OBSERVATIONS OF DIAPAUSE CHARACTERS IN THE WESTERN TARNISHED PLANT BUG, *LYGUS HESPERUS*

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Abstract

Knowledge of overwintering strategies of the western tarnished plant bug, *Lygus hesperus* Knight, is essential to the development of ecologically-based management strategies. The adult diapause of *L. hesperus* has been studied, but temporal patterns in the development and exhibition of morphological characters of diapause have not been defined. We reared *L. hesperus* from egg to adulthood under long (14:10) and short (10:14, L:D h) photoperiods at 28°C and dissected the resulting adults at various ages. While diapause characters could generally be distinguished at 3 d of age, the distinction between diapausing and reproductive bugs was not unambiguous because the morphological characters indicative of the respective conditions were not fully developed. Differences in the morphology of diapausing and reproductive bugs became more distinct with increasing age up to 10 d. In addition to the diapause characters previously reported (hypertrophied fat bodies and undeveloped ovaries) accessory gland condition in males was informative. Although we observed fat bodies characteristic of those previously reported (reproductive, translucent and sheet-like; diapause, white and in well-formed globules) appearance of the fat bodies was highly variable. Fat body condition alone was not an adequate criterion to distinguish diapausing bugs. These results provide baseline information to improve the accuracy of classification of physiological condition in future studies of *L. hesperus* diapause.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight, is a key pest of cotton (*Gossypium hirsutum* L.) and other crops in the San Joaquin Valley of California. At present, most management efforts in cotton involve the use of conventional pesticides. Development of more ecologically-based management strategies will require improved understanding of lygus ecology in the San Joaquin Valley. Of particular interest is overwintering ecology and the seasonal movement of lygus adults among cultivated and non-cultivated hosts.

One important aspect of the overwintering ecology of *L. hesperus* is a relatively brief adult diapause (Leigh 1966, Strong et al. 1970). Although it is well established that photoperiod influences the diapause response of *L. hesperus* (Beards and Strong 1966, Leigh 1966, Strong et al. 1970), some inconsistencies exist in reports of these effects. Beards and Strong (1966) conducted both field and laboratory investigations of the diapause. Based on field collections, they reported about half of adults were diapausing by mid-September, when the photoperiod was about 12.5 h. However, they also suggested the induction cues were received by the nymphal stage. Assuming a 30-d nymphal development period, Beards and Strong (1966) concluded the critical photoperiod was about 13.5 h, and all nymphs were expected to produce diapausing adults at photoperiods shorter than 13 h. In contrast, the laboratory studies of Beards and Strong (1966) indicated a high incidence of adult diapause in response to constant photoperiods between 7 and 11 h at 27°C, but lower incidences of diapause at photoperiods of 6, 12, and 13 h. Furthermore, it was concluded that the intensity of diapause induced in the laboratory was not similar to that of field-collected bugs. In addition, Beards and Leigh (1960) reported a laboratory rearing procedure for *L. hesperus* using a photoperiod of 13 h, with no mention of reduced reproduction or diapause. Finally, Leigh (1966) reported that more than half of field-collected diapausing bugs terminated diapause in one week when exposed to a 13-h day length at 80°F (26.7°C).

Striking among the various studies of *L. hesperus* diapause are differences in the morphological criteria used to distinguish diapause, and the ages of bugs at the time of dissection. Assessments of diapause by Beards and Strong (1966) focused on females. In their studies, females were considered reproductive if the ovaries contained at least one chorionated egg, diapausing if the ovaries were undeveloped and the fat body was hypertrophied, and either non-reproductive or intermediate otherwise. Bugs from field studies were of unknown age, while bugs in laboratory studies of photoperiod were dissected at a total age (combined nymph and adult stages) of 44 days. Studies of the influence of temperature on diapause used total ages of 73 (21°C), 44 (27°C), and 30 d (32°C), although bugs held at 21°C under a constant photoperiod of 10 h were considered diapausing if the ovaries were undeveloped regardless of fat body condition. Leigh (1966) considered female bugs to be diapausing if there was little or no ovary

development and well-developed fat bodies, and reproductive if distinct ovary development was present. The distinction between diapausing and reproductive males was less clear, but Leigh (1966) noted that diapausing males exhibited well-developed fat bodies and atrophied seminal vesicles. Leigh (1966) also used an intermediate classification which was interpreted to represent sexually immature females (little ovary development but poorly-developed fat bodies). Finally, Strong et al. (1970) reported that males collected from the field in October featured hypertrophied fat bodies, accessory glands of reduced size and development, and in many specimens, atrophied testes.

