TIMING OF SHORT-DAY EXPOSURE INFLUENCES DIAPAUSE RESPONSE OF WESTERN TARNISHED PLANT BUG

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Abstract

The western tarnished plant bug, *Lygus hesperus*, enters adult diapause in response to short photoperiods. However, the instars or stages responsive to the photoperiodic cue are poorly defined. Lygus were reared under short days (10 h) until they were dissected to determine diapause status as 10-d-old adults. For comparison, bugs similarly reared under short days were transferred to long days (14 h) at either 1st or 3rd instar, or as newly eclosed adults. In reciprocal treatments, bugs initially reared under long days were either maintained under long days or were switched to short days. Only one bug reared under short days and switched as a nymph (3rd instar) to long days exhibited diapause characters. Only a small portion of bugs (<10% of both genders combined) switched from short to long days as young adults retained the diapause characters by 10 days of adult age. Incidence of diapause was highest (55%) for bugs receiving only the short-day photoperiodic cue, or for those switched from long to short days as 3rd or earlier instars (40-53%). Diapause was not observed among bugs switched from long to short days as young adults. These results indicate the photoperiodic cue elicits a response in at least 3rd and earlier instars, and suggest the incidence of diapause increases slightly with increased exposure to the short day length. Exposure to long days was efficient at reversing diapause induction irrespective of earlier exposure to short days.

Introduction

Adults of the western tarnished plant bug (*Lygus hesperus* Knight) enter a photoperiodically-induced diapause in late-summer or fall (Beards and Strong 1966, Leigh 1966, Strong et al. 1970). Recent work has questioned the morphological criteria used earlier to distinguish diapause, and suggested revised criteria (Spurgeon and Brent 2010). These revisions prompted additional investigations that showed the diapause response is amenable to rapid selection when insects are cultured in the laboratory (Spurgeon 2012), and that the diapause response is highly variable and incomplete in at least some populations (Spurgeon and Brent 2015). Studies of *L. hesperus* development and survival at low temperatures have suggested that alternative overwintering survival strategies may complement or substitute for the adult diapause in some cases (Cooper and Spurgeon 2012, 2013, 2015). A more thorough and ecologically-useful understanding of *L. hesperus* diapause will require reevaluation of earlier experiments and their interpretations, and additional information to fill important gaps in the current state of knowledge. One of these gaps involves the timing at which the photoperiodic cue elicits a diapause response. Because no empirical evidence is available to indicate the stage(s) or instar(s) that respond to the diapause-inducing photoperiodic cue, experiments were conducted to evaluate the influence on diapause response of photoperiod switching during the early nymphal and early adult stages.

Materials and Methods

Each repetition of the experiment used bugs of the F_1 generation obtained as eggs from 400–500 field-collected adults, except for the first repetition in which F_2 bugs were used. Native adults were obtained from fields of alfalfa (*Medicago sativa* L.) in the vicinity of Maricopa, AZ during October (2015) and March, April, and June (2016). Each adult colony, maintained at about 27°C under a 14:10 (L:D) h photoperiod, was fed green beans (*Phaseolus vulgaris* L.) and raw seeds of sunflower (*Helianthus annuus* L.) as described by Spurgeon and Brent (2015).

Eggs were obtained by exposing eight green bean pods to the adult colony for 6–8 hours. This short oviposition time prevented exposure of eggs, once laid, to photoperiods other than those to which they were assigned. The eight bean pods were cut in half, the cut ends were sealed with melted paraffin, and one half of each pod was assigned to either short (10 h light) or long days (14 h light) within respective environmental chambers (model I30BLL, Percival Scientific, Perry, IA) maintained at 26.7±0.5°C. Each pod half was contained within a 100 × 15 mm plastic Petri dish with a 100-mm disk of Whatman no. 2 filter paper fitted in the dish bottom. The margins of dishes were then wrapped in Parafilm M (Pechiney Plastic Packaging, Chicago, IL) to prevent desiccation of the beans. The dishes were examined every 1–2 days and condensation was removed by blotting, after which the dishes were sealed again with Parafilm. Beginning at 5 days after oviposition, the dishes were observed at least twice daily for newly hatched nymphs.

Upon hatching, each nymph was placed individually in a 5-dram plastic vial (Thornton Plastics, Salt Lake City, UT) closed with a snap-cap lid. The lid was ventilated by a hole (1.2 cm diameter) closed with nylon organdy. Each vial also contained a section of green bean pod, and cut ends of the pods were sealed with paraffin to minimize desiccation and prevent entry by early instar bugs. The newly-hatched nymphs were then randomly assigned to an initial photoperiod (10 or 14 h), and an age at which the photoperiod was switched (not switched, 1st instar, 3rd instar, newly eclosed adult). For example, insects assigned to an initial photoperiod of 10 h and not switched were maintained under the 10-h photoperiod from oviposition until dissection of the adult. Insects assigned to an initial photoperiod of 10 h switched at 1st instar were switched at hatch, whereas those assigned to switch at 3rd instar were switched as soon as the molt from 2nd to 3rd instar was detected. These treatments were chosen on the basis of a preliminary experiment in which few nymphs reared under a 14-h photoperiod entered diapause after being transferred to a 10-h photoperiod as early 3rd instars. In summary, each repetition of the experiment used eight combinations of initial photoperiod and age of switching (Fig. 1), and the entire experiment was conducted four times, each time with a new cohort of insects.

