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Estimating the impact of parasitoids on the dynamics of populations of gypsy moths.

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ESTIMATING THE IMPACT OF PARASITOIDS ON THE DYNAMICS OF POPULATIONS OF GYPSY MOTHS

A Dissertation Presented

by

JULI R. GOULD

Submitted to the Graduate School of the University of Massachusetts in partial

fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 1990

Department of Entomology

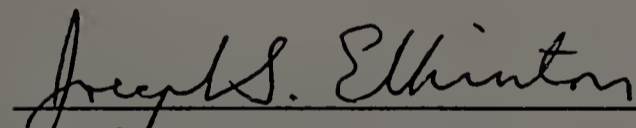ESTIMATING THE IMPACT OF PARASITOIDS ON THE DYNAMICS OF

POPULATIONS OF GYPSY MOTHS

A Dissertation Presented
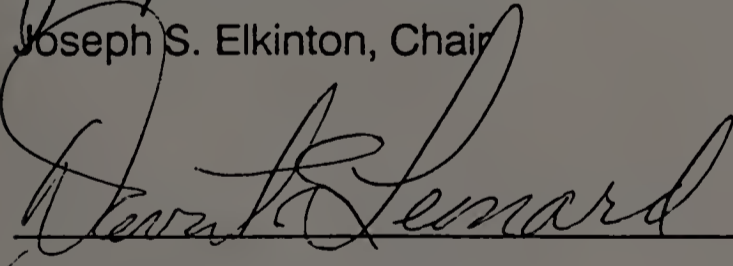
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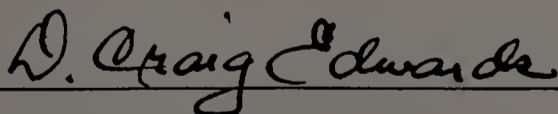
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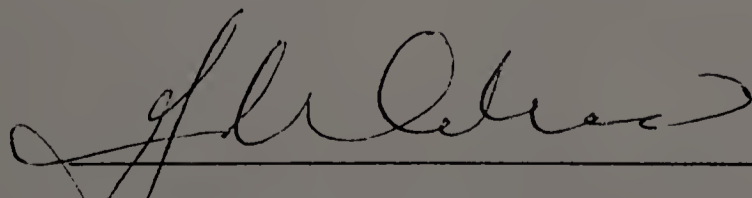_Joseph S. Elkinton, Chair_

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I finally come to the person to whom I owe the most thanks, my husband Brian Kowalski. Brian helped my research by designing and building essential cages and insectaries. He could also be pressed into service in the field, as long as he "didn't have to count things". Perhaps more important, however, was the moral support and encouragement Brian provided when things got rough. Thanks Brian!

ABSTRACT

ESTIMATING THE IMPACT OF PARASITOIDS ON THE DYNAMICS OF

POPULATIONS OF GYPSY MOTHS

MAY 1990

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Directed by: Professor Joseph S. Elkinton

To estimate the impact of parasitoids, one must be able to accurately measure the mortality they cause. I therefore investigated biases associated with several methods of calculating stage-specific and time-specific parasitism by Cotesia melanoscela, Parasetigena silvestris, and Brachymeria intermedia. I released laboratory-reared gypsy moths into the field to measure the timing of oviposition by parasitoids. I determined the timing of emergence of parasitoids by collecting naturally occurring gypsy moths. I also monitored the timing of host recruitment to and advancement out of the stage(s) that was susceptible to parasitism. I found that many of the methods used in previous studies of gypsy moth dynamics were affected by overlap of these processes and that several methods severely over- or underestimated parasitism. I estimated temperature-dependent development of C. melanoscela for use with the Southwood & Jepson method of calculating the number of parasitoids attacking hosts.

I also studied some effects of superparasitism by P. silvestris. The dispersion of eggs of P. silvestris on gypsy moth larvae collected in the field was more aggregated when larvae were collected from under burlap bands. This resulted in lowered percentage parasitism of hosts collected in this manner. In laboratory studies, deposition of more than one egg on a single host significantly increased host mortality, but had a negative effect on survival of the

immature parasitoid and the size of the puparium produced.

Experimental manipulations of densities of gypsy moths revealed a strong, positive spatially density-dependent reduction in gypsy moth populations. Positive density-dependent mortality occurred during the early and mid larval stages and was primarily due to Compsilura concinnata, a polyphagous parasitoid. Oviposition by P. silvestris, an oligophagous parasitoid, was initially inversely density-dependent, but became positively density-dependent during the late larval period. I conclude that if populations of gypsy moths increase and decrease in density asynchronously on a spatial scale of a few ha, the density-dependent responses of parasitoids could suppress the populations to a point where small mammal predation would be able to prevent population increase. This phenomenon may explain the apparent stability of gypsy moth populations on a region-wide basis for the years between outbreaks.

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CHAPTER 1

INTRODUCTION

Gypsy moths, <u>Lymantria</u> <u>dispar</u> (L.), are not native to North America. They were inadvertently released from the home of Etienne Leopold Trouvelot, an amateur entomologist residing in Medford, Massachusetts. Only a few individuals escaped from Mr. Trouvelot's home in 1868 or 1869, and although Mr. Trouvelot alerted the public to the potential dangers, no action was taken to find and eliminate the gypsy moths (Forbush & Fernald 1896). For 10 years, only those people residing near the Trouvelot home took any notice of the gypsy moth. Within 20 years, however, the gypsy moth had spread to 30 towns and was causing extensive defoliation.

North America provided a suitable climate and food supply for the gypsy moth, but the complement of natural enemies found in Europe was absent. Although some native species (birds, parasitoids, and small mammals) caused mortality of gypsy moths, they were unable to prevent outbreaks. Early strategies to control gypsy moths did not include importation of natural enemies, because efforts were focused on eradication; but in 1904, the federal government and the state of Massachusetts joined forces to begin importing and releasing such enemies.

The search for natural enemies was concentrated in Europe, where the strain of gypsy moth in North America originated. Between 1905 and 1914, six species of parasitoids were successfully established in North America: <u>Compsilura</u> <u>concinnata</u> (Meigen) (Diptera: Tachinidae), <u>Blepharipa</u> <u>pratensis</u> (Meigen) (Diptera: Tachinidae), <u>Cotesia</u> <u>melanoscela</u> (Ratzeburg) (Hymenoptera: Braconidae), <u>Phobocampe</u> <u>disparis</u> (Hymenoptera: Ichneumonidae), <u>Anastatus</u> <u>disparis</u> (Viereck) (Hymenoptera: Eupelmidae), and

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Ooencyrtus kuvanae (Howard) (Hymenoptera: Encyrtidae). Exploration in Europe was interrupted by World War I, but continued from 1922-1933. During this period two to three more parasitoids were established in North America: _Parasetigena silvestris_ (Robineau-Desvoidy) (Diptera: Tachinidae), _Exorista larvarum_ (L.) (Diptera: Tachinidae), and possibly _Brachymeria intermedia_ (Nees) (Hymenoptera: Chalcididae). Exploration and importation were conducted again in the early 1960's, but in spite of extensive efforts, no new parasitoids became established.

In North America, populations of the gypsy moth typically remain at low densities for eight to ten years and then increase rapidly to outbreak levels. After a few years of defoliation, epizootics of nuclear polyhedrosis virus cause the collapse of these populations. Because prevention of outbreaks in areas where the gypsy moth is well established is virtually impossible and attempts at control are extremely costly, an understanding of the dynamics of this species must be gained if there is to be any chance of managing this pest. In particular, we must identify the factors responsible for maintaining populations at low densities and the reason that they fail in certain years, resulting in outbreaks.

Campbell and Sloan (1976, 1977, 1978) hypothesized that positively density-dependent predation of late-instars and pupae by small mammals, especially _Peromyscus leucopus_, was responsible for maintaining low densities of gypsy moths during the years between outbreaks. When the density of gypsy moths exceeded some threshold, above which predation by small mammals became inversely density-dependent, populations "escaped" to an outbreak phase. Recent studies support Campbell's finding that rates of mortality are highest during the late instar and pupal stages of the gypsy moth (Elkinton et al. 1989, Liebhold & Elkinton 1989a) and that much of this mortality is attributable to predation by _P. leucopus_ (Elkinton et al. 1989). In spite of much effort,

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however, positive density-dependent predation by P. leucopus has yet to be shown conclusively.

Is it possible that parasitoids play an important role in suppressing or regulating populations of gypsy moths? Many researchers (e.g. Ticehurst et al. 1978, Campbell & Sloan 1977, Campbell et al. 1977, and Reardon 1976) have concluded that parasitoids do not cause sufficient mortality to limit the growth of gypsy moth populations. Unfortunately, these conclusions were typically based on values of percentage parasitism calculated using methods that can result in severely biased estimates of levels of parasitism (Gould et al. 1989, Van Driesche 1983). Whether or not biases exist in the use of other methods of calculating percentage parasitism has not been tested for parasitoids of the gypsy moth. Also, levels of parasitism were quite high in some gypsy moth populations (Blumenthal et al. 1979, ODell & Godwin 1979, Barbosa et al. 1975, and Doane 1971). When one is considering whether or not a natural enemy can control its host or prey, however, the critical issue is not necessarily the magnitude of mortality, but whether or not the mortality is density-dependent. The strength of the density-dependent response may determine the equilibrium density of the host (Varley et al. 1973) and whether the host population exhibits stability or large fluctuations in density (May 1986).

To accurately assess the effect of parasitoids on populations of the gypsy moth, I had to begin with the basics. How does one obtain unbiased estimates of levels of parasitism? Van Driesche (1983) has shown that values of percentage parasitism observed in samples collected over time reflect levels of parasitism for the stage(s) of the host that is susceptible to parasitism (stage-specific parasitism) only in specific instances. In general, samples must be collected when all hosts and parasitoids are available for sampling. If either hosts or parasitoids are entering (being recruited to) or leaving (advancing out

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of) the stage of the host that is susceptible to parasitism while samples are being collected, values of percentage parasitism in those samples do not accurately measure levels of stage-specific parasitism.

Some other approaches to calculating stage-specific parasitism include examination of remains (pupal exuviae in the case of the gypsy moth), using the graphical method of Southwood & Jepson (1962) to calculate the number of parasitoids attacking susceptible hosts (Bellows et al. 1989), and directly measuring the number of hosts and parasitoids that enter the system (Van Driesche & Bellows 1988). The first two of these techniques have been used to calculate parasitism by gypsy moth parasitoids, but the biases associated with these methods, as well as the methods using values of percentage parasitism in samples, had not been examined.