An astute understanding of the full repertoire of seasonal dispersal and survival mechanisms, including diapause and its controlling factors, is necessary to develop an ecologically-based management strategy for *L. hesperus*. Regarding diapause, detailed information on environmental induction and maintenance cues, factors responsible for diapause termination, and associated ecological implications (e.g., extended host-free longevity) are needed. Furthermore, many aspects of the diapause cannot be efficiently studied unless it is possible to produce diapausing insects in the laboratory that are comparable to those present in the field. Given the information available in the literature, it seems reasonable to examine the morphology associated with diapause to select an unambiguous suite of characters. Establishment of such a character set would facilitate comparison of results from respective studies, simplifying the interpretation of results. The objective of the work reported herein was to make a preliminary examination of the temporal development of morphological characters associated with diapausing and reproductive *L. hesperus* adults. These will be used to design a more detailed study of character development.

Materials and Methods

All stages of *L. hesperus* from eggs ≤24 h old to adults were maintained under diapause inducing (10:14, L:D h) or diapause suppressing (14:10, L:D h) photoperiods in environmental chambers maintained at 28°C. Several paired cohorts, each of ≥ 20 nymphs, were reared under each photoperiod.

Eggs were obtained by exposing fresh pods of green bean (*Phaseolus vulgaris* L.) to ovipositing adults for 24 h. Following oviposition, each bean was cut in half and each half was assigned to a different photoperiod. Each bean half was placed in a 100×15-mm Petri dish on a disk of filter paper. Petri dishes were sealed with Parafilm M (Pechiney Plastic Packaging, Chicago, IL) to prevent bean sections from shriveling excessively. Petri dishes were examined daily for the presence of nymphs, and on the fifth day after oviposition the Parafilm was removed.

Newly-hatched nymphs were transferred to individual 5-dram plastic vials (Thornton Plastics, Salt Lake City, UT) which were closed by a snap-cap lid ventilated by a 1.1-cm diam hole covered by nylon organdy. Nymphs were provided a fresh section of green bean pod three times weekly, and were examined daily to monitor development. When nymphs molted the cast skins were removed from the vials. On the day of adult eclosion bug gender was recorded.

Dissections were performed at adult ages ranging from 0 (the day of adult eclosion) to 14 d, but most bugs were dissected at 3, 7, 10, or 14 days of age. For each dissected bug, a brief description of fat body condition (abundance, color, and configuration) was recorded. Descriptions of the conditions of reproductive organs (males: testes, accessory glands, and seminal vesicles; females: ovaries) were also recorded.

Results and Discussion

Nymphal development time varied from 10 to 15 days, with both observed extremes sometimes occurring within individual cohorts. Most nymphs developed to adulthood in 11-13 d. About 5% of nymphs exhibited supernumerary molts. In these instances a 2nd, 3rd, or 4th instar molted on consecutive days without advancing developmentally to the next instar.

The fat body of reproductive bugs varied in color from white to aqua. In many reproductive bugs the fat body was sheet-like or sparse (Fig. 1a), but in some specimens it was more globular (Fig. 1b). In reproductive bugs with white, globular fat, hypertrophy of the fat body was less extensive than in diapausing bugs ≥ 10 d old. Hypertrophy of the fat body in diapausing bugs was apparent as early as the third day after eclosion. The fat body of diapausing bugs was white or whitish, sheet-like or in a solid column adjacent to the dorsal vessel, and becoming globular towards the lateral margins of the abdomen (Fig. 1c). Fat bodies in these diapausing bugs continued to increase in

apparent volume and opacity until about 10 d of age (Fig. 1d).

