# Diapause in the Parasite *Diolcogaster facetosa* (Hymenoptera: Braconidae)

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Environ. Entomol. 20(1): 65–70 (1991)

**ABSTRACT** The critical photoperiod for diapause induction was determined in the laboratory and validated in the field for *Diolcogaster facetosa* (Weed), a parasite of the green cloverworm. In the laboratory at constant 24°C, as well as in the field under natural conditions, the critical photoperiod for diapause induction is between 13:11 and 14:10 (L:D). Second instars are sensitive to diapause-inducing photoperiods, and diapause occurs in the final instar after it has emerged from the host and spun a cocoon. Study of diapausing individuals from the field during autumn and winter indicated that photoperiod has only a minor role, if any, in diapause maintenance or termination. The date of diapause termination was not determined, but the combination of diapause development and postdiapause development had not been completed in the field by the spring equinox.

**KEY WORDS** Insecta, *Diolcogaster facetosa*, diapause induction, photoperiod

THE SOLITARY ENDOPARASITE *Diolcogaster facetosa* (Weed) is one of several braconid wasps which attack the green cloverworm, *Plathypena scabra* (F.), in the eastern United States (Harper et al. 1983). The green cloverworm feeds primarily on herbaceous plants, especially wild and cultivated legumes. *D. facetosa* is also known to attack larvae of four other species of Lepidoptera (Krombein et al. 1979), all of which typically feed on trees, although one of them (the redbanded leafroller, *Argyrotaenia velutinana* (Walker)) sometimes feeds on herbaceous plants. None of these Lepidoptera appears to exceed the green cloverworm in terms of its length of seasonal availability as a potential host for the parasite.

*Diolcogaster facetosa* oviposits primarily in second and third instars of the green cloverworm. The second instar is the longest of the parasite's three instars, lasting about a week at 24°C in nondiapausing individuals (Yeargan & Braman 1986). A fifth-instar green cloverworm that is parasitized by *D. facetosa* leaves its host plant about 1 d before the third-instar parasite emerges. The parasite then spins a cocoon at or below the soil surface. We observed that *D. facetosa* larvae that emerged from hosts collected in the field during late summer sometimes entered diapause after they had spun cocoons.

Considerable research has been directed toward overwintering capabilities and migration of the green cloverworm (e.g., Wolf et al. 1987 and references therein), but little attention has been given to seasonal adaptations of its parasites. To understand overwintering and seasonal activity of *D. facetosa* better, we studied several aspects of diapause in this parasite.

Preliminary observations in the laboratory indicated that photoperiod was the primary cue for diapause induction in *D. facetosa*. Our objectives in this study were (1) to determine the critical photoperiod for diapause induction under controlled laboratory conditions, (2) to identify the stage or instar most sensitive to diapause-inducing cues, (3) to compare laboratory results on critical photoperiod with diapause-inducing photoperiods in the field, and (4) to determine the role of photoperiod in the maintenance and termination of diapause in a field population.

## Materials and Methods

For the laboratory experiments, a colony of *D. facetosa* was initiated in 1988 and supplemented in 1989 with parasites reared from green cloverworms collected in Fayette County, Ky. Similarly, a green cloverworm colony was initiated and periodically augmented with field-collected individuals during 1988 and 1989. These colonies were maintained as described by Yeargan & Braman (1986). Voucher specimens of *D. facetosa* were placed in the collection of the Department of Entomology, University of Kentucky, Lexington.

**Critical Photoperiod in the Laboratory.** Parasites were allowed to oviposit in hosts for a 4-hr period; in all cases, oviposition occurred at 24°C and under the same photoperiod as that used for subsequent growth and development of the parasite. Eight photoperiods, all at 24°C, were tested for effect on diapause induction: 9, 10, 11, 12, 13, 13.5, 14, and 15 h of light per 24-h day.

