EFFECT OF FOOD AND PERHAPS LARVAL CROWDING ON DIAPAUSE INITIATION IN TUFTED APPLE BUD MOTH (LEPIDOPTERA: TORTRICIDAE)[1]

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ABSTRACT

Larvae from a laboratory strain of tufted apple bud moth, *Platynota idaeusali* (Walker), were reared on semisynthetic diet and excised apple leaves while exposed to naturally-decreasing daylengths. Leaf-reared larvae showed a higher incidence of diapause than larvae reared on diet. The critical daylength for diapause induction in leaf- and diet-reared larvae occurred at ca. 14.8 and 14.3 h, respectively. Larvae reared on semisynthetic diet and exposed to naturally-decreasing daylengths were subjected to different levels of crowding. As the number of individuals reared together in the same container increased, the incidence of diapause increased. Separation of the effects of larval crowding from any possible effects of the quality of the semisynthetic diet on diapause induction was not attempted.

Key Words: Tufted apple bud moth, *Platynota idaeusalis,* larval diapause, food, larval crowding.

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INTRODUCTION

Diapause is an adaptive mechanism which enables an insect to synchronize its active stages or morphogenesis to suitable environmental conditions, thus facilitating the insect's survival during unfavorable periods. A knowledge of the processes involved in the onset, maintenance, and termination of diapause is important not only because it contributes to a greater understanding of insect population dynamics, but also because it may provide a focal point for the development of effective pest management strategies (Tauber and Tauber 1973a).

Of the many exogenous factors implicated as being involved in the induction of diapause of various insect species, photoperiod has been shown to be of the greatest importance (Beck 1980). The effects of photoperiod may be modified, however, when the photoperiodic response by an insect is evaluated in combination with other environmental factors (Beck 1980). Rock et al. (1983) demonstrated a photoperiodically-controlled larval diapause of the short-day, long-day type (Beck 1980) in the tufted apple bud moth, *Platynota idaeusalis* (Walker), an important insect pest of apple in North Carolina. Larval diapause was determined by the failure to reach larval-pupal ecdysis by a certain age and this age was temperature dependent. The relationship between photoperiod and temperature was examined

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in the laboratory, and a critical photoperiod for diapause induction (constant 21°C) was determined (Rock et al. 1983). Factors other than temperature may also serve to modify an insect's photoperiodic response, including nutrition (Morris 1967) and crowding (Iwao 1968). The purpose of this investigation was to observe the temporal patterns of diapause induction in *P. idaeusalis* under naturally-occurring daylengths, and how these patterns may be influenced by food and larval density.

MATERIALS AND METHODS

The initial stock of *P. idaeusalis* was obtained from egg masses collected in several apple orchards near Hendersonville, NC (35° 20′ N latitude, 82° 28′ W longitude). Larvae were laboratory-reared on a semisynthetic diet as described by Rock and Monroe (1983) for ca. 9 and 18 generations before use in the 1980 and 1981 experiments, respectively. A new stock colony was begun in the autumn of 1981 from the same collection sites, and maintained as described above for ca. 10 generations before use in the 1982 study.

On several dates during late summer to early autumn, unfed neonates were inoculated singly (unless indicated otherwise) into 10-ml plastic cups containing semisynthetic diet. The cups were then capped and transported to an open air shelter in an apple orchard near Clayton, NC (35° 39′ latitude, 78° 30′ longitude). Measurements of percent cumulative pupation among all inoculation dates were made periodically until November, in order to demonstrate the temporal pattern of development of *P. idaeusalis* exposed to naturally decreasing daylengths. Diapause was determined by the failure to reach larval-pupal ecdysis by 1 February the following year. The critical photoperiod for diapause induction refers to the amount of light per day at which diapause occurs in 50% of the individuals tested (Danilevsky et al. 1970), and was extrapolated from a linear regression of the data.

Daylength refers to the time measured in hours and minutes elapsing between sunrise and sunset, including civil twilights. Daylengths occurring in the experimental orchard were estimated from a graphical representation of the data presented by Beck (1980) at 35° N latitude.

Statistical analysis was performed using prepared computer programs of the Statistical Analysis System (SAS Institute 1982). The X-axis of all regression equations was scaled, so that an inoculation date of 30 July, 31 July, or 1 August would be represented as -1, 0, and 1, respectively.

