GENETIC SELECTION, EVALUATION AND MANAGEMENT OF NONDIAPAUSE *APHIDOLETES APHIDIMYZA* (RONDANI) (DIPTERA: CECIDOMYIIDAE) FOR CONTROL OF GREENHOUSE APHIDS IN WINTER

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Short title:

NONDIAPAUSING *APHIDOLETES APHIDIMYZA*

Linda A. Gilkeson

ABSTRACT

Ph.D. Linda A. Gilkeson Entomology

GENETIC SELECTION, EVALUATION AND MANAGEMENT OF NONDIAPAUSING *APHIDOLETES APHIDIMYZA* (RONDANI) - (DIPTERA: CECIDOMYIIDAE) FOR CONTROL OF GREENHOUSE APHIDS IN WINTER

The aphidophagous midge, *Aphidoletes aphidimyza* (Rond.), is a promising biological control agent, but in greenhouses its effectiveness is restricted by diapause during winter. Five nondiapausing lines of *A. aphidimyza* were selected under LD 8:16 (21°C). One selected line was maintained for 50 generations (3 yr); selection did not affect fecundity, sex ratio, or morphology. When selection was relaxed, no significant reversion to higher diapause incidence occurred until after eight generations. Simultaneous fluctuations in diapause incidence were observed in all lines. This may have been caused by variation in host plant quality, which was shown to affect quality of aphids sufficiently to influence diapause in midge larvae. Rearing larvae under LD 8:16 and thermoperiod 21:15°C, induced diapause in larvae from selected lines, however, low intensity radiation (<48 uW/cm^2) at night prevented diapause under these conditions. A release rate of 1 predator:10 aphids was sufficient to control aphids in cages, from November to January, in the greenhouse.

RÉSUMÉ

Ph.D. Linda A. Gilkeson Entomologie

SÉLECTION GÉNÉTIQUE, ÉVALUATION ET GESTION DE *L'APHIDOLETES APHIDIMYZA* (RONDANI) (DIPTERA: CECIDOMYIIDAE) SANS DIAPAUSE, POUR LE CONTROLE DES PUCERONS DAN LES SERRES, EN HIVER

Le moucheron aphidiphage *Aphidoletes aphidimyza* (Rond.) démontre un grand potentiel en tant qu'agent de contrôle biologique efficace. Son efficacité à l'intérieur des serres est cependant restreinte due à une diapause hivernale. Cinq lignées d'*A. aphidimyza* qui n'entrent pas en état de diapause furent sélectionnées sous un régime photopériodique de 8 heures de lumière (LD 8:16) à 21°C. Une de ces lignées fut maintenue pendant 50 générations (3 ans) sans qu'il y ait d'effets sur la fécondité, la proportion des sexes ou la morphologie. Lorsque la sélection fut stoppée, une augmentation significative du niveau d'incidence ne fut notée qu'après les 8 premières générations. Les fluctuations du niveau d'incidence de diapause se produisirent simultanément dans toutes les lignées et étaient possiblement attribuables à des variations de la qualité des plantes-hôtes, qui fut demontrée modifier suffisamment la qualité des pucerons pour influencer le taux de diapause des larves de moucherons. Un régime photopériodique LD 8:16 combiné à une thermopériode 21:15°C provoqua la diapause chez les larves provenant des lignées sélectionnées; cependant la faible intensité de

radiations la nuit (<48 $\mu W/cm^2$) empêcha la diapause en présence de telles conditions. En cages, un ratio de 1 prédateur relâché pour 10 pucerons permit de contrôler les pucerons de novembre à janvier dans les serres.

THE GALL MIDGE SONG

If I were a gall midge,
Aphidolete-lete-lete-lete-deedle-dee,
All day long I'd feast on Aphididae
If I were a predatory midge.

I'd eat the big ones, little ones,
Especially the juicy ones,
Green, blue, red, maroon and black.
Latching onto her sweet little knee joint,
What a tasty snack!

I'd inject my special paralysing toxin,
Making sure she couldn't get away.
Then I'd settle down to eat in peace.

Pigging out on aphids:
That's the surest way to heaven that I know--
Nab those knee joints--freeze 'em on the spot
Suck their juices--get 'em while they're hot!
Leave them lying shrivelled in a knot.

--If I were a predatory midge.

M. Klein & M. Sirois

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I appreciate the cooperation of Dr. Robert McClanahan, who provided the original FIN line of *A. aphidimyza*, Dr. Jeremy McNeil, who sent the clone of *Myzus persicae* used, and Don Elliott, who provided the predatory mite, *Phytoseilus persimilus*. I wish to thank Diane Matthews, Dr. Reese Sailor, and Dr. Alan Journet for their efforts to obtain southern races of *A. aphidimyza*, and Dr. Art Borkent, Dr. Carl Yoshimoto, and H.E. Bisdee for prompt

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ORIGINAL CONTRIBUTIONS

Of General Significance:

1. This is the first selection of a beneficial insect for nondiapause to improve its effectiveness for biological control.
2. This is the first demonstration that quality of a host plant can affect a phytophagous insect sufficiently to influence diapause incidence in its predator.

Pertaining to *A. aphidimyza*:

3. This is the first time that data on emergence, diapause, sex ratio, mortality, and pupal development in *A. aphidimyza* has been collected for a prolonged period (3 yr., >50 generations). It is also the first time that lines of *A. aphidimyza* from several different geographical areas have been collected and compared. Arising from this study are the following original contributions:
4. There is wide variation in sex ratio in *A. aphidimyza* from generation to generation; a stable sex ratio is an important attribute of lines used for mass-production. Maintaining an adequate sex ratio is a major problem in establishing wild lines.
5. There is a wide range in fecundity between *A. aphidimyza* females, with some individuals laying >200 eggs.
6. Diapause is dominant over nondiapause and is inherited in accord with elimination of paternal chromosomes.
7. Diapause oriented larvae have a longer developmental period than pupation oriented larvae.

8. There is an indication that liability to diapause and development rate are related at the genetic level.
9. Low intensity radiation (<48uW/cm^2) at night prevents diapause in *A. aphidimyza* under cool, short day conditions that would otherwise induce 100% diapause. This is applied to improving biological control of aphids in greenhouses during winter for the first time.
10. *A. aphidimyza* is able to control aphids in midwinter greenhouse crops at release rates of 1:3 and 1:10.
11. Previous work on the interaction between photoperiod and thermoperiod in inducing diapause, and the photoperiodic termination of diapause was expanded upon.
12. *A. aphidimyza* eggs and larvae are not affected by Safer's Insecticidal Soap sprays.
13. An efficient cage was developed for rearing *A. aphidimyza*, which is now used by a commercial insectary.

Other Contributions:

14. Green traps were found to attract certain aphid-parasitic Hymenoptera for the first time.

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INTRODUCTION

Before entering graduate school, I was a greenhouse grower. I produced nursery plants in my private business, and bedding plants, tomatoes and other vegetables at a solar greenhouse research project where I worked. Aphids were often a severe problem in both greenhouses, in a variety of crops. Because I was using biological control agents against other pests, I experimented with aphidophagous insects sold by commercial insectaries. Coccinellids were expensive and starved before aphids were eliminated; in three successive shipments of lacewing eggs, larvae emerged en route, and survivors of the ensuing cannibalism never became established. I collected syrphid larvae from aphid colonies outdoors, and native parasitic Hymenoptera were often found in the greenhouse, but neither had a significant impact on aphid populations. After two seasons of unsatisfactory, labor intensive control, suddenly, over a 10-day period in late March, a serious aphid infestation in the commercial greenhouse I was managing was eliminated by tiny, orange maggots. Thus, began my introduction to the aphid midge, *Aphidoletes aphidimyza*.

Over the next two years, I became familiar with this native aphid predator and developed simple field collection, rearing and management methods for local growers (Gilkeson and Klein, 1981; Gilkeson and Armstrong, 1983). Although control of aphids with this predator was superior to other natural enemies I had tried, one problem remained. Larvae in the greenhouse entered diapause during November, and adults did

not emerge until mid-March. Although aphid control was excellent from spring through fall, aphid populations were unchecked during winter.

I decided to address this problem for my doctoral research. Because *A. aphidimyza* was superior to other species of aphidophagous insects tested, and was gaining wider recognition and use in greenhouse industries around the world (USSR, Finland, England, the Netherlands), I decided to concentrate on it, rather than looking for another species.

The first section of this thesis describes research on genetic selection of *A. aphidimyza* for nondiapause under typical greenhouse winter conditions. Later, work on preventing diapause with very low intensity light at night is described. The final section deals with practical application of *A. aphidimyza*, in which diapause has been prevented, in winter greenhouses. I planned and executed all experiments described in this thesis.

Notation and Style

I have adopted the widely used notation for photoperiod (Tauber and Tauber, 1976; Saunders, 1982) in which light:dark cycles are designated LD, with hours of light followed by hours of darkness. Thus, LD 8:16 describes a cycle of 8 h light and 16 h darkness. A similar system is used for thermoperiod temperatures, with TC 21:15°C designating a thermophase temperature of 21°C and cryophase of 15°C. In all experiments, unless otherwise stated, hours of thermophase coincide with the light phase, and cryophase coincides with the dark phase.

Measurement of light intensity for low light experiments was made in micro-watts per cm^2 (uW/cm^2) to conform with measurements currently recommended for photoperiod experiments (Philogène, 1982), and in lux (lx), so that results could be compared with those in the earlier literature.

Style conventions followed were those given in the CBE style manual (1978), and published by the Entomological Society of America.

SECTION 1.

LITERATURE REVIEW

APHIDS AS PESTS ON GREENHOUSE CROPS

Aphids are among the worst pests in greenhouses (Miles and Miles, 1935; Hussey, et al., 1969; Hanan, et al., 1978). They thrive in the warm, humid environment, in the absence of the large complex of natural enemies that control them outdoors. Aphids usually reproduce parthenogenetically all winter in a greenhouse, without a sexual generation, which would occur in the fall outdoors. Therefore, all aphids present are viviparous females and populations are capable of expanding with astonishing speed (van Emden, et al., 1969; Hussey, et al., 1969).

The most common and destructive species is the green peach aphid, *Myzus persicae* (Sulzer), which is outstanding in its widespread distribution, host plant range and its ability to transmit over 100 virus diseases (Miles and Miles, 1935; van Emden, et al., 1969; Hussey, et al., 1969; Dixon, 1985). Heavy infestations of *M. persicae* distort leaves, buds and terminal growth and reduce growth. They also excrete heavy deposits of honeydew, which are colonized by sooty moulds (*Cladisporium* spp.). This decreases the photosynthetic area of leaves and reduces yields and market value of fruit and flowers. As a virus vector in greenhouses, *M. persicae* is particularly destructive to tomatoes, lettuce, chrysanthemums and carnations (Wyatt, 1965; Hanan, et al., 1978). It is a serious pest on green peppers in the Netherlands (Ravensberg, et al., 1983) and Finland (Tiittanen, pers. comm.[1]). Fecundity of *M. persicae* is highest at 15-20°C (Barlow, 1962), which is the temperature range employed in winter for most crops under glass.

The other most important species found in greenhouses are the cotton aphid, *Aphis gossypii* Glover, which is a serious pest of cucumbers and melons, the rose aphid, *Macrosiphum rosae* L., found on greenhouse roses, the potato aphid, *Macrosiphum euphorbiae* (Thomas), which attacks a variety of vegetable and ornamental crops, and the mottled arum aphid, *Aulacorthum circumflexum* Buckton, a widespread pest in British greenhouses on arums, chrysanthemum, cyclamen and certain other ornamentals (Hussey, et al., 1969). Many other species are also known to infest greenhouse crops, but are less important than those mentioned above.

With the development of resistance to pesticides, control of aphids has become increasingly difficult. Resistance to organophosphorus pesticides is already widespread (Wyatt 1965; Hanan, et al., 1978) and resistance is increasing to the most recently introduced pesticide, pirimicarb (van Lenteren, et al., 1980). Pesticide injury to greenhouse crops, particularly young cucumber plants and some chrysanthemum cultivars, is also a problem, since thorough, repeated applications are necessary to control aphids.

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[1]K. Tiittanen, Agricultural Research Centre, Department of Pest Investigation, Jokioinen, Finland.

TAXONOMY OF *A. APHIDIMYZA*

The Italian dipterist, Rondani, first described the aphid midge, *Aphidoletes aphidimyza*, in 1847. Subsequently, over 30 species of aphidophagous Cecidomyiidae were described, based on minor morphological differences and associations of larvae with different aphid species (Rübsaamen, 1891, 1892; Kieffer, 1896; Coquillett, 1900; Felt, 1911, 1912, 1914, 1918; Bagnall and Harrison, 1917a, 1917b, 1917c; Tolg, 1921; Barnes, 1927). Thirty-seven specific and 10 generic names of aphidophagous midges are listed in a taxonomic review by Barnes (1929), most of which are now synonyms for *A. aphidimyza*.

After Nijvelt (1954, 1955, 1957) and Milne (1960) reviewed adult morphology and established the fact that aphid midge larvae feed on several species of aphids, some of the taxonomic confusion was reduced. Nijvelt, however, later published a book on gall midges, which included numerous descriptions of aphidophagous species, all synonymous with *A. aphidimyza* (Nijvelt, 1969). Detailed descriptions of adult morphology were provided by Mamaeva (1964; 1981), and descriptions of four good species of aphidivorous midges were published by Harris (1966). Gagné (1971, 1973) revised the Nearctic species of aphidophagous Cecidomyiidae, defining three good species. Yukawa (1971) revised the Japanese gall midges, and Grover (1979) revised Indian Cecidomyiinae. A worldwide revision by Harris (1973) summarized distribution, host ranges and biology for all known species: *A. aphidimyza*, *A. urticariae* (Kieffer), *A. thompsoni* Mohn, *A. abietis* (Kieffer) and *Monobremia subterranea*

(Kieffer). The first three species are potentially useful as biological control agents.

Recently, using a transmission electron microscope, Dallai and Mazzini (1980) established that characteristics of cecidomyiid spermatozoa, including that of *A. aphidimyza*, could be used as taxonomic characteristics.

Full classification of *A. aphidimyza*, according to McAlpine, et al. (1981) is as follows: order, Diptera; suborder, Nematocera; infraorder, Bibionomorpha; superfamily, Sciaroidea; family, Cecidomyiidae; subfamily, Cecidomyiinae; supertribe, Cecidomyiidi. No tribe designation is used (Gagné, 1971).

GEOGRAPHICAL DISTRIBUTION OF *A. APHIDIMYZA*

A. aphidimyza is a common, holarctic species. Although it may have been indigenous to Europe initially, it was probably spread globally by humans, on plants, or in soil (Harris, 1982). It has been recorded from Czechoslovakia, Austria, England and Wales, Italy, Israel, Sudan, Canada, United States, USSR (Gagné, 1971; Harris, 1973), Japan (Yukawa, 1971), Hawaii (Swezey, et al., 1931), Finland (as far north as 68°N lat.) (Markkula, et al., 1979c), Egypt (Azab, et al., 1965a,b), Poland (Olszak, 1979), Turkey (Uygun and Özgür, 1980), Yugoslavia (Simova-Tošić and Vuković, 1980) and is probably present in the People's Republic of China (Qiu, pers. comm.[1]). The only records for the southern hemisphere are from Chile (Gagné, 1971).

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[1]Qiu, S., Biological Control Laboratory, Beijing, P.R.C.

BIOLOGY OF *A. APHIDIMYZA*

Information in the following section is from detailed descriptions of biology and external morphology given in Davis (1916), Roberti (1946), Milne (1960), Azab, et al. (1965a), Harris (1966, 1973), Markkula, et al. (1979c) and Bouchard, et al. (1981), as well as from my own research.

Adults

Adult *A. aphidoletes* are tiny, fragile insects (2 mm long) with long, slender legs (Figure 1.). The sexes are readily distinguishable, without magnification, by their differing antennal morphology. Antennae of males are longer and curved backward, with whorls of looped circumfilia at each flagellomere (Gagné, 1973). Females have shorter, thicker antennae, without circumfilia. Both sexes are predominantly dark grey, however, the abdomens of ovipositing females are larger and tinted orange.

The life span of midges is short, males living only 3–5 d and females 7–12 d. Feeding on honeydew increases adult life span (Uygun, 1971), the influence depending on both source and quality of the honeydew (Wilbert, 1977). Exposure to high temperatures and low humidity decreases life spans.

Adults are nocturnal; during the day they rest under leaves or in tall grass, where it is shaded and humid. Mating and oviposition usually take place at dusk. Females must mate to lay fertile eggs, although, contrary to El Titi (1974b), virgin females can lay eggs, albiet not viable ones (Bouchard, et al., 1981). Wood-Baker (1964) reported paedogenesis in *A. phidimyza* and several Soviet authors have speculated on the existence of parthenogenesis (Grinchuk, 1974; Chubareva and

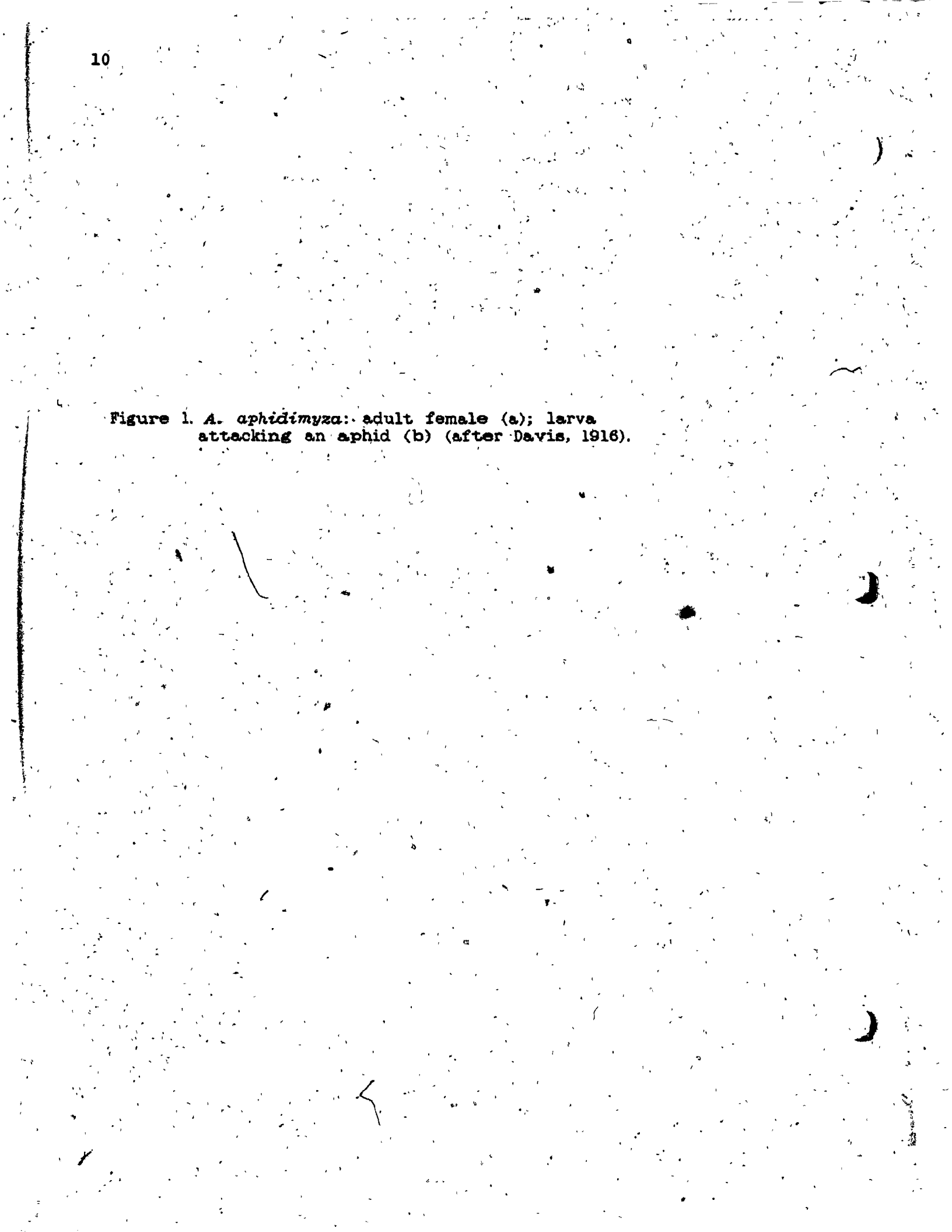
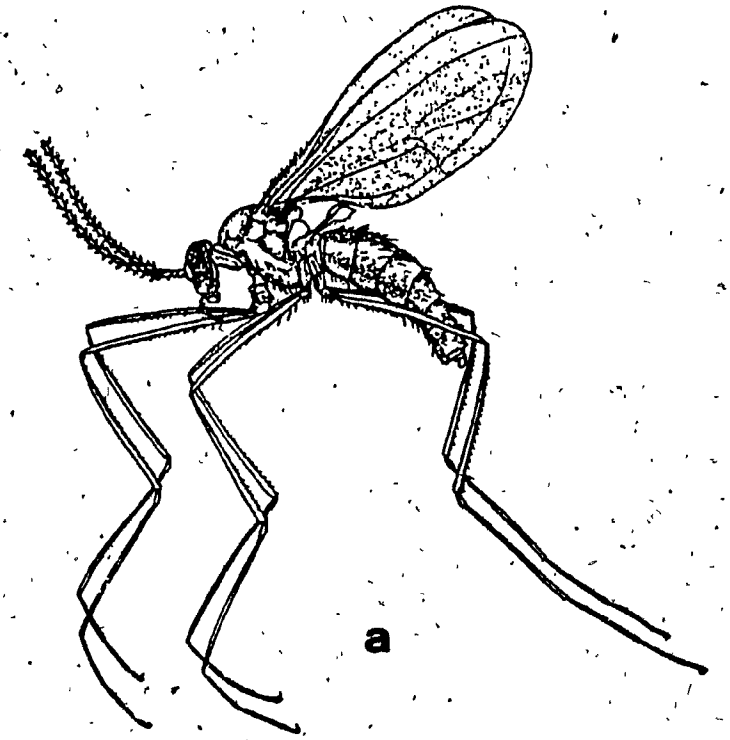
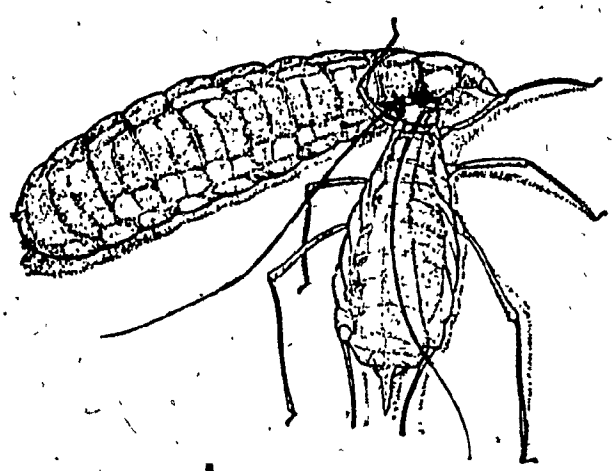


Figure 1. *A. aphidimyza*: adult female (a); larva attacking an aphid (b) (after Davis, 1916).



a



b

Koslova, 1980), however, no other authors have found evidence of either condition.

Reproduction is monogenic, thus all offspring of a single female are the same sex (Sell, 1976). In many Cecidomyiidae and some other families of Diptera, monogenic reproduction is associated with a peculiar chromosome cycle, in which paternal chromosomes are eliminated during early cleavage, leaving only maternally derived chromosomes in the germ lines. Thus, males contribute genes to somatic, but not germ line, cells of offspring and it is the genetic constitution of females that determines the sex of offspring (Metcalf, 1935; Geyer-Duszyńska, 1959, 1961; Crouse, 1960; Nicklas, 1960; Panelius, 1971; Ullerich, 1973; Mori, et al., 1979; Abbott and Gerbi, 1981). With the exception of Grinchuk (1974) and Chubareva and Koslova (1980), who investigated the morphology of polytene chromosomes, no analyses of *A. aphidimyza* chromosomes or inheritance have been reported. My studies on inheritance of diapause are reported in this thesis (pg. 116).

Sex ratio in experimental midge cultures has been given in the literature as 1 male:1 female (Sell, 1976), 1:1.7 (Uygun, 1971) and 1:2-3 (Ushchekov, 1975). In my studies, however, sex ratio varied from generation to generation (1:0.6-6.0); in some lines it was so unstable that eventually only one sex emerged. Such a shift to predominantly females was reported in laboratory cultures by Bondarenko and Kozlova (1982), however, in my cultures, a shift to predominantly males was equally common.

Females do not oviposit until 24 h after eclosion (Pritchard, 1961), when eggs are laid among aphids on

undersides of leaves. Females distinctly prefer to lay eggs under low light conditions (280 lx) rather than in bright light (>1100 lx) or total darkness, and also prefer lower leaves, probably because of the higher humidity (Mansour, 1976, 1980). Number of eggs laid is nearly proportional to aphid density (El Titi, 1972/73), and females are able to find isolated aphid colonies quickly (Ushchekov, 1975). El Titi (1974a) found that they were able to locate one infested plant among 75 aphid-free ones, however, if all plants were equally infested, edge plants received more eggs than central plants (El Titi, 1972/73). How females locate aphids is not clear, but, according to El Titi (1972/73; 1974b), a combination of olfactory and tactile stimuli from aphids, honeydew and exuviae release oviposition in *A. aphidimyza*. Some differences in host plant preference have been shown (Mansour, 1975, 1980). Although El Titi (1974b) stated that aphid species did not seem to affect oviposition, Havelka and Růžička (1984) found that ovipositing females showed a clear preference for *Aphis fabae* Scopoli over three other aphid species reared on broad beans, *Vicia faba* L.

Mean number of eggs laid per female has been reported as 6-38 eggs (Ushchekov, 1975), 70 eggs (Uygun, 1971) and 100 eggs (Harris, 1973), but environmental conditions were not stated. According to a detailed study on effects of environment on oviposition, temperature had the greatest influence, but photoperiod was also important (Bradovskaya, 1975). Under optimum conditions (16 h day, 25°C) females laid a mean of 109 eggs over 10 d. I obtained exactly the same results during my first tests of fecundity (17 h day,

21°C), however, a year later, the mean for the same line of *A. aphidimyza* had risen to 164 eggs per female (same environmental conditions), possibly because of improved larval nutrition. Nutrition of larvae has been shown to affect adult fecundity (Uygun, 1971; Kuo, 1975, 1976/77, 1982; Havelka and Růžička, 1984; Sell, 1984b, 1985) and the quality of honeydew available to adults can also affect fecundity (Wilbert, 1977).

Eggs

A. aphidimyza eggs are smooth, shiny and bright orange (0.3 x 0.1 mm wide). They are laid singly or in small groups near aphids, and occasionally on aphids. Larvae hatch from the anterior end of the egg in 2-3 d (21°C). Effects of temperature on development rates and lower threshold temperatures for egg (10.1°C), larval (4.3°C) and pupal (5.7°C) stages were investigated by Havelka (1980a).

Larvae

Larvae are 0.3 mm long at hatching and grow to 2-3 mm when ready to pupate (Figure 1.). They are typical dipterous maggots, elongated and narrow at each end with strong, hooked mandibles. Detailed internal and external morphology of head and mouthparts are illustrated in Solinas (1968). Larval color ranges from pale orange to dark red, depending upon the prey. Harris (1973) lists a host range of 61 species of aphids, including such serious agricultural pests as *Aphis fabae, A. gossypii* Glover, *A. pomi* De Geer, *Brachycaudus helichrysi* (Kaltenbach), *Brevicoryne brassicae* (L.), *Macrosiphum euphorbiae* (Thomas), *Myzus persicae*

(Sulzer), *Phorodon humuli* (Schrank) and *Rhopalosiphum padi* (L.).

Larvae develop in 7-14 d outdoors, depending on temperature. Although Uygun (1971) states that development took 3.8 d, at 21°C, other authors agree that ca. 7 d are required (Havelka, 1980a; Bouchard, et al., 1981). It is generally agreed that there are three larval instars, although Azab, et al. (1965a) report four.

Newly hatched larvae are able to locate aphids from a distance, over an area of 2.7 cm^2, mainly by olfaction, although vision may play a part (Wilbert, 1972, 1973, 1974). They attack aphids by piercing a joint in the integument, usually on the leg, with their curved mandibles. They then inject an unknown toxin from the salivary glands that paralyzes aphids in a few minutes (Mayr, 1975). Mayr (1975) suggested that phenol-oxidase in the saliva may be involved, however, the composition of this toxin has been studied by thin film chromatography and no compound has been found that would account for the strong paralyzing effect (Markkula, et al., 1979c; Laurema, pers. comm.[1]).

The contents of aphids are dissolved within 10 min and larvae then feed by extracting body fluids. Last instar larvae drain a small aphid in a few minutes, taking up to 15-30 min for a large one (Webster and Philips, 1912). After feeding, only the desiccated, empty husks remain, often hanging from stylets still embedded in the leaf (Bombosch, 1958).

The number of aphids consumed by one larva depends on

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[1] V. Laurema, University of Helsinki, Finland.

size, species, and density of prey. Individual larvae have been reported to eat, during their lives, 40-60 *B. brassicae* (George, 1957), 28 *Aphis pomi* (Adams and Prokopy, 1977b), 25 *A. fabae*, 28 small *M. persicae*, or 13 *Acyrthosiphum pisum* (Harris) (Uygun, 1971). The difficulty in assessing consumption by counting aphids is shown by Raworth (1984), who found predation was random with respect to aphid instar, and that total weight of aphids consumed was relatively constant (ca. 2.14 mg).

An extremely important characteristic of *A. aphidimyza* is their density dependent predation behavior, which is often overlooked when assessing potential predacity. When aphids are abundant, larvae kill many more than they can eat (Barnes, 1929; Dunn, 1949; Uygun, 1971). The number of aphids attacked is proportional to the logarithm of prey density (Uygun, 1971). Aphids may be uneaten, or only partially consumed, before larvae select another victim (Uygun, 1971). When aphids are scarce, however, one larva can survive and pupate on as few as 7 *M. persicae* (Nijvelt, 1966; Uygun, 1971).

During severe food shortages, young larvae are able to survive without food for 2 d, last instar larvae for 7 d (Uygun, 1971). While searching for prey, however, they resort to cannibalism of smaller larvae (Azab, et al., 1965a) and eggs. Larvae feed on aphids killed by cold, but larval development is prolonged and adult fecundity decreases (Sell, 1984b). They also attack scales of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, (Ushchekov, 1975; Pavlyushin, et al., 1982) and eggs of two-spotted spider mite, *Tetranychus urticae* Koch, but it is doubtful whether they

can survive to maturity on these prey. Larvae consume more aphids under cool conditions (15°C) (Uygun, 1971) and under dry conditions (56% relative humidity, RH) (Mayr, 1973), but are most active when relative humidity is highest (>90% RH) (Mayr, 1981).

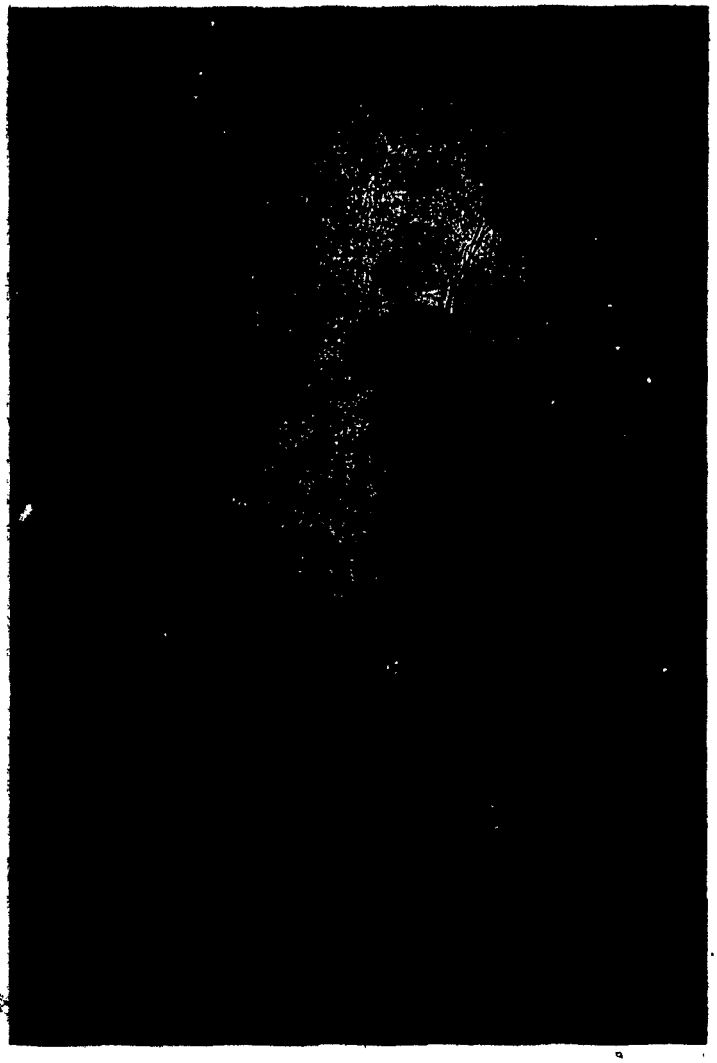
Kuo (1975, 1976/77, 1982) found that type of host plant and nutrition of aphids eaten by larvae affects both fecundity and longevity of adults, as well as larval and adult weights and predation behavior. Different species of aphids reared on the same host plants also affect larval development and adult fecundity (Havelka and Růžička, 1984).

Pupae

When larvae are ready to pupate, they leave the plant, usually by "jumping" (Milne, 1960; Osmers and Wilbert, 1979), and burrow 0.5-3.0 cm into the soil to spin cocoons (Figure 2.). They may pupate on leaves, particularly when there are many aphid husks and exuviae present to cover cocoons. Pupation begins within 2-4 d of spinning the cocoon; descriptions of pupal development are given by Azab, et al. (1965a) and Bouchard, et al. (1981). The greatest number of pupae survive in moist pupation substrates, with high relative humidity (80-90%) (Ushchekov, 1975).

Pupation lasts 11-15 d (21°C). Males tend to emerge a day or two sooner than females, and most eclosion takes place at dusk. Pupae work their way to the surface shortly before eclosion, and adults emerge head first from the split pupal case.

Figure 2. *A. aphidimyza*: pupa (a); cocoon (b); diapausing larva (c).



Diapause

In most temperate areas, there are 3-5 generations of *A. aphidimyza* in the summer. The last generation in fall diapauses as a prepupal larva in cocoon (Figure 2.); pupation takes place in the spring. Diapause induction occurs during the larval period, the degree of response being modulated by both photoperiod and temperature (Havelka, 1980b,c). In the laboratory, all larvae diapause under LD 8:16 and TC 25:10°C (Forsberg, 1979). A difference of 1.5 h in critical daylength for diapause induction was observed in two populations from different geographical areas of the USSR (Havelka, 1980b). Because diapause is facultative, a photoperiod regime of LD 16:8 (21°C) prevents diapause in laboratory cultures (El Titi, 1972/73), however, with lines originating in Canada, I found that a regime of LD 17:7 (21°C) was necessary to prevent diapause. Diapause in experimental populations can be terminated by photoperiod alone, without chilling (Havelka, 1980b).

NATURAL ENEMIES OF *A. APHIDIMYZA*

A. aphidimyza has few natural enemies. Voukasovich (1925) recorded a hymenopterous parasite, *Synopeas rhanis* Walker (Proctotrupoidea), and Harris (1973) mentions an unknown species of Braconidae emerging from a cocoon. I reared a single, female, *Gastranoistrus* sp. (Pteromalidae) from a larva collected in Quebec. Three other cocoons from the same site, containing parasitic wasp remains, were also observed. Because endoparasites appear to be rare, these may be cases of fortuitous parasitism. Under laboratory conditions, I observed that lacewing larvae, *Chrysopa* spp., collected from wild

aphid colonies, distinctly preferred *A. aphidimyza* larvae over aphids; therefore, it is likely that they feed on midge larvae in the field. Coccinellid larvae in the same test preferred aphids over midge larvae.

Perhaps a more important enemy under greenhouse conditions is the parasitic fungus, *Entomophthora apiculata* (Thaxter) M. Gustafsson, which has been observed attacking *A. aphidimyza* larvae in Finland (Kariluoto, 1982). A closely related midge species, *A. thompsoni*, is susceptible to *Beauveria bassiana* (Balsamo) and *Isaria farinosa* (Fries) Vuillemin (Smirnoff and Eichhorn, 1970).

APPLICATION OF *A. APHIDIMYZA* TO BIOLOGICAL CONTROL

Value as Natural Predator

In numerous studies of natural predator-prey systems, it has been shown that *A. aphidimyza* is the most effective native predator of *A. pomi* in orchards (Ross, 1917; Remaudière, et al., 1973; Adams and Prokopy, 1977a, 1977b, 1980; Olszak, 1979; Bouchard, et al., 1982; Tracewski, et al., 1984), of *B. brassicae* on cole crops (Hafez, 1961; Pollard, 1969; Raworth, et al., 1984) and various other aphid species (Davis, 1916; Azab, et al., 1965b; Harris, 1973; Whalon and Elsner, 1982; Lugovitsyna and Potemkina, 1983). Recently, its role in suppressing aphids in grain crops has been studied (Berest, 1980; Fougeroux, 1984).

In 1916, Davis concluded that *A. aphidimyza* was a potentially valuable biological control agent for aphids, but applied research was not conducted until over 50 years later. It is possible that the value of cecidomyiid larvae as predators of aphids was not fully appreciated until recently because eggs and larvae are inconspicuous and cannot be counted accurately in the field (Harris, 1973).

Basic studies in larval predacity and host finding ability of *A. aphidimyza* larvae and adults were carried out in the Federal Republic of Germany during the early 1970's (i.e., Uygun, 1971; El Titi, 1972/73, 1974a, 1974b; Wilbert, 1972, 1973, 1974; Mansour, 1975; Sell, 1975; Kuo, 1975). Despite reservations about production methods, Mayr (1973) concluded that effective control of aphids in greenhouses was possible with *A. aphidimyza*, but little applied research has been

published in Germany until recently (Kuo, 1982; Sell, 1984a,b, 1985).

Greenhouse Application

Practical application in greenhouse crops has been extensively investigated in the Soviet Union and Finland. In the Soviet Union, emphasis is on control of *A. gossypii* on cucumber plants (Asyakin, 1977; Bondarenko, 1975, Ushchekov, 1975, 1977; Adashkevich, 1975; Bondarenko and Moiseev, 1978; Storozhkov, et al., 1981). In Finland, research is directed toward control of *M. persicae* on peppers and tomatoes, and *Macrosiphum rosae* (L.) and *M. euphorbiae* on roses (Markkula, 1975, 1978; Markkula and Tiittanen, 1977, 1980; Markkula, et al., 1979c). *A. aphidimyza* pupae have been sold commercially to Finnish growers since 1978, when 70 growers applied over 100,000 pupae to crops (Markkula and Tiittanen, 1980). Studies have been conducted in Denmark (Hansen, 1980), Norway (Hofsvang and Hågvar, 1982) and in the Netherlands (van Lenteren, et al., 1980), where midge pupae have been sold to growers since 1980 (Ramakers, pers. comm.[1]).

Although a 1978 report to the Research Branch of Agriculture Canada recommended testing *A. aphidimyza* in Canadian greenhouses (Borden, et al., 1978), they have only been used on a small scale, or experimentally, in Canada (Gilkeson and Klein, 1981). Pupae have been available in Canada since 1983 from two commercial insectaries (Applied

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[1]P.J.M. Ramakers, Glasshouse Crops Research and Experimental Station, Naaldwijk, the Netherlands.

Bio-Nomics, Ltd., Sydney, B.C.; Better Yield Insects, Tecumseh, Ontario). A few trials have been conducted in the United States (Armstrong, 1983; Olkowski, et al., 1983; Meadows, et al., 1986), but no insectaries there supply midge pupae at this time.

Mass-production

Mass-production methods have been developed and refined, using *M. persicae* on green pepper plants as prey (Bondarenko and Asyakin, 1975; Markkula and Tiittanen, 1976a; Rimpiläinen, 1980). Neither larvae nor adults are suitable for shipping, but pupae survive handling very well (Asyakin, 1977; Markkula and Tiittanen, 1977), especially when sent in moist peat moss (Markkula, et al., 1979b). Prolonged cold storage of diapausing larvae promises to make mass-production more economical (Forsberg, 1980; Havelka, 1980b), but more research is needed to develop reliable storage methods. Accumulation of pupae in layers of cloth around the base of plants (Lukin, et al., 1983) or automatic collection of mature larvae in running water as they leave plants to pupate (van Leiburg and Ramakers, 1984) decrease rearing costs.

Release Rates and Methods

Several ways of introducing *A. aphidimyza* have been investigated, including attracting native populations into greenhouses using flowering plants and honey as lures (Ushchekov, 1977), saturating surrounding areas with massive releases of midges to establish permanent populations, which then enter greenhouses (Bondarenko, 1975), and accumulating midges directly in cucumber greenhouses on "banker" plants (Bondarenko and Moiseev, 1978). In the latter case, broad

beans were used, infested with *A. pisum*, or other aphid species not infesting cucumbers.

The most predictable control is achieved by releasing a known quantity of pupae, based on aphid density or greenhouse floor area, at regular intervals. Relatively low release rates (1 pupa:200 aphids) kept *A. gossypii* below economic injury level, when 2-3 releases, 7-10 d apart were used (Ushchekov, 1975). Higher release rates, such as 1:20 or 1:40 (Ushchekov, 1975; Storozhkov, et al., 1981), and in some cases 1:1 or 1:4 (Asyakin, 1977), were necessary for eradication of aphids. To control *M. persicae* on peppers in Finnish greenhouses, releases of 1:3 or 2-5 pupae per m^2 are recommended (Markkula and Tiittanen, 1977; Markkula, 1978). In practice, growers seldom use more than one release in early spring, although a second release may be necessary after two weeks (Tiittanen, pers. comm.). Scopes (1982) reports that three introductions, one week apart, of eight pupae per m^2, controlled *A. gossypii* on cucumber, which is twice as many as recommended by Bondarenko and Moiseev (1978). Discrepancies in release rates may be accounted for by differences in mean temperatures, and by the fact that overwintered midge populations contribute an unknown amount to aphid control the following spring.

Finnish growers, most of whom do not grow crops in midwinter, take advantage of permanent populations overwintering in soil (Markkula and Tiittanen, 1977). For other growers, such as those in Norway, the disappearance of the aphid predators as they enter diapause in late fall is a disadvantage (Hofsvang and Hågvar, 1982). This is also a

problem in Canada, where most growers produce crops year-round (Gilkeson and Klein, 1981).

Integration with Pesticides

The effect of pesticides on *A. aphidimyza* has been studied to find materials compatible with the use of midges in integrated control programs for greenhouses, orchards, and other crops. A wide range of pesticides have been tested (Markkula and Tiittanen, 1976b; Markkula, et al., 1979a; Warner and Croft, 1982; Whalon and Elsner, 1982; Sell, 1975, 1984a,b, 1985; David and Horsburgh, 1985) and it has been found that most fungicides and acaricides, and some insecticides, have little effect on *A. aphidimyza*. This may be because larvae have an active defense system, exuding a fluid that wets the surface of the body in response to irritation, such as poison particles (Ushchekov, 1975).

Some resistance to orchard chemicals was found in populations from cultivated orchards (Adams and Prokopy, 1977b; Warner and Croft, 1982). Moore (1976) provides a spray schedule that can be integrated with *A. aphidimyza* use in orchards; Tabashnik and Croft (1985) have published a model for predicting aquisition of pesticide resistance among apple pests and beneficial insects, including *A. aphidimyza*. Other work has been conducted on effects of pyrethroid compounds (Sukhoruchenko, et al., 1981), and insect juvenile hormones (El-Gayar, 1976), and I have investigated the effect of insecticidal soap on *A. aphidimyza* (pg. 203).

