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Research

Morphology, Diet, and Temperature-Dependent Host-Free Survival of the Boll Weevil, *Anthonomus grandis* (Coleoptera: Curculionidae)

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Abstract

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is an important pest of cotton (*Gossypium* spp. (Malvales: Malvaceae)) in South America, Mexico, and southernmost Texas in the United States. A key factor in the persistence of the boll weevil is its ability to survive the noncotton season. Mechanisms facilitating this survival in subtropical and tropical areas are incompletely known, and our understanding has been further complicated by recent reports of overwintering on noncotton hosts. In addition, the nature and even existence of the adult dormancy, and validity of the criteria used to distinguish it, have been questioned. We manipulated the boll weevil diet to produce a range of diapause responses and observed the corresponding host-free survival patterns. The estimated proportion of diapause was associated with subsequent survival. In addition, different diets producing similar incidences of diapause resulted in substantially different host-free longevity, and the longest survival was observed for weevils fed bolls as adults. Survival patterns exhibited a negative temperature dependence except for the lowest observed temperature (12.8°C), where survival was reduced compared with that at 18.3°C. This suggests chronic chilling injury in response to extended exposure to low, above freezing temperatures. All but the highest temperature (29.4°C) facilitated survival >6 mo, and survival of >1 yr was observed at 18.3°C. The temperatures permitting the greatest survival are typical for many subtropical and tropical regions during the noncotton season, indicating that diapausing boll weevils are capable of surviving the noncotton season in these regions without the benefit of presumptive noncotton hosts.

Key words: diapause, dormancy, temperature, cotton, weevil

The boll weevil (*Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae)) is a key pest of cotton (*Gossypium* spp. (Malvales: Malvaceae)) that has been eradicated from most of the United States. However, populations remaining in South Texas and adjacent Mexico present a constant threat to reinfest more northerly eradicated zones (TBWEP 2016). The weevil is also the major pest of cotton in many production regions of South America (Scataglini et al. 2006, Grilli et al. 2012, Paula et al. 2013).

In the standing cotton crop, chemical insecticides are the principal tools used to manage or eradicate the boll weevil. Some cultural practices, such as timely crop destruction (Hinds 1908, Fye 1968, Summy et al. 1986), management for short-season production (Heilman et al. 1979, Walker and Smith 1996), and in some regions uniform delayed planting (Fuchs et al. 1998), have long been recognized for their importance in reducing overwintering survival of the boll weevil. However, their implementation in the tropics and subtropics might be improved with better understanding of boll weevil overwintering ecology.

The ability of some boll weevil adults to survive the noncotton season is well documented (Hinds and Yothers 1909, Gaines 1959, Carroll et al. 1993, Spurgeon 2008). However, the mechanism by which such survival occurs has been subject to debate. Brazzel and Newsom (1959) first characterized the overwintering mechanism of the weevil as a diapause. Lloyd et al. (1967) subsequently identified the influence of photoperiod on the weevil larvae, and low nighttime temperatures on the adult, as important factors initiating diapause. But Lloyd et al. (1967) also acknowledged that the diets provided to the weevils influenced their responses. Numerous reports since have failed to demonstrate a consistent diapause response to photoperiod, temperature, or diet. Spurgeon and Raulston (2006) suggested many of the inconsistencies in the boll weevil diapause literature could be attributed to lack of control of the diet provided experimental weevils, inconsistencies in the morphological criteria used to distinguish diapause, and failure in the early literature to design experiments that could be properly analyzed or that demonstrated repeatable results. Spurgeon and Raulston (2006) conducted extensive studies with weevils from subtropical and southern temperate regions and failed to demonstrate diapause induction in response to either photoperiod or temperature. Instead, feeding regime imposed on the weevils elicited the most marked and repeatable diapause response.