To determine the effects of food source on diapause induction, neonates were inoculated on either excised apple leaves, or on semisynthetic diet. Leaves were collected from unsprayed trees in the Clayton orchard, rinsed in water, surface-dried, and placed into shell vials (25 × 95 mm). Following larval inoculation, the vials were sealed with a cork stopper and transported to the orchard open-air shelter. In 1981, an equal mixture of leaves collected from 'Golden Delicious' and 'Stayman' cultivars was used, while in 1982 leaves were used from the 'Delicious' cultivar. Larvae were transferred to fresh leaves every 10 - 14 days. Larvae in cups containing semisynthetic diet were not transferred to fresh diet-filled cups, unless the original diet showed signs of excessive drying. Transportation of leaf- and diet-reared larvae to the open-air shelter began on 28 July and continued at 3 - 5 day intervals until the end of August. Larvae were inoculated singly and in pairs per food source in 1981 and 1982, respectively.

The possible effect of larval crowding on diapause induction was evaluated in 1982. Neonates were inoculated in groups of 1, 2, or 4 individuals per cup containing semisynthetic diet and transported to the orchard open-air shelter. Four inoculation dates were used, beginning on 6 August and ending on 16 August. Approximately 60 cups per group were transported on each inoculation date.

As the number of neonates inoculated per cup did not necessarily equal the number in a group that survived within a few days following inoculation, all groups were examined after 20 days and reclassified according to the number surviving per cup. Although the choice of the 20-day examination date was arbitrary, laboratory observations have shown major mortality to occur within 1st-stadial larvae, even when reared singly (Boyne and Rock, unpublished data).Thus, any initial crowding effects, followed by the subsequent death of one or more individuals within a cup prior to the 20-day examination period, probably did not play a major role in the results obtained.

RESULTS AND DISCUSSION

The importance of food as a modifying influence on diapause induction was evident during both years (Table 1). Larvae maintained on excised apple leaves pupated later (thus, initiated diapause earlier) than those reared on semi-synthetic diet. Moreover, diapause was essentially determined in leaf-fed larvae by November, as indicated by the absence of further pupation between November and February. Diet-fed larvae, however, continued to pupate within this time period, suggesting that a less intense diapause occurred in these individuals.

The photoperiod at the time of diapause induction on each food source was predicted by fitting a regression line to the data (Fig. 1). For diet-reared larvae, the average critical daylength was 14.3 h (between 15 - 17 August) for both 1981 and 1982, the same value obtained in 1980 (the equations for 1981 and 1982 were: $Y = 95.25 - 2.67X$; $R^2 = 0.87$, and $Y = 90.65 - 2.56X$; $R^2 = 0.96$, respectively). The critical daylength for diapause induction in leaf-reared larvae larvae was 14.7 h (on 3 August) in 1981 and 14.9 h (on 29 July) in 1982 (the regression equations for 1981 and 1982 were: $Y = 66.06 - 5.28X$; $R^2 = 0.89$, and $Y = 39.05 - 4.83X$; $R^2 = 0.72$, respectively). Thus, the critical daylength for diapause induction in larvae maintained on foliage occurred at least 2 weeks earlier than larvae maintained on semisynthetic diet.

Reports in the literature suggest that food may be a major factor in modifying diapause incidence not only among phytophagous insects (Morris 1967), but also among predators (Tauber and Tauber 1973b) and parasites (McNeil and Rabb 1973). In some species, lack of food quality or quantity or both may act by inhibiting developmental rates, thus exposing the insect to a greater number of diapause-inducing photoperiods (Danilevsky et al. 1970). Recent laboratory investigations have shown that *P. idaeusalis* larvae reared on semisynthetic diet develop significantly faster than larvae maintained on excised apple leaves, when both are exposed to diapause-averting photoperiods (Boyne et al. 1985). Therefore, differences in food quality may increase in significance when experienced in combination with photoperiods approaching the critical daylengths for diapause induction. In the fall webworm, *Hyphantria cunea* Drury, larvae reared on late-

Table 1. Effect of food source on percent cumulative pupation in *P. idaeusalis* maintained under naturally-occurring daylengths.

Inoculation	1981						1982					
	Semisynthetic diet			Leaves			Semisynthetic diet			Leaves		
		% Pupation by:			% Pupation by:			% Pupation by:			% Pupation by:	
date*	N†	Nov. (81)	Feb. (82)	N	Nov. (81)	Feb. (82)	N	Nov. (82)	Feb. (83)	N	Nov. (82)	Feb. (83)
28 July	43	100	100	25	92.0	0	103	96.1	97.1	55	70.9	70.9
31 July	–	–	–	–	–	–	103	92.2	92.2	22	22.7	22.7
3 August	42	90.5	95.2	11	27.3	27.3	95	85.3	90.5	20	15.0	20.0
6 August	48	66.7	70.2	18	44.4	44.4	97	74.2	81.4	57	14.0	14.0
9 August	54	44.4	64.8	36	8.3	8.3	90	35.6	64.4	39	0	0
12 August	56	37.5	55.4	43	0	0	101	23.8	48.5	31	0	0
16 August	56	44.6	73.2	44	0	0	106	31.1	49.1	39	2.6	2.6
19 August	56	14.3	37.5	36	0	0	106	16.0	49.0	40	0	0
23 August	54	16.7	40.7	38	0	0	94	7.4	31.9	–	–	–
26 August	–	–	–	–	–	–	110	2.7	28.2	–	–	–
29 August	62	11.3	12.9	40	0	0	97	0	11.3	–	–	–

* Dates listed are within 1 day of actual inoculation date for either year.
† Number of individuals.

