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# N O T E

## Diapause Response of *Lygus hesperus* (Hemiptera: Miridae) at Different Temperatures<sup>1</sup>

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The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is an economic pest of numerous western crops (Nordlund 2000, Southwest. Entomol. Suppl. 23: 1–5). This pest is known to enter a relatively short-duration adult reproductive diapause in response to short days (Beards and Strong 1966, Hilgardia 37: 345–362; Leigh 1966, J. Econ. Entomol. 59: 1280–1281; Strong et al. 1970, Hilgardia 40: 105–147). Recent work indicates the diapause response is subject to selection under laboratory culture (Spurgeon 2012, Environ. Entomol. 41: 415–419), and in some locations only a portion of the field population exhibits the diapause response (Brent and Spurgeon 2011, Environ. Entomol. 40: 455–461; Spurgeon and Brent 2015, J. Entomol. Sci. 50: 238–247). These reports, combined with observations of diapausing adults in the field during late winter and an apparent absence of a maternal effect on diapause induction prompted Spurgeon (2017, J. Insect Sci. 17: 125) to suggest that the overwintering strategy of *L. hesperus* likely involves more than one diapausing generation. For diapause induced in the mid to late fall, or in a second overwintering generation of *L. hesperus* in winter, the short-day conditions would coincide with markedly lower temperatures compared with the late-summer and early-fall diapause induction period. Temperature is a known modifier of the photoperiodic response for many insect species (Denlinger 1986, Annu. Rev. Entomol. 31: 239–264; Lees 1956, Annu. Rev. Entomol. 1: 1–16; Mansingh 1971, Can. Entomol. 103: 983–1009); therefore, it seems reasonable to examine whether the induction of diapause in *L. hesperus* is influenced by temperature.

Beards and Strong (1966, Hilgardia 37: 35–362) reported the incidence of diapause induced by a 10-h day length was not influenced by temperatures of 21 or 27°C but was reduced at 32°C. However, the ages at which they assessed

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diapause by dissection (30–73 d) far exceeded the ages at which the diapause characters are reliably exhibited (Spurgeon and Brent 2010, J. Entomol. Sci. 45: 303–316). Also, their criteria for distinguishing diapause were not the same for all temperatures. Finally, the day length they used to induce diapause was shorter than the day length eliciting the maximal diapause response (Spurgeon and Brent 2015, J. Entomol. Sci. 50: 238–247). Spurgeon and Brent (2015, J. Entomol. Sci. 50: 238–247) estimated that a day length of ~12 h would induce diapause in half of the *L. hesperus* capable of exhibiting the diapause response. Furthermore, Spurgeon (2017, J. Insect Sci. 17: 125) showed that the fourth instar was the critical stage responding to photoperiod. Therefore, three temperatures (21.1, 26.7, or 29.4°C) combined with a 12-h photophase were examined for their influence on the incidence of diapause. These temperatures were selected to represent the range of average daily temperatures for Central Arizona during much of September through November (<https://azclimate.asu.edu/arizona-monthly-climate-summaries/>).

Experimental insects originated from adult *L. hesperus* collected from alfalfa (*Medicago sativa* L.) near Maricopa, AZ. Eggs were obtained either from these adults, or from their F<sub>1</sub> progeny. The parent colonies were maintained at ~27°C with a 14:10 (L:D)-h photoperiod on pods of green bean (*Phaseolus vulgaris* L.) and raw seeds of sunflower (*Helianthus annuus* L.) as described by Spurgeon (2017, J. Insect Sci. 17: 125). Because the *L. hesperus* diapause rapidly terminates in response to long days (Spurgeon 2017, J. Insect Sci. 17: 125), the experimental insects were maintained under the 12-h photophase from the day of oviposition until diapause status was assessed by dissection of adults.

On the day of oviposition, green bean pods bearing eggs were transferred to Petri plates lined with filter paper and sealed with Parafilm (Pachiney Plastic Packaging, Chicago, IL). The plates were held within an environmental chamber (I30-BLL, Percival Scientific, Perry, IA) maintained at 26.7 ± 0.5°C. Upon hatching, 50 first instars were randomly assigned to each experimental temperature (21.1, 26.7, and 29.4°C, ± 0.5°C). Each nymph was confined separately within an 18-ml plastic vial (Thornton Plastics, Salt Lake City, UT) closed with a screened snap-cap lid and provisioned with a section of green bean pod (~5 cm long) sealed with paraffin on the cut ends. Bean sections were replaced three times weekly until each insect was dissected.

Because reliability of the characters used to distinguish diapause varies at different ages of assessment (Spurgeon and Brent 2010, J. Entomol. Sci. 45: 303–316), the insects assigned to different rearing temperatures were dissected at the same physiological age (10 d at 26.7°C). This was accomplished by transferring newly eclosed adults from the chambers at 21.1 and 29.4°C to the chamber at 26.7°C for 10 d before they were dissected. This approach was supported by results of a preliminary experiment in which the incidence of diapause was similar among adults reared at 21.1°C whether they were held at that temperature and dissected as 20-d-old adults, or were transferred to 26.7°C and dissected as 10-d-old adults (unpublished data). Dissections were accomplished under physiological saline (0.7% NaCl [w/v]) and diapause was indicated by a well-developed fat body and absence of vitellogenic oocytes (females) or poorly developed medial accessory glands (males) as described by Brent and Spurgeon (2011, Environ. Entomol. 40: 455–461). The experiment was conducted three times, each time assigning the experimental temperatures to different environmental chambers.