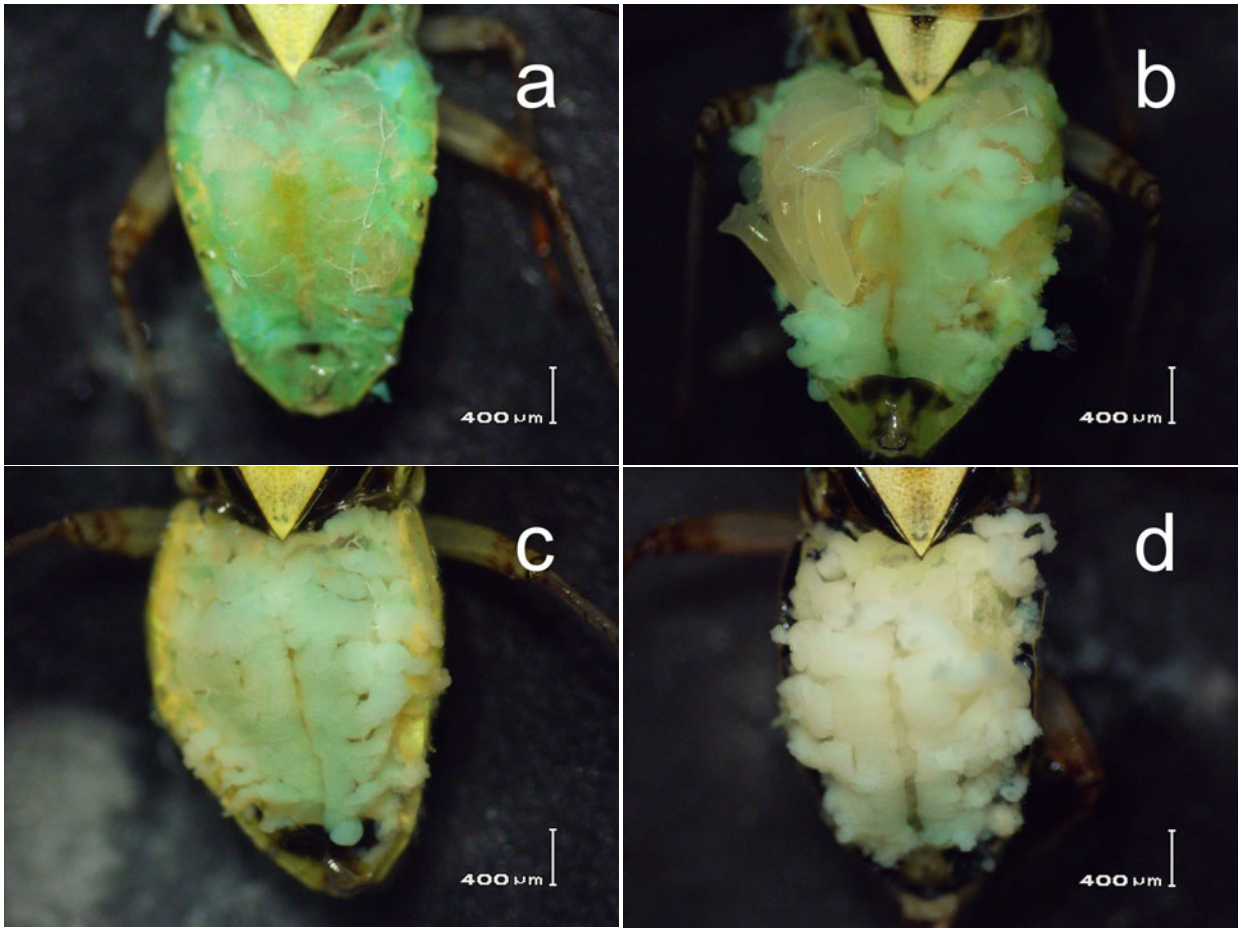

Figure 1. Fat bodies of *Lygus hesperus* adults: a) 7-d-old reproductive male, b) 3-d-old reproductive female, c) 3-d-old diapausing female, d) 10-d-old diapausing male.

