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E. Rolando López-Gutiérrez
University of Massachusetts Amherst

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BEHAVIORAL ECOLOGY OF *MYIOPHARUS DORYPHORAE* AND *MYIOPHARUS ABERRANS*, TACHINID PARASITOIDS OF THE COLORADO POTATO BEETLE.

A Dissertation Presented

by

E. ROLANDO LÓPEZ-GUTIÉRREZ

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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May 1995

Department of Entomology

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Approved as to style and content by:

David N. Ferro, Chair

David Leonard
David Leonard, Member

Joseph S Elkinton
Joseph S. Elkinton, Member

Gail L. Schumann
Gail Schumann, Member

Ring T. Cardé, Department Head
Entomology

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ABSTRACT

BEHAVIORAL ECOLOGY OF *MYIOPHARUS DORYPHORAE* AND *MYIOPHARUS ABERRANS*, TACHINID PARASITOIDS OF THE COLORADO POTATO BEETLE.

MAY 1995

E. ROLANDO LÓPEZ-GUTIÉRREZ. B.Sc., UNIV. DEL VALLE DE GUATEMALA
MA, UNIVERSITY OF MASSACHUSETTS AMHERST
Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST
Directed by: Professor David N. Ferro

The life history and behavioral ecology of *Myiopharus doryphorae* (Riley) and *Myiopharus aberrans* (Townsend), important parasitoids of the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say), were investigated through a series of field and laboratory studies.

The recruitment-recruitment method of determining percentage parasitism was compared with traditional methods in assessing population dynamics of the CPB and *Myiopharus*. Over the three-year period of the study, percentage parasitism calculated from traditional foliage sampling showed an erratic pattern. Revised estimates employing the recruitment method revealed a consistent 30-50% mortality of CPB larvae due to parasitism even when the CPB prepupal population density reached 80 per square meter per generation, showing that *Myiopharus spp.* can cause high levels of mortality to CPB larvae at higher host densities than has been reported in most previous field studies.

Field sampling demonstrated that *M. doryphorae* and *M. aberrans* overwinter as first-instar larvae within adult diapausing CPB and complete their development the following spring after the emergence of the parasitized beetles.

Growth-chamber studies were conducted to quantify development of summer-generation *M. doryphorae* at different life stages. During the first four days after being larviposited, these *M. doryphorae* remain as first-instar larvae but grow an average of 0.45 ± 0.03 mm prior to the prepupal stage of their hosts, in which the parasitoids complete development. During this latter period, development rates of *M. doryphorae* were found to track closely those of the CPB itself when modeled as a nonlinear function of temperature assuming cessation of growth outside the approximate range of 4 - 34° C. The model appears to require additional adjustment at temperatures below 10° C.

Laboratory studies showed that *M. doryphorae* do not discriminate between CPB larvae fed sublethal doses of *B. thuringiensis* and larvae not fed with *B. thuringiensis*. *M. doryphorae* appear more sensitive to CPB larval movement than to the presence or absence of *B. thuringiensis.*

Through field studies a series of behaviors was identified and their frequency and duration were quantified for the two *Myiopharus* spp., which appeared not to vary their allocation of time in response to each other's presence in the same field. A significant difference was found between the frequencies of larviposition by the two *Myiopharus* species across ranges of temperature and time of day.

Behavioral studies led to the discovery that larvipositing *M. doryphorae* and *M. aberrans* females discriminate between parasitized and non-parasitized host larvae, rejecting the former on contact and failing to larviposit in them when other potential hosts are available. This discrimination breaks down to some extent late in the growing season when CPB larvae of appropriate stages are rare; breakdown of host discrimination is accompanied, however, by the defense of recently parasitized hosts by females of both *Myiopharus* species, and is followed by the switch of larvipositing *M. aberrans* from larval hosts to adult CPB which are more common at this time of year.

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CHAPTER 1

LIFE HISTORY OF THE COLORADO POTATO BEETLE AND ITS NATURAL ENEMIES

Pest Status and Management Strategies of the Colorado Potato Beetle

The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) is the principal insect pest defoliating potato *Solanum tuberosum* L. in the northeastern United States and Europe (Ferro 1985, Hough-Goldstein *et al.* 1993). CPB primary hosts include buffalbur (*Solanum rostratum* Dunal), *Solanum elaeagnifolium* Cavanilles and *Solanum angustifolium* Miller, and it first shifted to potatoes in the southwestern United States some 150 years ago (Hare 1990, Casagrande 1985).

The life history characteristics of the CPB include its very high fecundity early in the season which can exceed 4000 eggs per female during its life-span (Brown *et al.* 1980). Additionally, it may migrate as much as 150 km per year (Hurst 1969, Jolivet 1991, Weber 1992) and it may overwinter through one, two, three and four years, allowing the beetle to emerge whenever the environment is most appropriate to complete its cycle (Ushatinskaya 1978). These characteristics, together with its great genetic variability (Hsiao 1984, 1985), contribute to the difficulty of controlling this pest.

Pest management strategies based on heavy use of insecticides led to the development of resistance to DDT by the CPB in the 1950s. Insecticides developed during the next 35 years were also rendered ineffective as the beetle successively developed resistance to each new insecticide in progressively shorter periods of time

(Forgash 1985). Ground water contamination and increasing costs as a result of these management strategies influenced growers as well as extension agents to reconsider and reevaluate the role of some natural enemies as part the CPB management strategies.

The use of natural enemies alone has not been satisfactory for controlling CPB in commercial crops. However, recent experience growing organic potatoes on small and medium size farms in northeastern USA has shown promising results (Ferro 1993). An essential ingredient in any program using natural enemies is the thorough knowledge of their life history and behavior. And in the case of CPB in particular, those natural enemies characteristics which might counteract the beetle fecundity, migration and/or overwintering habits.

Natural Enemies of the Colorado Potato Beetle.

Numerous Arachnida, Neuroptera, Hemiptera, Diptera and Hymenoptera species have been found to attack different stages of the CPB (Ferro 1993, Hare 1990). *Beauveria bassiana* (Balsamo) (Anderson *et al.* 1988, Hare 1990, Hough-Goldstein *et al.* 1993) and *Bacillus thuringiensis* Berliner var. *San Diego* and *tenebrionis* (both of them available in commercial formulations in the USA and in Europe) are the two entomopathogens most widely used to control the CPB. However, CPB resistance to *Bacillus thuringiensis* has already been documented (Whalon *et al.* 1993). One of the limitations for commercial biological control of the CPB is that many of the beetle's natural enemies are of tropical or subtropical origin with limited possibilities for surviving the winters of temperate zones where most of the potato crop in the world is produced

(Hough-Goldstein *et al.* 1993). This is the case with the extensively studied egg parasitoid of the CPB, *Edovum puttleri* Grissell (Hymenoptera: Eulophidae), that was introduced to USA from Colombia in 1981 (Grissell 1981). It has been found that *E. puttleri* kills CPB eggs by parasitism as well as by probing (Corriga *et al.* 1991, Lashomb *et al.* 1987a & b). In augmentative releases on eggplants this species has been shown to attack 71 - 91 % of egg masses to cause 67 - 79 % parasitism of eggs per egg-mass (Lashomb *et al.* 1987b, 1989). *E. puttleri* can not survive the cold winters of temperate zones, and it has to be maintained during the winter using laboratory rearing methods (Schroder *et al.* 1985). Because *E. puttleri* attacks only the egg stage of the beetle and releases are made only at commercial potato sites, it is very unlikely that *Edovum* would be able to cope with the beetle's migration abilities, and it would probably miss beetles reproducing in their many wild solanaceous host plants.

The only natural enemies known to attack mainly the CPB and already adapted to the temperate zones of the USA are the tachinid parasitoids *Myiopharus (=Doryphorophaga) macella* Reinhard, *M. (=Doryphorophaga) australis* Reinhard, M. *(=Doryphorophaga) doryphorae* (Riley) and *M. (=Adoryphorophaga) aberrans* (Townsend) (Diptera: Tachinidae) (Arnaud 1978). *M. doryporae* and *M. aberrans* have been the most studied as biological control agents, but unfortunately knowledge of their life history and behavioral ecology is still very limited.

M. doryphorae and *M. aberrans* are the only two indigenous parasitoids that are consistently found in northern USA potato regions. *M. doryphorae* had been reported in field populations of CPB since late 1800 (Riley 1869, 1871, 1872). After these pioneering studies, several other authors studied *M. doryphorae* (Biliotti and Persoons 1965, Feytaud 1938, Kelleher 1960 & 1966, Lipa 1985, Tamaki *et al.* 1983a, b, Trouvelot 1931). *M. doryphorae* larviposits on second through early fourth instar CPB larvae. Once the parasitoid larva is in the host, it remains undeveloped until the host completes its development and the CPB burrows into the ground for pupation (Tamaki *et al.* 1983a, b). At this point the *Myiopharus* larva resumes growth, killing its host and completing its development in about 8-10 days at mean summer temperatures of 24 to 28 ° C (Tamaki *et al.* 1983a & b, Kelleher 1960). Several studies have shown *M. doryphorae* to have two generations per year in northern regions (i.e. Canada: Manitoba and Ontario) (Kelleher 1966) and in northern USA (Horton & Capinera 1987, Tamaki *et al.* 1983a, b). In general, the number of *Myiopharus* generations will depend on the number of CPB generations per year which are related to the latitude and the length of the growing season (Hurst, 1975). Some studies in the USA have indicated an inverse relationship between population densities of *M. doryphorae* and its host (Kelleher 1960, Tamaki *et al.* 1983a), with low percentages of parasitism early in the season when beetle populations are the highest but an increasing percentage of parasitism later in the season when the beetle populations start declining and most of the potato crop has been already damaged by the beetles. However, in a few cases in which beetle populations were not very high, *Myiopharus* has reached high levels of parasitism throughout the entire season (Bjelgovic 1968, Horton & Capinera 1987).

Much less has been written about the biology and ecology of *Myiopharus aberrans* (Gollands *et al.* 1991, Hough-Goldstein *et al.* 1993, López *et al.* 1992). M. *doryphorae* and *M. aberrans* have been found sharing the same potato fields, and both appear to parasitize the same larval stages of their host. Due to the common way of determining *Myiopharus* parasitism in CPB (dissecting field collected CPB larvae under the microscope) and because the larvae of both *Myiopharus* species look very similar under a standard microscope, it is very likely that the parasitism often attributed to *M. doryphorae* alone may in fact be the combined effect of *M. doryphorae* and *M. aberrans*. This and some other possible assumptions surrounding *Myiopharus* parasitism needed clarification. This could be achieved only through the direct field observation and quantification of *Myiopharus* behavior.

Purpose of the Study.

It has become apparent that at the base of any sound biological control program is a knowledge of host and parasitoid behaviors and interactions. Host-parasitoid and parasitoid-parasitoid behavioral interaction studies under field conditions in tachinids are very limited (Clausen 1944), in spite of tachinid parasitoids being extensively used as biological control agents (Grenier 1988). More detailed behavioral studies under field conditions of these interactions have been recommended during the last decade (Van Lenteren 1981).

Tamaki *et al.* (1983a) suggested that before further work aimed to establish the actual role of *Myiopharus* in the population dynamics of the CPB is done, it would be necessary to determine their overwintering biology. Besides the knowledge of the overwintering biology of *Myiopharus*, several other areas of the biology, ecology and behavior of the CPB - *Myiopharus* relation needed investigation. In this endeavor, two basic lines of inquiry were undertaken: a biological approach and a behavioral ecology approach. Under the former approach the overwintering biology, as well as development rates and percentage parasitism were investigated. Under the latter approach, the behavioral ecology of M. doryphorae and *M. aberrans* and the beetles' response to *Myiopharus* attacks were thoroughly studied in the field. As part of the behavioral ecology approach, 8 different *Myiopharus* behaviors as well as the discrimination ability of both *Myiopharus* species were investigated under laboratory and field conditions. Because of the widespread use of *Bacillus thuringiensis* var. *tenebrionis* against CPB in potato fields where *Myiopharus* is also present, the behavioral response of *Myiopharus* toward immature stages of CPB treated with lethal and sublethal doses of *Bacillus thuringiensis* subsp. *tenebrionis* was also studied.

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CHAPTER 2

OVERWINTERING BIOLOGY OF *MYIOPHARUS ABERRANS* AND *MYIOPHARUS DORYPHORAE*

Introduction

Myiopharus species are the principal indigenous parasitoids of the Colorado potato beetle in North America, where they are widely distributed and are capable of overwintering in temperate areas. They appear to be relatively host specific and are able to locate hosts efficiently at low host densities (López & Ferro, 1990 unpublished data). *Myiopharus doryphorae* (Riley) has been reported to cause 30-70% parasitism in the second generation of field populations of the Colorado potato beetle (Horton & Capinera 1987, Tamaki *et al.* 1983, Kelleher 1960).

Flies of the spring-early summer generation insert first instar larvae subcutaneously into late first through early fourth instar Colorado potato beetle larvae. Parasitoid larvae remain in their larval hosts with little or no growth until the last instar of the host burrows into the soil to pupate. At this time, the parasitoid begins its larval development and consumes the internal organs of the host pre-pupa. The parasitoid pupates inside the remains of the pre-pupa and later emerges from the soil to parasitize second generation Colorado potato beetle larvae in late summer (Kelleher 1960, Bjegovic 1968 and Tamaki *et al.* 1983). In the first host generation flies emerge from the soil approximately 8 days (288 DD, López & Ferro 1990 unpublished data) after the parasitized 4th instar enter the soil.

Grenier (1988) cites the lack of knowledge of the biology and ecology of tachinid parasitoids as one of the main reasons for failure when using these parasitoids in applied biological control programs. This appears to be the case for *Myiopharus spp.* which were introduced into Europe as control agents for the Colorado potato beetle, but failed to establish (Briand 1935, Feytaud 1938, Lipa 1985).

Despite several studies on the biology and ecology of *Myiopharus spp.* (Franz 1957, Kelleher 1960, Billoti & Persoons 1965 and Tamaki *et al.* 1983) the complete life histories and in particular how the parasitoids overwinter were not identified. Kelleher (1960) dissected field-collected adult beetles in August and September and found about 5% to be parasitized by *Myiopharus spp.* He stated that these *Myiopharus spp.* first instars were ones which had not had sufficient time to complete their development and were, in his opinion, certain to die. Kelleher (1960) also collected adult beetles from the field the following June and dissected them but found no parasitoids. Franz (1957) suggested that the tachinids probably overwintered in their puparia in the soil. Kelleher (1960) tested the viability of *Myiopharus* puparia subjected to winter temperatures by placing fly puparia from field and laboratory samples, in moist sand at 40^{o} F. After about two months, he found no indication of diapause and no flies emerged from these puparia. Several other attempts to determine the overwintering biology of *Myiopharus spp.* were also unsuccessful (Tamaki *et al.*, 1983, Kelleher 1960).

We hypothesized that *Myiopharus spp.* overwintered either (1) as adults in undisturbed habitats adjacent to potato fields, or (2) as larvae inside Colorado potato beetle larvae or pre-pupae or (3) as pupae in puparia in the soil or (4) as larvae within overwintering adult Colorado potato beetles.

Methods

To determine if adult flies were the overwintering stage, observations were made in September of 1989 to locate night time resting sites used by flies. We reasoned that flies were likely to select similar places to overwinter and that such observations would indicate sites to monitor later in the year. During the first two hours after dusk, the foliage of a late planted potato field and that of the surrounding vegetation were thoroughly searched for flies. Sites included the foliage and bark of forest trees (maple, oak, birch, hemlock, cherry etc.) and the foliage of various herbaceous weeds.

To determine if *Myiopharus spp.* overwintered as larvae within Colorado potato beetle larvae, as pupae in their puparia in the soil or as prepupae or adults, soil samples were collected at South Deerfield, Massachusetts, USA, from mid-April to early May of 1990 in fields which had been planted to potato the previous year. Soil was removed from approximately 20 randomly selected sites (4 m^2 each) to a depth of 40 to 50 cm, the expected maximum depth of the majority of overwintering Colorado potato beetles. The soil was sieved and inspected for fly puparia and Colorado potato beetle adults, larvae and pupae. Soil temperatures had not exceeded 12° C prior to the date of our collections and it is unlikely that any significant parasitoid development would have occurred prior to the beetles being taken to the laboratory. No fly puparia or Colorado potato beetle larvae, prepupae or pupae were found, but a total of 379 overwintered adult Colorado potato beetles were collected. These adults were immediately placed individually into 30 ml ventilated plastic cups and covered with a 15 ml layer of moist, sterile, potting soil. A subsample of 67 beetles was used to detect overwintered *Myiopharus spp.* flies by placing the cups into a growth chamber (16:8, L:D) first at 17° C ± 2° C for 23 days and then at 24° C ± 2° C (16:8, L:D), for 28 days. The number of surviving adult beetles was recorded as was the number of emerged flies.

Results and Discussion.

During a total of 30 hours of evening field observations, only two flies were observed in early September, both on the lower leaves of potato plants. Because potato fields are cultivated at the end of the season, flies could not succesfully overwinter on crop plants. No flies were observed on vegetation surrounding the potato field nor were any flies observed flying from the potato field.

A total of five *M. aberrans* and two *M. doryphorae* flies emerged from seven of the subsample of 67 beetles collected from the soil in the spring of 1990 (Fig. 2.1). Because the beetles were collected before they had become active in the spring, it is unclear whether in nature beetles first move to the soil surface and flies then emerge from the beetles, or if the flies emerge from the beetles after they are completely out of the soil or if both behaviors occur. On one occasion an overwintered beetle emerged from the soil and actively fed before dying. Five days after the beetle died it was dissected and a fly puparium was found. Three adult Colorado potato beetle exoskeletons with parasitoid emergence holes were found on the soil surface of a commercial potato field on May 14, 1990, indicating that some beetles emerge from the soil before the parasitoids complete their development.

This is the first report of adult *Myiopharus spp.* flies emerging from overwintered Colorado potato beetle adults. It suggests that *Myiopharus spp.* larvae remain as undeveloped first instars within beetles from the previous summer. Colorado potato beetle adults that emerge after August 1 do not produce flight muscles or a reproductive system (Voss et al., 1988), and this response to a shortening day length appears to be regulated by juvenile hormone (JH) titer (de Wilde *et al.* 1968). After emerging from the soil, summer adult beetles feed for about two weeks, then burrow into the soil to overwinter or may first walk to overwintering sites outside of the potato field (Voss & Ferro 1990). The parasitoid larva could be responding to the low level of JH in the Colorado potato beetle hemolymph, or could be responding to some factor produced by larvipositing female flies which responds to the shortened day length.

Once diapause development is complete, and the soil temperature rises above 10° C, overwintered beetles regenerate their flight muscles and emerge from the soil in about 288 DD (Ferro unpublished). On the average it took 183 DD (ca. 12 days at 25° C) for flies to emerge from post-diapausing beetles after they had emerged from the soil and had become active. This is about the same amount of time it takes for *Myiopharus* development to be completed from the time Colorado potato beetle non-diapausing parasitized larvae burrow into the soil to fly emergence during the summer. Resumption of *Myiopharus* larval development within the post-diapausing beetle might possibly happens sometime after the adult Colorado potato beetle emergence from the soil.

Fig. 2.1 A *Myiopharus doryphorae* fly emerging from an overwintered Colorado potato beetle.

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CHAPTER 3

TEMPERATURE-DEPENDENT DEVELOPMENT RATE OF *MYIOPHARUS DORYPHORAE*, A PARASITOID OF THE COLORADO POTATO BEETLE

Introduction

The relationship between temperature and insect development has long been recognized (Sanderson & Peairs 1913, Uvarov 1931, Davison 1944, Richards 1957, Howe 1967) along with the consequent utility of this environmental parameter in modeling insect population dynamics. Accurate estimates of the development rate of both pest insects and their natural enemies are of obvious significance in the elaboration of control programs; during the past decade the use of phenological models for use in integrated pest management has increased considerably (Wagner *et al.* 1984b, Worner 1991, 1992). Linear degree-day models were some of the earliest to be developed and were applied to a large number of insect species (Howe 1967), but the non-linearity of growth found at high and low temperatures demonstrated these models to be often unrealistic. A number of nonlinear models, some of them empirical (Logan *et al.* 1976, Hilbert & Logan 1983) and others theoretical (Sharpe & DeMichele 1977, Schoolfield *et al.* 1981) have been elaborated for a wide variety of species and circumstances; further work (Gould & Elkinton 1990, Hébert & Cloutier 1990, Hilbert & Logan 1983, Wagner *et al.* 1984b) has applied and evaluated these models.

Among the nonlinear models referred to above, most have been found reliable in predicting development rates principally in the middle ranges of applicable temperatures. The Logan equation (Logan *et al.* 1976) is of the form: $r(\mathrm{T}) = \Psi(e^{\rho \mathrm{T}} - e^{\rho \mathrm{Tm} - \mathrm{T}})$ where Ψ is the development rate at base temperature, ρ is a composite Q_{10} for the critical enzyme-catalyzed biochemical reactions and the temperature (T) terms are determined by the experimenter. The Logan model normally overestimates growth at low temperatures, showing insect development at 0° C in some cases (Hilbert & Logan 1983). Sharpe &

DeMichele's model was based on a more elaborate equation that used absolute temperatures and incorporated the universal gas constant and the concept of enthalpy associated with rate-controlling enzyme activation or deactivation (Sharpe & DeMichele 1977). This model has been shown also to overestimate insect growth at certain temperatures: at low temperatures the model approaches zero development asymptotically and so predicts positive developmental rates even at 0° C in the cases modeled; it also predicts fairly high rates of developement at elevated temperatures which are known to be lethal (Hilbert & Logan 1983 and Gould & Elkinton 1990). The Hilbert & Logan Model is described by $r\,(T) = \Psi[\,(T^2/T^2+D^2) - e^{-(Tm-T)/\Delta T}]$ where $r(T)$ is the rate of development at temperature T; $T= T_o - T_b$ where T_o is the air temperature and T_b is the developmental base temperature; Ψ is the developmental rate at the base temperature T_b; Tm is the lethal maximum temperature; D is a "shape" parameter that control the inflection point of the sigmoid curve(it is an empirical parameter); and ΔT is the temperature range between developmental maximum and Tm.

The Hilbert & Logan model has been more realistic in not predicting growth at the low and high temperatures known to be lethal for the Colorado potato beetle (CPB) (Ferro *et al.* 1985). Based on this feature and on the success of this model in describing CPB larval and pupal development (Ferro *et al.* 1985, Logan *et al.* 1985), Hilbert and Logan's model was chosen for application in the present study to *Myiopharus doryphorae* Riley, an important parasitoid of the CPB in North America.

With very few exceptions (Gould & Elkinton 1990, Nealis 1984), these nonlinear temperature-dependent growth models have been tested in each instance on single, non-parasitic insect species. No temperature-related development rate studies have been conducted on *M. doryphorae*. The relationship of temperature to egg and larval development rates has been simulated for another tachinid, *Winthemia fumiferanae* Toth. a parasitoid of the spruce budworm, *Choristoneura fumiferana* Clem. (Lepidoptera: Tortricidae) (Hébert & Cloutier 1990). However, several important differences exist between the life cycles of *W. fumiferana* and *M. doryphorae*. *W. fumiferana* oviposits on a lepidopteran host and completes its pupal growth outside of the host. *M. doryphorae* has more than one generation per year: an overwintering generation placed as first-instar larvae within the host's larval stage late in the season which completes development when the adult beetles emerge in the spring (López *et al*. 1992 & Chapter 2) and a summer generation also larviposited into beetle larvae but completing development at the host prepupal stage within the same growing season. In both *M. doryphorae* generations, unlike *W. fumiferana*, parasitoid growth occurs entirely within the host. Due to the many complications inherent in the overwintering generation, the summer generation of M. *doryphorae* was selected here for initial evaluation of the development model.

It has been known for some time (Trouvelot 1931, Kelleher 1960) that *M. doryphorae* remain as first instar larvae throughout the larval development of their CPB hosts. Some authors have taken this to mean that no parasitoid growth occurs during this time, although Kelleher (1960) reported an average difference in length of 0.64 mm between recently laid first-instar *M. doryphorae* larvae and presumably older first-instar parasitoids found in "mature" CPB larvae. An initial aspect of this project was to establish whether the first instar grow while in its larval host before it host pupated. The second aspect was to model the temperature dependent growth of the remaining larval stage of the summer-generation *M. doryphorae* within pre-pupal CPB.

Methods

Larval Growth of *Myiopharus doryphorae* Before its Host Prepupal Stage

Early third instar CPB larvae from a laboratory colony were exposed to five fertile *Myiopharus doryphorae* flies in a 35 x 35 x 35 cm plexiglass-framed and metal screen cage under laboratory conditions. After each CPB larva was parasitized it was immediately removed from the cage using soft tweezers and placed in a 25 x 5 mm plastic petri dish with fresh potato foliage until 75 parasitized CPB larvae were obtained. Twenty-five of the parasitized CPB larvae were dissected immediately after parasitization and the length of the *Myiopharus* larvae found within them was recorded. The remaining 50 parasitized CPB larvae were separated into two groups of 25 each in petri dishes and simultaneously placed in a 27 ° C and 80% RH growth chamber. Fresh potato foliage was provided every day in the petri dishes as food for the larvae. Half (25) of the remaining parasitized larvae were dissected after 48 hours and the other half after 96 hours. In all cases parasitoid larvae were measured after dissection.

Using these measurements, the growth of the parasitoid first instars within third and fourth instar CPB of its host was regressed on hours after larvipositon using both a linear model and a quadratic regression model (Sigmaplot 2.0, 1994).

Temperature-Dependent Development Rate of Colorado Potato Beetle and *Myiopharus*

Colorado potato beetle adults and *Myiopharus* flies used in this experiment were collected in July-August 1990 from a potato field (CV. Katahdin) located at the University of Massachusetts Experiment Station in South Deerfield, Mass. and maintained under greenhouse conditions (25± 3°C, 80% RH, and constant photoperiod of 16:8 L:D).

From the first progeny of the beetles, 300 third-instars were exposed to fertile *Myiopharus* flies until each of them had been parasitized. All of these parasitized CPB larvae were kept in the greenhouse in ventilated plastic containers (7 x 7 x 14 cm) over a 2-cm layer of moistened vermiculite. A total of 10 CPB larvae were left in each container. Each of these containers was provided with fresh potato foliage which was inserted into a florist's water pick containing quarter-strength Hoagland's solution (Hoagland & Arnon 1950); Potato foliage was changed every day until CPB larvae completed their development and dug into the vermiculite for pupation.

Another 300 CPB larvae not exposed to the flies were kept simultaneously under the same conditions as the parasitized ones. At the onset of CPB pupation, a set of 60 prepupae from each group (parasitized and not parasitized) were left in the same containers in which they were reared and placed in each of five growth chambers at 17, 20, 24, 27, 30 and 32° C, all of them kept at 16:8 LD photoperiod and 80% RH, to complete their development until adult emergence.

During the 1990 trials, the CPB larvae may have been parasitized by either of two *Myiopharus* species, *M. doryphorae* and *M. aberrans*, and it was not possible to discriminate among parasitized larvae by parasitoid species. Therefore, in 1991 the experiment was repeated using only *Myiopharus doryphorae*.

The Hilbert & Logan Model described above (Hilbert & Logan 1983) was fit to values of the geometric mean rate of development at each temperature which was calculated as: Mean developmental rate = $1/\exp[\sum \ln(D_i)/n]$ where D_i is the observed developmental time and n is the sample size. Mean developmental rates were computed in this way to correct for the skewed frequency distributions of developmental time (Logan *et al.* 1976).

Initial estimates of the four parameters were determined graphically as described in Logan et al. (1976). Non-linear regression (SAS Institute 1988) was used to fit the model to values of mean development rate by an iterative least-squares procedure using the Marquardt algorithm (Marquardt 1963). The non-linear regression was performed on temperatures below 34°C because higher temperatures produced >80% mortality of parasitized CPB larvae.

Results and Discussion.

Larval Growth of *Myiopharus doryphorae* Before its Host Prepupal Stage

Myiopharus doryphorae larvae raised at a constant temperature of 27° C during their host's larval development were 0.69 ± 0.021 mm long for recently laid parasitoid larvae, 1.01± 0.02 mm after 48 h and 1.14 ± 0.061 mm after 96 hours. This was the approximate time it took CPB larvae raised at 27° C to go from late third instar to prepupal stage (Logan *et. al.* 1985, Walgembach & Wyma 1984 and Ferro *et al.* 1985). A quadratic regression curve was fit to all data points. The quadratic regression $Y = 0.698 + 0.422\,x - 0.115\,x^2$ gave a fit of $r^2 = 0.767$ to our data for the *Myiopharus* first instar larval growth (Fig 3.1). The fitted curve indicated a steady growth of the *Myiopharus* first instars from zero to 48 h after larviposition. From 48 to 96 h the rate of growth appeared to decrease (Fig. 3.1). However, these aspects of the shape of the curve could largely be artifacts of the quadratic stipulation of the model; a larger number of temporal points would help to establish more closely any changes in rate of growth through time. This result support the study of Kelleher (1960) which showed that first instar *Myiopharus* growth to double during its host larval development before it reached the prepupal stage.