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0046-225X/91/0065–0070$02.00/0 

Green cloverworm larvae were parasitized as second instars and reared on caged soybean plants (growth stage V4–V6 [Fehr & Caviness 1977]) until parasites emerged and formed cocoons. Parasite cocoons were placed individually in 30-ml plastic cups with paper lids. Cups were placed in a plastic box over moist paper towels and observed daily for emergence of adults. Nondiapausing parasites emerged from cocoons within about 1.5 wk after cocoon formation. Approximately 2 wk after last adult emergence, remaining cocoons were dissected to determine their contents. Presence of a live third instar at the time of dissection was interpreted as diapause. Any cocoons containing dead parasites were excluded from data analysis; mortality was low under all photoperiod regimes, averaging only 4.9% (range, 2.0–10.4%). The number of live parasites obtained (i.e., diapausing + nondiapausing) under the eight respective photoperiods listed above were as follows: 66, 33, 48, 46, 72, 43, 85, and 67.

**Sensitive Stage for Diapause Induction.** Procedures were similar to those used for the laboratory study of critical photoperiod, except for the exposure to photoperiods. In this experiment, rather than being reared under the same photoperiod throughout development, parasites were exposed to two different photoperiods during their development: 12 h of light per day (diapause-inducing) and 15 h of light (not diapause-inducing). A given set of parasitized hosts was switched from one photoperiod to the other only once; when one set of parasites was switched from 12 to 15 h of light, a paired set was switched from 15 to 12 h of light (reciprocal switches). The intent was to expose sets of developing parasites to one photoperiod up to a certain point in their development, then expose them to the other photoperiod for the remainder of their development. The number of parasites reared under the various regimes ranged from 18 to 56.

Three sets of reciprocal switches were made: at 5 d following oviposition, at 11 d following oviposition, and 1 d before cocoon formation (i.e., when parasitized green cloverworms left the host plant; hereafter called "wandering stage" hosts). The first of these switches corresponds with the approximate time of molt from first to second instar of the parasite at 24°C, and the second switch occurred during the late second instar. The third switch occurred at the time when the parasite molts from the second to the third instar, which is about 1 d before larval parasite emergence from the host (Yeargan & Braman 1986). Percentage diapause was determined for each set of parasites as described earlier for the laboratory experiments on diapause induction.

**Field Validation of Critical Photoperiod.** Parasitized green cloverworm larvae were collected by sweep net from alfalfa in Fayette County, Ky., on three occasions: 9–11 August, 30–31 August, and 13 September 1989. Only hosts that showed evidence of late stages of parasite development (slight swelling and yellowish coloration of the abdomen) were kept, and these were held in an outdoor cage for parasite emergence. By selecting only those hosts with late-instar parasites inside, we obtained a sample of parasites that varied no more than ±2.5 d in time of cocoon formation. Thus, all individuals in a given sample (e.g., 9–11 August) had experienced similar photoperiods during development.

Parasite cocoons were placed individually in 30-ml plastic cups and were left in the outdoor cage. They were observed daily for adult emergence. About 2 wk after last adult emergence had occurred, or should have occurred (13 September sample), cocoons were dissected as described earlier, and percentage diapause was calculated for each of the three samples.

**Diapause Termination.** Green cloverworm larvae that had been parasitized in the field by *D. facetosa* were collected in Fayette Co., Ky., during September 1989. They were held in an outdoor cage on soybean plants to allow parasite larvae to complete development, emerge from the caterpillars, and form cocoons; all cocoons were formed between 17 and 28 September.

Parasite cocoons ($n$ = 130) were left outdoors in the rearing cage until 12 October 1989, at which time they were randomly assigned to 13 groups of 10 cocoons each and moved to another field site. Each group of 10 cocoons was placed in a separate Petri dish; Petri dishes had screened holes in the tops and bottoms and were filled with soil. All Petri dishes were placed in the ground (tops flush with soil surface) in a soybean field that had been harvested; dishes were lightly covered with wheat straw mulch.

Three randomly selected dishes were brought from the field to the laboratory on 20 November, 20 December, 19 January, and 19 February. The 30 cocoons in each month's sample were randomly assigned in equal numbers to one of three photoperiods: 9.5, 12.25, or 15 h of light per day; all were held at constant 24°C. On 21 March, the one remaining group of 10 cocoons was brought indoors and held at 24°C and 15 h of light per day. Cocoons were checked daily, and dates of adult parasite emergence were recorded. This allowed us to follow progressive changes during autumn and winter in the amount of time required for adult parasite emergence after the cocoons were brought into the laboratory and to determine whether or not photoperiod affected the length of this period.

