NON-DESTRUCTIVE DETECTION OF DIAPAUSE IN MALES OF THE WESTERN TARNISHED PLANT BUG

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Abstract

Southern populations of the western tarnished plant bug, *Lygus hesperus* Knight, survive the late-fall and early-winter in adult diapause. Because this diapause is short, it is not clear how it integrates with other survival tactics to ensure overwintering survival of the population. Reports of extended development times and survival of immature stages and reproductive adults under low temperatures, and presence of springtime diapausing adults in the field, add to the potential complexity of *Lygus* overwintering ecology. A comprehensive picture of *L. hesperus* overwintering requires knowledge of the implications of diapause, including advantages from extended host-free survival. An obstacle to interpreting host free survival is lack of a non-destructive method to distinguish diapause in individual insects. Abdomen color appears to have utility for predicting diapause for females, but no comparable method is available for males. Morphological measurements were examined for association with diapause, based on observed abdominal distention in males possessing a hypertrophied fat body. Rearing insects at 26.7°C under short (10-h) days produced a mixture of reproductive and diapausing bugs. Measurements taken at 10 d of adult age were compared with diapause status determined by dissection. Corrected abdomen length (i.e., divided by head capsule width) was associated with reproductive and diapause status. Classification errors were minimized using a corrected abdomen length of 2.8, where accuracy was 90.6%. Once validated, this method should facilitate studies to unambiguously determine host-free survival of diapausing bugs, which will provide insights necessary to further examine the role of diapause in *L. hesperus* overwintering in southern climates.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight, is considered to overwinter in a state of adult diapause that is induced by short days (Beards and Strong 1966, Leigh 1966). In southern populations the diapause state is thought to terminate in late-fall to early-winter (Beards and Strong 1966, Strong et al. 1970), thus the duration of the diapause is relatively short. In Arizona, only a portion of the *L. hesperus* population can be induced to diapause in the laboratory (Spurgeon and Brent 2015). Cooper and Spurgeon (2012, 2013, 2015) found that all life-stages of this important pest of cotton (*Gossypium* spp.) are substantially extended by low temperatures. These findings suggest that at least a portion of *L. hesperus* population in the southern parts of its range may overwinter as slow-developing eggs, nymphs, or reproductive adults. Finally, some adults collected from alfalfa (*Medicago sativa* L.) in late-winter display a color phenotype reported by Brent (2012) as typical of diapause (unpublished observation). These collective observations suggest a more complex overwintering strategy for southern *L. hesperus* than is generally recognized. In particular, the ecological role of adult diapause and its implications to southern populations is poorly understood. Better understanding of *L. hesperus* overwintering ecology will require knowledge of the timing, duration, and termination of the adult diapause.

Useful estimates of the host-free longevity of diapausing *L. hesperus* are not available. Brent et al. (2013) compared host-free longevity of diapausing and non-diapausing *L. hesperus* adults but they used insects from a laboratory colony reported to exhibit a reduced diapause response (Brent and Spurgeon 2011). In addition, Brent et al. (2013) did not provide a source of moisture to the insects, which likely hastened their mortality. Although average longevity of diapausing insects was about three times that of non-diapausing insects, median host-free longevity was <200 hours at 28°C. In comparison, Cooper and Spurgeon (2015) provided unfed, newly eclosed, non-diapausing adults a source of moisture and observed host-free longevity averaging about 7 days at 26.7°C. Fye (1982) found that availability of moisture was important to survival of overwintering *L. hesperus*. Thus, it seems likely the host-free survival reported by Brent et al. (2013) underestimates that which is likely to occur in the field.

For a population of *L. hesperus* that does not uniformly exhibit diapause, a major challenge to estimating host-free survival is distinguishing diapausing individuals at the beginning of the host-free period. The diapause status of *L. hesperus* is generally determined by dissection. Although the incidence of diapause in a survival cohort could be estimated by dissection of a sample from that cohort, a non-destructive indicator of diapause would decrease the cost of the research and potentially improve accuracy of those estimates. Brent (2012) reported that diapausing females could be distinguished with about 84% accuracy based on abdominal color. However, similar criteria applied to males were only about 67% accurate. Spurgeon and Cooper (2012) applied the criterion of abdomen color to predict the reproductive status of adult males and reported similarly high error rates (40%). The objective of this work was to identify and make a preliminary evaluation of a non-destructive indicator of diapause in male *L. hesperus*.