SUITABILITY OF *A. APHIDIMYZA* FOR BIOLOGICAL CONTROL IN GREENHOUSES

Based on my work, and the literature cited in the previous review, the following advantages of using *A. aphidimyza* to control aphids in greenhouse crops are apparent.

1) Number of aphids killed by each larva increases with aphid density. Unlike coccinellids, they kill many more aphids than they eat when aphids are plentiful, yet can survive on very few aphids if necessary.
2) Adult midges are able to locate isolated foci of aphid infestation, even in large greenhouses.
3) Larvae feed on all species of aphids found in glasshouses, and are exclusively aphidophagous.
4) Unlike *Chrysopa* spp., larvae are not cannibalistic, except when in a state of extreme starvation.
5) Permanent populations can be established in greenhouses, even in those not heated during winter.
6) Mass production is easy and possible under a wide range of conditions.
7) Pupae stored in peat moss withstand shipping and handling well.
8) Short term cold-storage of nondiapausing pupae and long term cold-storage of diapausing larvae is possible.
9) Unlike parasitic Hymenoptera, midges can control aphids under cool, winter greenhouse conditions.
10) Use of *A. aphidimyza* can be integrated with insecticidal soap sprays, most fungicides, acaricides and selected other greenhouse chemicals.

The main disadvantage of *A. aphidimyza* as a biological control agent in Canadian greenhouses is that larvae diapause in response to short daylengths in the fall, even in a greenhouse, and do not emerge again until spring. This is the central problem addressed in my research. Relatively low fecundity has been cited as a disadvantage (Harris, 1982), however, this is mitigated by the functional response of larvae to high aphid densities. Synchronization of generations after one release in a greenhouse may also pose a problem, but this can be solved by successive releases.

DIAPAUSE INDUCTION IN INSECTS

Diapause has evolved as an escape from unfavorable environmental conditions (Dingle, 1978). Once thought to be a state of complete physiological rest, diapause is now known to be a genetically controlled state of low metabolic activity. During diapause, morphogenesis ceases, but physiological development continues in preparation for subsequent active phases. Unlike quiescence, or other semidormant states that occur in immediate response to adverse conditions, physiological preparations for diapause are made in advance, in response to environmental cues that signal a change in seasons (Mansingh, 1971; Tauber, et al., 1984). Induction of diapause by the interaction of several environmental and physiological factors assures synchronization of insect life stages with the seasons for which they are adapted (Danilevskiĭ, 1965). Therefore, in temperate and northern latitudes, where great seasonal differences in climate occur, diapause is an important aspect of an insect's ecology. Diapause, which may be facultative or obligatory, varies in intensity and duration, and occurs in egg, larval, pupal or adult stages, depending on species. The "diapause syndrome" (Tauber et al., 1984), has been reviewed by Andrewartha (1952), Masaki (1961), de Wilde (1962), Danilevskiĭ (1965), Lees (1968), Dingle (1978), Beck (1980) and Saunders (1982). The physiology of diapause has been reviewed by Lees (1956), Harvey (1962), Beck (1963), and Chippendale (1977); Mansingh (1971) outlines a classification system for diapause based on physiological differences.

Role of Photoperiod

Photoperiod is generally regarded as the most important environmental factor influencing diapause induction. Photoperiod changes with season and latitude, but it is predictable from year to year in the same locality, thus, it is a "noise-free" indicator of seasons (Saunders, 1982). Most temperate zone insects grow and reproduce during the long days of summer, and respond to decreasing fall daylengths by preparing to diapause. This "long day" photoperiodic response is the most common, but there are species, with "short day" responses, that diapause in summer and are active from fall to spring. Some exceptional species diapause in summer and winter, and have both types of photoperiodic response (Danilevskiĭ, 1965; Saunders, 1982).

An important concept is that of critical photoperiod, which is the photoperiodic regime that induces 50% of individuals in a population to diapause (Lees, 1956). For a particular species in a given locality, there is usually a well defined critical daylength that marks a shift in most of the population from diapause to nondiapause (Danilevskiĭ, 1965). In a few species, a difference of 10-15 min in daylength around the critical photoperiod is enough to shift most of the population from one developmental track to the other. In most, however, a change of one hour is necessary to effect this shift (Saunders, 1982).

In many species, the photoperiodic response actually depends upon changes in night length, rather than daylength (Lees, 1968; Beck, 1980). Thus, breaking up long nights, with light pulses at critical periods, prevents diapause in species

such as the turnip sawfly, *Athalia rosae* L. (Saringer, 1983), and the Indian meal moth, *Plodia interpunctella* Hübner (Kikukawa and Masaki, 1984). Daylength is important however, for if it is too short, the dark phase becomes ineffective as a signal (Lees, 1968; Beck, 1980). This was shown in *P. interpunctella*, in which full expression of the diapause response depends on both a minimum daylength and a minimum night length (Kikukawa and Masaki, 1984).

The concept of critical photoperiod is based on the assumption that the insect's receptor system reacts to absolute daylength, rather than to progressively increasing or decreasing daily changes in photoperiod (Beck, 1980). This assumption is probably valid for species with short photosensitive stages (de Wilde, 1962). In others, such as univoltine species with prolonged photosensitive stages, there is no characteristic critical photoperiod threshold, and it is the direction of changing daylength that becomes important (Danilevsii, 1965; Tauber and Tauber, 1970; Beck, 1980).

In most species, photosensitivity is limited to one, or a few, instars, and does not encompass the entire life cycle (de Wilde, 1962). When the sensitive stage is limited to one instar, only a few photoperiod cycles may be necessary to fix the developmental pathway. Photoperiods that promote growth and activity, rather than diapause, tend to override effects of diapause inducing photoperiods; a few long days at the end of the sensitive stage may cancel the effect of earlier short days (Dickson, 1949).

In some insects, the duration of the photosensitive period and the number of short day cycles necessary to induce

diapause, are separate factors. This was demonstrated in the flesh fly, *Sarcophaga argyrostoma* (Robineau-Desvoidy), by artificially prolonging and shortening the larval (photosensitive) period. A higher incidence of diapause occurred in larvae with prolonged development periods, because they experienced more short day, diapause inducing, cycles, than did larvae with shorter development periods (Saunders, 1975a).

It is important to distinguish between photoperiodic induction, which is a reversible process occurring during sensitive stages, and the photoperiodic response, which is the reaction within the insect ending in diapause (de Wilde, 1962). Sensitive stage and responsive stage are usually different, and may be widely separated. For example, eggs and young larvae of the vine tortricid, *Polychrosis botrana* (Schiffermueller), are photosensitive, but diapause occurs in the pupal stage (Lees, 1968). A more extreme example of this separation is maternal induction of diapause, in which environmental stimuli experienced by a female influences the developmental pathway of offspring (Rin[illegible], 1967).

Role of Light Quality

In addition to photoperiod, light intensity and spectral quality are involved in the photoperiodic induction of diapause. Early work on domestic silkworm, *Bombyx mori* (L.), showed that both eggs and larvae responded to light levels of 0.1–0.8 lx (Kogure, 1933). Harvey (1957) noted that low light intensity resulted in a higher diapause incidence in some lines of the spruce budworm, *Choristoneura fumiferana* (Clemens). Subsequent data from various experiments suggest that intensity thresholds for photoperiodic diapause response are as low as 0.025–25.0 lx in many insects (de Wilde, 1962; Lees, 1968; Saunders, 1982). The intensity threshold is usually higher than moon light (0.04–0.28 uW/cm^2, or 0.1–0.7 lx), while being below that of available light at sunrise and sunset (de Wilde, 1962; Saunders, 1975b, 1982). These thresholds are not symmetric, as shown by Takeda and Masaki (1979), who found that dawn thresholds (1 lx) were lower than at dusk (10 lx) in fall webworm, *Hyphantria cunea* Drury. A similar asymmetry occurs in the parasitic wasp, *Nasonia vitripennis* (Walker) (Saunders, 1975b). These thresholds occur approximately 40 min before sunrise and 20 min after sunset, thus, for practical purposes, effective photoperiod can be calculated by adding periods for civil twilight to the hours from sunrise to sunset.

Determination of light intensities and spectra actually experienced by insects is complicated by the fact that light must penetrate head capsule cuticles (and cocoons of pupating stages) to reach receptors in the brain. In the oak silkworm, *Antheraea pernyi* Guerin-Meneville, <0.5% of the light

striking the cocoon exterior reaches the brain (Williams, et al., 1965). For species pupating in soil or burrowing in fruit, light intensity is further reduced and spectral quality significantly altered in their habitat (Saunders, 1982). For larvae of the Oriental fruit moth, *Grapholitha molesta* (Busck), inside apples, Dickson (1949) found that an intensity of 32 lx, striking an apple surface, affected diapause induction. Later measurements of light levels inside apples showed that 32 lx on the outside declines to 1.2 lx at the interface of core and pulp (Prokopy, 1968).

Spectral quality of light is important in diapause induction because most species of insects are sensitive to blue light, from 365 nm near ultraviolet (UV) to 500 nm blue green, but not to longer wavelengths (de Wilde, 1962; Lees, 1968; Saunders, 1982). This is shown in the cabbage white, *Pieris brassicae* (L.), in which UV, green and yellow light spectra had the same effect as white light in inducing diapause, but red had no effect (Vuillaume, et al., 1974). In a related species, *Artogeia rapae* (L.), diapause was averted under long day, high UV conditions, but 10-30% diapause occurred when low UV was used with the same photoperiod (Thoms and Philogène, 1979). Although most insects detect only blue light, a few species respond to red light (>600 nm) (Saunders, 1982). Fourteen hours of red light (640 nm) induced 5% diapause in the pink bollworm, *Pectinophora gossypiella* Saunders, whereas 12 h (620 nm) induced 98% diapause, showing that larvae could detect the difference in length of light periods (Pittendrigh, et al., 1970). Similar results were obtained with *N. vitripennis* (Saunders,

1982). It is possible that infrared (IR) radiation also plays a role in diapause induction, since it penetrates leaves, vegetation, soil, water vapour in the air and some wood (Callahan, 1965)

Measurement of illuminance in lux is based on the spectral sensitivity of the human eye and is not a measurement of total energy, or irradiance, which is measured in watts/m^2 (W/m^2). The two measurements can only be equated at wavelengths of 400-700 nm (de Wilde, 1962). Because insects may respond to spectra outside this range, it is preferable to measure irradiance in intensity experiments (Philogène, 1982). This is important in experiments using artificial lighting, because light sources differ in spectrum and intensity.

Role of Thermoperiod and Temperature

The role of thermoperiod in diapause induction, recently reviewed by Beck (1983), is usually subordinate to, and modifies that of, photoperiod. A daily temperature cycle, however, in the absence of a light cycle, can simulate the effect of photoperiod in inducing or preventing diapause in several species (Saunders, 1975, 1982; Beck, 1982, 1983). Thermoperiodic control of diapause has been demonstrated in the southwestern corn borer, *Diatraea grandiosella* Dyar (Chippendale, et al., 1976), the pitcher plant mosquito, *Wyeomyia smithii* Coquillett (Bradshaw, 1980), *Pieris brassicae* (Dumortier and Brunnarius, 1981), *Plodia interpunctella* (Masaki and Kikukawa, 1981) and the European corn borer, *Ostrinia nubilalis* (Hübner) (Beck, 1982).

Photoperiod effects on diapause are usually intensified when the cool phase of a thermoperiod coincides with

scotophase, and are reduced when the warm phase coincides with scotophase (Roach and Adkisson, 1970; Gibbs, 1975; Beck, 1980, 1983). The effect of temperature on thermoperiodic determination of diapause is quite complex, but night temperatures appear to be more important than day temperatures (Saunders, 1982; Beck, 1983), although thermoperiod itself is also important. In *O. nubilalis*, diapause incidence depends on duration of the cryophase below a thermoperiodic response threshold temperature of 17.5°C (Beck, 1982), and this type of response may be true of other species (Saunders,1983). The diapause threshold temperature was shown to be separate from development rate in *O. nubilalis*, by rearing larvae under LD 12:12 and mean temperature of 20°C (TC 10:30°C), which resulted in 99% diapause, and compared to rearing them under constant 20°C, which resulted in only 5% diapause (Beck, 1982). Further investigation of temperature threshold effects on diapause in this species showed that induction of diapause was strongly influenced by cryophase temperatures below 17.5°C, when thermophase temperatures were relatively high, but was influenced by thermophase temperatures when cryophase temperatures were near 0°C (Beck, 1984).

A strong interaction between temperature and photoperiod is known in many species. Long days prevent diapause at low temperatures, and high temperatures counteract the effect of short days in species such as *C. fumiferana* (Harvey, 1957). In most insects, decreases in temperature tend to increase critical daylengths for diapause induction (Lees, 1968; Beck, 1980). In contrast, a few species, such as *P.*

brassicae, have a temperature compensated response over the normal range of temperatures experienced in nature (<30°C). Photoperiod response is independent of temperature, insuring that local populations enter diapause at the correct time, regardless of temporary weather conditions (Lees, 1968).

The complexity of temperature and photoperiod relations may be due to different temperature coefficients for light and dark phase physiological processes (de Wilde, 1962). Pittendrigh's (1981) pacemaker-slave oscillator model offers an explanation of why critical daylength is temperature dependent, because temperature affects phase relationships between pacemaker and slave oscillators and among slave oscillators.

Role of Diet

Diet is known to modify effects of photoperiod and temperature on diapause induction, particularly in phytophagous species, where it may be a major influence (Saunders, 1982). Dietary effects are often only detected in populations under critical photoperiods, when small physiological changes may make a large difference in expression of diapause. Food scarcity increases diapause incidence in the mosquito, *Aedes triseriatus* (Say) (Beck, 1980), the lacewing, *Chrysopa mohave* Stephens (Tauber and Tauber, 1973), the moth, *Ephestia cautella* (Walker) (Hagstrum and Silhacek, 1980) and the predatory mite, *Metaseiulus occidentalis* (Nesbitt) (Field and Hoy, 1985). Although these species responded to starvation with an increased tendency to diapause, in others, such as the

phytophagous ladybeetle, *Epilachna vigintioctopunctata* (F.), a poor quality diet prevented diapause, probably because nutritional deficiencies prevented complete fat body development (Kono, 1979).

Dietary components, as well as moisture content, may also affect diapause. Differences in starch content determined whether diets for the tobacco moth, *Ephestia elutella* (Hubner), induced a high incidence, low incidence, or absence of diapause (Waloff, 1949). Increasing lipid content in diets of *P. gossypiella*, increased diapause incidence (Adkisson, 1961; Bull and Adkisson, 1962; Foster and Crowder, 1980), as did decreasing moisture content (Raina and Bell, 1974b), when photoperiod approximated critical daylengths. Adding glycerol to diets for the Mediterranean flour moth, *Ephestia kuhniella* Zeller, decreased diapause incidence (Cox, et al., 1984). Sugar and protein levels in diets of the boll weevil, *Anthonomis grandis* Boheman, affected diapause incidence (Tingle nd Lloyd, 1969) and higher amounts of vitamin E and cholesterol in diet of the armyworm, *Heliothis armigera* (Hubner), prolonged the larval stage and increased diapause incidence (Nikishina, 1973).

There is some evidence that diapause incidence in predatory and parasitic species is also affected by quality of prey, and indirectly by diet of prey. Diapause incidence in the endoparasite, *Pimpla instigator* F., was markedly higher when they were reared on the cabbage moth, *Mamestra brassicae* L., than when reared on *P. brassicae*, which apparently resulted from nutritional differences rather than differences in host size (Claret and Carton, 1978). The

nutritional effect is more obvious in the predatory mites, *Amblyseius potentillae* (Garman), which were not able to diapause when fed on albino mutant spider mites, lacking vitamin A and carotenoids (Overmeer and van Zon, 1983; Veerman, et al., 1983).

A common difference in behavior and feeding between diapause and nondiapause individuals of the same species, is that those oriented toward diapause tend to eat more and feed longer than those not diapausing. This is consistent with the needs of diapausing individuals for extra accumulations of reserves in fat body and other storage tissues (Lees, 1968). Feeding of prediapause cereal leaf beetles, *Oulema melanopus* (L.), was significantly greater than that of nondiapause beetles (Wellso and Hoxie, 1981) and diapause oriented larvae of *Pectinophora gossypiella* were heavier and had a higher fat content than nondiapause larvae (Adkisson, et al., 1963).

DIAPAUSE DEVELOPMENT AND TERMINATION

Andrewartha (1952, pg. 43) defined diapause development as "physiological development or physiogenesis, which goes on during the diapause stage in preparation for active resumption of morphogenesis". This is synonymous with Beck's (1980) term, "diapause termination", and is influenced by the interaction of such rate controlling factors as photoperiod, temperature, moisture, sensory stimuli and nutritive factors. In many species, even those with marked photoperiodic responses for diapause induction, this response weakens or disappears during diapause and a period of chilling (0-10°C) is required for diapause development to proceed (Danilevskiĭ, et al., 1970). Photoperiodic sensitivity continues into diapause and governs diapause termination in some species (Lees, 1968; Saunders, 1982; Bunnarius and Dumortier, 1984). Development may resume in response to another critical photoperiod threshold in spring, or diapause may be photoperiodically reversible at any time (Lees, 1968). In *A. pernyi*, diapause can be terminated by one hour light breaks during the dark phase (Hayes, et al., 1974). Long days may hasten diapause development after chilling, as in *E. elutella*, in which there is a complex interaction between chilling, later development temperatures and photoperiod (Bell, 1983), or long days alone can terminate diapause without chilling, as in *O. nubilalis* (Mcleod and Beck, 1963), *W. smithii* (Smith and Brust, 1971), a pitcher plant midge, *Metriocnemus* sp. (Lees, 1968), and the bean bug, *Riptortus clavatus* Thunberg (Numata and Hidaka, 1982).

Chilling often synchronizes emergence after diapause, but it may not be necessary for diapause development. Instead, its effect may be to decrease the metabolic rate to conserve enough fat body reserves to insure morphogenesis after diapause (McLeod and Beck, 1963; Beck, 1980). Tauber and Tauber (1976) suggest that temperature thresholds for diapause development are very low at the beginning of diapause, rising as diapause proceeds, until they reach the thermal threshold for morphogenesis, at which time diapause is complete. For many species, diapause development may be complete before natural daylengths are long enough to terminate diapause, so photoperiods do not terminate diapause in the field (Beck, 1980). It is not known why species that do not rely on photoperiod for diapause termination in nature, such as *P. brassicae*, exhibit distinct photosensitivity under laboratory conditions (Brunnarius and Dumortier, 1984).

GEOGRAPHICAL VARIATION IN DIAPAUSE

Local differences in climatic conditions, acting on variations in genetic constitution of local populations, are usually responsible for geographical differences in diapause incidence among geographical races (Masaki, 1961). Differences in critical daylength for diapause induction are associated with latitudinal changes in photoperiod in many species; intensity of photoperiod response, and effect of temperature on that response, may also vary from area to area (Masaki, 1961; de Wilde, 1962; Beck, 1963; Danilevskiĭ, 1965; Lees, 1968; Danilevskiĭ, et al., 1970; Denno and Dingle, 1981; Saunders, 1982; Tauber, et al., 1984). The differences between geographic races was shown in a comprehensive study of 22

populations of *W. smithii*, which found that latitude accounted for over 80%, and altitude for over 15%, of variation in critical photoperiod (Bradshaw, 1976). Local populations adapt to increasing altitude because it is climatically analogous to moving north (Danilevskiĭ, et al., 1970). Temperature may be more important than photoperiod for diapause induction in local populations, as was found in two of ten races of *O. nubilalis* from different latitudes (Beck and Apple, 1961). Genetic divergence, rather than adaptation to different photoperiods, may also account for geographical variation in critical daylengths (Ando, 1979).

Differences in diapause response between geographical races has been studied in the European spruce sawfly, *Diprion hercyniae* (Hartig) (Prebble, 1941), *O. nubilalis* (Beck and Apple, 1961), *A. grandis* (Earle and Newsom, 1964), *Anopheles freeborni* Aitken (Depner and Harwood, 1966), *Manduca sexta* (Johannson) (Rabb, 1969), *P. gossypiella* (Raina and Bell, 1974a), *Sarcophaga peregrina* Robineau-Desvoidy (Kurahashi and Ohtaki, 1977), *Drosophila littoralis* Meigen (Lumme and Oikarinen, 1977), *P. interpunctella* (Bell, et al., 1979; Bell, 1982), *Atrachya menetriesi* (Ando, 1979) and *Calliphora vicina* Robineau-Desvoidy (Zinov'yeva, 1980).

Geographical variations in diapause are most likely to arise in species with a facultative diapause. They are often multivoltine in southern parts of their range, with the number of generations per season decreasing as latitude increases until they are univoltine in extreme northern ranges. Northern insect populations have a strong tendency to diapause over a

wide range of external conditions compared with southern populations (de Wilde, 1962). Higher temperatures may be required to avert diapause than in southern populations (Masaki, 1961), and photoperiod is usually a very strong cue because seasons are well defined at higher latitudes, where differences in daylength and its rate of change are relatively large (Saunders, 1982). With every 5° increase in latitude, Danilevskii (1965) found that critical photoperiod generally increased by 1.5 h.

Theoretical models for optimal timing of diapause are given by Cohen (1970) and Istock (1981).

GENETIC SELECTION FOR NONDIAPAUSE

Natural populations show a high degree of intrapopulation variation in their diapause response. The occurrence of a few nondiapausing individuals in a population is the basis for artificial selection for nondiapause (Lees, 1968; Danilevskii, et al., 1970; Herzog and Phillips, 1974; Hoy, 1978b; Tauber and Tauber, 1981), particularly since inadvertent selection of nondiapausing laboratory lines has occurred relatively frequently (Hoy, 1977). The spruce budworm parasitoid, *Pseudosarcophaga affinis* Fallen, lost its capacity to diapause during 200 generations of laboratory rearing (House, 1967), and diapause dropped from 96% to 10% in a line of *G. molesta* after 60 generations in the laboratory (Glass, 1970). A similar inadvertent loss of diapause has occurred in laboratory lines of *A. grandis* (Lloyd, et al., 1967; McCoy, et al., 1968), the blowfly, *Lucilia caesar* L. (Ring, 1971), the western spruce budworm, *Choristoneura occidentalis* Freeman (Lyon, et al., 1972), the pentatomid,

Aelia acuminata (L.) (Hodek and Honěk, 1970) and *E. kuhniella* (Cox, et al., 1984).

Intentional selection for nondiapause lines has been done for several reasons. They have been used in crosses with diapause lines to study diapause genetics (e.g., Ando and Miya, 1968; Sims, 1983), or to provide continuous laboratory cultures useful in other research (e.g., Harvey, 1957; Baerwald and Boush, 1967; Pickford and Randall, 1969; Hoy, 1977). Releasing nondiapausing lines of pest species in the field, to cross with local populations and produce offspring no longer adapted to the area, has been suggested as a potential control method by numerous authors (McCoy, et al., 1968; Rabb, 1969; Klassen, *et al*, 1970; Kurahashi and Ohtaki, 1977; Hoy, 1978b; Tauber and Tauber, 1979). This is not likely to work unless nondiapause is controlled by a single, dominant, fully penetrant gene (Klassen, et al., 1970), or unless it is sex-linked, as in *O. nubilalis* (Showers, 1981).

Genetic selection of diapause in natural enemies to enhance their usefulness may be a potentially valuable area of research, especially when species originating from different latitudes are to be released in the field. Poor host-parasite synchronization because of diapause, which can cause failure of a biological control program (Schlinger, 1960), could be avoided. Selection of nondiapausing natural enemies for use in greenhouses during the winter is the subject of this research project and has been recently accomplished in the predatory mite, *Metaseiulus occidentalis* (Hoy, 1984; Field and Hoy, 1985).

Nondiapause selection has been successful in a wide range of species (Table 1.). Although most of these species have a facultative diapause, nondiapause selection has been successful in species with obligatory diapause, such as *C. fumiferana*, (Harvey, 1957), *P. affinis* (House, 1967), *A. acuminata* (Hodek and Honěk, 1970), the gypsy moth, *Lymantria dispar* (L.) (Hoy, 1977) and the anise swallowtail, *Papilio zelicaon* Lucas (Sims, 1983). In these cases, rare individuals in the population capable of continued development without intervening diapause, apparently provided enough genetic variation for selection to operate (Hoy, 1978a).

Progress in selection for a high incidence of nondiapause is often rapid, especially in the first few generations (Barry and Adkisson, 1966; Lees, 1968; Glass, 1970; Herzog and Phillips, 1974). Diapause may be reduced to an extremely low or 0% level in selected lines (e.g., Glass, 1970; Nair and Desai, 1973); but may revert to a higher incidence after selection is relaxed (Barry and Adkisson, 1966). Because most nondiapause selection has been carried out under a single photoperiod regime and constant temperature, the resulting lack of diapause response may persist only under these conditions. A change in temperature, or addition of a thermoperiodic cycle, may dramatically affect diapause incidence (e.g., Waloff, 1949); lines selected for changes in temperature response are likely to differ genotypically from those selected for changes in photoperiod (Hoy, 1978b). In crosses of nondiapause and wild-type *L. dispar*, Lynch and Hoy (1978) found that chilling radically altered the

Table 1. Arthropod species intentionally selected for nondiapause.

SPECIES	COMMON NAME	REFERENCE
ORTHOPTERA		
Locusta migratoria gallica Remaudière	Locust	Le Berre (1953)
Melanoplus differentialis (Thomas)	Differential grasshopper	Slifer & King (1961)
M. sanguinipes F.	Migratory grasshopper	Pickford & Randell (1969)
GRYLLOPTERA		
Gryllus campestris L.	Field cricket	Ismail & Fuzeau-Braesch (1976)
COLEOPTERA		
Atrachya menetriesi Faldermann	False melon beetle	Ando & Miya (1968)
Trogoderma granarium Everts	Khapra beetle	Nair & Desai (1973)
Diabrotica vigifera LeConte	Western corn rootworm	Branson (1976)
HETEROPTERA		
Aelia acuminata (L.) *A. rostrata* Boheman	True bugs	Honěk (1972)
Oncopeltus fasciatus (Dallas)	Large milkweed bug	Dingle (1974)
Gerris spp.	Water striders	Vepsäläinen (1978)

Table 1. Continued

SPECIES	COMMON NAME	REFERENCE
HYMENOPTERA		
Diprion hercyniae (Hartig)	European spruce sawfly	Prebble (1941)
Pteromalus puparum (L.)	Chalcid wasp	Maslennikova (1968)
LEPIDOPTERA		
Ephestia elutella (Hübner)	Tobacco moth	Waloff (1949)
Choristoneura fumiferana (Clemens)	Spruce budworm	Harvey (1957); Lyon, et al. (1972)
Pectinophora gossypiella Saunders	Pink bollworm	Barry & Adkisson (1966)
Grapholitha molesta (Busck)	Oriental fruit moth	Glass (1970)
Galleria mellonella (L.)	Greater wax moth	Marini & Campadelli (1973/75)
Heliothis zea (Boddie)	Bollworm	Herzog & Phillip (1974)
Pionea forficalis (L.)	Garden pebble moth	King (1974)
Lymantria dispar (L.)	Gypsy moth	Hoy (1977)
Ostrinia nubilalis (Hübner)	European corn borer	Showers (1981)
Diatraea grandiosella Dyar	Southwestern corn borer	Takeda & Chippendale (1982)
Papilio zelicaon Lucas	Anise swallowtail	Sims (1983)

Table 1. Continued

SPECIES	COMMON NAME	REFERENCE
DIPTERA		
Rhagoletis pomonella (Walsh)	Apple maggot	Baerwald & Boush (1967)
Lucilia caesar L.	Blowfly	Ring (1971)
Wyeomyia smithii Coquillett	Pitcher plant mosquito	Istock, et al. (1976)
Poecilometopa spilogaster (Wiedemann)	Flesh fly	Denlinger (1979)
Sarcophaga bullata Parker	Flesh fly	Henrich & Denlinger (1982, 1983)
ACARINA		
Metaseiulus occidentalis (Nesbitt)	Predator mite	Field & Hoy (1985)

phenotypic expression of hatching time and proportion of hatch, both of which are related to the egg diapause. Other environmental factors, such as population density (Nair and Desai, 1973) and food quality (pg.37), can affect the expression of diapause.

An important part of nondiapause selection is the assessment of other characteristics that may have changed as a result of selection. Attributes, such as sex ratio, fecundity, searching ability, temperature tolerance and life span, may be affected by selection, as a result of inbreeding, inadvertent selection, random genetic drift, or because they are associated with diapause at the genetic level. Ando and Miya (1968) found that in a nondiapause line of *A. monetriesi*, fecundity was lower, egg mortality increased and female life spans were shorter than in unselected controls, probably because of inbreeding. Nondiapause *P. affinis* were not as cold-hardy as original stock (House, 1967), there were differences in sex ratio between control and nondiapause lines of *C. fumiferana* (Harvey, 1957), and a reduction in vigour, fecundity and viability of eggs occurred after 12 generations of nondiapause selection in the grasshopper, *Melanoplus differentialis* (Thomas), probably because of accumulated deleterious genes (Slifer and King, 1961). Selection of a nondiapause strain of *L. dispar*, however, did not result in significant changes in morphology, sex ratio, pupal weights or egg production (Hoy, 1978a).

GENETICS OF INSECT DIAPAUSE

Insects display an astonishing variety in their diapause response to photoperiod, temperature, and other environmental factors, and it is likely that modes of diapause inheritance are equally variable. Major reviews of diapause genetics are given by Lees (1955), Beck (1963, 1982), Danilevskiĭ (1965), and Hoy (1978b).

Characteristics, such as critical photoperiod, duration and intensity of diapause, temperature responses during diapause and temperature requirements for diapause termination, respond to intentional selection and are, therefore, genetically determined (Hoy, 1978b; Tauber and Tauber, 1979). Selection is possible because of the variation in diapause response, sometimes quite large, between individuals in populations. Large differences in diapause intensity and duration have been observed between individuals in populations of the garden pebble moth, *Pionea forficalis* (L.) (King, 1974), *M. sexta* (Rabb, 1969), the western corn rootworm *Diabrotica vigifera LeConte* (Branson, 1976), and in laboratory populations of the Khapra beetle, *Trogoderma granarium* Everts (Nair and Desai, 1973). In experimental populations of *O. nubilalis*, Beck and Apple (1961) found a range of photoperiodic sensitivity approximating a normal distribution, and concluded that corn borer populations were heterozygous for multiple genes controlling their response to photoperiod.

The previous examples demonstrate one of the difficulties in studying diapause genetics. Populations may exhibit a continuous distribution of diapause response, yet for an

individual, diapause is a threshold ("all-or-nothing") response (Falconer, 1981). There may be no way, with present techniques, to determine whether diapause characterstics are normally or multimodally distributed, because there could be many causes of variation, all with relatively small effects, involving many genes (Hoy, 1978b). Another complicating factor in studying diapause genetics is in defining diapause, particularly in species with an egg or pupal diapause, in which diapause and nondiapause states are defined by duration alone (Rabb, 1969).

The genetics of diapause are usually studied by crossing geographic races, or by crossing artificially or inadvertently selected lines. The diapause response of resulting hybrids is usually intermediate between that of parental lines. This was first demonstrated by Danilevskiĭ (1965) in crosses of geographical races of the moths, *Lucoma salicis* and *Acronycta rumicis* (L.). Similar results have been obtained with *S. peregrina* (Kurahashi and Ohtaki, 1977), *P. gossypiella* (Raina and Bell, 1974a), *L. caesar* (Ring, 1971), *A. acuminata* (Hodek and Honěk, 1970), *P. zelicaon* (Sims, 1983), and *Sarcophaga bullata* Parker (Henrich and Denlinger, 1983).

In some species, hybrids between diapause and nondiapause lines show a diapause response similar to one of the parental lines. For example, characters governing nondiapause were dominant in crosses of *A. grandis* (McCoy, et al., 1968). Rabb (1969) reported that diapause intensity in F_1 female *M. sexta*, (as measured by duration of pupal stage) was much greater than in either parent strain, possibly because of

cumulative effects of several genes. A more complex situation was demonstrated by Nair and Desai (1973) who suggested that major genetic factors determining density independent diapause in *T. granarium* were dominant, while those governing density dependent diapause were recessive.

Generally, the results of crossing experiments between nondiapause and diapause lines indicate that diapause is under polygenic control (Harvey, 1957; Danilevskiĭ, 1965; Nair and Desai, 1973; Rabb, 1969; King, 1974; Raina and Bell, 1974a; Kurahashi and Ohtaki, 1977). The type of genes and inheritance involved, however, is extremely variable. Several authors conclude that an initial rapid decline in diapause incidence during nondiapause selection, followed by a plateau before further selection progress, indicates that diapause is controlled by a few major and several minor genes (Harvey, 1957; Barry and Adkisson, 1966; Herzog and Phillips, 1974). A small number of interacting loci, or a single gene locus, may govern the metabolic, hormonal and developmental events connected with diapause in *S. bullata* (Henrich and Denlinger, 1983). Diapause appears to be controlled by supergenes, which show discrete inheritance, in *Gerris* spp. (Vepsäläinen, 1978) and *D. littoralis* (Lumme and Oikarinen, 1977). Tauber and Tauber (1979) demonstrated Mendelian inheritance of diapause in lacewings by crossing two sibling species, *Chrysopa downesi* Banks (univoltine) and *C. carnea* Stephens (multivoltine).

Diapause is sex-linked in some insects. It is inherited through the male parent (the homogamic sex) in such Lepidoptera as *P. forficalis* (King, 1974), *L. dispar*

(Lynch and Hoy, 1978), *B. mori* (reviewed by Lees, 1955) and *O. nubilalis* (Showers, 1981). Maternal influence has been found in *A. menetriesi* (Ando and Miya, 1968).

The relationship between development rate and tendency to diapause may occur at the genetic level, possibly resulting from the pleiotropic effects of diapause genes, or from linkage between genes associated with diapause and development (Henrich and Denlinger, 1982). Slower developing *G. molesta* larvae tend to diapause (Dickson, 1949; Glass, 1970), progeny of early emerging adults *P. forficalis* are least likely to diapause (King, 1974), and fast development is correlated with low diapause incidence in *W. smithii* (Istock, et al., 1976). Artificial selection of *S. bullata* for greater duration of larval stage resulted in a population that tended to pupariate later, and had a higher diapause incidence, than a line selected for fast larval development (Henrich and Denlinger, 1982).

Only in *B. mori* have diapause genes been extensively analyzed (reviewed by Lees, 1955). Because many genetics experiments were performed as afterthoughts in selection experiments, and often at single temperature or photoperiod regimes, the information obtained on diapause response is limited. No clear conclusions regarding diapause genetics are possible until more research is conducted.

BIOLOGICAL CLOCK MODELS

Several biological clock models have been proposed to account for photoperiodic responses observed in insects, including modelling the circadian component of seasonal rhythms such as diapause (recent reviews by Beck, 1980; Saunders, 1982). In the external coincidence model, first proposed by Bünning (1936), induction depends on a coincidence in time of a particular phase in the circadian cycle with a particular light phase. Some phases of the cycle that occur in darkness at one season, occur in light as daylength increases. Coincidence of these phases is necessary for the photochemical steps that initiate diapause. In the internal coincidence model, first proposed by Danilevskiĭ (1965), multi-oscillator systems are proposed that keep a temporal order within the circadian system itself and respond to changes in photoperiod. On one side of the critical photoperiod, the phase relationships between oscillators allow a particular sequence of reactions, whereas, on the other side of the critical photoperiod, different phase relations exist, which activate the metabolic pathway resulting in diapause (Pittendrigh, 1981). In both models, light has a circadian entraining role, which may be replaced by thermoperiod, but in the external model, light is also necessary for induction (Saunders, 1984).

Refinement of a pacemaker-slave oscillator model of circadian organization gives the internal coincidence model a more substantial foundation (Pittendrigh, 1981). It can be used to explain why critical daylength for photoperiodic induction of diapause depends on temperature in some species. The pacemaker in the system has a constant period and time

course with respect to temperature, but the phases of the slave oscillators, and their relationship to the pacemaker and other slave oscillators, are temperature dependent (Pittendrigh, 1981). As photoperiod increases, the phase of the slave oscillators (relative to dawn) shifts also, but at different rates.

Beck (1980) has proposed and refined a dual-system theory of diapause induction in which there are two main circadian oscillators. One system functions as a circadian pacemaker and determines the rhythm, the other functions as a gating system (Beck, 1980). He hypothesizes that photoperiodic determination of diapause involves a gating mechanism, similar to the gating of circadian rhythms. This theory has been criticized, however, because it does not account for, or predict, certain fundamental characteristics observed in circadian systems (Scopik, et al., 1981).

Evidence from the few species extensively studied suggests that both hour-glass timers and circadian oscillators may be involved in the photoperiodic response (Saunders, 1982). For example, Takeda and Skopik (1985) found that a northern strain of *O. nubilalis* showed an hour-glass diapause response at 20°C, but circadian periodicity at 30°C, whereas a southern strain exhibited the converse.

Superimposed on the photoperiodic clock is what Saunders (1982) calls the "photoperiodic counter", which adds up short day, or long day, cycles until the diapause pathway is determined. Some species may count both short days and long days; in others, one or the other is neutral, and may or may not be temperature compensated (Saunders, 1982). This is one

explanation of why insects reared at high temperatures, under short day cycles, do not diapause; they have not accumulated the required number of short day cycles to induce diapause.

SECTION 2.

MATERIALS AND METHODS

Materials and methods used throughout the research are described in this section. Methods unique to each experiment are decribed separately in each chapter.

INCUBATOR ENVIRONMENT

One large, controlled environment chamber, and numerous small, refrigerator-type incubators were used during this research. The large chamber (1.7 x 2.5 m) was used for all nondiapause selection exeriments under LD 8:16. Three sets of four, 40-W Vita-lite® (Duro-Test Corp., Montreal; Que.), full spectrum fluorescent tubes (120 cm) were installed in the chamber to simulate natural lighting. Two sets of tubes were suspended 55 cm above the floor, and a third set was installed the same distance above a second level shelf. The lighting system operated independently of the normal incubator wiring system and photoperiod was controlled by a timer that operated on both AC and DC power. An alternate 12-V DC light system, consisting of two full spectrum tubes (45 cm) per set of lights, powered by a heavy duty marine battery, automatically switched on when power failed (wiring diagram, Appendix A.) Because the same timer controlled both AC and DC lights, correct photoperiod was maintained regardless of the time of day when power failed. Even though electricity on campus failed 13 times in three years (once for 33 h), the emergency system was reliable and no selection data was lost as a result of disrupted photoperiod cycles. Temperature in the large chamber was maintained at 21±1°C, and a humidifier kept relative humidity (RH) at 80-90%.

Aphids were reared in plant growth cabinets with high light levels. Cages with control lines of *A. aphidimyza* were kept in open Climatoria for the first year. These were equipped with 40-W Vita-lite® fluorescent tubes, but, because chambers were open to the room, temperatures and RH were not

controllable. Room temperatures were 24-28°C. After a year, control lines were moved to refrigerator-type incubators so that 21°C and 80% RH could be maintained. All other experiments were conducted in the same type of incubator, which had photoperiod and thermoperiod controls.

EXPERIMENTAL GREENHOUSE

The Entomology greenhouse used during the research was a single section (4 x 9.2 m), sealed off from the adjacent section, with separate entrance and environmental controls. Joints in the glass partition were caulked to prevent pesticides from drifting into the greenhouse from the adjoining Horticulture section. Soil mixing, seedling transplanting, insect collection and counts for greenhouse experiments were done in the potting-shed laboratory attached to the greenhouse.

The greenhouse was equipped with two free-standing benches (1 x 6 m). Ventilation was provided by two ridge vents (one manually controlled, one automatic), two manually operated side vents, and a thermostatically controlled, variable speed, exhaust fan. Steam heat supplied from the college system was also thermostatically controlled. Glass was whitewashed annually, in late May, to reduce summer daytime temperatures. A floor-to-ridge blackout curtain between sections was drawn in winter when supplemental lighting used in the neighboring section would have interfered with diapause research.

Although cooling and heating systems were adequate, equipment was not as accurate or flexible as that employed by commercial growers. For example, it was not possible to set separate night and day temperatures, therefore, it was

necessary to make manual adjustments twice daily during experimental periods intended to duplicate commercial conditions.

HOST PLANT PRODUCTION

Aphids were reared on green peppers, *Capsicum officinale* cv. Early Canada Bell. Peppers are good host plants because they are compact, upright and support dense aphid populations with minimal leaf curling or dropping. The slow growth rate of peppers and their requirement for warmth were disadvantages, however, for winter aphid production. Therefore, the suitability of brussels sprouts, *Brassica oleracea* cv. Jade Cross, as host plants was investigated. They are compact, upright and grow quickly at lower temperatures than do peppers. Although their growth in winter was satisfactory, *M. persicae* was slow to colonize leaves and was more irritable and restless, dropping off easily when plants were moved. Perhaps brussels sprouts provided a poor diet (Dixon, 1985) or the waxy leaf surface may have repelled aphids (van Emden, al., 1965). Rearing the cabbage aphid, *Brevicoryne brassicae* (L.), might have been more successful. For the purpose of this research, however, which specifically addressed aphid control in greenhouses, I decided to continue with *M. persicae* on peppers. Recently, Havelka and Růžička (1984) found that *M. persicae* is one of the best quality food sources for *A. aphidimyza* larvae.

Plants were seeded weekly in the greenhouse (summer) or every two weeks in a growth chamber (winter). At four weeks, seedlings were transplanted to pots (12.5 cm) and grown for 3-4 more weeks, until they were 20-25 cm high and ready to be

infested with aphids. Every other week, 40-80 plants were transplanted, depending on experimental requirements. The soil mix was coarse grade vermiculite, horticultural peat and pasturized top soil (1:1:2). Lime and 20-20-20 fertilizer were added to the mix. When frequent watering was necessary in sunny weather, pots were watered weekly with liquid 20-20-20 to replace nutrients leached from soil.

Pest Control

Weekly or bi-weekly sprays of Safer's® Insecticidal Soap (Safer-Agro Chem. Ltd., Willowdale, Ont.) were necessary to control aphids and the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, on young plants. Controlling aphids on young plants in winter and spring became a serious problem because only nonresidual pesticides could be used. The insecticidal soap was too phytotoxic for frequent application and its effectiveness declined, probably because resistance developed in aphid populations. During the second year, Wilson's® wettable-powder rotenone sprays (Wilson Laboratories Inc., Dundas, Ont.), alternating with insecticidal soap sprays, provided satisfactory control. After a year of use, however, aphids were also less affected by the rotenone.

During summer, when aphids were controlled by high temperatures in the greenhouse, whiteflies were controlled with yellow sticky traps and pan traps on greenhouse benches. During the second year, bright green sticky traps and pan traps were also placed on benches to attract parasitic Hymenoptera that had entered from outdoors and had spread to aphid cultures in incubators (pg. 63).

In late summer 1982, severe infestations of the two-spotted spider mite, *Tetranychus urticae* Koch, on plants in both greenhouse and incubators were eliminated within a week by a shipment of predatory mites, *Phytoseiulus persimilis* Athias-Henriot (from Applied Bio-Nomics, Ltd., Sidney, B.C.).

APHID CULTURE

Green peach aphids, *Myzus persicae* (Sulzer), were propagated from a clone received in May, 1982. Initially, aphid cultures were kept in the greenhouse in large, nylon mesh covered, wood frame cages (60 x 75 x 44 cm high) with sleeve openings. Each cage held 20 plants. High temperatures inside cages suppressed aphid reproduction and escaping aphids infested seedlings growing in the same greenhouse. Consequently, aphid cultures were moved to controlled environment chambers under LD 16:8, TC 22:19°C.

