohorts held at 23.9 ± 1°C. Cohorts were designated by their approximate dates of adult eclosion, and were allowed to feed for 14 days on vegetative-stage regrowth cotton under (A) natural environmental conditions in 2002 or (B) controlled conditions (23.9 ± 2°C, 13:11 [L:D] h photoperiod) in 2003.

approach was predicated on the assumption that photoperiod was the dominant induction factor, and the morphological criteria they used to classify weevils were completely different from those previously used.

In contrast, Phillips (1976) opined that some weevils were already in a state of diapause at the time of adult emergence, although he provided no supporting experimental evidence. Taub-Montemayor et al. (1997) produced putative diapausing weevils by exposing unfed newly-emerged weevils to a photoperiod of 12:12 h and a thermoperiod of 26:21°C [L:D], but none of the weevils survived beyond 10 d. Finally, Spurgeon and Raulston (1998a) and Spurgeon and Esquivel (2000) found that feeding regimen could be manipulated to control the diapause response, and their techniques were used to produce high levels of overwintering survival (45-73%) in field studies (Westbrook et al. 2003), and to identify and characterize a storage protein diagnostic of diapause in boll weevils (Lewis et al. 2002). Spurgeon et al. (2003) suggested that failure to recognize the respective fat body types characteristic of newly eclosed, reproductive, and diapausing weevils has likely contributed to confusion in the literature. In particular, their observations make the occurrence of diapause in newly emerged, unfed weevils unlikely because such weevils are characterized by

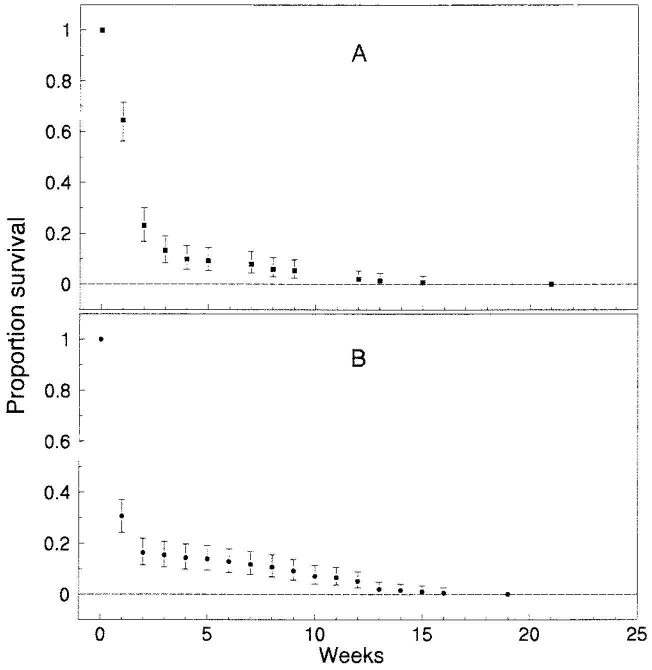

Fig. 2. Mean (±95% C.I.) host-free survival of boll weevils held at 23.9 ± 1°C. Weevils were allowed to feed for 14 days on vegetative-stage regrowth cotton under (A) natural environmental conditions in 2002 or (B) controlled conditions (23.9 ± 2°C, 13:11 [L:D] h photoperiod) in 2003.

a specific fat body type that is short-lived relative to other fat types. Because of the confusion in the literature regarding diapause status, we emphasized the conditions of fat bodies in our dissections rather than diapause per se. Dissections in 2002 indicated a small proportion (12%) of weevils fed regrowth cotton possessed the hypertrophied fat bodies that Spurgeon et al. (2003) associated with diapause. Therefore, we expected that at least some weevils would exhibit extended host-free longevity. Indeed, approximately 5% of the weevils survived ≥12 weeks beyond the feeding period. In comparison, Hunter and Hinds (1905) reported an average longevity of only 5.5 d for newly emerged unfed weevils.

