Diapause Response to Photoperiod in an Arizona Population of *Lygus hesperus* (Hemiptera: Miridae)[1]

D.W. Spurgeon[2] and C.S. Brent

USDA, ARS, Pest Management and Biocontrol Research Unit, 21881 N. Cardon Ln., Maricopa, Arizona 85138 USA

J. Entomol. Sci. 50(3): 238–247 (July 2015)

Abstract The western tarnished plant bug (*Lygus hesperus* Knight) is an important crop pest in the western United States that overwinters in an adult diapause. However, knowledge of *L. hesperus* diapause is incomplete. Eggs from field-collected adults were reared under photoperiods of 10:14, 11:13, 12:12, and 13:11 (L:D) h at 26.7 ± 1°C, and the diapause response for each gender was modeled by a logistic function. Incidence of a recently reported "glass bead" fat body type also was examined. Validation studies using the same methods were subsequently conducted using photoperiods of 10.5:13.5, 11.5:12.5, and 12.5:11.5 (L:D) h. No effects of bug gender, photoperiod, or diapause status on occurrence of the novel fat type were detected. Estimates of diapause in validation studies were within confidence intervals for initial predictions, but systematic deviations from initial predictions prompted refitting of the models to include validation data. Refitted functions estimated critical photoperiods of 11h44min for females and 11h21min for males. The maximum incidence of diapause was lower for males than for females and was $<100\%$ for both genders. Refitted functions also predicted 50% of the population-specific maximum diapause response corresponded to photoperiods of 11h54min (females) and 12h7min (males). These results, combined with other recent findings, suggest heterogeneity in diapause response likely enables *L. hesperus* populations to adapt to local conditions. The estimated functions relating photoperiod to diapause incidence provide baselines to facilitate future studies of environmental and geographical influences on diapause in this species.

Key Words *Lygus hesperus*, western tarnished plant bug, photoperiod, diapause

The western tarnished plant bug, *Lygus hesperus* Knight, is an important pest of horticultural and row crops in the western United States. Although *L. hesperus* remains an important pest in Arizona cotton (*Gossypium hirsutum* L.), its economic impact has declined because of improved management (Ellsworth 1998) and use of reduced-risk insecticides (UA 2013). The prevailing low-pesticide-use conditions in Arizona cotton suggest additional opportunity to improve management of *L. hesperus* through enhanced knowledge of its chemical and physiological ecology. In addition, such knowledge may be valuable to current efforts to apply transcriptomic resources (Hull et al. 2013, 2014) to identify and manipulate xenobiotic and environmental stress responses with the goal of developing environmentally based control tactics.

---

[1] Received 11 February 2015; accepted for publication 20 April 2015.

[2] Corresponding author (email: Dale.Spurgeon@ars.usda.gov).

One aspect of *L. hesperus* ecology that is poorly studied is its diapause. *Lygus hesperus* overwinters in a state of adult diapause that is induced by photoperiod (Beards and Strong 1966, Leigh 1966, Strong et al. 1970). Beards and Strong (1966), who conducted the most extensive studies of diapause, reported the diapause response was maximal at daylengths ranging from 7 to 11 h, whereas an intermediate response was observed at daylengths of 6, 12, and 13 h. Furthermore, they reported that when young adults reared under short-day conditions (10 h) were transferred to a long daylength (16 h) the diapause response was partially nullified, and that late instars were responsive to photoperiod but the photoperiodic environment of early instars also influenced the diapause response. Finally, Beards and Strong (1966) suggested that under field conditions, exposure of nymphs to a 13-h daylength would produce diapause in all subsequent adults, but the sensitivity to photoperiod of the different instars was not determined. None of these earlier studies featured experimental designs amenable to analysis. Nor was it demonstrated that results, which varied among individual studies, were repeatable.

