References

Alston, R. E., and B. L. Turner. 1963. Biochemical systematics. Prentice-Hall, Inc., New Jersey.

Atwood, C. E., and O. Peck. 1943. Some native sawflies of the genus *Neodiprion* attacking pines in Eastern Canada. *Can. J. Res.* Sect. D., Zool. Sci. **21**: 109–144.

Barlow, J. S. 1964. Fatty acids in some insect and spider fats. *Can. J. Biochem.* **42**: 1365–1374.

Brenner, M., and A. Niederwieser. 1960. Dünnschicht-Chromatographie von Aminosaüren. *Experientia* **16**: 378–383.

Fahmy, A. R. *et al.* 1961. Dünnschicht-Chromatographie von Aminosaüren auf Kieselgel G. Eine Schnellmethode zur Trennung und zum qualitativen Nachweis von 22 Aminosaüren. *Helv. Chem. Acta* **44**: 2022–2026.

Moffat, E. D., and R. I. Lytle. 1959. Polychromic technique for the identification of amino acids on paper chromatograms. *Anal. Chem.* **31**: 926–928.

Ross, H. H. 1955. The taxonomy and evaluation of the sawfly genus *Neodiprion*. *For. Sci.* **1**: 196–209.

Schaefer, C. H. 1964. Free amino acids of the Virginia pine sawfly. *Neodiprion pratti* Dyar: their chromatographic determination and biosynthesis. *J. Insect Physiol.* **10**: 363–369.

Schaefer, C. H. 1965. Fatty acids of the Virginia pine sawfly, *Neodiprion pratti* Dyar. *Can. Ent.* **97**: 941–945.

Wallace, D. R. 1964. Spectrophotometry in the taxonomy of the conifer sawflies. *Can. Ent.* **96**: 162.

Wallace, D. R., and I. M. Campbell. 1965. Method for characterizing sawfly egg pigmentation. *Nature, Lond.* **207**: 1363–1364.

West, A. S. *et al.* 1958. Systematics of *Neodiprion* sawflies. I. Preliminary report on serological and chromatographic studies. *Rep. ent. Soc. Ontario* **89**: 59–65.

Whittaker, J. R., and A. S. West. 1962. A starch gel electrophoretic study of insect hemolymph proteins. *Can. J. Zool.* **40**: 655–671.

(Received 28 December 1966)

THE EFFECTS OF PHOTOPERIOD, TEMPERATURE, AND FOOD SUPPLY ON RATE OF DEVELOPMENT AND DIAPAUSE IN *COCCINELLA NOVEMNOTATA*[1,2]

R. D. McMullen

Research Station, Canada Department of Agriculture, Summerland, British Columbia

Abstract *Can. Ent.* 99: 578–586 (1967)

The optimum temperature for rate of development and survival of immature stages of *Coccinella novemnotata* Herbst was found to lie between 70° and 80°F. Different photoperiods between 10 and 18 hours per day did not influence rate of development or survival. With a 16-hour photoperiod the mean number of eggs laid per female and mean longevity of females were greater at 70° than at 80°F, but not significantly so. At 90°F egg production was sharply curtailed and most eggs produced were infertile, probably due to inactivation of sperm in the male. Photoperiods of 10, 12, and 18 hours per day induced diapause in a large percentage of adult females; intermediate photoperiods of 14 and 16 hours per day were much less effective. Low temperature and lesser amounts of food available to adult females increased the effectiveness of the short and the long photoperiods for inducing diapause. The stage susceptible to induction of diapause, or conversely the initiation of gonad maturation, was determined to be the young adult from emergence to 7 days of age. The results of experimental data are related to a field study of the biology of this insect in California.

[1]Extracted from a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of California, Berkeley, 1964.

[2]Contribution No. 204 of the Research Station.

