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# ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

## Non-Destructive Detection of Diapause in the Western Tarnished Plant Bug, *Lygus hesperus* Knight (Hemiptera: Miridae)

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### ABSTRACT

**The western tarnished plant bug, *Lygus hesperus* Knight, overwinters in an adult diapause. However, the ecological implications of diapause to *L. hesperus* population survival are poorly understood. Enhanced understanding of *L. hesperus* overwintering ecology might reveal opportunities to develop ecologically-based management tactics. One factor limiting efforts to understand the dynamics of overwintering is lack of a non-destructive method to distinguish diapause. We evaluated a suite of external characters for utility in distinguishing diapausing from non-diapausing *L. hesperus*. Abdomen coloration of female *L. hesperus* was a highly reliable indicator of diapause status. During these studies, a single female (0.6%) was misclassified using the abdomen color criterion. For male *L. hesperus*, corrected abdomen length (abdomen length / head capsule width) was used to predict diapause status with ≈84% accuracy. Both criteria provided improved accuracy compared with earlier reports, in part because their application was limited to adults of a specific age (10 d) and reared at a specific temperature (26.7°C). Application of these criteria to individuals that were subsequently starved allowed us to unambiguously distinguish the survival functions of diapausing and non-diapausing males and females. Although neither criterion (abdomen color for females, corrected abdomen length for males) was error-free, both offer sufficient accuracy to justify their use in studies of the ecology, physiology, or molecular biology of *L. hesperus* diapause. These criteria provide the ability to non-destructively distinguish the diapause status of adult *L. hesperus* with reasonable accuracy, and should enhance efforts to better understand diapause and overwintering ecology in this important pest species.**

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The western tarnished plant bug, *Lygus hesperus* Knight, is a key pest of western crops including cotton (*Gossypium* spp.). Despite the economic importance of this pest, several aspects of its basic biology remain poorly investigated, including the ecology of overwintering. *Lygus hesperus* is considered to overwinter as diapausing adults in response to short days (Beards and Strong, 1966; Leigh, 1966). Diapause in southern populations is thought to terminate in late-fall to early-winter (Beards and Strong, 1966; Strong et al., 1970), but the mechanism facilitating population survival from early-winter until the availability of spring-time hosts has been a matter of conjecture.

In Arizona, only a portion of the *L. hesperus* population exhibits the short-day diapause response (Spurgeon and Brent, 2015), and the ecological fate of the reproductive (non-diapausing) insects is unknown. Cooper and Spurgeon (2012, 2013, 2015) found the durations of all *L. hesperus* life-stages were substantially extended by low temperatures. These findings suggest the potential for a portion of southern *L. hesperus* populations to overwinter as slow-developing eggs, nymphs, and reproductive adults. There is also potential for a second generation of diapausing adults. Brent (2012) illustrated a color phenotype associated with diapause in adult *L. hesperus*, and this phenotype has been observed in alfalfa (*Medicago sativa* L.) in late-winter (Spurgeon, 2018). Studies of diapause induction in the laboratory, using $F_1$ progeny of field adults collected throughout the year, have not exhibited seasonal patterns (Spurgeon and Brent, 2015; Spurgeon, 2017), which implies absence of a maternal effect on diapause induction. These observations prompted Spurgeon (2017) to suggest that some *L. hesperus* developing during winter and early spring may exhibit the same diapause response as insects developing in late-summer and fall. Collectively, these reports suggest a more dynamic overwintering ecology for *L. hesperus* than is generally recognized. Better understanding of *L. hesperus* overwintering ecology will require improved knowledge of the timing and duration of adult diapause, as well as its ecological impact on population survival.

Ecologically relevant estimates of host-free longevity of diapausing *L. hesperus* are not available. Brent et al. (2013) reported the host-free longevity of diapausing *L. hesperus* adults was about three times that of non-diapausing adults. However, the insects were obtained from an established colony known to exhibit a reduced diapause response (Brent and Spurgeon, 2011), and the starved adults were not provided a source of moisture. The importance of moisture to overwintering success of *L. hesperus* was previously reported by Fye (1982). Consequently, the median survival of diapausing adults observed by Brent et al. (2013) was <200 h at 28°C. In contrast, Cooper and Spurgeon (2015) provided newly-eclosed, unfed, non-diapausing adults a source of moisture and observed median host-free longevities of 6.1 (males) to 8.8 d (females) at 26.7°C. Therefore, improved estimates of host-free longevity of diapausing *L. hesperus* might be obtained in experiments where the insects are provided a moisture source.