Materials and Methods

Insects used in the study were the F_1 progeny of adults collected from alfalfa in the vicinity of Maricopa, AZ. The field-collected adults were maintained at about 27°C with a 14:11 (L:D) h photoperiod within a rearing cage provisioned with shredded paper, pods of green bean (*Phaseolus vulgaris* L.) and raw seeds of sunflower (*Helianthus annuus* L.). Bean pods containing eggs were confined in 1-gal. plastic buckets with screened lids. Nymphs were reared in these same buckets, which were provisioned with green bean pods and raw seeds of sunflower. Nymphs (1^{st} – 4^{th} instar) periodically obtained from the rearing buckets were confined individually within 18-ml plastic vials with ventilated snap-cap lids (Thornton Plastics, Salt Lake City, UT). Nymphs were held within an environmental chamber (I30-BLL, Percival Scientific, Perry, IA) at 26.7±0.5°C with a 10:14 (L:D) h photoperiod, where they were provided a fresh section of green bean pod three times weekly. For each insect, the date of adult eclosion was noted. Diapause assessments (measurements and dissection) were made on males that were 10-d-old.

The approach to the objective was motivated by the observation that male *L. hesperus* exhibiting a hypertrophied fat body typical of diapause also exhibit distention of the abdomen. This distension is easily observed from the dorsum once the wings are removed (Fig. 1), but spreading the wings sufficiently to observe the abdomen without injury to the specimen has proven difficult. However, it seemed likely that dimensions of the abdomen observed from the ventral aspect might provide a usable indicator of abdomen distention.

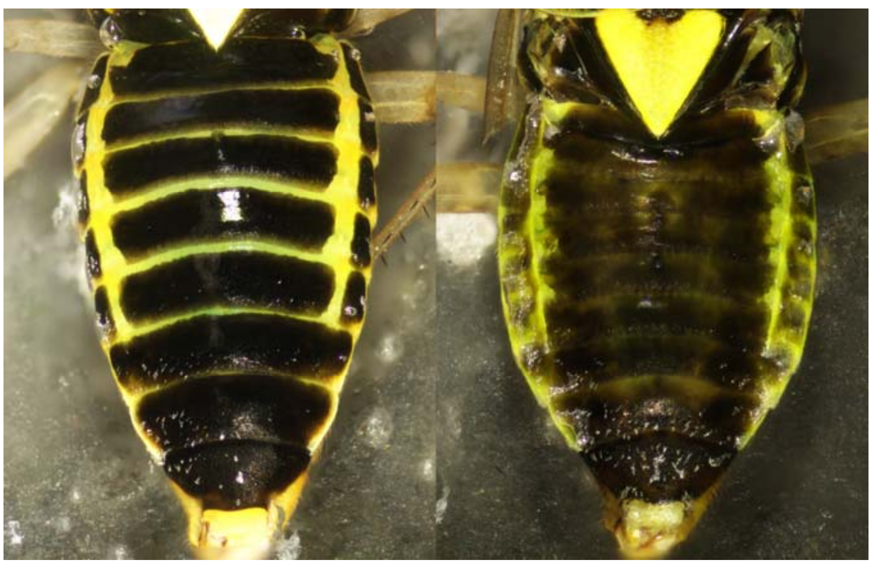

Fig. 1. Distended abdomen of a 10-d-old diapausing male *L. hesperus* (left) compared with a 10-d-old reproductive male (right).

Potential criteria that were examined for utility in predicting diapause included abdomen color, length, and width, and abdomen length and width each corrected for individual insect size (divided by head capsule width). Only results for corrected abdomen length are reported here.

At 10 d of adult age, each insect was photographed at a magnification of 45× using an Olympus DP-SAL stand-alone camera system mounted on a SZ-61 dissection microscope (Olympus Corp., Tokyo, Japan). During this process, each insect was enclosed within a clear, sealable plastic bag, and was positioned ventral side up on the microscope stage. Measurements were made using firmware of the DP-SAL system. To minimize error in measuring head capsule width, a parallel lines method was used by adding the respective distances to the outermost borders of the eyes from a longitudinal reference line positioned near the center of the insect. Abdomen length was measured directly from as close as possible to the posterior margin of the 1st abdominal sternite to the tip of the last abdominal sternite. The posterior margin of the 1st abdominal sternite generally lays near the metathoracic coxae.