Temperature-Dependent Development Rate of Colorado Potato Beetle and *Myiopharus*

Parameter values for the Hilbert and Logan model (defined above for the 1990 experimental data) were estimated by Marquardt's nonlinear least squares with the function fit to data in Tables 3.1a and 3.2a. For the Marquardt's nonlinear least squares; computing 250 iterations were chosen because it has been shown that after that number of iterations in most cases the parameters values change very little. The sum of squares of errors (SSE) is an estimate of the fit of the model. The smaller the SSE the better the fit. The parameters for *Myiopharus sp.* were T_b = 2.36525; D = 86.3803; Ψ = 1.2688; T_m = 34 ; and ΔT = 1.1072 with a SSE = 0.000162. For the CPB the parameters estimated for 1990 were: T_b = 2.26751; D = 83.52402; Ψ = 1.18252; T_m = 34; and ΔT = 1.0949 with a SSE = 0.000162. Fig. 3.2 represents the plots for functions and data. In 1991 parameter values were again estimated by Marquardt's nonlinear least squares with the function fit to data in Tables 3.1b and 3.2b for *Myiopharus doryphorae* and CPB respectively. The parameters for *Myiopharus doryphorae* were: T_b =4.55643; D = 89.963020; Ψ = 1.530223; T_m = 34; and ΔT = 1.951225 with a SSE = 0.000021958. For CPB the parameters were: T_b = 3.873746; D = 106.87168; Ψ = 2.36702; T_m = 34; and ΔT = 1.4855 with a SSE = 0.00008298. Figure 3.2 presents the empirical data and model output. The development rate using the Hilbert and Logan equation projects to zero at $T \leq T_b$. The sum of squares errors in 1990 indicated a similar fit of the curve to the values for both CPB and *Myiopharus* (SSE = 0.000162). The SSE for the 1991 data indicated a much better fit of the curve to the values for both, the host and the parasitoid. However, the fit seems to be slightly better for *Myiopharus* than for CPB with values of 0.000021958 for *Myiopharus* and 0.00008298 for CPB.

The empirical nonlinear model of Hilbert & Logan predicted a lower temperature threshold for development of both CPB and *Myiopharus* than has normally been assumed (Tb= 10° C for most insects); the model's predictions were: T_b = 4.5564 °C for *Myiopharus* and T_b = 3.87 °C for the beetles. In both cases the predicted T_b value is unrealistic. The model aproximated better development rate data at higher temperatures, at least up to those of maximum development; beyond this, the model appeared to be more reliable than the earlier ones in that it predicted decline in growth reaching zero at 34 ° C, a temperature known to be lethal to these insects. Temperatures during the summer of 1990 reached the 8-9° C range only one day in May and after that, average temperatures 10 cm under the soil surface rose but never exceeded the 34 ° C lethal threshold. During 1991 the lowest temperatures were 7 -8° C only during 4 hours on May 4 and 1 hour on May 5. The rest of the summer was much hotter than the 1990 summer. At the University of Massachusetts experimental farm at 10 cm depth May to September there was a total of 67 hours of temperatures above 34° C for 15 different days between June 20 and August 17.

Several points should be taken into consideration in relation with the recording of temperature data and the behavior of the insects involved. All probes and thermistors from the recording device were placed on bare soil with no shade of any kind. Under these conditions one might expect the soil to be drier and hotter than the soil beneath the potato foliage where the parasitized beetle larvae are usually found. Another important factor is that the soil is of a sandy loam texture within which beetle larvae easily penetrate deep in the ground for pupation. Pupating larvae are frequently found between 10 to 15 cm below the soil surface (Chapter 4). Behavioral mechanisms undoubtedly contribute to these insects' thermoregulation and the avoidance of extreme temperatures (May 1979). If temperatures are low the larvae expose themselves to direct sunlight, while if it gets too hot they seek shelter on the undersides of foliage where the temperature is considerably cooler, especially during the hottest hours of the day (personal observations; Ferro & Voss 1985).

Table 3.1 Development of *Myiopharus sp.* and *Myiopharus doryphorae* at 7 temperature regimes. a) 1990 & b) 1991.

a) *Myiopharus* sp. 1990.	Parasitoid	Temperature, °C				
Host stage	stage	17	24	27	30	34
Prepupa-pupa	larva-pupa					
Mean develop. time (days)		38.3	15.1	12.7	12	11
SE		0.8	0.3	0.3	0.1	0.0
Number		60	60	60	60	60
No. not completing development		7	10	5	9	58
Developmental rate		0.0262	0.066	0.0789	0.0833	0.075

b) *Myiopharus doryphorae* 1991 Host stage	Parasitoid stage	20	25	28	30	32
Prepupa-pupa	larva-pupa					
Mean develop. time (days)		28.6	16.5	14.4	12.1	14.1
SE		0.5	0.3	0.2	0.3	0.2
Number		60	60	60	60	60
No. not completing development		0	0	4	4	18
Developmental rate		0.0437	0.076	0.0872	0.968	0.0768

Table 3.2 Development of Colorado potato beetle at 7 temperature regimes. a) 1990 & b) 1991.

a) 1990.	Temperature, °C				
	17	24	27	30	34
Stage: Prepupa-pupa					
Mean develop. time (days)	30.3	12.5	11.6	8.43	9.3
SE	0.8	0.4	0.3	0.1	0.1
Number	60	60	60	60	60
No. not completing development	0	0	0	6	18
Developmental rate	0.0329	0.08	0.0863	0.1187	0.1076

b) 1991.	20	25	28	30	32
Stage: Prepupa-pupa					
Mean develop. time (days)	22.31	15.1	11.3	9.8	11.9
SE	0.35	0.3	0.3	0.3	0.1
Number	60	60	60	60	60
No. not completing development	0	0	0	2	5
Developmental rate	0.056	0.083	0.1109	0.1274	0.1053

In spite of many valid criticisms (Worner 1991, 1992) regarding the possible inaccuracies of non-linear models using constant temperatures versus variable temperatures and under laboratory versus natural conditions, there still exists a need to predict population trends of both host and parasitoids in a variety of conditions for which no adequate alternative methods exist.

Our findings indicate that *Myiopharus* summer generation larval development includes three periods of growth: one, when parasitoid larvae are recently laid and before their host larvae reach maturity; a second period with limited larval growth between host larval maturity and the host prepupal stage; and a third, between the host prepupal stage and final adult *Myiopharus* emergence. Several other factors besides temperature seemed to be acting simultaneously upon *Myiopharus* development rates during each of the three periods. The focus here has been upon the third period of *Myiopharus* larval growth and its development rate was modeled as a function of temperature. To improve the fit of the model during this growth period, more experimental data covering the temperature range from 7 to 15° C are needed.

The development rate curves developed herein for CPB and *Myiopharus* based on the Hilbert and Logan model appear to be a workable tool for temperatures from 15 - 34° C. Taking into account the limitations mentioned above, the results from the model might well be used in conjunction with the "graphical method" of Southwood and Jepson (1963) as modified by Bellows *et al.* (1989) to estimate numbers of hosts and parasitoids entering a given stage.

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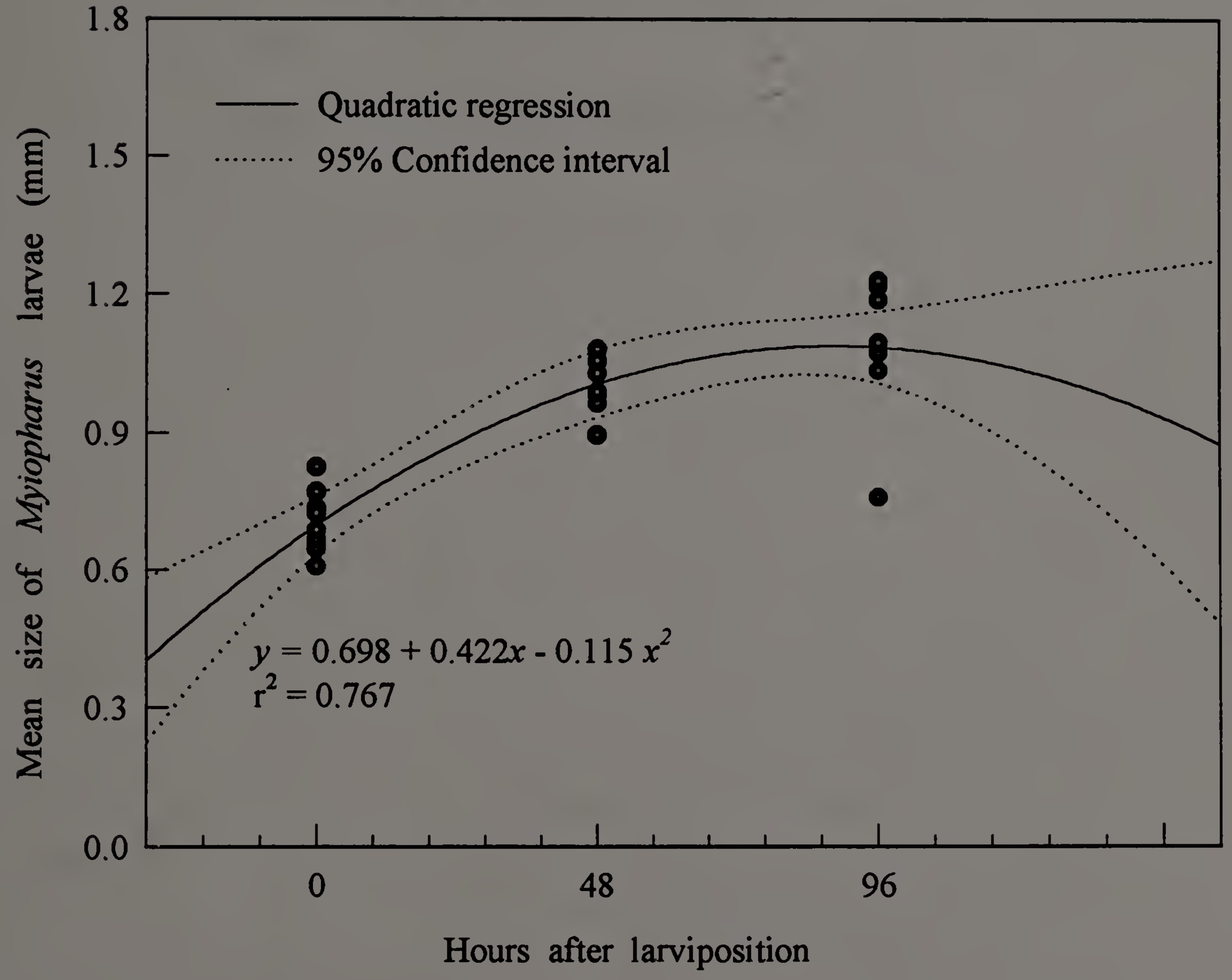

Fig. 3.1 Fitted quadratic regression curve to *Myiopharus doryphorae* first instar larval growth before CPB reaches the prepupal stage

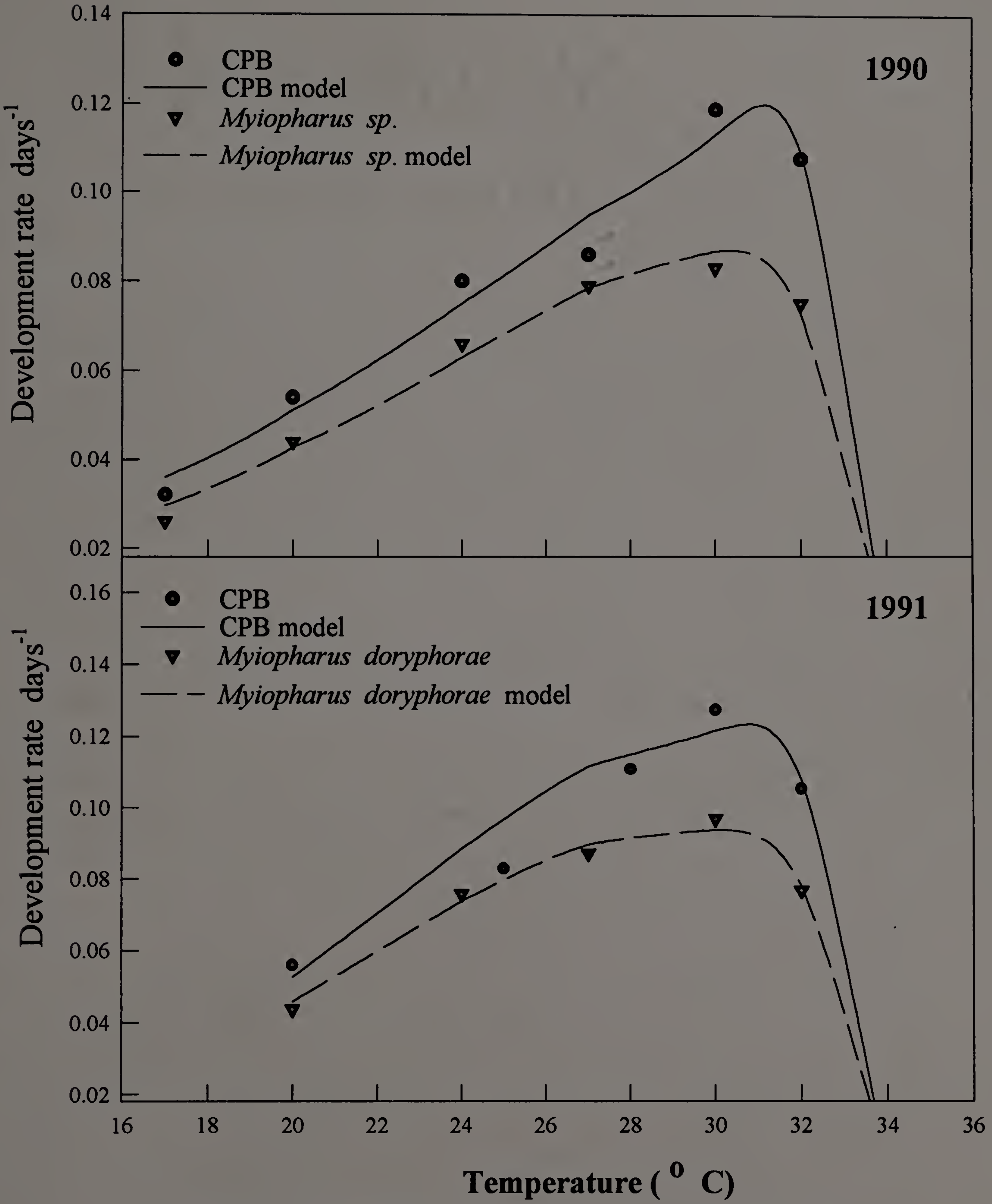

Fig. 3.2 Development rates of CPB and *Myiopharus sp.* modeled as a non-linear function of temperature.

CHAPTER 4

ASSESSMENT OF COLORADO POTATO BEETLE TOTAL LOSSES DUE TO PARASITISM BY *MYIOPHARUS SPP*.

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) is the most destructive insect pest of potato in the northeastern United States (Ferro 1985). Under favorable weather conditions, first generation CPB larvae and adults can cause up to 80-100% crop loss (Bjegovic 1968, Forgash 1981). *Myiopharus doryphorae* (Riley), together with *Myiopharus aberrans* (Townsend), are the most important parasitoids of CPB larvae in the United States (Riley 1869). However, it is not known why these parasitoids seem to be important control agents for CPB in certain regions of USA (Bjegovic 1968, Horton & Capinera 1987) but not in others (Kelleher 1960, Tamaki *et al.* 1983a).

The impact of *Myiopharus doryphorae* in past studies has been measured by dissecting CPB larvae collected periodically to assess larval density, determining the proportion parasitized. However, this approach fails to take into consideration some characteristics of the parasitoid's life history. The proportion of CPB larvae bearing *Myiopharus* maggots at any given time in field samples is the result of the cummulative maggot deposition in second through fourth instar CPB larvae because maggots defer development until the host digs into the soil and initiates pupation (Kelleher 1960, Tamaki *et al.* 1983b). This causes the proportion of host larvae bearing parasitoid maggots to be greatest in the final CPB instar (fourth). This distorts the estimates of the proportion of hosts attacked and erroneously suggests a preference for older larvae. Furthermore, as CPB larvae grow older, their defenses become more effective and by the time they reach fourth instar they tighten their cuticle during parasitoid's attack and become extremely difficult to penetrate (Tamaki *et al.* 1983b). Harcourt (1971) used life table methods to measure the impact of *Myiopharus* upon CPB populations and he concluded that Myiopharus was inversely densitiy dependent in its action with respect to CPB pupal densities.

The Colorado potato beetle *Leptinotarsa decemlineata* is a chrysomelid beetle native to south-central Mexico that entered the USA in or before the early 19th century and fed on wild solanaceous plants such as *Solanum rostratum* Dunal, *Solanum elaeagnifolium* Cav., *Solanum carolinense* L. and cultivated potato *Solanum tuberosum* L. (Jacques 1988, Gauthier *et al.* 1981). Adult beetles overwinter in soil. Female beetles emerge from soil in the spring, begin feeding on their host plant and lay up to 500 eggs in clusters of 25-50 on the undersides of leaves of potato or other crops. These eggs give rise to the first summer generation of larvae. Egg laying occurs over a 4-week period. Most adults die within 5 weeks after emerging from the soil (Jacques 1988). There are four larval instars. Mature larvae drop from plants, burrow into soil and construct a spherical cell 2-4 inches beneath the soil surface. In 4-7 days, larvae transform into yellowish pupae which persist for 5-10 days (Jacques 1988). In Massachusetts there are normally two CPB generations per year between May and September.

There are a number of native predators of CPB eggs and larvae in North America, including *Coleomegilla maculata* (De Geer), *Podisus maculiventris* (Say), *Perillus bioculatus* (Fabr.), *Lebia grandis* Hentz. In contrast the only indigenous larval parasitoids are *Myiopharus doryphorae* and *Myiopharus aberrans*, which are endoparasitic tachinids that larviposit directly into CPB larvae. Under field conditions, larviposition takes only 1-2 s when flies attack second through early fourth instars. Parasitoid maggots defer development until host larvae enter the soil to pupate. Parasitoids kill their hosts in the prepupal stage and complete their development in about 10 days (Tamaki *et al.* 1983b).

The objective of this research was to apply the recruitment method of Van Driesche and Bellows (1988) to the CPB - *Myiopharus spp.* system to obtain more accurate estimates of the impact of *Myiopharus* parasitism on CPB populations in Massachusetts.

Methods

Analytical Methods

The analytical approach used in this study was that of Van Driesche and Bellows (1988), taking into account that *Myiopharus spp.* are solitary parasitoids that normally do not superparasitize under field conditions. When more than one parasitoid maggot are deposited per host, only one survive finally emerging only one adult parasitoid from each host (R.L. unpublished).

Field Site Description

In 1989 and 1990 a 48 x 24 m field of potato *Solanum tuberosum* (c.v. Katahdin) was planted the first week of May at the UMASS farm in South Deerfield, MA, with 90 cm inter-row and 20 cm inter-plant spacings. Commercial practices were followed regarding hilling, fertilization, weed and disease controls (using standard dosages of metribuzin (4-amino-6-*tert*-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one) and metholachlor (2-chloro-6'-ethyl-N-(2-methoxy-1-methyl)acet-*o*-toluidide) for weed control and maneb (manganese ethylenebis(dithiocarbamate) (polymeric) or mancozeb (manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt) for disease control). M-One (Bacillus thuringiensis var. San Diego) was used once in each of the third and fourth weeks of June each year to keep CPB larval populations from defoliating the experimental plots. Early season potatoes such as c.v. Superior, planted in this area at the beginning of May normally become senescent a few weeks after the initiation of the second CPB generation. Late planted plots were established each year at the UMASS farm and in these plots the second generation CPB completed development by the end of August. In 1991, a 30 x 20 m plot of potatoes (c.v. Katahdin), with the same spacings among rows and plants and the same commercial practices, as above, was also established at the farm of Edward McGlew, in Hatfield MA, for the first beetle generation.

Estimating Colorado Potato Beetle Density and Percentage Parasitism

Study plots were sampled twice per week during the 1989 growing season and once per week during 1990-1991 growing seasons. To determine density of CPB larvae, 40 potato plants were randomly selected and examined. For the first beetle generation, the whole potato plant was searched for CPB egg masses, larvae and adults. For the second generation only the three main stalks on each plant were searched for CPB egg masses, larvae and adults. Counts were converted to numbers per m^2 by multiplying the number of CPB egg masses, larvae or adults per plant or stalks (for the second generation) by the number of plants per m^2, which was determined by counting the numbers of plants (or stalks) in a 1 m^2 wooden frame placed randomly in 20 different locations within the plot. All second through fourth instar CPB larvae were removed from the sample plants, placed in ventilated plastic petri dishes, and supplied with fresh potato foliage. Dishes were placed in an insulated chest together with ice packs and larvae returned to the laboratory for dissection. The experimental plot contained 1500-2000 potato plants such that the 40 plants from which larvae were removed on each sample occasion constituted only 2-3% of the plot in 1990-1991 and 4-6% in 1989 when sampling was done twice a week.

All CPB larvae collected from the field plots for dissections were second through fourth instars since first instars CPB larvae are too small for the parasitoid to larviposit in effectively (Tamaki *et al*. 1983a). Parasitism levels were assessed weekly in all three years by randomly selecting and dissecting 100 larvae from each larval instar present in samples on each sample date.

Assessment of Colorado Potato Beetle and Parasitoid Recruitment

The CPB prepupal stage and parasitoid maggots within prepupae were chosen as the stages into which CPB and parasitoid recruitment would be measured. Host recruitment for the first CPB generation was measured by placing metal screen cages under the ground individually surrounding 40 potato plants. Cages were designed so that all CPB larvae which dropped from the plant to the ground to pupate would be trapped in the cage and could be located when cages were unearthed and their contents examined. Before placing the screen cages in the soil, the top 8-10 cm of surface soil around each potato plant was removed with a shovel. In the first generation, cages were constructed in two halves and one half placed at each side of a potato plant. The two halves of the screen cage were put back together around the potato plant using and leaving an empty circle (5 cm diam.) in the center for the plant stalks. Each cage was them filled with the soil that had been removed and sifted to remove preexisting CPB stages and reshaped to form a hill. CPB prepupal and pupal stages together required 10 or more days in the ground prior to adult emergence at typical summer temperatures (Ferro *et al.* 1985). CPB prepupae and pupae found in soil in cages were placed in 4 x 8 x 5-cm ventilated plastic cages, covered with 1 cm of moist soil, returned to the laboratory where the larvae were dissected and examined for maggots. When more than 100 prepupae were recovered from all cages combined for the collection date, extra prepupae were reared in a growth chamber at 27°± 1 °C. Immediately after collecting prepupae from each screen cage, new plants for the recruitment estimation for the next week were selected and screen cages again buried surrounding these new plants as before. The number of pupae or prepupae in which parasitoid maggots were observed during dissection was taken as the estimate of parasitoid recruitment per unit area for the week. For sample occasions when prepupae and pupae were reared, counts of emerging adult flies and beetles were included in recruitment estimates.

To estimate CPB recruitment in the second generation, cages were placed in the ground between potato plants such that the equivalent of one whole plant was sampled. This practice was necessary because of the increased plant size and recumbent stature of mature plants. Cages were of the same size as those used in the first generation but were constructed as a single piece rather than two interlocking halves. Samples were processed as described for the first generation.

Results

Densities of CPB larvae (all instars combined) peaked at 182, 103 and 102 per m^2 on 2 July 1989, 1990 and 1991 respectively (Figs. 4.1c, 4.2c and 4.3c). Adult beetles per square meter peaked at 24, 17 and 27 on 6 August 1989 and 1990 and on 27 August 1991 respectively. For all three years, egg masses per square meter for the first generation exceeded the number in the second generation (Fig. 4.1a, 4.2a & 4.3a). In 1990, due to unusually high temperatures, adult CPB oviposition for the second generation was delayed and extremely reduced, resulting in the absence of a second generation of larvae.

Parasitism by *Myiopharus doryphorae* of CPB larvae collected in samples to determine larval density was always higher in the second than in the first generation for both large and small larvae. Percentage parasitism ranged from 1-4 % for second instar larvae and 2-12% for third and fourth instar larvae in the first generation and from 9-11% for second instar larvae and 10-37% for third and fourth instar larvae for the second generation during the three year period from 1989-1991 (Table 4.1 and Figs. 4.1c, 4.2c & 4.3c). Percentage parasitism from density samples for the same generation over the three year period showed a more erratic pattern than from the recruitment estimates (Figs. 4.1c-d, 4.2c-d & 4.3c-d).

The total number of CPB prepupae recruited to the population per m^2 ranged from 1 to 7 per day for the first generation and from 1 to 5 for the second generation. Parasitoid recruitment levels per square meter in the first CPB generation ranged from 0 to 0.5 per day and, for the second generation, ranged from 0.03 to 3.2 (Figs. 4.1d, 4.2d & 4.3d). Seasonal trends in host and parasitoid recruitments are shown in Figs. 4.1d, 4.2d, 4.3d and Table 4.2. In the first generation, mortality from parasitism was estimated by the recruitment method as 2.15, 34.33 and 33.91% for 1989, 1990 and 1991 respectively.

Recruitment estimates for CPB prepupae and *Myiopharus* for the second generations in 1989 and 1991 were 31.52% and 47.47% for CPB prepupae respectively.

Additional data from samples at four different organic farms in Franklin county in western Massachusetts, during 1990, also showed that beginning on 9 July *Myiopharus* was able to maintain 20% parasitism or higher with CPB larval densities ranging from 1-8 larvae/m^2 (Figs. 4.4a, 4.4b & 4.4c).

Discussion

Parasitism of second instar CPB larvae was lower than levels in large larvae (instars 3-4). While this could reflect a lower preference or attack efficiency on second instar larvae by *Myiopharus* flies, it could also be explained by noting that any *Myiopharus* maggots larviposited in second instar CPB larvae remain undeveloped until their hosts complete their development and drop to the ground for pupation. Thus, parasitized larvae within the large category (instars 3-4) represent the total parasitism accumulated over all four larval instars. Furthermore, field behavioral studies of *Myiopharus* confirm that the lower rate parasitism of first to second instar larval CPB is not because lower searching efficiency by *Myiopharus* but rather due to morphological and physiological limitations. *Myiopharus* needs to hold the CPB larva with its legs while larvipositing in order to successfully introduce its larvipositor into the host. It is difficult for *Myiopharus* to successfully grip first and early second instar CPB larvae because at this stage *Myiopharus*' larvipositor is almost as large as the CPB larva itself.

Seasonal trends in host and parasitoid recruitment indicated that *Myiopharus spp.* populations were able to parasitize 30-50% of hosts when CPB larval recruitment ranged from 1-5 larvae/m^2/day (Figs. 4.1d, 4.2d & 4.3d). This study demonstrates, contrary to the findings of other researchers, that *Myiopharus* can cause high levels of parasitism on CPB not only late in the season when beetle larval populations are lower but also through the season provided that beetle larval densities do not exccedd 5 larvae recruited/m^2/day.

At higher host densities, harcourt (1971) found substancially lower levels of *Myiopharus* parasitism.

Low levels of *Myiopharus* parasitism occurring early in first generation when CPB recruitments are 6 or more larvae per m^2/day still have to be explained. Several hypothesis have already been proposed. Possible differential mortality of overwintering parasitized beetles Tamaki *et al.* 1983b), which has not been tested yet in the field. It is known that CPB and *Myiopharus* differ in their lifetime fecundities. In the first generation, *Myiopharus* flies lay about 200 larvae over approximately 30 days (Tamaki *et al.* 1983b) compared to 450 eggs for CPB (D. N. Ferro unpublished information), placing flies in a reproductive disadvantage for the first generation of CPB. For the second generation the situation is reversed because flies continue to lay 200 larvae versus only 45 eggs per female beetle.

The difference between percentage parasitism determined from density samples and from recruitment data might be partially due to the eminently cumulative nature of parasitism at the prepupal stage (which was declared the recruitment stage) where parasitism is represented by the summation of all the maggots laid during the lifespan of each CPB larval instar from second through fourth. On the other hand, percentage parasitism from density samples was determined using CPB larvae that were at any point in their life history before they completed their development on each larval instar probably missing life-exposure time to the parasitoids.

The recruitment method for assessing total losses to parasitoids using metal screen cages buried around the potato plant and underneath its foliage was successfully applied to the *Myiopharus doryphorae* - Colorado potato beetle life-system. Furthermore, the recruitment method provides a more reliable index of percentage parasitism by *Myiopharus* on CPB adult generation than parasitism determined from density samples.

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Table 4.1 Percentage parasitism of CPB larvae by *Myiopharus doryphorae* in a potato field at South Deerfield MA, 1989-1991, summed across sample dates and divided by size classes.

	FIRST GENERATION			**SECOND GENERATION**			**SEASONAL AVE.**[b]		
1989	**%**	**SEM**	**(n)**	**%**	**SEM**	**(n)**	**%**	**SEM**	**(n)**
SMALL	3.59	3.5	(857)	10.8	3.2	(277)	7.4	2.4	(1134)
LARGE	12.00	3.8	(1761)	36.8	2.9	(430)	27.4	2.3	(2191)
AVERAGE[a]	3.00	1.3	(2618)	24.5	2.2	(707)	8.9	1.1	(3325)
1990									
SMALL	3.86	1.6	(1454)						
LARGE	2.80	0.7	(1621)	**No second generation this year.**					
AVERAGE[a]	1.19	0.4	(3075)						
1991									
SMALL	0.73	1.54	(295)	9.6	1.20	(146)	3.59	0.95	(441)
LARGE	1.71	0.89	(410)	17.9	0.91	(395)	6.90	0.64	(805)
AVERAGE[a]	1.57	0.49	(705)	15.7	1.54	(541)	2.84	0.41	(1246)

[a] *=Averages for combinations of CPB larvae sizes were obtained by weighting each category by its sample size.*

[b] *=Averages over time for the two generations were obtained by weighting the value for each sample date inversely to the sample variance.*

Table 4.2 Total host and parasitoid recruitment/m^2 for each generation in a potato field at S. Deerfield, MA. 1989-1991.