A major impediment to accurate assessment of host-free longevity is the inability to distinguish diapausing from non-diapausing individuals at the beginning of the survival period. The diapause condition in *L. hesperus* is usually determined by dissection, which precludes subsequent observations of the same individuals. Although estimates of the incidence of diapause based on a dissected subsample of each survival cohort are useful, this approach increases necessary sample sizes and introduces the potential for sampling error in the diapause estimate. A reliable non-destructive indicator of diapause would decrease the cost of the research while improving the accuracy of survival estimates.

Brent (2012) reported that diapausing *L. hesperus* could be distinguished from reproductive bugs by abdominal coloration, with average accuracies of 84% for females and 67% for males. Spurgeon and Cooper (2012) used abdomen color to predict reproductive status of adult *L. hesperus*, but error rates of those predictions were generally high and varied markedly with rearing temperature. In both of these studies, diapause or reproductive status was assessed at a variety of adult ages. However, Spurgeon and Brent (2010) recommended diapause assessments at adult ages ≥10 d at 26.6°C. In addition, fat body hypertrophy associated with diapause typically causes the dorsal plate of the male's abdomen to be less flattened, and the intersegmental membranes to be more prominent, compared with reproductive males (Fig. 1). However, these characters are difficult to observe from the ventral aspect, and they are obscured by the wings from the dorsal aspect. Displacement of the wings to allow observation from the dorsal aspect often results in injury to the insect. We hypothesized that the accuracy of color-based diapause assessments might be improved if temperature conditions and adult ages were narrowly restricted, and that compared with color classes, measurements of the abdomen taken from the ventral aspect might provide less subjective criteria for distinguishing diapause status of *L. hesperus* adults.

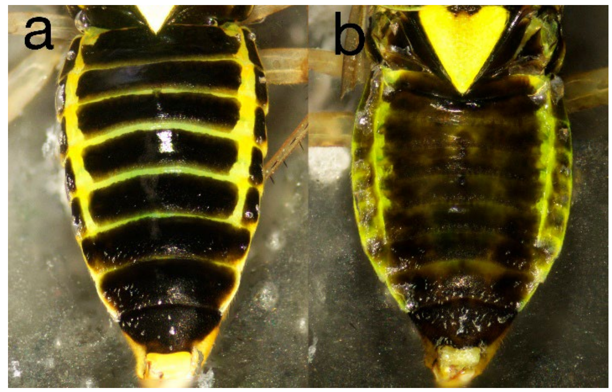


**Fig. 1. Illustration of abdomen distension caused by fat body hypertrophy in a diapausing male *L. hesperus* (a) compared with a reproductive male lacking fat body hypertrophy (b).**

## MATERIALS AND METHODS

We evaluated external morphological characters as indicators of diapause in three separate experiments: 1) an initial experiment to determine the most promising characters for further evaluation, 2) a survival experiment to determine whether application of the initial diagnostic rules were related to host-free survival, and, whether those rules could be modified to improve accuracy of classifications, and 3) a validation experiment utilizing an independent observer and small sample sizes typical of laboratory studies. Each repetition of each experiment used the $F_1$ progeny of about 500 *L. hesperus* adults collected from fields of alfalfa near Maricopa, AZ. Field-collected adults were maintained at 27±1°C with a 14:10 (L:D) h photoperiod within 0.03-$m^3$ screened cages. Cages were provisioned with shredded paper, pods of green bean (*Phaseolus vulgaris* L.), raw seeds of sunflower (*Helianthus annuus* L.), and a water-saturated cotton pad. The bean pods, which also served as oviposition substrate, were replaced three times weekly. Bean pods containing eggs were held on shredded paper within 3.8-L plastic buckets with screened lids under the same conditions as the parent adults.

Experimental insects were obtained as newly-hatched first instars which were held individually within 18-ml snap-cap vials (Thornton Plastics, Salt Lake city, UT). Vial caps were ventilated with nylon organdy. Each insect was provided a section of green bean pod (≈5 cm long) that was replaced three times weekly. Cut ends of the pod sections were sealed with paraffin to prevent entry by the early instars. Insects in all phases of each experiment were held at 26.7±0.2°C with a photoperiod of 10:14 (L:D) h. Nymphs were examined three times weekly until they developed to fifth instar, after which they were examined for adult eclosion at least once daily. Adults remained within the vials and were fed three times weekly until they were dissected or assigned to a survival cohort as 10-d-old adults.

