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## Voltinism and diapause in the oligophagous leafminer *Phytomyza chaerophylli* (Kaltenbach) (Dipt., Agromyzidae)

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### Abstract

*Phytomyza chaerophylli* (Kaltenbach) is multivoltine with two distinct population density peaks per year, the first in spring and the second in fall. The fly shows both a summer and a winter diapause, the most important cues for diapause regulation being temperature and photoperiod. Winter diapause is maintained by low temperatures and short photoperiod. High temperatures can cause winter diapause termination. Temperature can override the effect of photoperiod in winter diapause and seems thus to be the single most important signal for winter diapause maintenance and termination. Summer diapause is maintained by high temperatures and long photoperiod. Short photoperiod can cause summer diapause termination. The relative importance of photoperiod seems to be more pronounced in summer than in winter diapause. Diapause regulation mechanisms provide this species with a high degree of flexibility in its life cycle and allow close adaptation to the phenology of specific host plant populations.

### 1 Introduction

Environmental conditions change throughout the year for temperate zone insects. Diapause, a physiological stage in which development and reproduction is suppressed, is one way insects cope with such changes. The most important function of diapause is adaptation to specific habitat conditions such as synchronization of seasonal cycles and withstanding unfavorable seasons (TAUBER et al. 1982, 1983). Diapause is generally regulated primarily by external stimuli, mainly photoperiod and temperature, allowing the insect to anticipate approaching seasonal changes (HODEK and HODKOVÁ, 1988; SAUNDERS 1976; TAUBER and TAUBER 1973, 1981; MASAKI 1980).

Photoperiod, a relatively noise-free seasonal cue, is widely used among insects as the primary factor signaling initiation and maintenance of diapause. In Agromyzidae, the roles of temperature and photoperiod in the regulation of pupation and diapause have been studied in detail in *Agromyza frontella* (Dipt., Agromyzidae) (MELLORS and HELGESEN 1982; NECHOLS et al. 1983; TAUBER et al. 1982; THERRIEN et al. 1985). These studies suggest photoperiod as the single major stimulus for the initiation of diapause. Unlike most insects, however, maintenance and termination of diapause is regulated primarily by temperature in *A. frontella*.

In this paper, voltinism pattern, seasonality and the role of temperature and photoperiod in maintenance and termination of diapause in *Phytomyza chaerophylli* Kalt. is discussed. This oligophagous leaf-mining agromyzid fly is distributed over most of Europe on several species of Umbelliferae (SPENCER 1976). The two most important host plants in Central Europe are *Anthriscus sylvestris* Hoffm. and *Chaerophyllum temulum* L. Both host plants occur at borders of forests and in hedges, generally in small populations, but *A. sylvestris* expanded its habitat to include cultivated pastures where it is now widespread.

The paper addresses three main questions: 1. What is the voltinism pattern in *P. chaerophylli*? 2. What is the main parameter determining diapause period? 3. How do temperature and photoperiod affect pupation time and diapause incidence?

## 2 Materials and methods

### 2.1 Insect material

The populations studied are located in the proximity of Basel, Switzerland, at altitudes between 250–700 m and at distances between 0.5 to 40 km apart. Infested host plant leaves, mostly with second instar larvae, were collected in the field in small plastic bags and placed in a controlled environment at a fixed temperature of 16.5°C and a 12L:12D photoperiod. Bags were examined daily for pupae which were placed in small plastic tubes with moist plaster bottoms. Tubes were checked for emerged flies three times a week. Because no diapausing pupae were dissected, no distinction was made between diapause development and post-diapause development and the term diapause is used to refer to the period from pupation to eclosion.

### 2.2 Experiments

#### 2.2.1 Voltinism pattern

Voltinism pattern was determined by comparing field infestation to laboratory emergence data. To ascertain the degree of synchronization with the development of its host plant, these data were compared to host leaf supply. Leaf supply was measured using three characters, the number of leaves per plant, the height of the plant in generative individuals and the length of the longest leaf in vegetative individuals. Empirical observations suggested the choice of these characters as a simple but satisfying method of total leaf surface estimate (FREY 1986).