More recent work questions the interpretation of the earlier reports based on the morphological characters used to distinguish diapause. Spurgeon and Brent (2010) evaluated morphological characters for association with diapause and found that results of many previous reports were based on irrelevant characters, or inconsistent application of appropriate characters. Many of the earlier studies were also based on observations of insects from established laboratory colonies, and Brent and Spurgeon (2011) and Spurgeon (2012) demonstrated the diapause response of *L. hesperus* is subject to rapid selection under the conditions of laboratory rearing. Based on these questions, improved understanding of diapause in *L. hesperus* will require at least reexamination of the stages or instars receiving the photoperiodic cue, the influence of temperature on diapause induction, the consequences of diapause to survival and subsequent reproduction, and improved understanding of the relationship between daylength and the induction of diapause. Our objective was to quantitatively describe the relationship between photoperiod and diapause, and to estimate the critical photoperiod.

Materials and Methods

Each experimental repetition used F_1 insects obtained as eggs from a separate cohort of 400–500 adults collected near Maricopa, AZ. All but one cohort was collected from alfalfa (*Medicago sativa* L.); the additional cohort was collected from London rocket (*Sisymbrium irio* L.) in March 2013. Some collections included a small number of *Lygus lineolaris* (Palisot de Beauvois). No effort was made to remove the *L. lineolaris* from the adult colonies, and their progeny were included in the experiment. However, the adults, which are easily distinguishable from newly eclosed *L. hesperus* adults, were excluded from any analyses. The field-collected adults were held within a 0.03-m^3 screened cage containing shredded paper in an environmental chamber at 27–28°C, 40–60% RH, and with a photoperiod of 14:10 (L:D) h. The adults were provided a water-saturated cotton pad within a petri plate bottom, raw sunflower (*Helianthus annuus* L.) seeds, and 20 green bean (*Phaseolus vulgaris* L.) pods that were replaced three times weekly. The beans served both as food and oviposition substrate.

The initial experiment examined the diapause response of *L. hesperus* at photoperiods of 10:14, 11:13, 12:12, and 13:11 (L:D) h and a constant temperature of 26.7 ± 1°C. This temperature regime was selected because it is typical of temperatures used in previous laboratory studies of diapause in *L. hesperus* (Beards and Strong 1966, Brent and Spurgeon 2011, Leigh 1966, Spurgeon 2012) and *L. lineolaris* (Snodgrass 2003; Villavaso and Snodgrass 2004, 2005). In addition, the experimental temperature is between the respective mean daily temperatures for the months of September and October at Maricopa, AZ (UA 2015). These experimental treatments were maintained within four environmental chambers (model I30BLL, Percival Scientific, Perry, IA). Temperatures within the chambers were monitored twice weekly using portable loggers (model U10-003, Onset Computer, Bourne, MA). The experiment was conducted four times (repetitions) using progeny of parents collected on 14 September and 11 October 2012, and 6–7 March and 20 May 2013. Photoperiod treatments were randomly assigned to the four environmental chambers at the beginning of each experimental repetition.

When examination of green beans exposed to field-collected parents indicated high levels of oviposition, eight fresh beans were placed into the rearing cage. After an oviposition period of 18–24 h, the beans were removed, cut in half, and the cut ends were sealed with melted paraffin. Each bean half was placed in a filter paper–lined petri dish (100 × 15 mm) that was sealed with Parafilm M (Pechiney Plastic Packaging, Chicago, IL) to prevent desiccation of the eggs. Four bean halves were randomly assigned to each photoperiod treatment, with the restriction that no treatment received both halves of the same bean. The bean halves were examined daily for mold or excessive condensation, either of which prompted removal of the Parafilm. Otherwise, the Parafilm was removed on the fifth day after oviposition and a section of fresh bean was added to each plate as food for emerging nymphs.

Newly emerged nymphs (typically 6–7 d after oviposition) were individually transferred to 18-ml plastic vials (Thornton Plastics, Salt Lake City, UT), each containing a section of green bean (4–5 cm, with waxed ends) and closed with a screened snap-cap lid. Vials containing nymphs were then returned to the assigned photoperiod. In each experimental repetition, 50 nymphs were assigned to each photoperiod. Bean sections were replaced three times weekly, except the initial bean section was not replaced until the first nymphal molt to minimize mortality from handling. Nymph development was noted when bean sections were replaced until the fifth instar, after which nymphs were checked daily for adult eclosion. The date of adult eclosion was recorded, and adults were maintained using the same conditions as for nymphs until they were 10 d old.

