

- mm posterior to lambda, 3.4 mm lateral to the midline and 8.5 mm beneath the top of the skull. For RPC rats they were 2.0 mm posterior, 1.0 mm lateral, and 9.2 mm deep. The electrodes were then cemented in place on the skull and attached to a 22-gauge wire to which "bubble clips" or jeweler's "pin clutches" could be attached. Another 22-gauge wire was attached to a skull screw to serve as an indifferent electrode. After being tested, the rats were killed and perfused with 10 percent Formalin. The brains were removed and electrode placements in the CN or RPC were verified by examining serial histological sections stained with cresyl violet.
7. The apparatus to measure startle has been described [G. T. Weiss and M. Davis, *Pharmacol. Biochem. Behav.* 4, 713 (1976)]. Briefly, an 8 by 15 by 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame was used. Cage movement resulted in displacement of an accelerometer where the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered and was measured with a specially designed sample and hold circuit. The stabilimeter was housed in a lighted, ventilated, sound-attenuated chamber, 50 cm from a high-frequency speaker. The acoustic startle stimulus was a 50-msec, 110-dB burst of white noise having a rise-decay time of 0.1 msec. Background white noise was 80 dB.

- Sound-level measurements were made within the cages with a sound-level meter (General Radio 1551-C) (A scale).
8. Electrical stimuli were monophasic, negative-going single pulses 1 msec long delivered through a mercury slip ring from a constant-current stimulator. One or two days before the main experiment, rats were tested with a few bilateral shocks to determine the intensity required to produce a startle of about 20 to 40 units. These intensities, which ranged from about 25 to 100 μ A to each electrode, were then used in the main experiment. Subsequent histology showed that higher currents were generally required to elicit startle when electrode tips were somewhat off target. However, placements on target occasionally required higher currents, perhaps because of local tissue damage. The electromyographic topologies of the responses to acoustic stimuli were similar to those to electrical stimulation of the CN or RPC (5).
9. If rapid rates of stimulation through the RPC are used (for example, 1 stimulus per second), responses will decline. Hence a separation of habituation and sensitization may depend on the exact interstimulus intervals used.
10. J. N. Sanes, J. R. Ison, A. A. Adelson, *Neurosci. Abstr.* 4, 304 (1978).
11. Supported by NSF grant BNS-78-17421, NIMH grants MH-25642 and MH-18949, Research Scientist Development Award MH-00004 to M.D., and by the state of Connecticut.

14 May 1981; revised 13 January 1982

A New Role for Temperature in Insect Dormancy: Cold Maintains Diapause in Temperate Zone Diptera

Abstract. *In early autumn, high temperatures terminate diapause in the alfalfa blotch leafminer *Agromyza frontella*; low temperatures maintain diapause. These responses subserve a thermally malleable dormancy and allow flexibility in the annual number of generations. The view that favorable conditions cannot reverse the course of diapause are contradicted by the data on *A. frontella*. A better understanding of the diverse seasonal adaptations that insects have evolved may add precision to life history studies and phenological models in insect pest management.*

Diapause, a physiological state of developmental and reproductive suppression, is a prime synchronizer of insect seasonal cycles. It is regulated by token stimuli (primarily photoperiod and temperature) that allow the insect to anticipate approaching seasonal changes (1-4). The specific action of natural photoperiod and temperature has been characterized for very few natural populations, particularly as they undergo overwintering (3, 5, 6). This omission limits the interpretation of how life histories evolve, and it impedes the construction of accurate phenological models in insect pest management. For example, it is generally considered that once fully initiated, diapause is not reversible, even

under favorable conditions, until after certain physiological changes have occurred. In many species of insects from the temperate region these changes take place at low temperatures (compare vernalization) (1, 3, 7, 8). Despite evidence to the contrary (3), researchers and writers of general texts (9, 10) frequently assume that low temperatures are required to accelerate diapause termination. Our results with the alfalfa blotch leafminer *Agromyza frontella* (Rondani)