#### 2.2.2 Main diapause determining parameter

To find the main cue used by these flies for determination of diapause period, a comparison was performed between the peak infestation periods of spring and fall as observed in the field versus diapause periods observed in individuals reared as pupae under controlled laboratory conditions.

It has been shown that cold temperatures increase pupation time of diapausing pupae in members of the family Agromyzidae (TAUBER et al. 1982). Also, very hot temperatures can prolong diapause development in insects undergoing summer diapause (MASAKI 1980). Thus, there is a polynomial relation between diapause period and temperature. To visualize this relation, a second order polynomial regression was fitted to the laboratory data and the data from the field measurements were superimposed in fig. 2.

In the field, the number of days between observed infestation peaks of spring and fall for two and three years, respectively, was recorded and average temperature was measured at 5 cm depth in the soil (FREY 1986). Plant infestation was measured as the mean number of larvae per plant.

#### 2.2.3 Effects of temperature and photoperiod on diapause

The effect of temperature on pupation time and diapause incidence was determined by rearing the collected pupae under four different temperatures at 12L:12D photoperiod: 12.8°C, 16.5°C, 22.5°C and 24.0°C. The effect of photoperiod (12L:12D vs. 16L:8D) was tested at 22.5°C in pupae of the fall infestation period.

## 3 Results and discussion

### 3.1 Voltinism pattern

*Phytomyza chaerophylli* shows two distinct infestation peaks per year, one in spring and a second in fall (fig. 1). The period between the spring and fall peak infestations of one year averages to  $154 \pm 19$  days ( $N = 3$ ; measured 5 cm deep in the soil in 1983–1985), which is distinctly shorter than the period between the fall peak infestation and the spring peak infestation of the next year with  $213 \pm 20$  days ( $N = 2$ ), (Mann-Whitney  $U = 0$ ,  $p = 0.083$ ). This difference between winter and summer diapause periods indicates that the flies have two different diapause types, a summer diapause and a winter diapause, the latter requiring about 27% more days than the former. However, nondiapausing pupae can be observed in both peak infestation periods. Thus, *P. chaerophylli* is multivoltine with two periods of mass emergence.

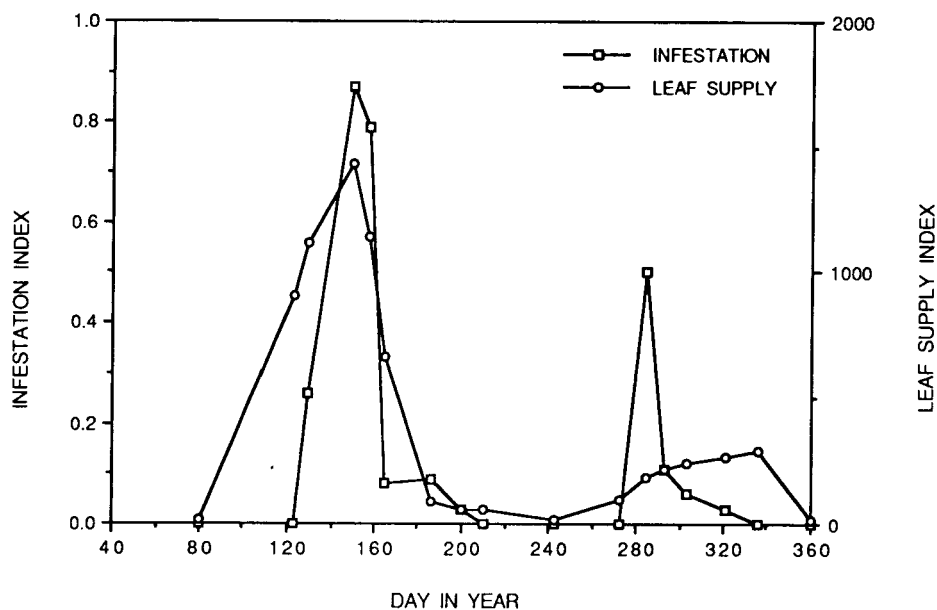


Fig. 1. Leaf supply of *Anthriscus sylvestris* and infestation by *Phytomyza chaerophylli* in one population in one year