Diapause and overwintering ecology in the subtropics are even less well understood than in the southern temperate regions. Rummel and Summy (1997) summarized perceived differences in the ecology of temperate and subtropical weevils, one key difference being the propensity for a portion of the subtropical weevil population to continue reproduction so long as fruiting cotton is available. However, similar observations from temperate production regions were common before the widespread adoption of mechanical harvest (Mally 1901, Sanderson 1907, Hinds and Yothers 1909, Reinhard 1943, Brazzel and Hightower 1960, Beckham 1962). Graham et al. (1979) examined the seasonality of diapause in the Lower Rio Grande Valley of Texas, reporting the first diapausing weevils appeared around the time of the first open cotton boll, but the incidence of diapause increased around the time the crop was defoliated. However, Summy et al. (1993) reported the incidence of diapause in the Lower Rio Grande Valley varied considerably from year to year. Graham et al. (1978) examined the overwintering habitats of the weevil, and concluded that breeding of the weevil in unplowed cotton, combined with a high incidence of diapause at that time, was responsible for the high overwintering population during the winter of 1973–1974. Guerra et al. (1982) acknowledged the occurrence of an adult dormancy in the Lower Rio Grande Valley, but suggested it was better characterized as a quiescence than a diapause. Their conclusion was based on the observation that all wintering, trap-captured weevils oviposited within 2–5 d of having access to cotton squares at 29.5°C. However, they distinguished reproductive from diapausing weevils by observation of the fat body through the dorsal abdominal cuticle; a method Spurgeon et al. (2003) deemed unsatisfactory for assessing fat body condition. Furthermore, Spurgeon and Raulston (1998) reported that newly-eclosed adult females required a median time of about 3 d to develop an egg at 29.4°C. This suggests that at least some of the weevils examined by Guerra et al. (1982) possessed developing oocytes or eggs at the time of capture, and therefore were not in diapause. Greenberg et al. (2007) conducted an experiment similar to that of Guerra et al. (1982), and reported that weevils captured in traps during November–February did not oviposit until squares had been provided for an average of 14–20 d. Paula et al. (2013) concluded the dormancy of the boll weevil in Brazil was a quiescence, but based their classifications of dormancy on fat bodies of weevils dissected dry, and characters of reproductive development considered by Spurgeon et al. (2003) as atypical of diapause.

Despite these reports of an adult dormancy in the subtropics, additional controversy has arisen from recent syntheses of the literature. These syntheses draw conclusions extrapolated from three key reports (Showler 2006, 2008; Showler and Abrigo 2007). Showler (2006) observed higher trap captures of weevils near Lower Rio Grande Valley citrus (*Citrus paradisi* Macfad. and *C. sinensis* L. Osbeck (Sapindales: Rutaceae)) during the springtime compared with other habitats, and hypothesized that the weevil was overwintering in citrus. Showler and Abrigo (2007) demonstrated extended adult longevity for weevils provided no-choice diets of sliced citrus or fruit of prickly pear cactus (*Opuntia englemannii* Salm-Dyck ex. Englem. (Caryophyllales: Cactaceae)), although the extent to which weevils might feed on these nonhosts in the field was not examined. Showler (2008) examined the propensity of boll weevil adults to develop eggs on diets of dissected parts of cotton squares and bolls. Based on the absence or scarcity of egg production by weevils fed dissected locules or carpal walls of some age classes of cotton bolls, and limited longevity of weevils fed dissected carpal walls of mature bolls, Showler (2008) concluded that diapause is not induced by diet. Showler (2008) supported this conclusion citing reports by Guerra et al. (1982) and Graham et al. (1978), incorrectly indicating that these authors reported ‘a rarity or absence of dormancy’ in the subtropical boll weevil.

In his first synthesis of the literature, Showler (2007) emphasized the important role of citrus and prickly pear to overwintering of subtropical boll weevils. However, utilization of these putative feeding hosts by weevils in the field has not been demonstrated, and prickly pear typically fruits in May in the Lower Rio Grande Valley, when fruiting cotton is widely available to the weevil. The role of noncotton hosts was expanded by Showler (2009a), who suggested that growth of the citrus industry may have facilitated the infestation of the Lower Rio Grande Valley by the boll weevil. However, early citrus types were poorly adapted to the Lower Rio Grande Valley, and the industry did not see major expansion until the 1920’s and 1930’s (Anciso et al. 2002, Sauls 2008). By that time the boll weevil had already reached the Atlantic coast (Hunter and Coad 1923). Showler (2009a) also provided a limited critique of the boll weevil diapause literature. For example, Suh and Spurgeon (2006) demonstrated extended survival of boll weevils feeding on vegetative-stage regrowth cotton. Showler (2009a) criticized their work, describing the plants as ‘actively fruiting’. However, the occurrence of squares (≤3 mm diameter) in their studies was limited to plants provided to only one of the 16 experimental cohorts of weevils (Suh and Spurgeon 2006). Esquivel et al. (2004) reported extended longevity of overwintered weevils provided vegetative-stage cotton. However, according to Showler (2009a), Esquivel et al. (2004) concluded that vegetative-stage cotton was nutritionally inadequate to facilitate extended survival. In addition, Showler (2009a) reported that diapause does not occur in the Lower Rio Grande Valley, citing reports by Graham et al. (1978) and Summy et al. (1988). In contrast, Graham et al. (1978) reported a high incidence of diapause in 1 yr of their study. Summy et al. (1988) documented reproduction on regrowth cotton plants by a portion of the overwintering weevil population, but did not exclude diapause as an alternative survival strategy.