Introduction

The results of a field investigation of the biology of *Coccinella novemnotata* Herbst and the physical and biological factors probably influencing diapause have been presented in an earlier paper (McMullen 1967). Briefly, there are two generations per year in the San Joaquin Valley of California. The adults of the spring generation pass the hot, dry, summer months in diapause and lay eggs in the early fall. The adults of the fall generation pass the winter in diapause and reproduce in the early spring. The spring generation is subjected to a regimen of increasing temperatures and long, increasing photoperiods. The fall generation is subjected to a regimen of decreasing temperatures and short, decreasing photoperiods. Prior to the advent of land cultivation and irrigation, native prey species of *C. novemnotata* were abundant only in the spring and fall. Hagen (1962) hypothesized that the diapause pattern of *C. novemnotata* is an adaptation to survive seasonal inclemencies and scarcity of prey. This paper presents data on the influence of photoperiod, temperature, and amount of food supply on rate of development, mortality of immature stages, adult fecundity, and induction of diapause.

Methods

The pea aphid, *Macrosiphum pisi* (Harris), was used exclusively as prey for rearing *C. novemnotata*. The aphids were reared in a greenhouse on alfalfa plants potted in vermiculite and watered with Hoagland's solution. For experiments requiring measured quantities of food, large mature aphids of uniform size were selected by passing them through two screens of different mesh sizes. Aphids of various sizes were used for mass rearing of the stock colony.

A stock colony of *C. novemnotata* was maintained in a rearing cabinet at $70° \pm 1.5°$F with a daily photoperiod of 16 hours. Relative humidity in the cabinet varied between 15 and 40%. Eggs were obtained by placing pairs of recently emerged males and females in 8-dram shell vials, one pair per vial, stoppered with a plug of nonabsorbent cotton. Pea aphids, as food, were supplied ad libitum. The males of each pair were removed after 48 hours to reduce cannibalism of eggs laid by the female. Once oviposition commenced the females were transferred daily to new vials and the old vials containing eggs were set aside in the rearing cabinet. As the larvae emerged from the eggs, sufficient small aphids were added to each vial to provide food for 1 or 2 days. When fresh aphids were supplied the larvae were usually transferred to clean vials. Eight-dram shell vials were suitable for rearing approximately 100 larvae per vial through the first and second instars. Third and fourth instar larvae were reared in clear plastic boxes, $1'7 \times 64 \times 64$ mm, with 2-in. diameter circular holes cut in either end. The holes were covered with nylon organdy. A small bouquet of alfalfa stems and leaves held in a water-filled 3-dram vial by a plug of nonabsorbent cotton was placed in each box. The bouquets served to maintain the aphids in a fresh state, and because the aphids were able to reproduce on the alfalfa bouquets, the bouquets also served to economize the aphid supply. The boxes were examined daily to determine if additional aphids were necessary. The larvae were transferred to clean boxes with fresh alfalfa bouquets every 3 to 5 days. These boxes were adequate for rearing 25 to 40 third instar larvae or 12 to 15 fourth instar larvae. Larger numbers of larvae per container resulted in excessive losses due to cannibalism. When mature, the larvae were removed from the boxes and placed individually in 8-dram shell vials. This was necessary because the pupal stage is particularly vulnerable to cannibalism by larvae.

The influence of temperature and photoperiod on the rate of development of immature stages and ovary maturation in female beetles was determined at 60°, 70°, 80°, and 90°F with a photoperiod of 16 hours per day, and at 70°, 80°, and 90°F with a photoperiod of 10 hours per day. Lots of newly oviposited eggs from females of the stock colony were placed in 8-dram shell vials, stoppered with a cotton plug, at the beginning of each test. The number of hours from oviposition to eclosion was recorded for each lot. Then, from each lot of eggs, 10 to 15 larvae were reared individually in similar vials. Each larva was provided with an excess of aphids daily and the dates of larval moults were recorded. The females obtained were mated and the longevity and number of eggs produced by each were recorded.

The effects of different levels of temperature, photoperiod, and the amount of food available to the recently emerged adult upon diapause induction in females were determined under the combinations of these factors (Table III). Except for three treatments, marked with a dagger in Table III, all of the individuals in each treatment were reared from egg to adult at the temperature and photoperiod particular to the treatment. The eggs used in the experiment were obtained from the stock colony which was maintained at 70°F with a 16-hour photoperiod. During the larval stages food was provided to excess. After emergence of the adult the quantity of food was restricted as noted. Each female beetle was confined individually in an 8-dram shell vial stoppered with a cotton plug, except for the first 24 hours after emergence when a male beetle was included for mating.