Determining stage-specific parasitism is not the only way of assessing the impact of parasitoids. One can also determine the proportion killed during certain intervals of time, i.e. time-specific parasitism (Elkinton 1990a). Calculation of time-specific mortality eliminates the need to calculate the number of individuals entering a stage, which is often difficult. Elkinton (1990a) proposed calculating survival from attack by a given agent during short intervals of time. Multiplying these survivorships results in the total survival from attack by a given agent over the entire interval. Elkinton (1990b) also advocates calculating the marginal probability of being killed by a given parasitoid in the absence of contemporaneous mortality agents, rather than the number that actually die. He presents techniques for calculating the marginal probability of being killed based on the number actually observed to die in samples. This technique is not, however, without biases (Elkinton 1990b).

The first goal of my research was to determine which, if any, of the methods used to calculate percentage parasitism are appropriate for use with parasitoids

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of the gypsy moth. Because data on the rate of development of the parasitoid is necessary for use of the Southwood & Jepson graphical method, I first conducted a laboratory study to determine the temperature-dependent growth rate of C. melanoscela. I then released laboratory-reared insects in the field to determine the phenology of four processes: (1) recruitment of hosts to the stage(s) susceptible to parasitism, (2) advancement of hosts from the susceptible stage, (3) recruitment of parasitoids (oviposition), and (4) advancement of parasitoids (emergence and death). These data, as well as data on rates of mortality of healthy and parasitized gypsy moths, allowed me to determine which of the methods of calculating stage-specific or time-specific parasitism were appropriate for calculating percentage parasitism by C. melanoscela, P. silvestris, and B. intermedia.

Once I had identified appropriate methods for calculating levels of parasitism, I was able to investigate other aspects of the impact of parasitoids on populations of gypsy moths. It has been argued that superparasitism (the deposition of more than one egg in a single host) by solitary parasitoids is a waste of eggs (Fiske 1910). Superparasitism, according to this viewpoint, reduces the effectiveness of the parasitoid as a control agent. It also is considered maladaptive from the perspective of the female parasitoid, owing to the reduced survivorship of her offspring (Salt 1961). Offspring developing in superparasitized hosts may also be smaller or less fecund (King et al. 1976). For the above reasons, it has been argued that selection of mechanisms to avoid superparasitism should be strong (Van Lenteren 1981, Rogers 1975).

In recent years, however, a revised view of superparasitism as an alternative reproductive strategy that can be advantageous under certain conditions, has developed (van Alphen & Visser 1990, Hubbard et al. 1987, Waage 1986, Bakker et al. 1985, Cloutier 1984, van Alphen & Nell 1982). For instance,

superparasitism may increase the probability of survival of the parasitoid (and thus death of the host) if more than one parasitoid is better able to overcome the defenses of the host (Beland & King 1976, Puttler 1974, Streams 1971). Also, superparasitism may be a better strategy than laying no eggs at all, when hosts are scarce or percentage parasitism is high.

I chose to study superparasitism by the tachinid, P. silvestris. This species lays large, macrotype eggs on the integument of the gypsy moth, so the distribution of eggs can be studied in the field. Also, superparasitism by this species is often reported (e.g. ODell & Godwin 1979, Weseloh 1974, 1976, Burgess & Crossman 1929, and Prell 1915), yet usually only a single parasitoid emerges (Burgess & Crossman 1929, Prell 1915). I decided to conduct field studies of the distribution of eggs of P. silvestris to determine whether superparasitism was the result of random oviposition or whether some hosts were more susceptible to attack than others. I conducted these studies in plots with high and low densities of gypsy moths and collected gypsy moths from both the general population and from under burlap bands. I hoped to determine if the density of hosts or the method of collection affected levels of superparasitism, and whether this in turn influenced levels of host mortality.

The field studies provided information on the occurrence of superparasitism, but I also wanted to know the effect of superparasitism on individual hosts. In the laboratory, I investigated the effect of an increasing number of eggs per host on three parameters: (1) percentage mortality of hosts, (2) probability of survival of the offspring of the parasitoid, and (3) size of emerging parasitoids.

It has been argued that for a parasitoid to regulate its host, the proportion of hosts attacked must change with host density in a predictable manner (Dempster & Pollard 1986). Some researchers claim to have found positive density-dependent parasitism (Furuta 1982, ODell & Godwin 1979, Sisojevic

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1977, Reardon 1976), and others have found a negative correlation between percentage parasitism and host density and/or percentage defoliation (Ticehurst et al. 1978, Reardon & Podgwaite 1976, Reardon 1976, Weseloh 1973). In most of these studies, however, the methods used to estimate host density, calculate percentage parasitism, or determine a statistical relationship were unsatisfactory. The usual method of assessing whether or not natural enemies cause density-dependent mortality has been to follow a population for many generations (Varley et al. 1973). For practical reasons, few long-term studies have been undertaken. Also, several statistical problems have been identified with the methodology (e.g. Pollard et al. 1987, Slade 1977, Bulmer 1975, Benson 1973). Stochastic variation (Hassell 1985, 1987) or monitoring the population on an inappropriate spatial scale (Hassell et al. 1987, Heads & Lawton 1983) may obscure underlying density-dependent processes.

Experimental manipulation of host populations has been proposed as an alternative to long term life-table studies (Murdoch & Reeve 1987, Hassell 1987). I therefore decided to augment populations of gypsy moths to four different densities in order to determine whether any of the gypsy moth parasitoids cause spatially density-dependent mortality (differential responses to different host densities within a generation). In contrast, temporal density dependence is differential mortality as host populations change in density between generations. Several mathematical models have shown that spatial density-dependence can promote population stability. Others (e.g. Dempster & Pollard 1986) argue, however, that spatial density dependence alone, without a temporal component, cannot regulate a population. The goal of my study was not to determine if populations of gypsy moths are regulated, but whether parasitoids cause spatially density-dependent mortality.

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CHAPTER 2

TEMPERATURE-DEPENDENT GROWTH OF COTESIA MELANOSCELA

(HYMENOPTERA: BRACONIDAE)

Introduction

Cotesia melanoscela (Ratzeburg) is one of the principal parasitoids attacking

early instar gypsy moths in North America. Estimating the effect of parasitism by

C. melanoscela and other parasitoids on populations of the gypsy moth is an

important research goal. Rates of development of C. melanoscela as a function

of temperature are needed for simulation models of the effects of this parasitoid

on the population dynamics of the gypsy moth, Lymantria dispar (L.). With these

rates, one can use the "graphical method" of Southwood and Jepson (1962) to

estimate the number of individuals entering a given stage. This method has

been extended by Bellows et al. (1989) to estimate the number of parasitoids

attacking the susceptible stage of the host and has been used by Kolodny-

Hirsch et al. (1988) to evaluate the impact of sequential releases of C.

melanoscela on populations of L. dispar.

Weseloh (1976) has shown that C. melanoscela is generally capable of

attacking only first- through third-instar gypsy moths; therefore, this study was

confined to these instars. I compared the development of parasitoid larvae

reared in host larvae feeding on oak foliage (as one would find under field

conditions) with that of larvae reared in hosts feeding on artificial diet

(conditions that would occur if larvae were collected in the field and reared in

the laboratory). I also determined the rate of development of parasitoid pupae.

Developmental rates of pupae would be required for a simulation of population

dynamics, because the timing of emergence of C. melanoscela adults of the

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second generation in relation to the developmental stage of their host is a critical factor in determining the magnitude of the impact of this parasitoid (Weseloh 1976).

Methods and Materials

Development of Parasitoid Larvae in Hosts Feeding on Foliage

Gypsy moth egg masses were collected from a moderately dense (approximately 200 egg masses per ha) population on Otis Air National Guard Base (ANGB), Cape Cod, Massachusetts, in the spring of 1985. Egg masses were dehaired and soaked for one hour in a 10% formalin solution (Bell et al. 1981) to inactivate nuclear polyhedrosis virus (NPV) on the surfaces of the eggs. Larvae that hatched from these eggs were placed in groups of ten in 100 mm diameter petri dishes and reared at $25^{o}C$ (60% RH and 16:8 L:D) until they were ready for parasitization. Larvae were fed black oak (Quercus velutina) foliage that was collected from a site on Otis ANGB with few gypsy moths and little or no defoliation for the previous 2 yr. The foliage was washed in a solution of 4% chlorine bleach and was rinsed with water to prevent infection of larvae by NPV. To reduce water loss, the tip of the petiole of each leaf was dipped in paraffin and the Petri dish was sealed with Parafilm. Because removing leaves from trees could affect levels of secondary plant compounds in the leaves, the foliage was replaced daily.

Parasitoids were obtained by rearing wild gypsy moth larvae collected from a moderately dense population on Otis ANGB. Cocoons produced by emerging parasitoid larvae were placed in open petri dishes in plexiglas cages (30 by 30 by 30 cm), and adult parasitoids were allowed to emerge and mate. Water was provided on cotton wicks placed in plastic cups (30 ml) with plastic lids, and

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honey was streaked on paper disks (10 cm diameter) which were suspended from the tops of the cages.

Parasitization by C. melanoscela was accomplished by placing 10 host larvae on a paper disk (10 cm diameter) and suspending the lid from the top of a cage containing parasitoids. The activity of the parasitoids was monitored continuously, and host larvae were removed from the cage as soon as an oviposition was observed. Late first, second, and third instars that were about to molt (the new head capsule was visible beneath the old head capsule) were chosen for the study because they were not very mobile and thus easier to manipulate during parasitization.

Parasitized larvae were placed in groups of 10 in Petri dishes (100 mm diameter) and placed in incubators at 10, 15, 20, 25, 30, 33, or 34° C (60% RH and 16:8 L:D). There was a total of eight Petri dishes (n_o=80) of first instar gypsy moths and five dishes (n_o=50) of both second and third instars at each temperature. Host larvae were checked every 24 h (when fresh foliage was provided) for parasitoid emergence and host mortality.

Development of Parasitoid Larvae in Hosts Feeding on Artificial Diet

Gypsy moth eggs were obtained from a laboratory colony at Otis ANGB. Late first instars were parasitized in the manner described above and were placed individually in plastic cups (30 ml) containing 10 ml of a wheat germ based artificial diet (Bell et al. 1981). Fifty cups (n_o=50) were placed in each incubator at 10, 15, 20, 25, 30, 32, or 34°C (60% RH and 16:8 L:D). Host larvae were checked daily for parasitoid emergence.