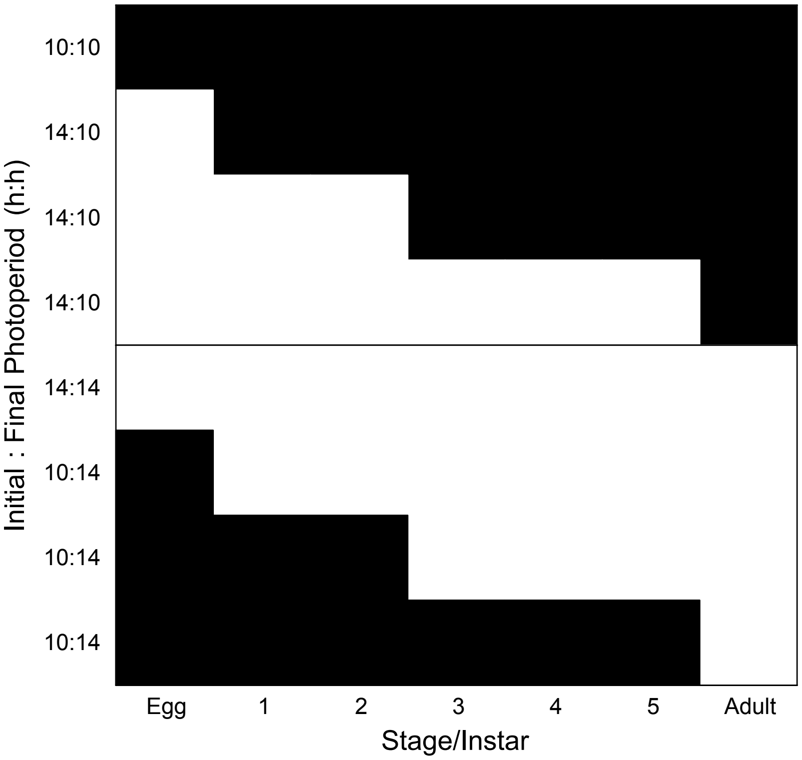

Fig. 1. Graphical representation of eight treatment combinations where *L. hesperus* were either maintained under short day (10 h, black bars) or long day (14 h, white bars) photoperiods, or were switched between photoperiods at 1st or 3rd instar or as newly eclosed adults.

Each nymph was provided a fresh bean pod section three times weekly, except for the newly-hatched nymphs. Replacement of the initial bean section for most nymphs was delayed until the molt to 2nd instar to minimize mortality from handling. All nymphs were observed at least three times weekly. In addition, nymphs designated to switch photoperiods were observed at least twice daily each day of the stadium preceding the switch, with the last daily observation after 15:00 h. The molt from 2nd to 3rd instar was identified by the presence of a cast skin or a change in head capsule width in comparison with the abdomen, and in comparison with the head capsules of other insects in the same treatment. Immediately before molting, the nymphal abdomen is conspicuously enlarged relative to the width of the head capsule whereas after molting this relative size difference is noticeably less. In later repetitions of the experiment, the molt from the 2nd to 3rd instar was also confirmed using head capsule width measured with an ocular micrometer (unpublished data). Fifth instars of all treatments were also observed twice daily to ensure adults assigned to switch photoperiods did so on the day of adult eclosion, and that adult age was accurately known. Gender of each insect, determined at adult eclosion, was also recorded.

Diapause or reproductive status was assessed by dissection of 10-d-old adults in 0.7% (w/v) saline. For females, diapause was indicated by a hypertrophied fat body combined with the absence of vitellogenesis as described by Spurgeon and Brent (2010). In males, diapause was indicated by a hypertrophied fat body and undeveloped or underdeveloped medial accessory glands as described by Brent and Spurgeon (2011).

Patterns in the frequency of diapause occurrence were analyzed separately by final photoperiod (the photoperiod the insects were switched to) and insect gender. Each analysis used a 2 × 4 contingency table with diapause status as columns and age (instar) at photoperiod switching as rows. In each analysis, repetitions of the experiment formed strata which served as blocks. Because the levels of rows and columns were ordinal, differences in the patterns of diapause occurrence within a table were assessed using the Mantel-Haenszel nonzero correlation statistic in PROC FREQ of SAS (SAS Institute 2012). In addition, Agresti-Coull 95% confidence limits (which are asymmetrical) were calculated for each combination of photoperiod treatment and insect gender.