Each host plant was inoculated by laying an aphid infested leaf on its top leaves. After a mean of 7 d, infestations reached 30-35 aphids per cm^2 of leaf. Aphid reproduction was slow and unsatisfactory on plants >10 wk old. On younger plants, aphid populations spread evenly over undersides of all leaves and checked plant growth. Plants kept in aphid incubators for longer than three weeks often developed sooty moulds, *Cladosporium* spp., on the heavy accumulation of honeydew, which interfered with movement of *A. aphidimyza* larvae on leaves. Excessive numbers of alatae were also generated and leaf drop was severe on these plants. Consequently, plants were either used or discarded within 10-14 d of infestation.

Parasite Control

Two species of Braconidae (*Aphidius matricariae* Haliday and *Praon* spp. nr. *P. unicum* Smith) interfered with aphid production. They entered the greenhouse every summer and were carried on pepper plants into aphid incubators, where they were very difficult to control. Parasitized aphids wandered from plants and were eventually mummified in corners and under racks and pots. Large numbers of wasps emerging from this unseen source of mummies periodically severely depressed aphid production. Neither white nor yellow traps attracted wasps and at times it was necessary to resort to crushing mummies by hand.

Two solutions were found to this problem. One was the discovery that wasps were attracted to bright green. Sticky traps, made from squares of green polyethylene (6 mil) spread with Tanglefoot® (The Tanglefoot Co., Grand Rapids, Mich.), caught many wasps in incubators. These were replaced with green plastic trays (30 x 44 x 6 cm) filled with soapy water, which were easier to maintain and permitted daily counts of wasp catches. Over a 10 d period, 817 wasps were caught in a green pan trap, compared with 76 caught in an identical yellow trap beside it.

The second solution was to code host plants as they entered aphid cultures with a different colored sticker for each day. This ensured plants were removed in the same order they entered, and none were left in aphid incubators longer than 10 d. The swift rotation eventually eliminated wasp populations because plants were not in incubators long enough for wasps to emerge.

ORIGIN OF *A. APHIDIMYZA* LINES

A. aphidimyza were obtained from different geographical areas to provide the greatest possible genetic variation in diapause response. Efforts to obtain larvae from Pennsylvania, Missouri and Florida were not successful, either because colleagues could not find larvae, or they collected the wrong species (e.g., *Lestodiplosis* spp.). The following list describes the geographical races collected for this research. Abbreviations used in the text are given in parenthesis before each description.

Laboratory Line

(FIN) A laboratory line, originating from Finland in 1971 (Tiittanen, pers. comm.), was received 23 August, 1982. An unknown number of adults emerged from the peat moss in which they were sent and eggs were obtained within three days of arrival. First generation larvae were laid on moist peat to pupate, starting 1 September. Over 200 adults emerged in the second generation. This line became well established, maintained a stable sex ratio and was used throughout the research.

Wild Lines

Aphid colonies, common in fields and roadsides, were examined for *A. aphidimyza* larvae (Gilkeson and Klein, 1981). When larvae were found, the plant material was placed in plastic bags and taken to the laboratory for sorting. Last instar larvae were either put directly on moist peat moss in trays or into sample vials. Younger larvae were reared in plastic bags on additional *M. persicae*, which they appeared to attack as readily as their original host

aphids. When larvae ceased feeding and began wandering, they were removed from the sides of bags with a fine, wet paintbrush and placed on peat moss. Initially, *A. aphidimyza* lines were kept in incubators with LD 16:8 (21°C). Daylength was increased to 17 h in late August, 1982, after problems occurred with establishing wild lines, which may have been due to a high incidence of diapause.

(ARB1) A total of 335 larvae was collected 20 July, 1982, from wild parsnip, *Pastinaca sativa* L., near the entrance to the Macdonald College Arboretum. These were kept in clear plastic boxes (4 x 4 x 4 cm) until ready to pupate, when they were either placed on a peat tray in a small cage (28 x 28 x 28 cm) or in vials for observation. A second collection of 61 larvae from the same site, made 27 July, was added to the cage. Only ca. 30 adults, (sex ratio: 1 male:1.8 females) emerged, possibly because last instar larvae escaped or because of high mortality from disease (larval mortalities of >50% occurred in some vials).

A third collection of 135 larvae was made at the same site 10 August. These were added to the ARB1 cage, except for 91 larvae put in vials. Of larvae in vials, 58% diapaused; sex ratio of emerging adults was 1:1.1. The line seemed well established by 20 September after two generations, but the third generation, emerging 5–10 October consisted entirely of males.

(QUE) Ca. 125 larvae were collected 10 August, 1982, from prickly lettuce, *Lactuca scariola* L., along the edge of a garden on the Macdonald College campus. From a sample of 20 larvae put in vials, 1 diapaused and 15 emerged (sex

ratio: 1:1.2). This line became well established by 7 September and was used for first year nondiapause selection experiments.

(DP) Over 380 larvae were collected 27 August, 1982, from Canada thistle, *Cirsium arvense* (L.) Scopoli, at Diamond Point, near Sequim, Washington. Only 20 adults emerged by 10-17 September, possibly resulting from a high percentage of diapause and larval mortality, which may have been induced by the short days and low night temperatures (10°C). The population was too small to maintain a balanced sex ratio; only five females were observed in the second, and final, generation.

(BC) Ca. 250 larvae and eggs were collected 28 August, 1982, from cultivated roses, *Rosa* spp., in Victoria, B.C. Over 100 larvae were put in peat the same day; the rest were reared in the laboratory. First generation adults, which began emerging 13 September, laid numerous eggs, however, the second generation consisted entirely of females.

(ARB2) A total of 218 larvae was collected 14-17 August, 1983, from valerian, *Valeriana officinalis* L., in the Macdonald College Arboretum. Only 68 adults emerged (sex ratio: 1:2), because 62% of larvae diapaused. Surprisingly, despite high larval mortality (100% in some vials) in the second generation, this line became well established, had a stable sex ratio and was used in 1984-85 nondiapause selection experiments.

Establishing Wild Lines

Because all offspring from one female are the same sex, it is a matter of luck, when collecting wild larvae, whether or not enough female- and male-producing females are obtained to maintain a satisfactory sex ratio. Collecting a large number of larvae did not necessarily guarantee an adequate sex ratio, but it probably helped. It is possible that laboratory conditions favored the inadvertent selection of one sex over the other in some lines.

Collecting larvae early in the season is advisable to avoid a high proportion of diapause. A portion of all larvae collected in August diapaused. By 26 July, at this latitude (45° 25'), daylength (sunrise to sunset) has decreased to 15 hours (on 21 June, daylength is 15 h 40 min) and by 18 August, it is 14 h. Even allowing for the addition of one hour of twilight, natural daylength is <15 h by late August. This is less than the critical photoperiod for diapause induction in many insect species from this latitude (Saunders, 1982).

Diseases in wild insects may present problems. High larval mortality occurred in vials from initial ARB1 and ARB2 generations, apparently as a result of bacterial infection. Sterilizing vials and equipment in 5% bleach solutions and storing forceps and brushes in alcohol prevented further problems.

Hymenopterous parasites were encountered in ARB1 and QUE larvae collected 10 August, 1982. A single female Pteromalid, *Gastrancistrus* sp., emerged from a 28-day-old *A. aphidimyza* cocoon. Three other cocoons,

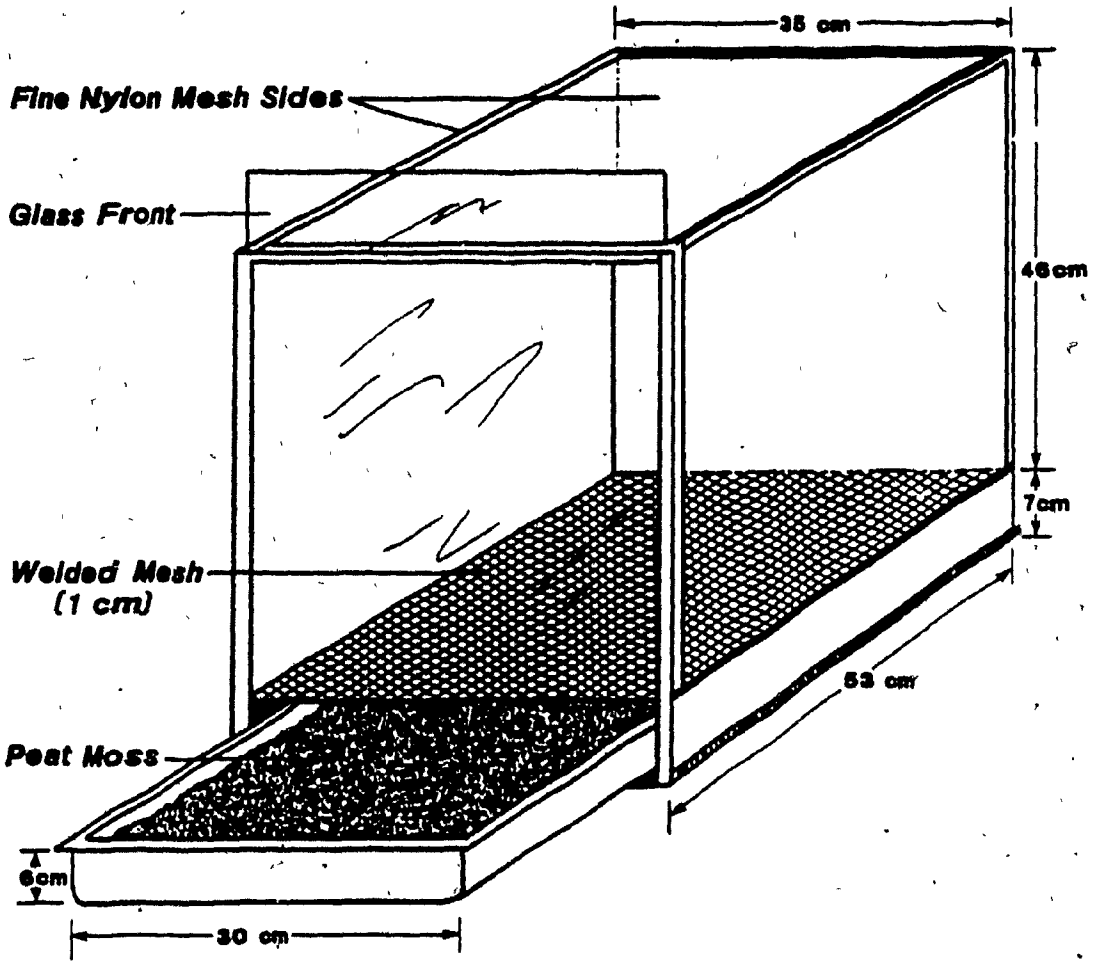
each containing remains of several wasps, were dissected. Since this is the only time parasites were encountered, they are probably not a problem.

REARING *A. APHIDIMYZA*

Lines of *A. aphidimyza* were kept in wooden frame cages (35 x 53 x 53 cm long) covered with fine nylon mesh (Figure 3.). Access was through a vertical sliding glass front. A sheet of welded wire mesh (1 cm^2), installed 7 cm above the cage floor, supported oviposition plants. A plastic tray (30 x 44 x 6 cm) filled with moist peat moss, which is an excellent pupation substrate, was placed in the bottom of each cage. When the glass was raised, the tray could be removed, or last instar larvae could be placed on the peat, without disturbing either plants or resting midges.

Aphid infested oviposition plants were left overnight in cages with 50-1000 adult midges (ca. 16 h) so that all larvae on a plant were the same age. The following morning, plants were removed and placed under desired experimental conditions for rearing larvae. It was not necessary to keep plants with developing larvae in cages because larvae did not leave plants unless they were starving, or until they were ready to spin cocoons. Care was taken to provide plentiful supplies of aphids during development and, if there were insufficient aphids on the original oviposition plant, it was cut and laid on another aphid infested plant. Larvae readily moved down to new sources of prey. When larvae reached last instar, plants or leaves were cut

Figure 3. Rearing cage for *A. aphidimyza*. Sliding glass front allows removal of tray without disturbing insects; potted oviposition plants rest on wire mesh.


35 cm
Fine Nylon Mesh Sides
Glass Front
46cm
7cm
Welded Mesh
(1 cm)
52 cm
Peat Moss
6cm
30 cm

and laid directly on peat. As larvae finished feeding, they burrowed into the peat to spin cocoons.

Control lines were renewed by placing oviposition plants in cages every 2-3 d. Thus, ca. 50-200 adult midges were present continuously. Often, more eggs were laid overnight than were needed to renew the colony. When that occurred, 2-5 leaves were picked randomly from the oviposition plant and all eggs on these leaves were reared. Peat moss in control cages was renewed at six month intervals.

ESTABLISHING THE SAMPLING PROGRAM

Suitability of Cotton Pupation Substrate

It was necessary to develop a method of sampling from each *A. aphidimyza* generation to provide data on incidence of diapause and mortality as well as emergence. Although emergence from peat could be determined, it was not possible to obtain a reliable count of diapause or mortality by extracting cocoons from peat moss. It was also extremely time consuming to search in peat, even though most cocoons were within 2-5 mm of the surface. Because larvae readily spun cocoons in dental cotton rolls put in rearing containers to provide moisture, sample vials were set up with moist cotton rolls for pupation substrates. As soon as rearing was well established, emergence from the cotton substrate was compared to peat to find out if placing larvae in clear vials, where they experienced more light during pupation than those in peat, had an effect on diapause incidence.

Materials and Methods. Three groups of larvae were tested: 1) 383 FIN control larvae, reared under LD 17:7 (24°), 2) 308 FIN control larvae, reared under LD 8:16 (21°C), 3) 376 FIN(ND) larvae from the F_3 generation, reared under LD 8:16 (21°C).

Groups of 25 last instar larvae were placed in shell vials (8 ml), with either peat or cotton substrates. Peat vials had 1 cm of moist peat moss pressed into the bottom; the bottom of the vial was wrapped in black electrician's tape to keep out light. Cotton vials had 0.5 cm pieces of dental cotton rolls wedged firmly accross the bottom. Distilled water (ca. 0.5 ml) was injected into the cotton with an eyedropper to provide

sufficient moisture. Using a fine, wet paintbrush, larvae were placed alternately in peat and cotton vials. Vials were set upright in holes drilled in thick wooden trays, to provide a darkened place for pupation in cotton vials. Vials were temporarily corked to prevent wandering larvae from escaping. Corks were removed daily for aeration and, after 2-3 d, when all larvae had spun cocoons, corks were replaced with squares of nylon mesh secured by elastic bands. Vials were then packed in culture dishes in plastic bags to maintain high humidity.

Vials were examined daily and, upon emergence, midges were sexed and removed. After 21 d all cocoons in cotton rolls were opened to count diapausing larvae and larval and pupal mortalities. Because most pupating midges emerged in 11-16 d, it was decided that larvae that had not begun pupation after 21 d were in diapause. Rare individuals that were pupating at the time cocoons were dissected, and that may, therefore, have had a short diapause, were recorded separately as late pupae. Using these methods, fewer than 1-3% of larvae were unaccounted for in each sample.

Results and Discussion. There was no significant difference (X^2 test, $p<0.05$) in emergence from cotton or peat substrates in any group (Table 2.). Midges in peat emerged 1/2-3/4 day earlier than those in cotton. This is consistent with the observation that larvae immediately entered the peat when placed in vials, whereas in cotton vials, a few individuals wandered for up to two days before spinning cocoons. Despite the apparent preference for a peat pupation substrate, percentage of emergence was the same from peat and cotton, therefore, it was assumed that data on

TABLE 2. Effect of pupation substrate (cotton versus peat, moist versus dry) on diapause and mortality of A. aphidimyza larvae.

Line	Replicate	Larval regime (21°C)	n	Pupation substrate[1]	% Emergence	% Diapause	Comparison between treatments[2]	% Mortality	Comparison between treatments[2]
FIN(ND)		LD 8:16	227	Cotton	84.6	3.1	NS[3]	12.3	-
			250	Peat	73.6	-		-	
FIN control		LD 8:16	296	Cotton	43.9	44.6	NS	10.1	-
			300	Peat	59.3	-		-	
FIN control		LD 17:7	230	Cotton	79.6	0	NS	20.4	-
			250	Peat	80.0	-		-	
FIN(ND)		LD 8:16	80	Moist	82.5	3.8	NS	13.8	NS
			95	Dry	82.1	8.4		9.5	
FIN control	1	LD 8:16	95	Moist	38.9	53.7	NS	7.4	NS
			94	Dry	30.9	61.7		7.4	
	2	"	64	Moist	56.2	37.5	NS	6.2	NS
			99	Dry	53.5	40.4		6.1	
FIN control	1	LD 17:7	90	Moist	91.1	0	NS	8.9	p<0.05
			87	Dry	77.0	0		23.0	
	2	"	139	Moist	89.2	0.7	p<0.05	10.1	NS
			158	Dry	77.8	4.4		17.7	

[1]Cotton substrate used in moist versus dry experiment.

[2]Chi-square test.

[3]Not significant, p<0.05.

percentage of diapause and mortality in cotton vials accurately reflected the fate of larvae in peat trays.

Dry versus Moist Cotton Substrate

In some insects, moisture in the diet or surroundings influences diapause incidence. According to Ushchekov (1975), dry pupation substrates increased mortality in *A. aphidimyza*. To determine whether moisture, or lack of it, in the cotton pupation substrate could affect results, the effect of dry cotton on mortality and diapause incidence was compared to moist cotton.

Materials and Methods. In the first replicate, two groups of larvae from the FIN control line were reared, one under LD 17:7 (24°C) and one under LD 8:16 (21°C). Along with the second replicate, a group of FIN(ND) larvae were reared under LD 8:16 (21°C).

When fully developed, larvae were placed in vials with cotton dental rolls that were either dry, or had been moistened with 0.5 ml distilled water, according to methods given above.

Results and Discussion. Larvae in dry conditions spun complete, tough, dark cocoons that were difficult to cut open. This contrasted with the minimal, delicate cocoons spun by larvae in moist conditions.

Mortality was not significantly different (X^2 test, $p<0.05$) between moist and dry substrates for FIN control larvae reared under LD 8:16 (Table 2.). Although diapause incidence was significantly different between replicates (because of other factors discussed pg. 138), it was not significantly different between moist and dry substrates.

In replicate 2 of FIN control larvae reared under LD 17:7, diapause incidence was significantly higher in dry than in moist cotton (X^2=4.4077, df=1, $p<0.05$); in replicate 1, mortality was significantly higher in dry cotton (X^2=6.6040, df=1, $p<0.05$) (Table 2.).

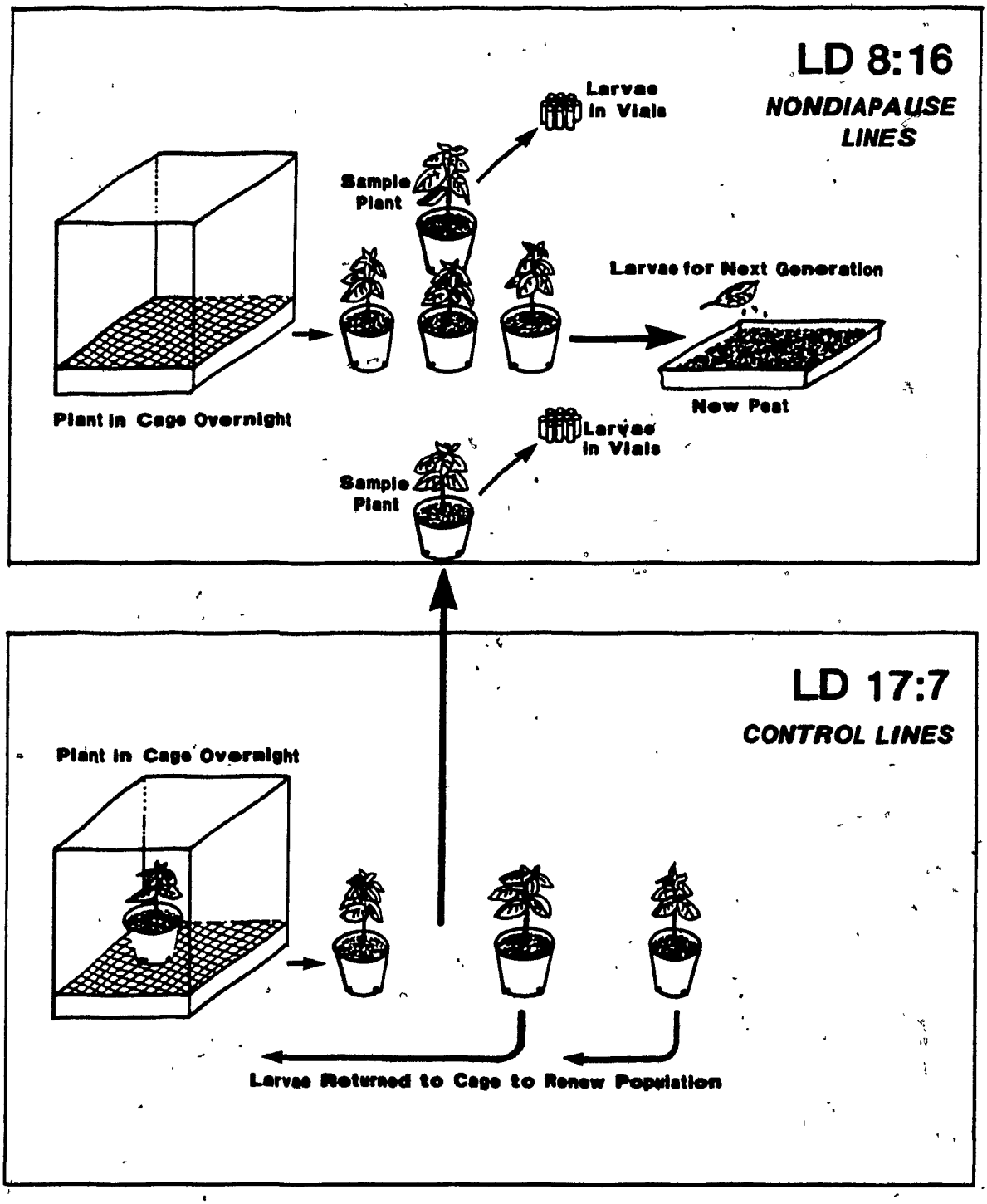
No significant difference was found in either diapause or mortality between moist and dry cotton in FIN(ND) larvae under LD 8:16.

The higher incidence of diapause and mortality under LD 17:7 may have been due to the higher temperature (24-28°C). Heat may have built up in vials and desiccated larvae, resulting in a higher mortality than occurred in LD 8:16 at 21°C. Although the higher diapause incidence in dry vials under LD 17:7 was statistically significant in replicate 2, it is probably not of practical importance because there was no difference in replicate 1. Also, in replicate 2, diapause in dry vials was <5% higher, which is a small difference. It was concluded that moisture levels in the substrate did not have a noticeable effect on diapause, although under certain conditions it did affect mortality.

There was no significant difference in percentage of adults emerging by day 13 from larval-pupal ecdysis for any group, contrary to Ushchekov (1975), who reported prolonged pupation in dry conditions. Leaving vials open to the air, rather than enclosed in plastic bags where humidity was relatively constant, might give different results.

It was concluded that a moist substrate was best, but that level of moisture was probably not critical, because dry cotton gave similar results.

Figure 4. Rearing and sampling plan for nondiapause and control lines of *A. aphidimyza*. All generations of nondiapause lines and sample plants from control lines, reared under LD 8:16 (21°C).


LD 8:16
NONDIAPAUSE LINES
Larvae in Vials
Sample Plant
Larvae for Next Generation
Plant in Cage Overnight
New Peat
Larvae in Vials
Sample Plant
LD 17:7
CONTROL LINES
Plant in Cage Overnight
Larvae Returned to Cage to Renew Population

Sampling Procedure for Each Generation

During the first year of research, 200-300 mature larvae from each generation were randomly collected from all oviposition plants, over a 2-3 d period. Because fresh oviposition plants were placed in cages daily for 3-4 d, sample larvae could come from eggs laid on any of those nights.

After it was found that development time of diapausing larvae was significantly longer by almost a day than that of nondiapausing larvae (pg.120), the sampling procedure was changed to avoid bias in samples resulting from collecting only early maturing larvae. Beginning with F_{14} of FIN(ND), one of the four oviposition plants from each generation was randomly designated the "check" plant (Figure 4.). All larvae (200-400) from this plant were reared and collected in vials over a 2-3 d period. If more eggs had been laid on this plant than were needed, 4-6 leaves were picked randomly and all eggs on these leaves were reared. On the sixth day of larval development, the entire "check" plant, bearing last instar larvae, was placed in an inflated plastic bag (29 x 34 cm) together with a supply of aphids. Thus, larvae ready to spin cocoons could not wander away during the last night of larval development. Mature larvae were collected in vials the following morning, and those not ready to spin cocoons were given more aphids and returned to the incubator to continue feeding. The same sampling procedure was followed in all experiments in which diapause incidence was determined for larvae reared under various conditions.

STATISTICAL TESTS

Results of experiments were analyzed using analysis of variance (ANOVA) and X^2 test for heterogeneity where appropriate. Pearson's product moment correlation coefficient (PPMC) (SAS User's Manual, 1982a; pp. 500-504) was used to determine correlations in data within lines and between lines. The log linear model for categorical data (Sokal and Rohlf, 1981; SAS User's Manual, 1982b; pp. 275-286) was applied to relaxed selection results. Means in the release rates experiment were compared using Tukey's Studentized range test for variable (SAS User's Manual, 1982b; pg. 123). Time series analysis (Legendre and Legendre, 1983, pg. 361) was performed on FIN(ND) data to check for cycles in diapause incidence. In all tests, the level of significance was set at $\alpha = 0.05$.

SECTION 3.

GENETIC SELECTION OF NONDIAPAUSING *APHIDOLETES APHIDIMYZA*

The following section describes genetic selection of nondiapausing lines of *A. aphidimyza* and the evaluation of nondiapause and control (unselected) lines with respect to morphology, sex ratio and fecundity. The effect of relaxing selection pressure on diapause incidence and the inheritance of diapause in *A. aphidimyza* are examined, followed by a series of experiments investigating the relationship between rate of larval development and diapause incidence.

The possible causes of large fluctuations in diapause response from generation to generation were also studied, including the influence of aphid host plant quality on diapause in *A. aphidimyza* larvae.

The concluding chapters describe nondiapause selection experiments under fluctuating thermoperiods and the testing of nondiapause lines under winter greenhouse conditions.

GENETIC SELECTION OF NONDIAPAUSE LINES

Once *A. aphidimyza* lines were established in the laboratory, selection for nondiapause was undertaken.

Materials and Methods. In a preliminary study, the same percentage of FIN larvae (80-90%) diapaused under LD 12:12, LD 9:15 and LD 8:16 (21°C). Therefore, lines selected for nondiapause (ND) were kept continuously in a large controlled environment chamber under LD 8:16 (21°C). This photoperiod regime was chosen as the target "photoperiod without diapause" to permit selected lines to be used during the shortest days (8 h) as far north as 50° N latitude. This is approximately in line with the Gaspe Penninsula (Que.), Regina (Sask.), Calgary (Alta.), and the Fraser River valley (B.C.); it encompasses most Canadian greenhouse regions. The shortest winter daylength in Montreal (lat. 45° 25'), where this research was conducted, is 8 h 44 min from sunrise to sunset. Because the photosensitive threshold for *A. aphidimyza* was not known at the time, civil twilight was not included in calculating minimum winter daylength.

Nondiapause selection was accomplished by breeding only from adults that emerged without diapausing under short day conditions. The peat moss substrate, containing diapausing larvae, was discarded at each generation (21 d) to prevent individuals that had diapaused from mating with nondiapause adults of a later generation.

Five nondiapause lines were selected: two FIN lines, two QUE lines and one ARB2 line. Enough larvae were reared (>1000) to ensure that >200 adults emerged in F_1

generations, which was sufficient to establish selected lines without intense inbreeding. The first 3-5 generations comprised 200-500 adults. Later generations comprised >1000 adults, because diapause incidence was low and most of the 1000-2000 larvae reared per generation pupated.

Ca. 200-500 larvae from each nondiapause generation were placed in sample vials, which were examined daily. After 21 d, cocoons remaining in cotton were dissected to determine whether they contained diapausing larvae, late developing pupae, dead larvae or dead pupae.

Up to August, 1983, diapause incidence in control lines was checked occasionally by rearing a sample of larvae under LD 8:16. Frequent sampling from control lines began after random checks showed that diapause in the FIN control lines had dropped from the initial 80-90%. After August 1983, oviposition plants were placed in control line cages on the same day plants were put in nondiapause line cages. Plants from the control cages were moved to LD 8:16 the following morning and larvae were reared and collected in sample vials under the same conditions as larvae from nondiapause lines (Figure 4.).

Pearson's product moment correlation coefficient was used to determine correlations between percentage of diapause, late pupation, larval and pupal mortality, percentage of adults emerging by day 13 from larval-pupal ecdysis and ratio of two kinds of females in the generation. To compare pupal durations, the percentage of adults emerging by the 13th day after larval-pupal ecdysis was used, rather than time to 50% emergence, because emergence

is not continuous over the day. Adults emerge 1-2 h before onset of darkness, therefore, those counted in vials every morning had all emerged at about the same time the previous evening. It was assumed that the ratio of male-producing to female-producing females in a generation was the same as the sex ratio in the following generation.

Results and Discussion. Results of nondiapause selection are discussed separately for each line. All generation data for control and selected lines is included in Appendix B; a summary for all lines is given in Table 3.

FIN(ND) (Figure 5.) Fifty generations were reared under LD 8:16. There was a nearly linear decrease in diapause of 15% per generation in the first five generations. Diapause incidence averaged 11% for F_6-F_{50}, which was significantly lower (ANOVA, $F=46.6$, $df=1,79$, $p=0.0001$) than the mean of 48% for the FIN control line. Detailed evaluation and genetics experiments were conducted on this line.

FIN2(ND) (Figure 5.) Selection of this line from the FIN control line began five months after beginning selection of FIN(ND). Six generations were reared, enough to demonstrate nondiapause selection from 36% diapause in F_1, to a mean of 11% for F_2-F_6.

QUE(ND) (Figure 6.) Percentage of diapause decreased rapidly from 61% to 3% over the first five generations, with a mean of 3% for F_5-F_9. Low initial diapause incidence may have been a result of inadvertent selection for nondiapause larvae during field collection or may reflect a weaker response to short day photoperiods as a cue

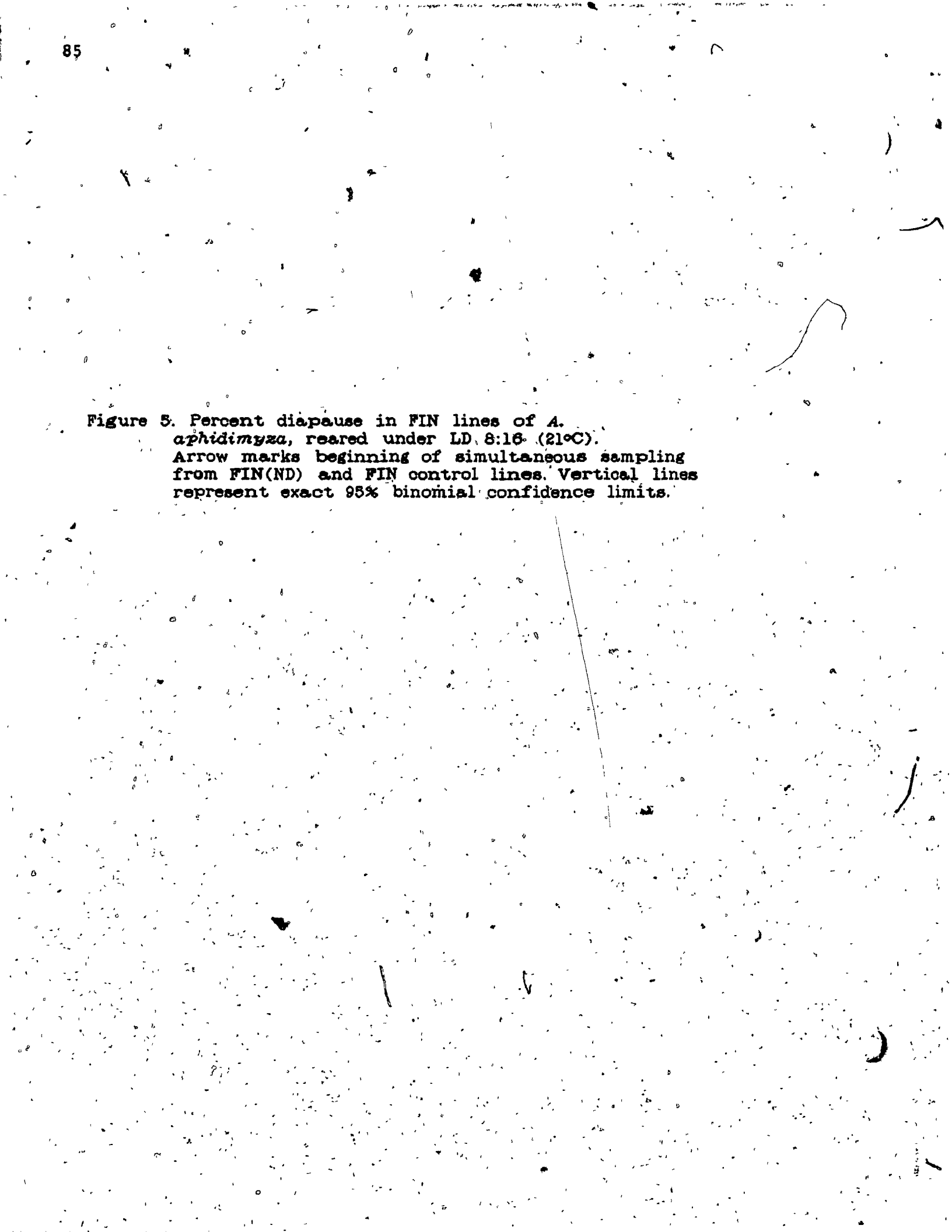
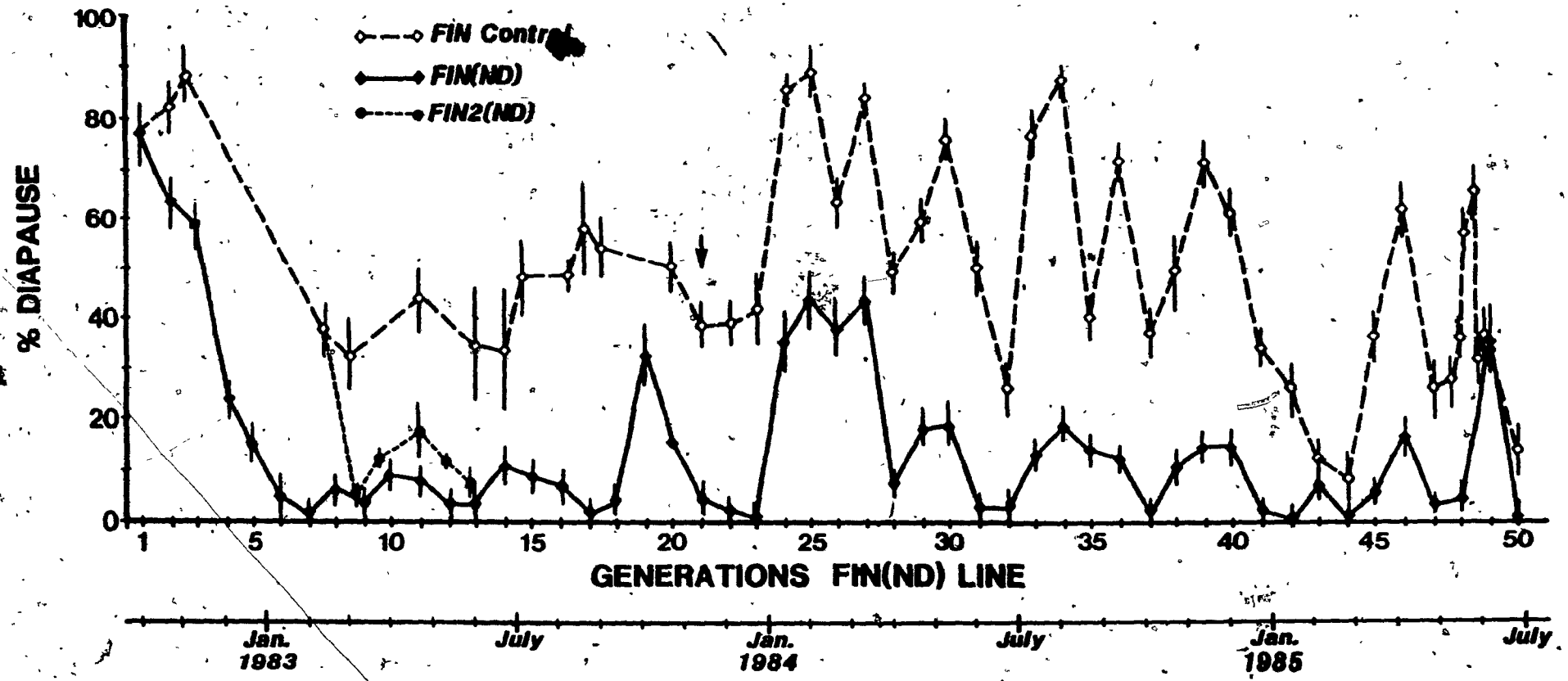


Figure 5. Percent diapause in FIN lines of *A. aphidimyza*, reared under LD 8:16 (21°C). Arrow marks beginning of simultaneous sampling from FIN(ND) and FIN control lines. Vertical lines represent exact 95% binomial confidence limits.


FIN Control
FIN(ND)
FIN2(ND)
% DIAPAUSE
100
80
60
40
20
0
1
5
10
15
20
25
30
35
40
45
50
GENERATIONS FIN(ND) LINE
Jan. 1983
July
Jan. 1984
July
Jan. 1985
July

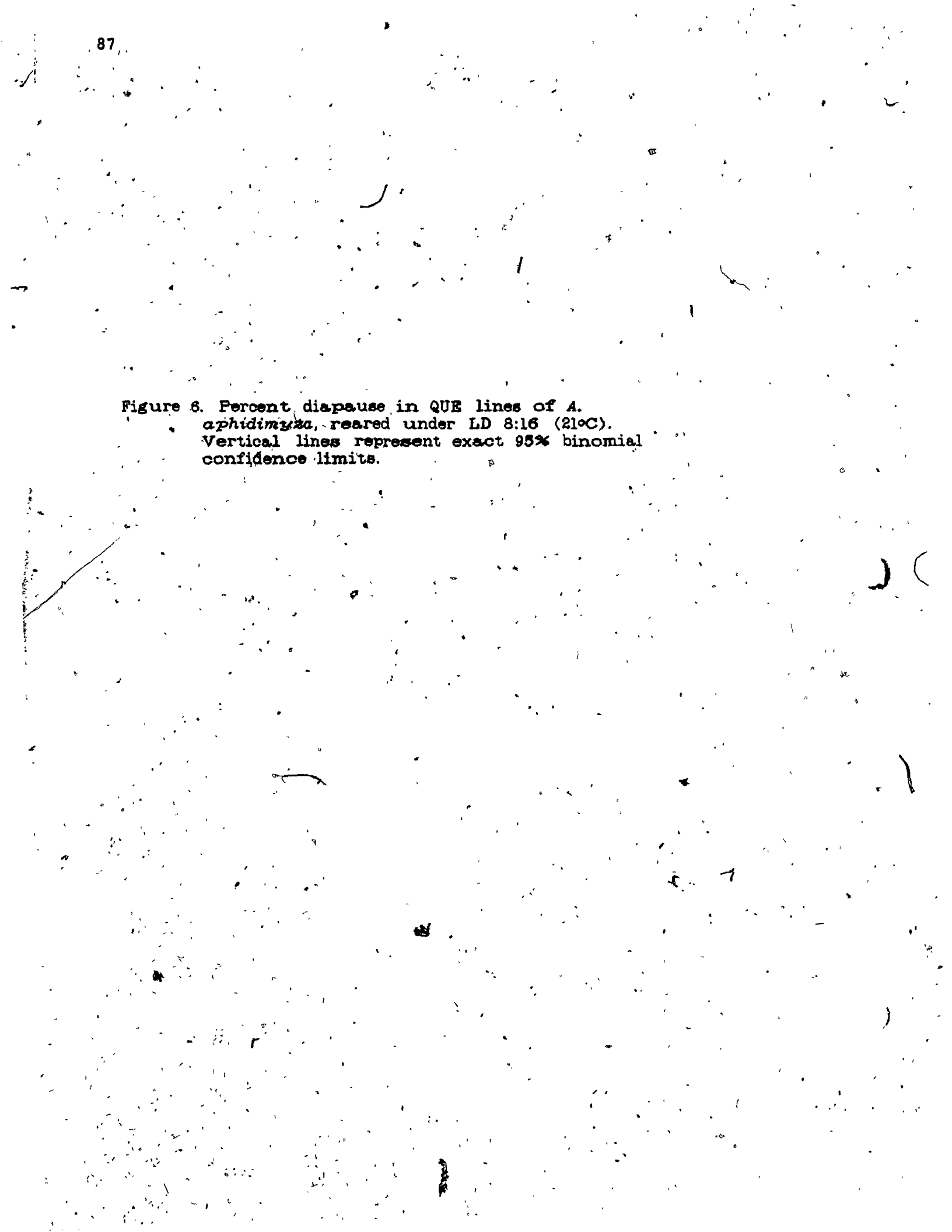
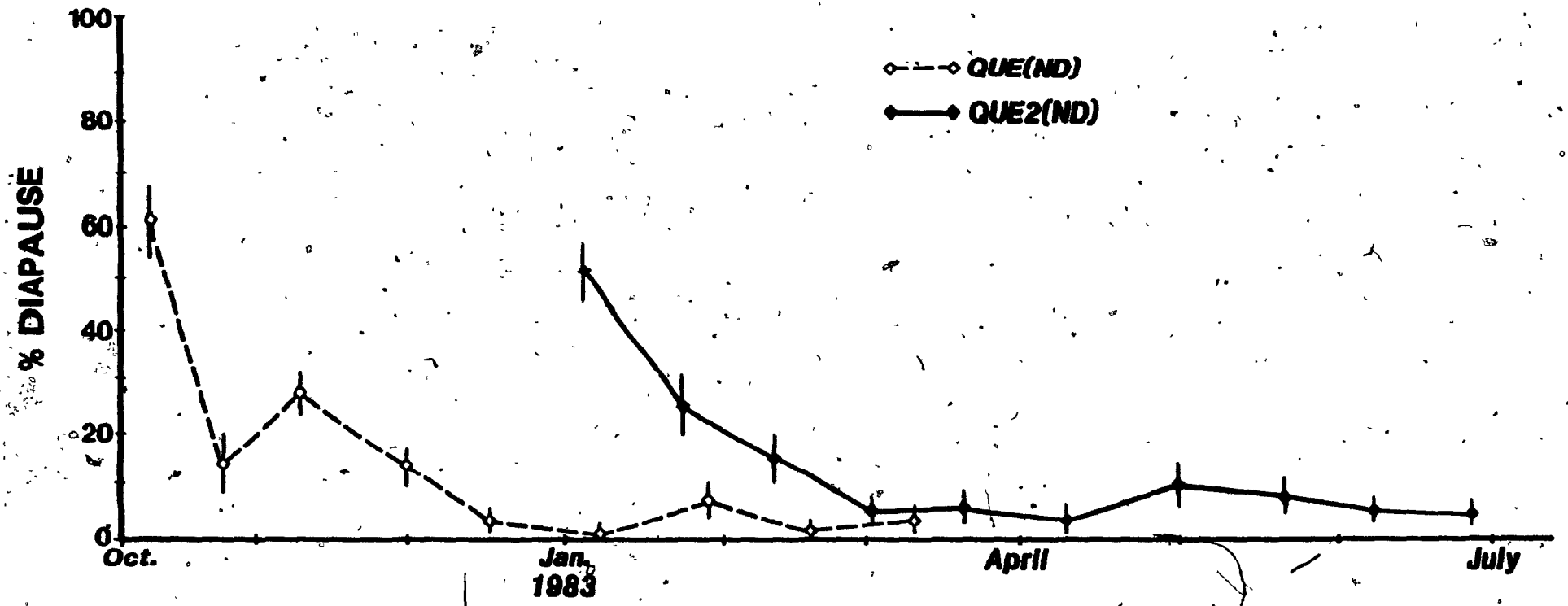


Figure 6. Percent diapause in QUE lines of *A. aphidimyza*, reared under LD 8:16 (21°C). Vertical lines represent exact 95% binomial confidence limits.