Showler (2009b, 2010) and Grilli et al. (2012) also report the importance of citrus to survival and range expansion of the boll weevil. Showler (2009b, 2010) cite Graham et al. (1978) and Summy et al. (1988) as providing evidence of the absence of diapause in subtropics, and added supporting reports by Graham et al. (1979), Guerra et al. (1982), and Showler (2003, 2006). However, Graham et al. (1979) reported the seasonal incidence of diapause was linked to the boll maturation period, and reports by Showler (2003, 2006) do not address diapause. Finally, Showler (2009b, 2010) similarly misinterpreted methods, conclusions, or both reported by Greenberg et al. (2007), Lewis et al. (2002), Sappington et al. (2004), Spurgeon et al. (2003), and Spurgeon and Raulston (2006), among others, and concluded that the boll weevil does not diapause. In particular, Showler (2009b, 2010) was critical of the morphological criteria used to distinguish diapausing from reproductive weevils, although these criteria are typical of those used to assess adult diapause in beetles (Hodek and Cerkasov 1961, McMullen 1967, Litsinger and Apple 1973, Clark 1975). In fairness, an association between these characters and an ecological consequence, such as extended survival, has not been established for the boll weevil. Our objectives were to 1) implement a range of diets to examine the relationship between the diapause criteria of Spurgeon et al. (2003) and subsequent host-free survival and 2) employ a diet known to induce a high incidence of diapause (Spurgeon and Suh 2017) to examine the influence of temperature on potential host-free survival.

Materials and Methods

Our objectives were addressed in two separate experiments. With the exception of the experimental treatments, both experiments used similar methods. Weevils were collected from the field as late-instar larvae within cotton squares. The field-collected squares were maintained within environmental chambers maintained at about 29°C with a photoperiod of 13:11 (L:D) h. The squares were periodically inspected for presence of pupae. Once pupae were found they were harvested daily, which involved transferring them from the squares to a 100 × 15 mm Petri plate containing a layer of moistened vermiculite. Up to 50 pupae were held in each plate, where they were inspected daily for adult eclosion. Newly eclosed adults were separated by sex using the tergal notch method (Sappington and Spurgeon 2000), and assigned to experimental treatments. In both studies, adult weevils were held in single-sex groups of 40, each group within a 473-cm^3 cardboard carton closed with a screened lid. In addition to the assigned diet, each carton was provisioned with a 29.5-ml plastic cup of water closed with a lid that was penetrated by a cotton wick. During the 14-d feeding period preceding diapause or survival assessment, adult weevils in both experiments were held at 29.4 ± 1°C with a 13:11 (L:D) h photoperiod. Although feeding period duration influences host-free survival (Spurgeon 2008; D.W.S., unpublished data), the 14-d period at temperatures ranging from 18.3 to 29.4°C has been shown to produce a high incidence of diapause and subsequent host-free survival (Spurgeon and Suh 2017). A putative diapause-suppressing photoperiod was used because the daylength in South Texas during the late-season, when diapause would be induced, is longer than 12 h.

In both experiments, the proportion of weevils in diapause from each carton was determined by dissection under water in a paraffin-lined Petri dish. Criteria indicating diapause for females included the combination of a hypertrophied fat body (fat white, in large globules, obscuring most or all other internal organs) and lack of vitellogenic oocytes, eggs, or follicular relics in the ovaries. Diapause in males was indicated by the combination of a hypertrophied fat body and small testes coated with an opaque layer of fat. These characters were illustrated and recommended by Spurgeon et al. (2003). Weevils exhibiting any other combination of characters of the fat body or reproductive organs were classed as nondiapausing.

Weevils selected to determine host-free survival were marked on one elytron with a nontoxic paint pen (Speedball Painters, Hunt Manufacturing, Statesville, NC). During the survival periods, the weevils were held within acrylic cages (20 × 20 × 20 cm) ventilated on four sides with aluminum screen. Each cage contained craft paper (30.5 × 45 cm) that was crumpled to provide resting sites, and water in a snap-cap vial fitted with a cotton wick protruding through the cap. Males and females within the same cage were marked with different colors and were numbered consecutively using a technical pen (Pigma Micron 005, Sakura Color Products, Osaka, Japan) to scribe the number on the painted elytron.

Diapause Morphology and Subsequent Survival

A range of diapause responses (proportions of weevils classed as diapausing) among the cartons of weevils was obtained by manipulating the adult diet, similar to the approach used by Spurgeon et al. (2003) to obtain specimens exhibiting a range of diapause or reproductive characters. We established 28 single-sex cartons of weevils (14 cartons of each sex). Four cartons of weevils were fed one boll (17–22 mm diameter) per 10 weevils thrice weekly. Weevils in twelve cartons were fed one large square (7–9 mm diameter) per five weevils daily, and weevils in eight cartons were fed one small square (5–7 mm diameter) per five weevils daily. Finally, four cartons of weevils were fed two small squares per five weevils daily. In each case, bracteoles were removed from the food items before they were provided to the weevils. When mortality occurred during the feeding period the number of food items per carton was adjusted to maintain as close as possible the desired feeding rate. At the end of the 14-d feeding period, 22–25 weevils were randomly selected from each carton for estimation of host-free survival, and remaining weevils (8–14 per carton, mean = 10.3) were dissected to obtain an estimate of the proportion of diapause for each carton.