	FIRST GENERATION		SECOND GENERATION	
1989	*(28 June to 9 August)*		*(18 August to 12 Sept.)*	
	Total recruitment	**SEM**	**Total recruitment**	**SEM**
CPB	178.76	13.06	4.79	0.76
MYIOPHARUS	3.84	2.12	1.51	0.41
GENERATIONAL PARASITISM	2.15%	0.67%	31.52%	4.84%
1990	*(25 June to 13 August)*			
CPB	79.49	10.03	**No second generation this year.**	
MYIOPHARUS	27.29	7.99		
GENERATIONAL PARASITISM	34.33%	5.80%		
1991	*(30 May to 23 July)*		*(30 July to 30 August)*	
CPB	20.73	3.06	23.70	3.03
MYIOPHARUS	7.03	1.98	11.25	1.80
GENERATIONAL PARASITISM	33.91%	5.47%	47.47%	7.36%

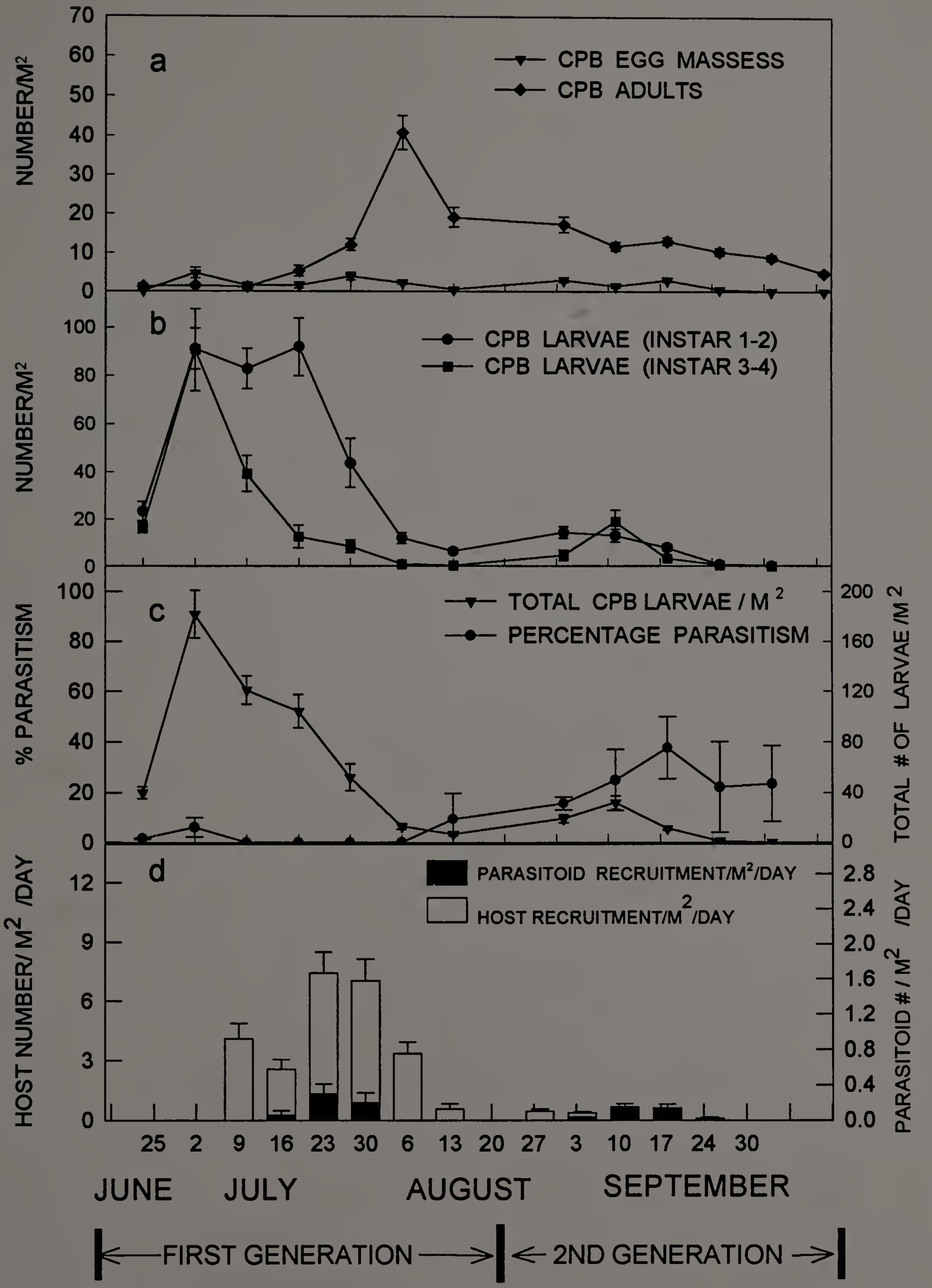

Fig. 4.1 Colorado potato beetle and *Myiopharus doryphorae* population dynamics. S. Deerfield, MA. 1989.

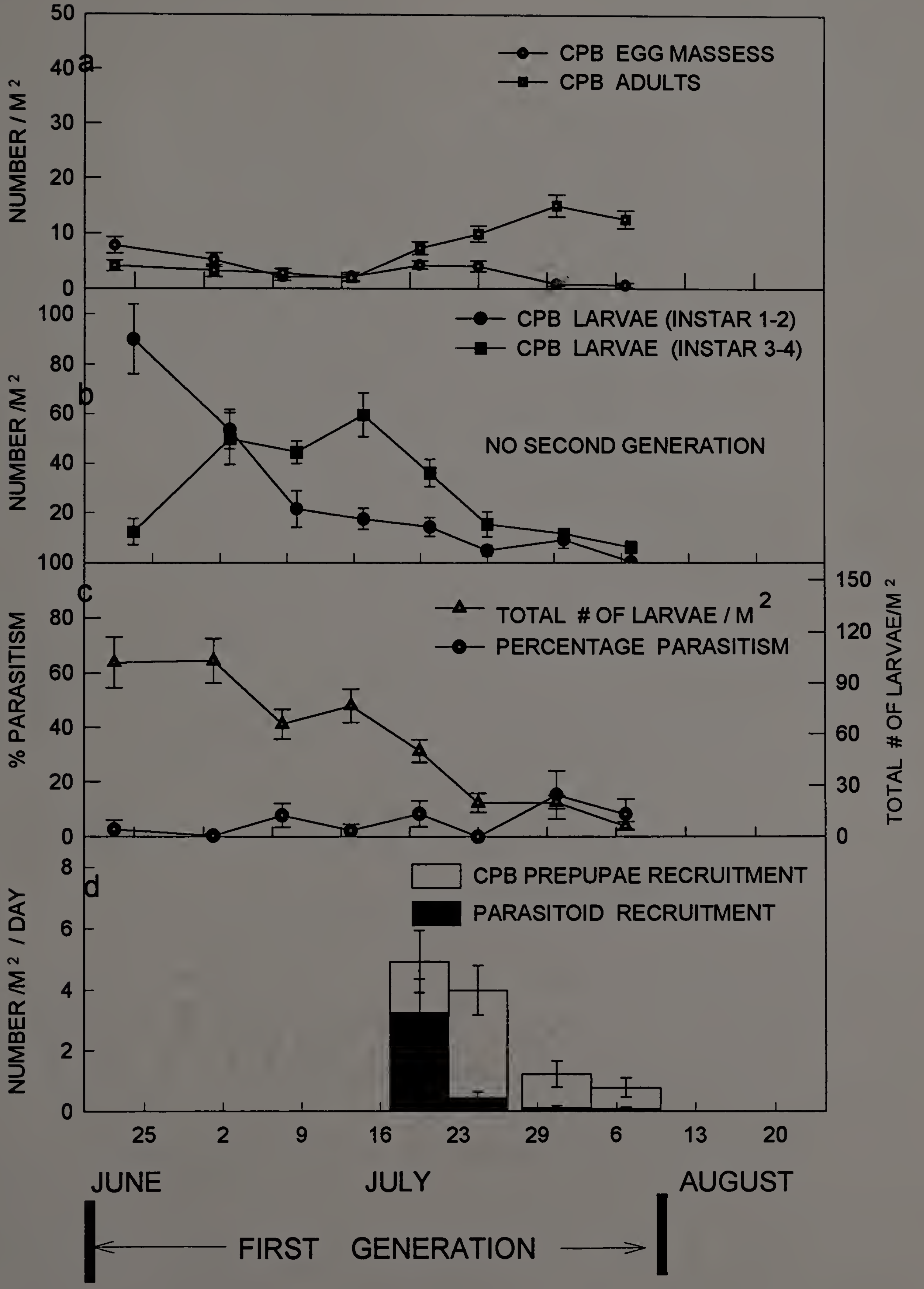

Fig. 4.2 Colorado potato beetle and *Myiopharus doryphorae* population dynamics. S. Deerfield, MA. 1990.

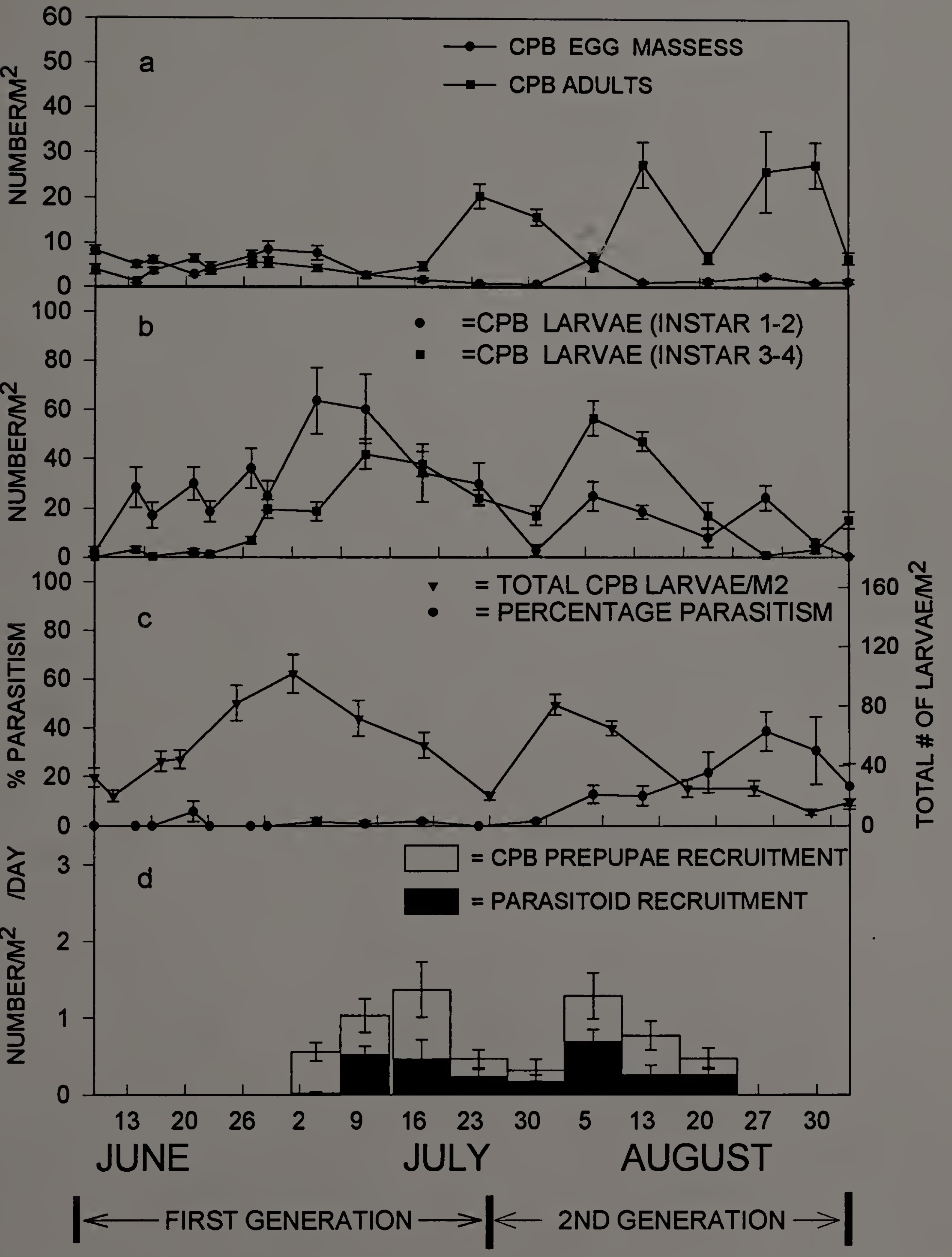

Fig. 4.3 Colorado potato beetle and *Myiopharus doryphorae* population dynamics. S. Deerfield, MA. 1991

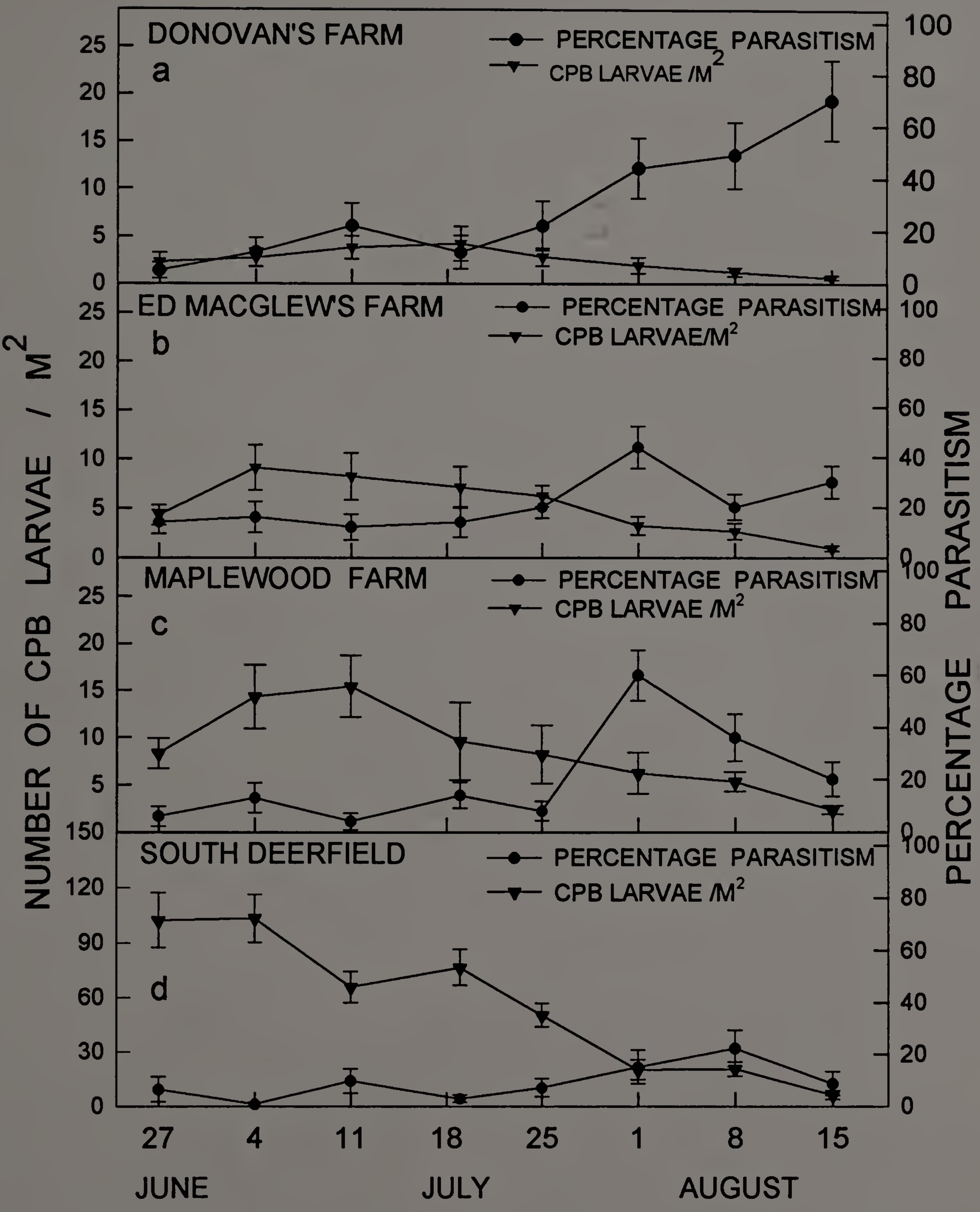

Fig. 4.4 Percentage parasitism and numbers of CPB larvae/m^2 at four organic potato farms in Franklin County, MA. 1990.

CHAPTER 5

HOST DISCRIMINATION ABILITY OF FEMALE *MYIOPHARUS SPP.*

Introduction

Host discrimination by a parasitoid is defined as its ability to distinguish non-parasitized from parasitized hosts (Salt 1934). Salt (1937) noted that the avoidance of superparasitization involves two distinct abilities: (1) the ability to discriminate between healthy and parasitized hosts, and (2) the ability to refrain from oviposition when suitable hosts are not available. However, Van Alphen *et al.* 1986, have shown that superparasitization by inexperienced parasitoids that encounter only parasitized hosts is adaptive from a foraging point of view and that the tendency to super parasitize may be influenced by experience (Van Alphen *et al.* 1987, Van Alphen & Visser 1990). Host discrimination by parasitoids prevents wastage of time and parasitoid offspring. It may also serve to cause adult parasitoids to move to new areas after finding several parasitized hosts in one place (van Lenteren 1981), thus distributing their offspring in space and time.

Host discrimination ability seems to be widespread among parasitic Hymenopotera (Salt 1961, Clausen 1940, Doutt 1959, Askew 1971, DeBach 1974, Huffaker & Messenger 1976, Ridgway & Vinson 1977). Askew (1971) wrote that discrimination had not been reported among parasitic Diptera and van Lenteren (1981) noted that there have been very few studies of ovipositional behavior of parasitic Diptera, especially under natural conditions. We believe this is the first report of tachinids being able to discriminate between parasitized and non parasitized hosts.

Myiopharus doryphorae (Riley) and *M. aberrans* (Townsend) are solitary tachinid parasitoids which attack second through early fourth instar larvae of *Leptinotarsa decemlineata* (Say), the Colorado potato beetle, in eastern United States (Kelleher 1960, Tamaki *et al.* 1983a & b, López *et al.* 1993). Only one adult parasitoid emerges from a parasitized host (Kelleher 1960). Kelleher (1960) found superparasitism in field-collected Colorado potato beetle larvae to occur very rarely and only late in the season. When it did occur only one parasitoid larva was found alive. Based on the behavior of other tachinids he presumed that the survivor actively killed the other larva although this has not yet been documented in *Myiopharus spp*.

The purpose of the present investigation was to study the larvipositional behavior of *M. doryphorae* and *M. aberrans* under laboratory and field conditions to examine their ability to discriminate between parasitized and non-parasitized hosts.

Methods

Preliminary Studies

The methods developed to answer the principal research question depended upon two preliminary studies aimed at establishing indicators of (a) the occurrence of successful larviposition and (b) the age of a developing parasitoid. These two studies and their results are presented prior to the description of the principal experiments.

Determination of Successful Larviposition. Under field conditions, *Myiopharus* flies larviposit in Colorado potato beetle larvae in 1-2 seconds and can be observed by eye from up to 80 cm (López *et al.* 1993). During the process of larviposition a *Myiopharus* fly mount a Colorado potato beetle larva, insert and remove its larvipositor while depositing its own larva, and a drop of hemolymph appear on the surface of the host larva. We adopted the observation of this series of behaviors as indications of successful larviposition. Rates of correct determination in the five studies that follow (based on subsequent dissections) were 100%, 85%, 83%, 96%, and 95%, respectively.

Red Pigmentation as Indicator of Parasitoid Larval Age. Kelleher (1960) reported the presence of bright red pigmentation in the lower body cavity of *Myiopharus* first instar. From the dissection of field samples of parasitized Colorado potato beetle larvae, we confirmed the presence of a bright red tissue in the lower cavity of the parasitoid larva, but we also found some larvae with no pigmentation at all or only a faint red pigment. We hypothesized that the intensity of pigmentation increased with parasitoid larva development; were this true, it would be possible to use presence or level of pigmentation as an indicator of time elapsed since larviposition.

To test our hypothesis, previously isolated early third instar Colorado potato beetle larvae were exposed to fertile *Myiopharus* flies under greenhouse conditions. Immediately after the larvae were parasitized they were placed under refrigeration (4° C) to minimize metabolism until 60 parasitized larvae were obtained. Ten of these larvae were immediately dissected and the larvae examined for red pigmentation. The remaining 50 parasitized larvae were placed over a 5 mm layer of moistened vermiculite together with fresh potato foliage on 150 mm diameter plastic petri dishes with ventilated lids. The dishes with the parasitized larvae were kept in an environmental chamber maintained at a constant temperature of 25 ± 2 ° C; L16:D8 and 80 ± 5% R.H. Every hour for five hours, 10 parasitized larvae were dissected. The inner body cavities of the parasitoid larvae were examined visually for the presence of red pigmentation. This procedure was repeated two more times under similar conditions. No red pigmentation was observed for the 120 parasitoid larvae dissected at 0, 1, 2 and 3 h after larviposition. All 60 larvae observed 4-5 h after larviposition clearly exhibited red pigmentation. We concluded that the presence of red pigmentation in the developing parasitoid indicates passage of at least 3 h since larviposition.

Laboratory experiments with *Myiopharus* were performed in a greenhouse located on the campus of the University of Massachusetts at Amherst. Cages were 35 x 35 x 35 cm Plexiglas-framed cages with metal screens for the top and three sides, a Plexiglas bottom, and an easily removable clear plastic sheet covering the fourth side to facilitate introduction and removal of larvae and flies. Field behavioral observations were made at the University Vegetable Research Farm in South Deerfield, Massachusetts and at Brookfield organic farm, Belchertown, MA.

Host Discrimination by *Myiopharus doryphorae* under Greenhouse Conditions

To obtain parasitized Colorado potato beetle larvae, previously isolated early third instar larvae were placed on potato foliage in a petri dish within a cage and exposed to fertile female *Myiopharus doryphorae* flies (8-12 days after emergence) under greenhouse conditions until 100 larvae were parasitized. These parasitized larvae were placed in two 150 mm diameter plastic petri dishes with ventilated lids, fed fresh potato foliage and kept overnight at 17± 1° C and 80± 5 % R.H. to allow parasitoids to develop. Another five hundred non-parasitized larvae (raised isolated from contact with parasitoids) of the same age as the parasitized larvae were kept overnight in separate cages from the parasitized ones but in the same growth chamber.

Twenty-four hours after parasitization, all Colorado potato beetle larvae were taken from the growth chamber and exposed in groups of 12 parasitized and 12 non-parasitized larvae at a time to different numbers of caged fertile female *M. doryphorae* flies. The larvae were placed in each of three 18 x 12 x 2.5 cm plastic trays having 24 individual wells, each 15 mm deep and 15 mm in diameter. The wells were half filled with jellied agar, and a 1 cm diameter disc of fresh potato foliage was placed on the agar as food for the larvae. One Colorado potato beetle larva was placed in each well, distributed by type within each tray as follows: a) 12 parasitized Colorado potato beetle larvae were placed randomly using a computerized random number generator on a 6 x 4 grid pattern and marked with a 1 mm permanent red ink dot on the border of the well, and b) one non-parasitized Colorado potato beetle larva (of the same instar as the parasitized larvae) was placed in each of the other 12 wells in the tray. One such tray of Colorado potato beetle larvae was introduced simultaneously into each of three cages containing 1, 2 and 4 fertile female *Myiopharus doryphorae* respectively. The flies had been kept isolated from Colorado potato beetle larvae for the 24 h prior to these exposures. Water and sugar cubes were placed on the floor of each cage for the flies.

Larvipositional behavior was carefully observed from the moment the trays were placed inside the cages. Each time a Colorado potato beetle larva was observed to be parasitized it was immediately removed using a different set of soft tweezers for each kind of larva (parasitized vs non-parasitized) and replaced with another larva of the same kind to maintain a constant proportion of parasitized and non-parasitized larvae within each cage. Physical contacts of flies with each larva (via fly tarsi or proboscis) as well as occurrence of larviposition were recorded.

After one hour, all flies in all cages as well as the 24 Colorado potato beetle larvae in the trays were replaced with new, fertile flies and with new parasitized and non-parasitized larvae. This process was repeated three times the daily. Flies and cages were combined such that each cage received one replicate of each number of flies (1, 2 , or 4).

Host Discrimination by *Myiopharus sp.* under Field Conditions: Experimental Test

Forty previously isolated early third instar larvae of Colorado potato beetle were parasitized in the laboratory by fertile *M. doryphorae* flies in late August of 1991. These parasitized larvae were kept together in a growth chamber maintained at 17° C; L16:D8 and 80 ± 5 R.H. but not in contact with the 120 non-parasitized larvae of the same age. Twenty-four hours later all larvae were taken to a potato field at the University of Massachusetts research farm. All parasitized larvae were marked on the pronotum with a 1 mm diameter yellow dot using a Speedball® non toxic Painters Opaque Paint Marker (Hunt manufacturing Co. Statesville, NC 28677) . Non-parasitized larvae were marked with a blue dot. Four replicates of the experiment were placed in the potato field by randomly choosing 4 potato plants and removing all Colorado potato beetle life stages. Ten parasitized larvae and 30 non-parasitized larvae were distributed on each of the four plants. The 3:1 ratio of non-parasitized : parasitized larvae was chosen because at the time of the year of the experiment this was the average percentage parasitism normally found in this area (López *et al.,* 1993). Colorado potato beetle larvae were left exposed to *Myiopharus spp.* flies under natural conditions for three hours after which all larvae from each replicate were collected and returned to the laboratory for dissection.

To avoid any possible effect of the paint on *M. doryphorae* behavior, a second method of marking Colorado potato beetle larvae was used in the field. Eighty early third instar Colorado potato beetle larvae were parasitized by fertile *M. doryphorae* flies in the greenhouse. After parasitism, the tarsa of the middle leg on the right side of all parasitized larvae was severed. The tarsa was also cut from the middle leg on the left side of another 80 non-parasitized Colorado potato beetle larvae of the same age.

The walking performance of these larvae was not inhibited when compared to intact larvae of the same age based on observations on plants. This was determined by taking an equal number of intact third. instar Colorado potato beetle larvae and those with their middle tarsi cut (30 of each) and forcing them to walk on top of a potted potato plant for 20 min by constantly stimulating them with a paint brush. The proportion of the larvae with cut tarsi falling to the ground was not greater than that of intact ones.

All non-parasitized as well as parasitized Colorado potato beetle larvae were placed in group in 150 mm diameter plastic petri dishes with ventilated lids and fresh potato foliage for food and kept overnight in a growth chamber at 17 ± 2° C; L16:D8 and 80 ± 5% R.H. The following day all larvae were taken to the Brookfield farm. At 10:00 am on a clear sunny day, August 28th 1991, 8 potato plants were chosen and all Colorado potato beetle life stages were removed from them. Ten parasitized and 10 non-parasitized Colorado potato beetle larvae were randomly distributed on each plant and left for 3 h and 15 min. This proportion was chosen to provide an equal probability of wild flies coming in contact with each experimental larva. After this exposure all larvae from these plants were placed in a cooler, returned to the laboratory and dissected and examined for fly larvae. The time it took to return the larvae to the laboratory and dissect them was no more than 20 min.

Discrimination Ability by *Myiopharus doryphorae* and *Myiopharus aberrans* under Field Conditions: Long-Term Behavioral Study

Observation of the customary searching and larviposition behavior of *M. doryphorae* female flies in the field was part of a three-year study described elsewhere (López *et al.* 1993, unpublished). Early in that study it was occasionally observed that a *M. doryphorae* fly landed on a Colorado potato beetle larva as if to larviposit but instead immediately flew away without completing larviposition. To test whether these apparent rejections constituted discrimination of already parasitized hosts, the following experiment was conducted in conjunction with the long-term behavioral observations.

During three years of general field behavioral studies (1989-1991), *Myiopharus* females were followed in their searching and larviposition behaviors by two observers every day Monday to Friday from 21 August to 15 September in 1989 and 1990 and three days a week, Monday, Wednesday and Friday from late June through 15 September in 1991. Every time a Colorado potato beetle larva was contacted (by landing on top of the larva as it was mentioned above) and larviposited, the larva was collected and the fly was followed; the next larva contacted and abandoned by the same fly was also collected. After collecting one contacted and larviposited and one contacted and abandoned beetle larva from a particular fly, different *Myiopharus* flies behavior were followed and if new pairs of contacted and parasitized and contacted and abandoned larvae occurred during the observation period, they were also collected. All contacted and parasitized larvae were kept together in the same petri dish but separate from "contacted and abandoned" ones. All larvae were placed in a cooler, taken to the laboratory and dissected to determine presence and pigmentation of *Myiopharus spp*. larvae. Dissections took place before 3.5 h had passed from the time when the first "contacted and abandoned larva" was collected. Fly larvae with red pigmentation were assumed to have been larviposited prior to our field observations, while those lacking the pigment were considered to have been newly deposited.