100
80
60
40
20
0
% DIAPAUSE
QUE(ND)
QUE2(ND)
Oct.
Jan.
1983
April
July

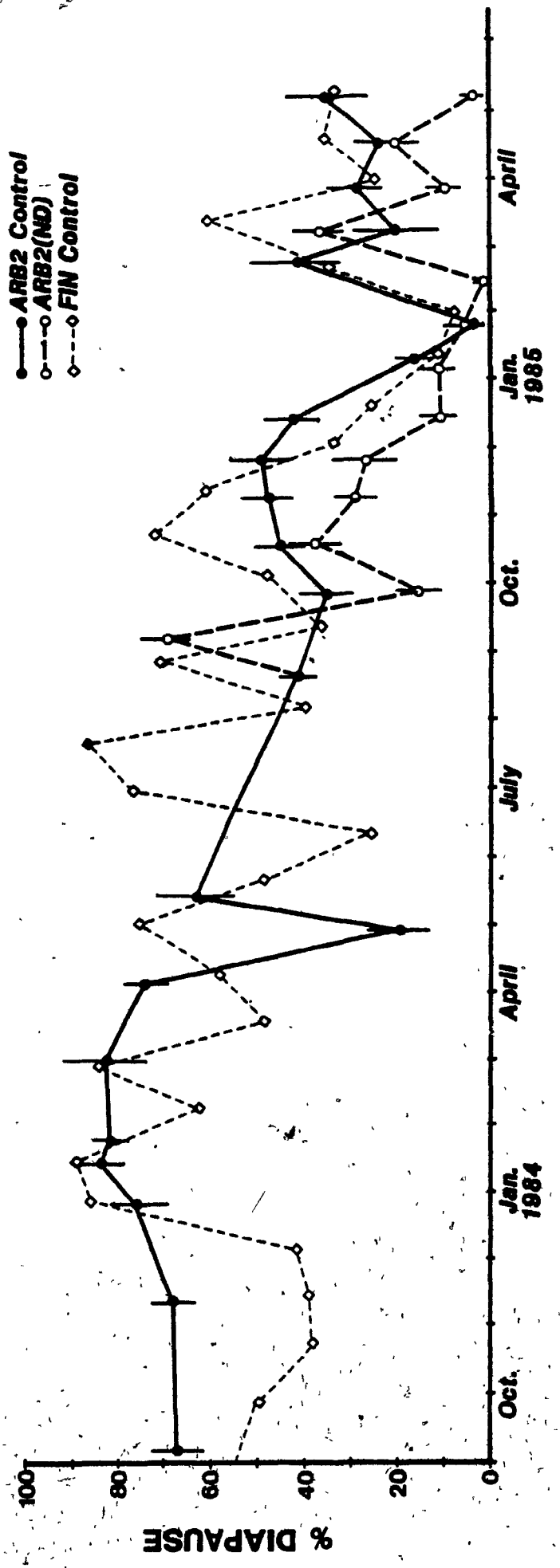
for diapause induction compared to FIN lines. Only 1 male (to 130 females) emerged in sample vials from F_6, therefore, ca. 20 males from the QUE control line were added to the cage. When only females emerged in F_9, no further efforts were made to continue this line.

QUE2(ND) (Figure 6.) Selection began simultaneously with F_5 of QUE(ND), after sex ratio became a problem. Sex ratio in QUE2(ND) remained stable (mean of 1 male:2.9 females) for 10 generations. Diapause incidence decreased an average of 15% per generation for the first 3 generation, and averaged 6% from F_4-F_{10}. Although a promising line, it was discontinued in favor of FIN(ND), in which sex ratios were always stable, when it became necessary to eliminate some lines to begin evaluation experiments.

ARB2(ND) (Figure 7.) Selection for nondiapause began after the ARB2 control line had been reared as part of another research project for 11 months. During the first seven months, diapause incidence in the ARB control line averaged 69% under LD 8:16, then began to drop. During the second year of rearing, diapause in ARB2 control averaged 33% under LD 8:16, significantly lower (ANOVA, $F=20.54$, $df=1,21$, $p=0.0002$) than the first year. The mean of 23% diapause for 14 generations of ARB2(ND) was lower, but not significantly so, than the ARB2 control line (33%) during the same period.

Although there was no clear response to nondiapause selection in the ARB2 line after a year of laboratory rearing, the swift decline in diapause incidence in FIN and QUE lines under nondiapause selection was similar to results obtained in other insect species (c.f. Barry and Adkisson,

Figure 7. Percent diapause in ARB2 lines of *A. aphidimyza*, reared under LD 8:16 (21°C). FIN control line data included for comparison. Vertical lines represent exact 95% binomial confidence limits.


ARB2 Control
ARB2(ND)
FIN Control
% DIAPAUSE
100
80
60
40
20
0
Oct.
Jan. 1984
April
July
Oct.
Jan. 1985
April

1966; Lees, 1968; Glass, 1970; Herzog and Phillips, 1974). Possible causes of inadvertent nondiapause selection in control lines are discussed on pg. 136.

There was a wide variation in percentage of adults emerging by day 13 in both FIN and ARB2 nondiapause and control lines (Table 3.). The means for FIN(ND) (46%) and FIN control (49%) did not differ significantly from each other, nor did means for ARB2(ND) (44%) and ARB2 control (46%). The mean for FIN2(ND) (68%) was not significantly different from FIN(ND). The QUE(ND) line was significantly slower to develop (ANOVA, $F=18.72$, $df=1,14$, $p=0.0007$) than the FIN(ND) line, with only 4% emerging by day 13. The QUE2(ND) line, started three months later, developed much faster than QUE(ND), with a mean emergence of 58% on day 13. This may reflect the rearing method used during the first three months, which was biased towards offspring of the first adults to emerge. As soon as the first midges were present, eggs were collected until enough were obtained for the next generation. To avoid further inadvertent selection for early emergence during subsequent generations, plants were not put into cages for oviposition until adults had been present for two days. Thus, eggs were obtained from as many different females as possible. Interestingly, it was the slowest developing line, QUE(ND), that died out due to an extremely unbalanced sex ratio. It is not known, however, whether these facts are connected.

Although correlations (PPMC) were run on all data from each line, only significant correlations are given in Table 4. Negative correlations between diapause incidence

TABLE 3. Means of data from all lines of *A. aphidimyza*.

Number of Generations or Samples	Line	% Diapause[1]	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂:♀	Emergence by day 13
			$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
30	FIN control[2]	48.4 ± 4.4	2.7 ± 0.5	2.2 ± 0.3	2.4 ± 0.2	1:2.5 ± 0.9	49.4 ± 9.8
50	FIN(ND)	11.1 ± 1.8	3.4 ± 0.4	3.0 ± 0.2	4.5 ± 0.4	1:2.5 ± 0.2	46.1 ± 3.5
6	FIN2(ND)	10.7 ± 2.3	2.5 ± 0.8	2.8 ± 0.8	4.1 ± 0.9	1:2.7 ± 0.4	68.2 ± 3.9
9	QUE(ND)	3.1 ± 1.2	1.1 ± 0.5	3.1 ± 1.1	5.6 ± 1.4	1:46.0 ± 20.1	4.5 ± 2.1
10	QUE2(ND)	6.1 ± 0.8	2.5 ± 0.9	3.4 ± 0.6	4.0 ± 0.8	1:3.0 ± 0.6	58.4 ± 6.8
9	ARB2 control (yr 1)[3]	68.7 ± 6.6	2.8 ± 1.9	3.7 ± 0.9	3.1 ± 0.7	1:11.4 ± 9.8	6.2 ± 2.4
13	ARB2 control (>yr 1)	33.4 ± 3.8	3.8 ± 0.7	2.4 ± 0.2	3.5 ± 0.6	1:1.6 ± 0.2	45.9 ± 6.4
14	ARB2(ND)	23.1 ± 5.0	5.1 ± 0.9	2.7 ± 0.4	4.3 ± 0.6	1:1.8 ± 0.3	44.0 ± 6.2

[1] In nondiapause-selected lines, $\bar{x} \pm SE$ calculated after diapause incidence appeared to stabilize [FIN(ND), F_{6-50}; FIN2(ND), F_{2-6}; QUE(ND), F_{5-9}; QUE2(ND), F_{4-10}; ARB2(ND), F_{1-14}].

[2] Data from samples matching FIN(ND) generations.

[3] First year of rearing, before nondiapause selection started.

TABLE 4. Correlations in data from generation to generation in lines of A. aphidimyza, reared under LD 8:16 (@1°C).

Line	Number of Generations	Correlation between % diapause and % emerged by day 13		Correlation between % diapause and % pupal mortality	
		r^1	p	r	p
FIN control	30	-0.2843	0.1278	-0.5671	0.0011
FIN(ND)	50	-0.4822	0.0004	-0.5096	0.0002
QUE2(ND)	10	0.6467	0.0433	-0.5949	0.0697
ARB2 control	13	0.4628	0.1112	-0.6412	0.0182
ARB2(ND)	14	-0.5363	0.0480	-0.4734	0.0875
REL2	12	-0.2382	0.4559	-0.7622	0.0040

[1] Pearson product moment correlation coefficient.

and percentage emerged by day 13 occurred in nondiapause lines, but not control lines; the implications of this are discussed on pg.133.Diapause incidence was negatively correlated, either significantly ($p<0.05$) or moderately ($p<0.1$), with pupal mortality in all lines. This is not surprising, since a lower diapause incidence means a higher proportion of pupae were present. Larval mortality did not increase, however, when diapause increased. In vials with only diapause larvae, mortality was extremely low or zero, whereas in mixed vials it was higher. Although there was little difference between mean larval and pupal mortalities, it appears that most fatalities occurred in pupation oriented larvae. Because pupation requires "switching on" a new set of genes, it is probably more frequenty fatal.

It is likely that selection for low diapause incidence, under LD 8:16 (21°C), succeeded because the genotype(s) resulting in nondiapause under these conditions occurred with intermediate (ca. 20%) frequency in the parental population. If nondiapause had been a rare trait, selection would have been unlikely to succeed (Falconer, 1981).

The fluctuations in percentage of diapause from generation to generation show that the nondiapause trait was not in equilibrium in the FIN(ND) population. Because diapause persisted in the population, even after 50 generations of selection, it is unlikely that a completely diapause-free line of *A. aphidimyza* can be obtained using these methods. This may be because diapause is a threshold character; there is an underlying continuous

variation, or liability, which is both environmental and genetic in origin, with a threshold at some point dividing the population into two phenotypes (Falconer, 1981). Within these two groups are individuals with different liabilities, but as long as individuals are in the desired phenotypic class (e.g, nondiapause), artificial selection cannot distinguish between them. Some individuals with a relatively high liability to diapause could be present in a nondiapausing population, perpetuating diapause genes. The persistence of diapause after many generations of selection may also have been because individuals with low liability to diapause were selected against. If low liabiity to diapause was associated at the genetic level with disadvantageous behavior (e.g., slow larval feeding rate, poor searching ability) or poor environmental adaptation, then they would be at a selective disadvantage compared to nondiapause larvae with a higher liability to diapause.

Because the original FIN line had been a laboratory line for 14 years, one might expect greater variability in diapause response between individuals in the population as a result of random genetic drift. Those individuals with low liability to diapause would not have been eliminated every 4-5 generations as occurs in nature every fall. The wild lines that became established in the laboratory, however, exhibited a similar, or lower, diapause incidence under LD 8:16 (21°C). Thus, the lack of diapause selection in the long-running laboratory line may not have affected the equilibrium level of diapause genes in the population.

MORPHOLOGY

Adults were examined for possible changes in external morphology that might result from inbreeding, genetic drift or selection pressure.

Materials and Methods. Samples of 20-50 adults of both sexes were taken from each generation of nondiapause lines and periodically from control lines. Specimens were preserved in 90% methyl alcohol and examined under the microscope (50X) for gross physical anomalies.

Results and Discussion. No morphological differences were found. Relatively large populations were maintained for most generations, which provided a large gene pool and prevented excessive inbreeding. Detailed examination of FIN(ND) specimens was discontinued after 21 generations.

SEX RATIO

It was important to monitor sex ratio in *A. aphidimyza* because several wild lines had shifted to a single sex under laboratory rearing. As in many other cecidomyiids, *A. aphidimyza* is monogenic, so all offspring from one female are of the same sex (Sell, 1976). Therefore, it is possible that artificial selection for nondiapause could change sex ratio if it affected male-producing and female-producing females differently.

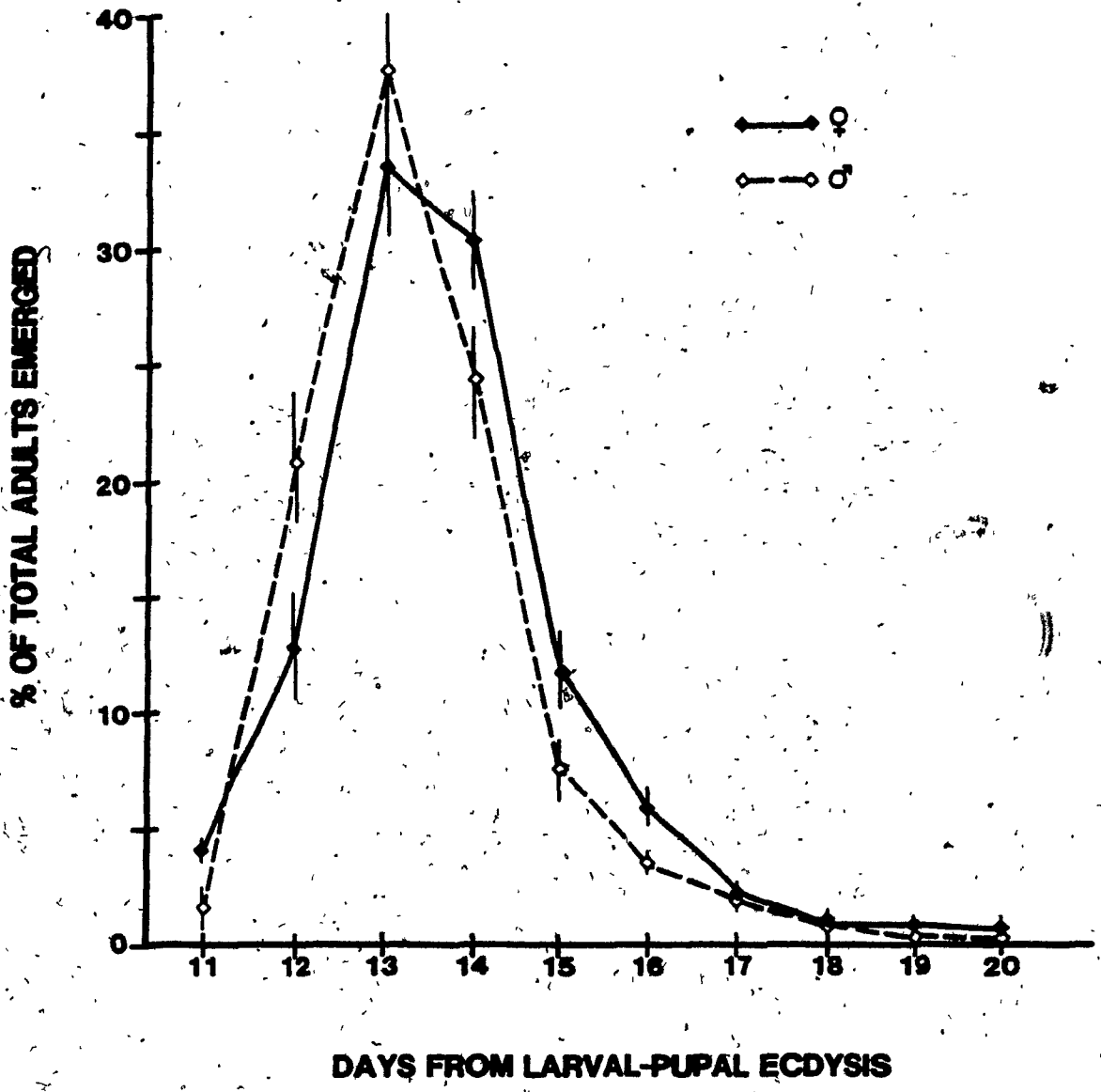
Materials and Methods. Sex of adults emerging from sample vials was recorded daily and used to calculate sex ratio for each generation and to determine emergence curves for each sex.

Results and Discussion. Mean sex ratio for all generations of FIN(ND) and FIN control averaged 1 male:2.5 females; mean for the six generations of FIN2(ND) was 1:2.7 (Table 3.). This agrees with the ratio of 1:2-3 reported by Ushchekov (1975), although it differs from the 1:1 ratio reported by Sell (1976) and the 1:1.7 ratio reported by Uygun (1971). Variability from generation to generation in sex ratio of all FIN lines was similar, with the exception of one FIN control sample collected 9 May, 1985 (1:28). Mean sex ratio in the QUE2(ND) line was 1:3, and appeared to be quite stable, unlike the first nondiapause line, QUE(ND), which ended in 100% females after 9 generations. Mean sex ratio of ARB2(ND) (1:1.8) was similar to that of the ARB2 control line (1:1.6) during the same period, although during the previous year, before nondiapause selection began, sex ratio varied widely from 1:1.2 to 1:90. It appears that if

wild colonies did not shift to a single sex during the first few generations in the laboratory, then sex ratio became more stable with time. This is consistent with the observation that wide, random fluctuations in gene frequencies occurring in small populations tend to reach equilibrium after several generations of random mating (Falconer, 1981). A sex ratio biased toward females is unusual, however, Hamilton (1967) draws a parallel between the unusual chromosome cycles found in Cecidomyiidae, in which paternal chromosomes are eliminated, and arrhenotokous modes of reproduction, in which males are haploid. In arrhenotoky, female biased sex ratios predominate, with sex of offspring dependent on the phenotype of females. It is further characterized by early emergence of males, the ability of males to mate many times, and the capability of females to store sperm, all characteristics of cecidomyiids such as *A. aphidimyza* (Hamilton, 1967).

The earliest emerging adults in a sample are usually males, and, although time to 50% emergence for both sexes is similar (Figure 8.), the sex ratio of adults emerging on each day changes, which may account for some of the variation in sex ratio reported in the literature. Since sex ratio also varies from generation to generation, reports in the literature based on one generation or on small samples may be misleading.

Figure 8. Comparison of emergence curves for males and females of the FIN(ND) line of *A. aphidimyza*. Data from 50 generations.


40
30
20
10
0
% OF TOTAL ADULTS EMERGED
♀
♂
11
12
13
14
15
16
17
18
19
20
DAYS FROM LARVAL-PUPAL ECDYSIS

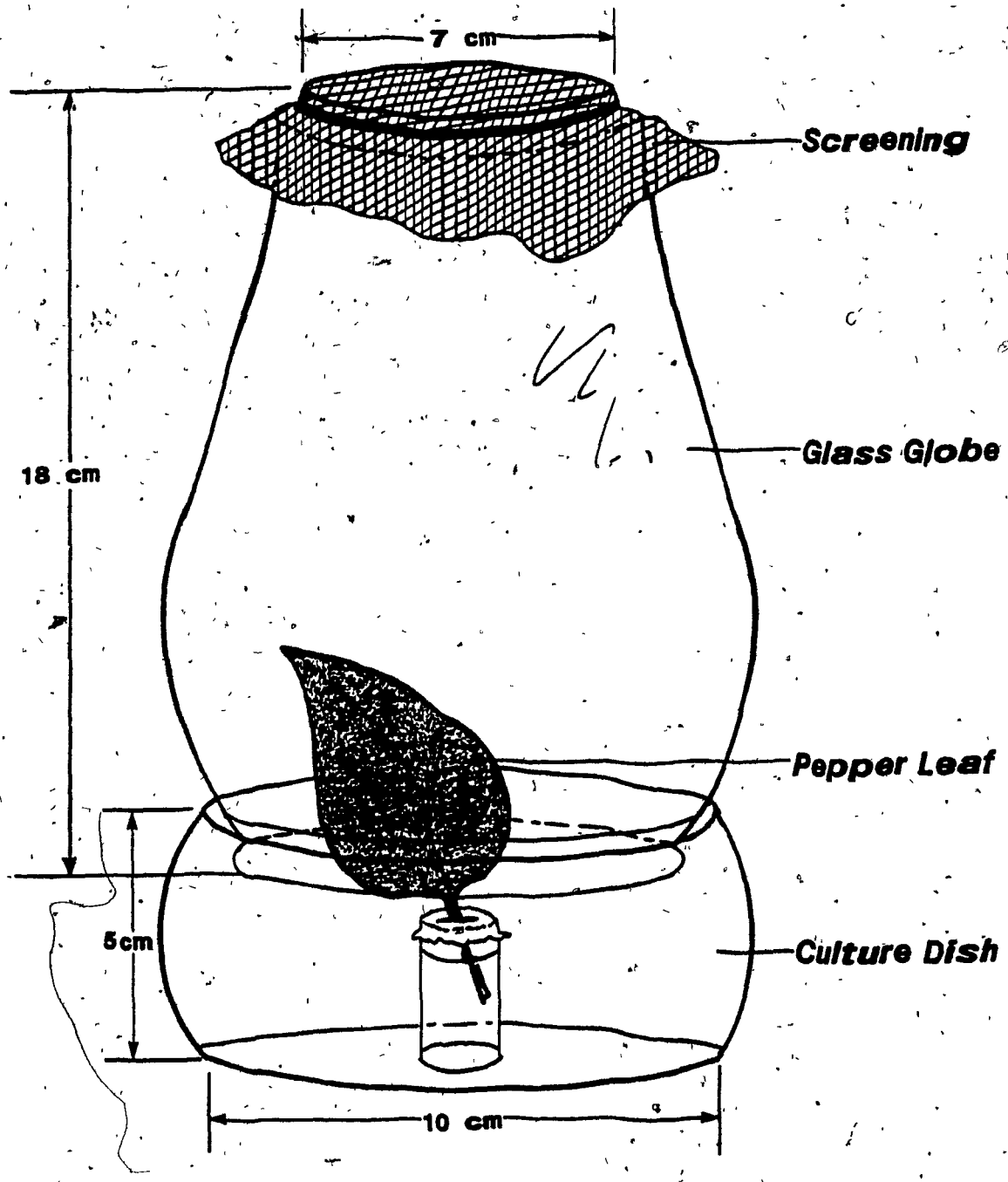
FECUNDITY

To determine whether fecundity had been affected by nondiapause selection, the number of eggs laid by individual females from FIN(ND) and FIN control lines was compared in 1983, after 18 generations of selection, and again in 1984, after 31 generations.

Materials and Methods. Oviposition cages were assembled from glass lamp globes (11 cm diam x 18 cm high), covered with nylon mesh and set in heavy glass culture dishes (Figure 9.). The importance of cage design is demonstrated by the fact that most females died in preliminary experiments using cages with hurricane lamp chimneys instead of globes, because they were caught in condensed droplets on the chimney "shoulders". Adults for tests came from FIN(ND) and FIN control larvae pupating in individual vials (4 ml) with squares of moist filter paper (0.5 cm) on the bottom. Upon eclosion, one female and three males were released in each cage. When insufficient males emerged in vials, additional males were added from main cages to make up the total of three.

Green pepper leaves with similar levels of aphid infestation were cut from host plants. The petiole of each leaf was inserted through a slit in parafilm, covering a vial (8 ml) of distilled water, and one leaf at a time was placed in each cage for oviposition. Leaves were removed and replaced by gently lifting globes without disturbing adults resting on the glass. All eggs on both leaf surfaces were counted under 25X magnification. Eggs did not hatch until the third day, which made it possible to replace leaves

Figure 9. Oviposition cage for individual females. Glass globe is raised to replace oviposition leaf.



OVIPOSITION CAGE

every two days, rather than daily, thus minimizing mortality and escapes resulting from handling.

Cages were maintained until females died (ca. 10 d), whether or not eggs were laid, because it had been observed that toward the end of their oviposition period, a pause of one or two days could occur between periods of oviposition. Most males died within 2-4 d and more were added only if females had not begun to lay eggs. In preliminary work, it was found that once mated, females would continue laying eggs throughout their life span, although later eggs were not viable unless females were mated again. Eggs were rarely laid the first night after eclosion, probably because ovaries were not developed (Pritchard, 1961), but most began laying the second night.

In 1983, FIN control females in oviposition cages were kept in LD 17:7 (21°C) and FIN(ND) females were kept in LD 8:16 (21°C). In 1984, both groups were kept in LD 8:16 (21°C) to conserve incubator space. This is not likely to have affected fecundity, according to Bradovskaya (1977), who found that there was little difference in fecundity of females kept under 10 h and 16 h photophases (20°C).

Two other oviposition tests were conducted in 1984 under conditions simulating winter greenhouse environments. Females from FIN(ND) line were kept under LD 9:15 with 21°C day and 18 or 15°C night temperatures. Thermophase was two hours shorter than photophase (pg.164).

Results and Discussion. Selection for nondiapause did not affect fecundity (Table 5.). There was no significant

TABLE 5. Comparison of fecundity in A. aphidimyza from FIN(ND) and FIN control lines.

Year	Line	Number of ♀♀ laying eggs	Egg production ($\bar{x} \pm SE$)	Life span (d) ($\bar{x} \pm SE$)	Number of ♀♀ not laying eggs
1983	FIN control	23	98.5 ± 8.8a[1]	6.6 ± 0.5a	5
	FIN(ND)	23	109.4 ± 7.6a	7.6 ± 0.6a	6
1984	FIN control	26	156.6 ± 11.3b	10.8 ± 0.9ab	16
	FIN(ND)	28	163.7 ± 10.4b	8.9 ± 0.4b	11
	FIN(ND)[2]	10	98.3 ± 13.7a	9.8 ± 0.6ab	4
	FIN(ND)[3]	10	118.4 ± 10.1ab	10.1 ± 0.6b	2

[1] Means comparisons using Tukey's studentized range test. Means with a common letter are not significantly different ($p<0.05$).

[2] Reared under LD 9:15, TC 21:18°C.

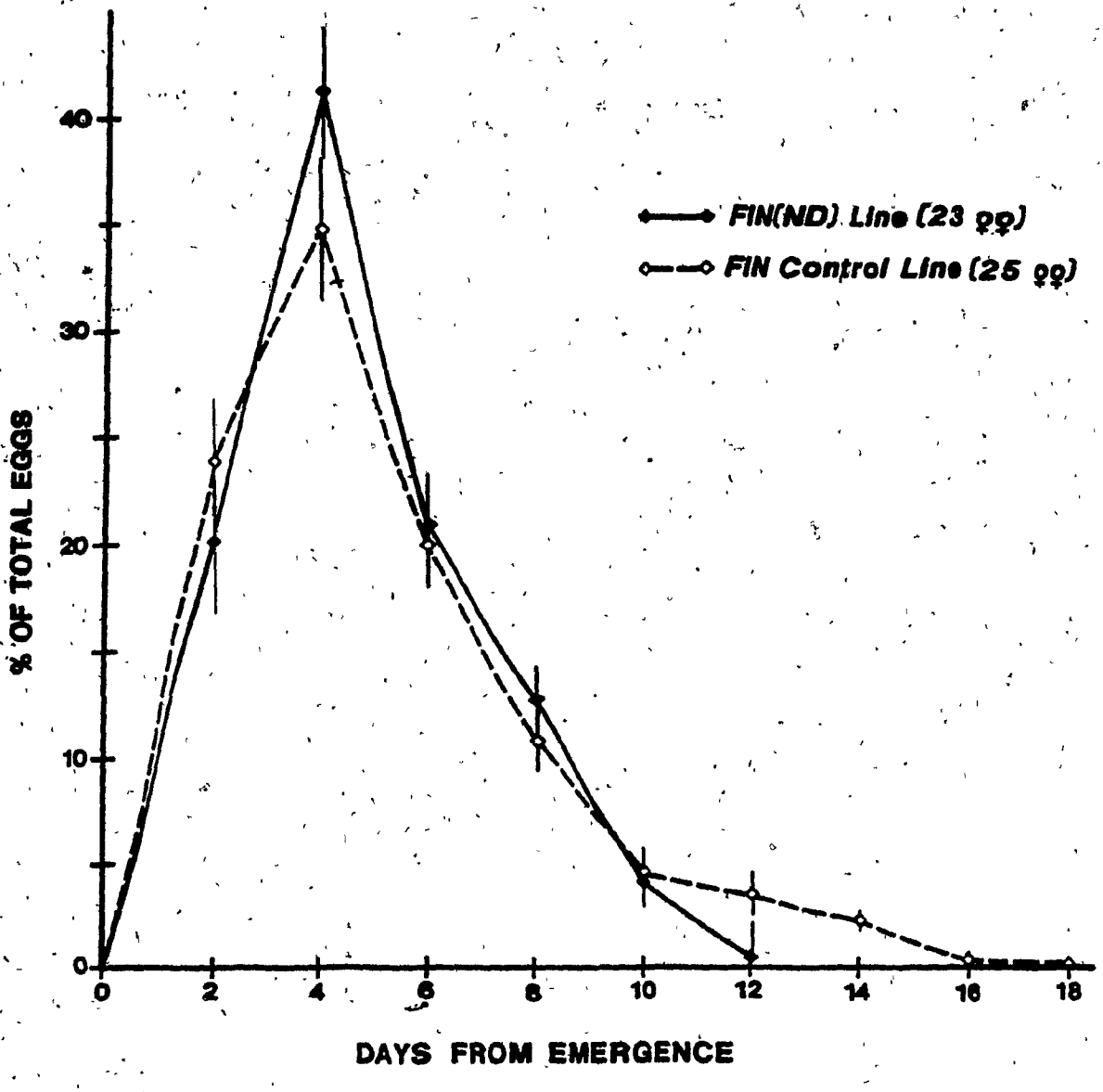
[3] Reared under LD 9:15, TC 21:15°C.

difference in mean number of eggs per female between nondiapause and control lines, at constant 21°C, in either 1983 or 1984; oviposition curves were also very similar (Figure 10.). Mean number of eggs per female, however, was significantly higher in 1984 than 1983 for both lines (ANOVA, FIN(ND): $F=15.3$, $df=1,49$, $p=0.0003$; FIN control: $F=15.9$, $df=1,47$, $p=0.0002$). Nutrition of *A. aphidimyza* larvae can affect fecundity of adults (Kuo, 1976/77, 1982; Havelka and Ruzicka, 1984) therefore, greater skill in mass-rearing later generations may have resulted in better quality adults. This conjecture is supported by the significantly longer life span ($\bar{x}$=11 d) among FIN control females in 1984 than in 1983 ($\bar{x}$=7 d) (ANOVA, $F=14.6$, $df=1,47$, $p=0.0004$).

The number of eggs laid under TC 21:18°C and TC 21:15°C were not significantly different from each other, however, number of eggs per female in both treatments was significantly lower (Tukey's studentized range test, $p<0.05$), than under constant 21°C. Life spans were not significantly different.

There was an extremely wide range in number of eggs per female (12-276) from both lines. Since the environment was uniform and sufficient prey were provided during larval development, differences in genotypes of individual females may largely account for the difference. In 1984, 20-30% of females, from both FIN lines, laid >200 eggs (LD 8:16, 21°C) and 23% of FIN control females lived >15 d (one female lived 24 d and laid 276 eggs). This is a large enough proportion of the population that genetic selection for

Figure 10. Oviposition curves for females from FIN(ND) and FIN control lines of *A. aphidimyza*.


FIN(ND) Line (23 ♀♀)
FIN Control Line (25 ♀♀)
% OF TOTAL EGGS
0
10
20
30
40
0
2
4
6
8
10
12
14
16
18
DAYS FROM EMERGENCE

lines with higher fecundity and longevity might succeed.

Although most females did not lay eggs unless mated, a few virgin females did eventually do so. They laid up to 60 eggs, usually in large haphazard clumps, unlike the normal pattern of oviposition, in which eggs are laid singly or in small clusters. It was also observed that individual females exhibited characteristic oviposition patterns, some leaving only single eggs, others leaving groups of 2-5. One female laid eggs only on the upper surface of leaves.

An average of 25% of females from both lines never laid eggs, despite repeated releases of males. Dissection showed that their ovaries contained ca. 40 developed eggs, but no satisfactory explanation was found for their inability to oviposit. Bradovskaya (1977) found that the proportion of fertile females varied with temperature and photoperiod, with ca. 8% of females being infertile under LD 16:8 and 20-25°C. In preliminary tests, I found that 12 of 14 females (86%) kept under LD 17:7 and 24-28°C for pupation never laid eggs. Temperatures inside vials may have been higher, so it is possible that high temperatures negatively affected fertility. This is supported by Bradovskaya (1977), who found that ca. 40% of females were infertile at 30°C, regardless of photoperiod regime. The infertile females obtained from larvae reared at 21°C may have been affected by this temperature, they may not have been sufficiently mated (although live males were always present), or something about the cage environment may have inhibited oviposition. Another possibility is that the absence of a crepuscular period,

which, according to some researchers (Tiittanen, pers. comm.) is necessary for successful oviposition in *A. aphidimyza*, affected some females and not others. Because numerous eggs were laid in incubators, without a period of dim light before full darkness, no provision was made for such a period, which would have required installation of additional timers and lights in all incubators.

RELAXED SELECTION

Two tests were conducted to find out whether diapause incidence would increase in the FIN(ND) line when selection pressure was relaxed.

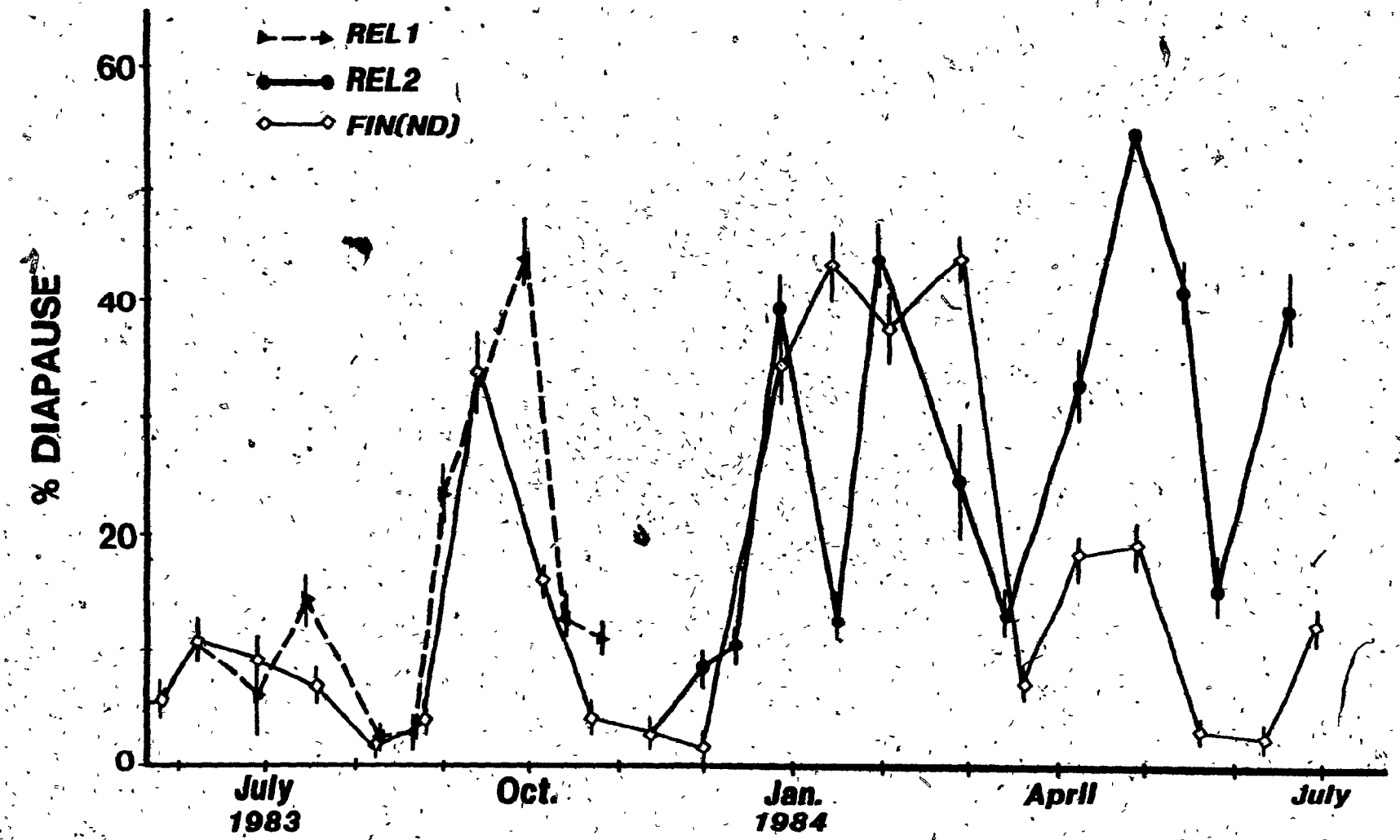
Materials and Methods. The first line (REL1), beginning with eggs from F_{14}, was reared for 10 generations. The second line (REL2) started with eggs from F_{22} and was reared for 12 generations.

To begin each relaxed selection line, ca. 1000 eggs on oviposition plants were taken from the FIN(ND) cage and placed in LD 17:7 for larval development. All generations of REL1, and the first four generations of REL2, were reared in Climatoria under 24-28°C to shorten generation time. Later REL2 generations were reared at 21°C to coincide with FIN(ND) generations.

Adults were kept in small, wood frame sleeve cages (28 x 28 x 28 cm) covered with nylon mesh, with trays of moist peat moss for pupation in the bottom. Succeeding generations were kept continuously in diapause preventing conditions, and at each generation an oviposition plant taken from the REL cage was placed under LD 8:16 (21°C) to check diapause incidence among REL larvae. All larvae were collected in vials according to methods described. Sample plants were not taken from the third and seventh REL1 generation because of a scarcity of aphids.

Results and Discussion. In both REL lines (Appendix B, Table B8.), diapause incidence departed significantly (X^2-test, $p<0.005$) from that of the FIN(ND) parent line at the eighth generation (Figure 11.). In REL1, diapause

Figure 11. Percent diapause in FIN(ND) line, after selection for nondiapause was relaxed. REL 1 line originating from F_{14}, REL 2 from F_{22} of FIN(ND); samples checked under LD 8:16 (21°C). FIN(ND) line data included for comparison. Vertical lines represent exact 95% binomial confidence limits.


REL1
REL2
FIN(ND)
% DIAPAUSE
60
40
20
0
July
1983
Oct.
Jan.
1984
April
July

decreased again in ninth and tenth generations. In REL2, diapause in generations 8-12 was significantly higher (X^2 test, $p<0.0005$) than in simultaneous FIN(ND) generations. It remained significantly lower (X^2 test, $p<0.0005$), however, than in simultaneous samples from the FIN control line. The log linear model for categorical data (Sokal and Rohlf, 1981) indicates that there is a significant difference in diapause ($p=0.0001$) between REL2 and both FIN(ND) and FIN control lines. The slow drift to a higher percentage of diapause when selection was relaxed is consistent with what would be expected when a dominant trait is not in equilibrium. These results suggest that a high proportion of nondiapause midges could persist in a greenhouse population for at least 12 generations (9-12 mo), as long as there were no genetic additions from wild *A. aphidimyza*.

RECIPROCAL CROSSES BETWEEN NONDIAPAUSE AND CONTROL LINES

Reciprocal crosses between FIN control and FIN(ND) lines to the F_2 generation were made to determine inheritance of diapause in offspring and demonstrate chromosome elimination in *A. aphidimyza*.

Materials and Methods.

Experiment 1:

Three oviposition plants were taken from each of FIN control and FIN(ND) cages. Two plants from each line were kept under LD 17:7 (21°C) to provide the parental populations. Because it is was not possible to sex larvae, they were placed in individual vials (4 ml) for pupation. They were reared under diapause preventing conditions so all larvae in the population, regardless of genotype with respect to diapause, could contribute genes equally to the next generation. All eggs on the other plant from each cage were reared under LD 8:16 (21°C) to determine diapause incidence in parental lines. Upon emergence, adults for the first cross (N ♀ x D ♂ , D ♀ x N ♂ , where N=nondiapause, D=control) were sexed, then released in small screened sleeve cages (28 x 28 x 28 cm) with oviposition plants. Eggs were reared under diapause inducing conditions (LD 8:16, 21°C), and all larvae were collected in sample vials.

Experiment 2:

It is necessary to obtain an F_2 generation to show the effect of male chromosome elimination on inheritance (Gallun and Hatchett, 1969), therefore F_1 reciprocal crosses were included in the second experiment.

First generation crosses were made as above, with the exception that four oviposition plants were placed in each F_1 cage. Two plants from each cage remained under diapause inducing conditions to determine F_1 diapause incidence, and two were moved to LD 17:7 (21°C) to provide adults for the next cross. Larvae pupated in individual vials and upon emergence were divided according to sex and released in cages. Crosses were ND♀ x ND♂, DN♀ x DN♂, ND♀ x DN♂, and DN♀ x ND♂, where the first letter designates origin of female parent and the second designates origin of male parent of that individual. Eggs laid on three oviposition plants from each cage were reared under LD 8:16 (21°C) and collected in sample vials.

Results and Discussion. Results of both crossing experiments between FIN(ND) and FIN control lines show that diapause in response to LD 8:16 (21°C) is dominant over nondiapause (Table 6.). In both crosses, diapause incidence in F_1 generations was not significantly different from that in the parental control generation when the female was from the control line. When the male was from the control line, diapause incidence was significantly lower (Exp.1: $X^2=3.90$, df=1, $p<0.05$; Exp.2: $X^2=11.26$, df=1, $p<0.005$) than when the female parent was from control line. Although, in this cross, diapause was significantly lower, it was still closer to that of the parental control line than the parental nondiapause lines. This depression in diapause may be explained if there were a preponderance of ND genotypes in the parental control line, because the elimination of paternally derived chromosomes from the germ

TABLE 6. Percent diapause in mass reciprocal matings between control (D) and nondiapause (N) A. aphidimyza from FIN lines, reared under LD 8:16 (21°C).

Generation	Cross	n	Sex ratio ♂ : ♀	% Diapause	Comparison to parental D line[1]	Comparison between reciprocal matings[1]
Parents	N	204		10.8[2]		
	D	127		48.8[3]		
F_1	N ♀ x D ♂	347	1:4.5	42.4	NS[4]	p<0.05
	N ♀ x D ♂	393	1:2.9	49.6	NS	
Parents	N	114		1.8[2]		
	D	127		53.5[3]		
F_1	N ♀ x D ♂	685	1:2.3	40.0	p<0.005	p<0.005
	D ♀ x N ♂	566	1:2.0	49.5	NS	
F_2	ND ♀ x ND ♂	587	1:3.8	31.5	p<0.005	p<0.05
	DN ♀ x ND ♂	460	1:2.0	38.6	p<0.01	NS
	DN ♀ x DN ♂	528	1:1.9	43.7	NS	NS
	ND ♀ x DN ♂	476	1:1.9	46.8	NS	

[1] Chi-square test.

[2] Percent diapause in same generation of FIN(ND) line.

[3] Percent diapause at same time in FIN control line.

[4] Not significant, p<0.05.

line in early cleaveage would result in a higher proportion of NN genotypes in offspring.