The criteria employed to diagnose diapause or nondiapause status in this experiment were (1) failure of a female to oviposit within a certain period after emergence and copulation with a male and (2) the condition of the ovaries and the degree of fat body development after a specified period. Females which had not produced eggs after 21 days at 60°F, 14 days at 70°F, 10 days at 85°F, and 7 days at 90°F were highly suspect of being in diapause. This preliminary diagnosis was checked by dissection and examination of the ovaries and fat body tissue. Absence of ovogenesis in ovules and presence of moderate to large amounts of fat body tissue confirmed a diagnosis of diapause.

To establish the stage, or stages of development responsive to stimuli that determine diapause induction or nondiapause development, eggs, larvae, pupae, and adults of various ages were transferred from an environment which previously had been determined to allow nondiapause development (70°F and 16-hour photoperiod), to one which had been determined to induce diapause (70°F and 10-hour photoperiod), and vice versa. Control treatments were reared without transfer under both of the treatment conditions. The number of beetles of each treatment in diapause at the end of the experiment was determined by examination of the ovaries on the fourteenth day after emergence from the pupal stage.

Results and Discussion

Effects of Temperature and Photoperiod on Rate of Development, Premature Mortality, and Adult Fecundity

No differences in rate of development were observed between treatments of a 10-hour photoperiod compared with treatments of a 16-hour photoperiod at equivalent temperature. This agrees with Hodek's (1957) results with *Coccinella septempunctata* L. The rate of development of all immature stages was greatest at 90°F and least at 60°F. The greatest increase of relative growth rate per 10°F temperature increment occurred between 70° and 80°F, and the least between 80° and 90°F. Since immature mortality was negligible at lower temperatures and

only 5% at 80°F, compared with 36.6% at 90°F, the optimum temperature for the development of immature *C. novemnotata* is near 80°F (Table I).

Data showing the effects of various temperatures at a 16-hour photoperiod on the preoviposition period, the number of eggs produced per female, and adult longevity are summarized in Table II. Data for a 10-hour period are not given because this short photoperiod induces diapause in the adult. The preoviposition period, number of eggs produced per female, and average longevity decreased with increase of temperature. The numbers of eggs produced per female and longevity were severely reduced at 90°F, compared with 70° and 80°F, and, as noted in Table II, the viability of eggs produced in the 90°F treatment was reduced. Eggs produced by females held at 90°F were normal in appearance for 1 to 2 days. After the second day they turned dark orange-brown, the contents coagulated, and the egg finally shrivelled.

To determine the cause of nonviable egg production, part of the experiment was repeated. Copulation and transfer of sperm was found to be normal at 90°F. However, sperm from males reared at 90°F were nonmotile while sperm from males reared at 70°F were motile. When females reared and held at 90°F were mated with males reared at 70°F, 87% of the eggs produced were viable. Females reared at 90°F until 1 week old and then transferred to 70°F and mated with males reared at 70° or 90°F produced eggs which were 100% and 7% viable, respectively. One?week-old females reared at 70°F, transferred to 90°F, and mated with males reared at 90°F produced eggs 100% nonviable. It may be concluded that a constant temperature of 90°F induces sterility in male *C. novemnotata.*

The Influence of Photoperiod, Temperature, and Food Supply on Diapause Induction

As shown in Table III, short photoperiods of 10 and 12 hours per day and long photoperiods of 18 hours per day induced diapause in a large percentage of the females in these treatments. Only a small percentage of the females in treatments with intermediate photoperiods of 14 and 16 hours per day entered diapause. In treatments with photoperiods of 10, 12, 14, and 18 hours per day, higher temperatures and larger food supplies reduced the number of females entering diapause.

Photoperiod has been recognized for a number of years as one of the chief environmental factors regulating diapause in insects. In the case of a species closely related to *C. novemnotata,* Hodek and Cerkasov (1961) reported that short photoperiods and low temperatures induced diapause in *C. septempunctata* L.; long photoperiods and high temperatures prevented diapause induction. Also, excess food tended to inhibit the influence of short photoperiods and low temperatures on diapause induction. Availability of food has also been shown to influence diapause induction in *Leptinotarsa decemlineata* Say by Faber (1949) and De Wilde *et al.* (1959). In this species the proportion of individuals entering diapause increased when the coincidence of photoperiod and availability of food was less than 10 hours per day. Masaki (1956) described a diapause pattern in *Barathra brassicae* L. that closely parallels that of *C. novemnotata.* A long photoperiod in the late spring induces a short aestival diapause in the pupal stage, and a short photoperiod in the late fall induces an hibernal diapause in the pupae of the second generation.