## Results and Discussion

**Critical Photoperiod in the Laboratory.** The critical photoperiod for induction of diapause in *D. facetosa* at 24°C was between 13 and 14 h of light per day (Fig. 1). The percentage of the population entering diapause decreased at photophases below 11 h. These results best fit the Type I diapause induction curve of Beck (1980).

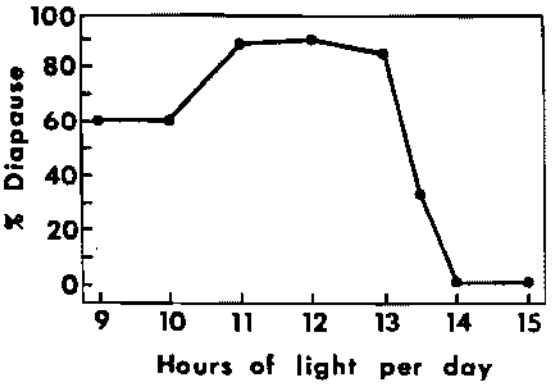


**Fig. 1.** Photoperiodic response curve for diapause induction in *D. facetosa*.

This type of photoperiod-based induction curve is common among insects that undergo autumnal–hibernal diapause in temperate regions of the world (Beck 1980). The decreased incidence of diapause induction at photoperiods below 11 h of light per day probably has no biological significance, because such photoperiods occur in Kentucky only from the end of October until mid-February, a time when neither the parasite nor its hosts are active. The results of this experiment indicate that diapause is likely to be induced in the field when photophases drop below 14 h, a period which corresponds with late August and early September in Kentucky.

It is not known whether photoperiod acts directly on *D. facetosa* to induce diapause or indirectly by altering the host's physiology. The green cloverworm is known to overwinter in Kentucky in the adult stage, apparently in reproductive diapause (Wolf et al. 1987), but diapause induction has not been studied in detail for this host.

**Sensitive Stage for Diapause Induction.** Parasite larvae did not enter diapause when their only exposure to a diapause-inducing photoperiod occurred before day 5 or after day 11 (Fig. 2). Conversely, diapause was observed when the parasites' exposure to a diapause-inducing photoperiod included the developmental period between days 5 and 11, which is during the first 6 d of the second stadium. Thus, the second instar of *D. facetosa* is sensitive to diapause-inducing photoperiods, whereas the eggs and first and third instars are not.

The highest incidence of diapause occurred when larvae were exposed to a diapause-inducing photoperiod for the entire period after day 5 (Fig. 2). This incidence (96.3%) was similar to the highest incidence (91.3%) obtained in the earlier experiment on diapause induction, in which parasite larvae were exposed to a 12-h photophase during their entire egg and larval development (Fig. 1).