Survival trends of weevils held under ambient temperatures (2002) did not strictly follow a seasonal pattern. In fact, maximum observed longevity occurred in the earliest replication (2 September) whereas the highest rates of survival beyond five weeks occurred in the latest replication (5 November). Based on the latter observation we suspected the temperatures experienced by weevils during the feeding periods may have strongly influenced subsequent survival. However, similar among-replication variation in survival was observed for weevils fed under controlled conditions. Furthermore, long-lived weevils were also observed in 2003 when photoperiod and temperature were controlled. These results suggest that factors in addition to temperature and photoperiod were responsible for the observed variation in weevil survival.

One plausible explanation for our observations is that a small proportion of the weevil population is predisposed to accumulate fat reserves, and either our sampling scheme (infested square collections) or sample sizes were not adequate to ensure their representation in each cohort. This explanation is consistent with the observations of Spurgeon and Raulston (1998b), who reported a low incidence of diapause characters in weevils exposed to feeding regimes designed to induce reproductive development. Regardless of the diapause status of weevils in our study, our results demonstrate that the boll weevil has the ability to feed on vegetative regrowth plants and accumulate fat reserves to permit extended host-free survival. However, our data are not extensive enough to accurately estimate the proportion of the population predisposed to do so.

In northerly regions of the cottonbelt the duration and extent of development of regrowth cotton is normally limited by low temperatures during the late fall and winter months. In more southerly regions the occurrence of regrowth is usually limited by postharvest stalk destruction. In either case, host-free periods are normally ≥5 mos., unless regrowth is allowed to persist in the fall. Therefore, the longevities we observed were not of sufficient duration to routinely ensure survival without supplemental feeding in most U.S. cotton production areas. Nevertheless, our results clearly illustrate the ability of a small proportion of the weevil population to accumulate substantial fat reserves by feeding on vegetative-stage regrowth cotton. This ability offers the potential to enhance winter survival of weevils when weather conditions or management practices permit the extended occurrence of regrowth plants.

Minimization of boll weevil overwintering survival is commonly regarded a critical goal of eradication programs. Accurate assessment of the risks posed by vegetative-stage regrowth cotton in active eradication programs would require not only an estimate of the propensity for weevils to acquire fat reserves on regrowth plants, but also of the extent to which overwintering populations use such plants. Limited field samples suggest that weevil population densities on vegetative regrowth plants are generally low (unpublished data). However, in some years hectarage of such plants can be vast, especially when stalk destruction efforts are hampered by inclement weather. In any event, our demonstration of extended host-free survival of weevils fed vegetative regrowth suggests such plants should not be disregarded in eradication zones, particularly in those experiencing unsatisfactory progress or resurgence of weevil populations.

Acknowledgments

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References Cited

Anonymous. 2002. Texas Register, Adopted Rules, Texas Department of Agriculture, Cotton Pest Management, Title 4, Chapter 20. http//texinfo.library. unt.edu/texasregister/pdf/2002/0816IS.pdf

Brazzel, J. R. and L. D. Newsom. 1959. Diapause in *Anthonomus grandis* Boh. J. Econ. Entomol. 52: 603-611.

Carter, F. L., T. C. Nelson, A. G. Jordan and J. R. Smith. 2001. U.S. cotton declares war on the boll weevil, Pp. 25-51. *In* W. A. Dickerson, A. L. Brashear, J. T. Brumley, F. L. Carter, W. J. Grefenstette and F. A. Harris [eds.], Boll weevil eradication in the United States through

1999. The Cotton Foundation Reference Book Series, No. 6. The Cotton Foundation Publisher, Memphis, TN.

Earle, N. W. and L. D. Newsom. 1964. Initiation of diapause in the boll weevil. J. Insect Physiol. 10: 131-139.

El-Lissy, O., F. Myers, R. Frisbie, T. Fuchs, D. Rummel, R. Parker, D. Dipple, E. King, G. Cunningham, F. Carter, J. Boston and J. Hayes. 1997. Boll weevil eradiction update—Texas, Pp. 973-979. *In* Proc. Beltwide Cotton Conferences, National Cotton Council, Memphis, TN.