Accessory glands of adult males 0 to 1-d-old were small and colorless and seminal vesicles were empty. The accessory glands of some 2-d-old males had begun to enlarge and white contents were visible in the medial glands. This early development of accessory glands is illustrated in Fig. 2a. Also, most 2-d-old males had visible sperm in the seminal vesicles. Accessory glands of most 3-d-old males were large with the medial glands filled with a white material. At that age 91% of males reared under the 14-h photoperiod (n = 11) had visible sperm in the seminal vesicles. Both the seminal vesicles and the basal chamber of the medial accessory glands were typically distended in 7-d-old reproductive males, and the bases and termini of the lateral accessory glands were nearly opaque (Fig. 2b). In all ages of reproductive males the sutures demarking testicular septa held a thin layer of fat. In reproductive males with white and globular fat bodies, the medial surfaces of the testes were often covered by a layer of fat similar to that in diapausing males. Increased age beyond 7 d was associated with continued distention of the seminal vesicles. Accessory glands of diapausing bugs remained small and undeveloped at all ages, and in those with well-developed fat bodies the testes were sheathed in a dense layer of fat (Fig. 2c). There were no apparent differences between reproductive and diapausing bugs in the sizes of testes or seminal vesicles.

Ovaries were undeveloped in 0 to 1-d-old reproductive females, and in all ages of diapausing females (Fig. 2d). Ovary development, indicated by the visible accumulation of yolk in oocytes, was apparent in reproductive bugs as young as 2-d-old. Chorionated eggs were present in 80% (n = 10) of 3-d-old reproductive females held in the 14-h photoperiod (Fig. 2e). At ages ≥7 d, ovaries often contained reduced numbers of eggs (Fig. 2f). However, the unmated females oviposited in the green bean pods as early as day 7 of adulthood, which corresponded with the

appearance of follicular relics in the ovaries. Therefore, the reduced egg complements appeared to result from recent oviposition.