One carton each of males and females, both fed the same diet, were combined in each of 14 survival cages. Cages were held in environmental chambers at 23.9 ± 1°C with a photoperiod of 13:11 (L:D) h. This temperature was selected based on results of Spurgeon et al. (2008) and Suh and Spurgeon (2006), because it produces mortality at a sufficiently low rate to distinguish relevant differences among treatments without unnecessarily prolonging the duration of the experiment. Each cage was inspected at 7-d intervals to remove dead weevils. Live weevils typically clung to the craft paper, or walked or sulled when disturbed. Weevils that showed no response to handling were stroked on the unpainted elytron with forceps, which elicited a walking response by live weevils.

Based on dissections from each carton, an analysis was conducted to evaluate differences in the proportions of diapause among the various diets. The conditional model used Laplace estimation and a binomial distribution with events/trials syntax (PROC GLIMMIX, SAS Institute 2012). Occurrence of diapause was considered an event. This syntax provided one observation for each carton and eliminated the need for an additional error term for testing other model effects. Because the design was completely randomized with unequal replication, only fixed effects of diet, weevil sex, and their interaction were included. Pairwise comparisons among the diets were adjusted for multiplicity using the SIMULATE option of the LSMEANS statement.

Three survival analyses were conducted using PROC LIFETEST (SAS Institute 2012). The first of these computed survival functions for each combination of weevil sex and survival cage. The estimates of median survival from this analysis were used in subsequent regression analyses and no experimental effects were tested. The second analysis compared survival functions corresponding to the different diets, adjusted for (stratified by) weevil sex. Pairwise comparisons among the respective survival functions were adjusted for multiplicity using the SIMULATE option. The third analysis examined the association between the proportion of weevils in diapause and subsequent host-free survival. That analysis was stratified by weevil sex and contained the proportion of diapause for each carton as a covariate (in the TEST statement). In the last two analyses, which involved hypothesis testing, the log-rank statistic was used because it places greater weight on later mortality events compared with the Wilcoxon statistic, which places greater weight on earlier mortality events (Stokes et al. 2012).

Finally, simple linear regression (PROC REG, SAS Institute 2012) was used to examine the relationship between proportion of diapause corresponding to each carton of weevils (the independent variable) and median survival in weeks (the response variable) previously estimated by the LIFETEST procedure. Median survival was modeled instead of mean survival because the survival curves are right-tailed and the median is a better measure of central tendency than is the mean for skewed distributions. These regressions included analyses of covariance to determine whether the regression lines were coincident or shared a common slope for the two weevil sexes (Littell et al. 2002).

Temperature Dependence of Host-Free Survival

A total of 28 single-sex cartons of 40 weevils each were used as in the previous study. All cartons were provided the same diet (cotton bolls, 17–25 mm diameter, at a rate of one boll per 10 weevils), which was the diet that maximized host-free survival in the first study. Bolls were replaced thrice weekly and the feeding period duration was 14 d at 29.4 ± 1°C with a 13:11 (L:D) h photoperiod. At the end of the feeding period, cartons were randomly assigned to survival temperatures (29.4°C, $n = 6$; 23.9°C, $n = 8$; 18.3°C, $n = 6$; 12.8°C, $n = 8$). These temperatures were selected for consistency with those used by Spurgeon and Suh (2017), with the addition of a lower temperature (12.8°C) that would be typical of daytime highs during much of the noncotton season in the tropics and subtropics. Half of the cartons at each temperature contained males and half contained females. After temperature assignments, 25 weevils were randomly selected from each carton for determination of host-free survival and the remainder (8–15 per carton, mean = 11.2) were immediately dissected to estimate the proportion of weevils in diapause as previously described. Weevils selected for survival estimation were marked, numbered, and paired by sexes exactly as in the previous study. Cages and refuges were as described for the previous study, as was the procedure for monitoring weevil mortality.

Initial analyses examined whether there were differences in the incidence of diapause among the weevils assigned to different survival temperatures, based on the dissections corresponding to each carton. The design was completely randomized with unequal replication and the analysis used Laplace estimation, a binomial distribution, and events/trials syntax (PROC GLIMMIX, SAS Institute 2012). Occurrence of diapause was considered an event. The model contained only fixed effects of assigned temperature, weevil sex, and their interaction. Pairwise comparisons of the incidence of diapause among assigned temperatures were adjusted for multiplicity using the SIMULATE option. This analysis was necessary to determine whether the proportion of diapause corresponding to each carton of weevils should be included as a covariate in survival analyses.

