WESTERN TARNISHED PLANT BUG EGG DEVELOPMENT AND HATCH UNDER CONSTANT AND VARIABLE TEMPERATURES

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Abstract

The western tarnished plant bug (*Lygus hesperus*) is presently the most important pest of Arizona cotton. Cotton culture in the arid West is highly dependent on the availability of irrigation water. As water supplies become more limited, water conservation techniques such as deficit irrigation will likely result in at least periodically increased cotton canopy temperatures. The Pest Management and Biocontrol Research Unit at Maricopa, AZ is studying the molecular bases of thermal stress responses in Lygus in order to design molecular-based management tactics to capitalize on these microclimatic changes. However, currently available information on Lygus thermal ecology is limited to results of studies conducted using constant temperatures. We examined the development time and diel pattern of hatch of Lygus eggs at combinations of moderate and high temperatures, either held constant or exhibiting a variable thermoperiod. Development time was shorter under variable temperatures compared with constant temperatures in the moderate temperature treatment, but the difference was small. In contrast, development was faster at high constant temperatures compared with high variable temperatures. The diel pattern of egg hatch also differed between constant and variable regimes at both moderate and high temperatures. Results provide baseline information on the thermal ecology of Lygus eggs in response to a variable temperature environment, and suggest high temperatures represent an ecological vulnerability that may be exploited through transgenic or other molecular-based tactics.

Introduction

The western tarnished plant bug (*Lygus hesperus*) is presently the most important pest of Arizona cotton (Asiimwe et al. 2014). However, improved management (Ellsworth 1998) combined with availability of reduced risk insecticides (Anonymous 2013) have diminished this pest's economic impact. These accomplishments in management of Lygus bugs in Arizona provide an opportunity to develop ecologically-based management tactics in an environment minimally disrupted by pesticides. Coincident with reduced pest pressure in Arizona, limited availability and high costs of irrigation water are increasing concerns to western cotton producers. The prospects of increasingly limited water supplies have prompted emphasis on development of heat- and drought-tolerant cotton varieties (Ulloa at al. 2009) and alternative water management practices such as deficit irrigation (Mahan et al. 2012, Wanjura et al. 2004, Wen et al. 2013). Adoption of these cultural changes are likely to at least periodically change thermal characteristics of the cotton canopy (Carmo-Silva et al. 2012, Gonzalez-Dugo et al. 2006, Sui et al. 2012). Whether these changes will increase or diminish pest status will depend on the thermal ecology and the behavioral and physiological responses of the pests and their natural enemies.

Temperature-dependent development times of *L. hesperus* eggs were originally described using linear models (Champlain and Butler 1967, Butler and Wardecker 1971). More recently temperature-dependence of *L. hesperus* egg development rate was described using a non-linear model that accounted for developmental inhibition at low and high temperatures (Cooper and Spurgeon 2013). However, these previous reports were based on constant temperature conditions, and their relevance to more natural, variable temperature conditions has not been examined. This is particularly interesting because Hagstrum and Milliken (1991) cited numerous cases in which insect development under variable temperatures was poorly predicted by estimates of development under constant temperatures. Our objective was to compare *L. hesperus* egg development and the diel pattern of hatch under constant temperatures with those under corresponding variable temperature regimes that are ecologically meaningful.

Materials and Methods

Eggs for the study were obtained from Lygus adults collected from local fields of alfalfa (*Medicago sativa*) and reared in the laboratory for ≤3 generations. Separate cohorts of eggs were reared in environmental chambers under a 14:10 [L:D] hour photoperiod and one of four different temperature regimes: 1) constant 22°C (moderate constant