**Initial character evaluation.** Each of the six repetitions of the experiment included 30–33 nymphs, which, after mortality during development, yielded a total of 152 adults (72 males, 80 females). Initial criteria evaluated for association with diapause included abdomen color, length, and width. Head capsule width was also measured in case it was correlated with other measurements and therefore was needed as a correction factor. At 10 d of adult age, each insect was confined within a sealable plastic bag while it was photographed, ventral side up, at 45× using an Olympus DP-SAL camera system mounted on a SZ-61 dissecting microscope (Olympus America, Center Valley, PA). Because the focal plane differed for each measurement (head capsule width, abdomen length, abdomen width), three separate pictures of each insect were recorded. Measurements were obtained using firmware of the DP-SAL system. Head capsule and abdomen widths were obtained using a "parallel lines" function to ensure width was measured perpendicular to the long axis of the body. Abdomen length was measured from as closely as possible to the posterior margin of the first abdominal sternite to the posterior tip of the abdomen. This was necessary because the coxae obscured the anterior margin of the abdomen. Abdomen width was measured at its widest dimension, which was the third or fourth abdominal segment. Abdomen color was classed as "green" (Fig. 2a, 3a) or "not green" (Fig. 2b-d, 3b-d). When the male ventral abdomen was substantially melanized (Fig. 3b), classification was based on the color of unmelanized areas or the intersegmental membranes.

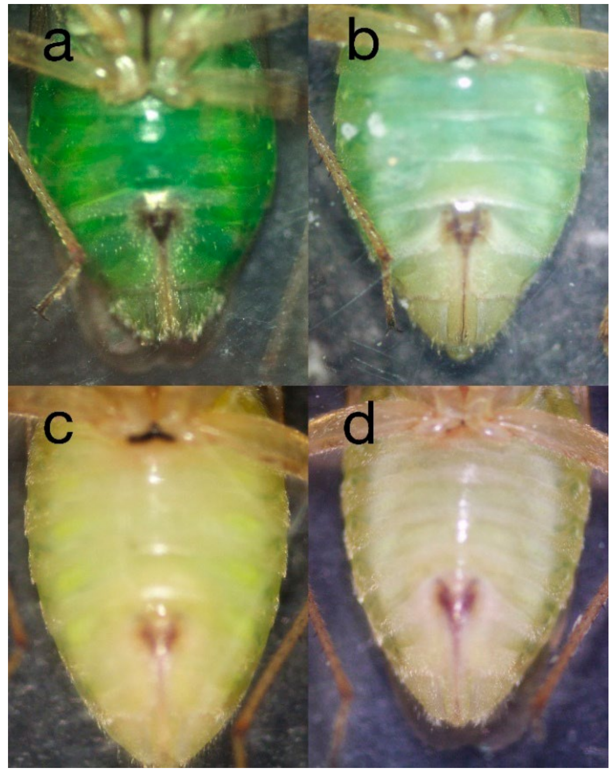


**Fig. 2. Color classifications of 10-d-old female *L. hesperus* representing "green" (classified as non-diapause; a) and "not green" (classified as diapause; b–d).**