The influence of temperature on host-free survival was assessed using the LIFETEST procedure (SAS Institute 2012). The analysis was stratified by weevil sex and cage within each temperature so the estimates of survival were adjusted for these factors. As in the previous study, tests of significance were based on the log-rank statistic. Pairwise comparisons among the survival functions corresponding to each temperature were adjusted using the SIMULATE option to control experimentwise Type-I error at α = 0.05.

Results

Diapause Morphology and Subsequent Survival

Estimates of the proportions of diapause corresponding to each individual carton of weevils ranged from 0.125 (diet of one small square per five weevils daily) to 1 (one large square per five weevils daily). Analyses indicated a significant influence of diet but no influence of weevil sex (Table 1). A negligible sex × diet interaction (Table 1) indicated both sexes of weevil responded similarly to the influence of diet. Comparisons among diets indicated the mean proportion of diapause for weevils fed one small square per five weevils daily was lower compared with other diets (Fig. 1).

Survival analyses indicated significant differences among the survival functions corresponding to diets (log-rank $\chi^2 = 157.90$, df = 3, $P < 0.001$). Comparisons of survival functions among diets indicated differences in all pairwise comparisons (adjusted-$P < 0.001$) except between the boll and large square diets (adjusted-$P = 0.899$). Survival tended to be greatest for weevils fed the boll diet and large square diet, followed by the diet of two small squares per five weevils daily (Fig. 2). These differences were not expected because the proportions of weevils in diapause were approximately the same on all three diets. Survival was lowest for the diet of one small square per five weevils daily (Fig. 2), which was expected because that diet produced the fewest weevils in diapause (Fig. 1). Irrespective of the effects of diet on survival, analyses examining the initial proportion of diapause as a covariate also indicated a significant association between proportion of diapause and subsequent survival (log-rank $\chi^2 = 31.00$, $P < 0.001$).

Initial regression analyses of the relationship between proportion of diapause for each carton of weevils and median host-free longevity did not indicate heterogeneity of the regression slopes (sex × proportion diapause interaction, $F = 0.40$; df = 1, 24; $P = 0.532$) or separate intercepts ($F = 2.61$; df = 1, 25; $P = 0.119$) for the weevil sexes. Therefore, a single regression was calculated to represent both weevil sexes. That simple linear regression was significant ($F = 7.15$; df = 1, 26; $P = 0.013$). Although predictive power of the model was relatively poor based on the R^2 (Fig. 3), the intercept of the regression (where proportion of diapause = 0) suggested a baseline median survival of about 3 wk in the absence of diapause, and median survival of about 14 wk if all of the weevils were in diapause (Fig. 3).

Table 1. Tests of model effects of the probability of diapause for adult boll weevils fed different diets for 14 d at 29.4 ± 1°C with a photoperiod of 13:11 (L:D) h

Model effect	*F*	df	*P*
Sex	1.02	1, 20	0.325
Diet[a]	8.70	3, 20	<0.001
Sex × Diet	1.43	3, 20	0.263

The analysis used a conditional model with a binomial distribution.

[a] Diets were one small square per five weevils daily, two small squares per five weevils daily, one large square per five weevils daily, or one boll per 10 weevils thrice weekly.

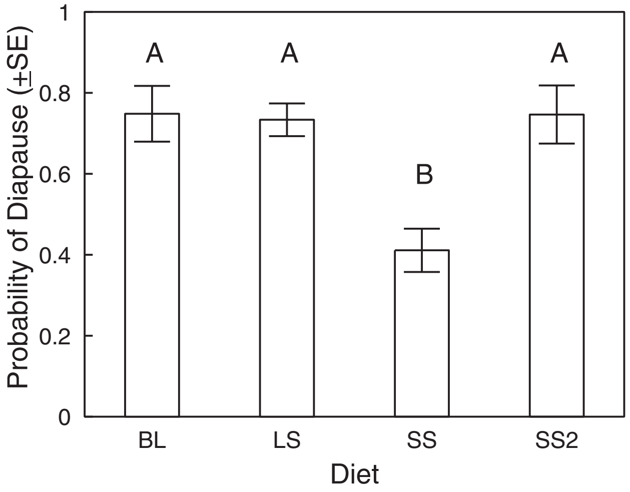

Fig. 1. Mean probability of diapause (±SE) for boll weevil adults fed four diets for 14 d at 29.4 ± 1°C under a 13:11 (L:D) h photoperiod. Diets are: BL, one boll per 10 weevils thrice weekly; LS, one large square per five weevils daily; SS, one small square per five weevils daily; SS2, two small squares per five weevils daily. Means with the same letter are not significantly different after adjustment for multiplicity (α = 0.05, SIMULATE option of SAS).