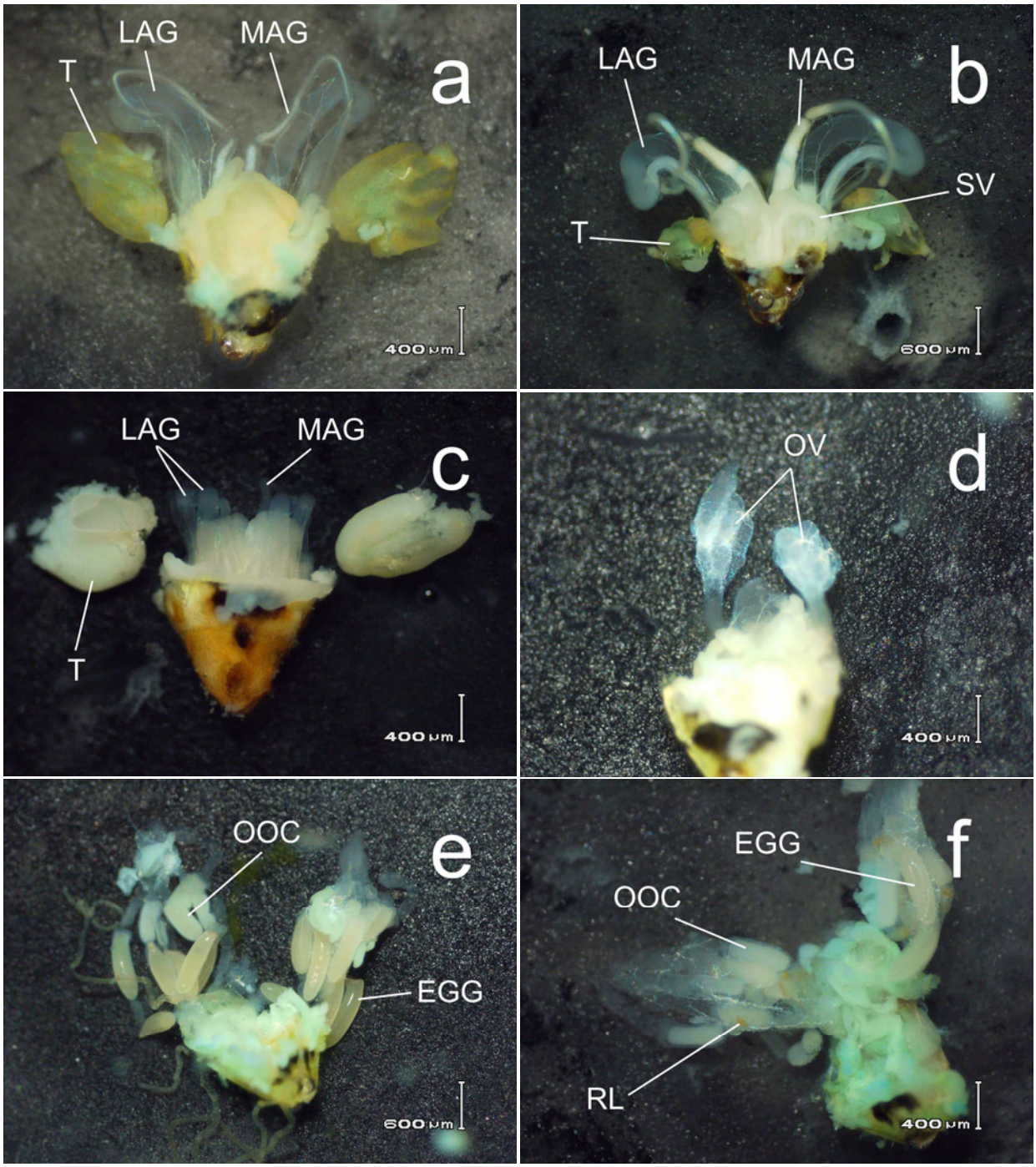

Fig. 2. Development of reproductive organs in adult *L. hesperus*: a) 3-d-old reproductive male, b) 7-d-old reproductive male, c) 10-d-old diapausing male, d) 3-d-old diapausing female, e) 3-d-old reproductive female, f) 10-d-old reproductive female. Labels are: T, testes; LAG, lateral accessory glands; MAG, medial accessory glands; SV, seminal vesicle; OV, ovary; OOC, oocyte; EGG, chorionated egg; RL, follicular relic.

No bugs reared under the 14-h-photoperiod were classed as diapausing at any age. However, the incidence of

diapause in response to the 10-h photoperiod was lower than expected. Bugs at 3, 7, 10, and 14-d of age were classed as diapausing using the criteria of obvious hypertrophy of the fat body (Fig. 1c, d) and absence of accessory gland development in males (Fig. 2c) or of ovaries in females (Fig. 2d). Only 65% of 3-d-old females (n = 17) and 23% of 3-d-old males (n = 11) reared under the 10-h photoperiod were classed as diapausing. For 7-d-old bugs the percentage classed as diapausing increased to 77% (females, n = 13) and 50% (males, n = 12). Only 25% (n = 12) and 50% (n = 10) of 10-d-old females and males, respectively, were classed as diapausing. The greatest percentage of diapause was observed in 14-d-old bugs (86% for both males [n = 22] and females [n = 7]).

Previous reports of *L. hesperus* omitted detailed descriptions of the fat bodies associated with diapause, referring to them only as being hypertrophied (Beards and Strong 1966, Strong et al. 1970) or well developed (Leigh 1966). *L. hesperus* fat bodies observed in this study were similar to those reported for *L. lineolaris* (Palisot de Beauvois) by Villavaso and Snodgrass (2004), except for the occasional occurrence of mildly hypertrophied, globular fat in some reproductive *L. hesperus*.