This emergence pattern corresponds closely to the availability of host plant leaves in natural habitats as well as in most cultivated pastures (fig. 1; FREY 1986). Both important host plants, *A. sylvestris* and *C. temulum*, produce a very restricted supply of fresh leaves during midsummer in natural habitats. Generative individuals of *C. temulum* die after seed ripening. However, leaf supply increases in fall due to growth of vegetative individuals. A similar seasonal variation in leaf supply was also found in *A. sylvestris*. This host occurs in many cultivated pastures in high abundances. The pastures usually are mowed before seed ripening of *A. sylvestris* and thereafter three to five times until snowfall. Because high mid-summer temperatures often cause dry soils which slow down growth, leaf supply of *A. sylvestris* is usually not recovered until late fall, when humidity is high enough for fast growth (fig. 1). However, small numbers of larvae from nondiapausing individuals can be found between the infestation peaks if host leaves are available.

Voltinism pattern in *P. chaerophylli* seems to be adapted to optimally use leaf supply of its host plant. The field data indicate that this is also true for cases where leaf supply is dependent on anthropogenous factors such as farming. Similar adaptations to farming practices have been reported for another agromyzid leafminer, *Agromyza frontella* (ANDALORO et al. 1983).

### 3.2 Main diapause determining parameter

Mean daily field temperature experienced by winter diapause pupae between the peak infestation periods of fall and spring was 4.8°C. Winter diapause in the field takes  $213 \pm 20$  days. This is shorter than would be expected from the extrapolation of the available laboratory data (fig. 2). However, for winter diapause there is a lower temperature threshold at which development stops completely. This means that accelerated development during relatively warm days is not compensated for in winter diapause which may explain the observed deviation from the expected value.

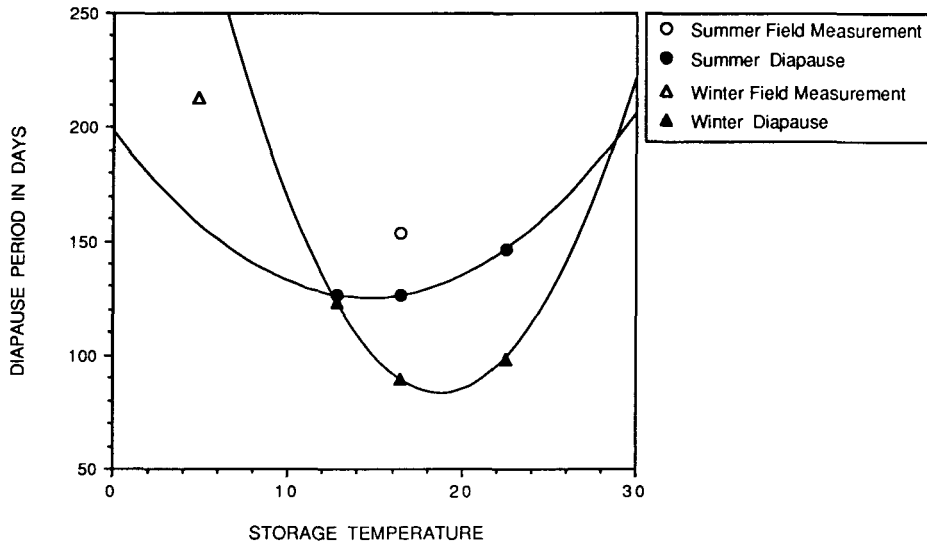


Fig. 2. Effect of temperature on diapause period. The difference between summer and winter diapause is visualized by fitting a second order polynomial regression on the laboratory data (summer diapause:  $y = 198.58 - 10.069x + 0.34364x^2$ ; winter diapause:  $y = 473.36 - 41.477x + 1.102x^2$ )

Laboratory conditions were kept constant for pupae until emergence. The flies could therefore not use any changes in temperature and/or photoperiod as a cue for diapause termination. This indicates that winter diapause termination does not depend on the incidence of high temperatures and long photoperiods, although in nature both signals may act as synchronizers for exact timing of emergence.