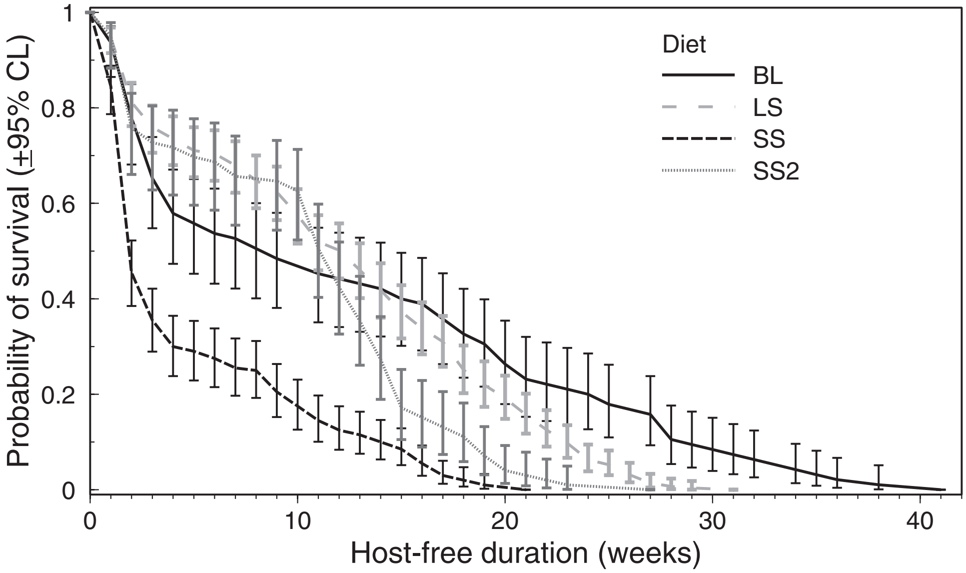

Fig. 2. Host-free survival functions (±95% CL) at 23.9 ± 1°C for boll weevil adults fed different diets for 14 d at 29.4 ± 1°C with a photoperiod of 13:11 (L:D) h. Diets were: BL, one boll per 10 weevils thrice weekly; LS, one large square per five weevils daily; SS, one small square per five weevils daily; SS2, two small squares per five weevils daily.

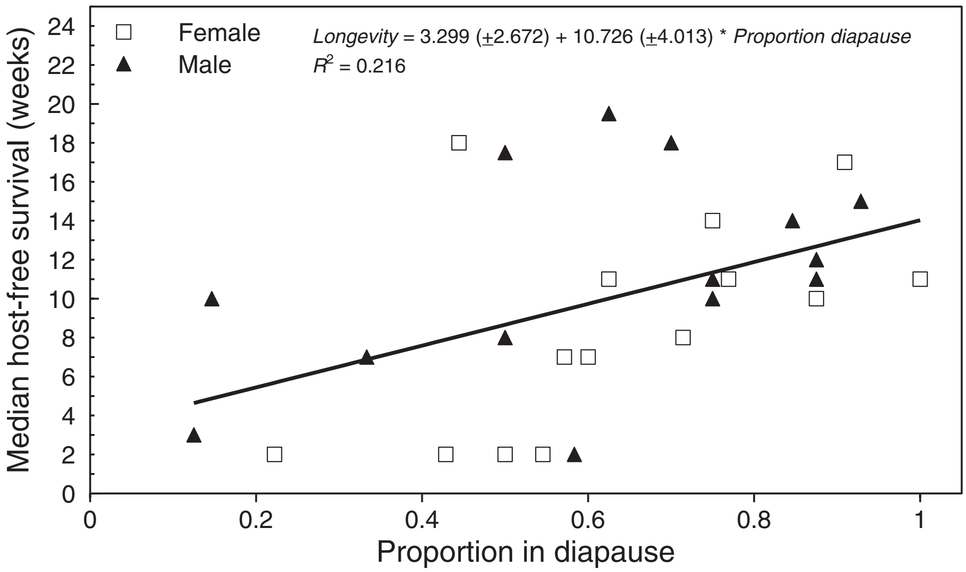

Fig. 3. Simple linear regression expressing the relationship between proportion of diapause of single-sex groups of adult boll weevils fed for 14 d at 29.4 ± 1°C under a 13:11 (L:D) h photoperiod, and median host-free survival at 23.9 ± 1°C. Standard errors of the regression intercept and slope are shown in parentheses.