The diapause status of 10-d-old adults was determined by dissection in saline (0.7% NaCl [wt:vol]), using the procedure described by Spurgeon (2012). The abdominal dorsal plate was removed to reveal the fat body, after which the alimentary tract was removed to reveal the developmental state of the ovaries (females) or medial accessory glands (males). Females exhibiting a hypertrophied fat body consistent with the description by Spurgeon and Brent (2010) and lacking evidence of vitellogenesis were considered in diapause (the stringent criteria of Brent and Spurgeon 2011). Males with hypertrophied fat body and undeveloped or underdeveloped medial accessory glands were considered in diapause (the relaxed criteria of Brent and Spurgeon 2011).

Spurgeon and Brent (2010) illustrated a novel “glass bead” fat body type previously unreported in *L. hesperus*. Brent and Spurgeon (2011) and Spurgeon (2012) subsequently reported on the incidence of the glass bead fat in California populations of *L. hesperus*. Although examining the incidence of this novel fat type and its association with environmental or physiological conditions was not a primary objective of this study, dissections to determine diapause status facilitated recording its presence in *L. hesperus* from central Arizona.

The proportion of insects classified as diapausing was calculated for each combination of photoperiod, insect gender, and experimental repetition. Using these proportions as inputs, the relationship between photoperiod and diapause was described by a weighted (by sample size) nonlinear function for each insect gender using the PROC NLIN procedure of SAS (SAS Institute 2012). The data for each gender were fitted to a logistic function of the form $Y = (a/[1 + e^{(b - c \times \text{photophase})}])$ (Sit and Poulin-Costello 1994), where $Y =$ the proportion of insects in diapause, photophase = daylength (h), $a =$ the upper asymptote, and $b/c =$ the inflection point of the curve; parameters *a*, *b*, and *c* were estimated from the observed data. Upper and lower 95% confidence limits of model-predicted responses were calculated, along with the critical photoperiod (photoperiod corresponding to 50% diapause) and the population-specific critical photoperiod (photoperiod corresponding to 50% of the maximal predicted occurrence of diapause).

The probability of occurrence of the glass bead fat type was estimated using the SAS procedure PROC LOGISTIC (SAS Institute 2012). The logistic regression model included terms for insect gender, photoperiod, their interaction, and experimental repetition. To minimize bias from small sample sizes and separability, Firth’s penalized likelihood was implemented (FIRTH option of the MODEL statement). Fit of the logistic regression was evaluated by the Hosmer and Lemeshow goodness-of-fit χ^2 statistic (LACKFIT option of the MODEL statement).

After completion of the initial experiments, two repetitions of an additional experiment were conducted to evaluate consistency of the diapause response with predictions from the fitted logistic functions. The validation experiment used the same procedures as the initial experiment except that photoperiod treatments were composed of 10.5:13.5, 11.5:12.5, and 12.5:11.5 (L:D) h, and occurrence of the glass bead fat was not evaluated. The experimental insects were obtained from parent collections from alfalfa on 13 August and 7 November 2013. Following comparisons of observed incidences of diapause to predictions from the initial logistic functions, the description of the relationship between photoperiod and diapause incidence, including predicted critical photoperiods, was refined by refitting the logistic function to the entire set of observations for each gender.

Results and Discussion

Of the 1,100 nymphs established on green beans in the collective experiments, 890 (80.9%) survived to 10 d of adult age. Of the survivors, 49 (5.5%) were *L. lineolaris*. The percentage of experimental adults that were *L. lineolaris* ranged from 0% (adult collections on 20 May and 7 November 2013) to 13.6% (adult collection on 7 March 2013). Sample sizes (10-d-old adults) for each combination of *L. hesperus* gender, photoperiod, and experimental repetition ranged from 12 to 27 for females and from 11 to 28 for males. Overall, 81% and 86% of sample sizes were $\geq$15 for *L. hesperus* females and males, respectively.