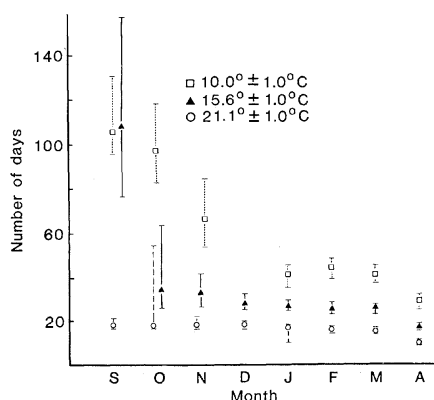
(Diptera: Agromyzidae) contradict the common finding that the course of diapause is irreversible under environmental conditions that favor growth and development. The results also show that low temperature can delay, rather than hasten, the completion of diapause in the temperate zone.

Agromyza frontella is a European species that was introduced into North America in the 1960's. Since then, it has become widespread in the northeastern United States and in Canada (11, 12), where it has caused significant damage to alfalfa. Females oviposit into the mesophyll of alfalfa leaflets, and the three larval instars mine and feed between the epidermal layers. Mature larvae exit from the mines and drop to the soil for pupation. In eastern North America, *A. frontella* produces three complete, and in some areas, a fourth or fifth generation per year (11-14). Overwintering is accomplished by partially developed pupae within puparia, at a soil depth of approximately 2 cm (13).

To investigate the overwintering of a natural population, specifically to establish the influence of naturally occurring temperatures on diapause maintenance and termination, we tested the thermal responses of a field population throughout dormancy (15). Our data (Table 1 and Fig. 1) (16) show that both photoperiod and temperature have roles in regulating diapause; however, temperature dominates. At 21°C, emergence of *A. frontella* individuals occurs within 18 to 19 days during all months from September through March; this means diapause is reversed at that temperature. During September and October, development at 10°C proceeds at approximately one-sixth the rate of that at 21°C; in November the rate of development at 10°C increases to about one-fourth of that at 21°C. Subsequently, in January, February, March, and April, development at 10°C stabilizes at approximately one-third the rate at 21°C—the level characteristic of postdiapause (17) and nondiapause (18) development.

Diapause in *A. frontella*, as in other insects (19), is a dynamic state, and the thermal responses of the insects change throughout its course. During September, diapause development is very sensitive to diapause-maintaining temperatures; both 10°C and 15.6°C retard the rate of diapause development. During October and November, thermal maintenance of diapause decreases; for example, by the end of October 15.6°C no longer decelerates diapause development, and by the end of November the

Fig. 1. Median time to emergence from September through April after transfer of overwintering *A. frontella* puparia from outside locations into three thermal conditions in a light-dark photoperiod of 16 hours of light and 8 of darkness (LD 16:8). Variation indicates emergence of individuals at 10th and 90th percentiles. Sample sizes 22 to 78; mean \pm 1 standard deviation = 43 ± 11 . See (16, 17) for details.



response at 10°C has decreased significantly (Fig. 1 and Table 1). The insects remain in dormancy after January, but mechanisms other than thermal reactions control the dormancy (17). Postdiapause pupal development begins when soil temperatures rise above the thermal threshold for development (3.9°C); in the Ithaca, New York, area this occurs during April (13, 17, 18).

Development in a number of species of insects from the temperate region contradicts the generalization that cold terminates diapause in that they end diapause more quickly under warm rather than cold conditions (3). However, these species generally rely on photoperiod as the primary diapause-maintaining factor. Although day length also has an important role in diapause maintenance during autumn in *A. frontella* (16, 17), the response is considerably less pronounced and can be overridden by temperature (17). Thus, we now have an example in which low temperature is the primary factor maintaining autumnal diapause in a species of insects from the temperate region (20). These data expand our conception of temperature's role in regulating insect dormancy. We can now regard high temperature not only as a primary factor in maintaining diapause in the fall, but also as a hastener of its completion.