Mean daily field temperatures for summer diapause, measured between spring and fall peak infestation in 1984 was 16.5°C. Under laboratory conditions at constant 16.5°C, summer diapause was somewhat shorter than in the field ( $126 \pm 38$  vs.  $154 \pm 19$  days). However, the difference is not significant ( $U = 101$ ,  $p = 0.189$ ) and since high temperatures generally decelerate summer diapause development (MASAKI 1980), this difference between field and laboratory data can be explained by the high temperatures pupae experience in the field during summer.

These results indicate that temperature is an important factor determining diapause period in *P. chaerophylli* (table 1, fig. 2). Temperature may provide more accurate information than photoperiod on the optimal time for eclosion for host-specific phytophagous insects. Average temperature may differ markedly between host plant populations depending on exposure and other microgeographic factors. This can cause large interpopulation differences in the developmental stage of host plants although photoperiod is the same in all populations (FREY 1986). Thus, a temperature-based regulation of diapause allows for very close synchronization of mono- or oligophagous insects with the phenology of their host plant as shown, e.g., for *Laspeyresia pomonella* (Lep., Olethreutidae; PHILLIPS and BARNES 1975) and for *Agromyza frontella* (NECHOLS et al. 1983).

The effect of temperature is more pronounced in winter than in summer diapause. At temperatures above 13°C winter diapause development is much faster than summer diapause development (fig. 2). Under field conditions, such a mechanism will allow for an earlier emergence period in spring. Furthermore, it will condense emergence period and thus synchronise eclosion. The high degree of synchronisation of emergence time in *P. chaerophylli* is reflected in the fast increase of infestation levels in the spring infestation period (fig. 1).

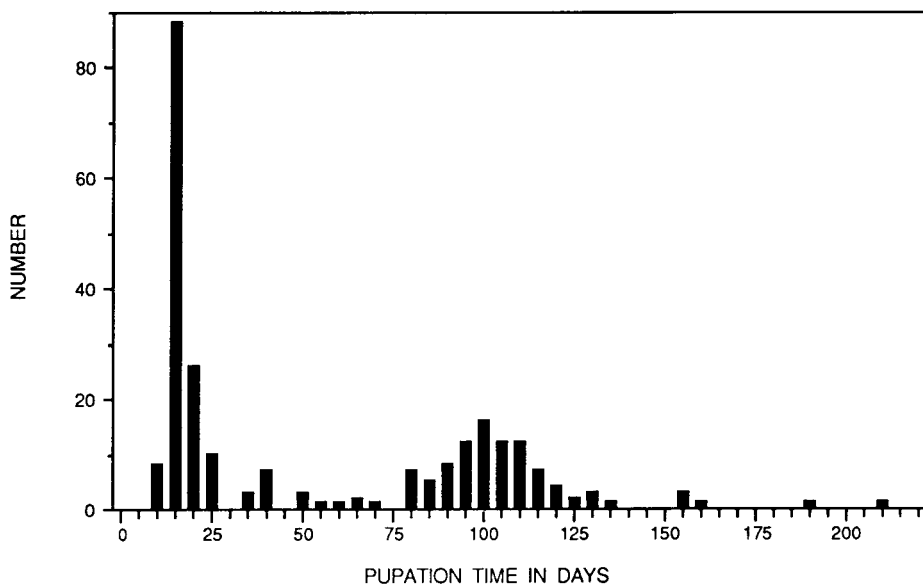
*Table 1. Pupation time in days of Phytomyza chaerophylli at different storage temperatures in spring and fall peak infestation periods (± standard deviation [STD])*

		Nondiapause pupae		Diapause pupae	
		Spring	Fall	Spring	Fall
12.8 °C	N	67	21	56	89
	Mean	35.6	35.3	126.2	122.9
	STD	4.3	5.7	32.4	38.0
16.5 °C	N	81	33	46	103
	Mean	25.6	29.0	125.5	89.2
	STD	6.3	11.6	37.8	19.2
22.5 °C	N	91	54	10	90
	Mean	17.3	16.4	145.9	97.3
	STD	8.1	7.2	35.6	14.8
24.0 °C	N		27		
	Mean		15.1		
	STD		3.8		