Development of Parasitoid Pupae

Parasitized third instar hosts were reared at 25°C in groups of 12 in 180 ml cups containing 85 ml of artificial diet until parasitoids were about to emerge (as determined by the results for developmental rates of parasitoid larvae). Host

larvae were then transferred individually to empty plastic cups (30 ml). These cups were checked every 24 h and were placed in an incubator once a parasitoid larva emerged, thereby avoiding disturbance of the parasitoid during cocoon formation. Fifty parasitoid pupae per temperature were reared at 15, 20, 25, 30, 32, 33 or 34° C and checked every 24 h until adults emerged, at which time the sex of the adult was recorded.

Data Analysis

Developmental rate was regressed on temperature using a linear model and the nonlinear models of Logan et al. (1976) and Sharpe & DeMichele (1977). Variances were found to be non-heterogeneous (Levene's test; Milliken & Johnson 1984), and I corrected for this by weighting each observation by 1/variance (within each instar and temperature).

Linear Model. Developmental rates (1/number of days) were regressed on temperatures below 32°C (SAS Institute 1987) because higher temperatures inhibited development. A test of significance of deviations from the regression model (Sokal & Rohlf 1981, pp. 482-483) was performed to determine if the data fit a linear model. Analysis of covariance (Sokal & Rohlf 1981) was used to test for differences in developmental rates among parasitoid larvae reared in three host instars feeding on foliage, differences between first instars reared on foliage versus artificial diet, and differences between male versus female parasitoid pupae. Partial and overall r^2 values also were determined.

Logan Model. The Logan model (Logan et al. 1976) was fit to values of mean rate of development at each temperature which were calculated as:

Mean developmental rate=$1/\exp[\ln(D_i)/n]$

where D_i is the observed developmental time and n is the sample size. Mean developmental rates were calculated in this fashion to correct for the skewed frequency distributions of developmental time (Logan et al. 1976). Another

method of compensating for the skewed frequency distribution would have been to take the median value of developmental rate as a measure of central tendency as recommended by Wagner et al. (1984a) and Casagrande et al. (1987).

The Logan model is described as:

$$r(T) = Phi^*[exp(Rho^*T) - exp((Rho^*T_m) - ((T_m - T)/\Delta T))]$$

where $r(T)$ is the rate of development at temperature T, T is the temperature in $^{\circ}C$ above a base temperature (T_b) (i.e., the lowest experimental temperature), Phi is the rate of temperature-dependent development at T_b, Rho is a composite Q_{10} value for critical enzyme-catalyzed, biochemical reactions, T_m is a thermal maximum (temperature at which life processes can no longer be maintained), and ΔT is the temperature range between developmental maximum and T_m. Initial estimates of the four parameters of the model were determined graphically as described in Logan et al. (1976). Nonlinear regression (SAS Institute 1987) was used to fit the model to values of mean developmental rate by an iterative, least squares procedure using the Marquardt algorithm. An F statistic was then calculated to test the significance of deviations from the regression model (Sokal & Rohlf 1981).

Sharpe & DeMichele Model. The model of Sharpe and DeMichele (1977), as modified by Schoolfield et al. (1981), was fitted to values of mean developmental rate. The modified six parameter equation is:

$$r(T) = \frac{RHO25^*(T/298.15)^*exp[(HA/R)^*(1/298.15 - 1/T)]}{1 + exp[(HL/R)^*(1/TL - 1/T) + exp[(HH/R)^*(1/TH - 1/T)}, $$

where $r(T)$ is the mean development rate at temperature T ($^{\circ}K$), R is the universal gas constant (1.987 cal degree^{-1} mole^{-1}), RHO25 is the

developmental rate at 25°C assuming no enzyme inactivation, HA is the enthalpy of activation of the reaction that is catalyzed by a rate-controlling enzyme, TL is the Kelvin temperature at which the rate-controlling enzyme is half active and half low-temperature inactive, HL is the change in enthalpy associated with low temperature inactivation of the enzyme, TH is the Kelvin temperature at which the rate-controlling enzyme is half active and half high-temperature inactive, and HH is the change in enthalpy associated with high-temperature inactivation of the enzyme.

The numerator of the equation describes the temperature-dependent developmental rate at moderate temperatures when high- and low-temperature inactivation are not important. The first exponential function in the denominator accounts for inhibition of development at low temperatures, and the second exponential function in the denominator accounts for inhibition at high temperatures. I used the computer program of Wagner et al. (1984b), which uses SAS procedures (SAS Institute 1987) to evaluate the significance of each portion of the equation (and thus to determine the number of parameters to be used). The program then determined the starting values of the parameters and computed estimates of the parameters with a least-squares procedure using the Marquardt algorithm. An F statistic was then calculated to test the significance of deviations from the regression model (Sokal & Rohlf 1981).

Cumulative Weibull Functions. To model the distribution of developmental times around the fitted values I used the computer program of Wagner et al. (1984a). This program normalizes the distribution of developmental times at each temperature by dividing each observation by the median developmental time at that temperature, identifies a single curve representative of all normalized distributions, and fits a cumulative Weibull function to this curve.

The form of the Weibull Function is:

$F(x) = 1 - \exp(-[(x - gamma)/eta]^{beta})$,

were F(x) is the probability of complete development at normalized time x, and gamma, eta, and beta are estimated parameters.

Environmental Temperature Data

For three years, I recorded temperatures at permanent study sites on Cape Cod, Massachusetts, during the period when gypsy moth larvae were parasitized by C. melanoscela. I used a Campbell Scientific CR21 weather station of standard design and measured ambient air temperature at 1 m above the ground with a thermistor probe. Temperature was recorded on a Campbell Scientific Data Logger as hourly averages of readings taken once per minute.

Results and Discussion

Development of Parasitoid Larvae

Developmental rate increased with temperature to 30°C and then declined rapidly to zero at 34°C (Fig. 2.1A and B), the temperature at which there was 100% mortality (Table 2.1) for parasitoid larvae reared in hosts of all instars. Developmental rate showed a significant linear relationship with temperature (Table 2.2) for temperatures <30°C. Analysis of covariance indicated that differences in development among parasitoid larvae reared in hosts of different instars were statistically significant (F = 20.499; df = 4, 524; P <0.001); however, considering instars separately accounted for <1% more of the variability than did the regression for all instars combined (overall r^2 improved from 0.955 to 0.961). Also, there was no consistent trend in developmental rates among instars (Table 2.1). For these reasons and because in some applications of these models (i.e., the graphical method of estimating numbers entering the

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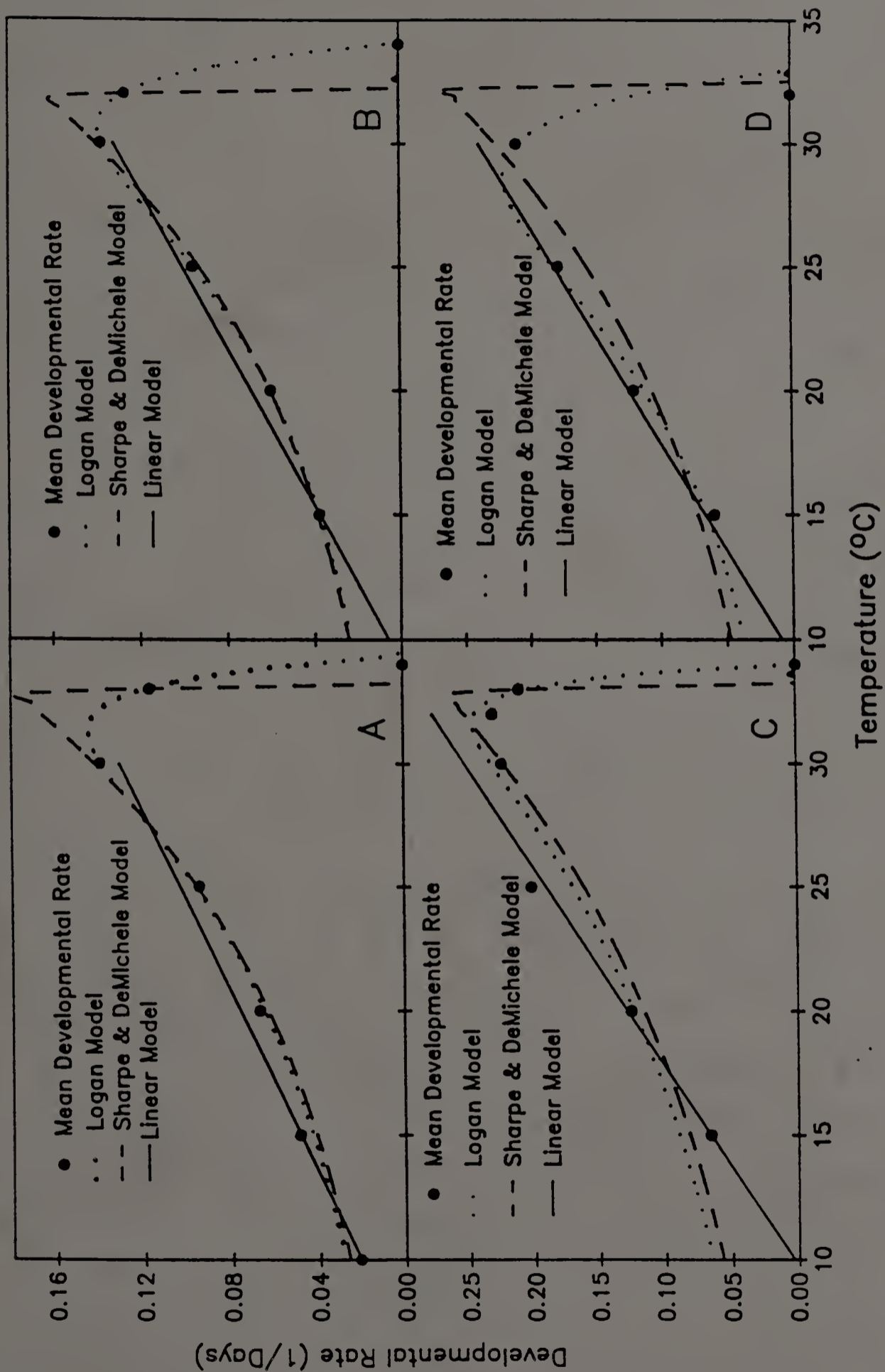
Figure 2.1. Three models of developmental rate of *C. melanoscela*. (A) Larvae developing in first through third instars of gypsy moth feeding on oak foliage. (B) Larvae developing in first-instars hosts feeding on artificial diet. (C) male *C. melanoscela* pupae. (D) female *C. melanoscela* pupae.