Diapause status was determined by dissection after measurements were taken. Males were dissected under 0.7% (w/v) saline in a depression in a paraffin-lined Petri dish. Diapause was evidenced by presence of a hypertrophied fat body and poorly developed medial accessory glands as described by Brent and Spurgeon (2011). Males exhibiting developed or developing medial accessory glands were classified as reproductive irrespective of fat body condition.

The relationship between corrected abdomen length and the incidence of diapause was described by logistic regression (PROC LOGISTIC, SAS Institute 2012). Diapause status was the response variable, and corrected abdomen length was the predictor. Significance of the logistic regression was assessed by the likelihood ratio statistic (Stokes et al. 2012), and profile likelihood 95% confidence intervals were calculated at 0.1 increments of the corrected abdomen length from 1.9 to 3.0 (corrected abdomen length is a unitless ratio). Following this analysis, accuracy of classifications based on corrected abdomen length, at increments of 0.1 from 2.1 to 2.8, were calculated from contingency tables with diapause classification from dissections as rows and classifications from corrected abdomen length as columns.

Results and Discussion

The logistic regression indicated a significant relationship between corrected abdomen length and the probability of diapause (likelihood ratio = 42.55, df = 1, $P < 0.01$, $r^2 = 0.526$). Model parameters (in logits, ± SE) were: intercept, -40.21 ± 12.58; slope, 16.35 ± 5.00. The shape of the relationship suggested an inflection point between corrected abdomen length values of 2.4 and 2.5 (Fig. 2).

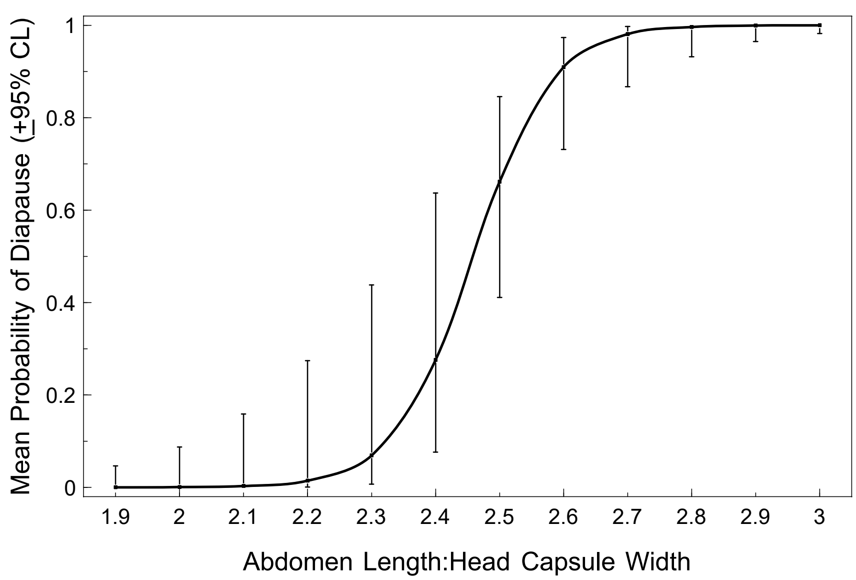

Fig. 2. Predicted probability of diapause (±95% confidence limits) for 10-d-old *Lygus hesperus* adult males based on corrected abdomen length (abdomen length / head capsule width).

Examinations of classification accuracy (concurrence between predictions using corrected abdomen length and determinations by dissection) suggested errors in classification were minimized near corrected abdomen length = 2.5 (Fig. 3). At lower levels of corrected abdomen length too many reproductive males were predicted to be diapausing, and at higher levels of corrected abdomen length too many diapausing males were predicted to be reproductive. The misclassification of reproductive males as diapausing at corrected abdomen lengths of 2.6 and 2.7 were unavoidable because those individuals possessed hypertrophied fat bodies that resulted in abdominal distension. However, they also exhibited early stages of medial accessory gland development that precluded their classification as diapausing.