In *A. frontella* the strong response to temperature during the initial stages of diapause provides between-year flexibility in the life cycle of an insect that also responds to photoperiod. That is, the dominance of temperature over photoperiod allows *A. frontella* to switch from a diapause mode to a nondiapause mode late in the season. Thus, if pupae in diapause experience temperature conditions favorable for growth and development, diapause can be reversed, and an additional, late-season generation can be produced (21). This type of malleable diapause could evolve only in a species with a short life cycle. In this case, it allows *A. frontella* to produce as many as four or five, rather than being restricted to three, generations in years or at localities with warm autumnal conditions. We expect, therefore, that when examined closely, *A. frontella* will exhibit considerable variability in the number of generations per year even among populations at the same latitude if local temperature conditions vary.

The data have implications for life history studies as well as insect pest management. The study of life history strategies has largely stressed variation in development, survival, and reproduc-

Table 1. Relative rates of development in *A. frontella* after transfer of overwintering puparia from outside into three thermal conditions in a light-dark photoperiod of 16 hours of light and 8 of darkness. Details for each temperature condition are described in (16, 17).

Month	Ratio of medians*		
	10.0° ± 1.0°C	15.6° ± 1.0°C	21.1° ± 1.0°C
September	5.8	6.0	1
October	5.4	1.9	1
November	3.7	1.8	1
December		1.6	1
January	2.4	1.6	1
February	2.8	1.6	1
March	2.7	1.7	1
April	2.9	1.7	1

*Calculated by dividing emergence time at each temperature by the emergence time at 21.1°C. Range of sample sizes 22 to 78; mean ± 1 standard deviation = 43 ± 11.

tion due to genetic variability and environmental diversity. Our data indicate that a third important factor is the physiological status of the organisms. For example, in *A. frontella* the relation between temperature and developmental rate varies during the season, as the insects pass through early diapause, late diapause, postdiapause, and nondiapause periods. This variation could strongly influence the calculation of developmental and reproductive capacity. Such studies may greatly improve predictions of growth, development, reproduction, and survival for some species under field conditions. This would result in pest management models of greater utility. In the case of *A. frontella*, these data are being incorporated into an existing pest management model (22) which can now accurately predict the number and timing of late-season generations and the timing of vernal emergence and activity.

MAURICE J. TAUBER
CATHERINE A. TAUBER
JAMES R. NECHOLS*
ROBERT G. HELGESEN†

Department of Entomology,
Cornell University,
Ithaca, New York 14853

References and Notes

1. A. D. Lees, *The Physiology of Diapause in Arthropods* (Cambridge Univ. Press, London, 1955).
2. —, in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1968), pp. 47–137.
3. M. J. Tauber and C. A. Tauber, *Annu. Rev. Entomol.* **21**, 81 (1976).
4. S. D. Beck, *Insect Photoperiodism* (Academic Press, New York, 1980).
5. M. J. Tauber and C. A. Tauber, *Nature (London)* **244**, 296 (1973).
6. —, *ibid.* **258**, 711 (1975).
7. H. G. Andrewartha, *Biol. Rev. Cambridge Philos. Soc.* **27**, 50 (1952).
8. J. R. Nechols, M. J. Tauber, R. G. Helgesen, *Can. Entomol.* **112**, 1277 (1980).