regime), 2) constant 29°C (high constant regime), 3) 14 to 30°C (moderate variable regime), or 4) 21 to 37°C (high variable regime). In each variable temperature regime the low temperature in the range was maintained from 0200–0600 h, increased to the high temperature by 1600 h and held at that temperature until 2000 h, and decreased to the low temperature by 0200h. The mean daily temperature of each variable regime corresponded to one of the constant temperature regimes. The temperatures used in the high variable regime were intended to be roughly similar to cotton canopy temperatures in response to limited irrigation during bloom on the Southern High Plains of Texas (Wanjura et al. 2004), in late August at Stoneville, MS (Sui et al. 2012), or during late-July and August in central Arizona (Carmo-Silva et al. 2012). The medium variable regime used the same temperature amplitude as the high variable regime (±8°C), and was intended to correspond to the linear portion of the development rate curve reported by Cooper and Spurgeon (2013).

Egg Development Time

When adults in the laboratory colony were 8–11 d old, females were aspirated individually into 18.5-ml plastic vials. Each female was examined for a recent mating based on observation of a spermatophore through the ventral abdominal cuticle (Cooper 2012). Mated females were confined with a section of green bean pod for oviposition substrate. Following a 6–8-h oviposition period, each bean section was examined for eggs, which were numbered on the bean pod using a permanent pen. The number of eggs monitored on any bean section was limited to five. Previous experience (W.R. Cooper, unpublished data) has suggested more variation in development time among eggs laid by different females than among eggs laid by a single female. Limiting the number of eggs contributed by any single female was intended to minimize the potential bias in egg development time caused by the more fecund individuals. Within an experimental repetition, a total of 50 eggs were monitored in each temperature regime.

Eggs were examined daily for development until extension of the operculum (Cooper and Spurgeon 2012), after which they were observed twice daily (morning and late afternoon) for hatch. Hatch was signified by dislocation of the operculum and extension of the serosal cuticle. Newly hatched nymphs were removed from the vials at each observation to avoid the potential for egg predation. The experiment was conducted three times.

Periodicity of Hatch

Diel periodicity of egg hatch was examined using the same temperature treatments and regimes as for egg development time. Eggs were obtained by exposing twelve green bean pods to the stock adult colony for 18–24 h. Three whole pods containing eggs were randomly assigned to each of the experimental treatments. The three pods assigned to each environmental chamber were held as a group within a 3.7-L plastic rearing bucket closed with a solid lid to maintain humidity. Condensation was removed from the inside of each bucket daily by blotting, which minimized deterioration of the beans from fungal growth. Beginning on the day before anticipated first hatch (day 5, high constant; day 6, high variable; day 8, moderate constant and variable) the buckets were inspected daily for newly hatched nymphs at 0700, 1300, and 1900 h until 24 hours after the last observed hatch. Three separate repetitions of the experiment were conducted.

Statistical Analyses

Development time was compared among the four temperature regimes using mixed-model ANOVA with heterogeneous variances (the GLIMMIX procedure; SAS Institute 2012). The model contained fixed effects of temperature (moderate, high), regime (constant, variable), and their interaction. Random effects included repetition of the experiment and the repetition*temperature*regime interaction which was used as the error term for tests of fixed effects. Degrees of freedom were corrected using the DDFM=KR option of the model statement and separate variances were obtained for different temperatures using the GROUP= option of the RANDOM _RESIDUAL_ statement.

The diel pattern of egg hatch was compared between regimes within each temperature. Because the number of observation periods over which eggs hatched varied among combinations of treatment and experimental repetition, the center nine observation periods (three days) for each experimental repetition within each temperature treatment were overlaid. Differences between patterns of hatch between temperature regimes were examined in contingency tables that controlled for experimental repetition using the Cochran-Mantel-Haenszel test of general association (Q_{GMH}; PROC FREQ, SAS Institute 2012). Although analyses used the observed counts without correction for duration of each period (the overnight period was 12 h compared with 6 h for other periods), results are displayed on the basis of the frequency of egg hatch per 6 h.