Results

Host Discrimination by *Myiopharus doryphorae* under Greenhouse Conditions

In the caged experiment under greenhouse conditions, acceptance of previously parasitized hosts by *M. doryphorae* flies ranged from 0% in a trial in which there was only one fly per cage, progressively increasing to a maximum of 53% when 4 flies were confined to the same cage (Fig. 5.1). Among the three replicates using 1, 2 and 4 *M. doryphorae* flies per cage, a total of 1, 7 and 24 cases of superparasitism were found respectively . A chi-square test on each treatment showed a significantly greater frequency of *M. doryphorae* larviposition in non-parasitized hosts (Fig.5.1). A logistic regression analysis was performed with a model including three variables: decision to larviposit in parasitized vs nonparasitized hosts, superparasitism in cages with one vs two and with one vs four parasitoids per cage. The analysis showed that all variables were significant in the model and that the rate of parasitisation of the non-parasitized hosts increases multiplicatively with the number of parasitoids in the cage. The overall odds ratio test and 95% CI for non parasitized hosts vs parasitized ones was 18.19 (8.93, 37.07), indicating that non-parasitized hosts were 18 times more likely to be parasitized than the parasitized ones (Table 5.4). At the same time odds ratios for 2 vs 1 and for 4 vs 1 parasitoids per cage were 1.59 (0.59, 4.25) and 3.71 (1.44, 9.54) respectively indicating that parasitized hosts in cages with two and four parasitoids per cage had progressively increasing chances of being superparsitized than hosts in the cage with only one parasitoid (Table 5.4).

Table 5.1 Parasitism by *Myiopharus spp.* in previously parasitized and non-parasitized Colorado potato beetle larvae (distinguished by paint markers) exposed to wild flies in a potato field, South Deerfield Massachusetts. 1991.

Treatments		Parasitized	Non-parasitized
No. of Colorado potato beetle larvae exposed		40	120
No. of Colorado potato beetle larvae recovered after 3 h		39	117
≥ 4 h old larvae	*a*	33	0
≤ 3 h old larvae	*b*	0	40
Superparasitized after field exposure	*c*	1	0
Not parasitized		5	77

a = Parasitoid larvae 4 h old or older had a red pigmented tissue developed within the body cavity as established in the preliminary studies.

b = Parasitoid larvae 3 h old or less did not have any red pigmented structure within their body as established in the preliminary studies.

c = Superparasitized larvae contained two parasitoid larvae, one ≥ 4 h and one ≤3 h.

Host Discrimination by *Myiopharus sp* under Field Conditions: Experimental Test

Out of the total of 160 Colorado potato beetle larvae exposed during the three-hour field test using paint markers and a 1:3 ratio of parasitized to non-parasitized hosts, only one larva was superparasitized with one old parasitoid larva and a new one (Table 5.1) (All superparasitized beetle larvae mentioned hereafter had only two parasitoid larvae in them: one old and one new). A chi-square test showed a significantly greater frequency of *Myiopharus* flies larvipositing in Colorado potato beetle larvae that had not been previously parasitized ($\chi^2 = 13.67$; with *df*, 1; and P = 0.0001).

In the test using Colorado potato beetle larvae marked by removing tarsi and a 1:1 ratio of parasitized to non-parasitized hosts, only four out of 160 Colorado potato beetle larvae were found superparasitized in the three-hour field test (Table 5.2). A chi square test again showed a significantly greater frequency of larviposition in non-parasitized hosts ($\chi^2 = 10.77$; with *df*, 1; and P = 0.0018).

Table 5.2 Parasitism by *Myiopharus spp.* in previously parasitized and non-parasitized Colorado potato beetle larvae (distinguished by tarsal excision) exposed to wild flies in a potato field, Belchertown, Massachusetts. 1991.

Treatments	Parasitized	Non-parasitized
No. of Colorado potato beetle larvae exposed	80	80
No. of Colorado potato beetle larvae recovered after 3 h	68	55
≥ 4 h old larvae *a*	62	0
≤ 3 h old larvae *b*	0	45
Superparasitized after field exposure *c*	4	0
Not parasitized	2	10

a = Parasitoid larvae 4 h old or older had a red pigmented tissue developed within the body cavity as established in the preliminary studies.
b = Parasitoid larvae 3 h old or less did not have any red pigmented structure within their body as established in the preliminary studies.
c = Superparasitized larvae contained two parasitoid larvae, one ≥4 h and one ≤3 h.

Discrimination Ability of *Myiopharus doryphorae* and *Myiopharus aberrans* under Field Conditions: Long-Term Behavioral Study

Over the three years, a total of 87 pairs of one "contacted and abandoned" Colorado potato beetle larva and one "contacted and effectively parasitized" larvae were collected and dissected within 3.5 h. Among a total of 47 Colorado potato beetle larvae contacted and abandoned by *M. doryphorae* , 44 (93.62%) had already been parasitized (i.e., the parasitoid larvae were ≥ 4 h old based on presence of red pigmentation). *M aberrans* showed a similar pattern. Of 40 Colorado potato beetle larvae "contacted and abandoned" by *M aberrans*, 38 (95%) contained larvae ≥ 4 h old. There were no superparasitized larvae found in 174 Colorado potato beetle larvae collected during the three year study. Nevertheless, *Myiopharus spp.* larviposited readily in previously non-parasitized hosts. Parasitoid larvae were encountered in 45 out of the 47 Colorado potato beetle larvae observed to have been larviposited in by *M. doryphorae* and these parasitoid larvae showed no sign of pigmentation, indicating that they were deposited within the observation period. Parasitoids were found in 38 out of the 40 Colorado potato beetle larvae observed to have been larviposited in by *M aberrans* and these larvae also showed no sign of pigmentation, indicating that they also were recently deposited.

Discussion

Both greenhouse and field experiments showed the occurrence of superparasitism to be rare and a highly significant behavioral selection by *Myiopharus spp.* of non-parasitized hosts for larviposition. In the long-term behavioral study under natural field conditions, no superparasitism by *M doryphorae* or *M. aberrans* was found despite high rates of larviposition in previously non-parasitized hosts. It appears that these *Myiopharus spp* possess the ability to discriminate between parasitized and non-parasitized hosts. In both species, discrimination occurred only after the fly contacted the integument of a parasitized larva with its tarsi. Thus it is probable that chemoreceptors and/or mechanoreceptors are involved in the process of discrimination. The average time the flies take to contact and abandon a parasitized larva was 1 (one) second.

Table 5.3 Number of Colorado potato beetle larvae found with *Myiopharus* maggots <3h old (*b*) and > 4 h old *(a)* that were avoided by larvipositing *Myiopharus* flies or parasitized by the same flies under field conditions (South Deerfield, MA. 1989-1991)

Myiopharus doryphorae	Contacted and avoided in the field	Contacted and larviposited in the field
Total Colorado potato beetle larvae collected after exposition	47	47
N° previously parasitized [a]	44	0
N° Newly parasitized [b]	0	45
N° Not parasitized	3	2
N° Superparasitized [c]	0	0

Myiopharus aberrans	Contacted and avoided in the field	Contacted and larviposited in the field
Total Colorado potato beetle larvae collected after exposition	40	40
No. previously parasitized [a]	38	0
No. Newly parasitized [b]	0	38
N° Not parasitized	2	2
N° Superparasitized [c]	0	0

a = *Parasitoid maggots 4 h old or older had a red pigmented tissue developed within the body cavity as established in the preliminary studies.*

b = *Parasitoid maggots 3 h old or less did not have any red pigmented structure within their body as established in the preliminary studies.*

c = *Superparasitized larvae contained two parasitoid maggots, one* ≥ 4 *h and one* ≤ 3 *h.*

In spite of the discrimination ability by these *Myiopharus spp*, a few superparasitized Colorado potato beetle larvae appeared in each of the laboratory tests. Superparasitism by *Myiopharus* has been reported under laboratory conditions by others (Tamaki *et al.* 1983b) although these workers were not specifically testing for discrimination ability. Superparasitism in our greenhouse experiments was probably caused by confining these trials with a limited ratio of available hosts relative to fertile parasitoids. This is supported by the progressively increasing percentage of superparasitism with increasing number of flies per cage in these experiments (Fig. 5.1,

Table 5.4). Increasing numbers of superparasitized hosts with increasing numbers of parasitoids in laboratory experiments has also been found with Hymenopteran parasitoids (Bakker *et al.* 1985, van Alphen & Visser 1990, Huffaker & Matsumoto 1982). An average number of .33, 1.17 and 2 superparasitized beetle larvae per fly were found in cages with 1, 2 and 4 flies respectively. Furthermore, in the greenhouse experiments, flies had been caged without hosts during the previous 24 h. By the time of the experiments those flies were probably more physiologically inclined to larviposit than *Myiopharus spp.* under usual field conditions. These results suggest that these occurrences of superparasitism by *Myiopharus* were a result of a breakdown of their tendency to refrain from larviposition when suitable hosts are unavailable or a possible adaptive strategy (van Alphen & Visser 1990), rather than a lack of host discrimination.

Table 5.4 Odds ratios obtained from the fitted logistic regression model from the greenhouse experiment with one, two and four *Myiopharus doryphorae* flies per cage respectively. Amherst, Massachusetts. 1991.

PREDICTOR VARIABLES	ODDS RATIO^{a}	95% Confindence Intervals
Parasitized vs Non-Parasitized	18.19	(8.93, 37.07)
Superparasitism in 2 vs 1 Flies/cage	1.59	(0.59, 4.25)
Superparasitism in 4 vs 1 Flies/cage	3.71	(1.44, 954)

a = See Hosmer & Lemeshow (1989) chapter 3 for a dicussion and interpretation of the odds ratios.

Caged experiments appeared to replicate the limited host conditions found in the field in late summer when superparasitism has been noted (Kelleher 1960). Our results might be due to the scarcity of Colorado potato beetle larvae relative to the number of larvipositing flies which normally occurs late in August.

In a separate three-year study based on regular bi-weekly field samples (López *et al.* 1993), five of 6347 Colorado potato beetle larvae were found superparasitized by *Myiopharus spp*., and all five cases occurred during the last two weeks of August. This strikingly lower rate of superparasitism in natural field conditions than in laboratory experiments illustrates the importance of direct field observations in behavioral studies.

If superparasitism is a parasitoid's "type II error," then contacting but avoiding a non-parasitized host would be the corresponding "type I error" in host discrimination which we also observed in a small number of cases. Seventeen of the 130 not-previously-parasitized Colorado potato beetle larvae contacted in greenhouse conditions were contacted and abandoned by *Myiopharus doryphorae* (Fig. 5.1), and five of the 87 Colorado potato beetle contacted and abandoned by *Myiopharus spp.* in the field turned out not to have been previously parasitized (Table 5.3). In routine work exposing third instar Colorado potato beetle larvae to both species of *Myiopharus* for parasitism in the laboratory, we have noted occasionally that apparently healthy, non-parasitized larvae are repeatedly contacted and abandoned by a given fertile fly. On five occasions such larvae were then exposed and rejected by *Myiopharus spp* (unpublished observations of authors). This behavior warrants further investigation to determine if, for example, the signal(s) used in host discrimination are sometimes produced by phenomena other than pre-existing parasitoids, or may be acquired by non-parasitized beetle larvae which come in contact with parasitized larvae. Factors other than previous parasitism (e.g. infirmity) might provoke rejection by potential parasitoids. Alternatively, Colorado potato beetle may have a defensive reaction that mimics the discrimination signal.

The discrimination ability of *Myiopharus spp.* increases their effectiveness at the population level and thus heightens their efficiency as natural enemies of the Colorado potato beetle. Together with their apparent capacity to migrate within their hosts in the spring (López *et al.* 1992), this characteristic suggests that they are promising agents for use in biological control of this insect pest.

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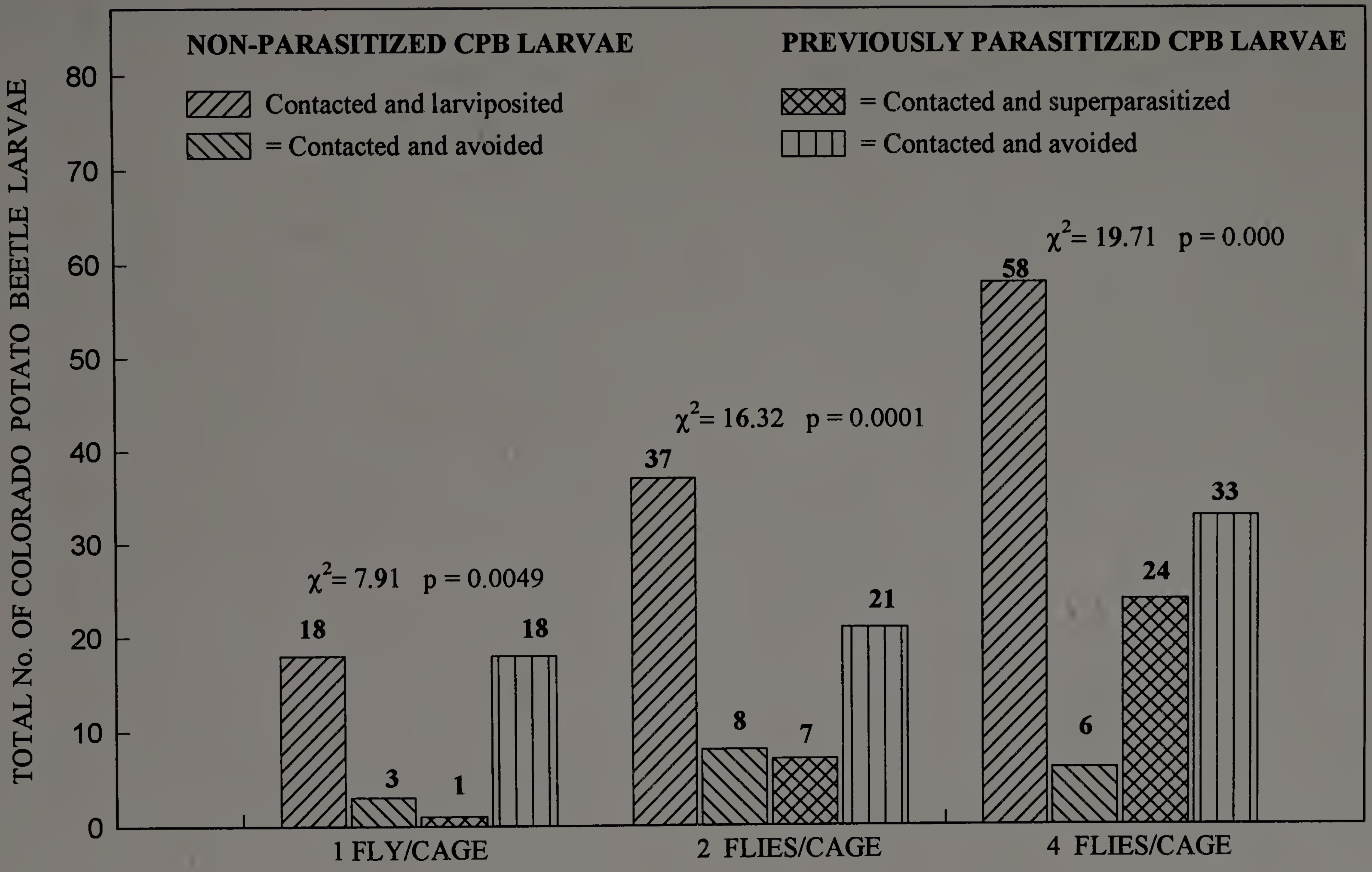

Fig. 5.1 Avoidance vs. Larviposition by *Myiopharus doryphorae* in non-parasitized and in previously parasitized Colorado potato beetle larvae under greenhouse conditions

CHAPTER 6

BEHAVIORAL ECOLOGY OF FEMALE *MYIOPHARUS DORYPHORAE* AND *MYIOPHARUS ABERRANS.*

Introduction

Understanding the behavior of parasitoids under field conditions is of great importance when assessing their potential as biological control agents. The behavior of many hymenopteran parasitoids has been extensively documented in the laboratory and under field conditions (Vinson 1981, 1976, Vinson & Iwantsch 1980; Waage 1978, 1979, Van Lenterem 1976, Salt 1958, Arthur 1981, Lewis *et al.* 1975, Van Alphen & Vet 1986, Vet & Groenewold 1990, etc.). Despite the use of numerous species of tachinids in biological control programs (Grenier 1988), only two behavioral studies of tachinids have been conducted under natural conditions (Herrebout 1967, Monteith 1958). The mobility and rapid movement of these insects make them difficult to follow while making behavioral observations in the field.

The tachinid flies *Myiopharus doryphorae* (Riley) and *Myiopharus aberrans* (Townsend) have been recognized as parasitoids of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), since the late 1800s (Riley 1869). *Myiopharus spp.* are found routinely in New England and other regions of the United States in potato fields that are infested with Colorado potato beetle and in which broad-spectrum insecticides have not been employed. Although they are the only known indigenous parasitoids of the

Colorado potato beetle in North America, it was not until recently that their life cycles were fully documented López *et al.* 1992).

This study was designed to identify and quantify the behavior of *Myiopharus* species under field conditions, taking particular note of any influences that these behaviors might have on the population dynamics of the Colorado potato beetle. The study also examined interactions between the two *Myiopharus* species when both were found together and their possible consequences in terms of overall parasitism.

Methods

Studies were performed on populations of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and of the tachinid flies *Myiopharus doryphorae* and *M. aberrans*, found during the growing season in organically grown potato (*Solanum tuberosum* cv. Kathadin) fields in western Massachusetts, U.S.A. Observations were made during the summers of 1989, 1990 and 1991 at the University of Massachusetts Experimental Farm (South Deerfield, MA) and in the summer of 1991 also at Brookfield Farm (Belchertown, MA) and at Hampshire College Experimental Farm (Amherst, MA). Potato plots were planted in early May with 90 cm inter-row and 20 cm inter-plant spacing. Standard practices of local commercial farmers were followed regarding hilling, fertilization, and weed and disease controls (using standard dosages of metribuzin and metholachlor for weed control and maneb or mancozeb for disease control). M-One (*Bacillus thuringiensis* Subsp. *tenebrionis*) was applied twice during the season on each plot to keep larval populations of the Colorado potato beetle from defoliating the experimental plots. The two *Myiopharus* species were identified by comparison with characteristics of specimens collected locally and determined by Dr. James O'Hara from the Biological Resource Division at the Center for Land & Biological Resources Research, Ottawa, Canada.

Identification of Behavioral Categories

The summers of 1989 and 1990 were dedicated to observing wild *Myiopharus* flies within the study plots and identifying their most common behaviors categories as a framework for subsequent quantification. During these observations, flies moved freely within or outside the field plots. Observations began at 0630 and ended at 2030 hours from 15 August to 15 September 1989 and 1990; they included a series of 2-h observation and recording periods separated by 30-min meetings among workers to share observations and refine behavioral categories. Time was divided equally between two complementary approaches to sampling fly activities. In both cases workers took haphazard walks through the study plots searching for *Myiopharus spp*. to observe; in the first approach only the behavior in which the fly was engaged when spotted was recorded. The second approach involved watching individual flies for as long as they could be followed and recording the duration of each separate behavior. Initial attempts at quantification employed digital stopwatches, however, hand recording proved cumbersome and was by the use of computerized data collection techniques which were employed in the principal study.

Some 20 different activities were identified during these two summers of preliminary observations; eight of the most common ones, recognized as together occupying close to 95% of female flies' time, were chosen for a more detailed quantitative study. These eight behaviors were defined as follows:

Resting : standing still except for occasional cleaning and grooming

Forays : meandering flight at about 0.5 m above the potato plants, normally oriented along a given row and lasting up to several seconds before alighting or disappearing from view

Localized searching: walking over a potato plant while palpating the surfaces of the leaves with tarsi; occasionally hopping from one leaf or stem to another and from one potato plant to another and continuing this exploratory behavior

Plant feeding: extension of the proboscis and contacting of the surface of the potato leaves with the labellum

Host contact and withdrawal: landing atop a beetle larva but flying away immediately upon tarsal contact with the larval integument

Pre-larviposition stimulus: landing atop a Colorado potato beetle larva, ostensibly attempting to larviposit, but withholding larviposition when the beetle larva shifts to a thanatosic ("playing dead") state; host "stimulation" involves the parasitoid pushing its larvipositor very slowly at the integument of the larva but stopping before actual penetration if the host still does not move, or sometimes hopping off the larva and poking at it with the foretarsi; these movements may be repeated several times before actual larviposition (when the host finally moves) or abandonment (if the larva remains quiescent)

Larviposition: landing atop a Colorado potato beetle larva and inserting the larvipositor through its integument

Feeding on host : extension of the proboscis until it contacts and absorbs the drop of hemolymph released upon larvipositing in a Colorado potato beetle larva

Male *Myiopharus spp.* engaged in only two of the above types of behavior: resting and plant feeding. They were far less active than the females, more difficult to find and observe, and as adults were not involved in direct interactions with their hosts. Consequently only female *Myiopharus* were included in this study.

In addition to the above eight categories, three types of behavior closely associated with larviposition and involving both the parasitoids and their hosts were singled out for a separate study because of their particular interest within the context of biological control.

Quantification of Eight Common Behaviors

Employing the typology of common behaviors defined above, a more precise behavioral study was conducted in the summer of 1991 using a computerized event recorder program. The study was performed in a 48 x 24 m plot located at the University of Massachusetts Experimental Farm, South Deerfield, MA; a 80 x 30 m plot at Brookfield Farm, Belchertown, MA; and a 50 x 50 m plot at Hampshire College Experimental Farm, Amherst, MA. Observations were made twice a week from 12 June to 15 September; on each day observations were made from 0800 to 1700 hours, based on the previous year's observation that the flies are mostly quiescent outside this time period. Again, all *Myiopharus* flies were naturally occurring and unrestricted in their movements.

Observers carried portable computers provided with the Quick Basic (Zanen *et al.* 1989) event recording software, with a different key from the keyboard assigned to each of the eight defined behaviors and a pre-set time limit of two minutes per subject. Two observers started from opposite ends of the potato field and walked randomly through the plot. For each *Myiopharus* fly spotted, its observer immediately pressed the key corresponding to its current behavior and continued recording each change in activity until the computer produced the sound signal indicating that a two-minute interval had elapsed. The observer then corrected any errors noted in data input, moved at least three rows away to reduce the probability of repeated sightings of the same fly, and resumed searching for new flies. The computer program registered each parasitoid entered as a separate record comprising all the behaviors noted within one two-minute observation period. After each 45 min of observing flies and recording behaviors, observers took 15-min breaks throughout each day of the study, leading to a total of 435 person-h of observation during 1991.

Temperature, rainfall and relative humidity were recorded continuously at a weather station located within the University of Massachusetts farm facility some 75 m from our study plot. For the data analysis frequency and duration were the main components computer recorded from *Myiopharus* behaviors. A consistent relation was apparent between these two components of *Myiopharus* behaviors and temperature since the initial preliminary field observations. Therefore, linear regression models were fitted to test frequency and duration of the behaviors across the temperature range experienced by the flies from 0800 h to 1700 h. Quadratic regression models were fitted to test the variation in frequency and duration of *Myiopharus* behaviors at the various times of day.

Larviposition-Related Behavioral Studies

Colorado Potato Beetle Defenses Against Larviposition. Our 1989 observations indicated that CPB larvae respond to *Myiopharus* attacks with a large repertoire of defensive behaviors, including: cycloalexy (circular defense), flailing of fore and middle legs , regurgitation, dropping from the potato plant, tanathosis, and tightening of abdominal muscles. In 1990 and 1991, studies were conducted to measure the effectiveness of such behaviors against *Myiopharus* attacks by recording observed occurrences of each defensive behavior along with their outcomes.

Every study day, one observer walked randomly through the potato plot in search of female *Myiopharus spp.* individuals which were then followed for as long as they made frequent larviposition attempts. Whenever a CPB larva approached by a fly reacted with one of the above defensive behaviors, the type of behavior was recorded along with the success or failure of larviposition. Larviposition was considered effective whenever a drop of hemolymph was observed exuding from the place of entry of the larvipositor (Chapter 5 and López *et al.* 1995); the fly was usually seen cleaning its larvipositor after these successful attempts. Larviposition failure was recorded when no drop of hemolymph appeared. When a *Myiopharus* under observation ceased to larviposit repeatedly or disappeared from view, the observer continued to walk until another fly was spotted. One-hour recording intervals were interspersed with 10-min break periods from 0800 to 1700 hours and an additional 30-min break at noon; the study was thus based upon a total of 109 h of observation.

Post-Larviposition 'Territorial' Behavior of *Myiopharus spp*. An unusual sequel to larviposition behavior was observed for *M. doryphorae* on nine occasions in the late summer of 1989. This apparent territorial behavior consisted of the fly remaining for several minutes on a single leaf of the same plant where the CPB larvae in which she had just larviposited were located and making quick darting flights at any other *Myiopharus* fly approaching that plant. The approaching fly was inevitably driven away by the encounter upon contact, after which the *Myiopharus* that had larviposited returned to the same potato leaf. This behavior was made the focus of a specific study in 1990 and 1991. Observations of "territorial" behavior were made between 15 August and 15 September of those years. Each day of the study was divided between hour-long recording periods and 10-min break periods from 0800 to 1700 with a 30-min break at noon, giving a total of 109 h of observation and recording. In addition, this behavior was watched for throughout the summers of 1990 and 1991 during the wider behavioral study described above.

Every study day, one observer walked randomly through the potato plot in search of larvipositing *Myiopharus* flies and hand-recorded their behavior after larviposition for up to one hour as long as each fly continued returning to the same leaf. Elements recorded for each episode were: species of *Myiopharus* engaging in defense, total number of larvipositions on hosts occupying a given plant before defense behavior began, number of repulsions of other *Myiopharus* flies realized before the defending *Myiopharus* abandoned its perch, and total time spent per defense episode.

Provocation of Pre and Post Diapausing Adult Colorado Potato Beetle by *Myiopharus aberrans*. During the three-year study individual *Myiopharus aberrans* females were frequently observed encircling individual adult CPB, jumping about with frenzied motion and poking at them continually with their tarsi to the extent that the beetles were prevented from eating for as long as the flies persisted. On a few occasions *M. aberrans* was observed having the same behavior toward beetle larvae. This behavior was mainly noted during the first days of the growing season (1-15 June) with post-diapause CPB adults and toward the end of the season (15-20 August through 15 September) with pre-diapausing CPB, although CPB adults were always present in the potato fields studied and in our caged greenhouse colonies of the flies.

Because this behavior coincided with the principal seasons of CPB flight and given that the only easily penetrable portions of the beetle's exoskeleton are beneath the elytra, we hypothesized that the *Myiopharus aberrans* fly might be attempting to provoke initiation of beetle flight to permit larviposition. One report (Biliotti & Persoons 1965) exists of two or more individuals of *M. doryphorae* larvipositing in adult CPB under laboratory conditions after the beetles' elytra and wings were removed; however, we never observed this species showing any interest in adult CPB and suspect that the flies in question might have been deprived of larval hosts before and during exposure to the adults. No other reports exist of either *Myiopharus* species larvipositing in adult beetles, and Biliotti and Persoons (1965) themselves regarded it an unlikely occurrence in natural conditions where presence of elytra and wings would prevent it. Houser and Balduf (1925) and Bussart (1937) do mention possible cases of the tachinid fly *Chaetophleps setosa* Coquillet larvipositing in adult cucumber beetles *Diabrotica vittata* Fabricius in flight, although the first of these reports describes an unlikely case of larviposition through the elytra and the second admits to having been unable to observe clearly the suspected insertion of the larvipositor from beneath the raised elytra. We investigated our hypothesis using the following procedures:

In the field, late in the 1990 season (20 August to 15 September) every study day two observers walked randomly through the potato plot in search of *Myiopharus aberrans* females in close proximity to adult CPB. An observer watched a given pair for as long as they remained together, hand-recording all activities of *M. aberrans* but paying particular attention to possible larviposition attempts. Observations were conducted from 0800 until 1200 hours every day with 10 min breaks every hour, leading to a total observation time of 60 hours.

Following these observations, workers spent up to one half hour seeking additional *Myiopharus*-CPB pairs in the field; all pairs up to a maximum of four encountered during this time were taken to a greenhouse at the University of Massachusetts and placed in 35-

cm cubical screened cages where their activities were observed and recorded for two hours.

In June of 1990, nine uninterrupted hours of video recording were made in the laboratory of two *M. aberrans* females interacting with three field-collected pre-diapausing adult beetles and five CPB larvae in a 35-cm cubical screened cage provided with sugar and water for the flies and potato leaves for the beetles. All insects were dissected afterward and examined for *M. aberrans* larvae.

In the laboratory also, several attempts were made in August-September of 1991 to record the response of *M. aberrans* females toward adult beetles with the abdominal dorsum artificially exposed. The elytra of 30 last-generation pre-diapausing adult beetles were removed without harming their wings. These winged, but elytra-less, beetles were exposed for a week simultaneously in groups of 10 each in three different 35-cm cubical cages as above to four field-captured fertile *M. aberrans* females per cage. The elytra and the wings of another 30 adult last-generation pre-diapausing beetles were removed and the beetles with the uncovered dorsal integument of the abdomen were also exposed simultaneously for a week in groups of 10 to three different groups of four fertile *M. aberrans* flies each in the same type of cage as above. Fresh potato foliage as well as sugar cubes and water were provided daily as food for beetles and parasitoids respectively. At the end of the week in both experiments all beetles and flies were dissected to check for the presence of parasitoid larvae, indicating parasitization and fertility respectively.

To induce flight in pre-diapausing CPB adults captured in flight in the field and then exposed in cages to *M. aberrans* females in the laboratory, several methods were tried during August-September of 1994, including placement of the beetles on narrow raised perches and placement of potted potato plants at some distance within an expansive (2.5 m x 3 m x 1.5 m tall) cage. Knowing from earlier work with the CPB that suspension by the thorax can provoke flight initiation in the beetles (Weber 1992), we attached one end of a #2 insect pin bent into a "Z" shape to the mesothorax of each of 10 of these pre-diapausing CPB adults with beeswax and tied a thin nylon filament to the other end of each pin; thus tethered and suspended in the air, the beetles were exposed to two female *M. aberrans* at a time in one of the screened plexiglass cages described above.