Esquivel, J. F., D. W. Spurgeon and C. P.-C. Suh. 2004. Longevity of overwintered boll weevils (Coleoptera: Curculionidae) on pre-fruiting cotton. J. Cotton Sci. 8: 13-16.

Fenton, F. A. and E. W. Dunnam. 1927. Winter survival of the cotton boll weevil at Florence. S. C. J. Econ. Entomol. 20: 327-336.

Hunter, W. D. and W. E. Hinds. 1905. The Mexican cotton boll weevil. U.S. Dept. of Agric. Bur. Entomol. Bull. 51: 44-48, 53-54.

Lewis, D. K., D. Spurgeon, T. W. Sappington and L. L. Keeley. 2002. A hexamerin protein, AgSP-1, is associated with diapause in the boll weevil. J. Insect Physiol. 48: 887-901.

Lloyd, E. P., F. C. Tingle and R. Gast. 1967. Environmental stimuli inducing diapause in the boll weevil. J. Econ. Entomol. 60: 99-102.

Phillips, J. R. 1976. Diapause as it relates to the boll weevil, *Anthonomus grandis* Boheman, Pp. 10-11. *In* Boll Weevil Suppression, Management, and Elimination Technology. Proceedings of a Conference, Memphis, Tenn. U.S. Agric. Res. Ser. ARS-S-71.

Rummel, D. R. and S. C. Carroll. 1985. Longevity of overwintered boll weevils (Coleoptera: Curculionidae) following emergence in spring and early summer. Environ. Entomol. 14: 127-130.

Rummel, D. R., S. C. Carroll and M. D. Arnold. 1999. A proposed rating system for estimating the winter survival potential of boll weevils. Southwest. Entomol. 24: 144-151.

Rummel, D. R. and K. R. Summy. 1997. Ecology of the boll weevil in the United States cottonbelt. Southwest. Entomol. 22: 356-376.

Sappington, T. W. and D. W. Spurgeon. 2000. Preferred technique for adult sex determination of the boll weevil (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 93: 610-615.

SAS Institute. 2002. SAS/STAT user's guide, release 9.1 ed. SAS Institute, Cary, NC.

Spurgeon, D. W. and J. F. Esquivel. 2000. Effect of food type and feeding rate on boll weevil diapause, Pp. 1073-1077. *In* Proc. Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Spurgeon, D. W. and J. R. Raulston. 1998a. Diapause induction in subtropical boll weevils, Pp. 1019-1026. *In* Proc. Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

1998b. Boll weevil (Coleoptera: Curculionidae) reproductive development as a function of temperature. Environ. Entomol. 27: 675-681.

Spurgeon, D. W., T. W. Sappington and C. P.-C. Suh. 2003. A system for characterizing reproductive and diapause morphology in the boll weevil (Coleoptera: Curculionidae). Ann. Entomol. Soc. Am. 96: 1-11.

Sterling, W. L., S. G. Wellso, P. L. Adkisson and H. W. Dorough. 1965. A cottonseed-meal diet for laboratory cultures of the boll weevil. J. Econ. Entomol. 58: 867-869.

Taub-Montemayor, T. E., K. H. Dahm, G. Bhaskaran and M. A. Rankin. 1997. Rates of juvenile hormone biosynthesis and degradation during reproductive development and diapause in the boll weevil, *Anthonomus grandis.* Physiol. Entomol. 22: 269-276.

Vanderzant, E. S. and T. B. Davich. 1958. Laboratory rearing of the boll weevil: a satisfactory larval diet and oviposition studies. J. Econ. Entomol. 51: 288-291.

Wagner, T. L. and E. J. Villavaso. 1999. Diapause in the boll weevil (Coleoptera: Curculionidae): seasonal occurrence in Mississippi populations. Ann. Entomol. Soc. Am. 92: 382-395.

Westbrook, J. K., D. W. Spurgeon, R. S. Eyster and P. G. Schleider. 2003. Emergence of overwintered boll weevils (Coleoptera: Curculionidae) in relation to microclimatic factors. Environ. Entomol. 32: 133-140.