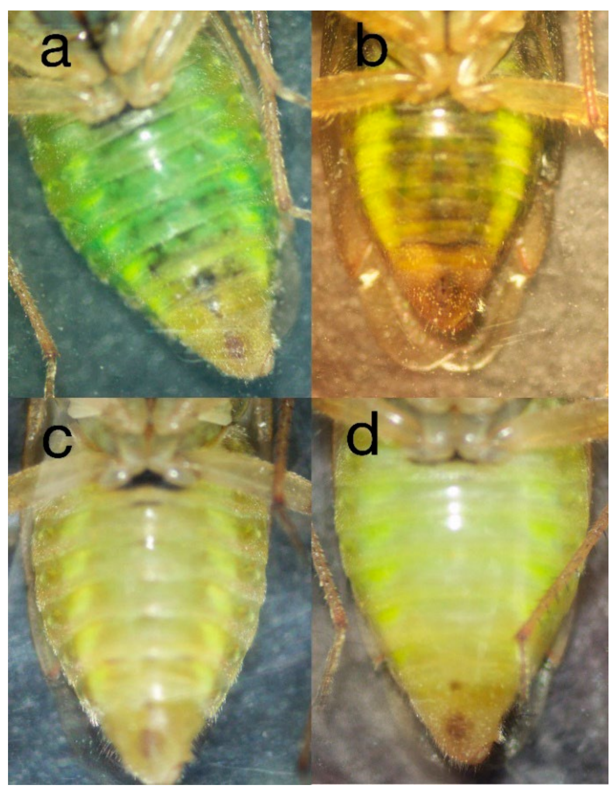


**Fig. 3. Color classifications of 10-d-old male *L. hesperus* representing "green" (classified as non-diapause; a) and "not green" (classified as diapause; b–d).**

Adults were dissected under 0.7% (w/v) saline immediately after they were photographed. Diapause was indicated by hypertrophy of the fat body and lack of vitellogenic oocytes and follicular relics (females), or presence of poorly developed medial accessory glands (males), as described by Brent and Spurgeon (2011). Females exhibiting vitellogenesis, or males with accessory glands exhibiting white contents within a basal section that was obviously elongated, were classified as reproductive irrespective of fat body condition.

All statistical analyses were conducted using SAS (SAS Institute, 2012). Preliminary analyses indicated significant correlations between head capsule width and abdomen length, or width, for some combinations of bug gender and diapause status. Therefore, subsequent analyses used head capsule-corrected measures (abdomen length or width divided by head capsule width). These unitless measures are henceforth referred to as corrected abdomen measurements. Each criterion (abdomen color, corrected abdomen width, corrected abdomen length) was examined for a relationship to diapause status using logistic regression (PROC LOGISTIC). Separate analyses were conducted for each sex. In each analysis, diapause status was the response variable and the selected morphological criterion was the independent variable. Significance of each regression was assessed by the likelihood ratio statistic (Stokes et al., 2012). The logistic regressions of color for both sexes used the Firth penalized likelihood because of quasi-complete separation (no individuals rated as "green" were diapausing). The criterion for each bug gender that most accurately predicted diapause status was then selected for further study. For males, the profile likelihood 95% confidence intervals were calculated for corrected abdomen lengths from 1.9 to 3.0 using increments of 0.1. Accuracy of male diapause status predictions was estimated using contingency tables (PROC FREQ) with diapause status from dissections as rows, and corrected abdomen length classes from 2.1 to 2.8 at intervals of 0.1 as columns.

**Host-free survival.** A cohort of 50 first-instars was established in each of two experimental repetitions, from which a total of 94 10-d-old adults (47 females, 47 males) was obtained for the survival assessments. On the tenth day of adulthood each insect was photographed as previously described. Females were classified according to abdominal color (Fig. 2) whereas corrected abdomen length was calculated for each male. Based on estimated error rates for corrected abdomen length in the initial experiment, males were classified as non-diapausing if the corrected abdomen length was <2.5, or as diapausing if corrected abdomen length was >2.5. After classification each insect was returned to the rearing vial, and the green bean section was replaced with a water-saturated cotton ball wrapped in tightly stretched Parafilm M (Pechiney Plastic Packaging, Chicago, IL). The Parafilm sachets, which were ~1.5 cm in diameter, were replaced at 10-14 d intervals. Survival was monitored daily until all adults had died.

Corrected abdomen length of predicted diapausing males was regressed against longevity (PROC REG) to examine the residuals for patterns indicating errors in classification. An identical regression was fitted for predicted reproductive males. Based on these residuals, the criterion for corrected abdomen length was modified, and both regressions were fitted again. Using color classifications for females and the refined corrected abdomen length for males, survival functions were compared among the combinations of bug gender and diapause status (PROC LIFETEST) using experimental repetitions as strata (a blocking variable). Differences in survival functions were assessed based on the Wilcoxon $\chi^2$ statistic, and the *p*-values corresponding to pairwise comparisons were adjusted for multiplicity (the ADJUST=SIMULATE option).