The goodness-of-fit test for the logistic regression examining the occurrence of the glass bead fat in the initial experiment failed to indicate lack of model fit ($\chi^2 = 5.92$; df $= 8$; $P = 0.66$). The analysis did not indicate influences of gender (Wald $\chi^2 = 0.33$; $P = 0.56$) or photoperiod (Wald $\chi^2 = 0.30$; $P = 0.96$). Furthermore, the gender by photoperiod interaction was not significant (Wald $\chi^2 = 6.51$; $P = 0.09$). Examination of simple effects of insect gender within photoperiod, and of photoperiod within gender, also failed to indicate significant gender ($0.26 < P < 0.43$) or photoperiod ($0.08 < P < 0.73$) effects. Results of these analyses were consistent with reports by Brent and Spurgeon (2011) and Spurgeon (2012), who found no evidence of association between the glass bead fat and diapause in *L. hesperus* from California. Overall, about 32% of the insects exhibited the glass bead fat. The observed frequency of occurrence of the glass bead fat was lower than that reported for insects from central California by Spurgeon (2012), but was consistent with the incidence of this fat body type reported by Brent and Spurgeon (2011). Although this novel fat type is frequently observed in the F_1 progeny of field-collected bugs, it seldom occurs in laboratory-reared insects (Brent and Spurgeon 2011). Also, it was not previously reported from *L. hesperus* in Arizona. The physiological or ecological implications of this fat body type are unknown. Regardless, recognition of its occurrence may be important to transcriptomic studies of native *L. hesperus*, either for association with transcripts of interest or as a source of within-population heterogeneity to be controlled in experiments.

The logistic functions describing initial estimates of relationships between observed proportions of diapausing female ($F = 102.1$; df $= 3, 13$; $P < 0.01$) and male ($F = 57.4$; df $= 3, 13$; $P < 0.01$) *L. hesperus* are illustrated in Fig. 1. The model for females (Fig. 1a) estimated a maximal diapause response of 79% and a critical photoperiod, at which 50% of the insects would enter diapause, of 11h52min. The population-specific critical photoperiod (inducing diapause in 50% of the estimated maximum proportion of diapause) was estimated as 11h57min. For males (Fig. 1b), the critical photoperiod was estimated as 11h25min, but the maximum predicted incidence of diapause was only 53%. The population-specific critical photoperiod was estimated as 12h9min. For either insect gender, as daylength declined from 11 to 10 h the increase in diapause incidence was negligible. Predictions from the models suggested the diapause response in males is initiated at longer daylengths than for females, but that a lower overall proportion of males enter diapause compared with female *L. hesperus*.

Estimated proportions of diapause observed for both females and males in the validation cohorts were within the 95% confidence limits estimated by the initial logistic functions (Fig. 1a, b). However, deviations of the diapause responses observed for the validation cohorts appeared systematic relative to predictions by the initial models, especially for female *L. hesperus* and at the longer daylength (12.5 h). Therefore, the logistic functions were refitted using the combined data from the initial and validation cohorts. Compared with the initial logistic function, the refitted model for females ($F = 135.0$; df $= 3, 19$; $P < 0.01$; Fig. 2a) reduced the slope of the logistic function, which was reflected in lower estimates of parameters *b* and *c* and their standard errors. The asymptote of the function (parameter *a*) was only slightly affected. The refitted function predicted a critical photoperiod of