Results and Discussion

Identification of Behavioral Categories

Observations made during the course of the principal behavioral study provided more detailed descriptions of each of the eight activities on which that study focused. These qualitative observations are summarized below for each of the behavioral categories; in all cases the behavior was similar for both *Myiopharus* species.

Resting: Early in the day, resting normally took place in full sunlight on the upper surfaces of potato leaves. At other times, the flies rested anywhere on the plants or on the ground. Gradually toward evening resting occupied an increasing proportion of time and the flies moved to the lower leaves of the plants, where they apparently spent the nights resting on the undersides of leaves.

Forays: These slow flights swept directly above the rows of potato plants and appeared to entail one form of searching, defined as the active movement by which an animal finds or attempts to find resources (Bell 1985, 1991). Initiation of flight was always noted to occur after a period of localized searching (below), often after an extended period without encountering hosts. The potato plant on which the fly landed at the conclusion of a foray frequently had more abundant and/or visible CPB larvae than surrounding plants, and forays were generally followed by localized searching upon this new plant.

Localized searching: Myiopharus spp. females spent much of their time in this characteristic activity involving quick walking and hopping motions across the upper and lower surfaces of potato leaves, along the vines, and among adjacent plants in movements that covered a large proportion of the plant foliage. Since males were never found engaged in this searching behavior, as well as the observation that flies invariably stopped searching and switched to a different activity upon encountering a CPB larva, it would appear that host larvae were the resource sought during this behavioral activity.

The flies' halting movements and their continual palpations of the plant surfaces suggested that tarsal chemoreceptors provided at least a portion of the cues used in these local searches. The possible role of various types of contact kairomones in the search was also indicated by the response to CPB spoor (feces, regurgitate, leaves damaged by beetle feeding, or CPB larvae themselves) encountered in their path: the fly usually stopped abruptly, followed by reorientation and apparently directed movement similar to the behavior termed "arrestment" by Waage (1978).

Plant feeding: Myiopharus spp. were observed feeding on two distinct categories of matter found on plant surfaces. During early morning hours the flies took in water from droplets of condensation remaining on the leaves. Dissolved deposits, including perhaps the various types of excretions left on leaves by the beetles and larvae were evidently one object of this consumption: flies often drank from the murky drops concentrated from water washed into pockets of curled leaves after ignoring the clear droplets generally found on flatter leaf surfaces.

Throughout the day, the flies fed on a second type of substance on the surfaces of potato plants: the exudates from edges of leaves recently damaged by feeding of CPB larvae or adults. It is interesting to consider that this may be an additional form of dependence by the flies on their hosts, given that these plant juices seem to be their only readily available source of water later in the day.

Host contact and withdrawal: Observations that some host larvae were "rejected" after being attacked by fertile females of *Myiopharus spp*. eventually led to an independent study of this behavior (López *et al*. 1995) in which it was shown that both *Myiopharus* species have the ability to discriminate between already parasitized and non-parasitized hosts. Discrimination is virtually instantaneous but occurs only upon direct tarsal contact with the host. Frequency of this behavior varies through time in conjunction with changing host-parasite ratios and other elements of behavior, as will be discussed later.

Prelarviposition stimulus: This behavior was employed only in cases of thanatosic defense behavior by their hosts (below), and it was therefore restricted to *Myiopharus* females attacking the third and fourth larval instars of CPB which are those that engage in tanathosis; for this reason it was not noted during the first few days of the growing season when only adults and early instars were present. The behavior may help the flies to distinguish live and healthy larvae from unhealthy hosts; conceivably also the limp state of a thanatosic larva prevents proper insertion of the larvipositor, and stimulus in this case might help restore an adequate degree of turgidity for larviposition to be completed.

Larvipositon: This behavior occurs during periods of searching activity when the *Myiopharus* female comes within a few cm of a CPB larva. Often it was initiated by a short period of "arrestment" (Waage 1978) before the fly jumped upon the dorsum of the larva and commenced insertion of the larvipositor. Evidence of successful insertion is manifest in the appearance of a drop of hemolymph which exuded from the wound. Laboratory trials followed by dissections (López *et al.* 1995) showed that observed insertion of the larvipositor was invariably accompanied by deposition of a parasitoid larva within the host. Flies larviposited only once within a given host but were able to larviposit in some 3-4 successive hosts, if available, within a few seconds; afterward they usually remained in one place grooming and cleaning for a few additional seconds before resuming localized searching behavior.

Feeding on host: Immediately after larviposition, flies sometimes consumed the drop of hemolymph exuded from the host larva. When this behavior occurred, it seemed deliberate and was unmistakable: the fly returned after jumping off the larva and proceeded directly toward the drop, which it took up quickly and completely. Patterns of occurrence of this behavior were difficult to determine in the field, but caged flies provided continuously with hosts ceased to feed on these exudations after approximately the third consecutive larviposition, indicating satiety (author's unpublished data).

This feeding could have been important for larvipositing females, which had no other apparent source of protein-rich nutrition. It was first reported by Bruneteau (1937) for *M. doryphorae* and according to Clausen (1940) has been recorded for *Anetia nigripes* Fallén as well. The only significant investigation of this behavior among tachinids was that of Nettles (1987), who attempted to analyze and replicate constituents of the hemolymph of *Helicoperva* sp. but found the resulting mixture unequal to natural hemolymph as a food for the tachinid parasitoid *Eucelatoria bryani* Sabrosky.

Quantification of Eight Common Behaviors

A total of 492 individuals of *Myiopharus* were recorded during the course of the quantitative study; of these, 174 were *M. aberrans* and 318 were *M. doryphorae*. The two species showed a markedly uneven distribution among the three study sites: at Brookfield Farm 99% of the *Myiopharus* flies recorded were of *M. aberrans*; at the Hampshire College Experimental Farm 99% were *M. doryphorae*; whereas at the University of Massachusetts Experimental Farm 74% were *M. doryphorae* and 26% were *M. aberrans*. Due to this variation and the possibility of shifts in behavior in the presence of a close competitor, it was decided to analyze data separately for each of the three fields. Henceforth the expression "separate fields" will refer to the Brookfield Farm (*M. aberrans* data only) and Hampshire College Experimental Farm (*M. doryphorae* only) study plots.

Data presented from the study plot at the University of Massachusetts Experimental Farm include both species, quantified separately but referred to as "within the same field."

A summary of the frequency and duration of the eight designated activities according to time of day as well as ambient temperature for *M. doryphorae* and *M. aberrans* is presented. For *Myiopharus spp.* found both in separate fields (Figs. 6.1- 6.4) and within the same field (Figs. 6.5-6.8).

A linear regression analysis comparing frequency and duration of each of the eight behaviors of each *Myiopharus* species occurring alone ("separate fields") versus those of the same species co-occurring with the other ("within the same field") did not show any significant differences. This situation is apparent in comparisons of the two series of graphs for each species, which show rather similar patterns across times and temperatures for each of the behavioral categories. Co-occurrence of these congeners in a given field does not, therefore, appear to affect these aspects of their behavior.

For the statistical analysis of all eight behaviors and their comparison between the two species of *Myiopharus* across temperature range and at different time of day, a Proc Reg procedure from SAS statistical analysis software was used where temperature and time were coded as follows: for temperature (° C), if temperature =16-19 then temp = 1; if temperature = 19-23 then temp = 2; if temperature = 23-27 then temp = 3; if temperature =27-31 then temp = 4; if temperature =31-34 then temp = 5; and for time the coding was: if time = 0800 - 1000 then time = 1; if time =1000-1200 then time = 2; if time = 1200-1400 then time = 3; if time = 1400-1600 then time = 4; if time = 1600-1800 then time = 5. Considering time = 1, 2, 3 ,4 and 5. Therefore, TIME2 = TIME X

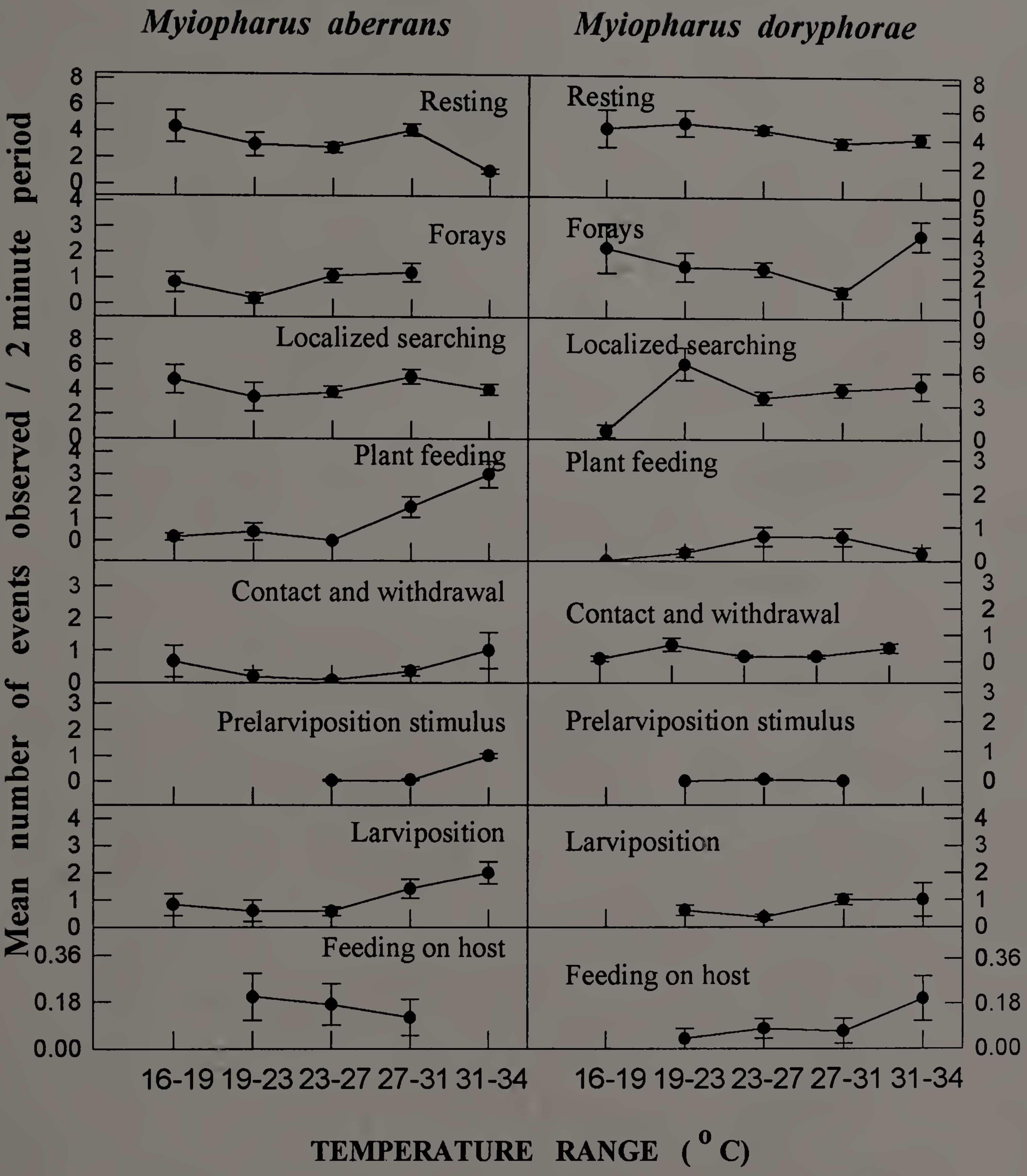

Fig. 6.1 Frequency of eight common behaviors across temperature range by *M. doryphorae* and *M. aberrans* found in separate fields.

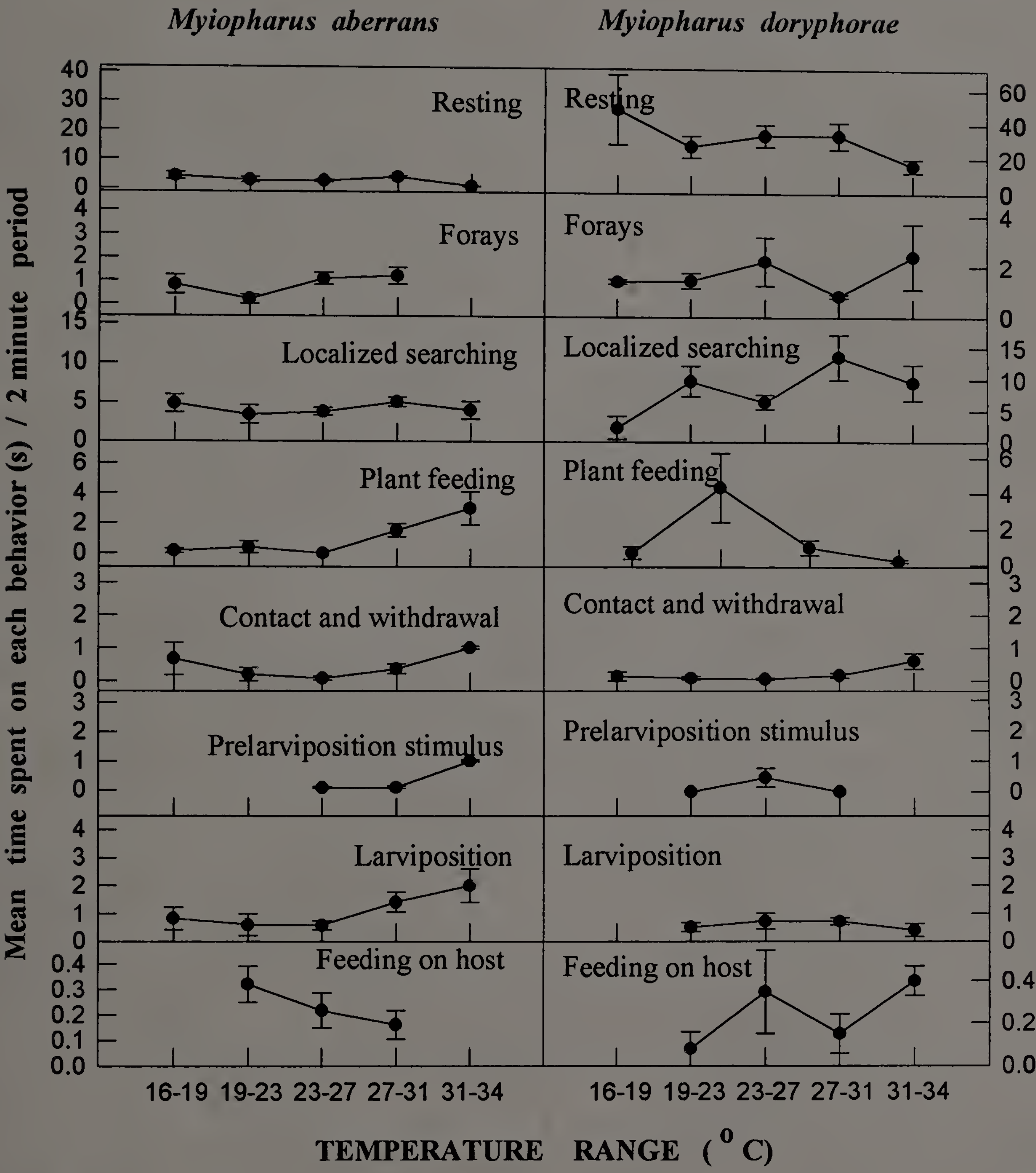

Fig. 6.2 Mean time spent on each behavior across temperature range by *M. doryphorae* and *M. aberrans* found in separate fields

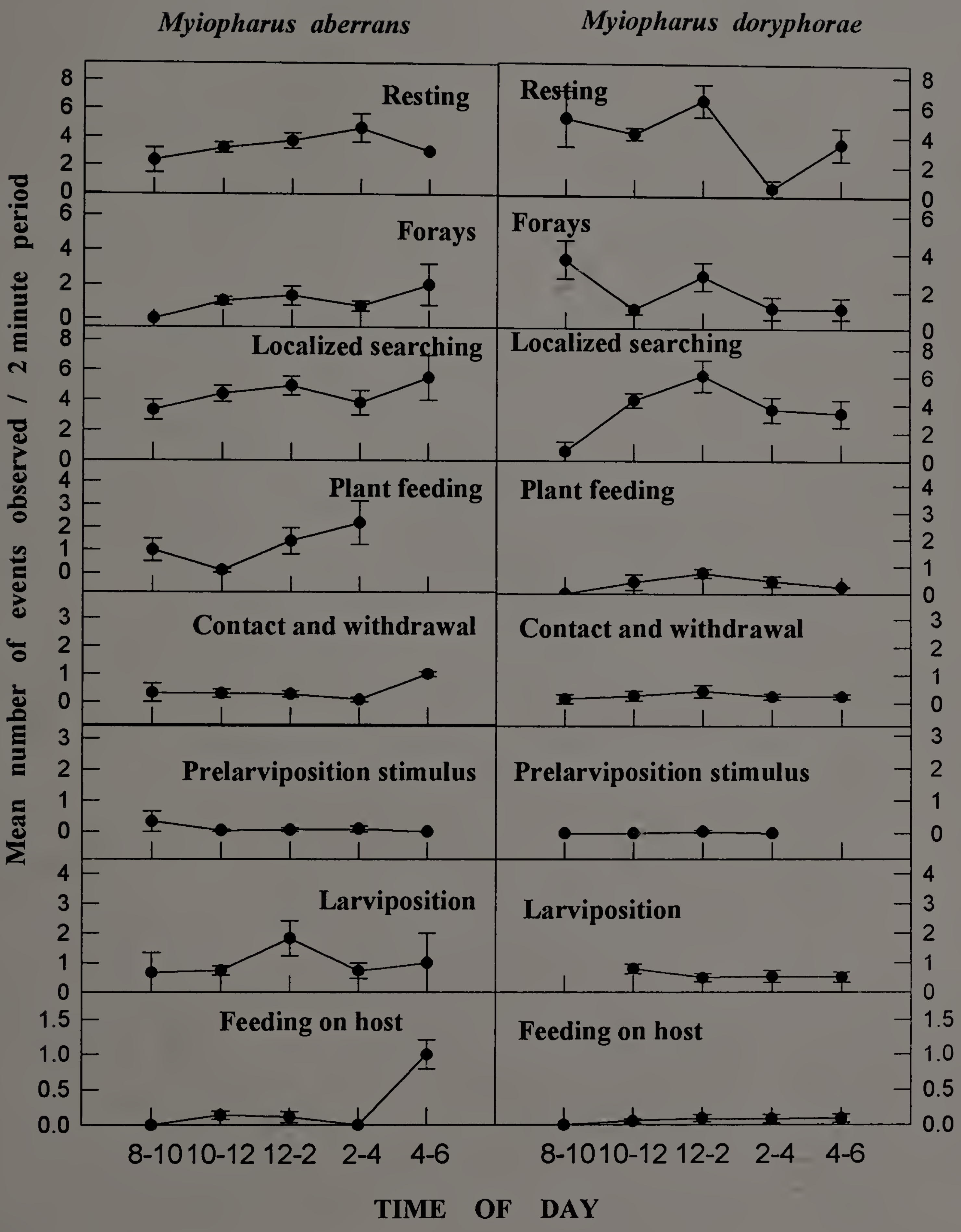

Fig. 6.3. Frequency of eight common behaviors across time of day for *M. doryphorae* and *M. aberrans* found in separate fields.

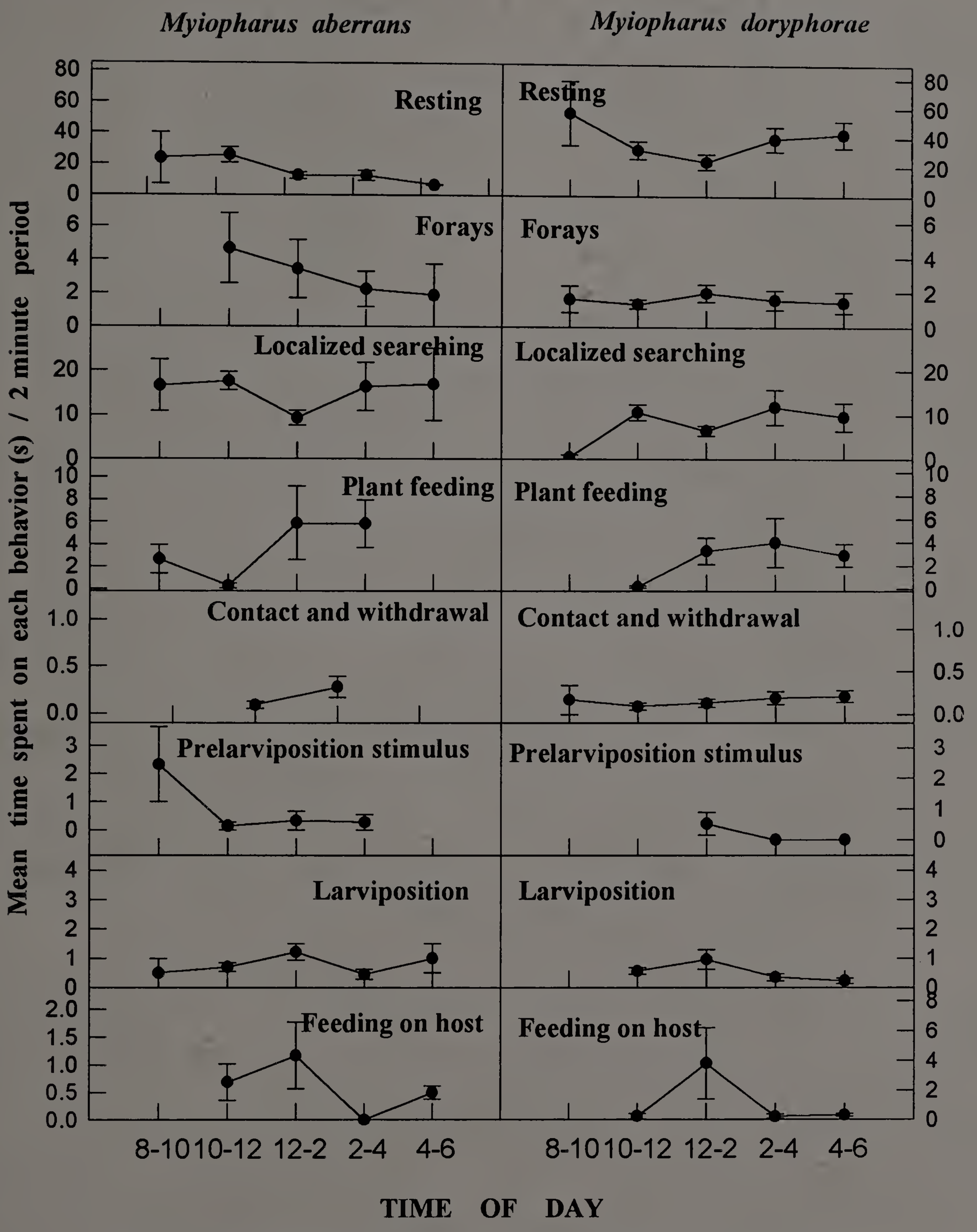

Fig. 6.4 Mean time spent on each behavior across time of day for *M. doryphorae* and *M. aberrans* found in separate fields

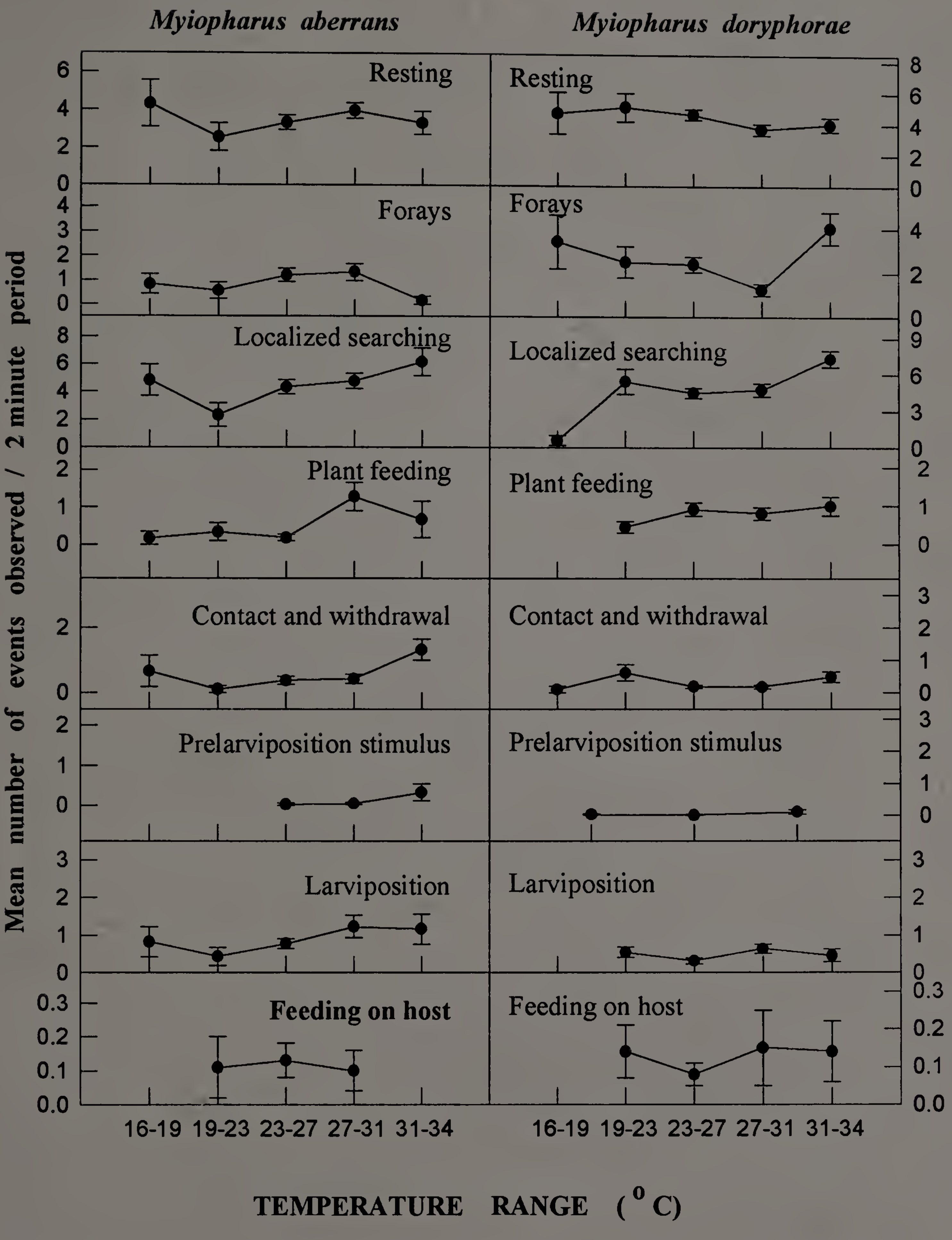

Fig. 6.5. Frequency of eight common behaviors across temperature range for *M. doryphorae* and *M. aberrans* found within the same potato field

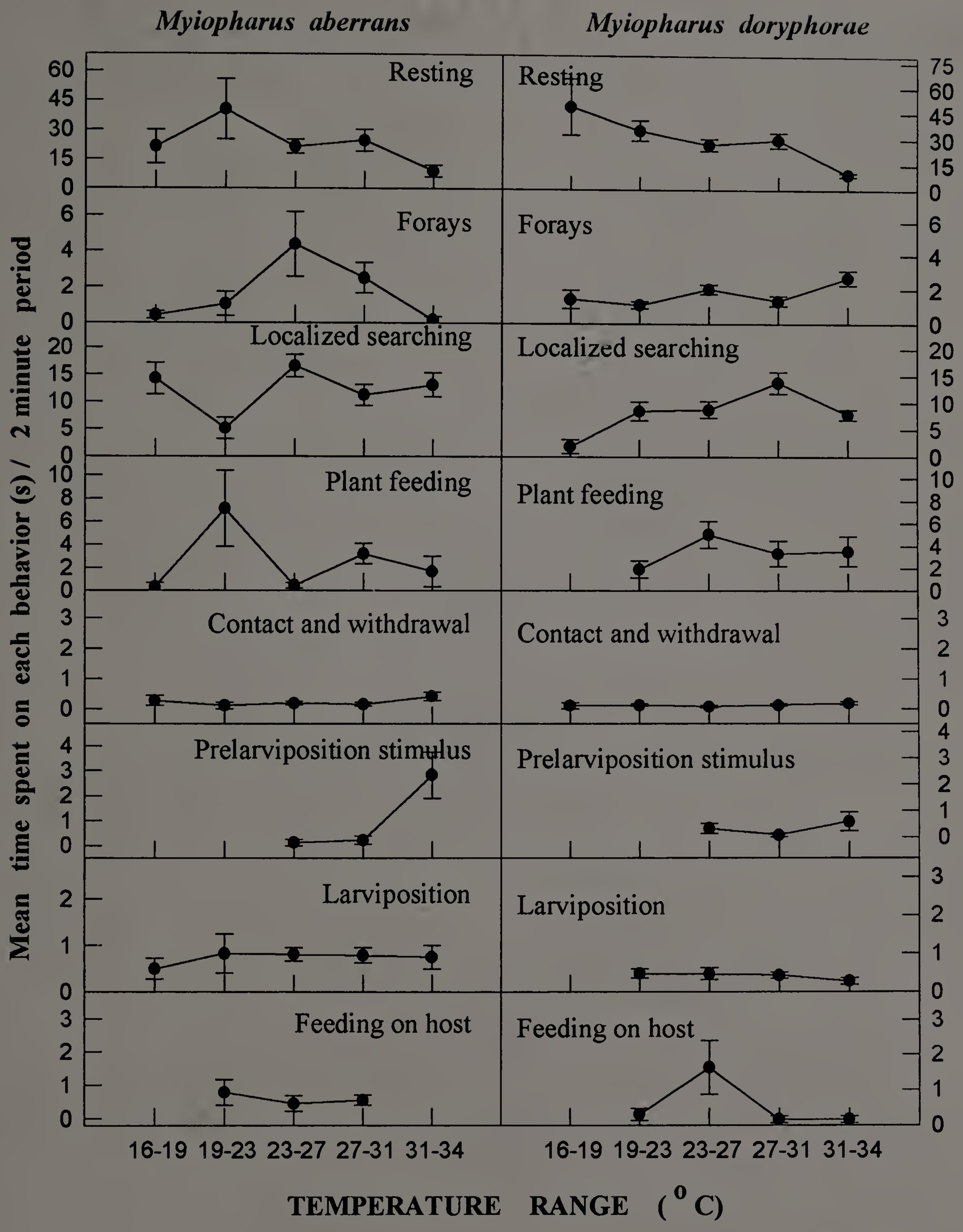

Fig. 6.6 Mean time spent on each behavior across temperature range by *M. doryphorae* and *M. aberrans* found within the same potato field