Results and Discussion

Egg development time varied between temperatures (F = 890.02; df = 1, 5.82; P < 0.01) and regimes (F = 25.45; df = 1, 4.67; P < 0.01). However, the temperature*regime interaction (F = 99.95; df = 1, 4.68; P < 0.01) indicated the effect of regime was not the same for both temperatures. Tests of simple effects indicated development time was shorter under the high temperatures compared with the moderate temperatures for both regimes (constant, F = 875.7; df = 1, 5.29; P < 0.01; variable, F = 291.4; df = 1, 5.34; P < 0.01). However, the influence of temperature regime differed between the moderate and high temperatures (Fig. 1). Development time for the variable regime was shorter than for the constant regime in the moderate temperature treatment (F = 11.69; df = 1, 5.18; P = 0.02) although the difference was small (≈0.4 d), whereas the opposite was true for the high temperature treatment (F = 119.0; df = 1, 4.19; P < 0.01) where the difference was ≈1.2 d.

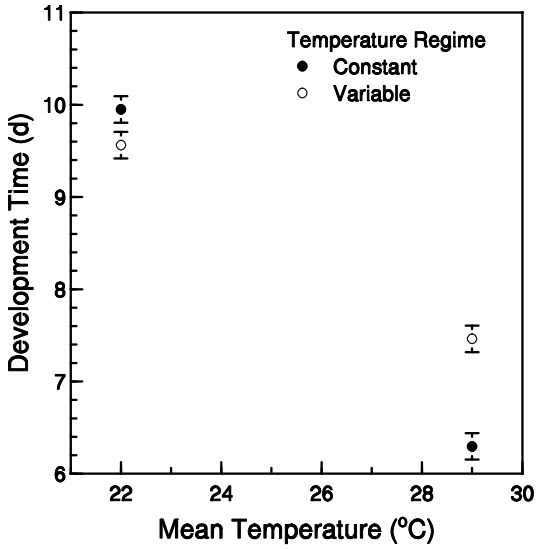

Fig. 1. Mean development time (day, ±SE) of *L. hesperus* eggs under moderate (22°C) and high (29°C) constant and variable temperature regimes. N = 521 eggs, which represents 87% survival to hatch.

These results are generally consistent with the conclusions of Hagstrum and Milliken (1991), who reported development times at constant temperatures above 25–30°C tend to be shorter than under variable temperatures with the same daily mean temperatures whereas the opposite is observed at lower mean daily temperatures. Although statistically significant, the difference in development time between temperature regimes under moderate temperatures in this study was small. A large difference was not expected because most of the temperature range encompassed by the variable regime is within the approximately linear portion of the non-linear development rate curve estimated by Cooper and Spurgeon (2013). In contrast, the difference between regimes under high temperatures was much greater. Although the mean daily temperature of both constant and variable high temperature regimes was well below the optimum temperature for *L. hesperus* egg development estimated by Cooper and Spurgeon (2013), the highest temperatures in the variable regime were above those where egg development to hatch has been observed, and approached a temperature known to disrupt normal egg development (Cooper and Spurgeon 2013). This suggests the developmental penalty imposed by the variable regime at the high temperature, compared with the constant regime, was caused by periodic inhibition of egg development during the warmer portions of the daily temperature cycle. Because of the observed influence of the high, variable temperature regime on egg development time, it became interesting whether these effects would be associated with an altered diel periodicity of egg hatch. Spurgeon and Mueller (1992) reported such an alteration in the periodicity of nymphal molting for the threecornered alfalfa hopper (*Spissistilus festinus* Say) in response to high temperatures in field-grown soybean.