**Diapause criterion validation.** A validation experiment using an independent observer and small samples typical of laboratory diapause experiments was conducted. In each of two experimental repetitions, 30 first instars were individually confined in vials as previously described. A total of 57 10-d-old adults (32 females, 25 males) was obtained for diapause classification. On the tenth day of adulthood the independent observer photographed each insect and assigned a predicted diapause status. Abdomen color was used to distinguish diapause in females, and a corrected abdomen length of 2.43 was used as the cut-point for separating diapausing from non-diapausing males. This cut-point represented a refinement of the previous cut-point (2.5), and was derived from results of the survival study. The insects were dissected to determine diapause status immediately after they were photographed. For each sex, association between predicted diapause status and diapause status determined by dissection was examined in contingency tables (PROC FREQ) using the Mantel-Haenszel nonzero correlation statistic ($Q_{CSMH}$, Stokes et al., 2012). In both analyses the tables were stratified (blocked) by experimental repetition. In addition, odds ratios and their 95% confidence limits were calculated.

## RESULTS

**Initial character evaluation.** Regressions relating adult size (head capsule width) to abdomen measurements provided variable results. The relationship between head capsule width and abdomen length for reproductive females was not significant ($F = 0.19$; df = 1, 39; $P = 0.67$). Lack of this relationship was caused primarily by the large variation in abdomen lengths among reproductive females with similar head capsule widths. Head capsule width and abdomen width were positively related ($F = 5.35$; df = 1, 39; $P = 0.03$) for reproductive females. For diapausing females, both abdomen length ($F = 16.15$; df = 1, 37; $P < 0.01$) and abdomen width ($F = 8.98$; df = 1, 37; $P < 0.01$) were positively related to head capsule width. For reproductive males, neither abdomen length ($F = 2.64$; df = 1,27; $P = 0.12$) nor abdomen width ($F = 2.46$; df = 1, 27; $P = 0.13$) were related to head capsule size. However, both abdomen measurements were positively related to head capsule size for males in diapause (abdomen length, $F = 8.45$; df = 1, 41; $P < 0.01$; abdomen width, $F = 11.44$; df = 1, 41; $P < 0.01$). As for the females, lack of a demonstrable relationship between head capsule width and abdomen measurements of reproductive males was caused by the variation in abdomen size among reproductive males with similar head capsule widths. However, lack of a statistical relationship at $\alpha = 0.05$ cannot be interpreted to mean that the independent variable (head capsule size) does not represent an important source of variation (Montgomery et al., 2012). This consideration, combined with the demonstrated relationships between head capsule width and abdomen measurements for other combinations of insect gender and diapause status, justified the use of the head capsule correction. Without such correction, the predicted diapause status would simply indicate insect size, with larger insects classed as diapausing and smaller insects classed as non-diapausing.

Logistic regressions of relationships between diapause status and either corrected abdomen length or corrected width were significant for females (length, LR $\chi^2 = 63.46$, df = 1, $P < 0.01$, $R^2 = 0.548$; width, LR $\chi^2 = 26.63$, df = 1, $P < 0.01$, $R^2 = 0.283$), but predictive power indicated by the $R^2$ values was relatively low. However, penalized likelihood analysis of abdomen color (LR $\chi^2 = 106.21$, df = 1, $P < 0.01$, $R^2 = 0.735$) was also significant, and no individuals were misclassified by this criterion. The analysis estimated the probability (±SE) of diapause for a female classified as "not green" was 0.994 (± 0.009). Therefore, remaining experiments used the criterion of abdomen color to distinguish diapausing from non-diapausing females.

The logistic regression relating male corrected abdomen width with diapause status was not significant (LR $\chi^2 = 3.76$, df = 1, $P = 0.053$, $R^2 = 0.051$). Although abdomen color was related to male diapause status (LR $\chi^2 = 34.36$, df = 1, $P < 0.01$) only 62.5% of observations were concordant and the $R^2$ (0.380) was relatively low. In comparison, corrected abdomen length (LR $\chi^2 = 69.47$, df = 1, $P < 0.01$, $R^2 = 0.619$) provided better prediction accuracy and 96.6% of observations were concordant. This latter logistic regression (Fig. 4) indicated classification errors should be minimized between corrected abdomen lengths of about 2.4 and 2.5. Calculated error rates at 0.1-intervals of corrected abdomen length indicated the observed rate of misclassification of diapause status was about 5.8% at a corrected abdomen length of 2.5 (Fig. 5). At that corrected abdomen length two diapausing males (4.6%) were misclassified as reproductive, and two reproductive males (6.9%) were misclassified as diapausing. All misclassified males exhibited hypertrophied fat bodies, but the reproductive males misclassified as diapausing also had developing medial accessory glands.