Diapause results in the F_2 generations are consistent with chromosome elimination (Table 6.) (c.f. Gallun and Hatchett, 1969). As expected, both crosses with males from the ND cage had a lower diapause incidence. Paternal D genes were eliminated, resulting in males that passed on only maternally derived N genes, which would produce a population with equal proportions of NN and DN genotypes. In crosses with males from DN lines, the paternally derived N gene would have been eliminated, resulting in offspring with either DD or ND genotypes, which are likely to be diapause phenotypes, according to F_1 results. Diapause incidence in crosses with DN males was not significantly different from the parental control lines, whereas diapause was significantly lower (X^2 tests, 1 df, $p<0.01$) in crosses with ND males. In crosses with ND males, there was a significantly higher diapause incidence ($X^2=5.1636$, 1 df, $p<0.05$) in the cross with DN females compared to the cross with ND females, however, no explanation for this is known.

COMPARISON OF LARVAL DURATION

This experiment was conducted at the beginning of the research to determine whether there was a difference in duration of larval stage between diapausing and nondiapausing larvae, as occurs in some other species.

Materials and Methods. Three replicates, with a total of 185 larvae, were made with eggs collected on different dates. For each replicate, a single, aphid infested leaf, with petiole in a vial of water, was placed in the FIN control cage for oviposition when numerous females (>50) were present. Because no oviposition plants had been in the cage for >24 h, females rapidly laid eggs on the leaf, which was left in the cage for only one hour to ensure eggs were the same age. At least 12 different females were observed ovipositing on each leaf.

Each leaf was placed in a covered petri dish on moist filter paper and incubated under LD 8:16 (21°C) until eggs hatched. The leaf was then placed across the top leaves of an aphid infested plant so larvae could move down to feed. On the sixth day after oviposition, larvae were placed individually in vials with moistened cotton in the bottom. Vials were checked twice daily and fresh pieces of aphid infested leaf were put in vials as necessary, until larvae had spun cocoons. The date larvae ceased feeding and commenced spinning a cocoon was recorded. Emergence of adults was recorded and remaining coccons were opened after 21 d to count diapausing larvae.

Results and Discussion. Data from replicates were pooled because ratio of pupating to diapausing larvae spinning cocoons each day was similar.

Duration of larval stage was significantly longer (ANOVA, F=31.78, df=1,183, p=0.0001) for diapausing larvae than for larvae that pupated (Table 7.). Twenty percent of larvae spinning cocoons on the seventh day after oviposition diapaused, compared with 86% on the tenth day. Results are presented in a slightly different way in Figure 12. These results are consistent with the greater needs of diapausing larvae for stored body reserves (Lees, 1968), and agree with results obtained for other insect species (Dickson, 1949; Glass, 1970; Henrich and Denlinger, 1982; Saunders, 1975).

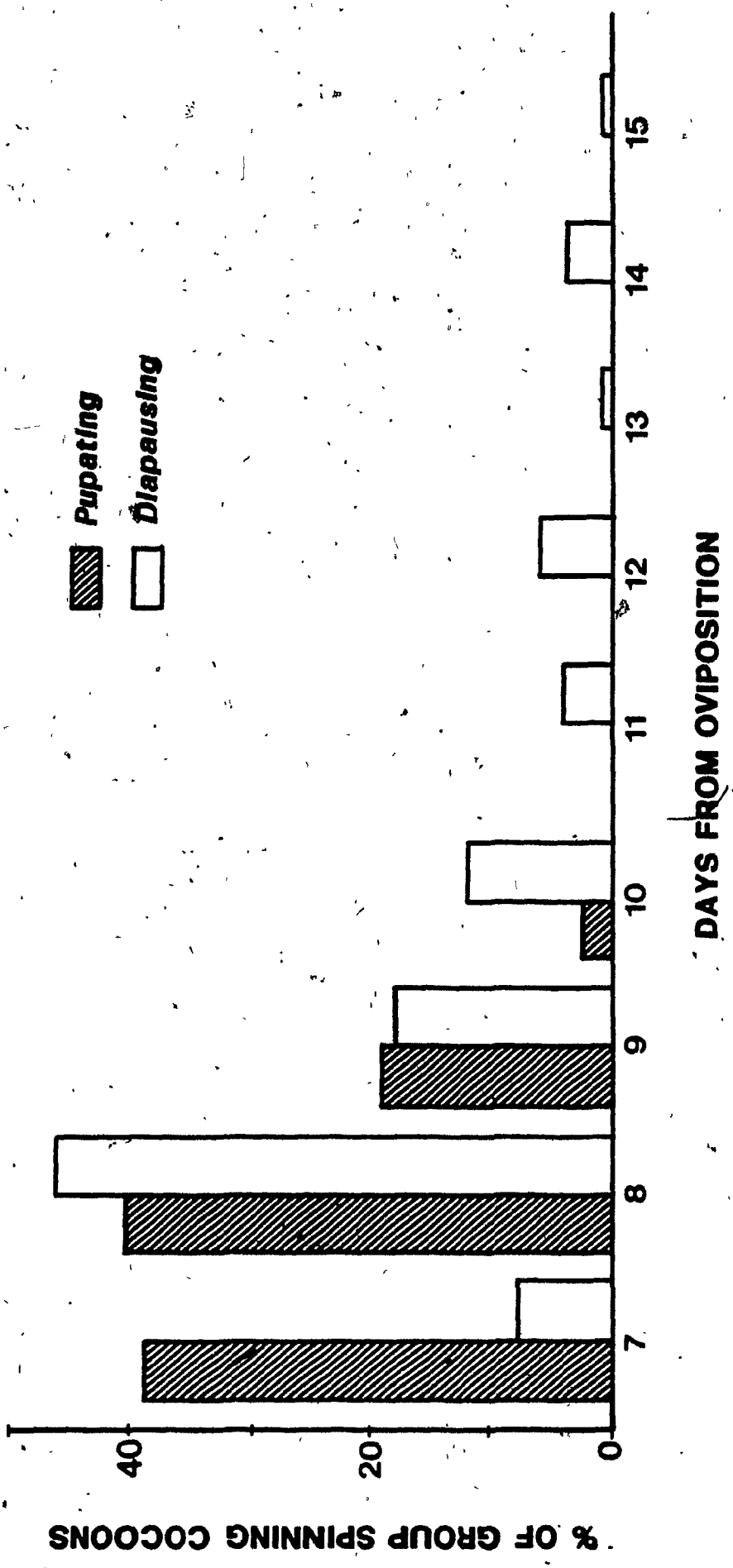
Three diapausing larvae never did spin coccoons, but wandered for up to 15 days before becoming stationary. Since fresh aphids were supplied continuously, it is not likely that they were searching for food, however, they may have been searching for more suitable diapause sites. Perhaps the choice of an overwintering site is more important than a pupation site.

As a result of this experiment, the sampling methods used to determine diapause incidence in each generation were changed to avoid bias toward faster developing larvae by rearing and collecting all larvae from a single plant, regardless of larval duration (pg.79). These results also led to the following experiment to determine whether artificially changing larval development rate affected diapause incidence.

TABLE 7. Larval duration, expressed as days from oviposition, for diapausing and pupating *A. aphidimyza* larvae from the FIN control line, reared under LD 8:16 (21°C).

Days from oviposition	n	% Larvae diapausing $\bar{x} \pm SE$	% Larvae pupating $\bar{x} \pm SE$
7	40	20.0 ± 6.9	80.0 ± 6.9
8	81	58.0 ± 7.2	42.0 ± 7.6
9	34	52.9 ± 19.1	47.1 ± 19.2
10	14	85.7 ± 8.2	14.1 ± 6.7
11	4	100.0	0
12	6	100.0	0
13	1	100.0	0
14	4	100.0	0
15	1	100.0	0

Figure 12. Duration of larval period in diapause and pupation oriented *A. aphidimyza* larvae. Bars represent percentage of each group spinning cocoons that day.


Pupating
Diapausing
0
20
40
% OF GROUP SPINNING COCOONS
7
8
9
10
11
12
13
14
15
DAYS FROM OVIPOSITION

EFFECT OF PROLONGING LARVAL STAGE

This experiment was designed to test whether artificially prolonging larval development, and thus the photoperiodically sensitive stage, increased diapause incidence. Saunders (1975) hypothesized that larvae of the fleshfly, *Sarcophaga argyrostoma* (Robineau-Desvoidy), that took longer to develop, had a higher incidence of diapause under short day conditions because they experienced more short day cyles. If this is so, then selection for nondiapause might favor larvae with faster development (shorter sensitive stage) relative to the number of days required at that temperature to induce diapause. Such larvae would complete their development before experiencing sufficient short days to reach the threshold for diapause response.

Materials and Methods. A single oviposition plant was placed overnight in the FIN control cage. The next morning, leaves were removed, divided randomly into two groups, and placed under LD 8:16 (21°C). One group, FAST, was reared with a maximum supply of host aphids, while the other, SLOW, was fed just enough aphids to maintain growth. Oviposition leaves for both groups were placed on aphid infested plants until eggs hatched and larvae began to feed. Those for the FAST group were distributed over 4-5 host plants to minimize competition for food, which might slow development. On the sixth day from oviposition, larvae were placed in inflated plastic bags (18 x 22 cm) along with sufficient aphids to complete development. All larvae were placed in sample vials according to established methods.

SLOW larvae were confined to one host plant for the first four days, then were placed in inflated plastic bags with a few aphid infested leaves. Leaves were renewed daily and bags were aired 4-5 times a day to reduce condensation and accumulation of CO_2. These methods resulted in longer larval development periods (and higher mortality) compared with the FAST group.

Three replicates, involving a total of 1310 larvae from the FIN control line, were tested. Eggs were collected on three different dates and, because diapause incidence varied widely between tests, data from replicates were not pooled.

Results and Discussion. No significant difference in diapause incidence was found between FAST larvae and SLOW larvae in the first two replicates (Table 8.). In the third test, there was a significantly lower incidence of diapause (X_2=42.04, df=1, $p<0.01$) among SLOW larvae. Development of SLOW larvae was delayed by an average of 2 d in the first two replicates, and by ca. 1 d in the third replicate.

Although physiological stresses due to prolonging larval development by semi-starvation might have acted counter to a trend toward diapause (larvae would not be able to accumulate necessary body reserves), it is not likely that increasing the number of short day cycles experienced by *A. aphidimyza* would increase diapause incidence. Therefore, selection for nondiapause under LD 8:16 is not likely to have resulted in a population of larvae with longer sensitive periods relative to development periods.

TABLE 8. Percent diapause in A. aphidimyza larvae with maximum development rate (FAST) versus larvae with development prolonged by semi-starvation (SLOW).

Replicate	FAST % Diapause	n	SLOW % Diapause	n	Comparison of FAST vs. SLOW[1]	Difference in larval development period (d)
1	15.7	(259)	24.6	(66)	NS[2]	2
2	6.1	(224)	9.5	(144)	NS	2
3	97.5	(368)	83.8	(249)	p 0.01	1

[1] Chi-square test.

[2] Not significant, $p<0.05$.

DIAPAUSE INCIDENCE IN OFFSPRING OF INDIVIDUAL FEMALES

The following experiment determined diapause incidence in offspring of individual females (mated to one male) and whether it was affected by maternal age.

Materials and Methods. Last instar larvae from FIN(ND) and FIN control lines were placed individually in vials (4 ml) for pupation. Upon emergence, they were sexed and 1 male and 1 female were released in each "globe" cage. (Figure 9.). All cages were kept in LD 8:16 (21°C). Because no additional males were released after the original male died, some later eggs were infertile.

Each day, until females died, oviposition leaves were removed and replaced with fresh leaves. After eggs were counted, each leaf was placed in a clear plastic vial (4 cm diam x 6 cm) and covered with Nitex nylon screen (64 micron). The screen was held on with a tight plastic cap that had a ventilation hole (2 cm diam) drilled in it. Larvae could not escape through the fine screen, therefore, it was possible to determine the fate of nearly every egg.

Unhatched eggs were counted on the third day after oviposition. Thereafter, vials were checked each day and aphids added when necessary. When larvae were ready to spin cocoons, they were collected in the usual sample vials containing moist cotton rolls. Data was recorded as for other samples (pg. 73).

In a preliminary test, only eggs laid every second day were reared from nine pairs of FIN control adults. In the later experiment all offspring from 11 pairs of FIN control and 6 pairs of FIN(ND) midges were reared to adulthood.

Results and Discussion. There was a wide range in diapause incidence among offspring from the FIN control line (2-94%) (Table 9., Figure 13.). There was less of a range in the FIN(ND) line (0-32%), which is consistent with selection for nondiapause. Because of the small number of pairs, results are not conclusive for FIN(ND). Most adults for this experiment were taken from the FIN control line at the same time the sample for comparison with F_{49} of the FIN(ND) line was taken, which had 36% diapause. Because this is similar to the mean diapause incidence of 28% for test offspring, it is likely that data from individual pairs accurately reflect proportions of diapause in offspring within the cage.

There was no evidence that level of diapause in offspring of FIN control pairs changed with maternal age. There was also no significant difference (ANOVA) in diapause incidence between offspring of female-producing females (34%) and male-producing females (27%), and no significant difference (ANOVA) in number of eggs or surviving larvae between both types of females.

It is surprising to find such a wide variation in diapause incidence among offspring from FIN control pairs. Perhaps, during the long period (>14 yr at the time of this experiment) of continuous laboratory rearing without natural selection, which would remove nondiapause individuals, random genetic drift resulted in a higher frequency of nondiapause genes. Inadvertent selection for nondiapause may also have occurred and a possible mechanism for this is discussed on pg. 133.

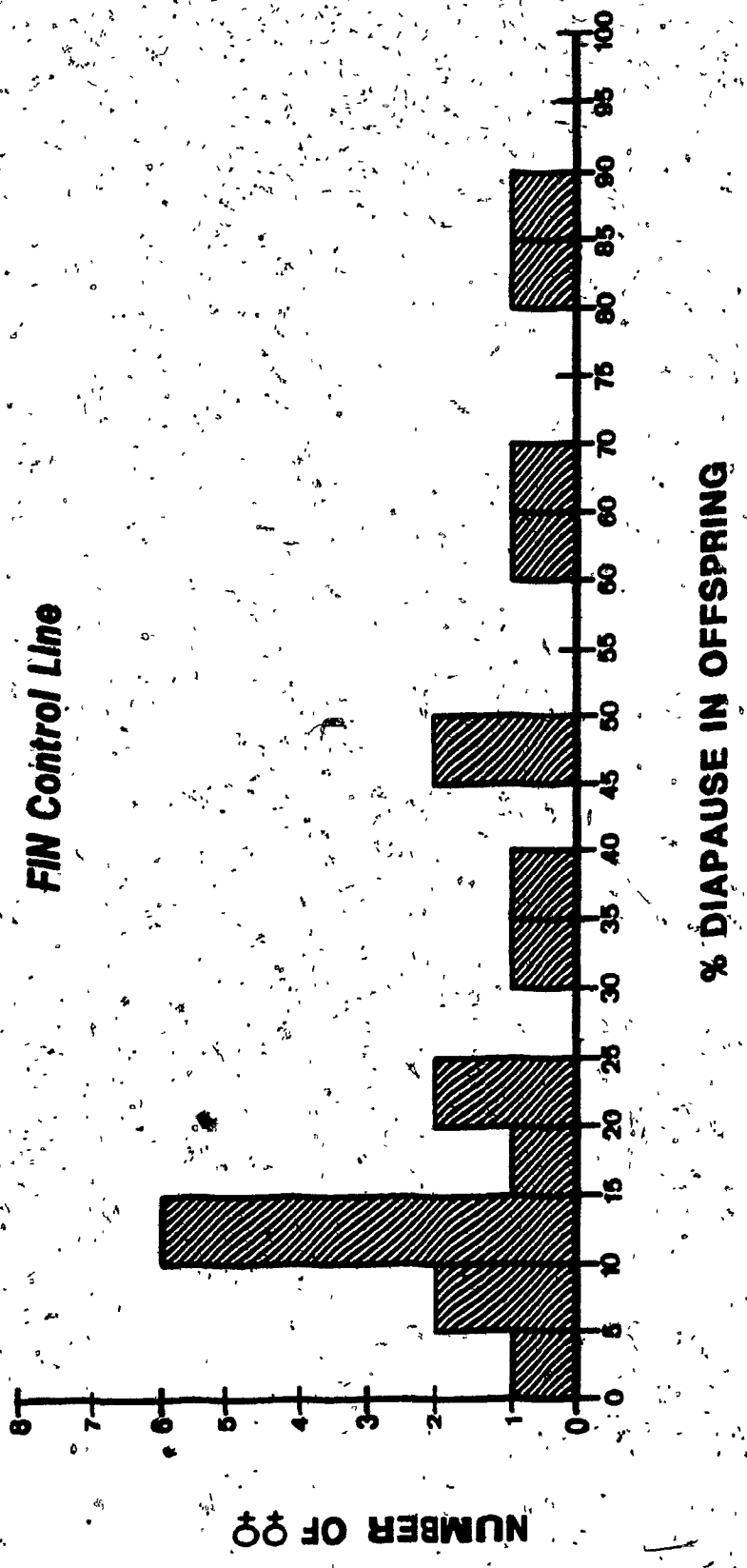
TABLE 9. Percent diapause in offspring of individual pairs of A. aphidimyza from FIN lines, reared under LD 8:16 (21°C).

Line	Number of eggs	Number of larvae reared	Sex of offspring	% Diapause
FIN control[1]	84	66	♀	81.5
	41	28	♀	7.1
	55	23	♂	17.1
	62	51	♂	63.0
	50	47	♂	48.0
	48	38	♂	11.1
	94	40	♀	25.0
	51	35	♀	50.0
	94	27	♀	10.3
FIN control[2]	125	97	♀	69.6
	122	83	♂	10.4
	141	108	♀	38.4
	101	37	♀	88.9
	72	64	♀	1.8
	40	21	♀	10.5
	127	96	♂	30.7
	98	74	♀	13.9
	87	49	♂	13.3
	75	59	♀	7.6
	75	52	♂	22.0
FIN(ND)	74	83	♀	0
	81	61	♂	16.1
	127	106	♂	11.5
	134	115	♀	1.0
	15	10	♀	0
	100	64	♂	32.4

[1] Eggs laid every second day reared.

[2] All eggs reared.

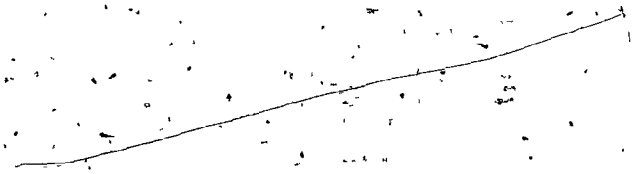
Figure 13. Percent diapause in offspring of pairs of *A. aphidimyza* from FIN control line. Bars represent number of females with offspring whose diapause incidence is in range designated.

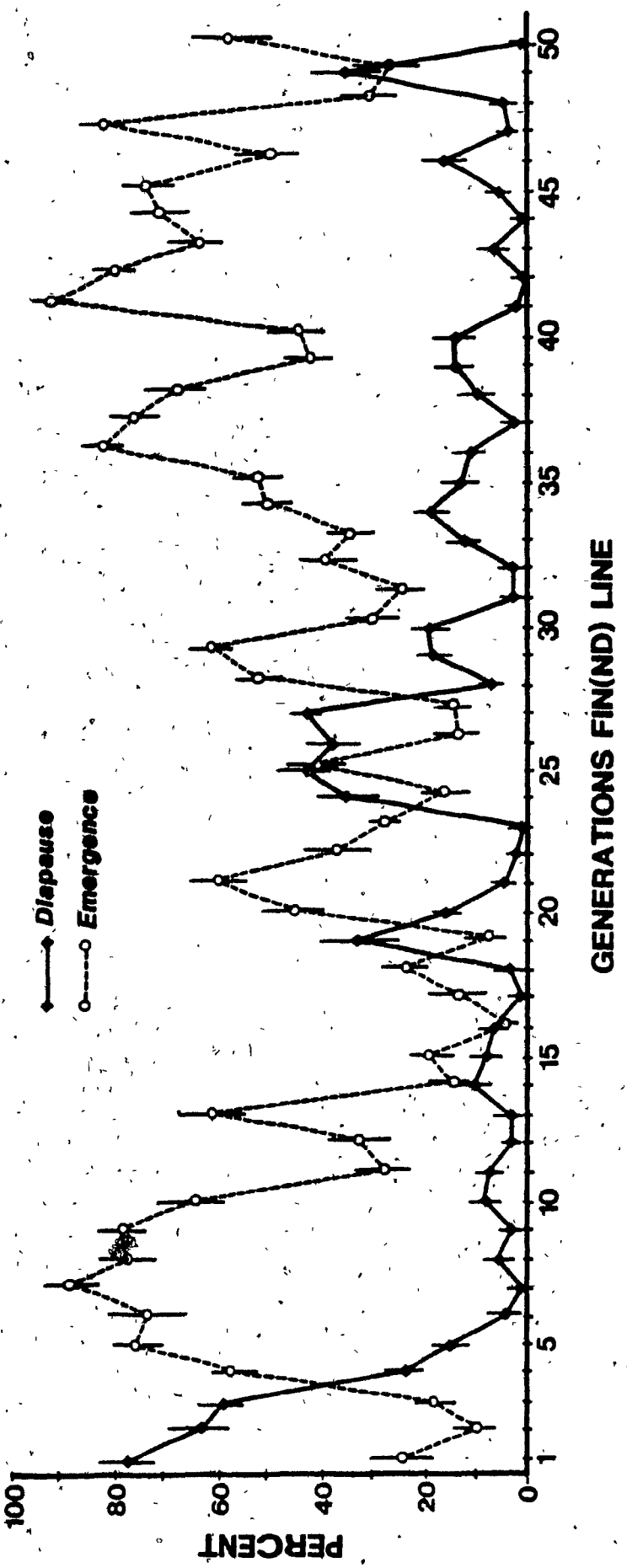

FIN Control Line
NUMBER OF ♀♀
% DIAPAUSE IN OFFSPRING
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

SECTION DISCUSSION

There is evidence that development rate and diapause induction are components of the same genetic system in the flesh fly, *Sarcophaga bullata* (Henrich and Denlinger, 1982), and the pitcher plant mosquito, *Wyeomia smithii* (Istock, et al., 1976). That this may also be the case in *A. aphidimyza* is shown by the inverse correlations between diapause incidence and percentage of adults emerging by day 13 in both FIN(ND) ($r=-0.48$, $p=0.0004$) (Figure 14.) and ARB2(ND) ($r=-0.54$, $p=0.048$) lines (Table 4). Diapause oriented larvae had a longer development period than pupating larvae, but artificially prolonging the larval stage did not increase diapause, which suggests a genetic basis for the relationship between development rate and diapause. There was no evidence of such a correlation in either FIN or ARB2 control lines or relaxed selection lines, and there were too few generations of both QUE(ND) and FIN2(ND) lines for data to be meaningful. The lack of significant correlation between diapause and pupal duration when there was no selection pressure, as in both control and relaxed selection lines, may indicate that linkage between gene loci affecting diapause and those governing development rate occurred under selection pressure. Linkage can be a cause of transient correlations as one combination of alleles is favored over another. When selection is relaxed, the correlation would be expected to decrease (Falconer, 1981), as it did. An alternative explanation that also fits the data, is that faster development rate is a pleiotropic effect of nondiapause genes. The clear response to

Figure 14. Inverse correlation between percent diapause and percent emerged by day 13 from larval-pupal ecdysis in FIN(ND) line of *A. aphidimyza*. Vertical lines represent exact 95% binomial confidence limits.




Diapause
Emergence
PERCENT
0
20
40
60
80
100
1
5
10
15
20
25
30
35
40
45
50
GENERATIONS FIN(ND) LINE

nondiapause selection in FIN and QUE lines in the first months of the project might be associated with the initial bias towards obtaining eggs from early emerging adults. Lack of response to selection in the ARB2 line, then, could be because later rearing methods were intended to prevent this bias. A better response to nondiapause selection might have been obtained by selecting simultaneously for early emergence (and possibly, faster larval development) and nondiapause.

Although obscured by fluctuations from generation to generation, there was a trend toward decreasing diapause incidence in the FIN and ARB control lines. Random genetic drift was ruled out because data from relaxed selection lines showed a slow increase in diapause incidence once selection pressure was relaxed. Therefore, it is likely that directional selection, toward nondiapause, did occur. During the first 16 mo of research, in five of ten samples of FIN control larvae, reared under LD 17:7 (21-28°C), a few larvae diapaused ($\bar{x}$=0.7±0.3%), or were slow in pupating ($\bar{x}$=1.2±0.7%), even under long day conditions. The steady removal of these few individuals with a high liability to diapause, over several years, may be sufficient selection pressure to account for the reduction in diapause. In addition, because control lines were maintained with adults emerging continuously to provide eggs daily for other experiments, larvae that developed fastest would have more descendants over several years than those with slower development. If genes for late development are associated with those for diapause (or early development is associated

with nondiapause), then a drift toward more nondiapause individuals would also occur (Barry and Adkisson, 1966; Glass, 1970; King, 1974). Regardless of how it occurred, offspring of individual pairs from the FIN control line, after 30 mo of rearing, showed the presence of a substantial number of individuals with low liability to diapause under LD 8:16 (21°C), which, presumably, was not the case earlier in the research when 80% diapause was obtained in control lines.

FLUCTUATIONS IN DIAPAUSE INCIDENCE

After five cycles of FIN(ND) were reared, diapause in the FIN control line was checked under LD 8:16 (21°C) and was found to have dropped to 50%. Further checks of diapause incidence in the FIN control line made during the next six months indicated that diapause incidence was fluctuating in a pattern similar to that of the nondiapause line. Because it was nearly a year after the original line was received, another shipment of *A. aphidimyza* pupae was obtained from the original source to see if diapause incidence had changed in that line as well. Diapause incidence in the new line under LD 8:16 (21°C) was checked twice, coinciding with F_{16} and F_{16} of FIN(ND). Percentage of diapause (17% and 27% respectively) was intermediate between that of the original FIN control and FIN(ND) lines, therefore, it was concluded that whatever the cause, changes in diapause were not unique to my cultures, although it did not rule out environmental conditions unique to my research, or the possibility of seasonal effects. Thereafter, frequent checks of diapause incidence in the control line were made, aphid supply permitting. Beginning with F_{21} of FIN(ND), eggs were taken from the FIN control cage on the same day eggs were laid for the next FIN(ND) generation (Figure 4.).

Starting with F_{21} of FIN(ND), there was a highly significant degree of correlation (PPMC, $r=0.6924$, $p=0.0001$) between the fluctuations in diapause incidence between generations of FIN control and FIN(ND) lines (Figure 5.). Correlations between other lines were checked, using the

closest generations in time for comparison (Table 10). There were significant correlations in diapause incidence between FIN control and both ARB2 control and ARB2(ND) lines as well as between FIN(ND) and QUE(ND) lines. While the latter correlation is probably mostly due to a similar decrease in diapause under selection pressure for the first few generations, the correlations between other lines are inexplicable.

The "damped" fluctuations in nondiapause lines, compared to the wide range (8-90%) in control lines, were probably a result of selection pressure. Nevertheless, whatever the cause of variation, it was sufficient to exert an influence on diapause levels in the population despite continuous nondiapause selection. The lack of correlation between FIN(ND) and both ARB2 lines may have been because the effects of nondiapause selection were superimposed on the overall pattern of fluctuation. Indeed, the question is not why these examples are not correlated, but rather, why the other lines are correlated, particularly when they originated from completely different geographical areas, with different laboratory histories.

Puzzling fluctuations in diapause incidence, occurring at the same time in both nondiapause selected and control lines of *Heliothis zea*, were attributed to diet or unknown environmental changes (Herzog and Phillips, 1974). Both possibilities were investigated in my research. Variations in the large incubator environment, where all nondiapause lines and check plants from control lines were reared, were unlikely because light controls, humidifier and

TABLE 10. Correlations in diapause incidence between lines of A. aphidimyza, comparing generations closest in time.

	FIN(ND)		QUE(ND)		QUE2(ND)		ARB2 control		ARB2(ND)	
	r[1]	p	r	p	r	p	r	p	r	p
FIN control	0.6924	0.0001			-		0.6539	0.0211	0.7757	0.0030
FIN(ND)			0.9212	0.0011	NS[2]		NS		NS	
QUE(ND)					-		-		-	
QUE 2(ND)							-		-	
ARB2 control									NS	

[1] Pearson's product moment correlation coefficient.

[2] Not significant, $p<0.05$.

thermometers were checked daily and no major adjustments or repairs had been necessary. To check this, temperature and relative humidity were recorded continuously for nine months on a strip chart hygrothermograph. No variations were found.

When it was discovered that exceedingly low radiation levels could prevent diapause in some *A. aphidimyza* larvae (pg. 169), the incubator was checked for light leaks. Only a barely discernible light leak around the view window cover was found. It was sealed with black tape, but this had no effect on subsequent generations.

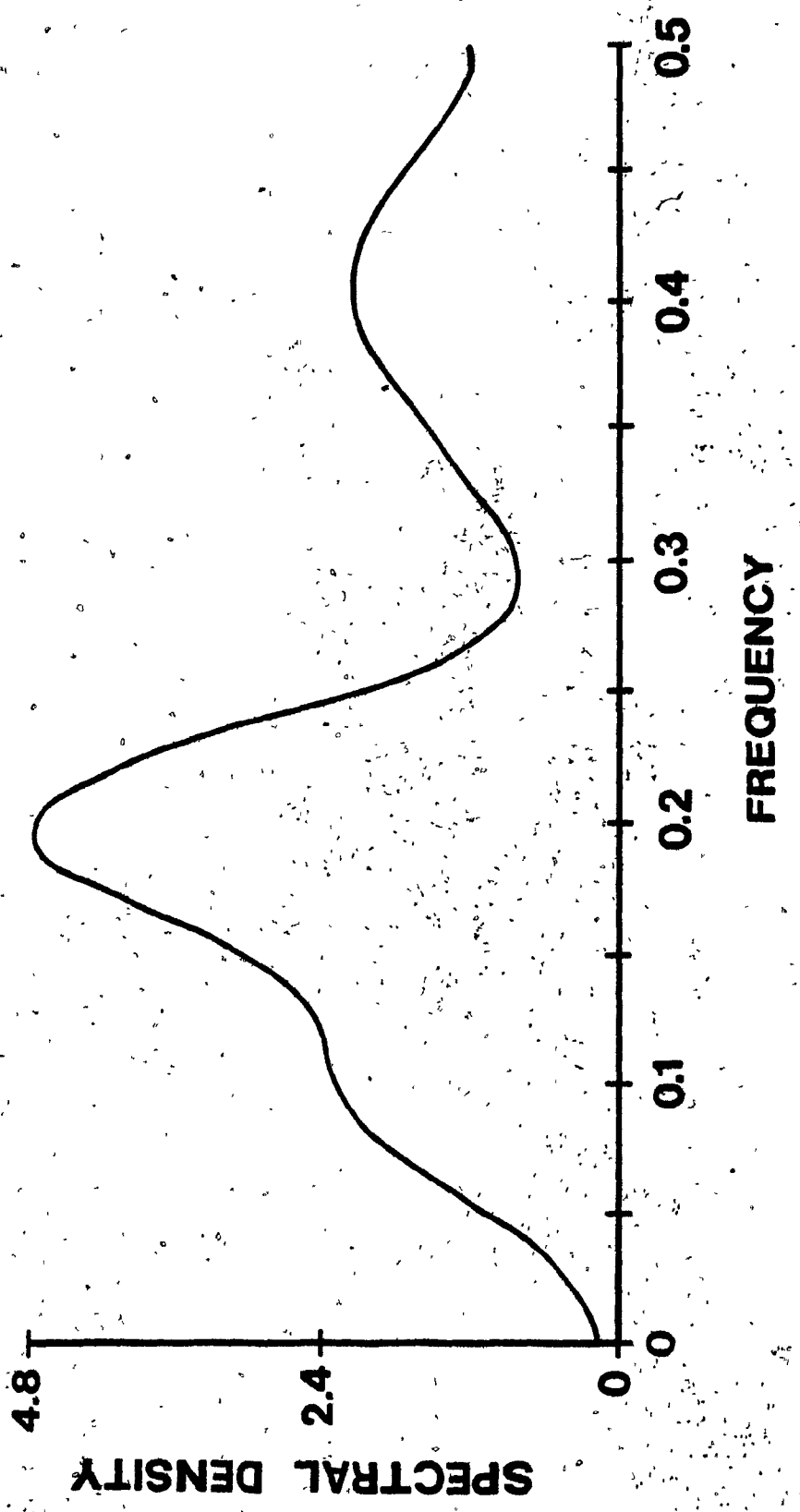
It is unlikely that larvae from different lines were mixed, since, throughout the research, care was taken to insure that larvae from one plant did not wander to another plant in search of food. Oviposition plants from the same line were kept together; they were separated from plants from other lines, which were either on a different shelf in the incubator or, if on the lower level, kept >1 m apart. To prevent larvae from wandering in search of prey, fresh host plants were provided before larvae had consumed all aphids present on leaves. On the last night of larval development, plants were cut and kept in plastic bags to prevent larvae from escaping. In addition, only FIN control and FIN(ND) lines were sampled at exactly the same time (and only after F_{21}) and rearing of larvae from other lines rarely coincided with FIN generations.

The possiblity of an endogenous cycle of diapause liability, which has been found in certain other insects and mites (Beck, 1980; Sapozhnikova, 1982), or a seasonal cycle, was investigated by using time series analysis (Legendre and

Legendre, 1983) to find out whether there was a statistically significant period to the cycles (Figure 15.). Data from generations 7-45 of the FIN(ND) line were analysed since it was the only line with sufficient data points. First a slight trend in the data was removed by fitting a quadratic equation and examining residual values. Time series analysis was performed on these values. While not statistically significant ($p=0.1796$), results suggest that cycles in diapause incidence, with a period of five generations, do exist. It is not likely, however, that these cycles are endogenous because, under natural conditions, *A. aphidimyza* has 3-4 generations per season in southern Quebec (Bouchard, et al., 1981) and probably fewer in Finland. Thus, a five generation cycle is not synchronized with seasonal rhythms and does not account for the synchrony in diapause incidence between lines that occurs regardless of generation. For example, because larvae developed slightly faster under long days than under short day at 21°C, FIN control generations were not synchronized with FIN(ND) generations.

With obvious sources of environmental influence on larvae ruled out, investigations were made into the effects of diet on larvae caused by changes in aphid host plant quality.

Figure 15. Time series analysis of diapause incidence in generations of the FIN(ND) line of *A. aphidimyza*. Peak frequency at five generations (1/20); width of spectral window, m=13; band width, b=0.1026.


SPECTRAL DENSITY
4.8
2.4
0
0
0.1
0.2
0.3
0.4
0.5
FREQUENCY

DIET AND DIAPAUSE

This study was conducted to determine whether fluctuations in diapause incidence common to all lines could be accounted for by differences in diet (i.e., differences in aphid host plants affecting aphid quality, in turn affecting *A. aphidimyza*).

Experiment 1:

Materials and Methods. Pepper plants were grown in two distinctly different environments. One group, SUM, was grown in the greenhouse during June and July, under warm (25-30°C day) summer conditions and full sunlight. The other group, WIN, was kept in an incubator simulating winter photoperiod (LD 9:15) and temperatures (TC 21:15°C), with thermophase two hours shorter than photophase, and full spectrum fluorescent lighting. To compensate for slower growth, WIN plants were started six weeks earlier than SUM plants. Soil from the same mix for WIN plants was saved and used for SUM plants. All plants were put in the same aphid incubator for colonization.

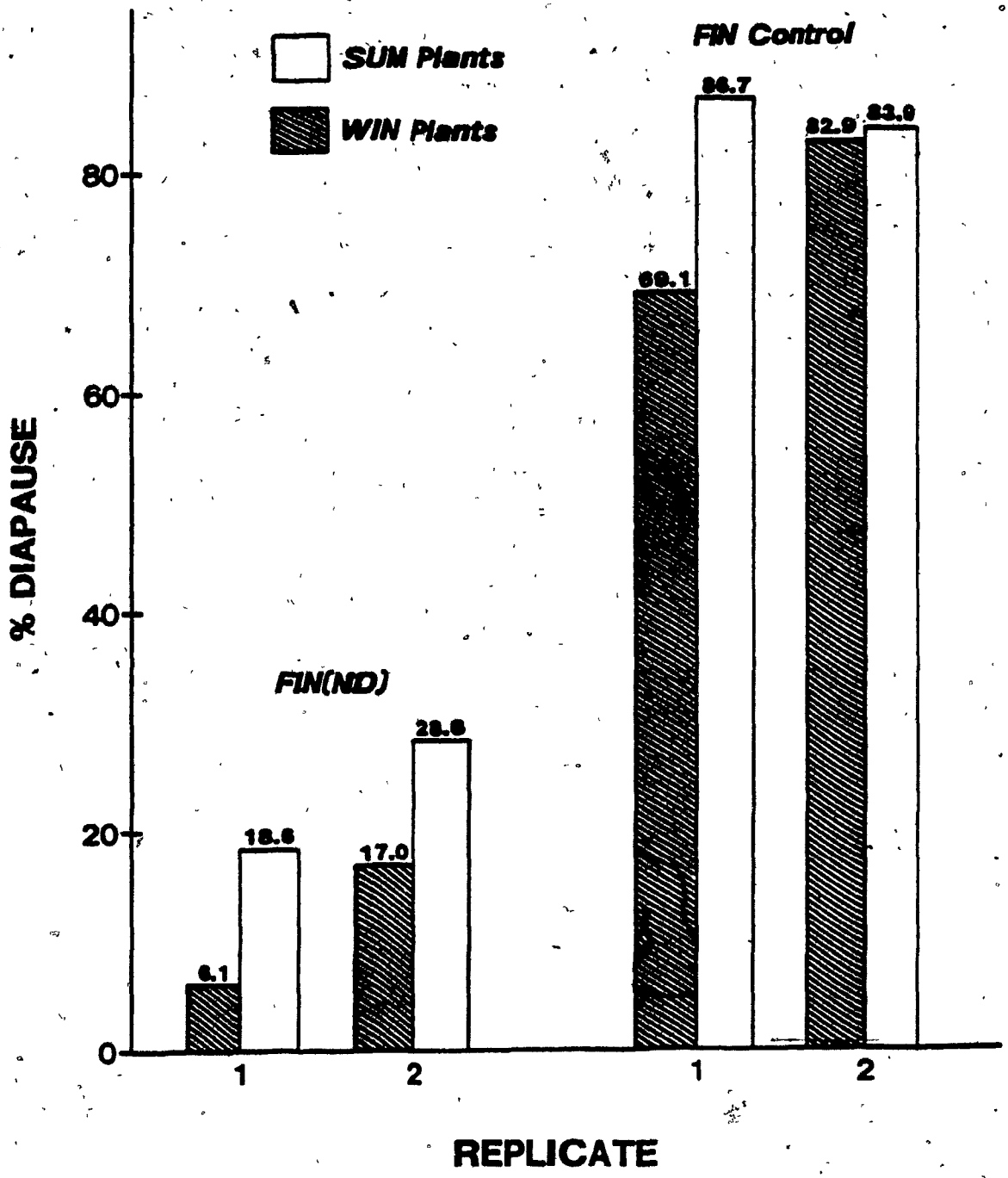
Pairs of plants (1 SUM, 1 WIN) were placed overnight for oviposition in midge cages. A second pair was placed in each cage the next night. Eggs were collected from both FIN(ND) and FIN control lines and all larvae were reared under LD 8:16 (21°C). Extra plants of both types were kept in aphid colonies until needed to supply additional prey for larvae. Larvae were reared and placed in vials according to methods described on pg. 73.

Results and Discussion. In both replicates from the FIN(ND) line, diapause incidence was significantly higher (X^2-tests, $p<0.005$) in larvae fed aphids from SUM plants (Figure 16.). In the FIN control line, diapause incidence was significantly higher ($X^2=34.987$, 1 df, $p<0.005$) in replicate 1, but not in replicate 2. Because diapause incidence in replicate 2 was near the maximum recorded for FIN control larvae under LD 8:16, it is possible that the maximum number of individuals inclined to diapause were already doing so and that changes in host plant quality could have no additional effect. These results led to a more detailed experiment to test effects of host plant quality.

Experiment 2:

Materials and Methods. For this experiment plants were reared under two different incubator conditions, which had been observed to produce different foliage and growth habits. SOFT plants (similar in appearance to SUM plants) were kept under full spectrum fluorescent lights at 24-28°C. HARD plants, which had darker, tougher foliage, and slightly smaller leaves, were grown under cool white fluorescent lights, at 21°C. Both incubators had photoperiods of LD 17:7. These two treatments were chosen because they were both used to hasten production of plants for aphid cultures during most of the fall to spring period. Both types of plants were color coded when they were put into aphid cultures, thus matching plants of each type could be paired when put in midge cages for oviposition.

Figure 16. Effect of aphid host plant quality on diapause in *A. aphidimyza*; first experiment. WIN plants grown under LD 9:15, TC 21:15°C; SUM plants grown in greenhouse June-July.


SUM Plants
WIN Plants
FIN Control
FIN(ND)
% DIAPAUSE
0
20
40
60
80
6.1
18.6
17.0
28.6
69.1
86.7
82.9
83.9
1
2
1
2
REPLICATE

Three replicates of paired plants (1 SOFT, 1 HARD), were placed in the FIN control cage for oviposition on three consecutive nights. Plants were all placed in LD 8:16 (21°C) for rearing larvae. Between 250 and 400 larvae were reared from each plant.

Eight plants of each type were cut and dried for tissue analysis of N, P, K, Fe, Ca, Mg and percent moisture. Extracts of plant tissue were analysed using an atomic absorption spectrophotometer (Perkin-Elmer 2380, Perkin-Elmer Corp., Norwalk, Conn.).

Results and Discussion. Differences in host plant quality were found to be related to differences in diapause incidence in *A. aphidimyza* larvae reared on aphids from those plants. In all three replicates, diapause incidence was significantly higher (X^2-tests, $p<0.005$) in larvae reared on aphids from SOFT plants than in larvae from HARD plants (Figure 17.). Diapause was 1.4 to 2.2 times higher on SOFT plants. Results of replicates were not pooled because they were significantly different (X^2-test, $p<0.05$).

Percentage of moisture was significantly higher in SOFT plants (ANOVA: $F=50.97$; $df=1,14$; $p=0.0001$), as was percentage of K (ANOVA: $F=23.06$; $df=1,14$; $p=0.0003$). Levels of N were higher in SOFT plants, but not significantly so. There was no difference in other nutrients tested (Table 11.).

Qualitative differences between aphid species have been shown to affect behavior, development and fecundity in aphidophagous insects such as coccinellids (Blackman, 1966) and *A. aphidimyza* (Havelka and Ruzicka, 1984). Weight

Figure 17. Effect of aphid host plant quality on diapause in *A. aphidimyza*; second experiment. HARD plants grown under cool white fluorescent lights, 21°C; SOFT plants grown under full spectrum fluorescent lights, 24-28°C.