Contingency tables indicated differences between constant and variable temperature regimes in the periodicity of egg hatch for both moderate (Q_{GMH} = 148.0, df = 8, P < 0.01) and high temperatures (Q_{GMH} = 74.1, df = 8, P < 0.01; Fig. 2). The moderate constant temperature regime resulted in a roughly bell-shaped frequency distribution of egg hatch over the 3-d hatch period (Fig. 2a). In contrast, the frequency distribution of the corresponding variable

temperature regime was punctuated with fluctuations caused by increased hatch frequency at the 1300- or 1900-h observations, and decreased hatch at the 0700-h observation. In contrast, under the high temperature the frequency of egg hatch under constant and variable regimes appeared similar except for the conspicuous absence of hatch observed at 1900 h in the variable regime (Fig. 2b). In the high variable temperature regime, the minimum temperature between 1300–1900 h was always above 32°C, which is within the temperature range producing high-temperature inhibition of development (Cooper and Spurgeon 2013). Therefore, it appears that the eggs of *L. hesperus* can avoid hatch under adverse, high-temperature conditions whether through direct thermal inhibition of development or through some indirect sensory mechanism.

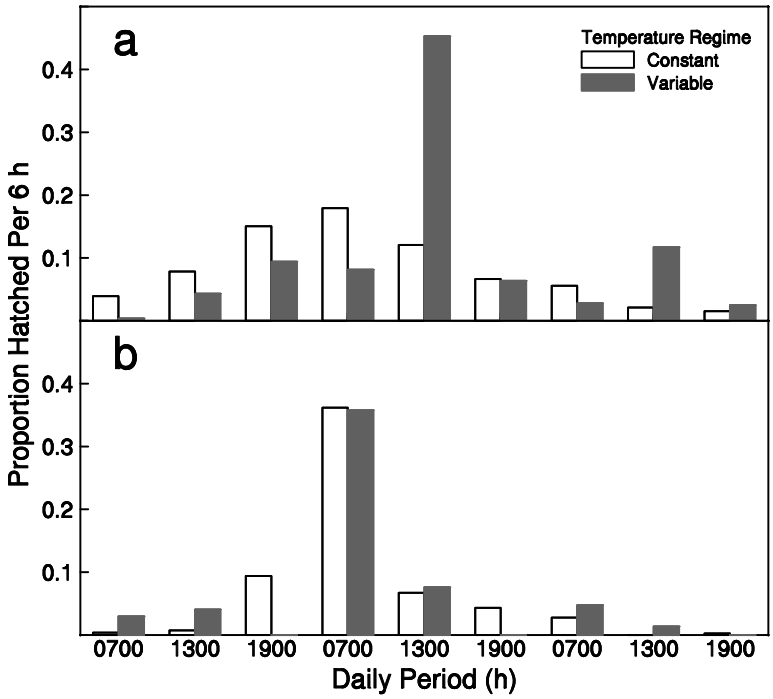

Fig. 2. Diel periodicity in *L. hesperus* egg hatch under moderate (mean, 22°C) and high (mean, 29°C) constant and variable temperature regimes. Amplitude of variable temperature regimes was ±8°C. *N* ranged from 332 nymphs (moderate constant temperature) to 416 nymphs (high constant temperature).

Conclusions

Our results show that estimates of *L. hesperus* egg development under constant temperatures, although valuable as baseline information, do not adequately represent development time in a variable temperature environment more typical of field conditions. In general, our observations were consistent with those of Hagstrum and Milliken (1991), that the divergence in estimates of development time are more pronounced at higher temperatures compared with more moderate temperatures. Continued investigation of *L. hesperus* development in variable temperature environments could result in improved ability to predict development and population phenology under field conditions. Whether the observed difference in development time between constant and variable temperature regimes under moderate temperatures was caused solely by thermally-moderated differences in development rate is not known. Corresponding differences in the diel periodicity of hatch would influence estimates of development time, which were small between regimes under moderate temperatures. Similar confounding influences may have occurred under the high temperature regimes, but the differences observed under high temperatures were too large to be explained by differing diel patterns of hatch. Regardless, these results expand our knowledge of *L. hesperus* thermal ecology and our findings may find application in current efforts of the Pest Management and Biocontrol Unit to identify and manipulate physiological or genetic mechanisms that *L. hesperus* employ to cope with a harsh thermal environment.

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