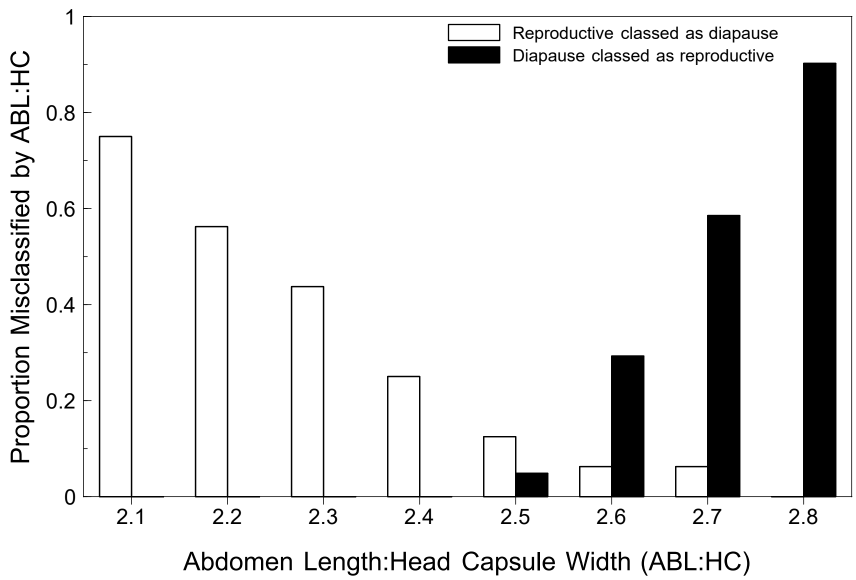

Fig. 3. Errors in diapause classification of 10-d-old *Lygus hesperus* adult males based on corrected abdomen length (abdomen length / head capsule width). Actual diapause status was determined by dissection.

Using a corrected abdomen length of 2.5, 12.5% of reproductive bugs were misclassified as diapausing, and 4.9% of diapausing bugs were misclassified as reproductive. The overall error rate at corrected abdomen length = 2.5 was 7%, but sample sizes of reproductive (n = 16) and diapausing (n = 41) males were unequal. Adjusting for equal sample sizes gave an overall error rate of 9.4%.

The sample size of reproductive males (n = 16) was too low for confident conclusions regarding the potential accuracy of corrected abdomen length as a predictor of diapause. Additional data collection is ongoing, and validation using independent samples and an examination of the association between predicted diapause status and subsequent host-free longevity are pending. However, the predictive accuracy of corrected abdomen length, even based on a small sample, is clearly superior to the 33% error rate reported for abdomen color by Brent (2012) or the 40% error rate for predictions of reproductive development reported by Spurgeon and Cooper (2012). One factor contributing to the higher accuracy of predictions in this study was the advanced age of the insects (10 d) at diapause assessment. The study by Brent (2012) included much younger ages of males, many of which may have been too young for diapause assessment even by dissection. Spurgeon and Brent (2010) previously recommended that at a temperature of 26.6°C, diapause assessments by dissection were best delayed until adult age was ≥10 d. Also, Spurgeon and Cooper (2012) found that development of abdominal coloration was temperature dependent, but its temporal pattern was different from the temperature dependent patterns of development for the reproductive organs. In their study, which assessed predictions of reproductive status based on abdominal color, rates of misclassification errors differed markedly among the different temperatures.

Predicted diapause status based on corrected abdomen length relies on abdominal distention caused by fat body hypertrophy. As such, this method cannot distinguish diapausing individuals from those with a hypertrophied fat body and developing medial accessory glands. However, whether these latter individuals should be classified as diapausing or as reproductive has not been conclusively demonstrated (Spurgeon and Brent 2010, Brent and Spurgeon 2011). Regardless, an estimated misclassification rate of <10% is likely superior to that achievable by dissection of a sample obtained from a cohort of insects where a non-destructive estimate of diapause status is desirable. In addition, predicted diapause status using corrected abdomen length assigns a classification on the individual level rather than providing an estimate for the population. These predictions should facilitate improved interpretation of studies of host-free longevity. Finally, if the experimental objective is simply to ensure that study insects are in the diapause state (or the reproductive state), individuals can be selected based on a corrected abdomen length other than the level that minimizes overall misclassification errors. For example, by using a higher value for corrected abdomen length one would sacrifice the diapausing insects that are misclassified as reproductive, but would also ensure the maximum probability of diapause in the selected insects. This method may be the best available alternative to dissection where diapausing males are needed for studies of endocrinology or molecular biology of diapause.

Summary

The results strongly suggest that use of corrected abdomen length has promise as a non-destructive indicator of diapause in male *L. hesperus*. Even with limited samples, the classification errors using corrected abdomen length were considerably lower than those previously reported for other non-destructive classification methods (Brent 2012, Spurgeon and Cooper 2012). Should additional data collection and validation confirm these preliminary findings, then use of corrected abdomen length will provide a relatively accurate, simple, and non-destructive method to assign diapause status on an individual level in other studies of *L. hesperus* overwintering ecology or physiology.

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