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Table 2.1. Development of C. melanoscela at eight constant temperatures.

Host stage	Parasitoid stage	Temperature, °C							
		10	15	20	25	30	32	33	34
		Host food -- Foliage							
1st-3rd instar	Egg-Larva								
x development time, days		47.6	20.6	15.1	10.8	7.2	NA	8.7	0.0
SE		0.5	0.2	0.2	0.2	0.1	--	0.2	--
No.		180	180	180	180	180	--	180	--
No. completing development		42	121	118	119	130	--	51	--
1st instar	Egg-Larva								
x development time, days		50.3	19.8	14.3	10.5	7.0	NA	8.7	0.0
SE		1.4	0.2	0.3	0.3	0.1	--	0.3	--
No.		80	80	80	80	80	--	80	--
No. completing development		7	58	52	57	58	--	37	--

NA, data not available. 0.0, no survivors at indicated temperature.

Continued, next page

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Table 2.1. Continued.

	Parasitoid stage	Temperature, $^{\circ}$C							
Host stage		10	15	20	25	30	32	33	34
2nd instar	Egg-Larva								
x̄ development time, days		48.5	21.4	14.3	11.2	7.1	NA	8.8	0.0
SE		1.0	0.4	0.3	0.4	0.2	--	0.3	--
No.		50	50	50	50	50	--	50	--
No. completing development		15	34	39	35	35	--	6	--
3rd instar	Egg-Larva								
x̄ development time, days		46.1	21.2	16.0	11.0	7.7	NA	8.5	0.0
SE		0.5	0.4	0.4	0.4	0.1	--	0.3	--
No.		50	50	50	50	50	--	50	--
No. completing development		20	29	27	27	37	--	8	--

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Table 2.1. Continued.

| Host stage | | | | Temperature, °C | | | | |
Parasitoid stage	10	15	20	25	30	32	33	34
			Host food -- Artificial diet					
1st instar Egg-Larva								
x̄ development time, days	0.0	26.6	16.8	10.7	7.3	8.1	NA	0.0
SE	--	0.4	0.4	0.6	0.2	0.3	--	--
No.	--	50	50	50	50	50	--	--
No. completing development	--	40	22	18	37	34	--	--
Male pupae								
x̄ development time, days	NA	15.2	8.1	5.0	4.5	4.4	4.8	0.0
SE	--	0.1	0.4	0.1	0.3	0.4	0.4	--
No.	--	NA	NA	NA	NA	NA	NA	--
No. completing development	--	15	13	24	12	8	5	--
Female pupae								
x̄ development time, days	NA	16.9	8.4	5.6	4.8	4.0	0.0	0.0
SE	--	0.3	0.3	0.1	0.1	NA	--	--
No.	--	NA	NA	NA	NA	NA	--	--
No. completing development	--	10	12	15	20	1	--	--

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Table 2.2. Estimates of parameters for linear models of development of C. _melanoscela_.

Host stage	Parasitoid stage	Intercept	Slope	Threshold, $^\circ$C	P_1	r^2	P_2
1st-3rd instar[a]	Egg-Larva	-0.0336	0.0055	6.11	<0.001	0.955	<0.001
1st instar[a]	Egg-Larva	-0.0390	0.0059	6.61	<0.001	0.959	<0.001
2nd instar[a]	Egg-Larva	-0.0310	0.0052	5.96	<0.001	0.937	<0.001
3rd instar[a]	Egg-Larva	-0.0289	0.0051	5.67	<0.001	0.961	<0.001
1st instar[b]	Egg-Larva	-0.0571	0.0063	9.06	<0.001	0.907	<0.001
--	Male pupae	-0.1208	0.0125	9.70	<0.001	0.912	0.016
--	Female pupae	-0.1074	0.0116	9.62	<0.001	0.961	0.003

P_1, significance of regression. P_2, significance of deviation from regression.

[a] hosts reared on oak foliage. [b] hosts reared on artificial diet.

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stage) knowledge of the instar in which oviposition occurred is usually not available, I present models based on all instars combined as well as separately.

A statistical improvement also was achieved by fitting separate linear models of developmental rates of parasitoids reared in first instar hosts feeding on artificial diet versus foliage ($F = 84.717$; df = 2, 345; $P < 0.001$). The differences between dietary treatments were more pronounced than for instar treatments, and developmental rates in first-instar hosts feeding on artificial diet were consistently slower than in hosts feeding on foliage (Table 2.1). The r^2 value increased from 0.909 for treatments considered together to 0.939 for treatments considered separately (partial r^2 = 0.329).

The differences in developmental rates observed for the diet versus the foliage treatments could have been because the gypsy moth hosts represented different strains (laboratory reared versus wild), but I do not feel that this is likely. Casagrande et al. (1987) concluded that there were no consistent differences in developmental rates among different populations of wild gypsy moths and that a single model was appropriate for describing developmental rate. Also, other experiments (unpublished data) indicate that at 25°C the development of first-third instar wild gypsy moths (from the same source as those used in this study) did not differ from that of the laboratory strain (J. A. Tanner personal communication). Although no differences in gypsy moth development have been found among gypsy moth strains, substantial differences have been observed for development on different host species (Casagrande et al. 1987), supporting my hypothesis that differences in parasitoid development were caused by differences in host nutrition rather than host strain.

Differences in developmental rates on foliage versus diet are important to consider when estimating the timing of parasitoid emergence from larvae collected in the field and those reared in the laboratory on artificial diet. One

solution would be to rear these larvae on foliage, but this would be quite labor intensive and time consuming. A more practical solution would be to collect field samples frequently and to rear the hosts on diet only until the next sample was taken. This procedure would minimize the error in estimating timing of parasitoid emergence caused by effects of rearing the hosts on artificial diet.

Although the r^2 values for the linear models were high, the deviations from linear regression were statistically significant (Table 2.2), which indicates that nonlinear models might be more appropriate. Both the Logan and the Sharpe & DeMichele models (Tables 2.3 & 2.4) had higher r^2 values than the corresponding linear model, and the deviations from regression were not significant. Inclusion of inhibition at higher temperatures significantly improved the fit of the Sharpe & DeMichele model for all treatments, but low temperature inhibition was not significant. Four parameter models (including high temperature inhibition) were therefore used. Both nonlinear models fit well for temperatures below 30°C; in fact, there was virtually no difference between the models for larvae reared on foliage (Fig. 2.1A). Figure 2.1B , however, indicates that the Logan model fits the data better, especially at higher temperatures, when host larvae were reared on diet.

Development of Parasitoid Pupae

The linear relationship between developmental rate of parasitoid pupae and temperature also was significant (Table 2.2). The model was improved by considering males and females separately (F = 14.916; df = 2,125; P < 0.001), although the partial r^2 value was only 0.193. It was necessary to consider male and female pupae separately because only one female pupa survived above 30°C (Table 2.1). The deviations from linear regression were significant (F = 6.510; df = 2,53; P < 0.003 and F = 3.690; df = 3,67; P < 0.016 for female and male pupae, respectively). Both nonlinear models had higher r^2 values than

Table 2.3. Estimates of parameters for the Logan model of development of C. melanoscela.

Host stage	Parasitoid stage	Phi	Rho	T_{max}	ΔT	P_1	r^2	P_2
1st-3rd instar[a]	Egg-Larva	0.0287	0.0811	24.33	1.21	<0.001	0.967	0.985
1st instar[a]	Egg-Larva	0.0293	0.0821	24.32	1.28	<0.001	0.967	0.993
2nd instar[a]	Egg-Larva	0.0277	0.0817	23.14	0.14	<0.001	0.955	0.997
3rd instar[a]	Egg-Larva	0.0276	0.0822	25.24	1.83	<0.001	0.973	0.998
1st instar[b]	Egg-Larva	0.0377	0.0937	19.06	1.53	<0.001	0.930	0.995
--	Male pupae	0.0894	0.0642	19.00	0.76	<0.001	0.927	0.999
--	Female pupae	0.0653	0.1116	18.00	2.40	<0.001	0.968	0.979

P_1, significance of regression. P_2, significance of deviation from regression.

[a] hosts reared on oak foliage. [b] hosts reared on artificial diet.

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Table 2.4. Estimates of parameters for the Sharpe & DeMichele model of development of *C. melanoscela*.

Host stage	Parasitoid stage	Rho25	HA	TH	HH	P_1	r^2	P_2
1st-3rd instar[a]	Egg-Larva	0.0955	13768	306.18	3680383	<0.001	0.967	0.985
1st instar[a]	Egg-Larva	0.0982	14196	306.17	3665918	<0.001	0.967	0.993
2nd instar[a]	Egg-Larva	0.0949	14262	306.18	3666383	<0.001	0.955	0.997
3rd instar[a]	Egg-Larva	0.0912	13128	306.19	3715936	<0.001	0.973	0.998
1st instar[b]	Egg-Larva	0.0925	14329	305.21	3665918	<0.001	0.930	0.995
--	Male pupae	0.1612	11034	306.22	3665918	<0.001	0.927	0.999
--	Female pupae	0.1557	12766	305.51	3665918	<0.001	0.968	0.979

P_1, significance of regression. P_2, significance of deviation from regression.

[a] hosts reared on oak foliage. [b] hosts reared on artificial diet.

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did the corresponding linear models, and the deviations from regression were not significant (Tables 2.3 & 2.4). As with larval development, the Logan model appears to fit the data on development of female pupae better than the Sharpe & DeMichele model at higher temperatures (Fig. 2.1C and D).

Environmental Temperature Data

Ambient temperatures exceeded 30°C, but not often (9 h in 1984, 8 h in 1985, and 14 h in 1986); however, the temperature probe was in a shaded, ventilated weather station. Lance et al. (1987) showed that when trees are defoliated, the internal temperature of gypsy moth larvae is often elevated 2-6°C above ambient temperatures. Also, early summer temperatures on Cape Cod are lower than those experienced by gypsy moths elsewhere. Thus, environmental temperature conditions may necessitate the use of the nonlinear models, which predict developmental rate at temperatures above 30°C.