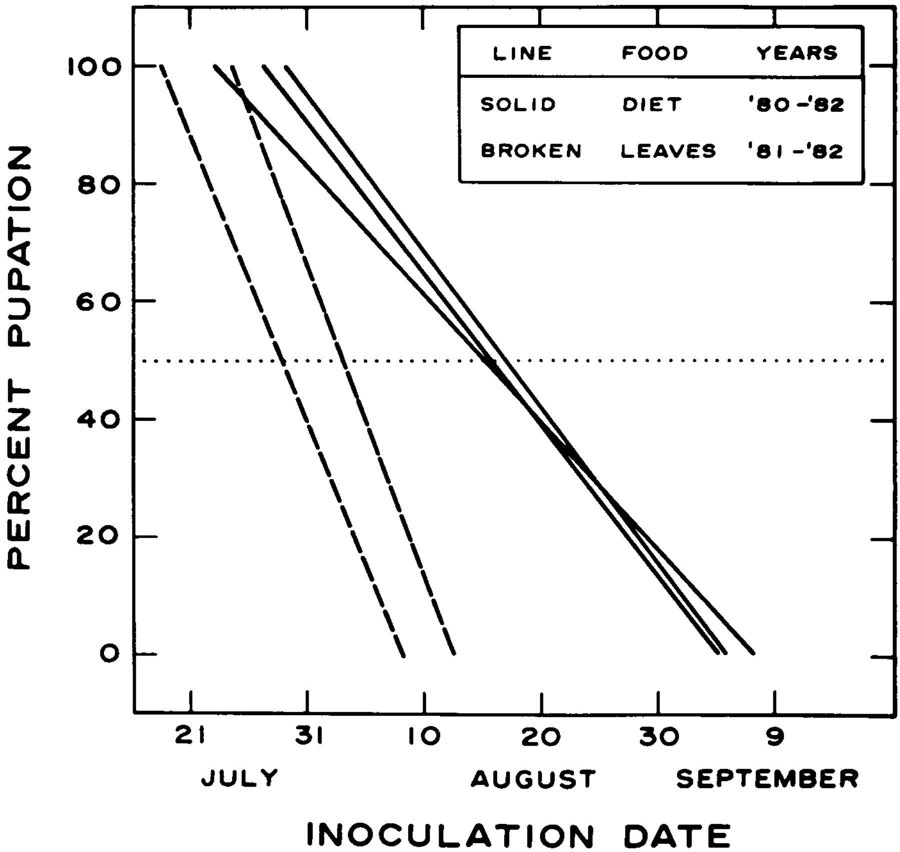

Fig. 1. Effect of food on diapause incidence in *P. idaeusalis* at different inoculation dates. Data and equations used to produce regression lines are discussed in text. The date of the critical photoperiod for diapause induction for each food source is determined by following the point of intersection of the regression line and the dotted horizontal line to the abscissa.

season apple foliage exhibited 100% diapause under the same photoperiodic conditions that would produce only 16 - 52% diapause in larvae maintained on early-season foliage (Morris 1967). Cox et al. (1981) reported that diapause incidence in the flour moth, *Ephestia kuehniella* Zeller, was greater when larvae were reared on natural food sources than when reared on artificial diet, similar to the results obtained in our investigation.

The relationship between larval density on semisynthetic diet and diapause induction at different inoculation dates is shown in Fig. 2. Diapause incidence increased as larval densities increased for each inoculation data. Using the data from Fig. 2, a multiple regression equation ($Y = 120.23 - 3.03X + 4.46Z$, $R^2 = 0.90$; where $Y = \%$ pupation, $X =$ inoculation date, and $Z =$ larval density per cup) was computed. The fitted response function was highly significant ($P <$