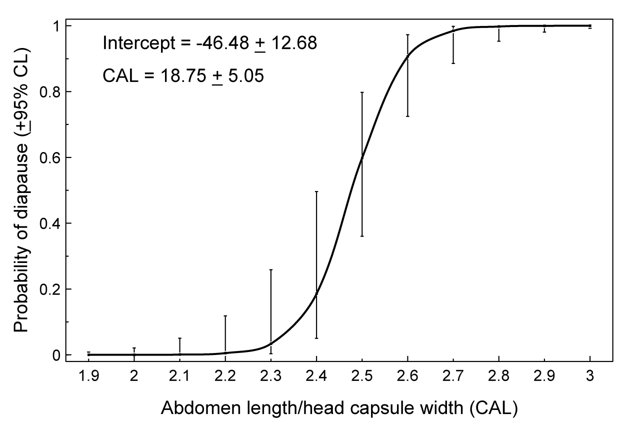


**Fig. 4. Probability of diapause (±95% CL) based on logistic regression of diapause status on corrected abdomen length (CAL, abdomen length/head capsule width) for 10-d-old adult male *Lygus hesperus* reared at 26.7±0.2°C with a 10:14 (L:D) h photoperiod.**

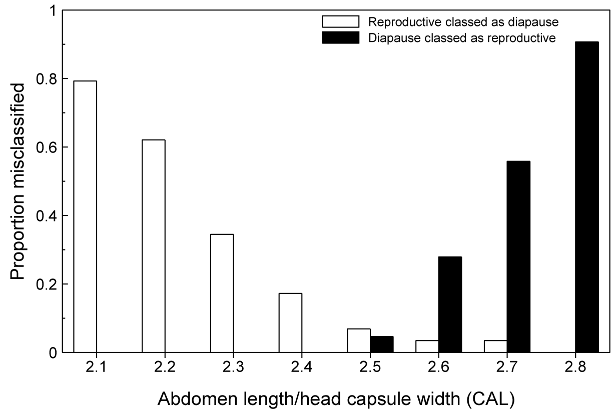


**Fig. 5. Errors in diapause classification based on corrected abdomen length (CAL, abdomen length/head capsule width) for 10-d-old adult male *Lygus hesperus* reared at 26.7±0.2°C with a 10:14 (L:D) h photoperiod.**

**Host-free survival.** The simple linear regression between corrected abdomen length and host-free longevity for males classed as non-diapausing (corrected abdomen length < 2.5) was significant ($F$ = 25.58; df = 1, 22; $P$ < 0.01), but the residuals displayed a distinct pattern suggesting an oversimplified model (Fig. 6a). In contrast, the regression for predicted diapausing males indicated no relationship between host-free longevity and corrected abdomen length ($F$ = 0.87; df = 1, 21; $P$ = 0.36; Fig. 6a). The observations responsible for the pattern in the residuals of predicted non-diapausing males all occurred near the abdomen length cut-off used to distinguish them from diapausing males. This suggested lack of a term for diapause (or inappropriate inclusion of diapausing males) in the model was responsible for the pattern in the residuals. When the corrected abdomen length criterion was reduced from 2.5 to 2.43, the relationship between longevity and corrected abdomen length for predicted non-diapausing males remained significant ($F$ = 5.09; df = 1, 17; $P$ = 0.04) but the pattern in the residuals was eliminated (Fig. 6b). The recalculated regression for males classed as diapausing remained non-significant ($F$ = 3.67; df = 1, 26; $P$ = 0.07) and the residuals did not display an interpretable pattern (Fig. 6b). Therefore, the refined criterion for distinguishing non-diapausing from diapausing males was used to classify male diapause status in comparisons of survival functions.

The stratified tests of equality indicated differences among the survival functions of non-diapausing and diapausing males and females (Wilcoxon $\chi^2$ = 115.25, df = 2, $P$ < 0.01). Pairwise comparisons did not indicate a difference between the survival functions of non-diapausing males compared with non-diapausing females after adjustment for multiplicity (adjusted-$P$ = 0.06; Fig. 7). Each of the remaining pairwise comparisons indicated a difference (adjusted-$P \leq 0.01$), with diapausing males surviving longer compared with non-diapausing males, and diapausing females surviving longer compared with non-diapausing females or diapausing males (Fig. 7). Although differences in the survival functions of diapausing and non-diapausing adults were easily distinguished, a single female classified as non-diapausing survived for 41 d, and was clearly a classification error. Therefore, one of the 47 females monitored for survival was misclassified.