Nonlinear models also are better predictors of developmental rate at low temperatures, because linear models predict developmental thresholds that are too high (Hilbert & Logan 1983). The result is that linear models underestimate development at low temperatures. In all cases, the nonlinear models predicted higher developmental rates than did the linear models for temperatures below approximately 15°C (Fig. 2.1). During the period when gypsy moth larvae were parasitized by C. melanoscela, temperatures frequently dropped below 15°C. Although simple linear models are fairly good predictors of developmental rates between 15 and 30°C, environmental temperatures frequently fall outside this range. One of the nonlinear models should, therefore, be used to predict temperature-dependent development of C. melanoscela.

Cumulative Weibull Functions

Parameter estimates of cumulative Weibull functions are given in Table 2.5. The r^2 values for all treatments were high (Table 2.5), and the curves fit the data

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Table 2.5. Estimates of parameters of cumulative Weibull functions. Data were fit to normalized cumulative probability distributions for development of parasitoid larvae and pupae.

Host stage	Parasitoid stage	Eta	Beta	Gamma	r^2
1st-3rd instar[a]	Egg-Larva	0.2870	1.8069	0.7678	0.949
1st instar[a]	Egg-Larva	0.2266	1.6452	0.8276	0.923
2nd instar[a]	Egg-Larva	0.3570	1.8600	0.7060	0.896
3rd instar[a]	Egg-Larva	0.2483	1.9500	0.7970	0.979
1st instar[b]	Egg-Larva	0.3329	2.3116	0.7207	0.920
--	Male pupae	0.3672	2.8590	0.6788	0.899
--	Female pupae	0.5372	6.8634	0.4898	0.948

[a]hosts reared on foliage. [b]hosts reared on artificial diet.

well (see Fig. 2.2 for two examples). Using these functions to model the distribution of parasitoid development is important in simulation models that are used to predict the age structure or frequency distribution of various life stages of the parasitoid.

Applications of the Models

Weseloh (1976) reared parasitized and non-parasitized hosts under three different variable temperature regimes and found that the mean developmental rate of C. melanoscela from egg to adult always was slower than the mean developmental rate of gypsy moths from first to fourth instar. Fourth instars generally are not suitable as hosts because of their long setae and vigorous defensive movements (Weseloh 1976). The second generation of C. melanoscela is thus not well synchronized with the availability of suitable hosts (Weseloh 1976). Treating hosts with Bacillus thuringiensis (Bt) retarded development of surviving gypsy moths compared with controls, and treated hosts were attacked by C. melanoscela to a greater extent than nontreated hosts (Weseloh & Andreadis 1982). This is because the former had not yet molted to the fourth instar at the time of parasitoid oviposition (Weseloh et al. 1983). Wollam & Yendol (1976) found that Bt and C. melanoscela acted synergistically and provided greater foliage protection than either treatment alone. If other treatments slowed the development of host larvae, my results could be used to predict the timing of the adult stage of C. melanoscela in relation to the host, and the potential impact of the parasitoid on populations of gypsy moths.

Another application of the models would be to predict the occurrence of various life stages of C. melanoscela to minimize the impact of management treatments such as pesticides. C. melanoscela is only susceptible to Dimilin during the early stages of its development (Granett & Weseloh 1975), and
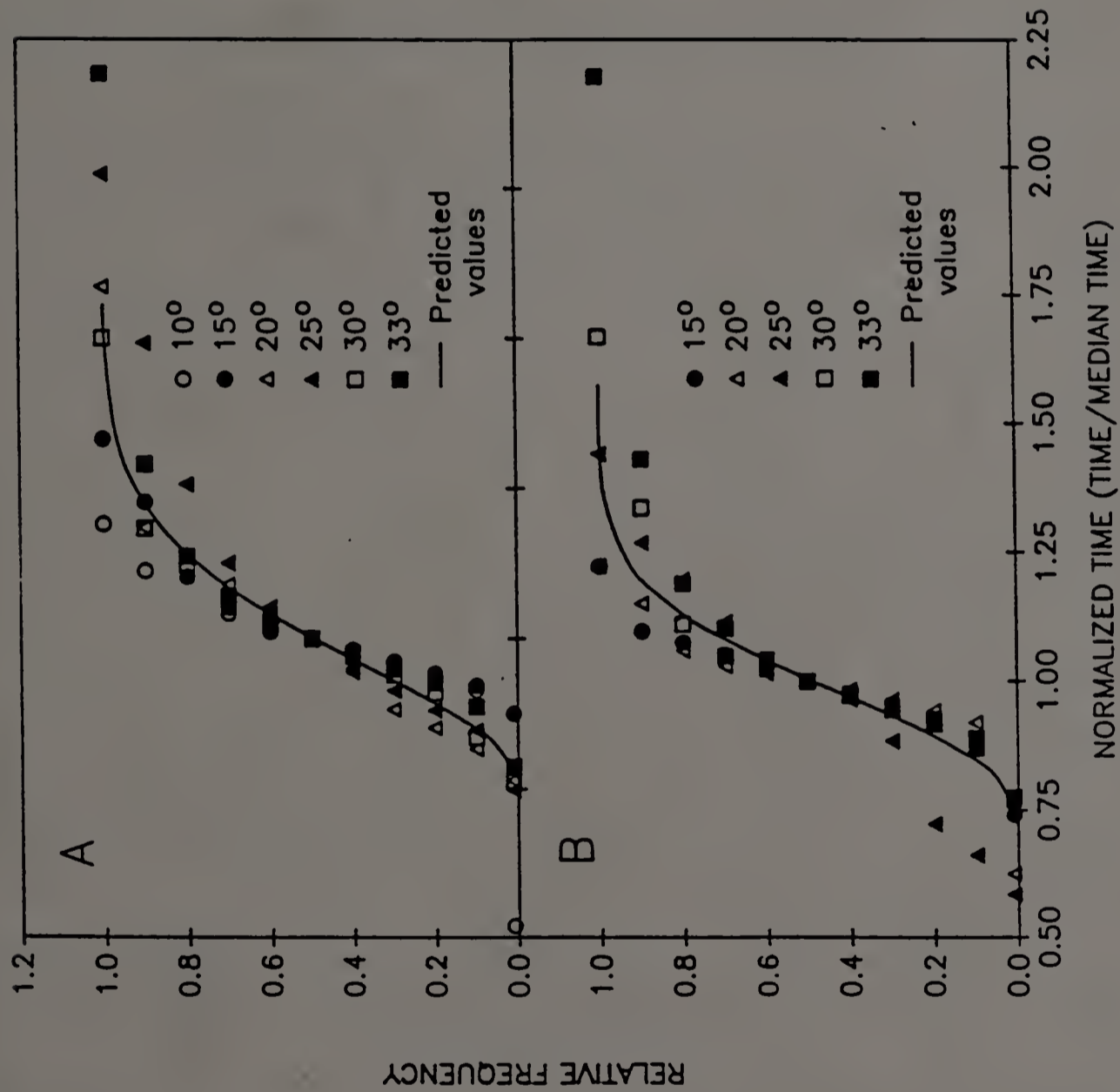
Figure 2.2. Cumulative Weibull functions. Data were fit to normalized developmental time for C. melanoscela larvae reared in first- through third-instar gypsy moths feeding on oak foliage (A) and artificial diet (B).

Granett et al. (1976) suggest that Dimilin sprays to control gypsy moth larval populations could be timed to avoid adversely affecting susceptible parasitoids. It is likely that adult C. melanoscela are susceptible to other pesticides and the timing of pesticide application could be adjusted to avoid harming adult parasitoids.

CHAPTER 3

ASSESSMENT OF POTENTIAL METHODS OF MEASURING PARASITISM BY

BRACHYMERIA INTERMEDIA (NEES) (HYMENOPTERA: CHALCIDIDAE)

Introduction

Brachymeria intermedia (Nees), a solitary endoparasitoid of lepidopteran

pupae, was first recovered from the gypsy moth in North America in 1965

(Leonard 1966). It is now found throughout the range of the gypsy moth in the

northeastern United States, and high levels of parasitism by B. intermedia have

been reported for some populations (e.g. Doane 1971, J.R.G. & J.S.E.

unpublished). Brachymeria intermedia is easily reared in the laboratory,

establishes well after release, and is a candidate for augmentative and

inundative releases (Leonard 1981, Blumenthal et al. 1981, Blumenthal et al.

1979). Because of the potential importance of this parasitoid, unbiased

methods of measuring levels of the parasitism it causes are needed.

Stage-specific parasitism in this paper is defined as the percentage of those

hosts entering a stage susceptible to parasitism that are subsequently attacked

by parasitoids. I will follow the guidelines of Varley et al. (1973) that a host

cannot be killed more than once and that death is attributable to the first agent

that attacks. Stage-specific parasitism has been calculated from field data in

several ways, but these methods do not always provide accurate estimates of

stage-specific parasitism levels (Van Driesche 1983, Gould et al. 1989 and

references therein). One method previously used in studies of B. intermedia

has been to calculate the percentage of parasitized hosts in either a single

sample or a series of samples (Ticehurst et al. 1978, Reardon 1976, Smilowitz

& Rhoads 1973, Doane 1971, Leonard 1966, 1967, 1971). Van Driesche

(1983) has shown, however, that the value of percentage parasitism in a given sample reflects the net result of four processes: (1) recruitment of the host to the stage that is susceptible to parasitism (2) recruitment of the parasitoid (i.e. oviposition), (3) advancement of the host out of the susceptible stage through death or molting, and (4) advancement of the parasitoid out of the host through death or emergence. The value of percentage parasitism in a single sample accurately estimates stage-specific parasitism only if all hosts have entered the stage of interest, all parasitoid oviposition is complete, and no hosts (parasitized or healthy) have been lost to death or molting to the next stage (Van Driesche 1983).

In some systems, evidence of the presence of the host (galls, mines, exuviae, etc.) remains after hosts have either died or advanced to the next life-stage. If the fate of these hosts can be determined by examination of the remains, percentage parasitism can be calculated by examining a single sample at the end of the host generation and determining the percentage of hosts from which a parasitoid emerged (Van Driesche 1983). Using this approach, samples of pupal exuviae collected at the end of the generation have been used to measure levels of parasitism of gypsy moth pupae by B. intermedia (Leonard 1966, Doane 1971). Predation, however, destroys all evidence that a particular host had become parasitized prior to being consumed. If levels of predation on parasitized and healthy hosts differ, examination of pupal exuviae does not accurately estimate stage-specific parasitism (Varley et al. 1973).