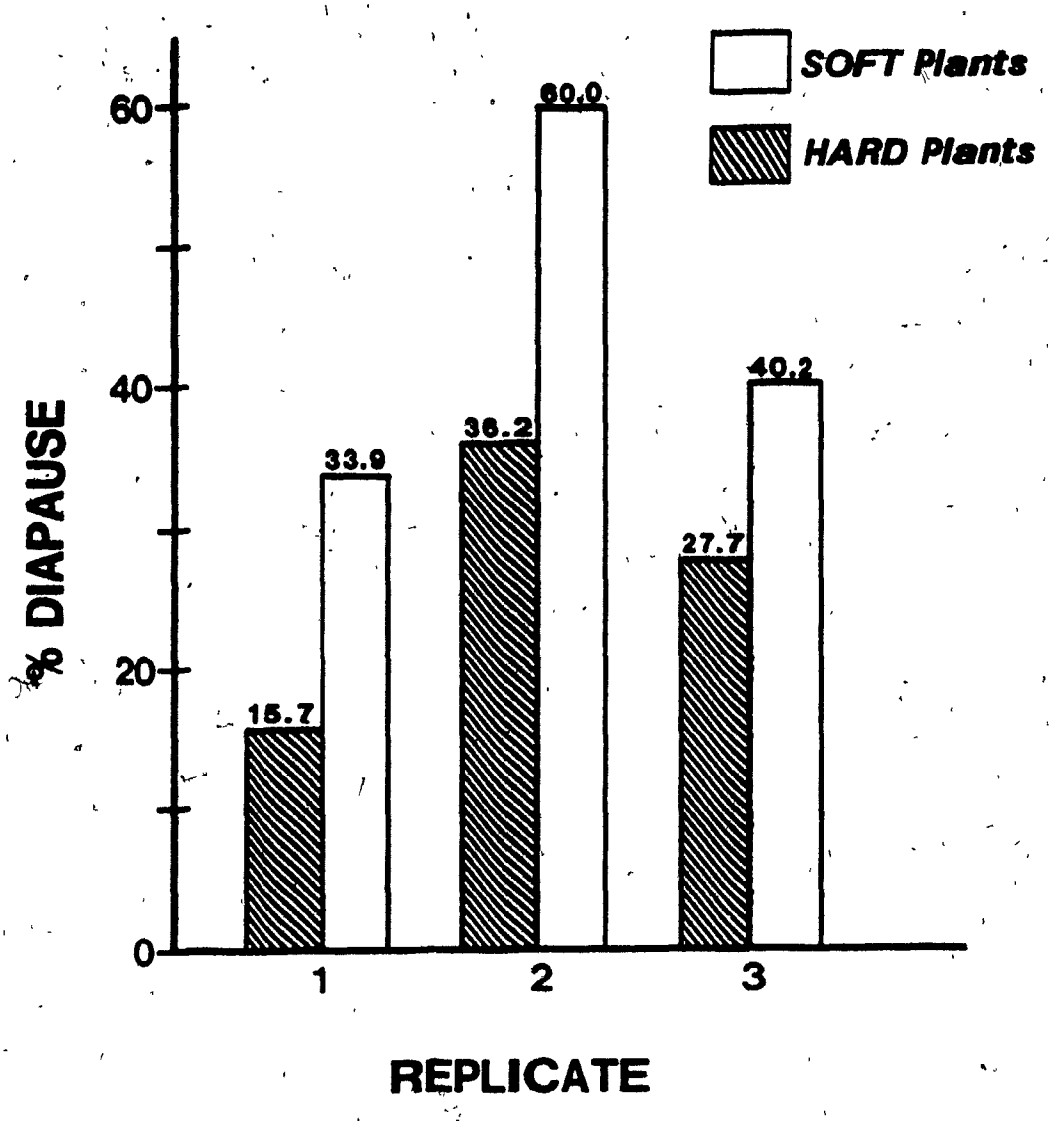

SOFT Plants
HARD Plants
% DIAPAUSE
0
20
40
60
15.7
33.9
36.2
60.0
27.7
40.2
1
2
3
REPLICATE

TABLE 11. Nutrient and moisture content of 8-week-old pepper plants grown in two different environments. Figures for nutrients expressed as percent of plant dry weight.

Nutrient	Plant type SOFT[2] $\bar{x} \pm$ SE	HARD[3] $\bar{x} \pm$ SE
N	7.59 ± 0.11	8.58 ± 0.53
P	0.32 ± 0.03	0.38 ± 0.05
K	4.77 ± 0.09	6.16 ± 0.08
Fe	0.30 ± 0.02	0.05 ± 0.01
Ca	1.68 ± 0.20	1.65 ± 0.15
Mg	1.37 ± 0.14	1.46 ± 0.12
% H_2O	85.70 ± 0.23	88.88 ± 0.46

[1] Calculated by $\frac{(\text{ppm} * 250\ \text{ml} * 1 \times 10^{-6}\text{g/ppm})}{\text{g of dry plant}} \times 100 = \%$ of plant dry weight.

[2] Grown under LD 17:7 (24-28°C) full spectrum fluorescent lights.

[3] Grown under LD 17:7 (21°C) cool white fluorescent lights.

and fecundity of *A. aphidimyza* are even affected by differences in host plants used for the same species of aphid, and it is possible that handling of plants (e.g., fertilizing and illumination) indirecty affects *A. aphidimyza* (Kuo, 1976/77). Although no previous studies examined effects of diet on diapause in *A. aphidimyza*, dietary effects on diapause (reviewed pg. 37) are well known in herbivorous insects and have also been found in the endoparasite, *Pimpla instigator* (Claret and Carton, 1975), and a saphrophagous insect, the tropical flesh fly, *Poecilometopa spilogaster* (Wiedemann). In the latter species, diapause incidence increased by 10% when moisture in larval diets increased 10% (Denlinger, 1979).

Aphid host plant quality could affect diapause response in *A. aphidimyza* directly through nutritional differences in the prey, or indirectly through changes in feeding behavior. Kuo (1982) demonstrated that changes in artificial diets of *M. persicae* significantly affected developmental time, larval and imaginal weights and fecundity of *A. aphidimyza*. Larvae compensated for reduced food quality by consuming up to twice as many aphids. On vitamin and amino-acid deficient diets this was adequate to sustain normal development and fecundity, however, in sucrose and some mineral deficient diets, larval development, larval and adult weight and fecundity were significantly affected (Kuo, 1975). On poor diets larvae would be unlikely to accumulate necessary body reserves to diapause and survive. Kuo (1975) also noted that the longer larval development observed in some treatments was related

to changes in feeding behavior. On amino-acid deficient diets aphids were more active, and harder for larvae to catch. Larvae attacked but did not consume more aphids on most deficient diets and left more aphids partly consumed. Because changes in both physiology and behavior of *A. aphidimyza* larvae have been demonstrated with variations in diet of host aphids (Kuo, 1975), it is likely that diapause could be affected as well. The higher diapause incidence on SOFT or SUM plants could have been because larvae were better nourished with enough body reserves for diapause.

Aphids feed by extracting amino-acids and some sugars from the phloem sap (Dixon, 1985). Despite the higher moisture in SOFT plants, which could indicate lower sucrose concentrations in phloem sap, (van Emden, et al., 1969), *M. persicae* reproduced faster on SOFT plants than on those under cool white fluorescent lights (HARD). This is an indicator of a good diet, which may be because translocation speed is greater in leaves in full light (Troughten, et al., 1977). The phloem in younger, expanding leaves and in older, senescing leaves, is richer in translocation materials, since they are the sites of synthesis and breakdown, respectively, of plant metabolites. The preference of *M. persicae* for such leaves is thought to be due to differences in soluble nitrogen levels, although secondary plant substances (gibberellins, abscisic acid, cytokinins, etc.) may also be involved (van Emden, et al., 1969). Levels of soluble nitrogen, osmotic pressure of sap, carbohydrate levels and pH are related in plants, and it is very

difficult to tell what is affecting aphids. Tissue analysis showed somewhat higher N in SOFT plants, which indicates that plants were more nutritious for aphids (Ziegler, 1975; Dixon, 1985). If aphids were more nutritious for *A. aphidimyza* larvae, then larvae would be able to accumulate necessary fat body reserves of glycogen and other compounds involved in cold hardiness and necessary for the physiological changes that occur in preparation for, and during, diapause (Wyatt, 1967).

Being fed upon by aphids affects a plant's physiology, usually by enhancing translocation to aphid feeding sites (van Emden, et al., 1969). Thus, control plants used in tissue analysis were not true indicators of the state of plants that had been infested with aphids for 6-8 d. Plants can respond within as few as three days to aphid infestations, with drastic reduction in sap flow (Forrest, et al., 1973). Therefore, although an explanation is proposed for the effect of host plants on diapause of *A. aphidimyza* larvae, much more work is necessary to elucidate this relationship.

SECTION DISCUSSION

Under short day (LD 8:16) conditions combined with relatively high constant temperature (21°C), *A. aphidimyza* larvae probably receive conflicting signals. In these unnatural conditions, diet could modify the effect of the other environmental cues. In the fall, back translocation from senescing leaves temporarily provides feeding aphids with more nutritious diets than experienced in midsummer. Possibly the presence of a nutritious food supply, which would ensure a better chance of survival and higher fecundity after diapause, acts, along with short days, to induce a higher incidence of diapause. A poor food supply, with the attendant risk of poor survival and reduced fecundity after diapause, might cause larvae to "overlook" the effect of short days when they occurred in conjunction with warm temperatures. Outdoors, photoperiod and temperature signals usually do not conflict (e.g., nights would be long and cool, or short and warm, but rarely short and cool). In cases of such mixed signals, the opportunity to accumulate sufficient body reserves might induce a larger proportion of the population to respond.

An alternative conjecture is that the diapause response of the *A. aphidimyza* larvae is not related to the nutritional value of aphids, but is related, instead, to the presence of secondary substances, such as gibberelins, which are generally in lower concentrations in plants under short days (Ziegler, 1975), or alkaloids, which can be absorbed by phytophagous insects from plants. For example, when the tomato plant alkaloid, tomentine, was ingested by

Heliothis zea, it was found to be toxic to the parasitoid, *Hyposoter exiguae* (Viereck), reducing size, development rate and longevity of adults (Campbell and Duffey, 1979).

That plant quality had a discernable effect on diapause under threshold photoperiod and temperature conditions was established. It was not established, although it is possible, that changes in host plant quality account entirely for the variation in diapause incidence. Plants used in the above experiments were uniform with respect to variety, soil mixture and duration of aphid infestation, yet a detectable difference in diapause was produced by different light regimes. It is possible, therefore, that differences in host plants might account for the environmental component of the variation in diapause from generation to generation. Over three years, host plants were more variable than those produced in these two experiments, being reared under a wide range of conditions, depending on time of year, availability of incubator space, and number of plants required. Photoperiod, light quality and intensity, thermoperiod and temperatures, soil mixture and fertilization, and watering, were not uniform thoughout the research. For a brief period (20-26 December, 1983) larvae from FIN and ARB2 lines were reared on aphids on brussels sprouts rather than peppers. Although aphids were reared in the same incubators, with the same photoperiod and thermoperiod throughout the research, the size and number of aphids per plant, and the length of aphid infestation, depended on whether aphid production was adequate for

experimental needs at that time. During the first 15 mo, recurring shortages of aphids caused problems, although aphid production was more stable after that. Since all larvae reared at the same time, regardless of origin, were fed aphids reared on plants with similar histories, host plant differences could be the main external, environmental factor causing correlations in diapause incidence between unrelated lines. Densities of aphids, or larvae, may conceivably have had an affect on diapause incidence in *A. aphidimyza* larvae, however, they are unlikely to be the same from plant to plant and line to line simultaneously. This is because differences in density of larvae depend on the number of eggs laid (number of females present) at the time oviposition plants were placed in cages, and this varied with the line but not with time.

To investigate this further, variables in plant production must be precisely controlled. The use of pre-mixes to assure consistent fertilization, and capillary matting, or drip irrigation, to ensure the same osmotic pressure in host sap would be necessary, as well as uniform light quality, light intensity, photoperiod and temperatures. Infestation with aphids for exactly the same length of time would also be required. Analysis of amino-acid and sugar concentrations in plants should be included in tissue analysis and it would be advisable to analyze nutrient content of plants that have been fed upon by aphids for the same period of time.

NONDIAPAUSE SELECTION UNDER FLUCTUATING THERMOPERIODS

Once a FIN line with low incidence of diapause was selected under LD 8:16 (21°C), the same line was subjected to selection under typical fluctuating temperatures experienced in greenhouses during winter. Most plant species, except for tropical foliage plants, need diurnal fluctuations for optimum growth; night temperatures are set 5°C lower than day temperatures for most crops (Hanan, et al., 1978).

Materials and Methods. Preliminary tests under LD 8:16 with TC 25:10°C and TC 23:16°C resulted in 99-100% diapause in all lines tested (QUE2(ND), FIN(ND), FIN2(ND), and FIN control). Consequently, it was decided to reduce the intensity of selection by starting with TC 24:18°C to obtain enough adults to continue the line. Ca. 700 eggs from F_{21} of FIN(ND) were collected on three oviposition plants to begin selection, which continued for eight generations.

A second experiment, starting with ca. 1000 eggs from F_{36} of FIN(ND), was conducted under a regime of LD 9:15 and TC 21:18°C. The thermophase was two hours shorter than the photophase to simulate thermoperiods recorded in the greenhouse.

Results and Discussion. No progress was made in selecting for diapause under LD 8:16 and TC 24:18°C (Table 12). Instead, diapause incidence tended to increase with succeeding generations. Results for two generations are excluded because, in F_3, the incubator thermostat malfunctioned and temperatures increased to 25-30°C,

TABLE 12. Percent diapause in A. aphidimyza larvae from the FIN(ND) line, reared under LD 8:16 and TC 24:18°C.

Generation	n	% Diapause	% Late pupation[1] + % diapause
1	405	14.2	89.9
2	351	38.6	86.3
3	346	1.0[2]	1.0[2]
4	286	7.7[3]	22.4[3]
5	297	54.5	79.3
6	200	54.5	79.3
7	204	65.4	81.9
8	16	75.2	83.3

[1] Percent of individuals pupating after 21 d in vials.

[2] Incubator thermostat malfunctioned, temperature 25-30°C.

[3] Incubator lights malfunctioned, on all night during pupation.

whereas in F_4, malfunctioning lights remained on at night during the pupal period, which terminated diapause (pg. 178). Percentage of late pupation (possibly larvae with a very short diapause) decreased with time, however, the combined total incidence of diapause and late pupation did not change (80-90%). There were insufficient eggs from F_8 to continue rearing this line.

In the second test, under LD 9:15 and TC $\overline{21:18}$°C, there was 99.7% diapause in the first generation and 100% diapause in the few larvae produced in the second generation.

Clearly, the addition of even a weakly fluctuating thermoperiod to short daylength was sufficient to induce diapause among most individuals from the FIN(ND) line, and it was not possible to use mass-selection to eliminate this response. Because night temperatures were the same in both tests, the higher daytime temperatures in the first temperature regime were probably enough to prevent diapause in a proportion of the population. For ca. 10-20% of individuals it may have provided enough developmental degrees above a thermal threshold to entirely prevent diapause. Results of another experiment (pg. 125) ruled out the possiblility that slower development rates under cooler thermoperiod regimes would result in higher diapause incidence because larvae were exposed to more short day cycles. It is more likely that, under environmental conditions tested, thermoperiod may be more important than photoperiod as a seasonal signal, at least in these geographical races of *A. aphidimyza*. It is possible that

the thermoperiodic response is genetically linked to developmental or behavioral characteristics not open to selection by these methods. If alleles for nondiapause under these conditions exist at all, they are rare and probably lost in heterozygous combinations; selection for such rare recessive genes is unlikely to suceed (Falconer, 1981). In the case of *A. aphidimyza*, sufficient adults must remain after selection to provide a balanced ratio of male-producing to female-producing females, and this was not possible.

It has been observed that thermoperiods may substitute for photoperiods in regulating activity patterns in far northern races of mosquitoes (Corbett, 1966), and that thermoperiod is possibly more important to northern races than to southern races of the same species. Thus, geographical races of *A. aphidimyza* from southern areas might be better candidates for diapause selection under fluctuating thermoperiods than the races studied, which were all from northern temperate regions. Unfortunately, efforts to obtain southern lines of *A. aphidimyza* for this research were unsuccessful (pg. 64).

GREENHOUSE TESTS OF NONDIAPAUSE LINES

After one year (20 generations) of nondiapause selection, the FIN(ND) line was tested in the greenhouse during winter to determine diapause incidence.

Materials and Methods. Ca. 700 eggs, on three oviposition plants from F_{20} of FIN(ND), were moved to greenhouse benches in late September, 1983. At the same time, two oviposition plants were moved to the greenhouse from F_6 of the FIN(ND) line re-selected under LD 8:16 and TC 24:18°C. Eggs on single plants were also taken from the FIN control line and reared in the greenhouse at 2-3 week intervals. Single oviposition plants from each greenhouse reared generation of FIN(ND) lines were reared in the large incubator under LD 8:16 (21°C) to find out how lines that had been selected for nondiapause under real greenhouse conditions responded in the incubator.

Daylengths, from sunrise to sunset, during larval development were ca. 11 h 45 m. Minimum night temperature was set at 15°C and maximum day temperature was 21°C. During larval development, plants were not kept in cages because daytime temperatures inside cages were up to 5°C higher than ambient air temperatures.

Larvae were placed on moist peat in foil trays to pupate and, after 10 d on greenhouse benches, trays were transferred to mesh covered sleeve cages (28 x 28 x 28 cm) for emergence. Samples of 200-300 larvae from each generation were collected in vials, which were kept in the shade on a shelf beneath one bench. Adults emerging from trays became parents for the next generation. Four

generations from each FIN(ND) line were reared, with the last generation being reared in early January, under daylengths of ca. 8 h. 45 m. All larvae from individual oviposition plants used to check results under LD 8:16 were collected in vials.

To check results of greenhouse rearing, larvae from FIN(ND) were reared in an incubator with temperature and photoperiod simulating greenhouse conditions. Data on greenhouse temperatures and thermoperiod patterns were obtained from hygrothermograph recordings taken continuously from late September to late December. The incubator was set at LD 9:15 and TC 21:15°C, with the warm phase beginning one hour after lights came on and ending one hour before dark. Ca. 600 eggs from FIN(ND), on two oviposition plants, were placed in this incubator on 29 December, and all larvae were reared and collected in sample vials.

Results and Discussion. Diapause incidence in both FIN(ND) lines and the FIN control larvae reared in the greenhouse averaged <4% for all generations (Table 13.). This seemed to show that nondiapause selected *A. aphidimyza* could be used successfully in winter greenhouses, since diapause incidence remained below that of incubator reared FIN(ND) generations under LD 8:16. The low incidence of diapause, however, in the FIN control larvae was surprising, since >50% were expected to diapause. These results were also inconsistent with the high level of diapause found in the FIN(ND) line re-selected in an incubator under fluctuating thermoperiods (Table 12.), in which temperatures were 3°C warmer than those in the

TABLE 13. Percent diapause in nondiapause and control FIN lines of A. aphidimyza under winter greenhouse conditions.

Line		Generation	n	% Diapause	% Late pupation
FIN(ND)[1]	Oct. 3-5/83	1	146	2.2	2.1
	Oct. 27-29	2	350	5.6	13.1
	Nov. 25-28	3	192	0.6	1.1
	Jan. 1-5/84	4	200	1.1	11.0
FIN(ND) RESEL[2]	Oct. 1-4/83	1	223	1.1	0.5
	Oct. 28-29	2	189	13.0	3.0
	Nov. 26-29	3	192	0	0.6
	Jan. 1-5/84	4	189	0.6	20.2
FIN control[3]	Oct. 28-29/83	-	267	1.2	2.8
	Nov. 13-15	-	427	3.4	5.4
	Dec. 7-10	-	262	0.9	5.6
	Dec. 15-16	-	162	2.0	9.5
	Dec. 20-22	-	345	0.3	1.9
	Jan. 9-12/84	-	422	1.3	6.3
	Jan. 17-20	-	214	1.9	2.5

[1] Beginning with eggs from F_{19} FIN(ND), subsequent generations reared in greenhouse.

[2] Beginning with eggs from F_5 of FIN(ND) re-selected under LD 8:16, TC 24:18°C, subsequent generations reared in greenhouse.

[3] Eggs taken from FIN control and reared in greenhouse each time.

greenhouse. In addition, 20-45% of larvae from greenhouse reared FIN(ND) generations diapaused under LD 8:16.

It was because of these anomalous results that FIN(ND) larvae were reared in an incubator with simulated greenhouse conditions. Results were consistent with all previous data obtained in incubators (of 511 larvae, 506 diapaused and 5 died). Because all FIN(ND) larvae exposed to greenhouse-like conditions diapaused, it was likely that an unknown factor in the real greenhouse environment prevented diapause. This is examined in the next section.

SECTION 4.

PREVENTION OF DIAPAUSE IN GREENHOUSE POPULATIONS OF *APHIDOLETES APHIDIMYZA*

The following section describes the investigation of extremely low intensity light at night to prevent diapause in *A. aphidimyza* under winter greenhouse conditions. Practical application of low light levels in commercial greenhouses and experiments on photoperiodic termination of diapause in *A. aphidimyza* are also given.

EFFECT OF LOW INTENSITY LIGHT AT NIGHT

This experiment was designed to test whether continuous illumination from a nearby parking lot at night could be the unknown factor preventing greenhouse reared larvae from diapausing.

Materials and Methods. Three aphid infested plants with eggs from the FIN control lines, laid the same night, were used. One plant was placed in an incubator simulating greenhouse conditions (LD 9:15 and TC 21:15°C, with thermophase two hours shorter than photophase). Two plants were placed in the greenhouse; one was covered with a double layer of black plastic between dusk and dawn, while the other remained uncovered. Daylength during larval development (12-30 January) was ca. 9 h 15 min, and maximum day temperature was 21°C, with a minimum of 15°C at night. Radiation intensity measured in the greenhouse at night was 25-35 uW/cm² (3-4 lx). All larvae (400-600 per plant) were reared and placed in sample vials. Since development was slower under the low temperatures, cocoons remaining in vials were dissected after 28 d to determine incidence of diapause and mortality.

Results and Discussion. Only 4% of larvae from the uncovered greenhouse plant diapaused, whereas 99% from the covered greenhouse plant diapaused. All (100%) incubator reared larvae diapaused. It was clear that low intensity illumination at night had prevented diapause in greenhouse larvae.

LIGHT INTENSITY THRESHOLDS

This experiment was designed to determine a threshold light intensity for preventing diapause under typical greenhouse winter conditions; also to investigate effects of red light and whether the response to low intensity radiation was influenced by low temperatures.

Materials and Methods. All tests were conducted in incubators set at LD 9:15 and TC 21:15°C, with thermophase starting one hour later and ending one hour earlier than photophase. In the test incubators, 48 uW/cm² (5.5 lx), 21 uW/cm² (2.5 lx) and 6.5 uW/cm² (0.7 lx) radiation was provided at night at plant level, from 7.5-W frosted incandescent bulbs (GE), controlled by rheostats, 50 cm above plants. The control incubator had no night light.

Irradiance and illuminance from light sources were measured using a LI-COR® meter (model LI 185; Li-Cor, Inc., Box 4425, Lincoln, Nebr.) with radiometer and photometer probes. Measurements were made in both uW/cm² (total radiant flux striking a surface) and lux (visible light striking a surface).

In the incubator used to test sensitivity to red light, a red 25-W incandescent bulb (GE) set at 20 uW/cm² (ca. 1.5 lx) replaced the frosted bulb.

The effect of lower temperatures was tested in an incubator under LD 9:15, TC 18:10°C, with thermoperiod two hours shorter than photoperiod. Irradiance at night was 48 uW/cm² (5.5 lx).

Each test was replicated twice, with oviposition plants taken from the FIN control cage two days apart. Larvae were placed in vials with either moistened cotton rolls or moist peat moss (1 cm deep) pressed into the bottom. The lower third of vials with peat was wrapped in black tape to exclude light. Emergence from vials with peat pupation substrates, which simulated natural conditions, was compared with results from vials with cotton, to ensure that results from the latter could be extrapolated to real conditions. Since larvae in cocoons are known to be photosensitive (Havelka, 1980c), it was possible, at the low intensities tested, that larvae in peat experiencing diminished radiation, would respond differently from those in cotton. For most replicates, last instar larvae were divided equally between peat and cotton vials.

Results and Discussion. Results of all treatments are given in Table 14., and those for radiation intensity are plotted in Figure 18. There was no significant difference (X^2-tests, $p<0.05$) between replicates of any test, and no significant difference between results from peat and cotton substrates in most tests. This is consistent with the finding that photoperiodic diapause induction is determined during the larval stage (Havelka, 1980c). Percentage of emergence from peat was significantly higher (X^2-tests, $p<0.05$) than from cotton in both replicates at 6.5 uW/cm^2. This is the reverse of what would be expected if larvae were affected by the reduction in radiation intensity experienced in peat vials, and may be related to better survival in peat.

TABLE 14. Percent diapause in A. aphidimyza larvae under short day (LD 9:15) conditions with low intensity radiation at night.

n	Radiation intensity at night. uW/cm^2 (lux) Upper leaf surface	Lower leaf surface	Temperature regime[1] Thermophase: cryophase (°C)	Type of pupation substrate	% Diapause of alive[2] $\bar{x} \pm$ SE	% Emergence of expected total $\bar{x} \pm$ SE
564	48 (5.5)	26 (2.5)	21:15	Cotton	1.1 ± 0.1	98.0 ± 0.3
400				Peat	-	100.0
400	21 (2.5)	11.5 (1.4)	21:15	Cotton	30.1 ± 1.0	6.7 ± 1.6
175				Peat	-	64.0
318	6.5 (0.7)	4.0 (0.5)	21:15	Cotton	67.1 ± 2.8	30.3 ± 2.7
300				Peat	-	43.6 ± 6.4
291	Dark	Dark	21:15	Cotton	100.0 ± 0	0
225				Peat	-	0
271	Red 20 (1.5)	Red 11 (0.9)	21:15	Cotton	88.6 ± 4.4	9.6 ± 3.3
225				Peat	-	6.0 ± 2.0
576	48 (5.5)	26 (2.5)	18:10	Cotton	88.7 ± 1.1	8.4 ± 0.5

[1] Thermophase 2 h shorter than photophase.

[2] Determined only from cotton substrates.

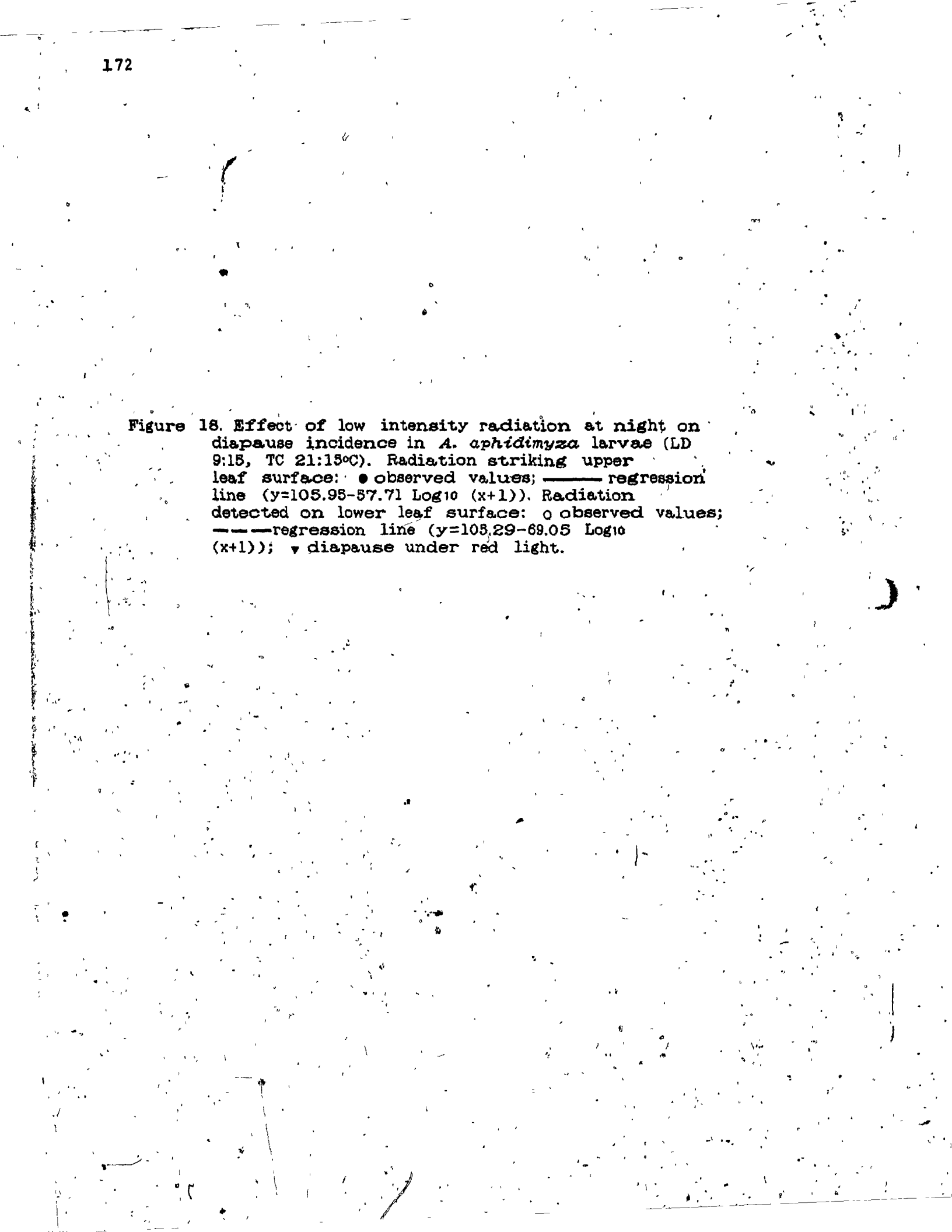
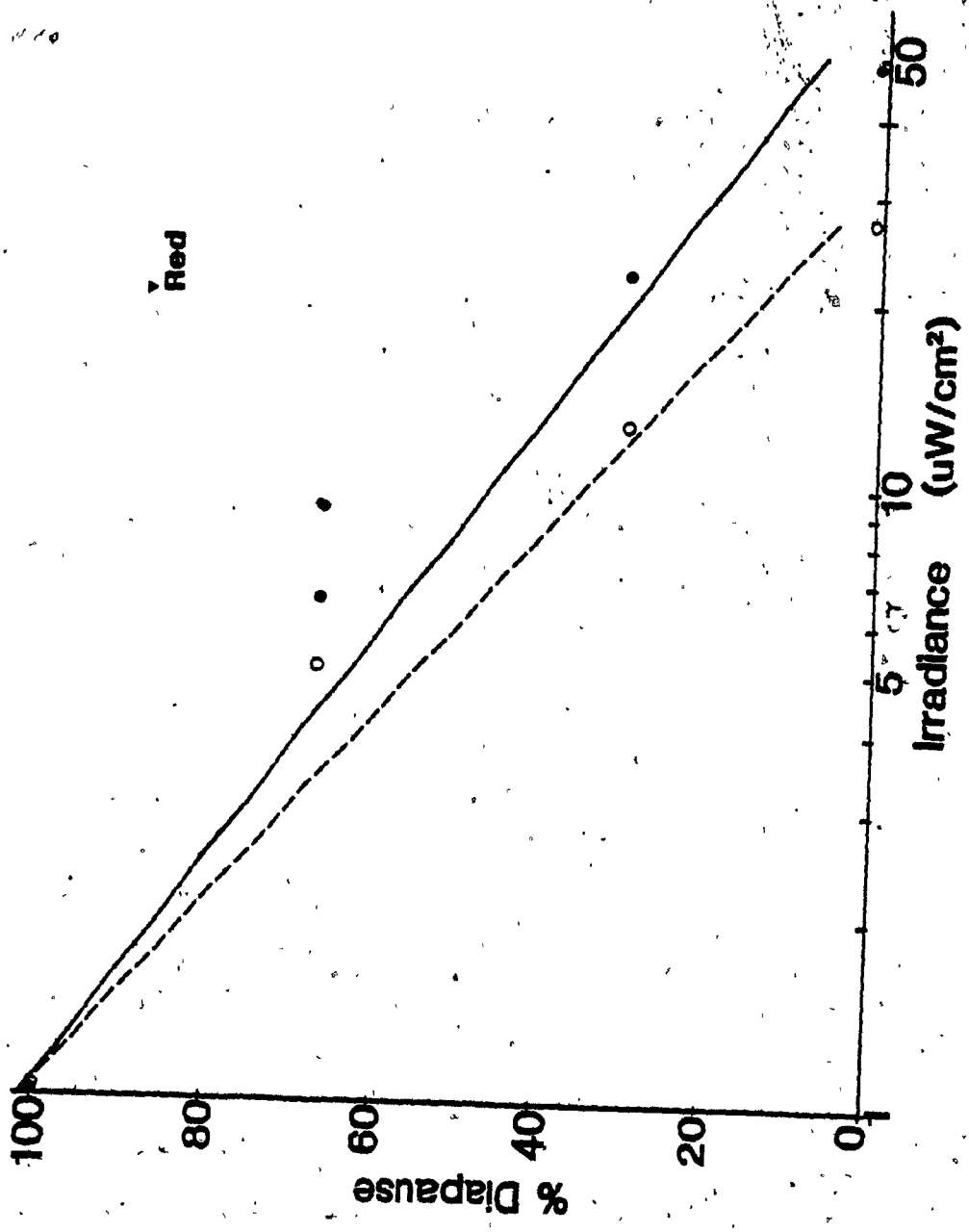


Figure 18. Effect of low intensity radiation at night on diapause incidence in *A. aphidimyza* larvae (LD 9:15, TC 21:15°C). Radiation striking upper leaf surface: ● observed values; ——— regression line ($y=105.95-57.71 \log_{10}(x+1)$). Radiation detected on lower leaf surface: ○ observed values; — — — regression line ($y=105.29-69.05 \log_{10}(x+1)$); ▾ diapause under red light.


Red
% Diapause
100
80
60
40
20
0
1
5
10
50
Irradiance (uW/cm²)

No threshold intensity for diapause induction was detected within the range of illumination and irradiance tested. Even at the equivalent level of visible light from the full moon (0.7 lx), under LD 9:15 (TC 21:15°C), 37% of larvae emerged without diapausing. Diapause incidence was linearly related to the logarithm of energy per unit area at levels <48 uW/cm².

Energy levels measured under single leaves, where larvae spend most of their development period, were approximately half of those above leaves (Table 14.), therefore, it is possible that diapause may be prevented in larvae actually experiencing 26 uW/cm² (2.5 lx) of radiation.

Radiation measurements in uW/cm² in incubators were over twice as high as figures obtained using mathematical conversions to lux for incandescent bulbs at full intensity (4.0 W/cm² per klux) (Biggs and Hansen, 1979). This is probably because, as voltage drops, an increasing proportion of energy emitted by incandescent bulbs is in the red to infrared (IR) range (Summer, 1962), which is detected by radiometric but not photometric sensors.

No sensitivity to red light was demonstrated, although sensitivity to IR, which may be involved in insect diapause response (Callahan, 1962), is not entirely ruled out, because all incandescent bulbs emit a high level of IR. If larvae were sensitive to red, then at least as many would be expected to respond to 20 uW/cm² of red wavelengths as to the same intensity of white (mixed spectrum) light. Only 30% diapaused under 20 uW/cm² from the white bulb, whereas 89% diapaused under the same intensity of radiation

from a red bulb. Even if larvae were unable to detect red light, sufficient white light may have leaked from flaws around the collar of the red bulb to saturate photoreceptors of a few extremely sensitive individuals. The extremely low level of radiation necessary to prevent diapause in an equivalent proportion of larvae can be calculated using the equation from the regression line (Figure 18.). Assuming that the log relationship holds between diapause incidence and radiation intensity below 6.5 uW/cm^2, calculations show that 1.2 uW/cm^2 (0.25 lx), would prevent diapause in 10% of *A. aphidimyza* larvae.

Under low temperature (TC 18:10°C) and 48 uW/cm^2 (5.5 lx) at night, diapause was induced in 89% of larvae. Thus, if a thermoperiodic response threshold for diapause induction exists (Beck, 1982; Saunders, 1983), then it differs with photoperiod. Because this thermoperiod regime is lower than that generally used in commercial greenhouses during winter (some lettuce varieties will grow under 18°C during the day, 10-12°C at night), these results do not detract from the usefulness of low light levels in maintaining reproducing *A. aphidimyza* populations during the winter.

To apply these results in a greenhouse, radiation measurements were checked at different distances from 60-W and 100-W incandescent bulbs to determine the area of effective illumination. In these measurements, 48 uW/cm^2 did not occur the same distance from bulbs as the 5.5 lx reading. This is probably because these lights were at full voltage, in contrast to the small bulbs used in

incubators, with reduced voltage and consequent proportionally higher levels of infrared. Also, the large bulbs were not frosted, which may have made a difference. For practical application of these results, the measurement in lux was used, since it is most likely that effective wavelengths for diapause induction lie within the visible spectrum (Chapman, 1982). For day-neutral crops, relatively insensitive to low light intensity (e.g., tomatoes, peppers, cucumbers), radiation from a single 60-W bulb could be used to prevent diapause in most *A. aphidimyza* within a circle 12 m in diameter, and would prevent diapause in >50% of the population in a circle >22 m in diameter. This would not be useful in an extremely light sensitive crop (e.g., poinsettias), however, most plants are insensitive to light below 2 lx (Hanan, et al., 1978), which occurs ca. 3 m from 60-W, or 4.5 m from 100-W incandescent bulbs.

Further work on preventing diapause could be directed toward determining whether *A. aphidimyza* larvae are more light sensitive at certain times during the night, as is known in other insects species, so that light pulses during scotophase could be used to prevent diapause (Lees, 1968; Beck, 1980; Brunnarius and Dumortier, 1984; Kikukawa and Masaki, 1984). The use of brief periods of illumination at night has been suggested as a way to control crop pests by preventing diapause in the field (Hayes, et al., 1974; Säringer, 1983). With *A. aphidimyza* in greenhouses, however, it could be used to obtain better biological control of aphids. This would only be feasible if the intensity or duration of light breaks did not disrupt dark

phase processes in crop plants.

Further investigation of spectral sensitivity in *A. aphidimyza* larvae also may be useful. If larvae respond most strongly to spectra not efficiently absorbed by plants (e.g., blue or blue-green wavelengths), continuous illumination at night could be of higher intensity, requiring fewer fixtures and less energy expenditure for a larger area, without disrupting plant processes.

The following section describes a related investigation into photoperiodic termination of diapause.

PHOTOPERIODIC TERMINATION OF DIAPAUSE

A series of tests was conducted under various environmental conditions for diapause induction and termination to find out under what conditions diapause can be photoperiodically terminated in *A. aphidimyza*.

Materials and Methods. In two tests, larvae remained under diapause inducing conditions (LD 8:16 and 21°C) until all diapausing larvae eventually resumed development and emerged. In three more tests diapause was induced under LD 8:16 (21°C) or LD 9:15 (TC 21:18°C) and then larvae were placed under LD 17:7 (21°C) until adults emerged.

In a further test, larvae, in which diapause was induced by rearing under LD 8:16 (TC 25:10°C), were divided into two groups, one of which was placed in LD 17:7 (TC 25:10°C). The other group remained under the original diapause inducing conditions. When no pupal development was observed after 42 d, this group was chilled (5°C) in darkness for 30 °d to hasten diapause development, then moved to LD 17:7 (24°C).

In a final experiment, replicated twice, larvae were reared under LD 9:15 (TC 21:15°C), to induce diapause. They remained under these conditions, but 48 uW/cm² (5.5 lx) of continuous illumination at night (from a 7.5-W frosted bulb) was added. When no emergence was observed after 31 d, half of the vials, randomly picked, were put in LD 17:7 (21°C), while the rest remained under LD 9:15, with low intensity illumination during the dark phase.

Thermophase was the same length as photophase in tests with LD 8:16 and LD 17:7. Thermophase was two hours shorter than photophase for LD 9:15 regimes, to simulate winter greenhouse conditions.

Results and Discussion. Results of all tests are given in Table 15. In the first three tests listed, larvae were left until all emerged (or died), thus time to 50% emergence is accurate. Remaining tests were terminated before all larvae had pupated and emerged, therefore, although time to 50% emergence is given, it is calculated from those that emerged within the test period. Therefore, it is early relative to time to 50% emergence for the total population.

In tests where larvae were moved to LD 17:7 (21°C) for diapause development, mean development time was 15 d, compared to 13 d for larvae reared entirely under LD 17:7, without diapause. Pupation apparently began within two days. Larvae that had been chilled for a month at 5°C took over twice as long to emerge. Diapause may have been more intense because they were reared under a cold night thermoperiod, or because the chilling deepened diapause (Havelka, 1980b).

Under LD 8:16 (21°C), diapause ended spontaneously in 33% of larvae within 33-50 d. Most of the rest emerged during the next 100 d, and a final few (11%) emerged up to 50 d later (200 d from the time they were placed in vials) (Figure 19.). Thus, although diapause eventually ended spontaneously under LD 8:16 (21°C), it was terminated swiftly, without chilling, by LD 17:7 (21°C). This is very useful for long term storage of mass-produced larvae,

TABLE 15. Diapause terminated under different environmental conditions in A. aphidimyza larvae.

Diapause induction regime (°C)	Days in diapause inducing regime	n	Diapause termination regime (°C)	Begin emergence (d)[1]	Time to 50% emergence (d)[1]	Last emergence or last observation (d)[1]	% Diapause at end of test	% Late pupation
LD 8:16 (21)	21	31	same	33[2]	74	200	0	0
LD 8:16 (21)	21	19	same	34[2]	42	194	0	0
LD 8:16 (21)	21	18	LD 17:7 (21)	12	15	23	0	0
LD 9:15 (21:18)	21	72	LD 17:7 (21)	12	>15	21	1.4	0
LD 9:15 (21:18)	28	176	LD 17:7 (21)	13	>15	28	2.5	7.4
LD 9:15 (21:18)	28	104	LD 8:16 (21)	15	>38	63	11.2	14.4
LD 8:16 (25:10)	21	87	LD 17:7 (25:10)	20	>31	42	7.5	12.5
LD 8:16 (25:10) + DD(5)	35 30	65	LD 17:7 (24)	17	>29	39	2.4	9.8
LD 9:15 (21:15)	21	247[3]	same + 48 uW/cm^2 night illumination	-	-	67	94.8	0
LD 9:15 (21:15)	53	247[3]	LD 17:7 (21)	12	19	35	0	1.0

[1] Days from change of regime.

[2] Days from larvae placed in vials.

[3] Results of two replicates pooled.

