Photoperiodic Responses of the Squash Bug (Heteroptera: Coreidae): Diapause Induction and Maintenance

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ABSTRACT   The squash bug, *Anasa tristis* DeGeer, undergoes a reproductive diapause from late summer to spring in northeastern Kansas. In the laboratory, diapause was induced in 100% of adult females reared under photoperiods shorter than 14:10 (L:D) and in a variably lower percentage of the population under all longer photoperiods. The critical photoperiod for diapause induction falls between 14:10 and 14.5:9.5; this range compares closely with prevailing natural daylengths when 50% of the adult population enters diapause in the field. Between October and March, short daylengths maintained, and long daylengths terminated, diapause in field-sampled adults. Under natural daylength at 26°C, the duration of diapause became progressively shorter with advancing sample date. In nature, the photoperiodic maintenance of diapause is completed in most of the population by late May. A prolonged diapause probably serves to prevent premature postdiapause development during the thermally variable spring conditions encountered in Kansas. Some implications of these findings for biological control and pest management programs are discussed.

KEY WORDS   Insecta, *Anasa tristis*, photoperiod, diapause

THE SQUASH BUG (SB), *Anasa tristis* DeGeer, is an important pest of cucurbitaceous crops throughout much of North America. Despite its broad distribution and relative importance, few studies have been made of this pest's biology (but see Worthley 1923, Elliot 1935, Beard 1940, Nechols 1987). Particularly lacking are experimental investigations of the factors that control the seasonal cycle. Such phenological studies provide the basis for understanding the seasonal synchrony of pests with natural enemies, host plants, and agronomic practices. Thus, this information is necessary for developing integrated or biological control programs for the SB.

Diapause is the primary means by which temperate zone insects and related arthropods synchronize their seasonal cycles with the availability of essential resources (see Tauber & Tauber 1976, Tauber et al. 1986) and with the occurrence of favorable physical conditions (see Tauber et al. 1985). Because photoperiod is the dominant environmental factor used by insects to regulate diapause (Tauber & Tauber 1973, 1976; Beck 1980; Tauber et al. 1986), I examined the influence of this factor on various aspects of SB dormancy. Specifically, I determined the critical photoperiod for diapause induction in the laboratory, provided evidence for photoperiodic maintenance of diapause throughout dormancy, and established when the photoperiodic regulation of diapause terminates naturally in the field.

Materials and Methods

Diapause Induction

All experimental animals were the F_1 progeny of field-collected, overwintered adults. Ovipositing stock adults were maintained on potted 'Green Zucchini' and 'Early Prolific Straightneck' squash (*Cucurbita pepo* L.; Harris Seed, Rochester, N.Y.) at 27 ± 1°C and a photoperiod at 16:8 (L:D).

Within 24 h of oviposition, egg masses were transferred to one of the experimental photoperiods (27 ± 1°C) (see Table 1). On the day of hatch, first-instar nymphs were moved into frame cages that contained potted straightneck squash plants. Plants were changed as needed and bugs were reared to the adult stage under their respective photoperiodic conditions.

The day after emergence, adult SB were transferred to individual squash plants inside cylinder cages. Each cage contained one to three pairs of adults (female and male). Daily, each cage in every condition was examined and plants were changed as needed. Dates of mating and oviposition were recorded. On day 10, SB females were dissected in a 3% solution of NaCl and examined for ovarial development; the degree of ovarial development was noted. Females that lacked mature or developing oocytes and showed no expansion of ovarioles were considered to be in, or entering, diapause.

The percentage of females in diapause was com-

puted for each photoperiod, and the critical pho-
toperiod was derived by standard procedures.

Diapause Maintenance and Termination

Field Collection and Storage. Large numbers of
SB adults were collected in early September 1984
from a pumpkin field located 2 mi south of Man-
hattan, Kans. These were transferred to screen-
covered cages that were sunk into the ground. Each
cage contained insulation material (scrap wood and
styrofoam sheets); a fresh pumpkin was provided
until early October as a source of food and water.

Experimental Procedure. Natural populations
of the SB enter a reproductive diapause by early
September (see Nechols 1987). To examine the
photoperiodic influence on diapause maintenance,
I sampled the SB monthly from October to May
(except in April) and subjected them to several
photoperiodic conditions, including natural day-
length.