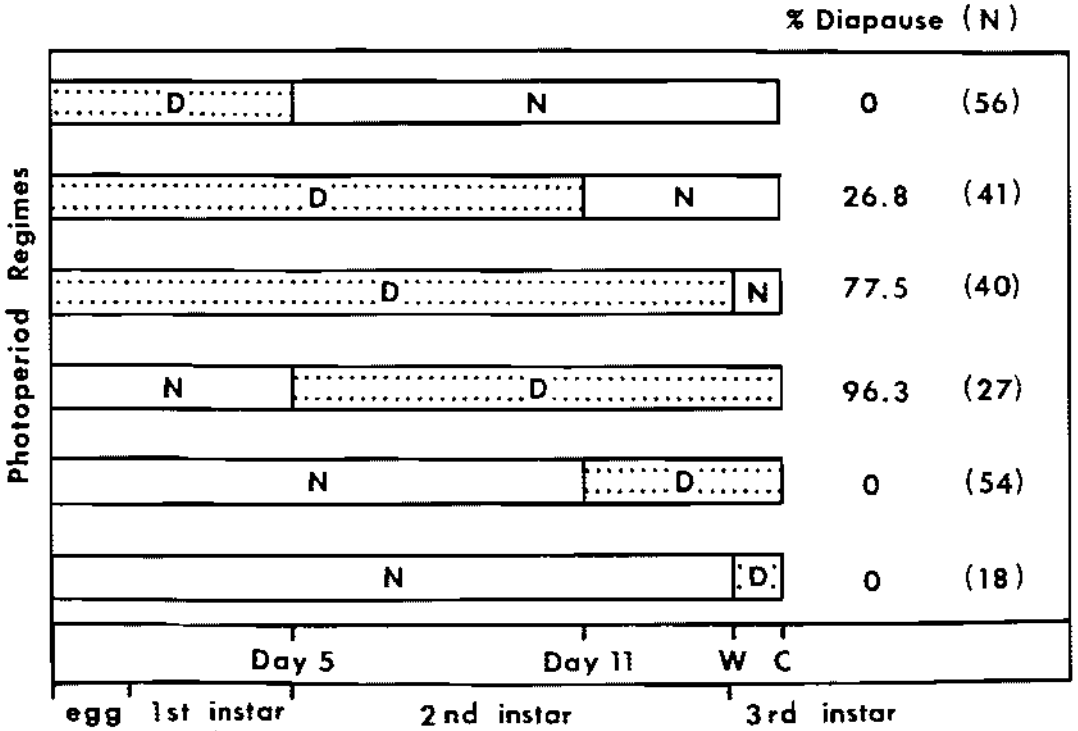


**Fig. 2.** Percentage diapause induction in *D. facetosa* when exposed to a diapause-inducing photophase during different portions of its development; D, diapause-inducing photophase (12 h); N, not diapause-inducing photophase (15 h); constant 24°C. Actual time of switch is shown on upper line at bottom (W, time when host wandered off plant; C, time when parasite cocoon was spun); approximate corresponding stage or instar of parasite is shown on lower line.

**Table 1. Percentage of *D. facetosa* population entering diapause when hosts (green cloverworms) that contained nearly full-grown parasite larvae were collected in the field and held outdoors following collection**

| Collection date(s) | H : min of daylight[a] | *n* | % Diapause |
|---|---|---|---|
| 9–11 Aug. | 13:49 | 67 | 6.0 |
| 30–31 Aug. | 13:04 | 30 | 73.3 |
| 13 Sept. | 12:32 | 44 | 100.0 |

[a] Median periods from sunrise to sunset on date(s) of field collection of parasitized hosts; exposure to ambient photoperiods of the stage of the parasite that is sensitive to diapause induction occurred over several days immediately preceding the respective collection dates.

Larvae that were switched from a diapause-inducing photoperiod to a noninducing photoperiod on day 11 had a much lower incidence of diapause than those similarly switched at the wandering stage (26.8 versus 77.5%). Incidence of diapause in those switched at the wandering stage also was slightly reduced compared with those which spent the entire period following day 5 under a diapause-inducing photoperiod (77.5 versus 96.3%). Keeping in mind that no diapause occurred when parasites were exposed to a diapause-inducing photoperiod only during and after the latter part of the second stadium (i.e., after day 11), it appears that second instars are sensitive to inducing photoperiods during the first 6 d of that stadium (at 24°C). However, induction may be reversed in some individuals by exposure to long daylengths during the latter part of the second stadium or early in the third stadium (Fig. 2).

This is apparently the first study of critical photoperiod and sensitive stage for diapause induction in the genus *Diolcogaster* (formerly *Protomicroplitis*). In several species of *Cotesia*, another genus in the same subfamily of braconids, prepupal diapause is induced by photoperiod acting on the developing parasite larvae (e.g., Rabb & Thurston 1969).