A close correlation exists between the data in Table III and field observations on the seasonal occurrence of diapause in *C. novemnotata* in relation to natural photoperiods and temperatures (McMullen 1967). Figure 1 of an earlier publication (McMullen 1967) illustrates the relationships between photoperiods based

TABLE I

Effect of temperature and photoperiod on rate of development and mortality of immature stages of *Coccinella novemnotata* Herbst

Temp. (°F)	Photo-period (hours)	No. of eggs tested	Mean incub. period (days)	No. of larvae tested	Mean duration of instar (days)					Mean total development time (days)	% mortality
					1st	2nd	3rd	4th	Pupa		
60	16	25	9.1±1.1	10	7.4 ±0.7	6.0 ±0.0	6.5 ±1.0	13.0 ±1.4	16.0 ±1.3	58.0±3.6	0.0
70	16	50	4.7±0.4	30	4.5 ±0.4	4.5 ±0.3	3.2 ±0.5	8.0 ±0.7	8.5 ±0.8	33.4±3.8	3.3
70	10	25	4.8±0.4	10	4.7 ±0.5	4.6 ±0.5	3.5 ±0.8	8.5 ±1.3	8.5 ±1.0	34.6±4.2	0.0
80	16	25	2.6±0.2	15	1.8 ±0.4	1.5 ±0.5	1.5 ±0.5	3.8 ±0.6	4.2 ±0.4	15.4±2.2	0.0
80	10	25	2.6±0.3	10	1.8 ±0.4	1.0 ±0.0	1.5 ±0.5	3.8 ±0.6	4.5 ±0.8	15.2±1.8	10.0
90	16	25	1.9±0.1	20	1.5 ±0.5	1.0 ±0.0	1.0 ±0.0	2.8 ±0.5	2.5 ±0.5	10.7±1.2	40.0
90	10	25	1.9±0.1	10	1.5 ±0.5	1.0 ±0.0	1.0 ±0.0	3.0 ±0.0	2.8 ±0.4	11.2±0.4	30.0

TABLE II

Effect of temperature on preoviposition period, fecundity, and adult longevity of *Coccinella novemnotata* Herbst

Temp. (°F)	R.H. (%)	No. of adults tested	Mean preovip. period (days)	Mean no. of eggs/female	Av. longevity (days)
60		5	20.4±0.8	N.D.	N.D.
70		10	12.3±2.9	473±146.0	62.0±13.0
80		5	6.4±0.6	322±134.7	47.6± 7.4
90	60	5	5.4±0.4	84± 58.1*	21.0± 8.1
90	20	8	7.5±0.8	16± 10.7†	11.2± 7.0

*72% of the eggs oviposited by females in this treatment failed to hatch.
†All of the eggs oviposited by females in this treatment failed to hatch.
NOTE: N.D., not determined.

on civil and astronomical twilights at 37° N. latitude and the dates of occurrence of the various developmental stages of *C. novemnotata*. It is important to note here, as shown in the next section of this paper, that the adult from emergence to 7 days of age is the critical stage susceptible to diapause induction. In the autumn of 1961 the natural photoperiod, based on astronomical twilight, during the period that teneral adults were present in the field decreased from 13 hours, 50 minutes to 13 hours, 5 minutes. This range of natural photoperiods approaches the 12-hour photoperiod found to induce a high incidence of adults in diapause at 60°F constant temperature in the laboratory. Considering that the lowest and highest mean weekly temperatures prevalent in the field during this period were 44° and 57°F, and that low temperatures tend to reinforce the effects of short photoperiods, then there is reasonable agreement between the results of the laboratory experiments and observations in the field. In the late spring of 1962 the natural photoperiod, based on astronomical twilight, during the period when teneral adults were present in the field increased from 17 hours, 30 minutes to 18 hours. These photoperiods closely relate to the 18-hour photoperiod found to effectively induce a high incidence of diapause in laboratory experiments conducted at 60° and 70°F constant temperature. In the field the lowest and highest mean weekly temperatures during the period when teneral adults were present were 60° and 75°F. Although the higher of these figures suggests that teneral adults in the field were subjected to temperatures which in the laboratory tended to effectively counteract the diapause-inducing influence of 18-hour photoperiods, there is still a reasonable positive correlation between the results of laboratory experiments and field observations.