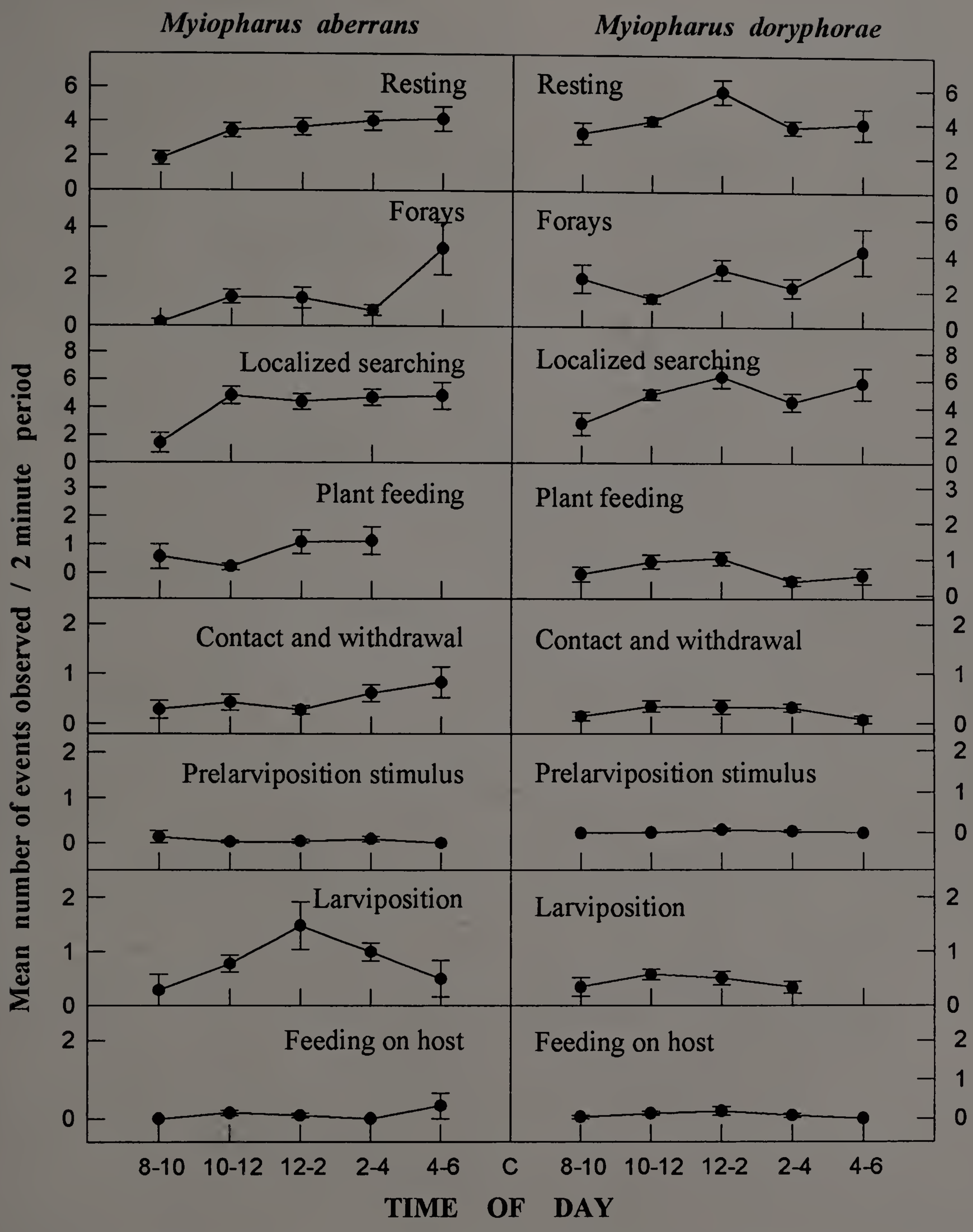

Fig. 6.7 Frequency of eight common behaviors across time of day for *M. doryphorae* and *M. aberrans* found within the same potato field

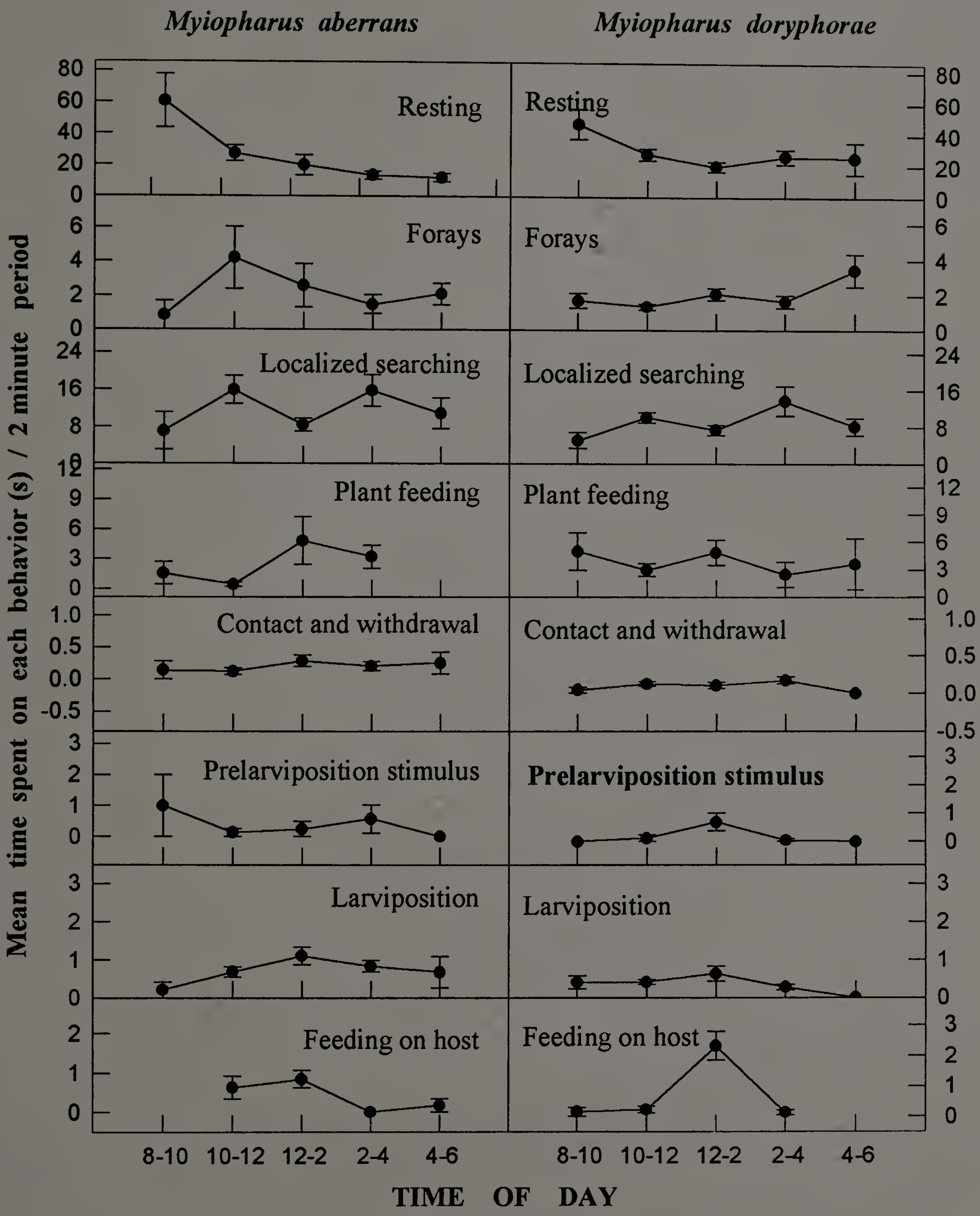

Fig. 6.8 Mean time spent on each behavior across time of day for *M. doryphorae* and *M.aberrans* found within the same potato field.

TIME= 1x1, 2x2, 3x3, 4x4 and 5x5 giving origin to a quadratic instead of a linear regression when analyzing the behaviors by time of day. For analysis purposes the two parasitoid species were also coded as *M. doryphorae* = 0 and *M. aberrans* = 1.

A linear regression analysis was performed for all eight behaviors of each *Myiopharus* species found in separate fields, to describe the effects of temperature and time of day on the flies' activities. The analysis showed that for *M. aberrans* there was a significant difference across the temperature range in frequency of both larviposition and plant feeding but not in frequency of the other six behaviors. There was no significant difference in the mean time spent by this species on any of the behaviors across temperature range or time of day. For *M. doryphorae* there was a significant difference in the frequency of larviposition, host feeding, plant feeding and resting across the temperature range; mean time spent in host contact and withdrawal also differed by temperatures. Searching frequency and mean time spent feeding on plants were the only two values which differed significantly across times of day for this species. (Figs. 6.9 - 6.12 and Tables 7, 8).

The same analysis as above was applied to the two species of *Myiopharus* when found within the same field, comparing the frequency and duration of each behavior across time and temperature ranges. A statistically significant difference was found between the two species in the frequency across temperatures of all behaviors except for host contact and withdrawal and feeding on host hemolymph (Table 6.1, Fig. 6.5). The mean time spent larvipositing, searching, resting and in prelarviposition stimulus across the temperature range were also significantly different (Table 2 and Fig. 6.6). A significant difference was also found between the two species in the frequencies of all behaviors except plant feeding, contact and withdrawal, and feeding on host hemolymph across time of day (Fig. 6.7). The mean time spent larvipositing and resting were also significantly different by time of day (Fig. 6.8).

Table 6.1 Linear regression comparison test for the frequency of each behavior per two-minute period recording across temperature range between the two *Myiopharus* species within the same field. **A** = Plant feeding, **B**= Forays, **C**= Feeding on host, **D** = Larviposition, **E** = Localized searching, **F** = Resting, **G** = Contact and withdrawal, **H** = Prelarviposition stimulus.

	A	B	C	D	E	F	G	H
Constant	-0.265	2.086	-0.015	0.027	-0.250	6.524	0.449	-0.145
Temp (° C)‡	0.040	0.012	0.005	0.016	0.203	-0.082	-0.005	0.007
Spec*	-2.037	-1.757	0.139	-0.927	1.126	-3.988	-1.031	-0.131
spec xTemp	0.074	0.017	-0.006	0.055	-0.065	0.123	0.045	0.005
Overall P	0.013	0.007	0.92	0.0001	0.017	0.091+	0.207	0.030

*= Spec.= species. Coded as *M. doryphorae* = 0 and *M. aberrans* = 1 (see text)
+=.05 < P < .10
‡= Temperature range (° C) coded as 1= 16-19; 2 = 19-23; 3 = 23-27, 4 = 27-30; 5 = 30-34 (See text).

Table 6.2 Linear regression comparison test for the mean time spent on each behavior per two-minute period recording across temperature range between the two *Myiopharus* species within the same field. **A** = Plant feeding, **B**= Forays, **C**= Feeding on host, **D** = Larviposition, **E** = Localized searching, **F** = Resting, **G** = Contact and withdrawal, **H** = Prelarviposition stimulus.

	A	B	C	D	E	F†	G	H
Constant	0.546	-0.660	0.582	0.427	-0.454	--	-0.090	-0.580
Temp. (° C)	0.117	0.093	0.005	-0.001	0.390	--	0.007	0.031
Spec. *	-2.593	2.352	-0.330	0.018	13.84	--	0.198	-1.609
spec.x Temp.	0.046	-0.049	0.003	0.013	-0.401	--	-0.004	0.065
Overall P	0.388	0.175	0.991	0.014	0.042	--	0.103	0.046

†= F could not be fit due to colinearity.
*= Spec.= species. Coded as *M. doryphorae* = 0 and *M. aberrans* = 1 (see text)

The following tables (Table 6.3 - 6.10) and corresponding figures (Figs 6.9 - 6.16) are representations of a model based on linear regression analysis of the frequency and duration of localized searching and larviposition behaviors of the two species across ranges of temperature and time of day. Figures 6.9 - 6.16 are based on the 1991 data when both *Myiopharus* species were found within the same field. These two behaviors were selected for modeling because of the consistent statistical differences found between the two species in regard to larviposition and the obvious connection of larviposition with searching behavior, as well as the likely importance in biological control of these two activities.

Table 6.3 Linear regression of larvipositing frequency by ambient temperature.

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	-1.4806	-1.1501	0.0271 *
Temp.(^{0} C)	0.099 +	0.070 +	0.0164 *
Spec.	--	--	-0.927
spec x Temp	--	--	0.0552 *
Overall P	0.0475	0.004	0.0001

*= $P < .05$

+=$.05 < P < .10$

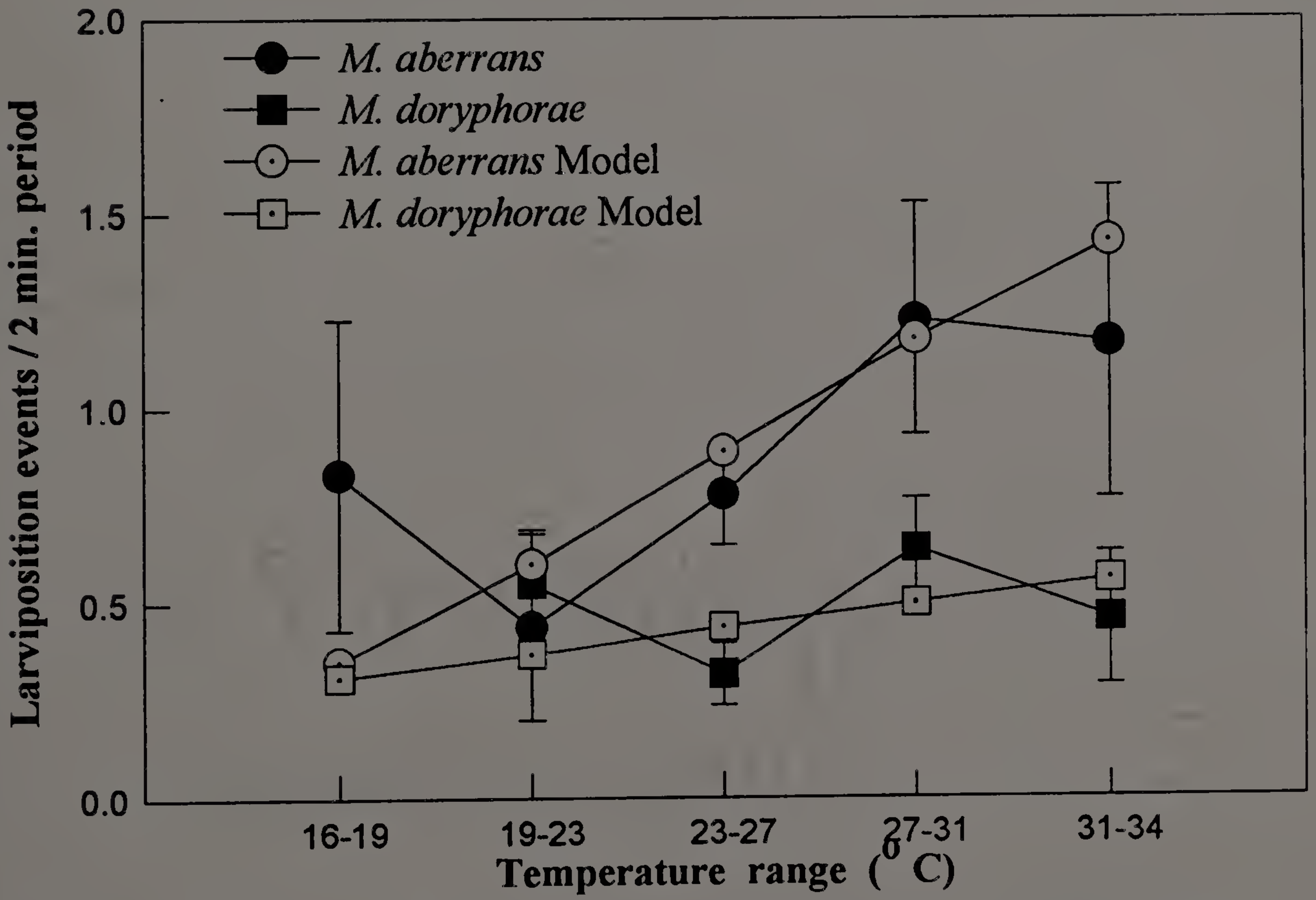

Fig. 6.9 Observed means and fitted values from the linear regression models of larviposition frequency for both *Myiopharus spp*. across temperature range. (1991 data)

Table 6.4 Quadratic regression of larvipositing frequency at 5 periods of time of day

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	-1.616	-0.078	0.034
Time†	1.864	0.573	0.437
Time2‡	-0.289	-0.111	-0.089
Spec.	--	--	-1.329
Spec x Time			1.162
Spec x Time2			-0.159
Overall P	0.239	0.581	0.0001

†= Time coded as 1 = 8-10, 2 = 10-12, 3 = 12-2, 4 = 2-4, 5 = 4-6;
‡ = Time2 = Time x Time = 1x1, 2x2, 3X3, 4x4 and 5x5 (See text)

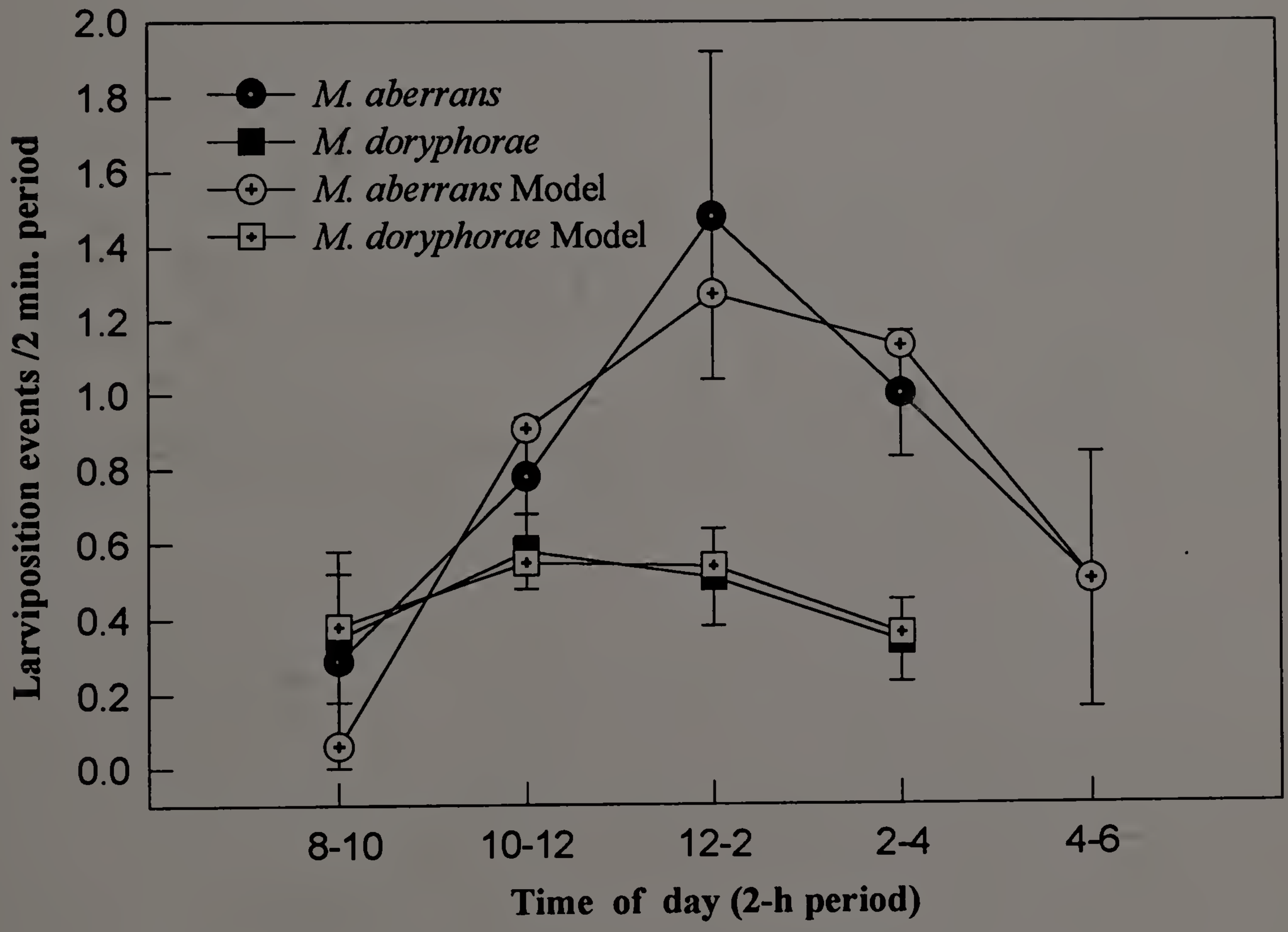

Fig. 6.10 Oberved means and fitted values of the quadratic regression modelsof larviposition frequency response to time of day by both *Myiopharus spp.* (1991 data)

Table 6.5 Linear regression of mean time (Sec) larvipositing by ambient temperature.

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	-0.0720	-0.573	0.427
Temp(^{0} C)	0.0344*	0.0472 *	-0.001
Spec.	--	--	0.0189 *
spec x Temp	--	--	0.0135*
Overall P	0.2586	0.164	0.0143*

*= P < .05

Table 6.6 Quadratic regression of mean time (Sec) larvipositing at 5 periods of time of day.

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	-0.4759	-1.518	-0.0469
Time	0.0932	1.666	0.4501
Time2	-0.1513	-0.297	-0.0889
Spec.	--	--	-0.6716
Spec x Time	--	--	0.5787
Spec x Time2	--	--	-0.0656
Overall P	0.413	0.1420	0.0013

Table 6.7 Linear regression of localized searching frequency by ambient temperature.

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	1.873	4.543	-0.2501
Temp(^{0} C)	0.1065	-0.003	0.2036
Spec.	--	--	1.1265
spec x Temp	--	--	-0.0651
Overall P	0.2773	0.982	0.017

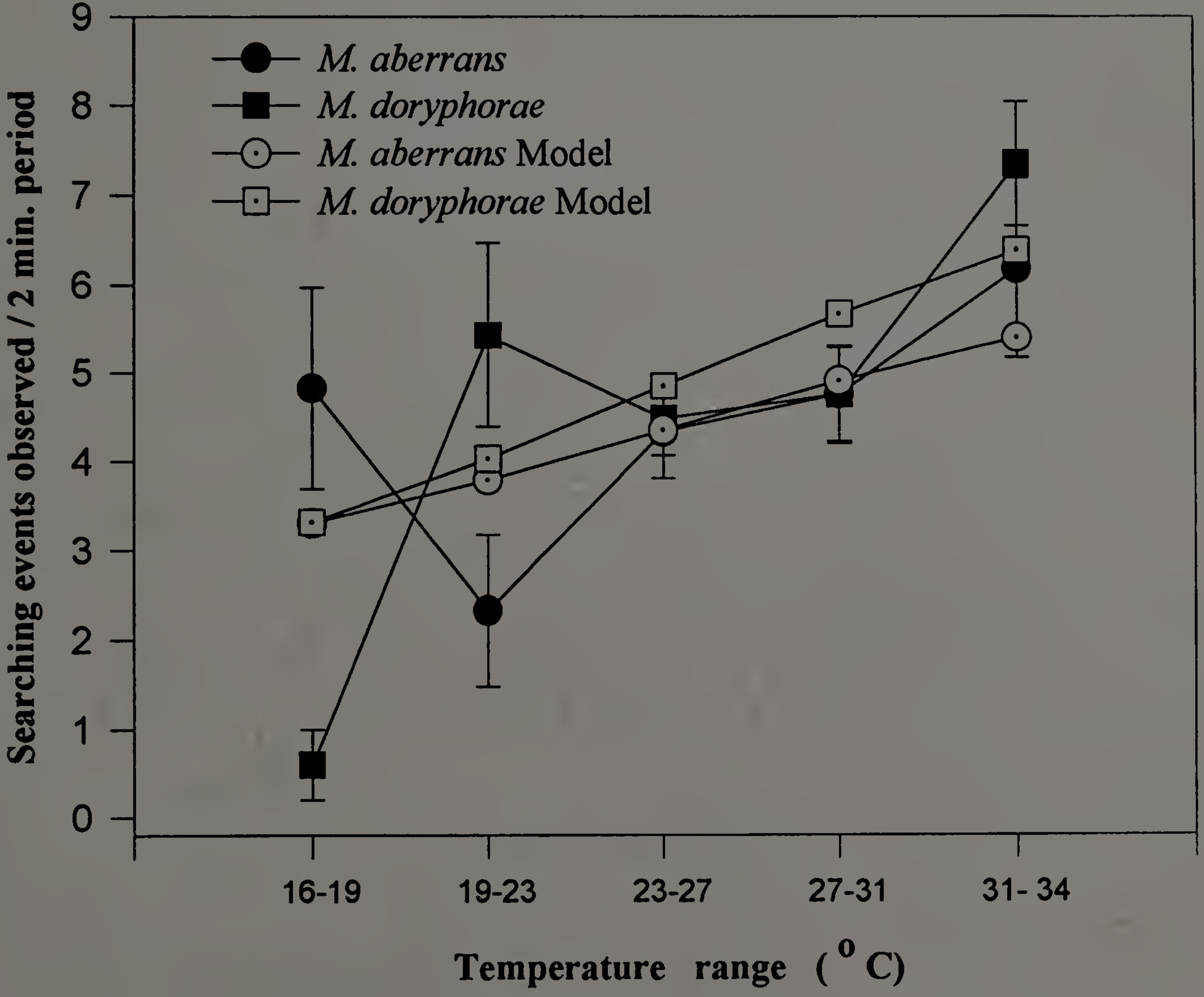

Fig. 6.11 Observed means and fitted values from the linear regression models of searching frequency for both *Myiopharus spp.* across temperature range (1991 data)

Table 6.8 Quadratic regression of localized searching frequency at 5 periods of time of day

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	2.259	-6.761	0.4343
Time	1.540	8.603	3.2172
Time2	-0.221	-1.494	-0.491
Spec.	--	--	0.057+
Spec x Time	--	--	-0.566
Spec x Time2	--	--	0.103
Overall P	0.706	0.0308	0.073+

+=.05 < P < .10

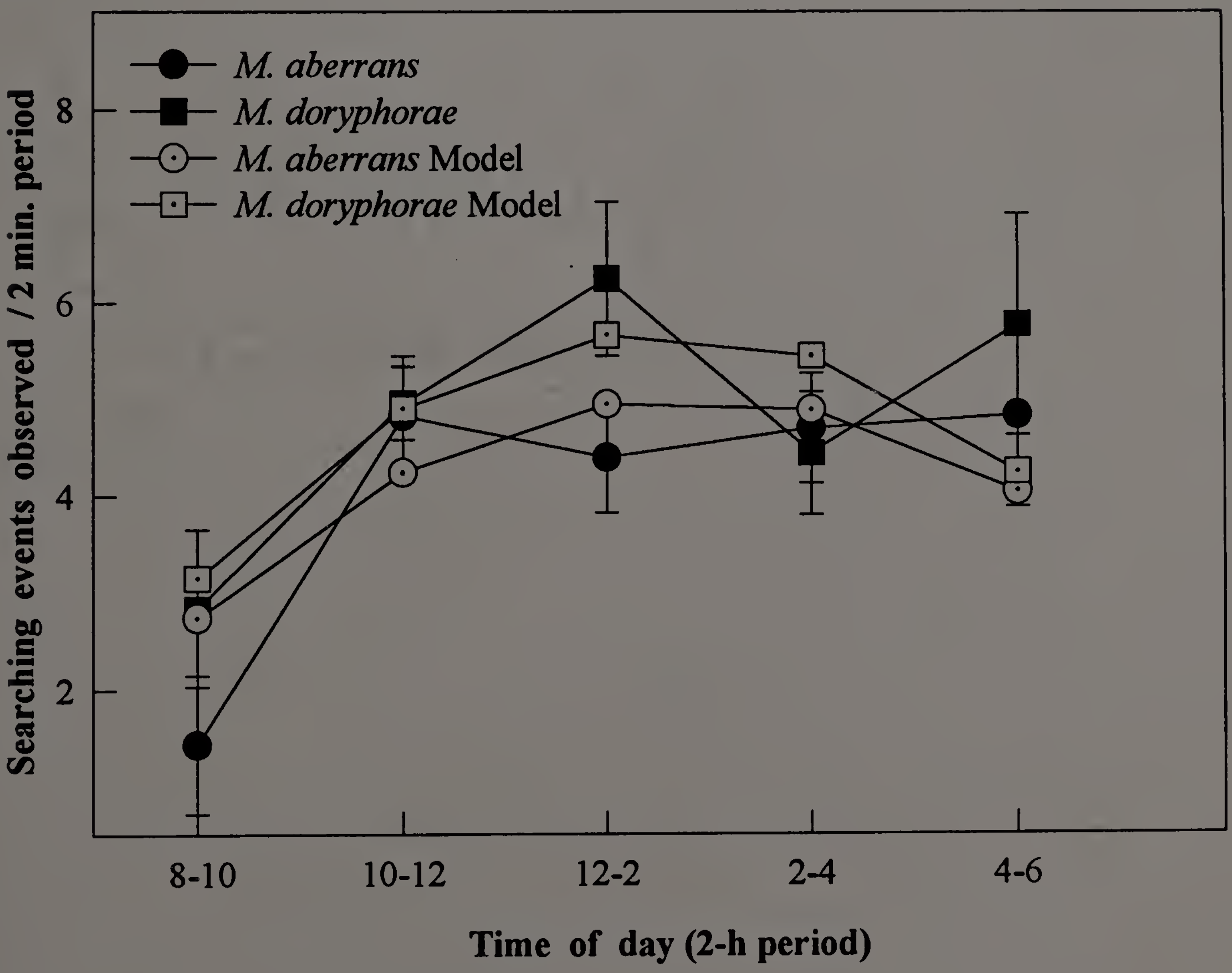

Fig. 6.12 Observed means and fitted values of the quadratic regression models of searching frequency response to time of day by both *Myiopharus spp.* (1991 data)

Table 6.9 Linear regression of mean time (Sec) localized searching at 5 temperature ranges

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	17.810	-2.087	-0.4541
Temp(^{0} C)	-0.111	0.4418	0.391
Spec.	--	--	13.843
spec x Temp	--	--	0.391
Overall P	0.779	0.192	0.0427

+=.05 < P < .10

Table 6.10 Quadratic regression of mean time (Sec) localized searching at 5 periods of time of day

	Separate fields		Within same field
	M. aberrans	*M. doryphorae*	*M. aberrans* + *M. doryphorae*
Constant	34.344	2.259	0.434
Time	-13.357	1.540	3.217
Time2	2.044	-0.221	-0.491
Spec.	--	--	0.057+
Spec x Time	--	--	-0.565
Spec x Time2	--	--	0.1029
Overall P	0.284	0.706	0.0731+

+=.05 < P < .10

Larviposition-Related Behavioral Studies

Colorado Potato Beetle Defenses Against Larviposition. Colorado potato beetle larvae responded to larviposition attempts by *Myiopharus aberrans* and *M. doryphorae* with a wide variety of behaviors hypothesized to deter the parasitoids from attacking or prevent them from larvipositing. These behaviors were not seen to be employed against the pentatomid predators *Perillus bioculatus* (Fabricius) and *Podisus maculiventris* (Say) or any other approaching insects besides *Myiopharus*, but appeared to be used indiscriminately against both species of *Myiopharus*. Total numbers reported (Table 6.11) were cumulative occurrences of the behaviors recorded during the 109 h of directed observations during the summers of 1990 and 1991. The description and effectiveness of each type of defensive is presented.