### 3.3 Effect of temperature and photoperiod

#### 3.3.1 Effect on pupation time

Most generations show a bimodal distribution in pupation time corresponding to non-diapausing and diapausing types of pupae (fig. 3). Pupation time for both types depends on storage temperature (table 1, fig. 2). Changing the photoperiod, in contrast, had no effect on pupation time at 22.5 °C storage temperature in nondiapause ( $U = 532.5, p = 0.325$ ) as well as in diapause pupae ( $U = 397.5, p = 0.927$ ). Pupation time data from 16L:8D



*Fig. 3. Distribution pattern of pupation time in Phytomyza chaerophylli*

photoperiod at 22.5 °C were therefore combined with data from 12L:12D photoperiod at 12.8 °C, 16.5 °C and 24 °C.

In nondiapauser pupae of both the spring and the fall generation, pupation time decreases significantly with increasing temperature (Kruskal-Wallis  $H = 138.3$ ,  $p < 0.001$  for spring;  $H = 57.3$ ,  $p < 0.001$  for fall). A comparison of the pupation time of nondiapauser spring vs. fall pupae revealed no differences at all temperatures (12.8 °C:  $U = 698.5$ ,  $p = 0.961$ ; 16.5 °C:  $U = 1290$ ,  $p = 0.771$ ; 22.5 °C:  $U = 2269$ ,  $p = 0.442$ ), indicating that the same mechanism may be responsible for determination of pupation period in nondiapausing spring as well as fall pupae.

Diapause period of fall pupae changes significantly with changing temperature ( $H = 46.3$ ;  $p < 0.001$ ), whereas in spring pupae, a change in diapause period occurs only above 16 °C (table 1, fig. 2). Diapausing spring pupae show a significantly longer pupation time than diapausing fall pupae at temperatures between 16 °C and 23 °C (16.5 °C:  $U = 909.5$ ,  $p < 0.001$ ; 22.5 °C:  $U = 96$ ,  $p < 0.001$ ) (see fig. 2). This corresponds to the two different diapause types, winter and summer diapause, as indicated above. Similar to the soil measurements, winter diapause pupae require about 30 % more days than summer diapause pupae.

### 3.3.2 Effect on diapause incidence

Diapause incidence decreased strongly with increasing temperature in both summer diapause pupae (Pearson  $\chi^2 = 34.1$ ,  $p < 0.001$ ) and winter diapause pupae ( $\chi^2 = 89.4$ ,  $p < 0.001$ ) (table 2). For winter diapause pupae this indicates that high temperatures cause winter diapause termination whereas low temperatures maintain winter diapause. The same response pattern for winter diapause was found in *Agromyza frontella* (NECHOLS et

Table 2. Percent diapause pupae of larvae collected in spring and fall at different storage temperatures and 12L:12D photoperiod

Numbers of emerged flies in parenthesis

Temperature	Spring	Fall
12.8	45 (123)	81 (110)
16.5	38 (127)	76 (136)
22.5	10 (101)	28 (32)
24.0	– (–)	0 (27)

al. 1983; TAUBER et al. 1982), and in many other insects [MASAKI 1980; THERRIEN et al. 1985; see HODEK and HODKOVÁ (1988) for a review].

Summer diapause pupae experience high temperatures for at least two thirds of their pupation period. In many insects, summer diapause is induced by high temperatures and long photoperiod (MASAKI 1980; MASAKI and SAKAI 1965; PAARMANN 1974; SARINGER and DESEO 1966; SULLIVAN and WALLACE 1965). Therefore, high temperatures can be excluded as a factor caus-

ing summer diapause termination in *P. chaerophylli*. However, spring larvae experienced a distinct decrease in photoperiod when put from field (15.5L:8.5D) into laboratory (10.6L:13.4D) conditions, indicating that short photoperiod can terminate summer diapause. The results suggest that photoperiod is an important signal regulating summer diapause maintenance and termination. The roles of temperature and photoperiod seem thus to be different in summer versus winter diapause regulation suggesting differences in mechanisms of diapause maintenance and termination between summer and winter diapause.