Temperature Dependence of Host-Free Survival

The analysis examining the incidence of diapause in cartons of weevils assigned to different temperatures failed to indicate differences corresponding to weevil sex, assignments to temperature treatments, or their interaction (Table 2). The difference in estimated probability of diapause between the groups of weevils with the lowest and highest diapause responses was less than 0.07 (Fig. 4). Therefore, the proportion of diapause for each carton was not entered into the survival analysis as a covariate.

Comparisons of survival functions adjusted for weevil sex indicated a significant effect of temperature during the survival period (log-rank χ^2 = 184.08, df = 3, P < 0.001). Pairwise comparisons

Table 2. Tests of model effects of the probability of diapause for adult boll weevils fed a boll diet for 14 d at 29.4 ± 1°C with a photoperiod of 13:11 (L:D) h and then assigned to different survival temperatures

Model effect	*F*	df	*P*
Sex	2.89	1, 20	0.105
Assigned temperature[a]	0.44	3, 20	0.724
Sex × Assigned temperature	0.15	3, 20	0.927

The analysis used a conditional model with a binomial distribution.

[a] Temperatures assigned for the subsequent survival period were 12.8, 18.3, 23.9, and 29.4 ± 1°C.

among survival functions for each temperature indicated differences at adjusted-$P < 0.001$ except for the comparison of 12.8 and 23.9°C, where adjusted-$P = 0.999$ (Fig. 5). On average, greatest host-free survival occurred when the temperature was 18.3°C, followed by 12.8 and 23.9°C, which were not different (Fig. 5). Also, there was little difference between either the mean (±SE; 12.8°C, 16.0 ± 0.68 wk; 23.9°C, 15.2 ± 0.75 wk) or median survival times for these two temperatures (12.8°C, 16 wk; 23.9°C, 17 wk). However, maximum observed survival at 12.8°C was 54 wk, compared with 48 wk at 18.3°C. In contrast, survival at 29.4°C was clearly reduced compared with survival at the other temperatures (Fig. 5).

Discussion

The variable diets provided a wide range of diapause responses, as intended, yet most of the estimated proportions of diapause were more than 40%. On average, only the diet of a single square daily per five weevils produced a diapause response that was lower than for the other diets. However, the experiment was not designed to evaluate diapause responses to the diets, otherwise the numbers of replicates for each diet (ranging from 2 to 6 for each sex) would have been more equitable. Instead, these results document the observed proportions of diapause in the study, but do not necessarily reflect precise estimates of the general responses to all of the diets. Still, survival analysis with proportion of diapause as a covariate, and the regression of median longevity on proportion of diapause, indicated a significant association between host-free survival and the morphological characters used to detect diapause. Predictive power of the regression was poor, but we had not anticipated a strong quantitative relationship because the physiological classification of individual weevils is categorical whereas longevity is quantitative. These findings should allay the concerns expressed by Showler (2009b, 2010) regarding the relevance of the morphological characters used to distinguish diapause.

In addition, survival functions differed among all of the diets despite the similar diapause responses observed for diets of bolls, large squares, or small squares fed at the higher rate (two squares per five weevils daily). This suggests that diet influences not only the incidence of diapause (Spurgeon and Raulston 2006), but also the subsequent host-free longevity of diapausing individuals. This finding has important implications for investigations of potential host-free survival in response to factors not studied here, and should be a consideration in comparisons among results of other studies of host-free survival.

Several studies have demonstrated extended host-free survival attributed to diet-induced diapause (Suh and Spurgeon 2006, Spurgeon et al. 2008, Spurgeon and Suh 2017). In addition, the duration of the induction period during which the weevils feed (D.W.S., unpublished data) and the temperature during this feeding period (Spurgeon and Suh 2017) have been shown to influence subsequent survival. Summy et al. (1993) reported overwintering survival of the boll weevil was highest for weevils fed bolls compared with those fed squares. However, their feeding period duration was too long (48 d) to produce a good estimate of potential host-free longevity because many of the weevils that did not enter diapause would have died before the end of the feeding

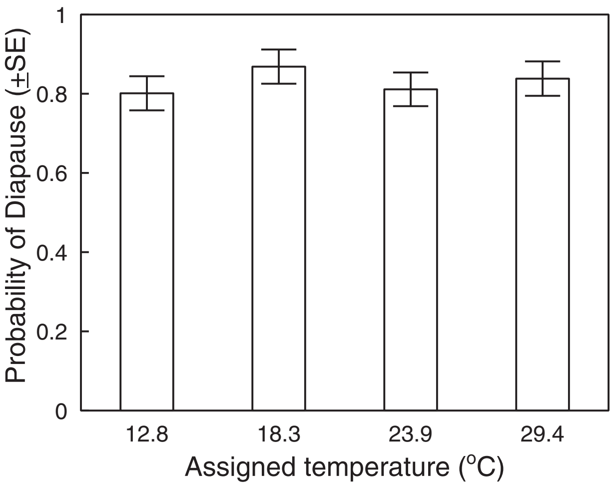

Fig. 4. Mean (±SE) probability of diapause for adult boll weevils fed a boll diet for 14 d at 29.4 ± 1°C with a 13:11 (L:D) h photoperiod prior to their transfer to assigned survival temperatures.