A comparison of natural photoperiods during the periods when teneral adults were present in the field in the spring and fall, with artificial photoperiods that effectively induced diapause in laboratory experiments, suggests that the threshold of illumination influencing diapause regulation in *C. novemnotata* is very low. In the fall the range of photoperiods, based on civil twilight, during the critical period was 11 hours, 50 minutes to 11 hours, 5 minutes. This compares even more closely with the short photoperiods effective for diapause induction in the laboratory than the natural photoperiods based on astronomical twilight. But, in the case of the spring generation of the range of photoperiods based on civil twilight during the critical period was 15 hours, 15 minutes to 15 hours, 30 minutes. This is considerably shorter than the long, 18-hour photoperiod required to induce aestival diapause in the laboratory. Therefore it is probably correct to base the natural photoperiod responsible for inducing diapause in *C. novemnotata* on the

TABLE III

The percentage of female *Coccinella novemnotata* Herbst entering diapause under different treatment levels of temperature, photoperiod, and food supply*

Photoperiod (h):	10			12			14			16			18		
No. of aphids per day per adult:	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20
60°F	100	100	100	100†	100†	89†	86†	78†	50†	10	0	0	100	100	82
	(10)	(8)	(22)	(10)	(9)	(10)	(7)	(9)	(18)	(10)	(10)	(20)	(10)	(10)	(22)
70°F	100	100	96	100†	100†	70†	10	30	5	10	0	0	90	80	20
	(10)	(10)	(24)	(9)	(9)	(10)	(10)	(10)	(20)	(10)	(10)	(37)	(10)	(10)	(20)
80°F	100	64	40	70	11	10	0	0	0	0	0	0	90	50	7
	(8)	(11)	(10)	(10)	(9)	(10)	(9)	(8)	(10)	(12)	(15)	(25)	(10)	(8)	(14)
90°F	70	40	20	56	33	10	0	0	11	0	0	0	80	20	10
	(10)	(10)	(10)	(9)	(9)	(10)	(8)	(8)	(9)	(10)	(10)	(10)	(10)	(10)	(10)

*The figures in parentheses indicate the number of individuals in each lot.
†Beetles used in these treatments were reared to the pupal stage at 80°F, 16-hour photoperiod.

TABLE IV

Effect of stage of development at time of transfer from diapause-inducing conditions to non-diapause-inducing conditions and vice versa on diapause induction in adult *Coccinella novemnotata* Herbst

Stage when transferred	Photoperiod prior to transfer	Photoperiod after transfer	% diapause	% diapause in control (not transferred)
Egg	16	10	100	0
	10	16	0	100
All larval instars	16	10	100	0
	10	16	0	100
Pupae	16	10	100	0
	10	16	0	100
Teneral adults (less than 1 day old)	16	10	100	0
	10	16	0*	100
Adult 3 days old	16	10	80	0
	10	16	0*	100
Adult 7 days old	16	10	0	0
	10	16	10*	100
Adult 10 days old	16	10	0	0
	10	16	100	100

*Ovogenesis even in nondiapause individuals under these treatments was retarded compared with the controls.

occurrence of astronomical twilight. The average light intensity at civil twilight is 0.4 ft-c and at astronomical twilight 0.02 ft-c (Marvin and Kimball 1931). Lees (1955) generalized that the threshold of response for arthropods lies just above the intensity of direct moonlight which is in the range of 0.01 to 0.05 ft-c. De Wilde (1962) set a threshold value of illumination responsible for diapause induction in adult *Leptinotarsa* at less than 0.01 ft-c and postulated that relatively low thresholds of sensitivity render photoperiodic responses independent from irregular factors such as cloudiness, haze, and fog.