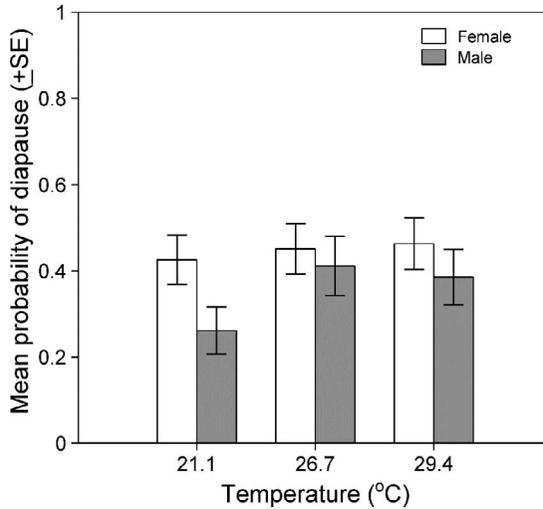
The estimated probability of diapause was compared among rearing temperatures by a generalized linear mixed model using a binomial distribution, events/trials syntax, and Laplace estimation (PROC GLIMMIX 2012, SAS Institute Inc., Cary, NC). Experimental temperature, bug gender, and their interaction were fixed effects, and repetition of the experiment was a random effect. Because the 50-insect cohort was the experimental unit for the fixed effect of temperature, the random effect of temperature  $\times$  repetition was included to serve as the error term for this effect. Residual was the error term for gender and the temperature  $\times$  gender interaction.

Of the initial 450 experimental insects assigned to the temperature treatments, 390 (86.7%) survived to be dissected as 10-d-old adults. Subsample sizes within each combination of temperature and experimental repetition ranged from 13 to 23 (mean = 19.2) for males and from 21 to 30 (mean = 24.1) for females.

Analyses of the diapause response did not indicate clear effects of rearing temperature ( $F = 1.42$ ;  $df = 2, 4$ ;  $P = 0.34$ ), bug gender ( $F = 3.69$ ;  $df = 1, 6$ ;  $P = 0.10$ ), or their interaction ( $F = 0.66$ ;  $df = 2, 6$ ;  $P = 0.55$ ; Fig. 1). Although the respective probabilities of diapause for male and female *L. hesperus* were not statistically different, males tended to exhibit a numerically lower incidence of diapause compared with females. This trend was consistent with observations in previous reports of *L. hesperus* diapause (Brent and Spurgeon 2011, Environ. Entomol. 40: 455–461; Spurgeon 2012, Environ. Entomol. 41: 415–419; Spurgeon and Brent 2015, J. Entomol. Sci. 50: 238–247). There was also an apparent trend for a lower incidence of diapause in males reared at 21.1°C compared with other temperatures. However, the relatively low mean diapause response by males at the lowest temperature was caused in part by an unusually low diapause response (21%) in the second experimental repetition at 21.1°C.

Where temperature during the induction period modifies the diapause response, it is usual for lower temperatures to result in increased diapause incidence compared with higher temperatures (Denlinger 1986, Annu. Rev. Entomol. 31: 239–264; Lees 1956, Annu. Rev. Entomol. 1: 1–16; Mansingh 1971, Can. Entomol. 103: 983–1009). Spurgeon and Brent (2015, J. Entomol. Sci. 50: 238–247) estimated the mean maximal incidence of diapause was ~51% for males and 80% for female *L. hesperus* from central Arizona. The mean ( $\pm$ SE) incidences of diapause in this study (males,  $0.35 \pm 0.04$ ; females,  $0.45 \pm 0.03$ ) were well below corresponding maximums. Therefore, the incidences of diapause observed in this study were not so high as to preclude modification of the diapause response by temperature.

It was expected that a temperature modification of the diapause response might be mediated through the numbers of photoperiodic cycles received by the critical stage, which is the fourth instar (Spurgeon 2017, J. Insect Sci. 17: 125). Based on estimated durations of the fourth stadium (Cooper and Spurgeon, J. Econ. Entomol. 105: 860–865), fourth instars in this study would have experienced five to six photoperiodic cycles at 21.1°C, two to four cycles at 26.7°C, and two to three cycles at 29.4°C. However, no response to temperature was observed. The lack of response to the lowest temperature (21.1°C) was consistent with the report by Beards and Strong (1966, Hilgardia 37: 35–362), but these authors also reported a reduction in the diapause response at 32°C. Although the highest temperature in this study was 29.4°C, there was no evidence of high-temperature modification of the diapause response. However, it is not possible to directly compare the results of



**Fig. 1. Mean probability ( $\pm$ SE) of diapause for *Lygus hesperus* reared to adulthood at different temperatures with a 12:12 (L:D)-h photoperiod and then held until 10 d of adult age at 26.7°C.**

the two studies because of methodological differences and because the experimental treatments by Beards and Strong (1966, *Hilgardia* 37: 35–362) were neither replicated nor statistically analyzed. Therefore, results reported herein indicate the photoperiodic diapause response of *L. hesperus* is largely temperature insensitive over the range of thermal conditions typical of early to late fall in central Arizona.

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