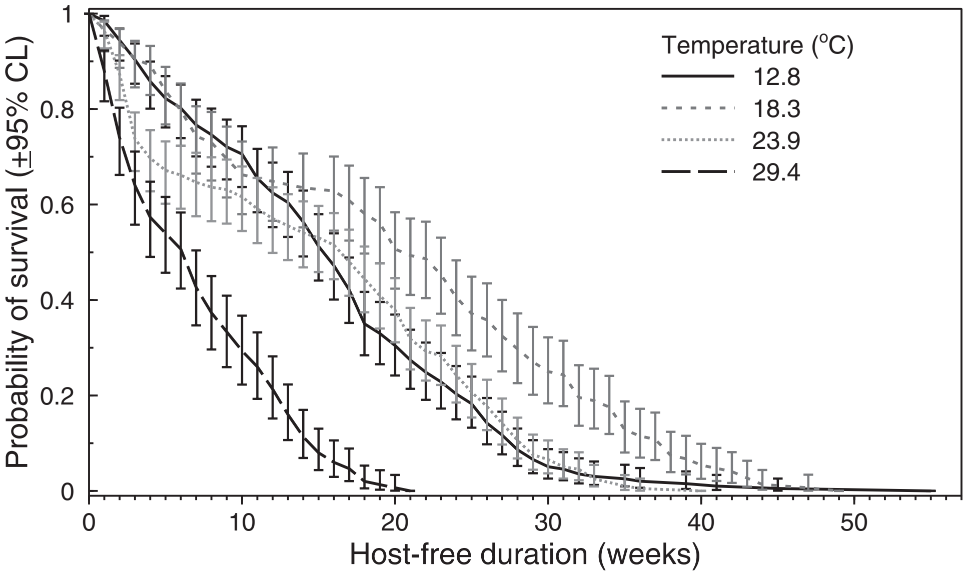

Fig. 5. Mean probability of survival (±95% CL) for boll weevil adults fed a boll diet for 14 d at 29.4 ± 1°C under a 13:11 (L:D) h photoperiod, then transferred to host-free conditions under different temperatures from 12.8 to 29.4 ± 1°C.

period. Most other studies of overwintering survival have been conducted under uncontrolled field conditions or used a mixture of food types (Fenton and Dunnam 1927; Gaines 1959; Rummel and Carroll 1983; Carroll et al. 1993; Parajulee et al. 1996, 1997; Westbrook et al. 2003; Spurgeon 2008), so comparisons with our results are not possible. In contrast, Spurgeon and Suh (2017) fed a cotton boll diet similar to the diet we used, and observed host-free survival at 23.9°C that was very similar to the survival we observed in this study. Based on these observations, it seems that survival studies that lack careful control of diet, feeding period duration, and feeding period temperature are unlikely to provide information that is as ecologically interpretable compared with more tightly controlled experiments.

Differences in weevil longevity among the survival temperatures suggest three important things. First, prolonged exposure to low but above freezing temperatures (e.g., 12.8°C) appears to be associated with reduced survival potential, which could be the product of chronic chilling injury. These low but above freezing temperature conditions often occur in southern temperate or northern subtropical areas during the noncotton season. Secondly, survival potential is substantially curtailed under relatively high temperatures, which is likely the product of temperature-dependent consumption of fat stores necessary for survival. Daily average temperatures in the range of 29.4°C are unlikely to occur during the noncotton season except perhaps in the early fall in areas such as the desert southwest of the United States. However, average daily temperatures between 18.3 and 23.9°C during the noncotton season would be common in many subtropical and tropical cotton production systems. For example, the average winter temperature in the Midwest of Brazil is about 18°C (Paula et al. 2013). Potential survival we observed in this temperature range would be sufficient to ensure substantial weevil survival between cotton cropping cycles even in the absence of presumptive alternate hosts.

The patterns of survival we observed at different temperatures, combined with our findings indicating an influence of diet on subsequent survival, have important implications for the management or eradication of the weevil. These implications are especially relevant when timely and thorough destruction of the harvested crop is hampered by inclement weather, or when fall rains promote regrowth or development of volunteer plants. In those instances, when resources available to management or eradication programs are limited, efforts focused on reducing potential overwintering weevil populations should give priority to cotton fields where green bolls or large squares are available compared with volunteer or regrowth plants that are not yet fruiting or are early in the fruiting cycle.

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