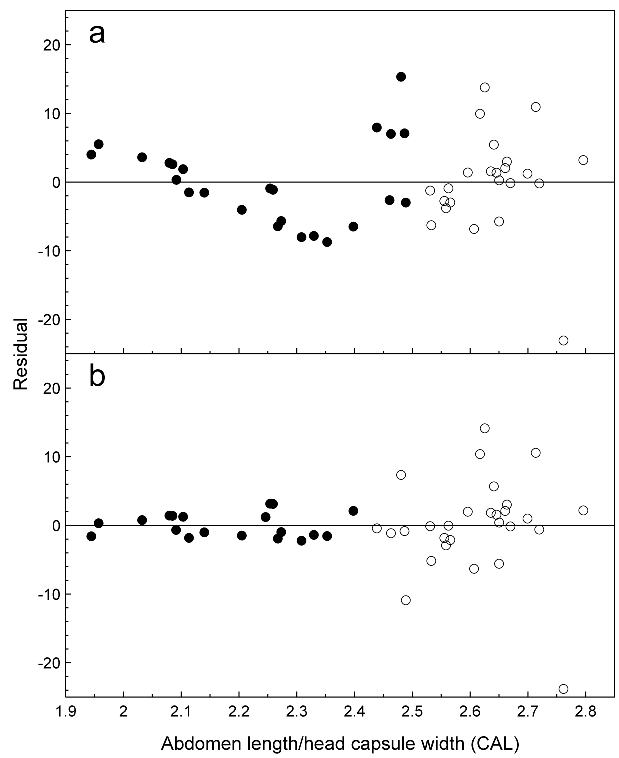


**Fig. 6. Residuals from simple linear regressions of corrected abdomen length (abdomen length/head capsule width; CAL) against host-free longevity for male *Lygus hesperus* classified as non-diapausing (•) or diapausing (○) using CAL=2.5 (a) or CAL=2.43 (b) to distinguish non-diapausing from diapausing males.**

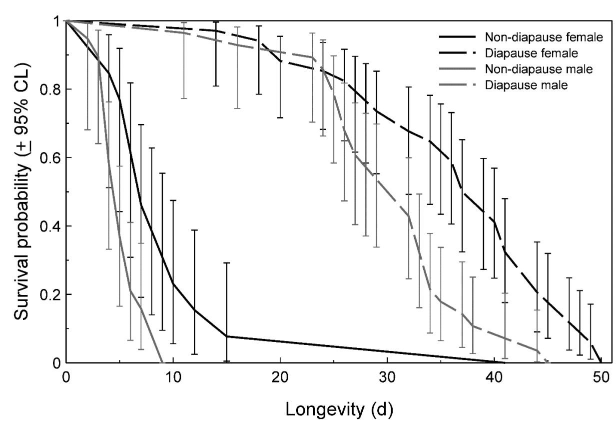


**Fig. 7. Host-free survival functions (±95% CL) for *Lygus hesperus* adults non-destructively classified at 10 d of age as non-diapausing or diapausing. Males with corrected abdomen length (abdomen length/head capsule width) > 2.43, and females with a "not green" abdomen coloration were classified as diapausing.**

**Diapause criterion validation.** The independent observer correctly assigned diapause status to all 32 females on the basis of abdomen color, and the Mantel-Haenszel non-zero correlation statistic reflected this high level of accuracy ($Q_{CSMH}$ = 17.00, df = 1, P < 0.01). The odds-ratio indicated a female with a "non-green" abdomen was 325× (95% CL, 5.76 – 18338) more likely to be in diapause compared with a female exhibiting the green abdomen coloration. Male classification based on corrected abdomen length was also associated with diapause classification based on dissection ($Q_{CSMH}$ = 11.41, df = 1, $P$ < 0.01). Males with corrected abdomen lengths >2.43 were 23.7× (95% CL, 2.3 – 239.9) more likely to be in diapause compared with males whose corrected abdomen length was <2.43. Using this corrected abdomen length cut-off, 21 of 25 males were correctly classified (84%). All of the diapausing males were correctly classified, but four of 18 reproductive males (22%) were misclassified as diapausing.