Laboratory experiments show that a lesser quantity of food available to newly emerged adults reinforces the influence of photoperiod on diapause induction (Table III). McMullen (1967) observed a drastic reduction in prey species populations, particularly the pea aphid, in alfalfa fields coincident with the occurrence of teneral adult *C. novemnotata*. However, Hagen (1962) observed that *C. novemnotata* invariably enters diapause even when there are dense populations of the spotted alfalfa aphid available as prey. Without further research, particularly on the nutritional suitability of various prey species, it is difficult to assess the importance of food as a diapause-regulating mechanism under natural conditions. It is apparent from the data discussed earlier in the paper that photoperiod is the prime factor regulating diapause induction in *C. novemnotata* and that temperature and food supply are secondary factors.

The Developmental Stage Susceptible to Diapause Induction

When eggs, larvae, pupae, or 1- and 3-day-old adults were transferred from a 10-hour to a 16-hour photoperiod none entered diapause as adults (Table IV). Among adults transferred when 7 or 10 days old, 10% and 100% respectively entered diapause. Also, the degree of ovary development which was assessed by measuring the length of ovarioles and comparing the relative amounts of yolk in developing ova was not as great in nondiapause females that were transferred when 1, 3, and 7 days old as in females of equivalent ages that were reared under a continuous 16-hour photoperiod. The retardation of ovary maturation was greatest

in the nondiapause females that were transferred when 7 days old and least in those that were transferred when 1 day old.

In treatments where groups of different developmental stages and ages of adults were transferred from a 16-hour to a 10-hour photoperiod the latest stage transferred in which diapause was induced was the 3-day-old adult. When the females of the various treatments were dissected at 14 days of age and the degrees of ovary maturation compared, no evidence was encountered to suggest that the 10-hour photoperiod had reduced the rate of ovary maturation of the nondiapause females in treatments where the transfer was made at 3, 7, and 10 days of age.

These results suggest that the critical period for induction of diapause, or conversely the initiation of gonad maturation, is the young adult between the ages of 1 and 7 days. From similar experiments with *Leptinotarsa decemlineata*, De Wilde (1958) concluded that the photoperiodic induction of diapause occurred immediately after the emergence of the adult and that possibly there was also some effect on the late larval stages.

References

Faber, W. 1949. Biologische Untersuchungen zur Diapause des Kartoffelkafers (*Leptinotarsa decemlineata* Say). *Pflanzenschutzberichte* **3**: 65–95.

Hagen, K. S. 1962. Biology and ecology of predaceous Coccinellidae. *A. Rev. Ent.* **7**: 289–326.

Hodek, I. 1957. Influence of temperature, relative humidity and photoperiodicity on the speed of development of *Coccinella septempunctata* L. *Acta Soc. ent. czech.* **55**: 121–141.

Hodek, I., and J. Cerkasov. 1961. Experimental influencing of the imaginal diapause in *Coccinella septempunctata* L. (Coccinellidae: Coleoptera). 7th contribution to the ecology of Coccinellidae. *Acta Soc. zool. bohemoslov.* **25**: 70–90.

Lees, A. D. 1955. The physiology of diapause in arthropods. University Press, Cambridge.

Marvin, C. F., and H. H. Kimball. 1931. Smithsonian Meteorological Tables. Fifth revised edition. *Smithson. misc. Collns*, Vol. 86.

Masaki, S. 1956. The local variation in the diapause pattern of the cabbage moth, *Barathra brassicae* Linne, with particular reference to the aestival diapause (Lepidoptera: Noctuidae). *Bull. Fac. Agric. Mie Univ.* **13**: 29–46.

McMullen, R. D. 1967. A field study of diapause in *Coccinella novemnotata* (Coleoptera: Coccinellidae). *Can. Ent.* **99**: 42–49.

Wilde, J. De. 1958. Perception of the photoperiod by the Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Proc. 10th int. Congr. Ent.* (1956). Vol. 2. pp. 213–218.

Wilde, J. De, C. S. Duintjir and L. Mook. 1959. Physiology of diapause in the adult Colorado potato beetle. I. The photoperiod as a controlling factor. *J. Insect Physiol.* **3**: 75–85.

Wilde, J. De. 1962. Photoperiodism in insects and mites. *A. Rev. Ent.* **7**: 1–26.

(Received 20 January 1967)