A third method of estimating stage-specific parasitism is direct measurement of host and parasitoid recruitment (Van Driesche & Bellows 1988). This method is unaffected by the timing of host and parasitoid recruitment to and advancement out of the sampled stages or by subsequent mortality of healthy or parasitized hosts. Values of percentage parasitism are calculated by dividing

the number of hosts that become parasitized by the number of hosts that enter the susceptible stage. If the biologies of the species involved are such that techniques can be devised to measure host and parasitoid recruitment, this method provides an unbiased estimate of stage-specific parasitism (Van Driesche & Bellows 1988).

This study was designed to compare the estimates of parasitism of gypsy moth pupae by B. intermedia provided by three methods (peak sample percentage parasitism, examination of exuviae, and direct assessment of host and parasitoid recruitment). The timing of host and parasitoid recruitment to and advancement out of the pupal stage of the gypsy moth were measured in the field to determine if peak sample percentage parasitism would reflect stage-specific parasitism. I also investigated the possibility that predation of parasitized and healthy pupae would differ and would thus bias estimates of parasitism based on examination of exuviae. Lastly, the feasibility of directly measuring recruitment of hosts and parasitoids was determined.

Methods and Materials

Description of Field Site

The study was conducted between 10 July and 18 August, 1986 near Otis Air National Guard Base in Falmouth, Massachusetts, a site dominated by oak, Quercus spp (a favored food of the gypsy moth). The density of gypsy moths at the study site was extremely high, and ca. 70% defoliation of the oak trees had occurred by the beginning of the study. The study plot measured 120 m X 50 m and had a 17 X 9 grid of sampling points (7 m between columns and 5 m between rows). The oak tree closest to each sampling point was numbered, and trees for the various sampling regimes were selected at random from the

153 sampling trees. Some gypsy moths had pupated prior to the beginning of sampling. The density of gypsy moth pupae and percentage parasitism of those pupae present at the beginning of the study were determined and used as starting values for calculating total numbers of hosts and parasitoids recruited.

Description of Sampling Regimes

Three types of observations of gypsy moth pupae were made: (1) semiweekly collections of field pupae to estimate values of sample percentage parasitism and the timing of host and parasitoid advancement, (2) repeated examination of sample trees and marking of field pupae to estimate recruitment of hosts to the pupal stage, and (3) exposure of insectary-reared pupae to estimate recruitment of parasitoids. In all cases, the sampling units were the trunk and branches of sampling trees between 0 and 3 m above the ground. Because the sizes of the trees differed, the diameters of the trees at breast height were measured and parameters are expressed as numbers per m^2 of trunk surface.

Collection of Pupae in the Field. Twice each week I collected 30 gypsy moth pupae from each of ten sample trees (n=300). Trees were never sampled more than once. Pupae that showed evidence of parasitoid emergence, adult gypsy moth emergence, or predation were not collected. The pupae were placed in 30 ml plastic cups containing tissue paper, reared in an outdoor insectary, and checked daily for emergence of adult gypsy moths or parasitoids. Dead hosts from which no parasitoids emerged were dissected, and those containing a B. intermedia pupa were considered parasitized by B. intermedia. The percentage of pupae that died or advanced to the adult stage prior to the next sampling occasion and the total percentage of pupae that died or advanced to the adult stage were calculated for each sample.

Repeated Examination of Sample Trees. At the beginning of the study, all the gypsy moth pupae found on 30 permanently tagged sample trees were marked with a dot of acrylic paint on the tip of the abdomen. A dot of paint was also placed on the tree next to each pupa. Every 3-4 days I returned and marked all new pupae that had appeared on the same trees (i.e. were recruited) since the previous sample occasion. After emergence of adult B. intermedia and gypsy moths was complete, the fates of all pupae were determined.

Exposure of Reared Pupae as Trap-Hosts. Recruitment of B. intermedia (through oviposition) was measured by placing unparasitized gypsy moth pupae in the field as trap-hosts for short intervals of time. To rear pupae for use as trap-hosts, egg masses of gypsy moths were collected from a moderately dense population of gypsy moths in Falmouth, Massachusetts in the spring of 1986. These masses were soaked for 1 h in an aqueous solution of 3.7% formaldehyde to destroy nuclear polyhedrosis virus (NPV) particles on the outside of the eggs and were rinsed for 1 h with water (Bell et al. 1981). Gypsy moth larvae hatched from these egg masses in cages (1 by 1 by 1 m), which had wooden frames, floors, and roofs. The cages were covered with screening (mesh size = 0.5 mm) to prevent parasitoid attack prior to exposure of trap-hosts in the field.

Trap-host larvae were fed black oak, Quercus velutina Lam., collected from an area with a low density of gypsy moths. This was done because host kairomones are important for host acceptance by B. intermedia females (Leonard et al. 1975, Tucker & Leonard 1977, Cardé & Lee 1990), and it is not known whether gypsy moths reared on artificial diet produce natural kinds or levels of kairomones. The branches of foliage were soaked for 1 h in a 4% solution of sodium hypochlorite to kill NPV and were then rinsed with water. Branches of foliage were placed in 4 liter jars of water equipped with foam

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stoppers (to prevent water loss and drowning of larvae) and were replaced with fresh foliage every two to three days. Larvae were transferred with camel's-hair brushes to new foliage at each change.

When the gypsy moth larvae were about to pupate, small oak twigs with leaves were placed on the bottom of the cages. Larvae spun webs of silk around these leaves and pupated in the webbing. Only larvae that pupated in the webs and were hence properly positioned to exert natural host defensive movements (Rothery & Barbosa 1984) were used as trap-hosts. On each sampling occasion, twigs containing a total of ten pupae were stapled to each of ten sample trees (n=100), using different trees on each occasion. Ten naturally occurring pupae were removed from each tree so that pupal density would not be altered. A dot of paint was placed on the tips of the abdomens of all trap-host pupae so that they could be distinguished from pupae of naturally occurring gypsy moths that used the twigs as pupation sites. Three to four days after the release, trap-host pupae were collected and placed in 30 ml plastic cups, containing pieces of tissue paper. The pupae were reared in an outdoor insectary and were checked daily for emergence of B. intermedia.

Calculation of Host and Parasitoid Recruitment and Advancement

Host Recruitment. Recruitment of hosts during interval i (HR_i) equalled the number of new pupae per m^2 of trunk surface that appeared on the thirty sample trees during the interval. An interval consisted of the three to four days between sampling occasions. The total number of gypsy moths that were recruited over n intervals to the pupal stage (HR) was calculated as:

$$HR = H_0 + \sum_{i=1}^{n} HR_i$$

where H_0 is the number of pupae per m^2 present at the beginning of the experiment, HR_i is the number of pupae per m^2 recruited during interval (i), and n = 9.

Parasitoid Recruitment. Recruitment of the parasitoid, B. intermedia during a given interval (PR_i) was calculated as:

$$PR_i = PTH_i * \{[(GMP_i * (1-HI_i)) + (GMP_{i+1} * (1-H_{i+1}))]/2\}$$

where PTH_i is the proportion of trap-hosts parasitized by B. intermedia during interval (i), GMP_i is the number of gypsy moth pupae per m^2 present at the beginning of interval (i), and HI_i is the proportion of hosts infected by disease or parasitoids at the beginning of interval (i). The term 1-HI was used to denote the proportion of hosts that were not already infected by parasitoids or disease. Mortality of these hosts would be attributed to the first agent that attacked (Varley et al. 1973). The proportion of pupae infected by parasitoids or disease (HI_i) was calculated by collecting and rearing samples of pupae. Because hosts were both being recruited and advancing during the intervals when parasitism was occurring, the number of hosts available for attack by B. intermedia during the interval was taken to be the average of the number of healthy hosts at times (i) and (i + 1).

The number of gypsy moth pupae present, GMP_i, was estimated by first summing the number of hosts recruited (HR_i) over all previous intervals and then subtracting the total number of hosts that had been killed by predators, advanced to the adult stage, or died prior to the beginning of the interval. The total percentage of pupae consumed by predators was estimated at the end of the experiment by examination of exuviae. The total number of pupae per m^2 consumed by predators was divided equally among intervals. A constant number of pupae consumed was used instead of a constant rate of predation

because it was felt that at the extremely high densities of gypsy moths in the plot, predators would be saturated.

The total number of $\underline{B}$. $\underline{intermedia}$ recruited during the pupal stage of the host (PR) was calculated as:

$$PR = P_0 + \sum_{i=1}^{n} PR_i$$

where P_0 is the number of pupae per m^2 parasitized by $\underline{B}$. $\underline{intermedia}$ at the beginning of the experiment, PR_i is the number of $\underline{B}$. $\underline{intermedia}$ per m^2 recruited during interval (i), and n = 9.

Host and Parasitoid Advancement. Advancement of hosts out of the pupal stage occurred when pupae died (due to disease, predation, or emergence of other parasitoid species) or when adults emerged. Advancement of $\underline{B}$. $\underline{intermedia}$ was in the form of emergence of adult parasitoids or predation of pupae parasitized by $\underline{B}$. $\underline{intermedia}$. Because the temperature conditions of the insectary were somewhat different from those in the field plot, and I was interested in the timing of emergence under natural conditions, I only monitored emergence of hosts and parasitoids for 3-4 days after pupae were brought to the insectary. I calculated the proportion of pupae in sample (i) that died or emerged prior to sample (i + 1). I then monitored emergence from pupae in sample (i + 1), and so on, for (i) = 1 to 9.

The number of hosts not parasitized by $\underline{B}$. $\underline{intermedia}$ (I will call them non-parasitized hosts) advancing during a given interval (HA_i) was calculated as:

$$HA_i = (PHA_i * H_i) + HP_i$$

where PHA_i is the proportion of non-parasitized hosts in sample (i) that advance prior to (i + 1), H_i is the number of non-parasitized hosts per m^2 present at the

beginning of interval (i), and HP_i is the number of nonparasitized hosts per m^2 that are consumed by predators during interval (i).

The number of B. intermedia advancing during a given interval (PA_i) was similarly calculated as:

$$PA_i = (PPA_i * PH_i) + PP_i$$

where PPA_i is the proportion of hosts collected at the beginning of interval (i) from which B. intermedia emerged prior to the beginning of interval (i + 1), PH_i is the number of hosts per m^2 parasitized by B. intermedia at the beginning of interval (i), and PP_i is the number of hosts per m^2 parasitized by B. intermedia that are consumed by predators during interval (i).