On each sample date, 5 to 12 pairs (female and
male) of adult bugs were confined together in frame
cages on potted zucchini plants (cv. Zucchini Elite)
with access to moistened cotton wads as a source
of free water. The cages were then placed in growth
chambers under constant short or long daylength
(27 ± 1°C), or under natural daylength in a room
that was maintained at 26 ± 2°C (Table 2).

Plants were changed weekly or when signs of
wilting or yellowing appeared. Detailed inspec-
tions were made twice a week until reproductive
activity (mating or oviposition) was noticed, and
then daily thereafter. Mating pairs were isolated
in plastic cylinder cages over individual zucchini
plants. When egg masses were found in the group
cage, each female was isolated with a male and
these pairs also were placed in individual cylinder
cages. If there were fewer males than females,
males were rotated among the females every other
day.

On each inspection date, the number and sex of
dead bugs were recorded. Fertility was established
by holding egg masses in shell vials until hatch.
Estimates of diapause duration included the post-
diapause reproductive developmental period and
were computed as the number of days between the
date of collection in the field and the date of first
oviposition. Mating was not used as an indicator of
diapause termination because it was not possible
to make observations frequently enough to deter-
mine all matings that occurred. Also, some females
that copulated appeared to remain in diapause.

Differences in time to oviposition under long and
short daylength conditions were considered as evi-
dence for a photoperiodic maintenance of dia-
pause. Conversely, the photoperiodic maintenance
of diapause was considered terminated when uni-
form oviposition times were observed under all
conditions. The Wilcoxon two-sample test was used
to analyze for differences in preoviposition periods
among photoperiods (Sokal & Rohlf 1981).

**Table 1. The percentage of *A. tristis* females in repro-
ductive diapause 10 d after emergence when reared in the
laboratory under the indicated photoperiods at 27 ± 1°C**

Photoperiod (L:D)	No. adults tested	No. in diapause	% diapause
13:11	16	16	100
13.5:10.5	15	15	100
14:10	14	13	92.9
14.5:9.5	17	2	11.8
15:9	43	5	11.6
15.5:8.5	16	2	12.5

Results

Diapause Induction. Between photoperiods of
14:10 and 14.5:9.5, there was a large decrease in
the percentage of SB females entering diapause (93
to 12%, respectively) (Table 1). All females entered
diapause under photoperiods shorter than 14:10.
On the other hand, under photoperiods of 14.5:9.5
or longer, a high percentage of the females avoided
diapause and reproduced. A relatively low per-
centage of the females that were maintained under
long days did not develop ovaries, and these ap-
peared to be in diapause regardless of photoperiod
(Table 1).

Diapause Maintenance and Termination. Un-
der laboratory conditions, short days (11:13 pho-
toperiod) maintained, and long days (16:8 photo-
period) terminated, diapause in SB females that
were sampled from the field between October and
March (Table 2). Under long daylength (16:8 pho-
toperiod), the median number of days to oviposi-
tion was uniformly short (18 to 24 d) in all samples.
In contrast, the times to oviposition under short
days were very long, ranging from 190 d in October
to 158 d in December. These values were signifi-
cantly (Wilcoxon two-sample test, $P < 0.05$) longer
than those under long days (Table 2). In all samples
after December, most of the individuals died be-
fore terminating diapause.

In February, SB adults that were transferred to
a 14:10 photoperiod terminated diapause and ovi-
posited as quickly as under a 16:8 photoperiod,
whereas under a 13:11 photoperiod, diapause was
maintained for a long time (112 d) (Table 2). Under
natural daylengths, there was a progressive decline
in the duration of diapause with advancing sample
date. The median time to oviposition ranged from
165 d in October to 60 d in March (Table 2).

The photoperiodic maintenance of diapause had
ended in most of the population by the May sample
date; only in this sample were the preoviposition
periods similar under constant long and short day-
lengths and under the natural daylength condition
(Table 2).