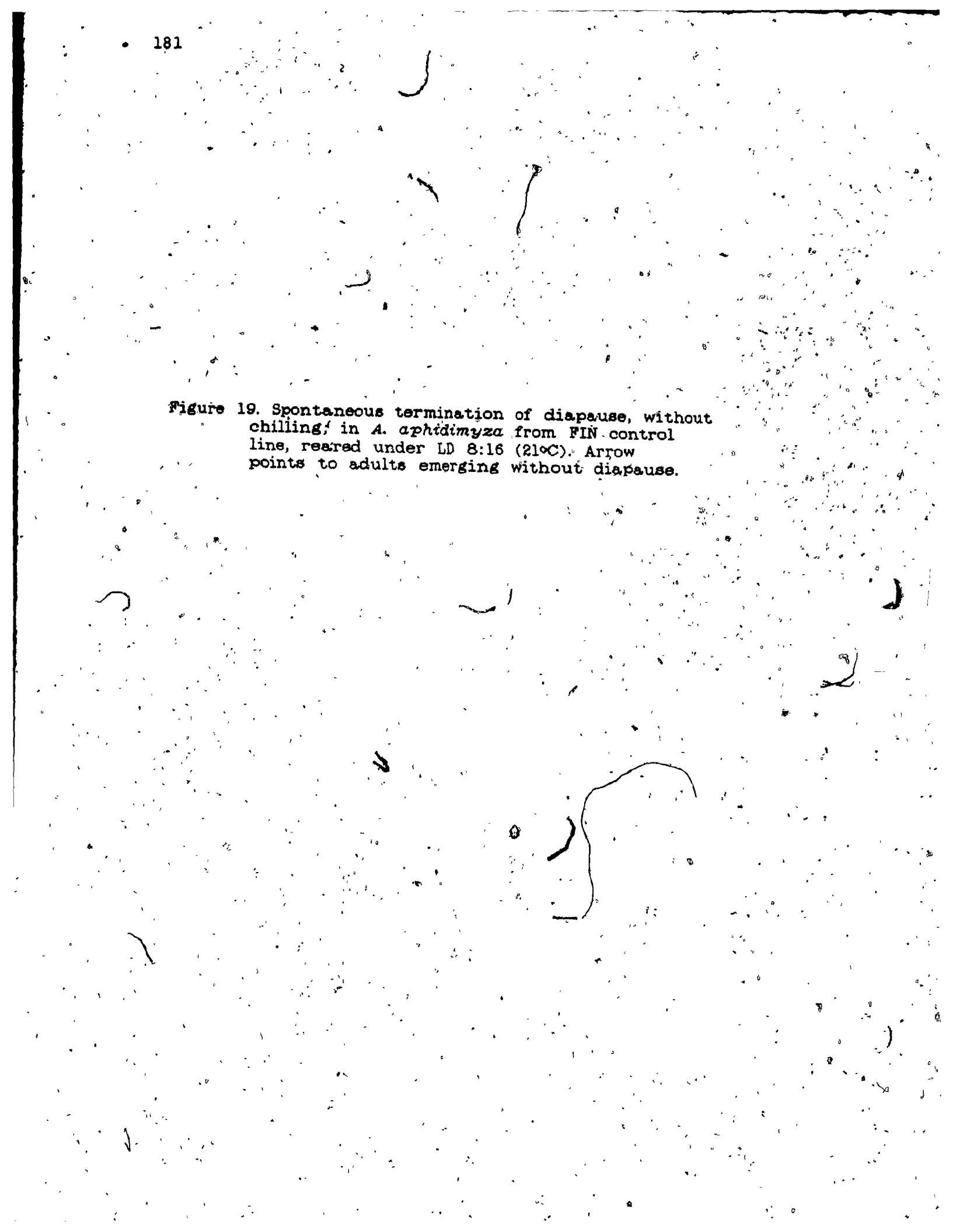
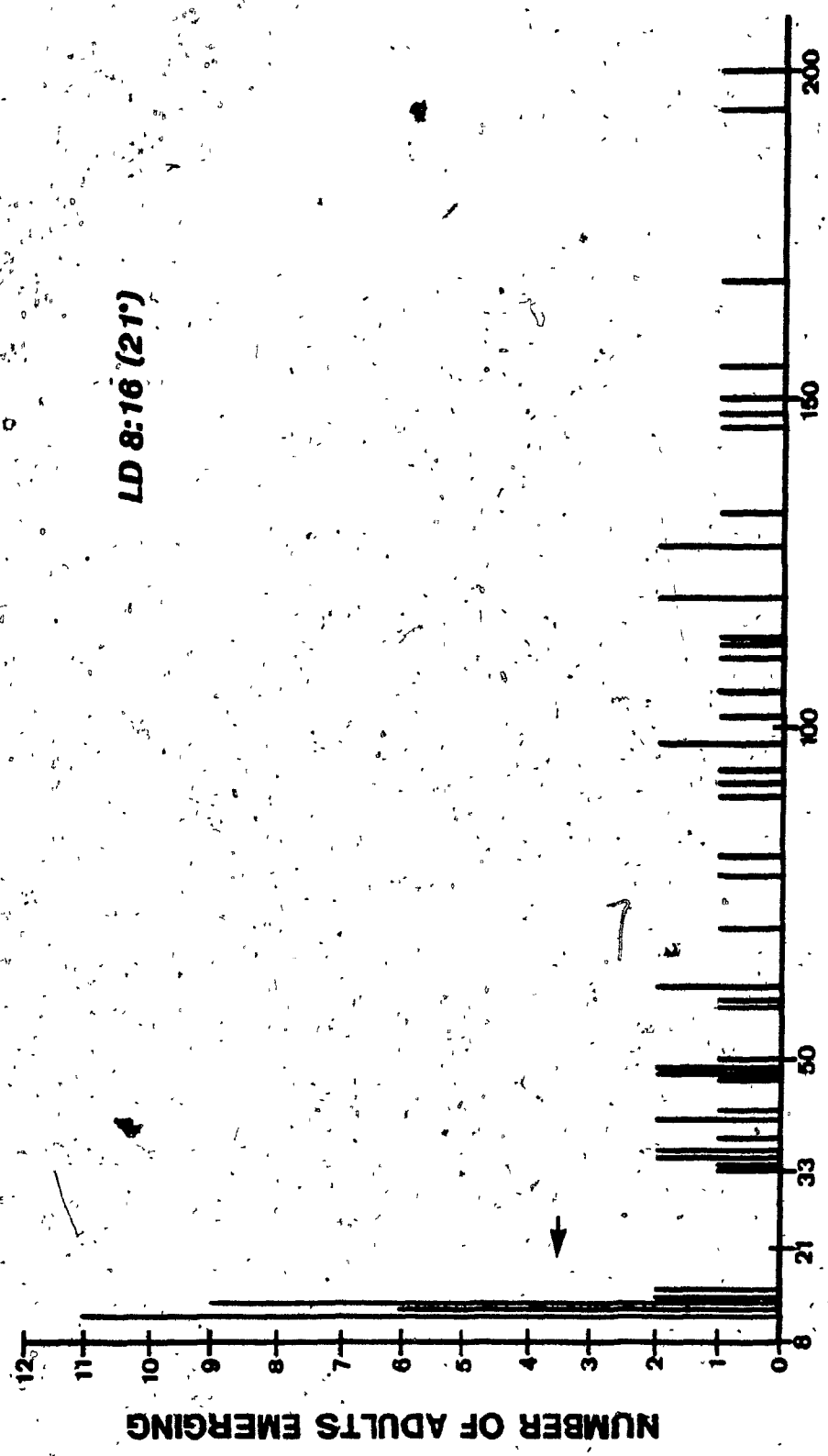


Figure 19. Spontaneous termination of diapause, without chilling, in *A. aphidimyza* from FIN control line, reared under LD 8:16 (21°C). Arrow points to adults emerging without diapause.


LD 8:16 (21°)
NUMBER OF ADULTS EMERGING
0
1
2
3
4
5
6
7
8
9
10
11
12
8
21
33
50
100
150
200
DAYS FROM LARVAL-PUPAL ECDYSIS

because there is only a slight delay in development once diapausing larvae are placed under long day conditions. The larvae in these tests may have been in what Mansingh (1971) terms "ateleo-diapause", a low intensity diapause that can be terminated precociously, because diapause was induced under relatively high temperatures. This may explain the swift termination of diapause under long day conditions.

Diapause induced under winter greenhouse conditions could not be terminated by exposure to 48 uW/cm²C at night, which has been shown to prevent diapause, therefore, it is likely that light intensity thresholds are higher for diapause termination than for diapause prevention.

Larvae reared under LD 8:16 (TC 25:10°C) took about twice as long to emerge under LD 17:7 (TC 25:10°C) (mean 21°C), than other diapausing larvae placed under LD 17:7 (21°C). Because mean temperatures were the same, larval and pupal development rates should have been the same, since all temperatures were well above larval and pupal development thresholds (4.3 and 5.7°C respectively) (Havelka, 1980a). This may be evidence of a thermoperiodic threshold for diapause development (Beck, 1983), in that the reactivating effect of long days was delayed when temperatures dropped below a certain threshold at night. There is no evidence of such a threshold in diapause induction, because in preliminary tests, <1% of larvae diapaused when reared entirely under LD 17:7 and TC 25:10°C.

Although these results have practical applications in commercial production of *A. aphidimyza*, they do not

explain the interaction between photoperiod and thermoperiod in diapause termination. Furthermore, results reported here are not necesarily true for other *A. aphidimyza* lines; Havelka (1980b) found differences in photoperiodic reactivation between two different geographical races of *A. aphidimyza*.

SECTION 5.

USE OF *APHIDOLETES APHIDIMYZA* IN GREENHOUSE CROPS DURING WINTER

Once a method of preventing diapause in *A. aphidimyza* larvae was found, experiments were done to determine whether they could control aphids under winter greenhouse conditions and, if so, at what release rates. Tolerance of *A. aphidimyza* to Safer's Insecticidal Soap (used to control aphids) was also studied and, in a concluding section, recommendations are given for the use of *A. aphidimyza* in Canadian commercial greenhouses.

MIDWINTER RELEASE RATES EXPERIMENT

With low intensity radiation preventing diapause in *A. aphidimyza*, release rates experiments were undertaken in the greenhouse to determine whether larvae could control aphids during winter and, if so, what release rates should be used.

Materials and Methods. Green pepper plants were used because the clone of *M. persicae* reproduced rapidly on them. If *A. aphidimyza* can control *M. persicae* on this host, it is likely that they can control *M. persicae*, or another, slower reproducing species, on other crop plants, At the temperature range maintained during the experiment (21°C daytime maximum, 15°C nighttime minimum), *M. persicae* reproduces 2-4 times more quickly than at higher or lower temperatures (Barlow, 1962), therefore, this experiment involved "worst case" condition for aphid control.

Twelve, wood-frame cages (45 x 90 x 60 cm high), covered with nylon mesh, were built into one greenhouse bench. Access to each cage was through a flap on one side fastened with Velcro® strips. Each cage contained 20 pepper plants in 12 cm diam pots. At the beginning of the experiment, plants had an average of nine expanded leaves and mean infestation level of 50 aphids per plant (1000-1200 aphids per cage). Because temperatures inside cages were ca. 3°C warmer during the day than outside cages, the greenhouse thermostat was set accordingly, to provide TC 21:15°C inside cages.

A randomized complete block design was used because it was suspected that temperatures at night varied from one end of the bench to the other. Three replicates of four release rates (1 predator:3 aphids, 1:10, 1:50, 1:100) were used. Over 3180 larvae from the FIN control line were placed in vials 29-31 October, and released in cages 11-13 November. Adults were sexed and released in a ratio of 1 male:2-2.5 females, which was the mean sex ratio in this line. A second release of adults, from 3750 larvae collected 13-15 November, was made 14 d later.

Six leaves from each cage were randomly picked at five day intervals and all living aphids, *A. aphidimyza* eggs and larvae were counted under 25X. After counting, leaves were laid on top of plants in cages, to minimize the impact of sampling on predator and prey populations. Although picking leaves retarded plant growth, there was a net increase in number of leaves per plant by the end of the experiment.

Mean aphid density per leaf was used as a criterion for success of a release rate, rather than fruit yield, because plants were confined to small pots at high density, and were unlikely to yield well. The number and weight of fruit, number of leaves, and height of plants, was recorded at the conclusion of the exeriment (65 d).

Results and Discussion. The experimental layout of cages is shown in Figure 20. Changes in the number of aphids and predator eggs and larvae for each replicate of the 1:3 and 1:10 release rate are shown in Figures 21. and 22. The mean of all replicates are shown for the 1:50 and 1:100

Figure 20. Experimental layout of cages for midwinter release rates experiment in greenhouse. North end of bench warmest, south end coolest.

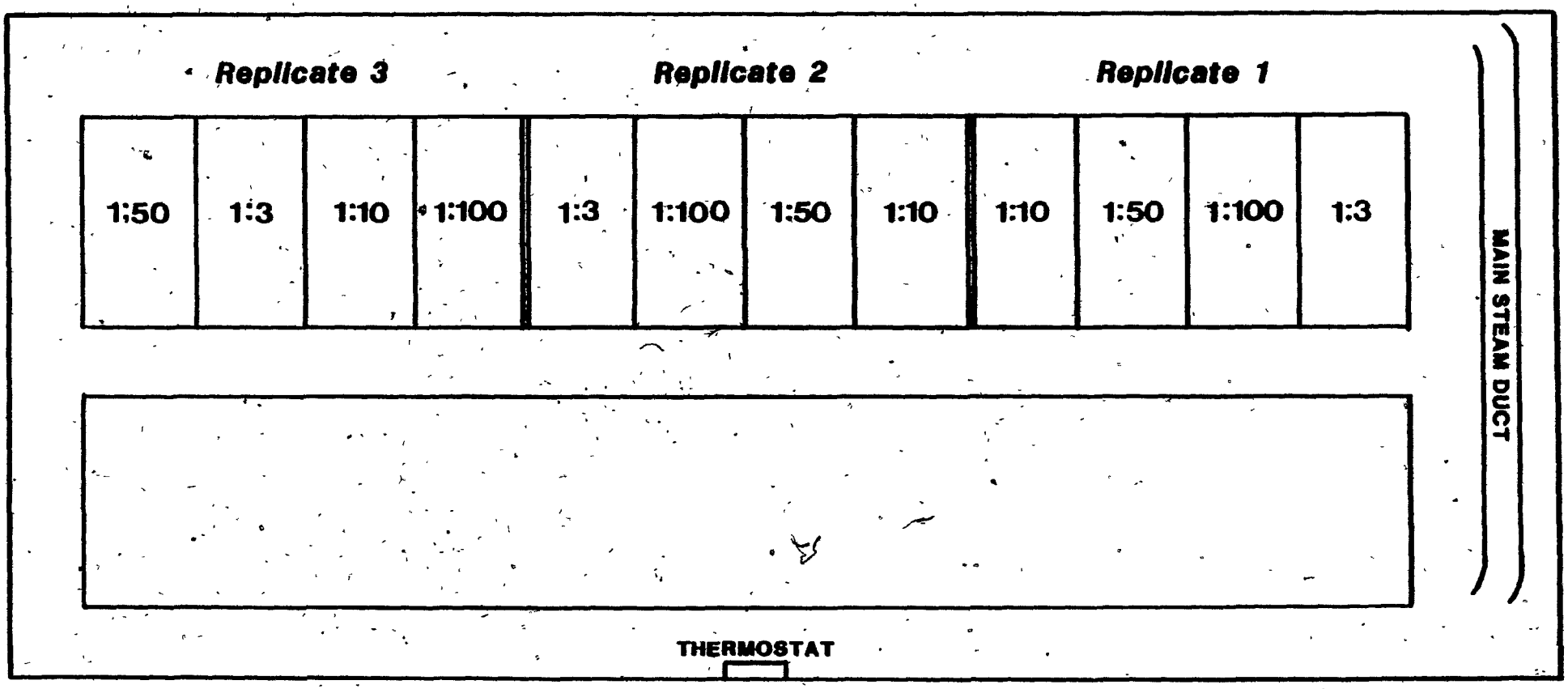

Replicate 3
Replicate 2
Replicate 1
1:50
1:3
1:10
1:100
1:3
1:100
1:50
1:10
1:10
1:50
1:100
1:3
MAIN STEAM DUCT
THERMOSTAT
N

Figure 21: Results of releasing 1 predator:3 aphids in midwinter greenhouse conditions. Mean number of aphids, *A. aphidimyza* eggs and larvae from six sample leaves. Arrows mark predator release dates.

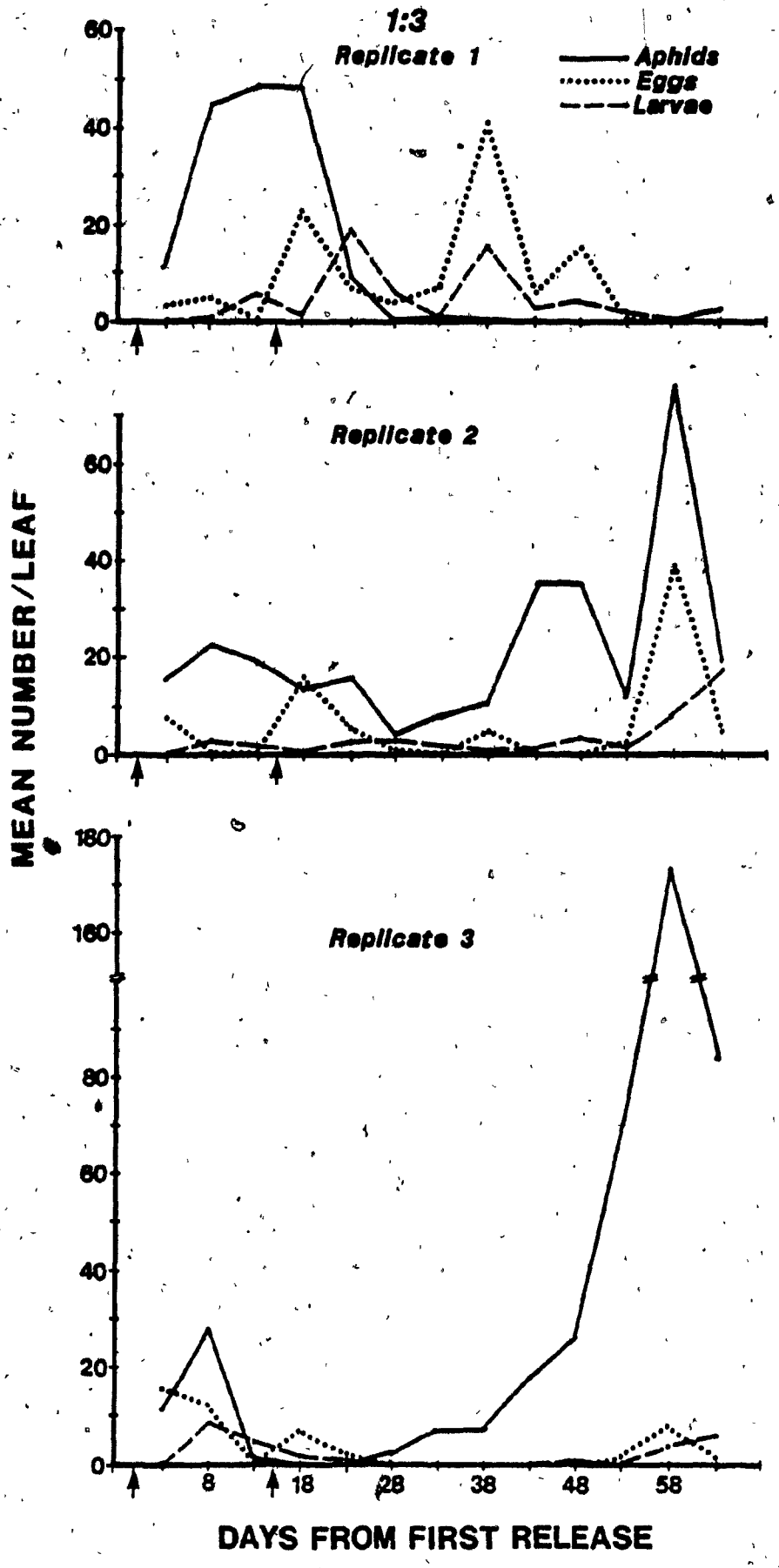

1:3
Replicate 1
Aphids
Eggs
Larvae
Replicate 2
Replicate 3
MEAN NUMBER/LEAF
DAYS FROM FIRST RELEASE
0
20
40
60
80
160
180
8
18
28
38
48
58

Figure 22. Results of releasing 1 predator:10 aphids in midwinter greenhouse conditions. Mean number of aphids, *A. aphidimyza* eggs and larvae from six sample leaves. Arrows mark predator release dates.

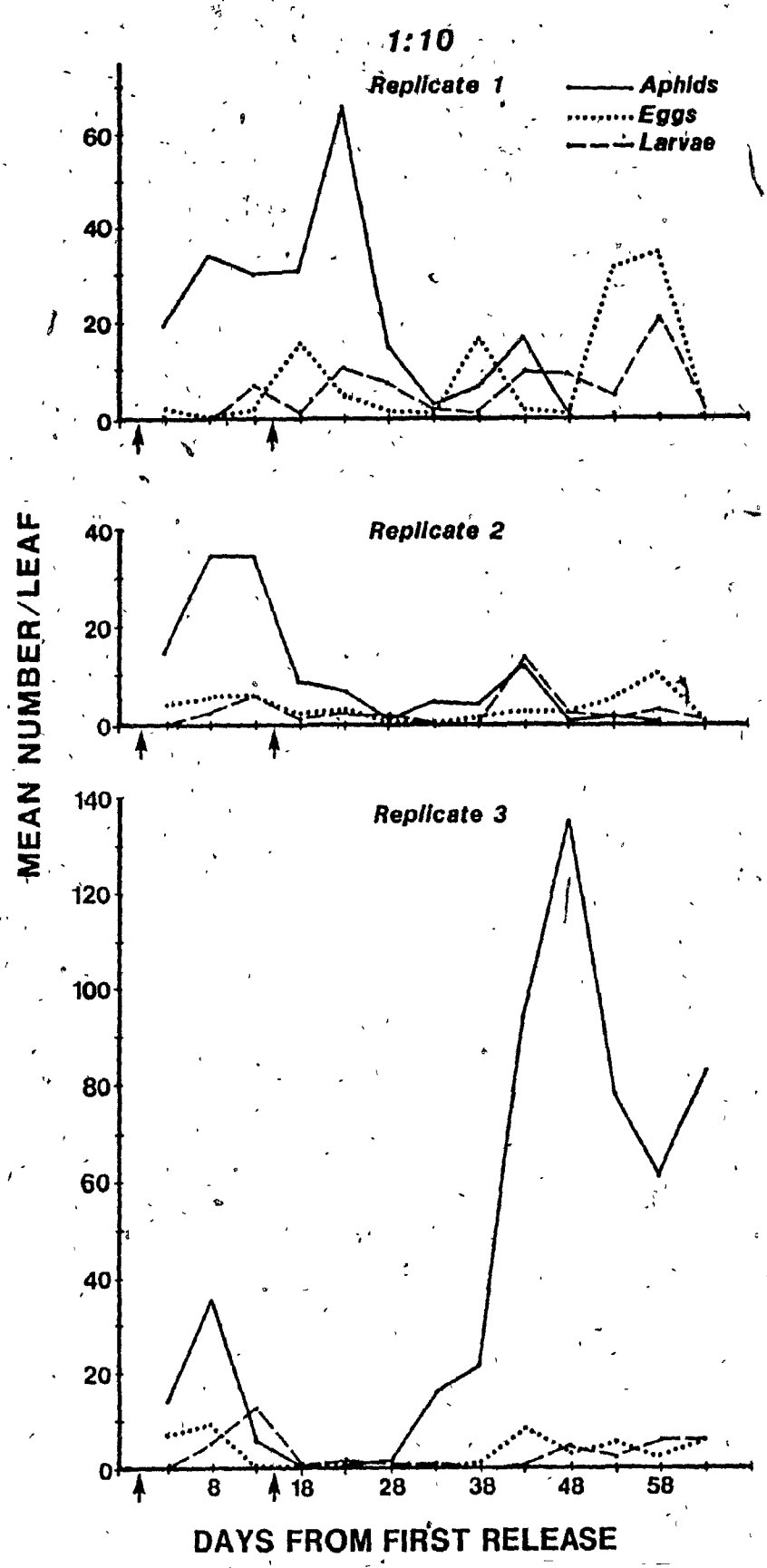
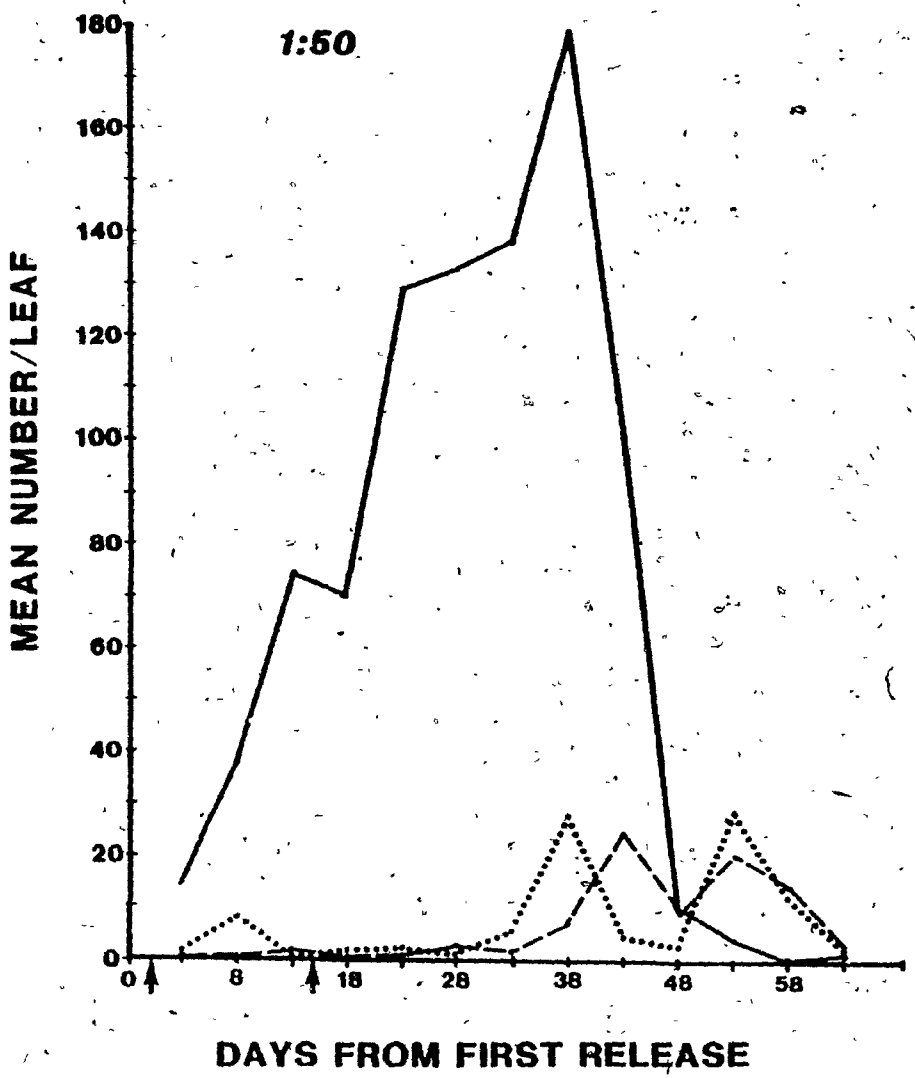
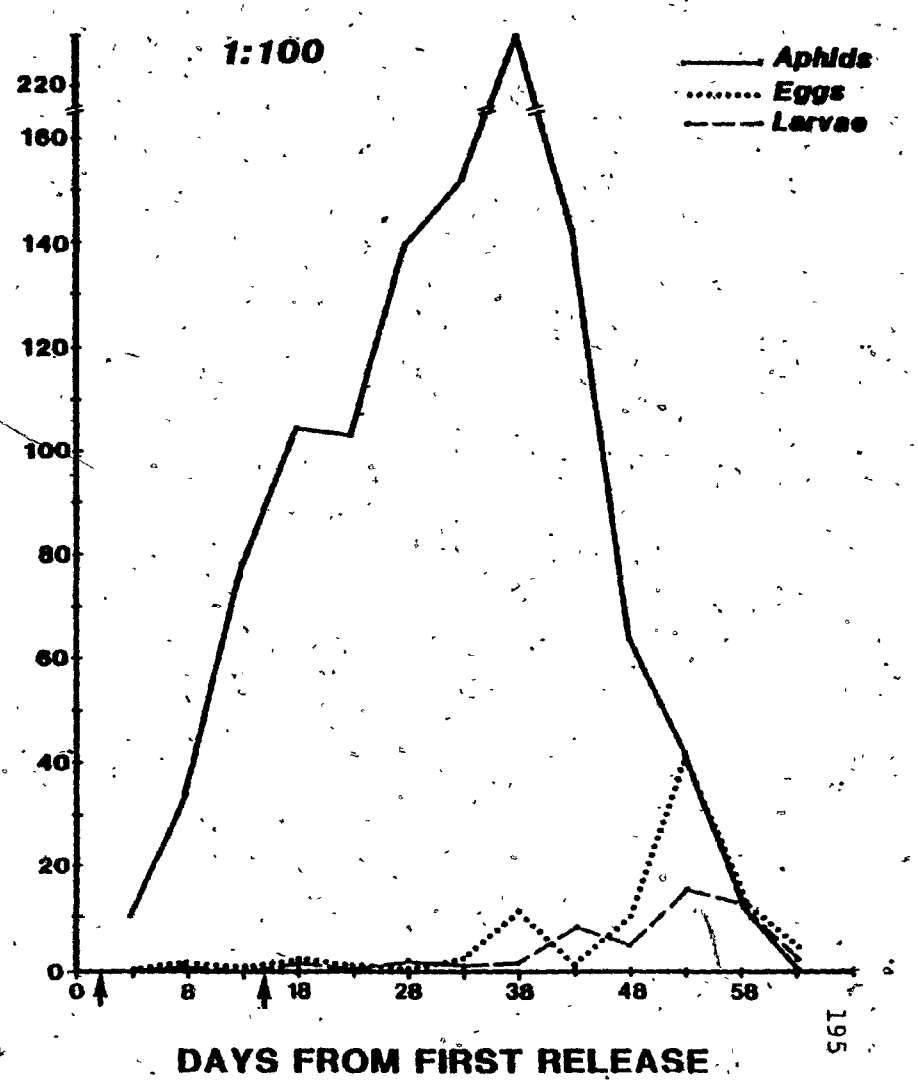

1:10
Replicate 1
Aphids
Eggs
Larvae
Replicate 2
Replicate 3
MEAN NUMBER/LEAF
0
20
40
60
80
100
120
140
8
18
28
38
48
58
DAYS FROM FIRST RELEASE

Figure 23. Results of releasing 1 predator:50 aphids and 1:100 in midwinter greenhouse conditions. Mean number of aphids, *A. aphidimyza* eggs and larvae from three pooled replicates of six sample leaves. Arrows mark predator release dates.


1:50
MEAN NUMBER/LEAF
180
160
140
120
100
80
60
40
20
0
0
8
18
28
38
48
58
DAYS FROM FIRST RELEASE


1:100
Aphids
Eggs
Larvae
220
160
140
120
100
80
60
40
20
0
0
8
18
28
38
48
58
DAYS FROM FIRST RELEASE

release rates in Figure 23. Data for each treatment are compiled in Appendix C.

Statistical analysis confirmed that there was a significant effect due to treatment (ANOVA, $F=33.03$; $df=3,102$; $p=0.0001$) and block (ANOVA, $F=6.31$; $df=2,102$; $p=0.0026$). There was a greater number of fruit in 1:3 and 1:10 cages, but not significantly so (Appendix C, Table C5.). There was also no significant difference in height of plants at the end of the experiment. Number of leaves in 1:3 and 1:10 treatments was significantly higher than in 1:50 and 1:100 treatments (Tukey's studentized range test for variable; $p<0.05$), showing that these plants were more vigorous as a result of lower aphid infestations.

The two lowest release rates, 1:50 and 1:100, were clearly inadequate, with aphid populations reaching >150 aphids per leaf in all replicates, before midge larvae brought the population under control. Even at these release rates, however, aphid populations were reduced to <3 per leaf in all replicates by the end of the experiment, demonstrating the predator's numerical (and functional) response to prey density.

Results of 1:3 and 1:10 releases were similar, and any differences between them were probably related more to the temperature gradient along the bench than to the different number of predators released. The north end of the greenhouse (Replicate 1 = R1), where the main steam pipes entered, was consistently warmer at night than the south end (R3). Because thermostats controlling heating and ventilation were near the centre of the bench, R2 cage

conditions were closest to TC 21:15°C. Replicate 1 was 1-3°C warmer at night and R3 was 1-3°C cooler, depending on weather outdoors. The effect of these temperature differences on *A. aphidimyza* development time is visible on the graphs; 30 d elapse between oviposition peaks for each generation in R1, 35-40 d elapse in R2, and 30 d elapse in R3.

At the 1:3 rate, aphid control was good in R1, with a mean of <3 aphids per leaf during the last five weeks of the experiment, but the other two replicates were not satisfactory. In both R2 and R3, aphid populations were reduced to <5 per leaf within four weeks, however, aphid populations rebounded. An explanation for this can be found by examining graphs for each replicate. In R2, a surprisingly low number of larvae resulted from the first release, although there appeared to be an adequate number of eggs, and larvae had little impact on aphids. The second release of midges was well timed and the second generation from this release was on the verge of decimating aphids by the end of the experiment (Figure 21.). In R3, offspring from the second release starved because aphid populations had been reduced to 0-1 per leaf, therefore, no larvae were present to control aphids. Although it is not clear whether the last oviposition peak is from offspring of the first or second release, it is most likely to be from the first release, because there were sufficient aphids between days 38 and 53 to support a large number of larvae, had they been present. Larvae from the first release may have had such a large impact on aphid populations because aphids reproduced

slower, relative to *A. aphidimyza*, under the slightly cooler conditions at the south end of the bench.

The best overall control was achieved using a 1:10 release rate (Figure 22.). When treatments were assigned to cages, R1 and R2 ended up side by side so cage temperatures would have been the same, which probably explains the similarity in results. In R3, aphids escaped control by the end of the experiment in a pattern very similar to that of R3 of the 1:3 rate, which was beside it on the bench. The second release of midges was evidently wasted in this replicate, as it was in the neighboring 1:3 treatment.

Results of this experiment show that *A. aphidimyza* can control *M. persicae* in the winter under conditions favorable for aphid reproduction. The 1:10 release rate appeared to be more effective than the 1:3 rate under midwinter greenhouse conditions. The second introduction of midges, 14 d after the first, had an impact in R1 and R2 of both treatments, but was wasted in R3, showing the advisability of basing releases on aphid counts rather than on a predetermined schedule. In R3 of both treatments, the second release would have been more effective four weeks after the first release.

The ability of adults to find mates, and females to find aphid colonies, under winter greenhouse conditions, was not tested in the small cages and must be checked in larger greenhouses. Also, air circulation in modern commercial greenhouses is well controlled, and temperatures are more uniform than those in the experimental greenhouse, further emphasizing the need for research on a greenhouse scale.

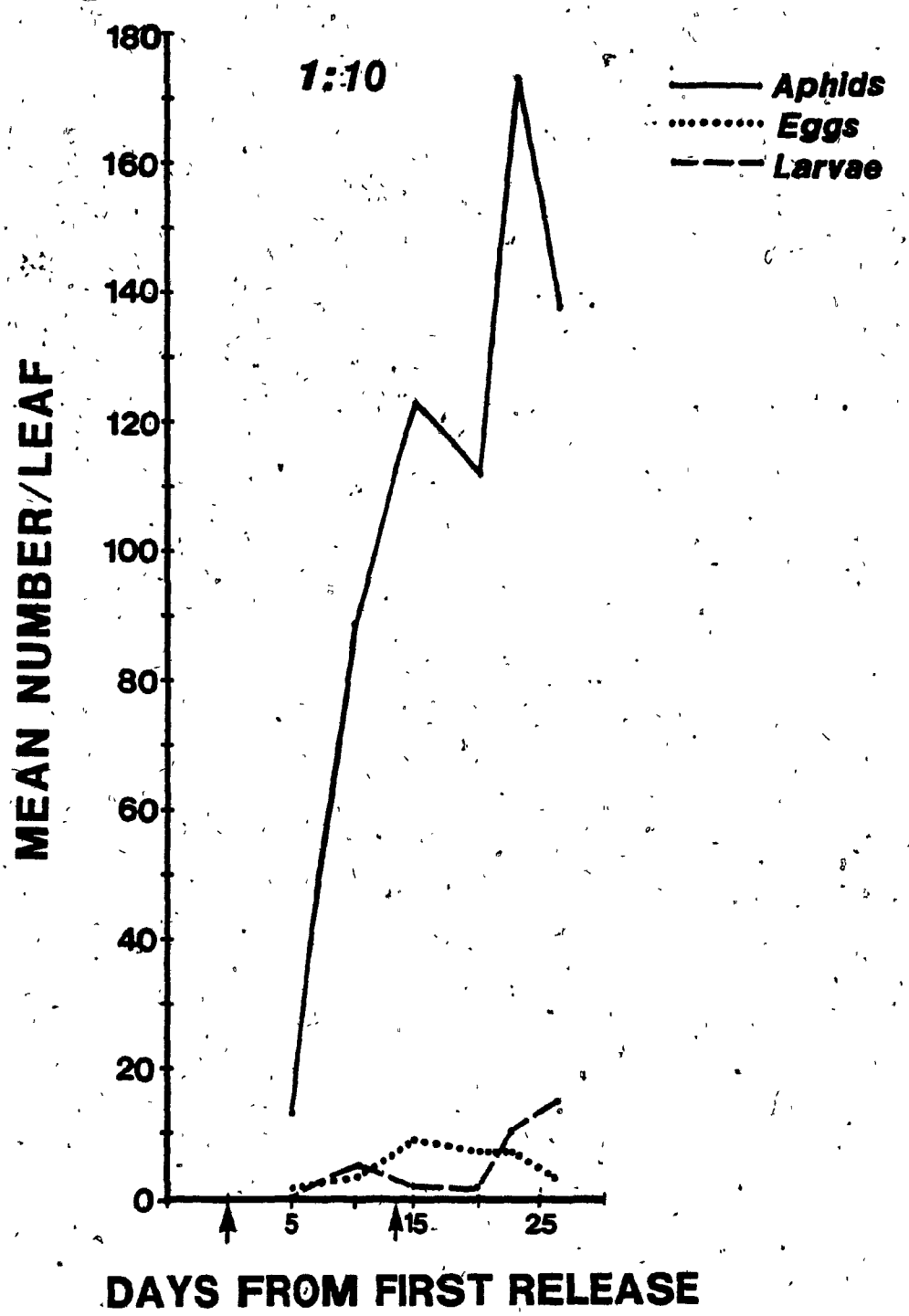
LATE WINTER RELEASE RATE EXPERIMENT

Based on results of the first release rates exeriment, a second experiment was conducted, using 1 predator:10 aphids. **Materials and Methods.** Methods were the same as in the previous experiment. Three replicates of the 1:10 release rate were set up in greenhouse cages. To minimize the effect of temperature differences along the bench, only centre cages were used, separated by an empty cage between replicates. Twenty pepper plants were placed in each cage and infested with 1300-1400 aphids per cage (65-70 per plant). Adults were released in cages on 27-28 February and again 14 d later. Sampling was conducted as in the previous experiment.

During the experiment, daily air temperatures rose to 23-26°C and night temperatures were 15°C.

Results and Discussion. The experiment was terminated after 26 d because aphid populations had reached destructive levels (>170 aphids per leaf) (Figure 24.). For unknown reasons, adults from the first release produced a very low number of eggs and the first larvae were not observed until the 10th day, unlike the previous experiment, in which larvae were counted on the 5th day. The larvae seemed to have little, if any, impact on aphid populations, which reached >120 per leaf by the time of the second release. The higher daytime temperatures and longer days during this experiment undoubtedly increased the rate of aphid reproduction, confirming the need for the higher release rate (1:3) in late winter and early spring recommended in Finland (Markkula, et al., 1979c). Poor reproduction from

Figure 24. Results of releasing 1 predator:10 aphids in late winter greenhouse conditions. Mean number of aphids, *A. aphidimyza* eggs and larvae from three pooled replicates of six sample leaves. Arrows mark predator release dates.


1:10
Aphids
Eggs
Larvae
MEAN NUMBER/LEAF
180
160
140
120
100
80
60
40
20
0
5
15
25
DAYS FROM FIRST RELEASE

the first midge release was probably an important factor in the failure to control aphids during this test, and further research is needed on methods to ensure uniform oviposition and larval survival in releases of *A. aphidimyza*. Because more work on release rates must be done before success is predictable, pesticides for controlling aphids that do not harm *A. aphidimyza* are needed; this is the subject of the following experiment.

INSECTICIDAL SOAP TESTS

Insecticidal soaps (potassium salts of fatty acids) are a naturally occurring, biodegradable alternative to chemical control for some pests (Parry and Rose, 1983). They can be integrated with biological control programs because they are nonresidual. In greenhouses, insecticidal soaps are used to control aphids and can be integrated with the parasite, *Encarsia formosa* Gahan, used to control whitefly, *T. vaporariorum* (Puritch, et al., 1982). This experiment tested the effect of a widely available brand, Safer's® Insecticidal Soap (Safer Agro-Chem Ltd., Willowdale, Ont.), on eggs and larvae of *A. aphidimyza*.

Materials and Methods. Two ages of larvae and 1-day-old eggs were sprayed in a Potter spray tower with two concentrations of Safer's soap: a 2% solution (0.04 mg/cm^2 active ingredient, AI, recommended rate), and a 20% solution (0.40 mg/cm^2 AI) Controls were sprayed with distilled water. Four replicates of 25 eggs or larvae were made of each treatment. Eggs were tested twice; 2-3-day-old larvae and last instar larvae (ready to pupate) were each tested once.

Oviposition plants, with eggs from the previous night, were taken from *A. aphidimyza* cages in the morning. For spray tests, eggs on individual leaves were counted under 25X and surplus eggs were removed, leaving 25 eggs per leaf on the lower leaf surface. Leaves were placed, eggs upwards, on dry filter paper in petri dishes, sprayed, then covered and incubated under LD 17:7 (21°C). Observations were made at 24, 48 and 72 h intervals.

The 2-3-day-old larvae were also treated while on leaves (25 larvae per leaf) because they were too small to handle. After treatment, fresh leaves, infested with aphids, were added to petri dishes to feed larvae. Observations were made as for eggs, with fresh aphids added as necessary.

Using a fine, wet paintbrush, last instar larvae were placed on dry filter paper in petri dishes just before being sprayed. Because larvae escape from petri dishes, they were placed in vials with cotton rolls for pupation immediately after treatment. Observations of larval mortality were made at 24 and 48 h; adult emergence was recorded and all remaining cocoons were opened after 21 d.

Results and Discussion. Neither concentration of Safer's Soap had a significant effect on hatchability of eggs (ANOVA on pooled tests, $p<0.05$), or on survival of last instar larvae (Table 16.). Adults from the latter larvae looked normal, and were kept in small cages for 10 d to make sure that mating and oviposition were normal (>95% of eggs hatched in all cages). No significant effect was found on 2-3-day-old larvae within 24 h. Analysis of data from observations at 48 h, however, was not possible since 49 larvae (16%) escaped and the remaining larvae were probably mixed. Because so few dead larvae were found (Table 16.), it is likely that the spray did not affect larvae. This may be because larvae are able to exude fluid over their body when they come in contact with chemicals, and this probably helps them resist poisoning (Ushchekov, 1975).

These results show that Safer's Insecticidal Soap can be used to control aphids when *A. aphidimyza* eggs and

TABLE 16. Results of spraying eggs and larvae of A. aphidimyza with two concentrations (2, 20% A.I) of Safer's Insecticidal Soap. Each treatment replicated four times (n = 25).

	Eggs % hatched		2-3-day-old larvae	Last instar larvae
	Test 1	Test 2	% Mortality (24 h)[1]	% Adult emergence
Control	95	92	1	80
2%	90	90	2	85
20%	94	89	4	84

[1] Percent survival not calculated because 16% of larvae escaped.

larvae are present. This pesticide joins the list of acaracides, fungicides and some insecticides compatible with the use of the predator. This is particularly useful because foci of aphids, or other pests susceptible to insecticial soap, can be spot sprayed without harming *A. aphidimyza* and disrupting a biological control program for aphids.

RECOMMENDATIONS FOR APPLICATION OF *A. APHIDIMYZA* IN COMMERCIAL GREENHOUSES

For Growers:

1) Release 1 pupa:3 aphids in spring and fall, 1:10 in midwinter if greenhouse is on a cool production schedule (TC 21:15°C), otherwise use 1:3 rate.
2) An additional release may be necessary, usually in 14 d during spring and fall, and after 21-28 d in midwinter, in cool greenhouses.
3) Use Safer's® Insecticidal Soap to control aphids, either before release of *A. aphidimza*, to bring populations to low levels, or to control "hot spots" of aphid infestation after midges have been released.
4) Leave container(s) of pupae in greenhouse, lid off, among plants where they won't be disturbed and out of the full sun. Ensure peat does not dry out completely for 10 d, the maximum emergence period.
5) Pasteurizing or fumigating greenhouse soils will kill pupating midges, preventing permanent establishment. Some fungicide soil drenches (e.g., for damping-off, collar rot) can be used without harming pupae in soil.
6) To maintain midge activity during winter when no supplemental light is used to extend daylength for crop, leave a 60-W or 100-W incandescent bulb on all night in the centre of each section (10 x 30 m) or 22 m apart each way in larger open-span houses.
7) Biological control of whitefly (*Trialeurodes vaporariorum*) and two-spotted spider mite (*Tetranychus*

urticae) can be integrated with the use of *A. aphidimyza*.