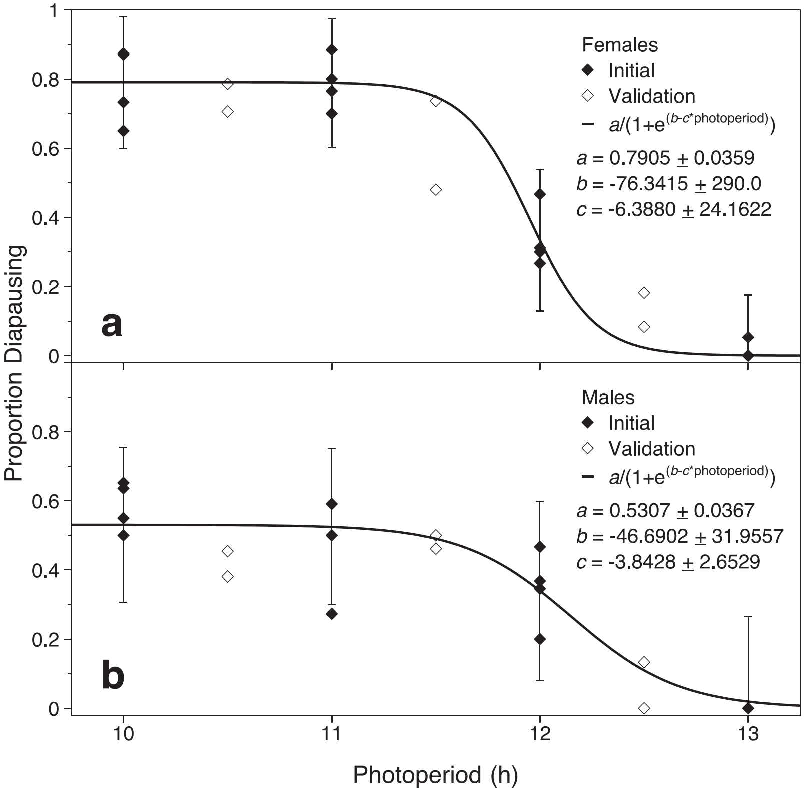

Fig. 1. Initial estimated logistic functions relating photoperiod to incidence of diapause for (a) female and (b) male *Lygus hesperus*. Closed diamonds indicate cohorts used to fit the initial model; open diamonds represent validation cohorts. Vertical lines indicate 95% confidence intervals; *a*, *b*, and *c* in the legend are model parameter estimates ± SE.

11h44min and a population-specific critical photoperiod of 11h54min, which were only modestly shorter than the estimates from the initial model. In contrast to results for female *L. hesperus*, refitting the logistic function to the male data produced only small changes in model parameters ($F = 70.14$; df = 3, 19; $P < 0.01$; Fig. 2b). Compared with the initial model, the asymptote (parameter *a*) decreased from 0.53 to 0.51. Although estimates of parameters *b* and *c* differed between the initial and refitted models, the respective standard errors and predicted inflection points (*b*/*c*) of the two functions were similar. The refitted function for males predicted a critical photoperiod of 11h21min, and a population-specific critical photoperiod of 12h7min, both of which were close to predictions from the initial function. As in the case of the initial logistic models, the refitted models continued to suggest male *L. hesperus* respond to somewhat longer photoperiods than do females, and that the maximum diapause response of males results in a lower proportion of diapause than for females in the central Arizona population of *L. hesperus*. In addition, the refitted

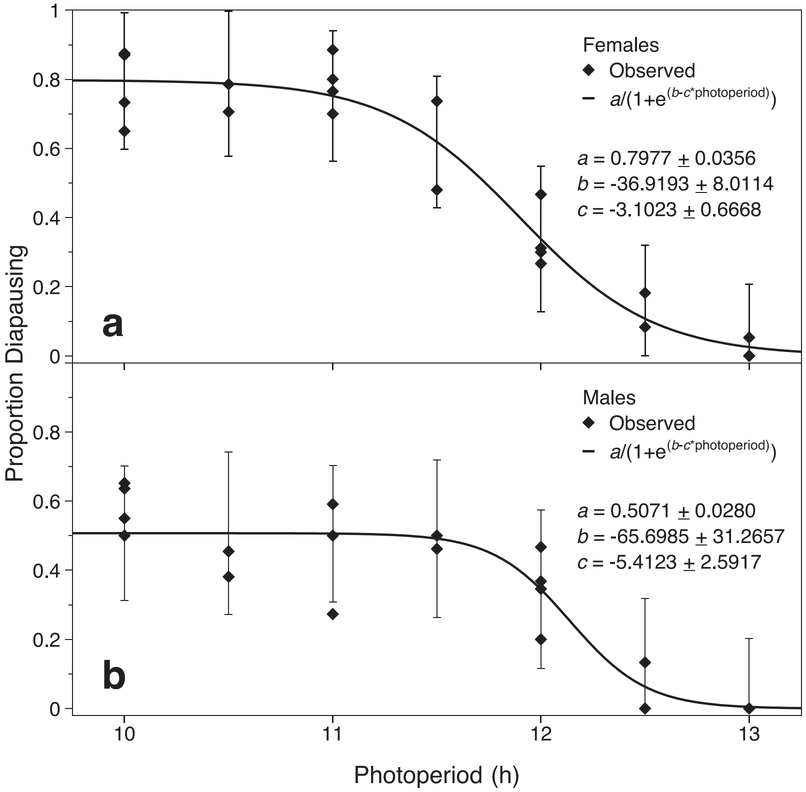

Fig. 2. Estimated logistic functions relating photoperiod to incidence of diapause for (a) female and (b) male *Lygus hesperus*. The refitted models include initial estimates of diapause (at 10-, 11-, 12-, and 13-h daylengths) and those from validation cohorts (at 10.5-, 11.5-, and 12.5-h daylengths). Vertical lines indicate 95% confidence intervals; *a*, *b*, and *c* in the legend are model parameter estimates ± SE.

models suggested the transition from non-diapause-inducing to diapause-inducing photoperiods involves a more narrow range of daylengths for male *L. hesperus* than for females.