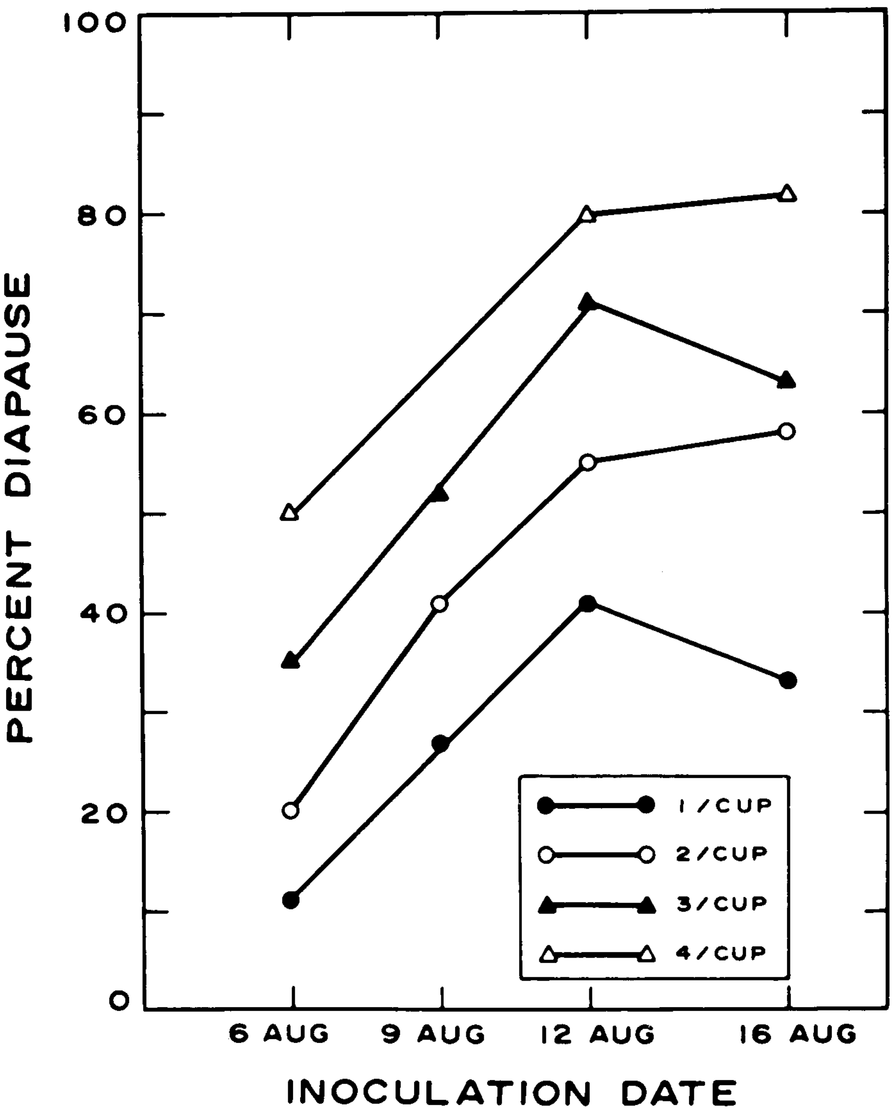

Fig. 2. Relationship between larval density on semisynthetic diet and diapause incidence in *P. idaeusalis* at different inoculation dates. Approximately 60 cups per group were transported on each inoculation date.

0.0001), indicating that development in *P. idaeusalis* is related to both larval density and time of inoculation. The relationship between larval crowding and incidence of diapause was most evident at the earliest inoculation dates (6, 9, 12 August), implying that the addition of one larva per cup (up to at least 4 larvae per cup) has roughly the same effect as delaying inoculation in the field by 2 - 3 days (Fig. 2). By 16 August, however, the rate of diapause incidence began to decelerate, suggesting that crowding effects may be more pronounced during photoperiods prior to the critical daylength for diapause induction.

Only a few studies on the effects of crowding on diapause induction have been reported. Iwao (1968) obtained increases in diapause incidence when rice green caterpillars, *Nagranga aenescens* Moore, were aggregated into rearing containers. Similarly, Tsuji (1963) demonstrated that a proportion of individuals in moderately crowded populations of the Indian meal moth, *Plodia interpunctella* Hubner, would enter diapause, even under diapause-averting photoperiods and temperatures. Development is accelerated by moderate crowding in some species, but retarded in others (Iwao 1968). Comparisons of density effects among *P. idaeusalis* larvae reared in our laboratory under diapause-averting conditions, have shown that developmental rates are reduced at a density of 4 per cup as compared to 1 per cup on semisynthetic diet (Smart, unpublished data). Therefore, a delay in development would be more significant if it occurred near the critical photoperiod, since a larva would be exposed to a greater number of decreasing daylenghts, which would enhance the probability of diapause induction. Separation of the effects of crowding from the possible associated effects of reduced food quality or quantity or both is difficult (Tauber et al. 1984). We did not attempt to separate larval crowding from the effects, if any, of the quality or quantity of the semisynthetic diet.

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