8) Control may be enhanced by providing pollen and nectar flowers for female midges to enable them to live longer and lay more eggs. Dill, or other small flowered Umbelliferae, are good sources of nectar and can be sown in soil beds, or at the head of the house in planters. Single plants at intervals (3-6 m) are likely to be sufficient.

For Suppliers:

To ensure the best control, two ages of pupae (5-7 d apart) should be included in the shipment. This is to compensate for early emergence and short life span of males, because females emerging from the earlier group will be re-mated by males emerging later, enabling them to lay fertile eggs during their entire life span.

CONCLUSIONS

The central aim of this research, to provide effective aphid control in winter crops in greenhouses using *A. aphidimyza*, was accomplished, although not in the way originally intended. Selection for nondiapausing lines under LD 8:16 was possible in four out of five lines (two from the FIN line, two from the QUE lines), but only under constant 21°C. No selection progress was made when larvae were exposed to a thermoperiodic cycle, even at relatively high temperatures (TC 24:18°C). The discovery that extremely low intensity radiation at night prevented diapause under LD 9:15 and TC21:15°C, made it possible to continue with release rates trials, because *A. aphidimyza* emerged without diapause all winter in the greenhouse. This was very important because it was not known whether *A. aphidimyza* could control aphids under winter greenhouse conditions, particularly *M. persicae*, with its high reproductive rate under cool conditions. Although the release rates experiment was, of necessity, a cage experiment, it showed that *A. aphidimyza* was capable of controlling aphids under cool, short day conditions at release rates (1 predator:10 aphids) lower than that recommended later in the spring (1:3). This confirms the value of this predator and its potential for controlling aphids in winter, giving further incentive to renew the study of preventing diapause.

The diapause response in *A. aphidimyza* was found to be very complex. An interaction between photoperiod and thermoperiod was demonstrated, with long day photoperiods preventing, or terminating, diapause under most temperatures,

and thermoperiodic cycles inducing diapause under short days, even in nondiapause selected lines. That low intensity radiation (<48uW/cm^2) at night prevented diapause was not previously known for this species. The evidence that under certain environmental conditions, the quality of the host plant affected the quality of aphids sufficiently to influence diapause in predator larvae was unexpected, and without precedent in other predator species.

A relationship between development rate and tendency to diapause was observed. Diapause oriented larvae took longer to develop than pupation oriented larvae; the correlation of early emergence with low diapause incidence in generations of nondiapause selected lines indicates there may be a genetic basis for the relationship, possibly through genetic linkage during nondiapause selection, or as a pleiotropic effect of nondiapause genes. The dominance of genes for photoperiodic induction of diapause, over those for nondiapause, was demonstrated; inheritance of diapause was found to be in accordance with a chromosome cycle in which paternal chromosomes are eliminated.

Variability in important attributes of *A. aphidimyza* reared in the laboratory were found in the course of the research. Variations and imbalances in sex ratio in some lines underscored the necessity of obtaining a line with stable sex ratio for mass-production. Wide intrapopulation variation in fecundity and life spans of individuals were also observed, suggesting that genetic selection for more vigorous lines may be possible.

FUTURE RESEARCH

As in many research projects, this study raised more questions than it answered. Although the use of low intensity light at night to prevent diapause works, and does not require additional equipment, because most greenhouses are equipped with light fixtures at intervals, it is not a universal solution. It cannot be employed in light sensitive crops and does require some effort and expense to the grower. A truly nondiapausing line of *A. aphidimyza* would be a better solution, if it can be found. I suggest that further research should be directed toward selection for nondiapause. Geographical races of *A. aphidimyza* from southern areas should be sought and screened quickly, once lines are established in the laboratory. Several generations of exposure to desired photoperiod and thermoperiod regimes is enough to determine whether any progress will be made in selection.

Further work on preventing diapause with low intensity radiation could concentrate on determining whether larvae are sensitive to wavelengths not efficiently absorbed by plants, such as blue or blue-green. If they are, diapause could be prevented over a larger area, with higher intensity illumination, without affecting plant dark phase processes. It might also be worth ascertaining whether pulses of light at night are effective in preventing diapause in *A. aphidimyza*.

Much more work on release rates and timing of releases of *A. aphidimyza* is needed, and on a greenhouse scale. Whether mating, or host-finding efficiency in females, is

reduced under winter greenhouse conditions should be investigated. Timing releases of the predator, based on aphid counts, or on ambient temperatures, also ought to be studied.

Another interesting avenue of investigation opened by this research is the possibility of genetic selection of a line with high fecundity. The variability in fecundity, with a substantial proportion of females laying over 200 eggs, might provide an adequate basis for genetic selection. Selection for increased life span might also be worth while.

Termination of diapause photoperiodically, without chilling, has greatest application in commercial production of midges for growers and, in conjunction with research on cold storage methods, would make midge production more economical. Reliable storage and reactivation methods free suppliers to produce pupae seasonally, while providing shipments of pupae on demand.

One of the most intriguing areas for further investigation, is determining how the quality of host plants affects aphids, which in turn affects diapause in predator larvae. Such third order effects have rarely been examined and may shed light on the subtle interactions between plants, phytophagous species and their predators.

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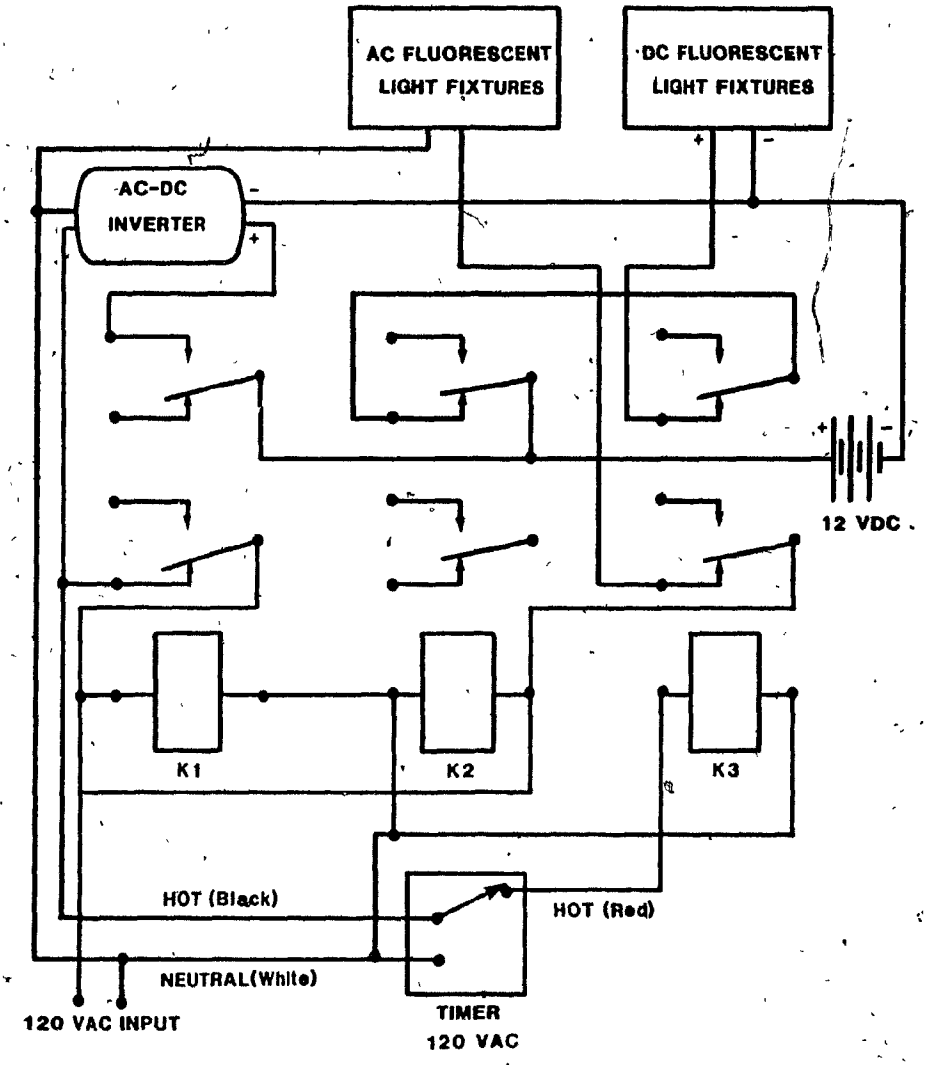
APPENDIX A.

Figure A1. Wiring diagram for timer and 12-V light system used for emergency lighting in incubator.



K1,K2 and K3 are 120 VAC DPDT Relays

APPENDIX B.

Data on percent diapause, late pupation, larval and pupal mortality, sex ratio and percent emerged by day 13 from larval-pupal ecdysis, for each generation, or sample, from *A. aphidimyza* lines.

TABLE B1. Data from the FIN control line of *A. aphidimyza*; samples reared under LD 8:16 (21°C).

Date collected	Generation[1]	n.	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Sept. 25-27/82	1	190	77.5	0	4.2	0	1:0.8	-
Oct. 12-16	2	185	81.6	0	1.1	0.5	1:0.6	-
Oct. 26	-	118	89.0	0	7.6	0	1:0.5	-
Feb. 6/83	-	270	37.9	0.7	3.3	1.9	1:1.9	-
Feb. 24	-	136	30.8	0	2.2	2.2	1:2.5	-
Apr. 13	-	185	43.3	2.9	3.8	3.8	1:0.6	-
May 16-18	13	61	33.9	3.4	3.3	0	1:1.8	-
June 6	14	52	31.9	2.1	1.9	7.7	1:3.4	-
June 22-23	-	138	48.8	2.4	2.9	5.1	1:4.1	-
July 31-Aug. 1	-	805	48.5	20.9	4.2	13.0	1:1.0	-
Aug. 7-8	17	96	57.3	1.1	4.3	3.1	1:2.1	-
Aug. 15	-	167	53.5	1.6	18.0	6.0	1:1.5	-
Sept. 24-26	-	296	49.6	1.5	3.7	6.4	1:1.1	-
Oct. 22-23	21	350	38.2	0.9	2.0	4.6	1:1.6	68.9
Nov. 11-13	22	454	38.8	3.0	5.3	5.5	1:1.6	73.3
Dec. 3-4	23	159	41.6	0.7	1.9	4.4	1:1.5	14.0
Dec. 22-23	24	462	85.5	2.0	0.9	0.6	1:1.4	24.6
Jan. 13-14/84	25	136	89.5	0	1.5	0.7	1:0.6	71.4
Feb. 6-7	26	371	62.5	2.3	3.2	1.9	1:0.8	40.3
Feb. 26	27	487	83.6	0.6	2.0	1.4	1:1.2	71.6
Mar. 18-19	28	455	48.5	2.3	3.3	2.0	1:1.2	41.0
Apr. 8-9	29	462	58.4	2.3	2.6	2.6	1:1.6	77.9
Apr. 29-30	30	525	75.4	0	2.1	1.1	1:0.9	42.4
May 19-20	31	286	49.2	6.8	3.2	3.8	1:1.6	48.7
June 9-10	32	258	25.4	5.6	0.8	3.1	1:0.8	18.1
June 28-29	33	337	76.7	0	0.3	0.3	1:1:0	30.8

TABLE B1. (Continued)

Date collected	Generation[1]	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
July 18-19	34	393	86.7	1.3	1.8	0.5	1:2.2	10.9
Aug. 6-7	35	348	39.9	3.3	0.9	2.6	1:1.2	34.6
Aug. 23-24	36	412	71.1	2.3	3.4	1.0	1:1.2	47.6
Sept. 12	37	391	36.4	6.6	1.8	5.4	1:1.7	49.3
Oct. 2-3	38	149	48.1	1.5	8.7	2.0	1:2.2	55.2
Oct. 20-21	39	302	70.9	3.0	0.7	1.3	1:1.6	41.6
Nov. 10	40	312	60.8	0.6	1.0	2.6	1:1.7	39.6
Nov. 30-Dec. 1	41	320	34.8	1.6	0	2.2	1:1.4	58.8
Dec. 18-19	42	273	25.5	1.1	0.7	1.5	1:5.1	59.6
Jan. 8-9/85	43	227	11.5	1.4	4.0	4.0	1:2.4	53.8
Jan. 28-29	44	219	8.0	2.8	1.4	1.8	1:1.4	83.6
Feb. 18	45	284	35.3	2.9	0.7	2.5	1:1.3	45.9
Mar. 9-10	46	295	61.3	1.4	2.4	1.7	1:1.2	41.5
Mar. 30-Apr. 1	47	197	25.0	1.0	0	2.5	1:2.5	78.9
Apr. 14-15	-	239	25.4	1.3	1.7	2.9	1:1.2	-
Apr. 18-19	48	361	35.5	1.4	0.6	1.9	1:1.7	58.6
Apr. 21-22	-	302	53.9	1.7	1.0	2.0	1:1.9	-
Apr. 26-27	-	271	65.0	0.4	1.8	0	1:3.0	-
Apr. 30	-	188	29.7	0	1.6	0	1:1.2	-
May 4-5	-	232	37.1	1.8	0.9	3.9	1:1.4	-
May 9	49	172	33.5	11.8	2.9	3.5	1:28.0	37.5
May 29-30	50	113	13.2	9.4	4.4	1.8	1:1.9	62.2

[1] Sample coincides with generation of FIN(ND) line.

TABLE B2. Data from the FIN(ND) line of *A. aphidimyza*, selected for nondiapause under LD 8:16 (21°C).

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Sept. 25-27/82	1	190	77.5	0	4.2	0	1:0.8	24.4
Oct. 16-22	2	238	63.4	1.3	4.2	0.4	1:3.2	10.0
Nov. 2-13	3	436	59.0	5.3	3.4	1.4	1:3.6	17.6
Nov. 28-Dec. 5	4	364	24.1	1.4	1.9	1.9	1:4.6	57.3
Dec. 18-26	5	262	15.1	2.0	2.7	1.5	1:2.2	76.0
Jan. 5-8/83	6	120	4.4	0	3.3	1.7	1:5.7	73.4
Jan. 24-26	7	160	0.6	2.6	1.9	1.9	1:5.7	88.6
Feb. 12-13	8	214	5.7	1.9	1.4	0.9	1:4.4	77.2
Mar. 2-5	9	222	2.4	0.5	3.2	1.4	1:2.5	78.2
Mar. 21-24	10	229	8.4	4.6	3.1	3.1	1:2.1	64.7
Apr. 4-11	11	268	7.3	8.5	1.9	5.6	1:4.3	27.9
Apr. 30-May 2	12	224	2.9	7.1	5.8	6.7	1:1.7	32.4
May 19-23	13	165	3.3	3.3	3.0	6.1	1:1.6	61.4
June 6-10	14	224	10.8	5.9	3.1	5.8	1:3.0	14.1
June 27-29	15	235	8.7	3.9	4.3	8.1	1:5.9	18.9
July 17-19	16	315	6.6	7.0	6.4	7.0	1:4.1	3.8
Aug. 6-7	17	122	1.8	0.9	0.8	5.7	1:5.9	13.5
Aug. 25-26	18	259	3.9	2.1	3.5	6.2	1:2.3	23.2
Sept. 13-16	19	146	33.6	11.2	1.4	0.7	1:2.2	7.5
Oct. 3-6	20	240	15.8	6.2	2.1	3.3	1:2.2	45.2
Oct. 22-24	21	274	4.4	0.4	5.1	4.7	1:3.1	59.6
Nov. 11-24	22	200	2.7	2.2	3.0	5.5	1:2.4	36.8
Dec. 1-2	23	196	1.8	1.8	5.6	8.8	1:5.6	27.2
Dec. 22-24	24	208	34.8	9.1	3.8	6.2	1:4.6	15.7

TABLE B2.. (Continued)

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Jan. 13-15/84	25	271	43.4	1.2	2.6	3.0	1:1.2	40.8
Feb. 2-7	26	251	37.6	5.6	3.2	3.6	1:1.0	13.5
Feb. 25-26	27	536	43.9	6.5	5.0	2.8	1:0.9	14.3
Mar. 18-19	28	515	7.1	4.1	4.7	4.8	1:1.5	51.4
Apr. 8-9	29	392	18.3	3.0	2.6	4.1	1:1.4	61.5
Apr. 29	30	337	19.0	7.5	5.0	4.4	1:3.1	50.4
May 19-20	31	291	2.7	7.0	2.8	8.9	1:2.1	24.6
June 9-10	32	227	2.4	2.9	1.3	6.2	1:1.5	38.7
June 29-30	33	389	12.1	0.8	1.0	3.3	1:2.1	34.2
July 18-19	34	491	18.6	4.6	1.8	4.9	1:1.2	49.7
Aug. 7-8	35	350	13.0	3.7	1.7	6.3	1:1.1	51.5
Aug. 23-24	36	350	11.5	1.3	2.0	1.1	1:2.7	82.3
Sept. 11-12	37	402	1.7	1.7	3.2	8.9	1:2.6	75.7
Oct. 2-3	38	283	9.8	2.3	2.1	4.2	1:0.9	67.8
Oct. 20-21	39	405	14.4	4.0	2.0	5.6	1:1.6	42.0
Nov. 10-11	40	310	14.1	2.8	3.9	4.5	1:1.8	44.5
Nov. 30-31	41	301	1.4	0.4	2.0	5.3	1:2.3	92.0
Dec. 18-20	42	286	0	1.9	3.5	5.6	1:1.6	79.9
Jan. 8-9/85	43	280	6.7	1.1	2.1	1.8	1:1.4	63.7
Jan. 28-29	44	276	0.4	0	1.8	4.0	1:1.7	70.8
Feb. 17-18	45	301	5.6	2.8	1.3	3.3	1:1.6	73.5
Mar. 9-10	46	285	16.4	2.6	2.1	3.9	1:1.2	50.2
Mar. 30-31	47	295	3.6	1.8	1.7	4.1	1:0.9	81.8
Apr. 17-18	48	277	4.5	1.2	5.1	6.1	1:1.8	31.0
May 8-9	49	147	35.5	6.4	1.4	2.7	1:1.5	27.1
May 28-30	50	156	2.2	5.2	5.8	8.3	1:1.7	57.3

TABLE B3. Data from the FIN2(ND) line of A. aphidimyza, selected for nondiapause under LD 8:16 (21°C).

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Feb. 6/83	1	270	37.9	0.7	3.3	1.9	1:1.9	65.6
Feb. 25-26	2	276	24.3	0.7	3.6	3.6	1:2.7	78.6
Mar. 15-19	3	269	13.3	3.1	1.5	1.5	1:4.5	63.7
Apr. 5-6	4	141	17.5	0.8	7.1	7.1	1:3.1	59.2
Apr. 26-27	5	247	11.0	5.5	2.0	2.0	1:1.8	60.4
May 13-18	6	214	7.2	4.1	2.8	6.5	1:2.4	81.4

TABLE B4. Data from the QUE(ND) line of *A. aphidimyza*, selected for nondiapause under LD 8:16 (21°C)

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13	% Emergence by day 14
Oct. 6-8/82	1	188	61.0	0.6	3.2	2.7	1:1.4	0	5.9
Oct. 16-22	2	109	13.9	0	1.8	4.5	1:35	0.01	17.5
Nov. 4-14	3	487	27.1	0	0.1	6.0	1:35.2	0	3.1
Nov. 27-Dec. 5	4	341	13.4	3.7	3.3	9.4	1:38.5	4.2	21.2
Dec. 13-24	5	298	3.3	0.4	5.0	3.0	1:130.0	6.8	39.3
Jan. 7-8/83	6	94	0	3.2	1.1	1.1	1:9.9	3.8	35.0
Jan. 28-29	7	299	7.3	1.4	1.7	2.0	1:20.6	19.8	80.2
Feb. 17-18	8	108	1.2	0	4.6	14.8	1:2.3	1.7	17.2
Mar. 7-15	9	215	3.8	0.9	7.0	7.4	1:173.0	1.7	17.7

TABLE B5. Data from the QUE2(ND) line of *A. aphidimyza*, selected for nondiapause under LD 8:16 (21°C).

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂: ♀	% Emergence by day 13
Jan. 4-5/83	1	244	51.1	0.4	2.9	2.9	1:2.0	80.9
Jan. 22-25	2	253	24.6	0	2.0	0	1:7.0	82.4
Feb. 10-13	3	214	14.9	0.5	1.4	1.4	1:3.7	79.5
Mar. 1-4	4	244	4.8	0.4	2.9	2.5	1:1.2	64.4
Mar. 19-20	5	235	5.7	2.6	1.7	3.0	1:5.2	60.9
Apr. 7-10	6	187	3.6	2.4	3.2	8.1	1:2.6	19.4
Apr. 30-May 1	7	178	10.1	9.5	4.5	6.7	1:1.3	61.4
May 19-23	8	312	8.0	4.1	3.3	5.2	1:3.4	54.3
June 7-9	9	360	5.6	3.1	4.2	6.4	1:1.6	44.9
June 25-28	10	196	5.0	2.2	7.6	4.1	1:2.1	36.3

TABLE B6. Data from the ARB2 control line of *A. aphidimyza*; samples reared under LD 8:16 (21°C).

Date collected	Generation[1]	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Sept. 2-7/83	-	225	67.5	17.8	9.8	2.7	1:0.8	3.4
Nov. 8-9	-	333	67.9	1.5	5.1	6.0	1:0.1	0
Dec. 21-23	-	186	75.7	0	3.8	3.2	1:0.2	12.5
Jan. 11-12/84	-	190	84.1	0	2.6	1.6	1:1.6	0
Jan. 22-23	-	340	82.1	1.2	4.1	2.4	1:2.3	0
Feb. 27-28	-	62	83.3	0	0	3.2	1:0.1	10.0
Apr. 3-4	-	343	74.6	0.3	2.0	1.5	1:4.9	2.4
Apr. 27-28	-	132	19.7	2.6	4.6	6.8	1:90.0	20.9
May 10-12	-	140	63.5	1.5	1.4	0.7	1:2.2	6.4
Aug. 19-20	1	498	41.4	1.3	3.2	3.6	1:2.4	70.7
Sept. 26-27	3	267	35.4	6.7	1.1	3.7	1:1:9	81.6
Oct. 15-16	4	315	45.5	7.7	3.8	1.9	1:2.8	37.4
Nov. 5-6	5	360	48.3	2.3	1.9	1.9	1:2.1	32.7
Nov. 24-25	6	261	49.0	5.1	1.2	1.2	1:1.3	47.0
Dec. 16	7	233	42.5	5.4	3.0	2.2	1:1.1	72.1
Jan. 7-8/85	8	197	16.8	5.8	2.0	1.0	1:3.0	40.1
Jan. 25-26	9	178	3.1	3.1	2.8	6.7	1:1.4	14.6
Feb. 22	10	92	42.0	1.1	3.3	1.1	1:1.1	72.0
Mar. 7	11	70	20.3	1.6	2.9	5.7	1:0.3	20.0
Mar. 26-27	12	307	28.9	1.4	2.3	4.1	1:0.7	58.5
Apr. 15-17	13	248	24.3	6.2	1.6	7.3	1:2.0	35.7
May 6-7	14	130	36.1	1.6	1.5	4.6	1:1.0	14.5

[1] Sample coincides with generation of ARB2(ND) line.

TABLE B7. Data from the ARB2(ND) line of *A. aphidimyza*, selected for nondiapause under LD 8:16 (21°C).

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
Aug. 19-20/84	1	498	41.4	1.3	3.2	3.6	1:2.4	70.7
Sept. 6	2	338	69.5	3.8	3.6	2.4	1:4.3	2.4
Sept. 25-26	3	268	15.6	6.7	2.2	7.1	1:0.9	34.4
Oct. 15-16	4	275	37.6	13.4	4.4	3.6	1:0.8	23.4
Nov. 5-6	5	353	29.3	3.6	1.4	2.8	1:1.0	27.8
Nov. 24-25	6	156	28.0	5.3	0	3.9	1:1.0	24.0
Dec. 15-16	7	293	10.9	3.5	1.4	1.7	1:2.8	79.8
Jan. 5-6/85	8	277	10.8	6.4	3.6	6.1	1:1.0	35.1
Jan. 25-26	9	126	5.7	3.8	6.4	9.5	1:2.7	28.1
Feb. 12	10	194	1.1	0.6	1.6	4.1	1:1.6	83.9
Mar. 6-7	11	336	36.7	5.9	1.5	2.7	1:1.5	46.5
Mar. 26-28	12	268	10.3	8.3	2.6	3.4	1:1.6	51.0
Apr. 19-17	13	217	22.6	6.5	3.7	4.6	1:2.0	53.2
May 5-6	14	189	4.0	2.9	2.6	4.8	1:1.0	55.2

TABLE B8. Data from generations under relaxed selection of the FIN(ND) line of *A. aphidimyza*, reared under LD 8:16 (21°C).

Date collected	Generation	n	% Diapause	% Late pupation	% Mortality larvae	% Mortality pupae	Sex ratio ♂ : ♀	% Emergence by day 13
REL 1:								
June 28-29/83	1	55	6.2	2.1	0	12.7	1:3.3	-
July 13-14	2	303	14.8	12.7	5.9	13.5	1:2.4	-
Aug. 7-9	4[1]	259	2.3	0.5	4.2	11.6	1:2.9	-
Aug. 21	5	89	2.6	3.8	2.2	10.1	1:0.9	-
Aug. 29-30	7[1]	255	23.5	3.0	1.6	6.7	1:4.3	-
Sept. 29-30	8	352	44.5	6.9	4.5	5.4	1:1.7	-
Oct. 12-13	9	335	12.7	2.0	3.9	6.9	1:2.6	-
Oct. 25-26	10	486	11.0	2.8	4.1	8.0	1:1.1	-
REL 2:								
Nov. 29-Dec 1/83	1	302	8.3	1.8	3.6	4.3	1:2.1	64.4
Dec. 10-12	2	327	10.2	4.2	5.5	8.0	1:1.5	20.2
Dec. 27	3	427	38.9	2.5	2.1	4.0	1:2.4	9.4
Jan. 15/84	4	400	12.9	2.0	5.0	8.0	1:0.9	29.0
Feb. 2-3	5	327	44.1	1.7	5.2	3.4	1:1.0	21.0
Feb. 24	6	69	24.5	11.5	7.2	4.4	1:1.3	35.9
Mar. 13-14	7	256	13.0	2.5	2.3	4.7	1:1.2	34.8
Apr. 6-7	8	216	33.0	0.5	4.6	4.2	1:1.3	48.8
Apr. 25-26	9	379	54.6	0.8	2.1	1.3	1:1.4	13.5
May 13-14	10	364	40.8	1.7	1.6	0.8	1:1.5	67.6
May 25-26	11	229	15.4	3.6	5.7	9.2	1:2.2	47.5
June 17-19	12	230	34.7	2.3	0.4	4.4	1:2.8	54.4

[1] No data for generations 3 and 6 because of insufficient aphids.

APPENDIX C.

Data from release rates experiments using *A. aphidimyza* to control *M. persicae* in the greenhouse during winter.

TABLE C1. Results of releasing 1 predator : 3 aphids.

Day	Replicate 1 aphids $\bar{x} \pm SE$	Replicate 1 eggs $\bar{x} \pm SE$	Replicate 1 larvae $\bar{x} \pm SE$	Replicate 2 aphids $\bar{x} \pm SE$	Replicate 2 eggs $\bar{x} \pm SE$	Replicate 2 larvae $\bar{x} \pm SE$	Replicate 3 aphids $\bar{x} \pm SE$	Replicate 3 eggs $\bar{x} \pm SE$	Replicate 3 larvae $\bar{x} \pm SE$
3	11.8 ± 3.4	3.1 ± 2.0	0	15.7 ± 5.5	7.7 ± 3.8	0	11.8 ± 4.6	15.8 ± 5.5	0
8	44.0 ± 17.9	4.7 ± 4.1	0.7 ± 0.5	22.7 ± 7.3	0.3 ± 0.2	3.3 ± 2.1	27.8 ± 12.2	12.0 ± 5.6	8.5 ± 3.7
13	48.2 ± 15.6	0.2 ± 0.2	5.5 ± 2.9	19.5 ± 9.1	0.3 ± 0.3	1.8 ± 0.9	1.3 ± 0.9	0.7 ± 0.6	4.8 ± 1.3
18	47.7 ± 20.1	22.5 ± 8.8	1.2 ± 0.6	13.5 ± 6.2	16.3 ± 7.2	1.0 ± 0.6	0	6.7 ± 2.7	1.7 ± 0.5
23	8.8 ± 4.8	6.7 ± 1.1	18.8 ± 5.7	16.2 ± 6.8	5.3 ± 3.4	2.8 ± 1.2	0	2.3 ± 1.6	0.7 ± 0.5
28	0	4.0 ± 1.4	6.8 ± 2.1	4.3 ± 1.3	1.0 ± 1.2	3.2 ± 1.2	2.2 ± 2.0	0	0.5 ± 0.5
33	0.8 ± 0.8	7.2 ± 3.8	0.7 ± 0.3	7.8 ± 5.0	0	2.0 ± 1.6	7.0 ± 1.2	0	0
38	0.3 ± 0.2	41.2 ± 19.4	15.7 ± 1.7	10.7 ± 5.8	5.3 ± 3.8	0.7 ± 0.3	6.7 ± 3.8	0	0
43	0.2 ± 0.2	5.7 ± 4.7	2.5 ± 1.0	34.5 ± 19.9	0	1.0 ± 1.3	18.0 ± 7.7	0	0
48	0	15.2 ± 5.3	0.7 ± 0.3	35.0 ± 23.4	0	3.7 ± 1.8	25.8 ± 8.5	0	1.0 ± 0.5
53	0.5 ± 0.5	0.2 ± 0.2	2.0 ± 0.9	12.2 ± 8.0	2.7 ± 1.8	2.0 ± 1.1	71.0 ± 25.1	1.5 ± 1.1	0.2 ± 0.2
58	0.3 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	76.3 ± 38.2	38.8 ± 28.2	8.3 ± 7.1	173.0 ± 39.4	7.8 ± 4.9	3.7 ± 2.7
63	2.7 ± 2.1	0.5 ± 0.3	0.2 ± 0.2	21.3 ± 6.9	4.8 ± 1.5	17.0 ± 6.0	84.8 ± 10.2	1.7 ± 0.8	5.7 ± 2.1

TABLE C2. Results of releasing 1 predator : 10 aphids

	Replicate 1			Replicate 2			Replicate 3		
	aphids	eggs	larvae	aphids	eggs	larvae	aphids	eggs	larvae
Day	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
3	19.6 ± 7.6	2.0 ± 1.7	0	15.0 ± 5.1	4.2 ± 2.9	0	14.2 ± 5.6	7.2 ± 3.8	0
8	34.3 ± 14.0	0.2 ± 0.2	0	34.8 ± 12.4	5.5 ± 3.0	2.5 ± 1.6	36.3 ± 13.6	9.3 ± 3.1	5.3 ± 2.5
13	30.3 ± 7.3	1.8 ± 1.2	7.0 ± 1.2	34.7 ± 18.4	5.7 ± 4.7	6.0 ± 1.8	6.0 ± 2.2	0.2 ± 0.2	12.8 ± 8.8
18	31.2 ± 13.3	15.8 ± 6.2	1.5 ± 0.8	8.5 ± 3.6	2.2 ± 1.5	1.0 ± 0.6	0.2 ± 0.2	0.2 ± 0.2	0.7 ± 0.3
23	65.8 ± 22.0	4.7 ± 1.9	10.2 ± 5.1	6.5 ± 3.5	2.7 ± 1.4	2.3 ± 1.9	0.3 ± 0.3	0.7 ± 0.4	0.8 ± 0.3
28	14.8 ± 9.0	1.2 ± 1.0	7.5 ± 1.4	0.7 ± 0.4	0.3 ± 0.3	2.0 ± 1.2	1.3 ± 1.1	0.3 ± 0.3	0
33	2.7 ± 1.4	0.7 ± 0.7	2.0 ± 0.3	4.5 ± 4.3	0	0.3 ± 0.2	16.2 ± 9.7	0	0.3 ± 0.3
38	6.7 ± 3.7	16.5 ± 8.2	1.7 ± 0.5	4.0 ± 1.5	1.5 ± 0.7	0.2 ± 0.2	21.8 ± 11.8	1.2 ± 1.0	0
43	16.8 ± 8.2	1.7 ± 0.5	9.8 ± 4.1	12.0 ± 6.0	2.5 ± 1.3	13.7 ± 9.4	94.2 ± 28.5	8.5 ± 3.9	0.3 ± 0.3
48	0.3 ± 0.3	1.0 ± 0.8	9.0 ± 5.6	0.3 ± 0.2	3.0 ± 1.8	2.2 ± 1.1	135.3 ± 30.2	3.2 ± 1.2	4.5 ± 3.0
53	0	32.2 ± 14.1	4.5 ± 2.1	1.3 ± 0.7	5.5 ± 1.6	1.5 ± 0.4	78.3 ± 28.4	5.8 ± 3.1	2.5 ± 1.1
58	0	34.8 ± 20.4	20.8 ± 8.2	0.5 ± 0.3	10.0 ± 3.7	3.3 ± 1.0	61.5 ± 13.8	2.5 ± 1.3	6.0 ± 2.6
63	0	0.3 ± 0.3	2.8 ± 0.9	0.5 ± 0.4	0.3 ± 0.2	0.7 ± 0.5	82.5 ± 22.1	1.0 ± 1.0	2.7 ± 0.8

TABLE C3. Results of releasing 1 predator : 50 aphids

	Replicate 1			Replicate 2			Replicate 3		
	aphids	eggs	larvae	aphids	eggs	larvae	aphids	eggs	larvae
Day	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
3	9.0 ± 2.4	0	0	6.5 ± 1.6	1.2 ± 0.8	0	28.5 ± 8.0	2.3 ± 1.6	0
8	28.8 ± 13.3	0	0	31.0 ± 13.0	1.8 ± 1.3	0.3 ± 0.3	52.7 ± 12.7	0.7 ± 6.5	0.2 ± 0.2
13	86.3 ± 18.7	0	0	54.3 ± 11.6	0.2 ± 0.2	0.3 ± 0.2	81.0 ± 21.4	0	1.2 ± 0.6
18	91.0 ± 29.8	1.3 ± 0.7	0.2 ± 0.2	53.5 ± 19.6	1.5 ± 1.0	0.2 ± 0.2	64.5 ± 20.4	1.2 ± 0.7	0.8 ± 0.5
23	181.2 ± 64.9	3.4 ± 1.3	1.2 ± 1.0	79.5 ± 14.2	0	1.5 ± 0.8	127.0 ± 18.7	3.2 ± 2.2	1.0 ± 0.5
28	120.7 ± 28.6	3.5 ± 2.3	3.3 ± 1.1	79.8 ± 23.7	0	1.3 ± 0.6	199.0 ± 3.5	1.3 ± 0.3	2.5 ± 0.4
33	108.3 ± 18.9	0	1.7 ± 1.1	133.8 ± 42.9	3.7 ± 2.4	0.7 ± 0.7	171.0 ± 18.8	15.8 ± 4.4	3.2 ± 1.6
38	152.3 ± 62.0	8.3 ± 3.0	1.2 ± 0.7	151.3 ± 22.9	12.5 ± 2.9	1.8 ± 0.4	233.2 ± 33.5	63.0 ± 11.7	17.5 ± 4.4
43	134.7 ± 23.5	0.5 ± 0.3	5.0 ± 1.8	123.7 ± 23.4	5.8 ± 2.7	25.7 ± 7.0	44.5 ± 18.9	6.3 ± 2.9	44.7 ± 22.9
48	28.0 ± 11.7	5.2 ± 2.6	5.2 ± 1.7	1.8 ± 2.6	1.3 ± 1.1	10.5 ± 3.1	0.3 ± 0.2	1.8 ± 0.8	12.5 ± 2.0
53	12.7 ± 11.1	64.3 ± 28.8	6.3 ± 3.1	0	3.2 ± 1.6	5.8 ± 1.6	0.2 ± 0.2	18.8 ± 9.8	8.3 ± 2.7
58	0	33.3 ± 10.7	32.3 ± 12.8	0	3.0 ± 2.2	6.0 ± 3.1	0.2 ± 0.2	0	35.0 ± 0.8
63	0.3 ± 0.3	5.2 ± 2.9	5.2 ± 1.8	0	0.2 ± 0.2	2.7 ± 0.7	2.8 ± 2.4	2.0 ± 1.1	1.5 ± 0.3

TABLE C4. Results of releasing 1 predator : 100 aphids

	Replicate 1			Replicate 2			Replicate 3		
	aphids	eggs	larvae	aphids	eggs	larvae	aphids	eggs	larvae
Day	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
3	5.8 ± 2.2	0	0	14.7 ± 1.6	0.2 ± 0.2	0	11.5 ± 3.7	0	0
8	18.0 ± 8.3	0	0	38.3 ± 15.2	1.2 ± 1.2	0.3 ± 0.2	48.2 ± 17.4	4.0 ± 1.6	0
13	82.7 ± 16.3	0	0	75.3 ± 13.2	0.5 ± 0.5	0.2 ± 0.2	76.5 ± 7.8	1.5 ± 0.9	1.0 ± 0.4
18	168.3 ± 41.0	3.3 ± 1.0	0	69.2 ± 15.0	3.3 ± 1.2	2.5 ± 1.6	77.2 ± 27.5	0.2 ± 0.2	2.7 ± 1.5
23	183.0 ± 36.9	1.5 ± 0.5	1.3 ± 0.9	60.0 ± 30.5	0.5 ± 0.5	0.2 ± 0.2	65.8 ± 14.2	0	0
28	186.2 ± 41.0	1.2 ± 0.6	1.2 ± 0.3	129.8 ± 36.4	0	4.5 ± 1.9	103.0 ± 33.4	0.8 ± 0.4	1.0 ± 0.6
33	208.7 ± 16.4	3.8 ± 2.5	1.0 ± 0.8	127.8 ± 28.4	0.7 ± 0.3	1.7 ± 0.7	119.3 ± 25.7	2.7 ± 1.3	1.2 ± 0.6
38	221.8 ± 34.3	9.0 ± 2.9	3.0 ± 1.7	147.7 ± 30.4	20.7 ± 11.0	0.7 ± 0.5	260.7 ± 37.8	5.7 ± 2.5	1.8 ± 0.8
43	78.3 ± 30.6	0.2 ± 0.2	13.7 ± 5.0	139.7 ± 19.8	3.2 ± 1.2	12.3 ± 5.9	210.7 ± 51.1	1.5 ± 0.7	3.5 ± 1.5
48	31.3 ± 25.1	6.5 ± 2.9	4.3 ± 1.6	28.3 ± 15.0	7.2 ± 4.4	5.7 ± 1.8	32.6 ± 13.3	17.3 ± 4.0	6.2 ± 2.0
53	25.7 ± 19.7	83.5 ± 29.1	29.3 ± 11.5	11.7 ± 8.6	18.2 ± 6.2	6.0 ± 1.5	56.8 ± 23.2	20.8 ± 5.0	12.0 ± 3.1
58	0	18.2 ± 3.1	17.5 ± 4.8	2.0 ± 1.0	9.0 ± 3.7	7.7 ± 2.6	37.0 ± 22.8	15.7 ± 6.4	16.3 ± 4.3
63	0	11.3 ± 6.0	3.3 ± 1.8	0	0.5 ± 0.3	12.7 ± 0.7	0.7 ± 0.6	4.3 ± 1.7	5.7 ± 0.5

TABLE C5. Fruit yield, number of leaves and height of plants at conclusion of first release rates experiment.

Replicate	midge : aphids	fruit/cage[1]	Weight of fruit/cage (g)	Leaves ($\bar{x} \pm$ S D)	Height (cm) ($\bar{x} \pm$ S D)
1	1 : 3	4	28	14.1 + 0.9	32.2 ± 1.5
	1 : 10	2	36.3	15.0 + 0.8	29.6 + 1.3
	1 : 50	0	0	10.4 + 0.6	24.2 + 0.8
	1 : 100	0	0	9.2 + 0.5	25.0 + 0.9
2	1 : 3	6	131.5	14.8 + 0.9	27.2 + 1.2
	1 : 10	9	172.2	16.7 + 0.9	30.3 + 1.5
	1 : 50	0	0	13.8 + 0.7	27.1 + 0.9
	1 : 100	0	0	13.4 + 0.7	24.9 + 0.7
3	1 : 3	9	132.3	15.8 + 0.8	29.0 + 1.2
	1 : 10	2	29.1	16.1 + 0.9	28.2 + 1.2
	1 : 50	1	27.9	14.8 + 0.8	28.2 + 0.8
	1 : 100	1	18.2	10.8 + 0.7	24.1 + 1.0

[1] Twenty pepper plants per cage.

TABLE C6. Results of second release rates experiment (1 predator : 10 aphids).

	Replicate 1			Replicate 2			Replicate 3		
	aphids	eggs	larvae	aphids	eggs	larvae	aphids	eggs	larvae
Day	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
1	15.8 ± 7.3	1.0 ± 0.8	0	9.5 ± 2.9	0.2 ± 0.2	0	15.5 ± 8.6	1.8 ± 1.2	0
5	99.7 ± 20.8	1.2 ± 0.9	4.8 ± 2.3	81.7 ± 40.4	6.2 ± 2.9	4.5 ± 4.3	85.2 ± 24.7	1.2 ± 0.8	6.5 ± 5.2
10	115.7 ± 29.5	7.5 ± 2.9	2.0 ± 1.3	74.2 ± 28.0	4.2 ± 2.0	2.2 ± 1.0	178.2 ± 45.5	14.8 ± 6.2	2.5 ± 1.5
15	124.2 ± 24.8	6.2 ± 2.3	2.0 ± 0.9	107.7 ± 26.9	8.5 ± 2.9	1.3 ± 1.1	102.8 ± 20.4	6.3 ± 2.2	1.5 ± 0.6
18	157.2 ± 39.4	6.7 ± 2.8	6.7 ± 3.2	167.5 ± 30.9	8.7 ± 3.3	12.0 ± 4.1	194.8 ± 25.3	6.5 ± 1.8	11.7 ± 4.2
21	101.3 ± 29.2	4.3 ± 1.4	3.8 ± 1.3	163.8 ± 44.0	4.2 ± 2.0	13.8 ± 5.5	146.5 ± 36.1	1.8 ± 0.7	26.8 ± 8.5

APPENDIX D.

TALKS AND PUBLICATIONS ARISING FROM THIS RESEARCH

Publications

Gilkeson, L.A., and S.B. Hill. 1986. Genetic selection for and evaluation of a nondiapause lines of predatory midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Can. Entomol. (In press).

Gilkeson, L.A., and S.B. Hill. Diapause prevention in *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Environ. Entomol. (submitted).

Gilkeson, L.A., and S.B. Hill. Role of aphid diet in influencing diapause in the predatory midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). (In preparation).

Gilkeson, L.A., and S.B. Hill. Biological control of aphids by the predatory midge, *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidomyiidae), under winter greenhouse conditions. (In preparation).

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Talks

Gilkeson, L.A. 1986. Biological control of pests in Canadian greenhouses. Agric. Can. Res. Station, Ste. Foy, Quebec. Jan. 9.

Gilkeson, L.A. 1985. Biological control of aphids in winter greenhouse crops using the midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Entomol. Soc. Can., Annual Meeting, Ottawa, Ont. Sept. 24.

Gilkeson, L.A. 1985. Biological control of aphids with the predatory midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Biol. Control Lab., C.A.A.S., Beijing, P.R.C. May 28.

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Gilkeson, L.A. 1982. Biological control of aphids in greenhouses with *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae). Joint Meeting Entomol. Soc. Amer., Can. and Ont., Toronto, Ont. Nov. 31.