Diapause incidence is significantly lower in spring pupae than in fall pupae at all storage temperatures (table 2;  $\chi^2$ : 12.8 °C at 12L:12D: 30.9,  $p < 0.001$ ; 16.5 °C at 12L:12D: 41.6,  $p < 0.001$ ; 22.5 °C at 116L:6D: 68.0,  $p < 0.001$ ). This pattern allows for a flexible number of nondiapausing summer generations where local environmental conditions are favorable.

Changing photoperiod had a strong effect on diapause incidence. At 12L:12D photoperiod and 22.5 °C (group 1), 28 % ( $N = 32$ ) of the pupae from larvae collected in the fall

infestation period were diapausing whereas at 16L:8D photoperiod at the same temperature (group 2), 63 % (N = 144) were diapausing ( $\chi^2 = 12.6$ ,  $p < 0.001$ ). This result seems to suggest that long photoperiod maintains winter diapause which contradicts our earlier results. However, fall larvae of the first group experienced only a slight increase in photoperiod but a distinct increase in temperature when transferred from field ( $7.2 \pm 2.6^\circ\text{C}$ , 10.6L:13.4D) to laboratory conditions ( $22.5^\circ\text{C}$ , 12L:12D). As shown above, high temperatures cause winter diapause termination which explains the low incidence of diapause in this group. The second group experienced distinct increases in both temperature and photoperiod. The individuals of this group were thus reared under typical summer conditions. Stable conditions typical for either summer or winter diapause conditions may thus be a prerequisite for diapause maintenance. Although these data are not conclusive, they demonstrate a strong effect of photoperiod on winter diapause incidence.

#### 4 Conclusion

*Phytomyza chaerophylli* is multivoltine with two periods of mass emergence. There are two modes of pupation, a nondiapause mode and a diapause mode which consists of two different types, a summer and a winter diapause. Short photoperiod and/or low temperatures maintain winter diapause. High temperatures may cause winter diapause termination. Summer diapause, in contrast, is maintained by high temperatures and long photoperiods and terminated by short photoperiod. Both temperature and photoperiod are very important signals in diapause regulation. However, data suggest that temperature can override the effect of photoperiod in winter diapause. This pattern was also found in *Agromyza frontella* (TAUBER et al. 1982). The major advantage of such a regulation mechanism is considered to be an increase in flexibility in the life cycle of an insect, allowing a flexible number of generations per year and close adaptation to the phenology of host plants (TAUBER et al. 1982). The results outlined in this paper support this hypothesis. Generation numbers are variable in *P. chaerophylli*, and peak infestation periods coincide closely with the availability of host plant leaves. The relative importance of photoperiod seems to be more pronounced in summer than in winter diapause, indicating differences in mechanisms of diapause maintenance and termination between these two diapause types.

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#### Zusammenfassung

*Diapause regulation bei der oligophagen Minierfliege Phytomyza chaerophylli (Kaltenbach)*  
(Dipt., Agromyzidae)

*Phytomyza chaerophylli* (Kaltenbach) ist multivoltin mit zwei deutlichen Perioden hoher Populationsdichte, der ersten im Frühling und der zweiten im Herbst. Die Fliege weist sowohl eine Sommer- als auch eine Winterdiapause auf, wobei die wichtigsten Faktoren bei der Regulation die Temperatur und die Photoperiode sind. Die Winterdiapause wird durch tiefe Temperaturen und kurze Photoperioden aufrechterhalten. Hohe Temperaturen können einen Abbruch der Winterdiapause bewirken. Die Temperatur kann den Effekt der Photoperiode in der Winterdiapause überdecken und scheint daher das wichtigste Einzelsignal für die Aufrechterhaltung und den Abbruch der Winterdiapause zu sein. Die Sommerdiapause wird durch hohe Temperaturen und eine lange Photoperiode aufrechterhalten. Eine kurze Photoperiode kann einen Abbruch der Sommerdiapause verursachen. Die relative Bedeutung der Photoperiode scheint in der Sommerdiapause stärker zu sein als in der Winterdiapause. Durch ihre Mechanismen der Diapause-Regulation verfügt diese Species über einen hohen Grad an Flexibilität in ihrem Lebenszyklus und kann dadurch eine gute Anpassung an die Phänologie spezifischer Wirtspflanzenpopulationen erreichen.

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