Recent work suggests the proportion of male *L. hesperus* that enters diapause tends to be less than for females, even though differences are not always statistically significant and depend on the criteria used to distinguish diapausing from reproductive bugs (Brent and Spurgeon 2011, Spurgeon 2012). Brent and Spurgeon (2011) advocated using the “stringent” diapause criteria for females and the “relaxed” criteria for males, in part because this approach diminished the observed differences in the diapause response between the two genders. Use of the recommended criteria in this study did not produce estimates of diapause response that were similar for the two sexes (Fig. 2a, b). Also, the frequencies of diapause observed for both genders in this study were less than those previously reported from populations in California (Beards and Strong 1966, Leigh 1966, Spurgeon 2012) or from Arizona (Brent and Spurgeon 2011). Differences between the extent of diapause observed in this study and that previously reported for bugs from Arizona may be at least partially explained by differences in methodology between the two reports. Estimates of diapause reported for Arizona bugs by Brent and Spurgeon (2011) were based on dissections of insects held in mixed-sex groups, which may have allowed mating. During mating the male accessory glands are emptied (Brent 2010). Therefore, for some reproductive individuals, mating shortly before dissection may have influenced the perceived extent of accessory gland development and resulted in their subsequent classification as diapausing. Dissimilarity in the diapause response of *L. hesperus* from Arizona compared with previous reports from California was not completely unexpected because the species inhabits a wide range of habitats in the western United States, and it is well documented that the diapause response to photoperiod varies among geographical populations of some species (Tauber et al. 1986).

Beards and Strong (1966) reported that, based on field-collected adult *L. hesperus*, the critical photoperiod inducing diapause was about 12.5 h, but based on the fate of field-collected nymphs the critical photoperiod was 13.5 h. They also reported that 2 September, representing a daylength of 13 h at Davis, CA, was the date after which all nymphs in the field would produce diapausing adults. Beards and Strong (1966) did not define “critical photoperiod” in the context of their report, nor did they explain how it was calculated. Therefore, it is not possible to make meaningful comparisons between their estimates and the critical photoperiods estimated in our study. No other estimate of the critical photoperiod inducing diapause in *L. hesperus* exists for comparison with our results. The report by Beards and Strong (1966) also indicated only limited influence of temperature on the diapause response of *L. hesperus*. However, their diapause-inducing photoperiod was 10 h, which is in the range of photoperiods producing maximal diapause response. If temperature is an important modifier of the photoperiodic response in *L. hesperus*, it seems this effect would be more easily detected and quantified near the population-specific critical photoperiod.

The wide confidence intervals of all the models fitted in this study, combined with the demonstrated ability to rapidly select for a reduced diapause response (Spurgeon 2012) probably reflects a high degree of genetic heterogeneity or plasticity in natural populations of this species. Such heterogeneity would seem ecologically beneficial, or even essential, to a polyphagous species with a wide geographical range. It would also imply that *L. hesperus* populations have the genetic ability to adapt to local environments in response to selection pressures favoring either reproduction or diapause. Maintenance of heterogeneity within a given population may serve as an ecological bet-hedging strategy, especially in southern portions of the insect’s range where fall and winter conditions are often favorable for reproduction. Because diapause is currently distinguished based on internal morphology revealed by dissection, heterogeneity in photoperiodic response poses a potential obstacle to studies of the ecology, physiology, or molecular biology of *L. hesperus* diapause unless a nondestructive and highly accurate means of distinguishing diapause can be devised.