9. C. Gillott, *Entomology* (Plenum, New York, 1980).
10. D. J. Borror, D. M. Delong, C. A. Triplehorn, *An Introduction to the Study of Insects* (Holt, Rinehart, & Winston, New York, 1981).
11. D. G. Harcourt, *Ann. Entomol. Soc. Quebec* **18**, 49 (1973).
12. R. M. Hendrickson, Jr., and S. E. Barth, *Ann. Entomol. Soc. Am.* **71**, 295 (1978).
13. W. K. Mellors, thesis, Cornell University, Ithaca.
14. J. C. Guppy, *Can. Entomol.* **113**, 593 (1981).
15. During late summer, alfalfa infested with third-instar larvae was gathered and moved to outdoor cages in Tompkins County, New York. Larvae completed development and the resulting puparia were collected and maintained in the outdoor cages. Each month (September through April), we transferred groups of approximately 80 puparia to each of various temperature regimes (Fig. 1). High relative humidity was maintained (17). Puparia were examined daily. The number of adults that emerged under each condition ranged from 22 to 78 (mean ± standard deviation = 43 ± 11); the remainder of the pupae were not viable. This level of mortality is typical of that in both diapause and nondiapause field-collected pupae [see W. K. Mellors and R. G. Helgesen, *Environ. Entomol.* **9**, 738 (1980)]. There was no correlation among level of mortality, date of sample, or condition. Emergence was not normally distributed; therefore, we used the Wilcoxon rank-sum test to analyze the data. In September, October, and November, some individuals (mean ± standard deviation = 38.8 ± 20.4, 2.2 ± 2.7, and 2.2 ± 3.4, respectively) emerged under almost all conditions within a relatively short time of sampling. We concluded that these early-emerging flies had not entered diapause, and they were therefore not included in our analysis of diapause maintenance and termination.
16. Median emergence of flies transferred in September to a light-dark photoperiod of 12 hours of light and 12 of dark (LD 12:12) at 10°C was 146 days (10th and 90th percentile = 127 and 163 days), compared with a median of 104 days at 10°C under LD 16:8 (Table 1); see also (17).
17. J. R. Nechols, M. J. Tauber, C. A. Tauber, R. G. Helgesen, *Ann. Entomol. Soc. Am.*, in press.
18. W. K. Mellors and R. G. Helgesen, *ibid.* **71**, 886 (1978).
19. M. J. Tauber, C. A. Tauber, S. Masaki, in *Environmental Entomology*, C. B. Huffaker and R. L. Rabb, Eds. (Wiley, New York, in press).
20. It has been demonstrated that brief exposure to relatively high temperatures in spring (about 3 days above 20°C) is required to terminate diapause in overwintered *Teleogryllus commodus* [S. Masaki, Y. Ando, A. Watanabe, *Kontyû* **47**, 493 (1979)]. There is other evidence for the termination of hibernation diapause by elevated temperatures in the spring [A. G. L. Wilson, T. Lewis, R. B. Cunningham, *Bull. Entomol. Res.* **69**, 97 (1979)]. These cases differ from *A. frontella* in that temperature can act to complete diapause only in spring after the insects have already overwintered. In several species of Sarcophagidae diapause is induced and maintained by low temperature; such response to low temperature occurs only in tropical species within 10°C of the equator where photoperiod provides a weak seasonal signal [D. L. Denlinger, *Biol. Bull. (Woods Hole, Mass.)* **156**, 31 (1979)].
21. The seasonal pattern of *A. frontella*'s thermal response during diapause maintenance appears analogous to the seasonal pattern of photoperiodic responses (3) shown by insects with photoperiodically maintained autumnal diapause. In both types of diapause maintenance, reaction to the abiotic factor is most pronounced during the initial phases of diapause, when environmental conditions are most variable. However, the apparently similar patterns of response to photoperiod and temperature have very different effects in nature. Photoperiodically induced and maintained diapause is tuned to day length—an invariable annual cue—and relatively little between-year variation can be expected in insects with strong reaction to photoperiod during autumnal diapause.
22. W. K. Mellors, *Search Agri. (Geneva, N.Y.)* **20**, 1 (1981).
23. We thank W. F. Ables and J. Knapp for their cooperation.

* Present address: College of Agriculture, UOG Station, University of Guam, Mangilao 96913.

† Present address: Department of Entomology, Kansas State University, Manhattan 66506.

17 May 1982; revised 1 September 1982