Results and Discussion

Both sexes of bugs exhibited an association between diapause occurrence and age at which the bugs were switched from a 14- to a 10-h photoperiod (female, $Q_{CSMH} = 41.67$, df = 1, $P < 0.01$; male, $Q_{CSMH} = 21.16$, df = 1, $P < 0.01$; Fig. 2a). However, in contrast to the observations from preliminary experiments, the association appeared to be driven by the absence of diapause among insects switched as adults. Although results from the females suggested a decline in diapause incidence with increasing nymphal exposure to long days during the early stadia, the confidence intervals corresponding to those treatments were broadly overlapping. In addition, trends in the results from males were not suggestive of a similar decline (Fig. 2a). A major experimental difficulty in establishing clear and repeatable trends in the photoperiodic response of *L. hesperus* stems from the plasticity of the diapause response (Spurgeon 2012) and the inherent variability in the diapause response within this central Arizona population (Spurgeon and Brent 2015). In the preliminary experiment (unpublished data) few insects switched from the 14-h to the 10-h photoperiod at 3rd instar exhibited the diapause response. In the results reported here, diapause response of insects assigned to this switching treatment ranged from 0% (both genders, 3rd experimental repetition) to 75% (females, 4th repetition) or 62% (males, 2nd repetition).

Insects switched from the 10- to the 14-h photoperiod exhibited a more consistent response compared with the reciprocal switching treatments. Of the 337 insects receiving long days (14-h) as the only or final photoperiod, a total of 2 males and 7 females exhibited the diapause characters as adults (Fig. 2b). Of these 9 insects, 8 of them did not experience the long-day conditions until adulthood. In addition, 7 of the bugs retaining the diapause characters were females, for which there was an association between diapause incidence and age at which they were switched to long-day conditions ($Q_{CSMH} = 9.01$, df = 1, $P < 0.01$). Numbers of males exhibiting diapause were too low to detect a pattern in diapause occurrence ($Q_{CSMH} = 3.58$, df = 1, $P = 0.06$). In general, exposure to long days following exposure to short days was effective in terminating the diapause program irrespective of earlier photoperiodic conditions.

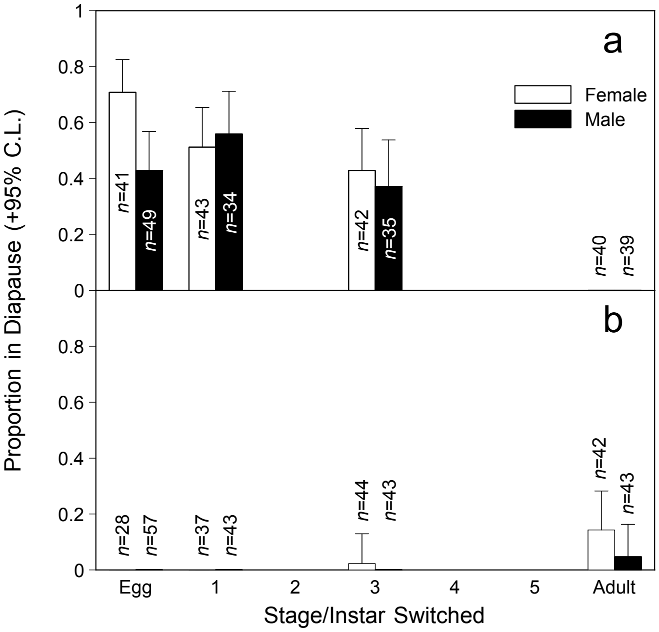

Fig. 2. Diapause response of *L. hesperus* a) reared under a 10-h photoperiod (egg) or switched from a 14-h photoperiod to a 10-h photoperiod at selected stages of development, or b) reared under a 14-h photoperiod (egg) or switched from a 10-h to a 14-h photoperiod at selected stages. Sample sizes and Agresti-Coull 95% upper confidence limits are indicated.

Summary

The results clearly show the terminating effects of long day conditions on early-instar *L. hesperus* previously exposed to short day, diapause-inducing conditions. Less clear is the diapause response of bugs switched from long day to short day conditions. A preliminary experiment, as well as some repetitions reported here, implied a declining diapause response as the delay in transfer from the long to the short photoperiod increased. However, marked variability among repetitions of the experiment, which is typical of the diapause response in this species (Brent and Spurgeon 2011, Spurgeon and Brent 2010, 2015, Spurgeon 2012), did not permit unambiguous conclusions regarding the influence of nymphal exposure to long days on subsequent diapause response to short days. The work does, however, demonstrate that in the early instars of *L. hesperus*, previously received photoperiodic cues, whether long or short days, can be substantially reversed by subsequently imposing the opposite photoperiodic cues. Additional investigations of the influences of intermittent disruptions of the photoperiodic program, and of the responses of later instars, is warranted.

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