**Field Validation of Critical Photoperiod.** Parasites used in this study were collected when they were late second instars still inside their hosts. We know this because visible external symptoms of parasitism were required for inclusion in our sample, and these first become apparent late during the parasite's second stadium (unpublished data). Hosts containing third-instar parasites would not have been susceptible to collection by sweep net because the host descends from the plant at about the time the parasite molts from second to third instar. Thus, it is reasonable to assume that the sensitive stage (early second instar) of the parasite had occurred under the daylengths prevailing in the field during the days immediately preceding collection. By comparing those photoperiods with the observed incidence of diapause in our sample, we were able to determine the validity of our laboratory-derived critical photoperiod for prediction of diapause induction under field conditions.

**Table 2. Time necessary for *D. facetosa* adults to emerge after cocoons were brought from the field to the laboratory and placed at constant 24°C**

| Date of field sampling | Mean no. d (SEM) required at photophases of | | |
|---|---|---|---|
| | 9.5 h | 12.25 h | 15.0 h |
| 20 Nov. | 46.5 (5.5) ($n$ = 2)[a] | 43.0 (10.0) ($n$ = 2) | 46.0 (—) ($n$ = 1) |
| 20 Dec. | 29.3 (1.6) ($n$ = 6) | 33.9 (2.5) ($n$ = 7) | 30.7 (1.6) ($n$ = 7) |
| 19 Jan. | 25.0 (1.2) ($n$ = 7) | 25.9 (1.1) ($n$ = 7) | 23.7 (0.8) ($n$ = 9) |
| 19 Feb. | 19.1 (1.2) ($n$ = 8) | 17.1 (0.6) ($n$ = 9) | 17.0 (1.6) ($n$ = 5) |
| 21 March | — | — | 8.4 (0.4) ($n$ = 7) |

[a] On 23 February 1990, 95 d after field collection, those cocoons in the November sample from which nothing had emerged were dissected; the cocoons contained 7, 8, and 6 live, diapausing larvae at the three respective photoperiods shown above. Most or all live parasites in the subsequent samples completed diapause and emerged as adults in the laboratory.

Only 6% of the parasites collected during the second week of August entered diapause (Table 1). During their sensitive stage, these parasites had been exposed in the field to daylengths (sunrise to sunset) between 13.75 and 14 h. Hosts collected at the end of August contained parasites whose sensitive stage had been exposed to daylengths of slightly >13 h, and about 73% of those parasites entered diapause. All parasites collected in mid-September entered diapause; their sensitive stage had been exposed to daylengths of <13 h.

It is well known that critical photoperiods can vary with temperature (Tauber et al. 1986). For insects with autumnal-hibernal diapause, lower temperatures typically cause the critical photoperiod to shift toward higher daylengths, and vice versa. During this study, mean daily temperatures near the site where parasitized hosts were collected averaged 22.8°C during the period from first to last sampling date. Thus, mean temperatures in the field during this period were similar to the temperature (24°C) used in the laboratory study of critical photoperiod. The field study supports our conclusion that the critical photoperiod for diapause induction in *D. facetosa* is between 13 and 14 h of light per day.

**Diapause Termination.** Of the 30 cocoons removed from the field in November, only five produced adult parasites in the laboratory, and those required an average of about 45 d for emergence (Table 2). Of the remaining cocoons in the November collection, 21 still contained live parasite larvae when the cocoons were dissected 95 d after they were brought indoors (4 contained dead parasites, 3 of which were immature at time of death). Those 21 larvae were evenly distributed among the photoperiod regimes, with 7, 8, and 6 found in cocoons held under photophases of 9.5, 12.25, and 15 h, respectively. These results suggest that the

larvae were in diapause when removed from the field in November and that the majority had not been able to complete diapause development at 24°C under any of the photoperiods used. Diapause development may have been retarded by the warm temperature (24°C) under which those parasites were held in the laboratory.

More than 70% of the cocoons brought indoors in December, January, and February produced adult parasites within 3–5 wk, with the average time until emergence decreasing about 1 wk for each additional month that the cocoons remained outdoors before being brought into the laboratory. These results contrast markedly with those from the November sample, in which few parasites completed diapause within a 3-mo period. Tauber et al. (1986) noted that, in general for insects which undergo autumnal–hibernal diapause, lowering of thermal thresholds is most pronounced during early autumn. As autumn proceeds, these thresholds often rise. Perhaps the upper threshold for diapause development was below 24°C for the parasites in the November sample.