Methods of Estimating Percentage Parasitism

Peak Sample Percentage Parasitism.
Pupae that had been collected in the field were kept in the insectary until all pupae had either died or emerged as adults. Percentage parasitism in the sample taken at the beginning of interval (i) (% PA) was calculated as:

$$\% PA = PH_i / PT_i$$

where PT_i is the number of hosts in sample i parasitized by B. intermedia and TH_i is the total number of hosts collected. Values of sample percentage parasitism were graphed and the peak value was determined.

Examination of Exuviae.
Gypsy moth pupae that had been marked with paint as they were recruited were examined after all mortality and adult emergence was complete. The fates of the pupae could be determined in most cases by the characteristics of the exuviae (Tigner 1974). Missing pupae were identified by the dots of paint on trees and were assumed to have been removed by predators. Emergence holes produced by B. intermedia are similar to those produced by the ichneumonid, Theronia atalantae fulvescens (Cresson), but the two were distinguished by breaking open the exuviae to examine the meconia.

The meconium of B. intermedia is compact and gray; the meconium of T. atalantae is white and sinuous. Pupae with no sign of parasitoid emergence or predation were dissected, and those containing pupae of B. intermedia were considered parasitized. Percentage parasitism by B. intermedia was calculated by dividing the number of exuviae containing emergence holes or pupae of B. intermedia by the total number of pupae originally marked.

Direct Assessment of Host and Parasitoid Recruitment. I estimated the total number of gypsy moth pupae (HR) and the total number that became parasitized (PR) by summing host and parasitoid recruitment values over all intervals. Percentage parasitism was calculated by dividing PR by HR.

Some of the parameters I calculated were obtained by multiplying several random variables. For example, estimates of PR_i were obtained by multiplying three random variables (PTH_i, GMP_i and HI_i). Confidence limits associated with parameters such as PR_i, HR_i, HA_i, PA_i, H_i, and PH were therefore calculated with a bootstrap approach (Buonaccorsi & Liebhold 1988, Efron & Tibshirani 1985). I randomly sampled the raw data with replacement 1000 times and calculated an estimate of the parameter at each iteration. A frequency distribution of estimates of the parameter was thus generated, and the mean and 95% confidence interval of this distribution were determined.

Results

Host and parasitoid recruitment in the study population occurred simultaneously (Fig. 3.1A and B), from the beginning of the study period until ca. 26 July. However, some host and parasitoid recruitment occurred before sampling began. Advancement of hosts not parasitized by B. intermedia was also high during this period, peaking on 19 July and declining after that date
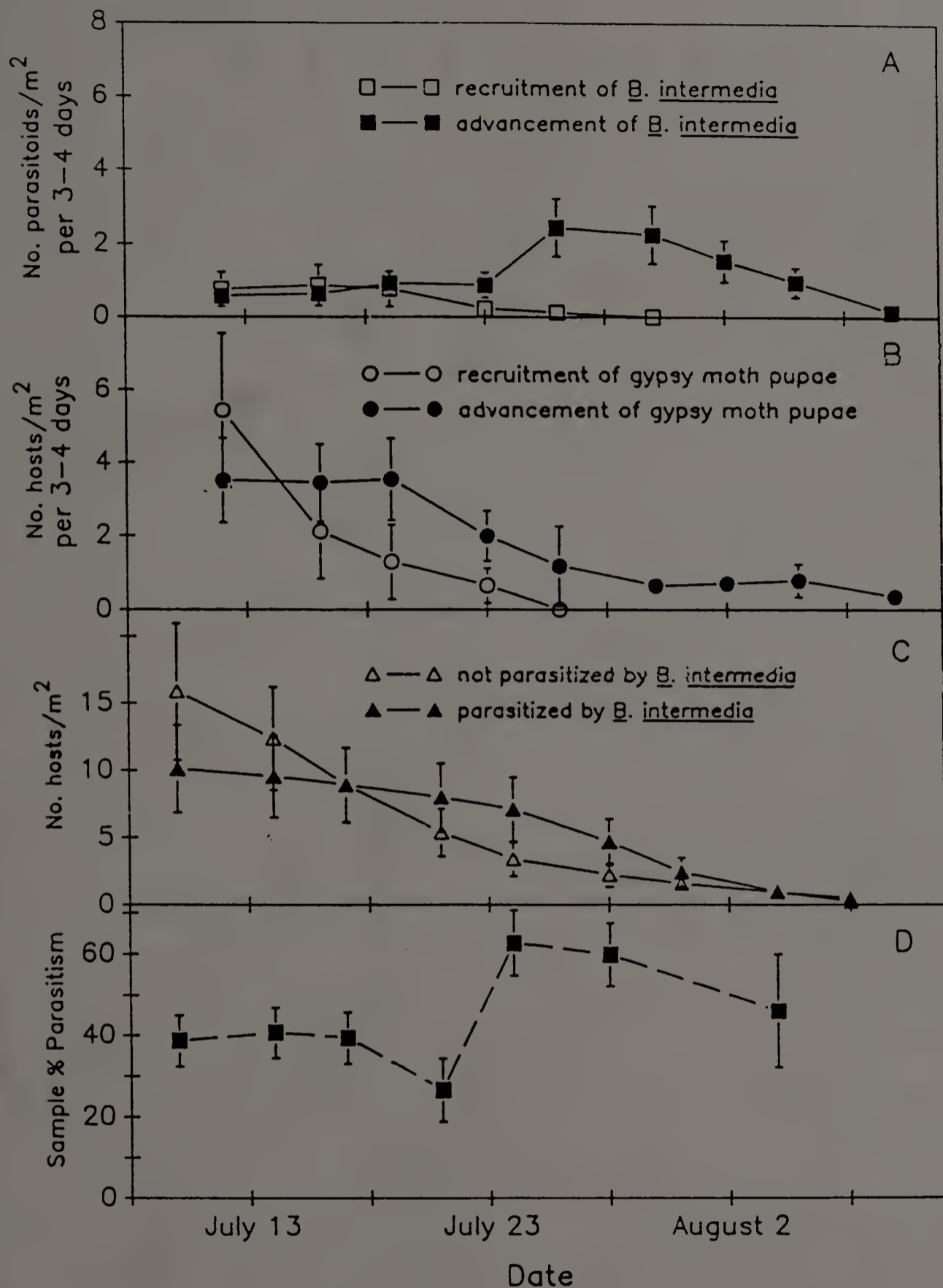
Figure 3.1. Timing of life-history processes of B.
intermedia and gypsy moths. (A) recruitment and advancement
of B. intermedia, (B) timing of host recruitment to and
advancement out of the pupal stage, (C) densities of
parasitized and nonparasitized hosts, and (D) percentage
parasitism in samples of gypsy moth pupae. For a population
of gypsy moth pupae parasitized by Brachymeria intermedia at
Otis Air Force Base, Cape Cod, Massachusetts in 1986. Bars
represent ± 95% CI.

(Fig. 3.1B). Peak advancement of B. intermedia did not occur until later, 26 July

(Fig. 3.1A). This was when emergence of B. intermedia adults began;

parasitoid advancement earlier in the season was due entirely to predation. As

hosts not parasitized by B. intermedia advanced early in the season, the

number remaining declined, and by 21 July, there were more hosts parasitized

by B. intermedia than there were hosts not parasitized by B. intermedia (Fig.

3.1C).

Sample percentage parasitism peaked on 24 July (Fig. 3.1D) at 62.7%. I

estimated that 36.3 gypsy moths per m^2 entered the pupal stage and that 13.1

per m^2 (36.1%) became parasitized by B. intermedia. I examined 1179 exuviae

at the end of the season, and 234 (19.8%) contained emergence holes of B.

intermedia. Of the remaining pupae, 223 (18.9%) had emergence holes

produced by T. atalantae, 137 (11.6%) had produced tachinid flies, 115 (9.8%)

died of unknown causes, and 435 (36.9%) had been consumed by predators.

Only 35 (3.0%) of the gypsy moths in this pupal population became adults.

Discussion

The estimate of parasitism based on the peak value of sample percentage

parasitism was high relative to estimates calculated using the other two

methods (Table 3.1). It has been shown using simulation models that the peak

value of sample percentage parasitism is a good estimator of stage-specific

parasitism only if samples are collected after all hosts and parasitoids have

been recruited and prior to host and parasitoid advancement (Van Driesche

1983). In the study population, there was no time when all hosts (healthy and

parasitized) were available for sampling. Recruitment and advancement of

gypsy moth pupae and B. intermedia overlapped (Fig. 3.1), and as a result,

Table 3.1. Percentage parasitism ($\pm$ SE) by <u>Brachymeria</u>
<u>intermedia</u> on pupae of the gypsy moth calculated using three
methods. For a population at Otis Air Force Base, Cape Cod,
Massachusetts, in 1986.

Method	% Parasitism
Peak Percentage Parasitism	62.7 $\pm$ 1.2
Direct Assessment of Recruitment	36.6 $\pm$ 8.0
Examination of Exuviae	19.8 $\pm$ 1.2

values of sample percentage parasitism fluctuated in a manner unrelated to stage-specific parasitism (Fig. 3.1D). Of particular note is the fact that hosts not parasitized by B. intermedia (Fig. 3.1B) advanced earlier in the season than those parasitized by B. intermedia (Fig. 3.1A) and were no longer in the population of hosts available for sampling. Approximately halfway through the sampling period, the number of hosts not parasitized by B. intermedia became lower than the number of hosts parasitized by B. intermedia, and values of sample percentage parasitism increased sharply. The peak value of sample percentage parasitism that occurred at this time did not reflect levels of stage-specific parasitism but rather was a consequence of the early advancement of hosts not parasitized by B. intermedia. When nonparasitized hosts remain available for sampling for a shorter period than do parasitized hosts, as in this study, the peak value of sample percentage parasitism overestimates stage-specific parasitism (Van Driesche & Taub 1983).

The estimate of parasitism based on examination of exuviae at the end of the season was low relative to estimates calculated using the other two methods (Table 3.1). Predation removes evidence that a particular host had been previously parasitized. Estimates based on examination of exuviae in populations where predation levels are high are therefore expected to underestimate stage-specific parasitism if parasitized hosts are exposed longer to predation because of longer developmental period. In the population on Cape Cod in 1986, 36.9% of the pupae were consumed by predators by the end of the season. When levels of predation are high, estimates of parasitism based on examination of exuviae can be adjusted. This is accomplished by partitioning hosts consumed by predators into the categories of parasitized and non-parasitized hosts based on the proportion of hosts that were not predated that fall into each of these categories (Varley et al. 1973). The adjusted value

that I calculated in this manner was 27.2%, a value that is still lower than the estimate based on host and parasitoid recruitment.