Although estimates of the proportions of diapausing insects in validation cohorts were within the confidence intervals of the initial logistic functions, refitting of the models to include the validation data substantially changed parameter estimates and their standard errors for female *L. hesperus*. This suggests that predictions from the refitted models are preferable to those from the initial models. The relevance of predictions from these models to *L. hesperus* from other geographical areas is not known. However, the relationships reported herein provide baseline information to facilitate investigations of the influences of environment and geography on the adult diapause of *L. hesperus*.

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. USDA is an equal opportunity provider and employer.

References Cited

Beards, G.W. and F.E. Strong. 1966. Photoperiod in relation to diapause in *Lygus hesperus* Knight. Hilgardia 37: 345–362.

Brent, C.S. 2010. Reproductive refractoriness in the western tarnished plant bug, *Lygus hesperus*. Ann. Entomol. Soc. Am. 102: 300–306.

Brent, C.S. and D.W. Spurgeon. 2011. Diapause response of laboratory reared and native *Lygus hesperus* Knight (Hemiptera: Miridae). Environ. Entomol. 40: 455–461.

Ellsworth, P.C. 1998. Integrated Lygus management in Arizona, Pp. 403–407. *In* Silvertooth, J. (ed.), Cotton: A College of Agriculture Report for 1998. Publication AZ1006. College of Agriculture, Univ. of Arizona, Tucson.

Hull, J.J., K. Chaney, S.M. Geib, J.A. Fabrick, C.S. Brent, D.J. Hawthorne, D. Walsh and L.C. Lavine. 2014. Transcriptome-based identification of ABC transporters in the western tarnished plant bug *Lygus hesperus*. PLoS ONE 9: e113046.

Hull, J.J., S.M. Geib, J.A. Fabrick and C.S. Brent. 2013. Sequencing and *de novo* assembly of the western tarnished plant bug (*Lygus hesperus*) transcriptome. PLoS ONE 8: e55105.

Leigh, T.F. 1966. A reproductive diapause in *Lygus hesperus* Knight. J. Econ. Entomol. 59: 1280–1281.

SAS Institute. 2012. SAS Release, Ed. 9.4. SAS Institute Inc., Cary, NC.

Sit, V. and M. Poulin-Costello. 1994. Catalogue of curves for curve fitting. Biometrics Information Handbook Series, W. Bergerud and V. Sit (eds.). Province of British Columbia Forest Science Research Branch, Victoria, Canada.

Snodgrass, G.L. 2003. Role of reproductive diapause in the adaptation of the tarnished plant bug (Heteroptera: Miridae) to its winter habitat in the Mississippi River Delta. Environ. Entomol. 32: 945–952.

Spurgeon, D.W. 2012. Physiological consequences of laboratory rearing of *Lygus hesperus* (Hemiptera: Miridae). Environ. Entomol. 41: 415–419.

Spurgeon, D.W. and C.S. Brent. 2010. Morphological characters of diapause in *Lygus hesperus* Knight (Hemiptera: Miridae). J. Entomol. Sci. 45: 303–316.

Strong, F.E., J.A. Sheldahl, P.R. Hughs and E.M.K. Hussein. 1970. Reproductive biology of *Lygus hesperus* Knight. Hilgardia 40: 105–147.

Tauber, M.J., C.A. Tauber and S. Masaki. 1986. Seasonal adaptations of insects. Oxford Univ. Press, New York.

[UA] University of Arizona. 2013. Cotton IPM: A quiet revolution reduces costs, losses and risks for Arizona's cotton growers. University of Arizona, College of Agriculture and Life Sciences, 2013 Impact Report. 8 December 2014. (http://cals.arizona.edu/apmc/docs/CottonIPM-2013_impacts.pdf).

[UA] University of Arizona. 2015. AZMET: The Arizona Meteorological Network. 8 December 2014. (http://ag.arizona.edu/AZMET/az-data.htm).

Villavaso, E.J. and G.L. Snodgrass. 2004. Diapause in tarnished plant bug (Heteroptera: Miridae) reared in dynamic photoperiod environmental cabinets. J. Agric. Urban Entomol. 21: 87–97.

Villavaso, E.J. and G.L. Snodgrass. 2005. Diapause in tarnished plant bug (Heteroptera: Miridae): Simulated seasonal occurrence in a Mississippi population. J. Entomol. Sci. 40: 10–15.