Table 6.11 Colorado potato beetle defensive responses to *Myiopharus* attacks under field conditions.

CPB Defensive Behavior	Events observed (n)	Larvipositions n (%)	Successful CPB escapes n (%)
Leg-flailing	124	68 (55)	56 (45)
Regurgitation	86	49 (57)	37 (43)
Dropping to the ground	72	64 (89)	8 (11)
Thanatosis	34	25 (74)	9 (26)
Tightening of abdominal muscles	92	2 (2)	90 (98)
Defecation	15	15 (100)	0 (0)

Flailing of fore and middle legs: This direct form of defense was used by individual CPB larvae from second to early fourth instar. The larvae were evidently able to detect approaching parasitoids from any direction at a distance some 0.5 m, at which point they reared up on abdomen and hind legs and began flailing their anterior pairs of legs. To test whether other approaching objects would induce leg-flailing, during one study day we tried moving several other small objects toward a number of CPB larvae which had shown this reaction to the tachinids (n=25 trials); we moved the approaching objects at different speeds, distances and angles, but were unable to provoke the reaction brought about by *Myiopharus*. Leg-flailing behavior was observed on 124 occasions during the study and was effective in preventing larviposition in 45 % of cases (Table 6.11).

Cycloalexy or circular defense. First- and second-instar CPB larvae often maintained themselves in tightly concentrated groups in which the limited exposure of any given larva by itself presumably conferred some degree of protection to these vulnerable early stages. In addition, the larvae in these groups commonly reacted to the approach of parasitoids with a collective manifestation of leg-flailing behavior as described above. This kind of circular defense, or "cycloalexy," has long been recognized in insects from several families (Wheeler & Mann 1923, D'Azevedo Marquez 1933) and was recently reassessed by Vasconcellos-Neto & Jolivet (1988) with respect to larval defenses of chrysomelids and by Weinstein (1989) for sawflies. Cycloalexic behavior of CPB larvae toward *Myiopharus* females was often dramatic, the flailing activity involving all or nearly all members of a given group and becoming increasingly intense with the approach of the parasitoid. Undoubtedly both the grouping of larvae and their defensive movements contributed to the effectiveness of this behavior in preventing larviposition; because of our inability to separate these effects cycloalexy is included with leg-flailing by later-instar larvae in the data in Table 6.11.

Regurgitation: Third- and some fourth-instar CPB larvae commonly responded to attacking *Myiopharus* females by ejecting from the mouth a large drop of brown liquid. When regurgitation occurred in this context it took place immediately upon physical contact by the parasitoid and before larviposition had occurred. Eighty six instances of regurgitation were observed, with 43% were successful in causing the tachinid to withdraw without completing larviposition (Table 6.11). All successful escapes occurred when beetle larvae were attacked while they were situated on the undersides of inclined potato leaves and positioned with head uppermost; from that position, the disgorged liquid ended up covering the body of the larva and as soon as this liquid was contacted by a parasitoid it flew away.

Dropping to the ground. Another common reaction of principally third-instar larvae at the instant parasitoids landed upon them was to drop from the potato leaf on which they had been feeding, landing on lower foliage or falling to the ground. Its success was limited, however; in nearly 90% of the 71 cases when this behavior was observed (Table 6.11) the parasitoids never released the seized larva and were able to complete larviposition.

Thanatosis. Threatened late-instar larvae and adults often adopt a type of passive posture (thanatosis, or "playing dead") long recognized in a large number of insect species. Thanatosis occurred in CPB larvae only at the time of approachment or contact by *Myiopharus* flies. The larva would become limp and motionless and cease to respond to most stimuli. The attacking fly, in turn, invariably responded by engaging in the behavior referred to above as "prelarviposition stimulus," which in most cases eventually succeeded in stirring a response by the host larvae. Thanatosis occurred in only 34 of the larviposition attempts recorded in our study and ultimately deterred larviposition in only 26% of these cases (Table 6.11).

Tightening of the abdominal muscles and integument. By the time well-fed late fourth-instar reached maximum size their abdomen became nearly spherical in shape and comprises over 90% of body volume. Morphological constraints prevent rearing of the body and the leg-flailing defense characteristic of early instars. Meanwhile the abdominal muscles of these larvae have thickened and strengthened, as was evident in the many dissections performed in the course of these studies. The most common defensive behavior observed in fourth-instars attacked by *Myiopharus* was for the larvae to further curl the body into an almost a perfect sphere and tighten the abdominal muscles, as evidenced in a change in the aspect of the integument from its normal dull cast to a shinier, smoother-appearing surface. This was the most effective defensive behavior recorded in our study for fourth instars, deterring larviposition in 98% of observed cases (Table 6.11). The importance of the active tightening of the musculature was observed in several caged larvae in the laboratory in which *Myiopharus* females were unsuccessful in inserting the larvipositor for as long as the larva maintained this tensed posture, but completed larviposition immediately if the larva relaxed its abdomen and continued walking. It was also noticed during the performance of injections into CPB larvae (author's unpublished data), when fourth-instars required prior chilling to prevent their tightening of abdominal muscles which impeded insertion of the syringe.

On five different occasions in late August (1989-1991), *M. doryphorae* females attacking fourth-instar CPB larvae in the field were observed to persist in their larviposition attempts for 5-7 min but eventually to desist without completing parasitization of these larger hosts. This species has a sharper larvipositor than *M. aberrans* (Bruneteau 1937) and, if it is unusual for the former to succeed in larvipositing in CPB of this instar, it would appear very unlikely that *M. aberrans* is ever successful in doing so under natural conditions.

Defecation. Although defecation by CPB larvae upon attack by female *Myiopharus* was noted in a small proportion of cases, it was ineffective in preventing larviposition in any instances observed (Table 6.11) and, in fact, was observed always to occur when the fly's larvipositor was already penetrating the integument of the host larva. Rather than a defense, therefore, defecation should probably be viewed as a traumatic reaction to the execution of larviposition itself.

Collectively, the defensive behaviors detailed above occurred only in about one half of the larviposition attempts by *Myiopharus spp.* which were observed during the course of the study; because unresisted larvipositions were not recorded, the global effects of these defenses upon CPB population dynamics remain to be ascertained. Of the instances of defense behavior observed, 49% overall were successful in preventing larviposition. Although CPB larvae are not infallibly recognizable in the field, our observations suggested that defense reactions of these larvae vary in a loose relation to the larval growth stage in the following sequence: leg flailing is used in conjunction with clustering in the first two instars; in the third instar individual leg-flailing is common as are regurgitation and dropping to the ground; thanatosis and finally abdominal muscle tightening become more prevalent means of defense during the fourth larval instar. Given this progression, our observations of the relative effectiveness of these different defenses--of which muscle-tightening and leg-flailing (particularly in combination with clustering) showed the highest rates of success--may account in part for our findings (López *et al.* 1993) of highest *Myiopharus* recruitment in the second and third larval instars of the beetle in field conditions.

Post-Larviposition 'Territorial' Behavior of *Myiopharus spp*. A total of 85 instances of post-larvipositional territorial behavior by *Myiopharus spp.* were observed during 109 h of observation in late summer of 1990 and 1991 (Table 6.12). As is evident in the table, both *Myiopharus doryphorae* and *M. aberrans* engaged in this behavior, with no significant differences between the two species with regard to the aspects recorded. No instances of this behavior were noted during our general behavioral studies at any other time of the growing season (i.e. mid-June through early August).

Table 6.12 Territorial behavior of *Myiopharus doryphorae* and *Myiopharus aberrans* in potato fields at South Deerfield, MA (1990 and 1991 data combined).

	M. doryphorae	*M. aberrans*
Defense episodes observed in 109 h	46	39
Larvipositions before defense began (Mean ± SE)	3.25 ± 1.12	3.3 ± 1.2
Repulsions per defense episode (Mean ± SE)	4.25 ± 1.2	3.2 ± 1.1
Mean time spent per defense episode (min ± SE)	7.23 ± 2.2	6.3 ± 2.2

A given territorial defense episode normally began after larviposition by the same fly in 3-4 larvae located on the same potato plant. The fly then remained based on a leaf of this same plant for some 6-7 minutes during which it drove away an average of 3-4 approaching flies of either or both species of *Myiopharus* (Table 6.12).

Two initially puzzling aspects of this defense behavior are probably interrelated. One of these is the question of why the defense of newly parasitized host larvae would occur only during the final weeks of the growing season, given that both *Myiopharus* species are engaged in larviposition into most larval instars of the same host throughout most of the summer. The other peculiar feature of such defensive behavior, whose only apparent advantage is that of preventing the superparasitization of individual larvae, is that defending a parasitized host should be redundant in species such as *Myiopharus aberrans* and *M. doryphorae* which discern and reject already parasitized CPB larvae (López *et al.* 1995). Time spent protecting already deposited progeny reduces opportunities for further larvipositions and would appear maladaptive given that the discrimination ability of both species works adequately to preclude superparasitization.

Two additional observations from our studies help to explain these apparent anomalies. One was that host discrimination (or at least the rejection of previously parasitized hosts) breaks down to some extent during the last weeks of the summer. Dissections from CPB population samples (López *et al.* 1993) revealed five superparasitized CPB larvae containing two *Myiopharus* larvae apiece (less than 1% of any given sample); all of these came from samples taken after August 15. This in turn appears to be related to the lowered host: parasitoid ratio found late in the summer (López *et al.* 1993), which drastically reduce the probability of the flies encountering acceptable host larvae; caged experiments in which host availability was kept artificially low have brought about superparasitization at other times of the summer (López *et al.* 1995).

A superparasitized CPB larva gives rise to at most one live *Myiopharus* adult; the cause of death of the additional larva has not been determined. Our untested hypothesis concerning the possible adaptive advantage of larvipositing *Myiopharus* females' defense behavior is that the 6-7 additional minutes thereby provided for establishment within the host confers some sort of advantage upon the first *Myiopharus* maggot deposited within a given CPB larva.

Provocation of Pre and Post Diapausing Adult Colorado Potato Beetle by *Myiopharus aberrans*. In Massachusetts, field collected beetles after the photoperiod had shortened to 14 h of light or less are considered prediapausing beetles (Voss *et al.* 1988). All beetles that emerge from the soil in the spring and colonize the potato fields from the end of May to the middle of June are considered postdiapausing beetles (Voss & Ferro 1990a). In the laboratory, beetles reared under short photoperiod conditions (i.e. 13:11, LD) will enter diapause. Beetles raised under long photoperiod regime would never enter diapause (Kort 1990).

No larvipositions were observed during the 60 h of field observation of *M. aberrans* stimulating adult beetles at the end of the 1990 growing season. Routine observations of this species during the other behavioral studies also revealed no examples of larviposition in adult beetles at the beginning of the 1990 summer nor during early or late summer of 1991. However, on 12 different occasions in 1990 *M. aberrans* females were seen to jump atop pre-diapausing beetles and hit them with the tip of their abdomen. This behavior occurred only after the parasitoids had spent 8-15 minutes stimulating the beetles with no apparent effect.

An additional 36 h of observations of caged *M. aberrans* with CPB adults also produced no larvipositions and no distinguishable differences in behavior of flies or beetles from those noted in field conditions.

Detailed examination of the videotape made of *M. aberrans* females caged with CPB larvae and adults showed that the flies continued provoking the adult beetles uninterruptedly for the entire 9 h of filming except for short (5-10 sec) visits to the water wick and sugar cubes. CPB larvae were disregarded by the flies, whereas interference with all three adults was such that the beetles were prevented from eating for several hours at a time. After the video recording was made the two *M. aberrans* females were dissected and shown to contain 9 and 11 mature parasitoid larvae. The two CPB adults and five CPB larvae were also dissected, but none were found parasitized.

Removal of elytra alone or elytra and wings to expose the abdomens of the beetles did not induce *M. aberrans* females to larviposit in pre-diapausing CPB adults in our caged trials, although the flies continued to pursue the beetles and were engaged in the same provocation behavior described above whenever the cages were examined. After a week of exposure to the flies, all 60 beetles were dissected but none of them were found to contain parasitoids.

Attempts to provoke flight in caged pre-diapausing CPB by provision of perches and other alterations of their environment were all unsuccessful. However, when these beetles were suspended by the thorax above they finally "flew" (opened elytra and spread wings and began beating them). *M. aberrans* flies on the floor of the same cage responded immediately, directing themselves toward the beetles with accelerated jerking movements but never taking flight to approach the beetles in the air. As the tethered beetles were lowered close to the flies and again provoked into initiating flight movements, the *M. aberrans* appeared increasingly excited and curved their abdomens downward, protruding their larvipositors as soon as the beetles began opening their elytra (Fig. 6.13). The instant that a suspended beetle spread its wings, the *M. aberrans* fly jumped upon the beetle's abdomen and with a quick movement hit the exposed dorsal wall with the tip of its abdomen and introduced its larvipositor in the soft abdominal integument. Insertion of the larvipositor was extremely swift, the fly escaping just before the beetle was able to fold back its wings and close its elytra (Fig. 6.14).

Of the 10 CPB adults exposed to the flies in this manner, only six could be induced to initiate flight, but of these all six were attacked by the larvipositing *M. aberrans*. The larviposition attempts took two different forms: one in which the fly jumped facing forward from behind the beetle, stung it and flew away through the opening between the beetle's raised elytra; and a second in which the fly jumped from beside the beetle to a position facing its rear, grasped the last segment of the beetle's abdomen, inserted the larvipositor in an anterior segment and escaped still facing in the same direction. Attacked beetles always folded back wings and elytra immediately, stopping short of actually taking flight. The six stung beetles were dissected within one hour of exposure to the flies; *Myiopharus* larvae with no red coloration (López *et al.* 1995)were encountered in three of these and the others contained no larvae. That the parasitoid larvae found within the dissected CPB were not pre-existing larvae from earlier larvipositions is established by their lack of red coloring, which would have appeared in older parasitoid larvae (López *et al.* 1995).

It can be concluded from the above that *Myiopharus aberrans* will parasitize adult Colorado potato beetles and are able to do so when the beetles initiate flight, allowing the flies to reach the exposed soft abdominal dorsum. The three unsuccessful "larvipositions" may have been cut short by the beetles' reactions; presence of wings and the movements involved make both precise observation and, evidently, larviposition itself more difficult than in the case of CPB larvae. In addition, the drop of hemolymph which signals a completed larviposition into larval CPB was not an applicable indicator of successful larviposition in the case of the winged adults. If insertion of the larvipositor did occur in these three cases, failure of parasitization may have been due to the age and possible overmaturity of the *M. aberrans* females available for this test.

This is the first account of *M. aberrans* larvipositing in adult CPB. The speed and short duration of the larviposition act, its infrequency relative to the time the flies spend provoking the adult hosts, and its evident restriction to short periods at the beginning and end of the growing season help explain why this behavior has not been witnessed under field conditions. This finding has important implications for the assessment of biological control programs. Past determinations of percentage parasitism by *Myiopharus spp.* on

CPB (Kelleher 1960, Harcourt 1971, Tamaki *et al.* 1983a, Horton and Capinera 1987, López *et al.* 1993) often did not distinguish between *M. doryphorae* and *M. aberrans* and did not take into account the latter species' parasitization of the beetle's adult stage. We now know that correct determination of parasitoid impact on CPB populations will need to include sampling of pre- and post-diapausing adult beetles in the process of migrating.

We take the "provocation" behavior discussed earlier to be a prelude to larviposition attempts during the two periods of the summer when it occurs, which coincide with the two main periods of CPB flight. Although it is not clear what causes the marked switches in attention of larvipositing *M. aberrans* from adult to larval hosts and back again during the course of each growing season, the series of behavioral studies described above gives some indication of the proximal cues required for parasitization of adult CPB. Successful larviposition in a given beetle appears to constitute a two-step process of pursuit and attack requiring different stimuli for the two steps.

Timely and close pursuit of appropriate adult hosts would seem essential given the very limited time span (less than a second during the life of a typical pre-diapausing beetle) in which the conditions for larviposition are met. *M. aberrans* can only insert its larvipositor when the beetle's raised wings uncover its soft abdominal dorsum; the fly is evidently incapable of larviposition when the beetle's wings are actually beating; it must thus sting its host during flight initiation , or the instant between opening of the wings and active flight; a pre-diapausing beetle normally initiates flight only once.

Not all adult CPB incur pursuit by *M. aberrans*; for most of the summer, adults are ignored in favor of CPB larvae, but adult stages as well as larvae were observed to be disregarded by *M. aberrans* in favor of pre- and post-diapausing adults. We hypothesize that some aspect of the development or regeneration of flight muscles, a principal physiological change occurring in circum-diapausing adult CPB, might provide the stimulus for this pursuit behavior by *M. aberrans*. Reaction to sex pheromones of the adult CPB would be insufficient to account for this behavior given its failure to occur toward reproductive adults in midsummer.

For the "attack" stage or larviposition itself to occur, exposure of the beetle's abdomen was a necessary but insufficient requirement, as evidenced by our experiments involving removal of elytra and wings. Given the visual acuity of most Diptera and their particular sensitivity to movement and contrast (Dethier 1963, Schmid 1992), it is likely that the movement of the elytra and the sharp difference between their striped exterior and plain white underside serve as the immediate triggers for attack by *M. aberrans*.

Whatever its proximal and distal causes, the switch to adult hosts early and late in the growing season holds interesting ecological and management implications. As congeners, *Myiopharus doryphorae* and *M. aberrans* could be expected by prevalent niche and competition theories to have become diversified in their resources or behavior or to maintain allopatric distributions, yet the two species share a single principal host in overlapping ranges and, as reported above, show minimal behavioral differences. However, the replacement of adult hosts for larvae by *M. aberrans* comes at precisely the times during the growing season when beetle larval populations are lowest and adults are a more available resource, reducing host competition between the two species. Late-season larviposition in adults greatly reduces the parasitoids' exposure to the CPB larval predators (pentatomids, carabids, cicindelids, etc.) which are most abundant during late summer.

These same features may lend *M. aberrans* added value as a biological control agent and as a complement to *M. doryphorae* for this purpose. The different reproductive strategies by these two tachinids, and by *M. aberrans* within the course of a summer, resemble and parallel somewhat the risk-reducing or "bet-hedging" patterns of the beetle's own diapause and migration behavior (Voss and Ferro 1990b). In early summer they may also help to counteract the lack of synchronization with the CPB population which has been cited as a cause for low parasitization rates by *M. doryphorae*.

Parasitism at flight-initiation of CPB adults also means that close to 100% of the late-summer larviposited *M. aberrans* undoubtedly migrate along with their hosts at the end of the growing season, as contrasted with a probability closer to 85% (Voss *et al.* 1990a and 1990b) for *M. doryphorae*. The migration of insect pests traditionally presents serious difficulties for their natural enemies. The case of *Myiopharus* is a rare one in which principal parasitoids of a significant economic pest migrate within their host, and *M. aberrans* would appear especially likely to economize on parasitoid releases and contribute to their moving with the beetle. This should make *M. aberrans* a particularly promising species for release at the edges of CPB distributions in areas such as Eurasia where the beetle is a rapidly expanding exotic pest (Jolivet 1991).

Conclusion

Studying the behavior of *Myiopharus doryphorae* and *M. aberrans* under field conditions has helped to demonstrate and explain their effectiveness in locating and parasitizing their CPB hosts during most of the growing season. Adult females of both tachinid species spend a large portion of their time engaged in a combination of local searching and longer forays that together seem highly efficient at encountering available host larvae, as illustrated in data from the site where both species occurred (Table 6.13a). Maximum number of larvipositions recorded per 2-min observation period were 13 for *M. aberrans* and 11 for *M. doryphorae*. Given that the maximum larval load of a fertile female *Myiopharus* is normally between 17 and 25 larvae/day (author's unpublished data and Tamaki *et al.* 1983b, Kelleher 1960), there would seem to be little doubt that *Myiopharus spp.* are able to allocate virtually all of their progeny when the host population is adequate.

Table 6.13 Larval host searching and larviposition efficiency of *M. doryphorae* and *M. aberrans* in a potato field in South Deerfield, MA, 1991.

	a) Throughout season of activity		b) After 1 August only	
	% of time spent searching	larvipositions/ min search time	% of time spent searching	larvipositions/ min search time
M. doryphorae	37	0.65	38	0.58
M. aberrans	48	0.97	56	0.30

This situation is illustrated in the center portion of Fig. 6.15, a composite graph in which the seasonal sequence of larviposition-related behaviors of *Myiopharus spp.* from the present study are juxtaposed in time with densities of CPB larvae and adults for the same year at the study site (data presented in Table 6.13). CPB larval density ranged between 40 and 100 individuals/m^2 from mid-June to mid-August (López *et al.* 1993), and the activity of both parasitoid species focused upon location of and larviposition in CPB larvae during this period.

As the CPB larval population became markedly reduced to 20-30 individuals/m^2 later in the growing season (López *et al.* 1993), both *Myiopharus* species began engaging in "territorial" defense behavior near their most recently parasitized host larvae (Fig. 6.15). Such activity might be expected to reduce the time available for searching and perhaps the total number of larvipositions accomplished but should raise the chances for survival of any defended offspring, given the breakdown of restraint from superparasitization which accompanies the lowered host to parasitoid ratios at this time. Table 6.13 portrays the actual situation at the site where the two tachinid species occurred together: *M. doryphorae* continued to spend approximately the same proportion of its time searching with slightly reduced success, while *M. aberrans* spent a somewhat greater proportion of its time in searching but with larviposition efficiency cut to less than one-third.

Soon after this, however, *M. aberrans* ceased parasitizing and defending the beetle larvae as it switched its attention to adult CPB (Fig. 6.15), coinciding with a rise in abundance of pre-diapausing adult CPB as well as the near-absence of any CPB larvae, other than fourth-instars. The late fourth-instar CPB larval integument appears impervious to the larvipositor of *M. aberrans*, but this species is able to larviposit in migrating adult CPB at the very moment that these beetles initiate flight.

As the growing season came to an end, *M. aberrans* continued ignoring CPB larvae in favor of pre-diapausing adults, yet *M. doryphorae* did not cease its protection of the CPB larvae in which it larviposited (Fig. 6.15). This "territorial" behavior was always observed to be directed indiscriminately toward conspecifics as well as females of the other *Myiopharus* species, and the survival advantage it may confer upon a given *Myiopharus* larva would seem independent of the species of any potential superparasitoid.

A rather curious situation was found at the very beginning of the growing season; for most of June, no *M. doryphorae* were found at the site and CPB larvae were far more plentiful than adults, yet *M. aberrans* females pursued adult beetles just as they did at the end of the season (Fig. 6.15). Possible advantages of parasitizing adult beetles at this time can be construed. The CPB larvae present early in the season are necessarily small early-instar ones which seem difficult for the larvipositing parasitoid to grasp properly (Chapter 4 and López *et al.* 1993); they also commonly engage in the rather effective cycloalexic flailing defense. In addition, if parasitoid development that begins in an early-season CPB adult takes appreciably less time than it requires when starting in a larval host (which must pupate before parasitoid larval development begins), larviposition in adult CPB at this time of year may enable *M. aberrans* to build up its population more quickly and take better advantage of the abundance of larval hosts. However, in such speculation it is good to be cautious of the "Panglossian paradigm" (Gould and Lewontin 1979); it is possible that this early period of pursuit of adult hosts results less from some direct selective advantage than from a physiological constraint imposed by this parasitoid's attraction to circum-diapausing adult beetles. By the last week of June in our study site the principal migration of post-diapausing CPB adults was over and *M. aberrans* had begun attacking larvae for a week before *M. doryphorae* appeared.

This earlier appearance of *M. aberrans* than of *M. doryphorae*, noted in at least one other study (Bruneteau 1937), appears anomalous given the congruencies in their life histories. Both *M. doryphorae* and *M. aberrans* overwinter as larvae within diapausing adult CPB (López *et al.* 1992); yet one species is found nearly a month before the other appears. Whether this relates in some way to the stage of the CPB originally parasitized or reflects some other factor of the insects' biology is yet to be determined.

What is perhaps most striking overall in Fig. 6.15 and the results of the present study is the apparent complementarity of these two parasitoids as controlling influences upon a given CPB population. The two species of *Myiopharus* attack the same host stages (CPB larvae) only during midsummer when larvae are most abundant, and for most of this time their discrimination ability keeps losses to superparasitization at close to nil. For perhaps a week toward the end of the season the larval host population plummets and competition apparently becomes acute enough to cause breakdown of the restraint against superparasitization but at this same time territorial behavior around parasitized hosts helps prevent superparasitization and may also reduce wastage of time searching among already parasitized larvae. Before the larval population becomes too drastically reduced, one species shifts to adults of the host species which are by this time more abundant. The same species emerges first the following year and attacks adult hosts while larval hosts are still of suboptimal size (Tamaki *et al.* 1983b, Fig. 6.15).

Improved knowledge of the life cycle of *Myiopharus doryphorae* helped in the design of a methodology for calculation of percentage parasitism that led to recognition of a greater role for that species in controlling CPB populations than was previously recognized (López *et al.* 1993). The findings of the present field study of the behavioral ecology of *Myiopharus spp*. lend further support to Salt's (1958) assertion that:

> *It is naive to suppose that natural enemies can be effectively used for control with inadequate knowledge of their biological nature, especially their physiology and behavior.... The behavior of insect parasites plays a great part in fixing their values as pest controls; and it follows that we must know their behavior before we can use them effectively.*

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Fig. 6.13 *Myiopharus aberrans* fly closely following the movements of an adult prediapausing CPB. (Pursuit by *Myiopharus* commenced prior to beetle's suspension by thorax as depicted)

Fig. 6.14 *Myiopharus aberrans* fly in the act of larvipositing in a prediapausing CPB at the moment it raises its elytra and expands its wings but immediately before the beetle starts beating its wings.