The percentage of adult SB mortality varied
among sample dates and between sexes, but it was
consistently higher in both sexes under short days
than under long days (Table 3). With the exception
of the February sample, mortality also was high

Table 2. Median number of days for *A. tristis* females to oviposit after transfer from the field to various laboratory photoperiods on the indicated dates

Photoperiod (L:D)	Sample dates (1984–85)						
	22 Oct.	21 Nov.	22 Dec.	22 Jan.	23 Feb.	22 Mar.	21 May
11:13	190a	162a	158a	—	181	—	10b
	[131–220]	[154–165]	[127–187]	—	—	—	[5–13]
	(4/9)	(4/9)	(3/9)	(0/9)	(1/12)	(0/8)	(6/6)
13:11	—	—	—	—	112	—	—
					(1/12)		
14:10	—	—	—	—	17.5b	—	—
					[11–20]		
					(4/12)		
16:8	19b	20b	22b	19	17b	24b	7b
	[19–33]	[15–55]	[17–31]	[16–26]	[12–19]	[20–27]	[6–10]
	(7/9)	(7/9)	(10/10)	(7/9)	(3/12)	(5/7)	(5/5)
Natural	165a	119a	127a	100	79a	61a	10b
	[130–182]	[92–171]	[100–138]	[92–108]	[66–89]	[58–76]	[8–34]
	(5/9)	(5/9)	(4/9)	(2–10)	(11/12)	(4/8)	(5/5)

Medians within columns followed by a different letter are significantly different at the 5% level (Wilcoxon two-sample test). [N–N], range of values; (N/N), number of ovipositing females/the total number of females per condition. Temperatures: constant photoperiods, $27 \pm 1°C$; natural daylength, $26 \pm 2°C$.

under natural daylengths; these rates were generally similar to those observed under short days (Table 3). Most female mortality in these cases occurred after a relatively long period in the laboratory. By contrast, when mortality did occur under long-day conditions, it nearly always did so within 1–2 wk of collection (Table 3). Under all photoperiods, adult mortality was lowest in the May sample.

Discussion

Diapause Induction. The critical photoperiod for diapause induction in adult female SB falls between photoperiods 14:10 and 14.5:9.5 at 27°C. Although an insect's photoperiodic responses under laboratory conditions may differ from those in nature (see Tauber et al. 1986), it is noteworthy that this laboratory-derived critical photoperiod is very close to the natural daylengths (including civil twilight) experienced by SB adults in the field in northern Kansas during late August. At that time, 50% of the emerging females enter reproductive diapause (Nechols 1987). Thus, although the specific SB stages that respond to diapause-inducing conditions are not known, the combined field and laboratory data suggest that the adult (and possibly late nymphal) stages are sensitive.

The adult is the most sensitive stage in many other insects that undergo reproductive diapause (e.g., de Wilde 1954, Hodek & Cerkasov 1961, Tauber & Tauber 1970; for review, see also Tauber et al. 1986). It is also a sensitive stage in another coreid, the bean bug (*Riptortus clavatus* Thunberg) (Numata & Hidaka 1982, 1983).

A low percentage of the females that were maintained under long days (i.e., 14.5 h or more of light) did not oviposit within 10 d of emergence under 27°C (Table 1). Dissections revealed ovaries with no signs of oogenesis. The condition of the fat body was variable but, in general, it was not well developed; thus, these individuals appeared to be in diapause. Beard (1940) reported a postdiapause preoviposition period of 1–2 wk for an overwintered field population of the SB in Connecticut. However, no published data are available on the preoviposition period of nondiapause SB. In our cultures, SB females usually oviposit within 7–10 d of emergence under temperatures comparable with those used in the experiment. Thus, the complete absence of oocyte development in some females was probably not related to premature dissection times.

The observed variation in the incidence of diapause under long days implies that intraspecific genetic variability exists in the SB's response to diapause-inducing stimuli. This variability may be directly related to the photoperiodic response or to factors that modify this response (e.g., food quality, humidity, etc.). It is not clear whether this variability is expressed under field conditions.

Diapause Maintenance and Termination. The findings (Table 2) indicate that the SB remains sensitive to photoperiod throughout diapause. Thus, although the specific mechanisms and responses underlying diapause cannot be elucidated from the present data, short daylengths appear to play an important role in maintaining diapause. These results are supported by the mortality data (Table 3), which show that SB females that died before ovipositing lived for much longer periods under short daylengths than under long daylengths.