Strong et al. (1970) reported that field-collected diapausing *L. hesperus* males contained small, nearly empty accessory glands and atrophied testes. Leigh (1966) reported no apparent differences in testis size for reproductive and diapausing males, but indicated the seminal vesicles of diapausing males were atrophied. Villavaso and Snodgrass (2004) reported the only apparent difference in the testes and seminal vesicles of reproductive and diapausing *L. lineolaris* was the presence of a fatty sheath enclosing the testes of diapausing bugs. Also, Snodgrass (2005) considered field-collected *L. lineolaris* males to be reproductive if accessory glands had begun to enlarge and contained white fluid. Testes and seminal vesicles of *L. hesperus* observed in the current study were consistent with the descriptions of Villavaso and Snodgrass (2004). In addition, observations of *L. hesperus* in the current study suggested accessory gland condition, especially in males ≥3-d-old, was a reliable criterion for distinguishing diapausing bugs. Villavaso (2007) indicated depletion of the accessory gland contents in *L. lineolaris* was a reliable indicator of mating, and Strong et al. (1970) reported the genital pouch of mated female *L. hesperus* contains a fluid similar to that in the medial accessory glands of males. Therefore, recent mating may be responsible for previous observations of incompletely developed accessory glands in studies using field-collected adults, or in which adults were held in mixed-sex groups. Observations of *L. hesperus* reported herein were for bugs held individually, and therefore denied the opportunity to mate. As a consequence, accessory gland development was distinctly different for reproductive and diapausing males ≥3-d-old.

Some reports of *Lygus* diapause in females include classifications of "intermediate" (Beards and Strong 1966, Leigh 1966) or "unclear" (Villavaso and Snodgrass 2004) based on either the simultaneous occurrence of a hypertrophied fat body and some ovary development, or the lack of fat body hypertrophy combined with little or no ovary development. These previous reports were based either on field-collected insects of unknown age, or on bugs held in mixed sex groups. In the current study no female *L. hesperus* >3-d-old was observed lacking both ovary and apparent fat body development. Mildly hypertrophied fat bodies were observed in reproductive females, some of which contained reduced egg complements. However, in those cases, presence of follicular relics or previous oviposition in the green bean sections indicated a status of reproductive.

In summary, observations of *L. hesperus* adults at different ages suggest presence of a hypertrophied fat body combined with absence of accessory gland or ovary development are reliable criteria for distinguishing diapause. Under the temperature conditions studied (28°C), diapause status could be identified in adults as young as 3 d old, but the distinction was less ambiguous in bugs ≥7 d old. Also, continued development of fat bodies with increasing adult age suggested that ecological studies of host-free survival and diapause termination should use avoid using adults exposed to diapause-inducing conditions for <10 d. Observed incidences of diapause, as indicated by the suggested criteria, were lower and less consistent than expected compared with previous reports. However, the results reported herein are preliminary. Conclusions regarding the influences of daylength on diapause induction in *L. hesperus* should be avoided until more extensive, replicated studies are conducted.

Summary

The results reported herein document the development of morphological characters associated with reproduction or diapause in adult *L. hesperus* held at a constant temperature of 28°C. The findings of this work suggest the presence of a hypertrophied fat body combined with absence of accessory gland development (in males) or ovary development (in females) are satisfactory criteria for the identification of diapausing *L. hesperus* adults ≥3 d old,

although complete development of the diapause morphology was not observed in adults <10 d old. These criteria are not completely consistent with those of earlier reports which examined field-collected adults or adults held in mixed sex groups. However, inconsistencies in distinguishing criteria also existed among these earlier studies. The use of unmated adults of known age in diapause assessments appears to reduce or eliminate ambiguities in diapause classifications reported in earlier studies. Assessments of diapause using the suggested criteria indicated lower and less consistent percentages of diapausing adults than was expected. These assessments lacked statistical replication and were based on limited sample sizes. Therefore, conclusions regarding the extent of the diapause response await more complete studies. The results reported herein suggest the need to re-examine the seasonality and associated photoperiodic control of diapause in *L. hesperus*. Improved understanding of the diapause phenomenon in *L. hesperus* should provide insights into seasonal movement patterns and contribute to the development of ecologically-based management strategies.

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