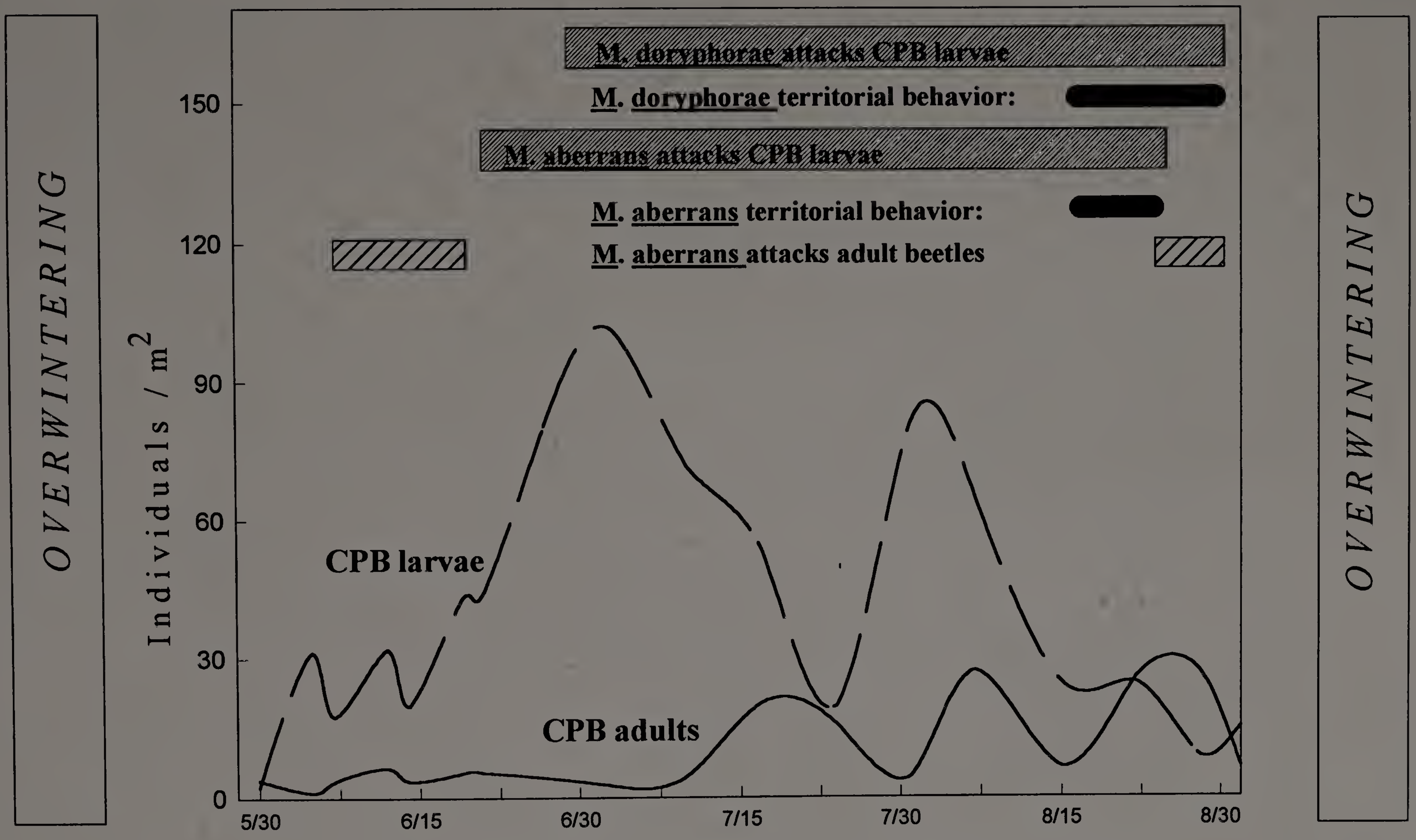

Fig. 6.15 Calendar of larviposition-related behaviors of *Myiopharus doryphorae* and *Myiopharus aberrans* in relation to population dynamics of host larvae and adults (South Deerfield, MA, 1991)

CHAPTER 7

MYIOPHARUS BEHAVIORAL RESPONSES TO COLORADO POTATO BEETLE LARVAE TREATED WITH M-ONE ® (*BACILLUS THURINGIENSIS* SUBSP. *TENEBRIONIS*).

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most damaging defoliator of potato worldwide (Ferro 1985, Gauthier *et al.* 1981). Control this insect pest over the last 30 to 40 yr with insecticides has been variable because the beetle has developed resistance to almost all insecticides used against it (Forgash 1985, Casagrande 1987). In addition, in many instances the insecticides have been shown to be more damaging to insect pests' natural enemies and other beneficial insects than to the insect pests themselves (Ehler & Endicott 1984).

Bacillus thuringiensis Berliner subsp. *tenebrionis* has been used successfully against immature stages of the Colorado potato beetle (Ferro & Lyon 1991, Ferro 1993). However, because under laboratory conditions it has developed resistance to the delta-endotoxin produced by *B. thuringiensis* (Wahlon *et al.* 1993), there is a concern that the beetle will develop this same resistance under field conditions (Ferro 1993). The integration of natural enemies of the Colorado potato beetle with judicious applications of *B. thuringiensis* subsp. *tenebrionis* could be important in delaying the development of insecticide resistance by the beetle while at the same time preventing the decline of populations of its insect predators and parasitoids.

The only endemic parasitoids of the Colorado potato beetle in North America are the tachinid flies *Myiopharus* (=*Doryphorophaga*) *doryphorae* (Riley) and *Myiopharus* (=*Adoryphorophaga*) *aberrans* (Townsend); occasionally *Myiopharus* (=*D.*) *australis* Reinhard and *Myiopharus* (=*D.*) *macella* Reinhard have also been found to attack Colorado potato beetle larvae (Arnaud 1978). During the first beetle generation each summer, adult female *Myiopharus* flies, which are obligate solitary parasitoids, larviposit within 2nd to early 4th instar Colorado potato beetles. First instar parasitoid larvae remain undeveloped until parasitized beetle larvae borrow into the ground to pupate. At this time the parasitoid kills its host and completes its development in about 10 days at 28° C (unpublished data). During the last summer generation of the beetles, the parasitoid larvae remain undeveloped within the Colorado potato beetle larvae, allowing beetle larvae to complete their development and emerge as adults. After generating flight muscles, the parasitized adult beetles migrate to overwintering sites, or remain in the field to diapause if they do not generate flight muscles. The first instar parasitoids overwinter within the beetles and complete development the following season after the beetles emerge from overwintering sites (López *et al.* 1992).

Low beetle populations in some localities coincide with relatively high densities of these parasitoids (Horton & Capinera 1986, Bjegovic 1968), and continuing studies of the biology and ecology of *Myiopharus* spp. (López *et al.* 1992, López *et al.* 1993) may lead to their applicability in integrated pest management programs. Studies concerning the behavioral response of these parasitoids to Colorado potato beetle larvae fed foliage treated with *B. thuringiensis* have not been reported. If the fly is unable to discriminate between healthy larvae and larvae intoxicated with the *B. thuringiensis* delta-endotoxin, parasitoid larvae deposited within lethally intoxicated larvae will not survive.

In this study, we investigated the larvipositional response of *M. doryphorae* flies presented with Colorado potato beetle larvae fed foliage treated with different concentrations of *B. thuringiensis* subsp. *tenebrionis* for different periods of time.

Methods

Colorado potato beetle larvae used in these experiments were from a colony started from beetles collected from the field the previous July. Populations from the area of collection have high levels of resistance to organophosphate and carbamate insecticides and lower levels of resistance to pyrethroids (Argentine *et al.* 1989). The *M. doryphorae* flies used in these experiments were 8 to 20 day old females from a greenhouse colony started from parasitized beetle larvae collected from the field the previous September. The quality of light is extremely important in maintaining this parasitoid under artificial conditions, and for this reason, the colony was kept in screened cages in a greenhouse rather than in the laboratory.

All behavioral observations were made in the greenhouse (Fernald Hall, University of Massachusetts, Amherst, MA) during the spring of 1990. The greenhouse was maintained at 70 ± 5° C and 90% RH during the experiments. Potato trifoliates were excised from greenhouse grown potato plants, *Solanum tuberosum* L. 'Superior', and placed immediately into floral pics (5.5 ml) containing one-quarter strength Hoagland's solution (Hoagland & Arnon 1950). The environmental chamber used in these experiments was maintained at 27° C, 75% RH, with a photoperiod of 16:8 (L:D)h.

Instar-specific Lethal and Sublethal Concentrations

Dose-mortality curves were determined for early first instars (1.0 mg body weight), late first instars (2.3 mg), early second instars (4.0 mg), late second instars (7.8 mg) and early third instars (15.6 mg) exposed to potato foliage dipped in different concentrations of M-one® (*Bacillus thuringiensis* subsp. *tenebrionis*, ref. MYD 810c, 11,354 μg toxin/ml; Mycogen Corporation, San Diego, CA). A minimum of 30 larvae were tested for each concentration (3 or 4 replicates per concentration). The control consisted of distilled water. Leaflets were dipped in the appropriate concentration, air dried, and placed into ventilated plastic boxes (12 by 7 by 6 cm). After 10 larvae were introduced into each box, the boxes were placed in the environmental chamber. Larvae were checked daily and Hoagland's solution was replenished as needed, as was fresh foliage. Mortality was assessed 96 hours after the larvae were placed on the foliage; twice the time needed for larvae to molt to the next instar at 27° C (Ferro *et al.* 1985). The criterion for mortality was failure of tarsi to move when larvae were prodded gently with a moistened paint brush. Polo-PC (Le Ora Software 1987) was used for probit analyses. The data are presented as percentage mortality as a function of mg toxin/liter of water/mg body weight.

Behavioral Response of Larvipositing *M. doryphorae*

The *M. doryphorae* flies used in this experiment had been kept in colony with Colorado potato beetle larvae, a dental wick saturated with distilled water and sugar cubes. Late second instars were used for all of the experiments. Dilutions of M-one® were made with distilled water. The lethal dose (10 mg toxin/liter of water/mg body weight) was the LC_{90}, and the sublethal dose (0.3 mg toxin/liter of water/mg body weight) was the LC_{20}.

Trifoliates of potato foliage were dipped in the appropriate concentrations and allowed to air-dry before placing the larvae on them. Half of the larvae were allowed to feed for 1 h before being removed from the treated foliage and placed on fresh foliage. The other half were left on the treated foliage for 24 h before being removed and placed on fresh foliage.

Each observation consisted of introducing a single female fly into the observation cage with three larvae, representing a larva exposed to a lethal or sublethal concentration of M-one, or to distilled water (control). Three potato trifoliates were placed in water pics and the bottom of the pics were inserted into a hole in a wooden stand so that trifoliates were 4 cm apart. One larva of each kind was placed on each trifoliate. Larval position was randomized among the three positions for each trial. Thirty-nine and 36 different female flies were observed for the larvae fed treated foliage for 1 h and 24 h respectively.

Each fly was placed inside the 25 by 25 by 25 cm screened observation cage 30 min before initiating the experiment to acclimate it to the arena. The wooden stand holding the foliage and larvae was then introduced into the cage, and observations were made for 15 uninterrupted min. Every larviposition by the flies was recorded. At the end of each trial, all larvae were collected and held separately in plastic cups (35 ml) before being dissected to determine the number of parasitoid larvae in each host larva.

Results and discussion

Instar-specific Lethal and Sublethal Concentrations

The LC_{50} values for the different larval stages were 2.03 mg toxin/liter/mg body weight for early first instars, 1.7 mg for late first instars, 1.09 mg for early second instars, 1.85 mg for late second instars and 0.95 mg for early third instars (Fig. 7.1). The LC_{90} values ranged from 3.0 mg (early second instars) to 10.1 mg (late first and second instars). When the data were converted to mg toxin/liter/mg body weight there was little difference between the different stages and percentage mortality.

Behavioral Response of Larvipositing *M. doryphorae*

A Chi square did not show a preference by *M. doryphorae* flies for Colorado potato beetle larva in either the 1 h or the 24 h treatment ($\chi^2 = 0.159$ and $P = 0.9261$ for 1 h treatment, and $\chi^2 = 1.15$ and $P = 0.5617$ for the 24 h treatment, Table 1). This indicates that *M. doryphorae* did not distinguish among larvae that have ingested a lethal or sublethal dose of *B. thuringiensis* toxin or no toxin. However, these observations were made with larvae that had been intoxicated for 24 h or less.

Under field conditions, *Myiopharus* flies in the process of larvipositing encountered various defensive reactions by the beetle larvae. Including flailing of fore and middle legs, dropping to the ground, and thanatosis ("playing dead"). Our field and laboratory behavioral studies with this parasitoid have shown the fly to be sensitive to such reactions by host larvae. *Myiopharus* readily larviposit on walking larvae, but were never observed to larviposit in dead larvae. The flies reaction to a thanatoid larva was normally to spend several minutes stimulating the larva until it moved before finally larvipositing. Defensive movements such as flailing of legs also effectively delayed larviposition (Chapter 6).

Colorado potato beetle larvae which fed for 1 h on treated foliage (lethal and sublethal doses), although still moving, reacted sluggishly to the presence of the parasitoids compared with the control larvae whose reaction was to quickly and strongly flail their fore and middle legs. *Myiopharus* readily larviposited in sluggish larvae but took more time to overcome the strong defensive reactions of healthy control larvae. This is reflected in the summary table of the mean time elapsed before the first larviposition in each treatment type in the larvae fed for 1 h (Table 1). For those larvae, an ANOVA showed a significant difference in the mean time elapsed before the first larviposition among these treatments when compared to the control (F= 6.77 and P= 0.0032, n=39).

When larvae fed for 24 h and were exposed to *Myiopharus* on treated potato foliage (lethal and sublethal doses), the larvae reacted more sluggishly to *Myiopharus* flies approaching them than the larvae fed for 1 h on these treatments. The *Myiopharus* larvipositing behavior was similar to their reaction to larvae with a thanatosis defense; they spent up to several minutes stimulating the larvae until they moved. In the case of the control larvae, *Myiopharus* also spent more time before larvipositing in them, but for the opposite reason; these larvae were more active and more defensive. An ANOVA did not show any difference in the mean time elapsed before larvae from the different treatment types were stung for the first time (Table 1. F=0.60 and p=0.5568, n= 36), although the average time in the case of the larvae fed a sublethal dose of *B. thuringiensis* was lower.

The chi square test showed that *M. doryphorae* was unable to discriminate between hosts intoxicated and not intoxicated with *B. thuringiensis*. Although flies were not deterred from larvipositing into host larvae intoxicated at different levels, their reactions to the larvae differed. There was equal probability of flies larvipositing into beetle larvae fed a lethal or sublethal dose of *B. thuringiensis* or control larvae. Larvae deposited by parasitoids within hosts fed with lethal doses of *B. thuringiensis* are certain to die because the hosts will never complete development; this would reduce the number of parasitoids surviving to the next Colorado potato beetle generation. However, when sublethal doses of *B. thuringiensis* allow Colorado potato beetle larvae to reach the prepupal stage, the parasitoid larvae within them will also complete development.

For purposes of biological pest control it is of interest to examine the population dynamics of *M. doryphorae* used in conjunction with *B. thuringiensis* against the Colorado potato beetle. Our results show that *M. doryphorae* does not discriminate between *B. thuringiensis* intoxicated hosts and non intoxicated hosts. In either case the larvae deposited by parasitoids within hosts fed with lethal doses of *B. thuringiensis* will die if the host dies before reaching the prepupal stage. However, sublethal doses of *B.*

thuringiensis do not stop the Colorado potato beetle larvae from reaching its prepupal stage. In a side experiment in the laboratory testing the survival of parasitoid larvae deposited in host larvae treated for 1 h with a sublethal concentration of *B. thuringiensis*, 39 out 50 third instar parasitized previously treated larvae produced healthy adult parasitoids versus 45 out of 50 for the controls. From an evolutionary perspective, tachinidae arose from a group of taxa whose larvae generally feed in decomposing materials, and therefore have long coexisted with bacterial and fungal toxins in their diet (Gauld *et al.* 1992). Thus, it is possible that the parasitoids do not react to the presence of *B. thuringiensis* because of their familiarity with bacterial environments. Once *Myiopharus* parasitoids are within their host, the only other requirement the flies need to molt and complete their development is the rise in ecdysone levels and low titers of juvenile hormone within the hemolymph of its host (unpublished data) and this naturally occurs when the host reaches the prepupal stage.

The behavior of parasitoids during the 15-min exposure period was not quantified except for the elapsed time before larvipositions and the number of larvipositions themselves. However, our observations showed that the host larva's defensive behaviors - especially regurgitation, flailing of their legs in the presence of parasitoid flies and falling to the ground when contacted by parasitoids- were readily exhibited by control larvae when compared with the larvae fed foliage treated with *B. thuringiensis*, especially those fed treated foliage with lethal concentrations which after 24 hours were not feeding anymore and were generally sluggish in their responses. If sublethal doses of *B. thuringiensis* retard larval development, then low doses of *B. thuringiensis* could allow more time for *Myiopharus* to act upon the larval population. This is particularly true if these larvae are more readily parasitized by *M. doryphorae* than either healthy or lethally intoxicated larvae, as indicated by average time elapsed before first larviposition for both 1 h and 24 h experiments (Table 7.1).

The lower percentage of larvipositions in the group of larvae fed treated foliage after 24 h than in those fed for 1 h (Table 7.2) suggests that the length of time after larvae fed on treated foliage may be important to inundative control programs; i.e. it may be best to introduce these parasitoids 24 h after the application of *B. thuringiensis* to ensure maximum establishment.

In regard to natural populations of *M. doryphorae*, the present studies showed *B. thuringiensis* did not seem to directly affect *M. doryphorae* parasitoids unless their larvae are laid within Colorado potato beetle larvae lethally intoxicated with *B. thuringiensis*. Superparasitism rarely occur in the field and when it happens only one adult parasitod survive per host (Kelleher 1960). Field and laboratoty studies have shown that *M. doryphorae* flies are able to discriminate between parasitized and nonparasitized hosts (López et al. 1995). Therefore, superparasitism in the cages (Table 7.2) was probably more a cage effect rather than the normal way of *Myiopharus* behavior. Therefore, time elapsed before first larviposition in the Colarado potato beetle larvae seems to be the most important factor. This time was not statistically different between controls and lethally treated larvae when exposed after 24 h. In the case of the control larvae it took the flies 5.1 ± 1.2 min and sometimes after two or three attempts before larvipositing them due to their strong defenses. However, the 5.4 ± 1.5 min before larvipositing lethally treated larvae were spent repeatedly avoiding them and larviposition occurred only after they have larviposited the sublethally treated and the control larvae within a cage(Table 7.1). The avoidance was related to the immobility of the treated larva because flies spent part of the time stimulating them and did not larviposit unless they moved. Under field conditions, *Myiopharus* flies avoid their host larvae once and normally do not attack them a second time unless the avoidance was due to strong defense from the larva (Chapter 6). Therefore, in release programs, lethally treated larvae after 24 h encountered by *Myiopharus* are more likely to be avoided but not after 1 h when they still have some mobility (Table 7.1). Because *Myiopharus* flies are not normally active during late afternoon and evening (López et al. 1992), if *B. thuringiensis* is applyed late in the afternoon the following day by the time *Myiopharus* becomes active again, lethally intoxicated larvae from the previous day will be paralyzed (personal observation) and will most likely to be avoided by naturally occurring *M. doryphorae* minimizing *B. thuringiensis* impact on the natural populations of *Myiopharus*.

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Table 7.1 Larvipositional response of *M. doryphorae* toward 2nd instar Colorado potato beetles fed foliage treated with lethal and sublethal doses of *B. thuringiensis* subsp. *tenebrionis* or fed untreated foliage for either 1 h or 24 h

Treatments Fed for 1 h	N (39)	Mean time elapsed in min before first larviposition	Standard error
Control	12	5.5^{a}	1.0
Lethal	14	2.5^{b}	0.6
Sublethal	13	2.0^{b}	0.4
Treatments Fed for 24 h	N (36)	Mean time elapsed in min before first larviposition	Standard error
Control	11	5.1^{ab}	1.2
Lethal	10	5.4^{a}	1.5
Sublethal	15	3.7^{b}	1.0

Mean times followed by different letter are significantly different; Tukey's procedure ($P\leq$ 0.05) (SAS Institute 1988])

Table 7.2 Number of *Myiopharus doryphorae* larvae found in Colorado potato beetle larvae fed foliage treated with a lethal or sublethal doses of *Bacillus thuringiensis* subsp. *tenebrionis* or fed untreated foliage for either 1 h or 24 h.

Treatment:	Number of parasitoid larvae found per host			
Fed for 1 h	0	1	2	3
Lethal	11	24	3	1
Sublethal	15	21	3	-
Control	12	22	5	-
Percentage	32.8	57.8	9.5	0.7
Fed for 24 h				
Lethal	21	13	1	-
Sublethal	16	12	7	-
Control	16	17	2	-
Percentage	50.5	40.0	9.5	0

χ^2 test for 1h vs 24h, 3 df [SAS Chisq. procedure, 1988]; Lethal $\chi^2 = 8.2$, P= 0.04; Sublethal $\chi^2 = 3.9$, P= 0.144; Control $\chi^2 = 2.3$, P= 0.312.

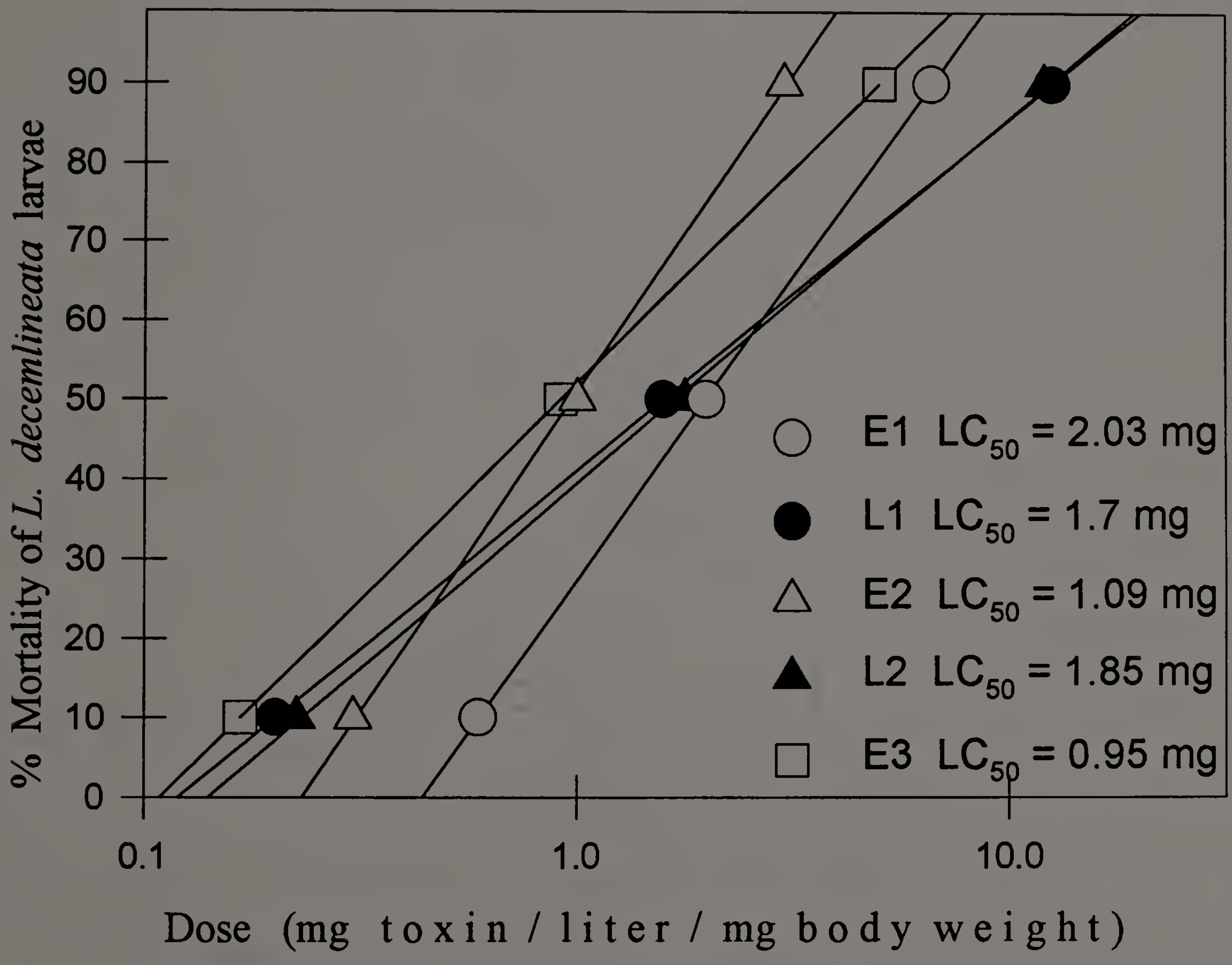

Fig. 7.1 *Bacillus thuringiensis* subsp. *tenebrionis* LC_{50} values for Colorado potato beetle larvae. E1 = early first instar: L1 = late first instar; E2 = early second instar; L2 = late second instar; E3 = early third instar.

CHAPTER 8

CONCLUSIONS AND PRACTICAL APPLICATIONS

Decades of insecticide use have been unable to provide adequate control of the Colorado potato beetle. The CPB has developed resistance to all synthetic insecticides presently registered for its control (Roush *et al.* 1990). Meanwhile, the very high fecundity of beetles colonizing potato fields early in the spring (Peferoen *et al.* 1981, Voss *et al.* 1988) and the pest' s migration potential (Voss & Ferro 1990a, b; Weber 1992) contribute to the high CPB populations that make it the most damaging insect pest of potato in the northeastern USA.

Given the defeat of synthetic insecticides by the beetles, an approach integrating multiple elements including biological and cultural control tactics seems the only viable alternative. In this context, the present study' s findings concerning the life histories of *M. aberrans* and *M. doryphorae* may significantly improve our ability to employ these tachinid species as biological control agents of CPB in the eastern United States.

The discovery that both *Myiopharus* species overwinter within adult overwintering CPB (López *et al.* 1992) suggests that the flies overwintering mortality, previously thought prohibitively high for successful CPB control (Tamaki *et al.* 1983), in fact might not be greater than the mortality of the beetle itself. Late-planting potato rows sporadically interspersed within potato fields might offer refugia for CPB larvae to complete their development late in the season (late August-September). Since 70-80% of these CPB larvae become parasitized (López *et al.* 1993), increasing the number of these parasitized CPB larvae through provision of late-season refugia might result in a significant increase in the overwintering population of *Myiopharus* and its subsequent emergence in the spring.

The finding that *M. aberrans* attacks beetles at the moment of flight initiation (Chapter 6) introduces important elements to take into account when using *Myiopharus* spp. as biological control agents. Normally, *M. aberrans* begins attacking adult colonizing beetles in early June, gradually switching to attack CPB larvae by the end of the month (Fig 6.15). As CPB larvae become more abundant *M. doryphorae* appears late in June and in early July and joins in parasitizing larvae. Therefore, any control strategy based on killing adult beetles early in the spring (i.e. butane flames or synthetic insecticide application) threatens also the *Myiopharus* overwintering population within the beetles plus the *Myiopharus* larvae recently larviposited by *M. aberrans* within colonizing beetles.

A biological control-based integrated pest management would require recognition of the rapid buildup of CPB larval populations in early June resulting from the high reproduction rates of colonizing beetles (López *et al.* 1993, Voss *et al.* 1988, Peferoen *et al.* 1981). *Myiopharus* spp. on the other hand, have a fixed reproduction rate of no more than 25-30 larvae per day (López unpublished). Consequently, attacks on colonizing CPB reproductive females by *M. aberrans* would be more cost effective than waiting for CPB females to lay their eggs and then trying to eliminate eggs or larvae. A mass release program of fertile *M. aberrans* flies early in the season when colonizing beetles are arriving might significantly impact beetle larval populations during the remaining part of the summer. Food sources for adult flies and water supply in or near potato fields would probably increase their survival rate during early releases. Grass left growing at the edges of fields seems to be a good source of water from nighttime condensation; hedgerows provided as shade might extend its availability longer through the day.

Additional control can be achieved using *Coleomegilla maculata* as a CPB egg predator either by improving natural populations of this coccinellid through habitat manipulation (i.e. planting corn and potato crops beside each other; Hazzard *et al.* 1991) or by direct mass release early in the season. In our experimental plots, a single well timed application of *Bacillus thuringiensis* subsp. *tenebrionis* provided an adequate complement to natural populations of *Myiopharus* for controlling the CPB. Appropriate timing of such an application should be based on sampling of beetle and fly populations at least three times a week during the first month after potato plant emergence given the explosive nature of early-season CPB population growth.

Based on results of our field studies to date, recommendations for integrated control strategy for CPB in western Massachusetts might consist of the following steps:

- Leave grass border by woody hedgerows around sections of potato field.
- Plant a corn crop nearby to provide pollen as food complement.for *Coleomegilla.*
- Sample three times a week to monitor colonizing beetles and flies as well as CPB egg-masses and larval densities.
- Apply *Bacillus thuringiensis* subsp. *tenebrionis* when CPB larval density reaches 20-30 /m^2 .

Based on future studies of the effectiveness of *M. aberrans* releases against early season adult CPB and of C. maculata releases against CPB egg-masses, it should eventually be possible to add specific recomendations regarding levels of application of these natural enemies as a function of sampled CPB densities.

It remains to determine the cost effectiveness of such a program in comparison with traditional chemical contro, which will depend in part upon availability of parasitoid and predator colonies for mass releases. However, long-term effectiveness of the combination of *Myiopharus* spp. and *B. thuringiensis* is indicated by our observations of organic farms in which adequate control has been achieved after several years without spraying.

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