The percentage of female SB mortality was variable, but generally was much higher under short-day conditions (i.e., 11:13 photoperiod and natural daylengths) than under long days (Table 3). This

Table 3. Percentage of adult SB mortality and median number of days to female death under various laboratory photoperiods (27 ± 1°C) for the indicated sample dates

Photoperiod (L:D)	Sex	Sample dates (1984-85)						
		22 Oct.	21 Nov.	22 Dec.	22 Jan.	23 Feb.	22 Mar.	21 May
		% mortality[a]						
16:8	♀	0 (0/8)	22 (2/9)	0 (0/9)	0 (0/8)	73 (8/11)	14 (1/7)	0 (0/5)
	♂	0 (0/7)	0 (0/7)	40 (2/5)	0 (0/3)	58 (7/12)	71 (5/7)	0 (0/9)
	Total	0 (0/15)	12.5 (2/16)	14 (2/14)	0 (0/11)	65 (15/23)	43 (6/14)	0 (0/14)
11:13	♀	37.5 (3/8)	37.5 (3/8)	62.5 (5/8)	100 (7/7)	75 (9/12)	86 (6/7)	0 (0/16)
	♂	50 (3/6)	71 (5/7)	50 (3/6)	50 (4/8)	90 (9/10)	86 (6/7)	17 (1/6)
	Total	43 (6/14)	53 (8/15)	57 (8/14)	73 (11/15)	82 (18/22)	86 (12/14)	4.5 (1/22)
Natural	♀	37.5 (3/8)	44 (4/9)	62.5 (5/8)	67 (4/6)	17 (2/12)	50 (4/8)	17 (1/6)
	♂	12.5 (1/8)	0 (0/7)	25 (2/8)	0 (0/6)	58 (7/12)	86 (6/7)	0 (0/8)
	Total	25 (4/16)	25 (4/16)	44 (7/16)	33 (4/12)	37.5 (9/24)	67 (10/15)	7 (1/14)
		No. d to female death[b]						
16:8		—	15 [2] (7-32)	—	—	6 [8] (3-33)	57 [1] —	—
11:13		124 [3] (124-157)	21 [3] (18-94)	69 [5] (19-126)	120 [7] (17-198)	8 [9] (6-121)	35 [6] (6-84)	—
Natural		140 [3] (122-140)	145 [4] (82-181)	121 [5] (5-138)	43 [4] (20-83)	37 [2] (6-67)	14 [4] (6-39)	13 [1] —

[a] Numbers in parentheses denote the number dying/total number.

[b] Numbers in parentheses denote the range of values; numbers in brackets are the number of females.

mortality may be related, in part, to the depletion of metabolic reserves during the SB's prolonged diapause under the warm experimental conditions (27°C). Short daylengths probably had a direct influence on the very slow rate of diapause development. They also may have had an indirect effect by maintaining a low thermal threshold for diapause development (see Tauber & Tauber 1976, Beck 1980). The high mortality in the February sample under both long and short daylengths is unexplained but, apparently, is related to at least one factor in addition to diapause duration (cf. Tables 2 and 3). The very low mortality observed under all conditions in the May sample is most likely associated with the short preovipositional periods of nondiapausing adults (Table 2).

The progressive decline in preoviposition times under natural daylengths indicates that the intensity of diapause diminishes gradually in the SB throughout autumn and winter (Table 2). Because the duration of diapause in this insect extends well beyond the winter solstice, it is possible that long—or increasing—daylengths play an active role in terminating diapause by accelerating the rate of diapause development. In contrast, diapause in the bean bug (*R. clavatus*) ends during the winter, and long days are apparently not important for its termination (Numata & Hidaka 1984).

The results (Table 2) show that reproductive diapause in the SB persisted through late March, but that its photoperiodic regulation had terminated in most of the population by late May when responses to long, short, and natural daylengths were similar. Thus, unlike many temperate zone insects that end diapause by late autumn or early winter (see Tauber et al. 1986), this species remains in diapause throughout the winter and early spring. Because

temperature conditions are highly variable in Kansas during late winter and early spring (National Oceanic and Atmospheric Administration 1985), a long diapause in the SB probably serves an adaptive function by preventing untimely postdiapause development. For example, natural host plants are not available until late May in Kansas (Barkley 1983), and temperature-sensitive cultivated hosts are not planted until after the frost-free date in early May.

This finding is relevant to the development and implementation of a biological control program for the SB, because it can contribute to an understanding of parasitoid-host synchrony (see Tauber et al. 1983). A knowledge of SB phenology also has useful implications for IPM (e.g., for developing reliable temperature-dependent predictive models [see Tauber et al. 1986]).

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