## DISCUSSION

Abdomen coloration in the initial evaluation and the small-sample validation experiment was highly accurate for determining diapause status of adult female *L. hesperus*. Although observed accuracy was 100%, females exhibiting fat body hypertrophy and the "not green" abdomen coloration occasionally exhibit vitellogenic oocytes (DWS, unpublished observation). Classification of these individuals based on abdomen color would not be consistent with classification based on dissections using the diapause criteria of Brent and Spurgeon (2011). Therefore, we acknowledge that the color-based criterion for distinguishing diapause in female *L. hesperus* is not completely accurate despite this failure to misclassify females when diapause status was confirmed by dissection. In addition, the long-lived female that was apparently misclassified as non-diapausing in the survival study represents an error we had not anticipated based on previous experience. Because this female was not dissected to determine condition of the ovaries, its morphological status was not determined.

Spurgeon and Cooper (2012) reported an association between the green abdomen phenotype and ovary development, but the strength of that association varied with temperature. Ovary development occurred more rapidly compared with abdomen coloration at temperatures ≤21.1°C, whereas the opposite was observed at temperatures ≥32.2°C. These observations suggest that development of the ovaries and the abdominal coloration are not directly linked. Therefore, it is possible that the single female misclassified as reproductive was diapausing but exhibited an aberrant abdomen color phenotype. Regardless, the accuracy of female diapause classifications achieved in these studies represents an improvement compared with the report of Brent (2012). Compared with the study by Brent (2012), the improvements we demonstrated were likely facilitated by the limits we imposed on the experimental conditions, especially regarding temperature (26.7°C) and adult age (10 d).

The observation of an association between male abdomen color and diapause was consistent with the report by Brent (2012). However, corrected abdomen length proved a better predictor of diapause status for 10-d-old males. Following an adjustment of the corrected abdomen length criterion from 2.5 to 2.43, classification errors were limited to males with hypertrophied fat bodies, but also some development of the medial accessory glands, that were incorrectly classified as diapausing. Such individuals have been observed in other diapause studies at a rate similar to what was observed (DWS, unpublished data). Although the rate of misclassification that was observed was lower compared with the error rate observed by Brent (2012), it appears that no abdomen length or color criterion will eliminate the classification errors observed. However, use of the corrected abdomen length criterion offers the ability to manipulate the nature of the misclassification errors, depending on individual study objectives and the type of error that can be most tolerated. For example, if the abdomen length criterion is increased the probability of misclassifying a reproductive male as diapausing is reduced and the probability of classifying a diapausing male as reproductive increases. Alternatively, an excess of insects can be exposed to diapause inducing conditions and error rates of both types can be minimized by excluding individuals with a corrected abdomen length within a range centered on about 2.4. As this range is widened, more insects are excluded but fewer misclassification errors occur. Where misclassifications can compromise interpretation of experimental results, such as in studies of the physiology or molecular biology of diapause, knowledge of the expected rate and nature of misclassification errors may be useful in identifying observations as outliers.

Although the criteria for distinguishing diapause in adult *L. hesperus* cannot be adjusted to exclude all classification errors, dissections of population subsamples to estimate the incidence of diapause are subject to sampling error, and the status of any individual is unknown. Based on observations, classification errors did not diminish the ability to distinguish the survival functions corresponding to combinations of insect gender and physiological state. Further, the errors that likely occurred would have only minor influences on estimated median survival times. Therefore, the selected criteria of abdomen color phenotype for females, and corrected abdomen length for males, appear to have utility in a variety of research avenues where non-destructive classification of individual physiological status is desirable. This is particularly relevant for studies that seek to elucidate the molecular pathways controlling diapause given that, at least for some species, induction involves differential expression of diverse mRNA transcripts or microRNAs (Zhu et al., 2017a, 2017b; Batz et al., 2017), and diapause development appears to proceed via a defined transcriptomic profile (Koštál et al., 2017). The ability to distinguish the diapause status of adult *L. hesperus* with reasonable accuracy is also expected to enhance efforts to examine the survival potential afforded by diapause and thus better the understanding of the ecology and physiology of diapause in this important pest species.

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