For each monthly sample after November, cocoons from which no parasites had emerged were dissected at 7–9 wk after they were brought indoors; this was long after adult emergence had ceased. Most of these residual cocoons contained dead parasites, but three in the December sample and one in the January sample (all from the 9.5-h photophase) contained live, presumably diapausing, larvae at the time of dissection. No other such cases were found in the subsequent samples. Overall, the hyperparasite *Mesochorus discitergus* (Say) (Hymenoptera: Ichneumonidae) emerged from three of the 130 cocoons, one each from the January, February, and March samples.

Based on the samples taken from the field during November through February, there was no evidence that photoperiod affected the time required for parasite emergence (Table 2). These results contrast with those from the limited number of similar studies of diapausing parasitic Hymenoptera sampled periodically from the field, in which photoperiod generally played an important role in diapause maintenance and termination (Claret 1973, Obrycki & Tauber 1979, Nechols et al. 1980). In those studies, parasites placed under short daylengths during autumn or winter or both required much longer to resume postdiapause activity than those placed under long daylengths. This was interpreted as evidence that short daylengths maintain diapause. In one of those studies (Nechols et al. 1980), temperature also played a role in diapause maintenance. Temperature, rather than photoperiod, may be the primary factor responsible for diapause maintenance in *D. facetosa*. Other possibilities not addressed in our investigation include gradually changing daylengths rather than absolute daylength, and interactions between temperature and photoperiod.

The group of 10 cocoons that was brought indoors on 21 March was placed under a 15-h photophase. Parasites in those cocoons required only 8.4 d to emerge, on average. Nondiapausing *D. facetosa* require 9–10 d to complete development from cocoon formation to adult emergence at 24°C and 15 h of light per day (Yeargan & Braman 1986). It must be pointed out, however, that developmental thresholds and thermal requirements for postdiapause development may not be the same as those for development of nondiapausing individuals. Thus, we do not know exactly when diapause terminated in the field. We can, nonetheless, conclude that the combined period for completion of diapause and postdiapause development in *D. facetosa* under field conditions lasted throughout the autumn and winter and had not been completed by the spring equinox.

Even though parasites in the 21 March sample emerged in about 8.4 d at constant 24°C when brought into the laboratory, it is likely that several weeks would be required before adult emergence occurs in the field. The parasite normally forms cocoons at or below the soil surface, and soil temperatures remain relatively cool during March and April (monthly soil temperatures beneath grass typically average about 7 and 12°C, respectively, during those months in central Kentucky).

One of this parasite's few known hosts, the green cloverworm, overwinters in Kentucky as an adult moth (Wolf et al. 1987). Green cloverworm larvae are available to the parasite as early as the end of April in central Kentucky, and we have collected green cloverworms parasitized by *D. facetosa* as early as 10 May (K.V.Y., unpublished data). As noted in this study (Table 1), a few adult parasites (<30%) emerge in the field in early September; because green cloverworm oviposition continues into early September (Sloderbeck & Yeargan 1983), young larvae suitable for attack by this parasite are available during this month. Although we occasionally find late instars of the green cloverworm in the field in Kentucky during October, these are generally too large for *D. facetosa* to attack (Yeargan & Braman 1986).

Thus, instars of the green cloverworm suitable for ovipositon by this parasite are available in Kentucky from late April through September. Diapause in *D. facetosa* provides an effective mechanism for seasonal synchronization with the green cloverworm, permitting the parasite to exploit this host from spring until early autumn.

**Acknowledgment**

P. M. Marsh (Systematic Entomology Laboratory, USDA) kindly provided identification of *D. facetosa*. M. J. and C. A. Tauber provided helpful suggestions on the diapause termination portion of this study. We thank D. L. Dahlman and D. A. Potter for critically reviewing an earlier version of the manuscript. The investigation reported in this paper (90-7-115) is in connection with a project of the Kentucky Agriculture Experiment Station and is published with approval of the director.

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*Received for publication 10 July 1990; accepted 28 August 1990.*