The method of Varley et al. (1973) assumes that the probability of predation is the same for parasitized and non-parasitized hosts. In the population I studied, hosts that were not parasitized by B. intermedia left the system much earlier than hosts parasitized by B. intermedia (Fig. 3.1A and B), and were therefore available to predators for a shorter period of time. The explanation for this phenomenon is that the developmental rate of gypsy moth pupae is less than that of B. intermedia. For example, it takes 12 days for female gypsy moth pupae to become adults and 17 days for male pupae at 23°C (Casagrande et al. 1987), while it takes 24 and 27 days for male and female B. intermedia to develop in gypsy moth pupae reared at 23°C (Minot & Leonard 1976). Even in the absence of selective daily rates of predation of parasitized pupae, therefore, pupae parasitized by B. intermedia suffered higher stage-specific rates of predation because they were exposed to predation longer. Examination of exuviae at the end of the stage, consequently, underestimated stage-specific parasitism.

When calculating percentage parasitism, I followed the guidelines of Varley et al. (1973) that no host may be killed more than once. If a host is attacked successively by two parasitoids (or by a parasitoid then a predator), death of the host is attributed to the first parasitoid. If the second parasitoid succeeds in emerging from the host at the expense of the first parasitoid, the second species is considered to have killed the first parasitoid species, not the host. If B. intermedia can develop and emerge from hosts previously infected by other agents (and already dead by this scenario), values of percentage parasitism based on sample percentage parasitism or examination of exuviae could be high relative to stage-specific parasitism levels. This problem does not occur for

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the method of directly measuring recruitment because I calculated parasitoid recruitment only in uninfected hosts.

The method of directly measuring recruitment is not affected by the timing of host and parasitoid recruitment and advancement. Also, because numbers of hosts and parasitoids are measured and summed over short intervals of time, subsequent predation of parasitized or non-parasitized hosts is not a concern. Potential biases associated with this method arise if the rates of parasitism of trap hosts differ substantially from parasitism of naturally occurring hosts. Care was taken, therefore, to simulate natural conditions when placing trap-host pupae in the field. I accomplished this by rearing trap hosts on oak foliage, by not altering the density of pupae in the field, and by only using trap-hosts that were encased in webbing. Levels of parasitism of trap-hosts were, therefore, probably quite similar to those for unparasitized pupae in the field. Given the above, I feel that the method of directly measuring host and parasitoid recruitment provides a relatively unbiased estimate of stage-specific parasitism.

In conclusion, I feel that estimates of parasitism of gypsy moth pupae by B. intermedia, based on values of peak sample percentage parasitism, overestimate stage-specific levels of parasitism. Collections of pupae timed to occur during the late pupal-stage (i.e. Leonard 1967) or after adult emergence is complete (i.e. Leonard 1966) will also result in overestimations of parasitoid impact. In contrast, estimates of parasitism based on examination of exuviae at the end of the season underestimate stage-specific parasitism because of overall differences in predation of parasitized and non-parasitized hosts. Because of the particular life-histories of B. intermedia and gypsy moth pupae, direct assessment of host and parasitoid-recruitment is feasible and provides the least biased estimate of stage-specific parasitism. This method is the most

time consuming of the three methods, but is superior to the other methods

examined in its freedom from major biases.

CHAPTER 4

TEMPORAL PATTERNS AND MORTALITIES OF HOST AND PARASITOID

POPULATIONS: IMPLICATIONS FOR MEASURING PARASITOID IMPACT

Introduction

Many attempts have been made to estimate the impact of parasitoids on the dynamics of the gypsy moth. The approach most commonly employed has been to measure stage-specific parasitism (i.e. the 'apparent' parasitism of life-table analysis), which may be defined as the percentage of hosts that enter the stage susceptible to parasitism that are killed by a given species of parasitoid.

Researchers often attempt to estimate stage-specific parasitism from data on trends of percentage parasitism in samples collected over time, selecting either the average value (Pooled Percentage Parasitism) or the highest value (Peak Percentage Parasitism) as their estimate. Van Driesche (1983) has shown that these two methods accurately measure stage-specific parasitism only if specific conditions are met. These methods can be severely biased by the timing of samples in relation to four life-history processes: (1) recruitment of hosts into the stage susceptible to parasitism, (2) advancement of hosts out of the susceptible stage due to death or molting, (3) recruitment of parasitoids (oviposition), and (4) advancement of parasitoids out of the sampled stage due to death or molting.

To accurately measure stage-specific parasitism, two parameters must be precisely estimated: (1) the number of individuals that enter (i.e. are recruited into) the host stage susceptible to parasitism and (2) the number of healthy hosts that are subsequently killed by parasitoids. The number of individuals entering a given stage differs from the number of individuals per sample unit

(i.e. host density) at a given moment. Many techniques have been developed to estimate numbers entering a stage from stage-frequency data (reviewed in Southwood 1978). To date, however, only one of these methods, which I will term the Southwood & Jepson (1962) Graphical Method, has been extended to provide estimates of both host and parasitoid recruitment (Bellows et. al. 1989). Mortalities that occur during the period when stage-frequency data are collected are major sources of biases in this process, when this method is used to measure numbers of hosts, parasitoids, or both (Bellows et al. 1989).

Direct measurement of host and parasitoid recruitment is an alternative approach for estimating stage-specific parasitism (Van Driesche & Bellows 1988, Lopez & Van Driesche 1989). I will call this the Recruitment Method. It consists of measuring the number of hosts and/or parasitoids entering the system over short intervals of time, and then summing these values over the entire sampling period to estimate the total number of individuals of each type that are recruited. This method has the advantage that the desired quantities are measured directly, rather than being inferred from stage-frequency data. The feasibility of this method depends on being able to design techniques to measure recruitment of hosts and parasitoids, given the biologies of the species involved.

Another approach, which I will term Time-Specific Death-Rate Analysis (Elkinton 1990a), offers some alternatives to using stage-specific parasitism to evaluate the impact of parasitoids. This method measures mortality rates over short, contiguous time intervals, rather than stages, and does not require estimates of either host densities or host or parasitoid recruitment. Percentage parasitism during an interval is calculated in terms of the number of individuals (regardless of stage) present at the beginning of a time interval that die from a given agent during the interval. Percentage parasitism over a series of intervals

is calculated as 1 - the product of the survivorship from parasitism in each interval. This technique is especially appropriate when estimates of host density are difficult to obtain.

The Time-Specific Death-Rate Analysis method solves some of the problems associated with estimating stage-specific parasitism, when several sources of mortality are operating contemporaneously in a population. In most populations it is the rule, not the exception, that periods of mortality by different agents overlap. Assuming that attack by two or more species is sequential, when it is not, results in errors in estimating mortality by these agents (Varley et al. 1973, Elkinton et al. 1990b). Alternatively, if one assumes the agents act entirely contemporaneously, by considering them to act as one "factor", one cannot estimate the impact of individual species on the dynamics of the host population.

Royama (1981a) resolved the problem of separately quantifying mortalities from contemporaneous agents by calculating the probability of hosts dying from each mortality agent in the absence of other agents (termed the marginal probability of death). For parasitoids, the marginal probability of dying is greater than the proportion actually killed because some individuals bearing immature parasitoids will die from other causes prior to parasitoid emergence. Marginal probabilities of two or more mortality agents acting together do not sum to the total percentage mortality during a stage or interval (as is the case for stage-specific percentage parasitism calculated in the usual manner). Instead, survivorships from the different agents multiply to equal the total survivorship from all agents (1 - percentage mortality). If the parasitoid is the only source of mortality or is the first in a series of factors acting sequentially within a stage or interval, then the marginal probability of dying from parasitism will equal the stage- or interval-specific proportion killed . Elkinton et al. (1990a) present

arguments that it is the marginal probability of dying and not stage-specific mortality that represents the most unbiased estimate of parasitoid impact on a population.

The goal of this study was to determine the timing of recruitment and advancement of hosts and parasitoids, as well as the timing of mortality from sources other than parasitism, for the second generation of Cotesia melanoscela Ratzeburg (Hymenoptera: Braconidae) and for Parasetigena silvestris Robineau-Desvoidy (Diptera: Tachinidae), parasitoids of the gypsy moth, Lymantria dispar (L.) (Lepidoptera: Lymantriidae). Based on my results, I evaluate the five methods (Pooled Percentage Parasitism, Peak Sample Percentage Parasitism, the Southwood & Jepson Graphical Method, the Recruitment Method, and Time-Specific Death-Rate Analysis) for calculating parasitism of gypsy moths by these two species. The first three of these methods estimate stage-specific parasitism. The Time-Specific Death-Rate Analysis method yields an estimate of the marginal probability of dying. Data on recruitment can be used to estimate either stage-specific parasitism or the marginal probability of dying, depending on the details of how the data are collected and the recruitment rate is estimated.

Methods and Materials

Field Sites

Field studies were conducted on two 9 ha plots (300 X 300 m) on Otis Air Force Base on Cape Cod, Massachusetts in 1984 and 1985. Initial densities of gypsy moth egg masses in the spring were 149 ± 14 SE egg masses per ha for Plot 1 (1985), and 200 ± 57 SE egg masses per ha for Plot 2 (1984). The most abundant tree species in both plots were oak (Quercus spp.) and pitch pine

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(Pinus rigida Mill.), and blueberry (Vaccinium spp.) dominated the understory. The plots were divided into nine, square 1 ha subplots, and forty sampling points were established in a 4 X 10 grid in each subplot.

Description of Sampling Regimes

In 1985, field observations were made on five parameters: (1) the density of egg masses per ha and numbers of larvae hatching per egg-mass, (2) the timing of hatch of egg masses in the field, (3) the density of gypsy moth larvae and pupae throughout the season, (4) rate of parasitism of insectary-reared larvae exposed in the field, and (5) sample percentage parasitism and the timing of host and parasitoid advancement in weekly collections of gypsy moth larvae and pupae. In 1984, I only measured the timing of parasitoid recruitment and advancement.

Density of Egg Masses and the Number of Larvae Hatching per Egg-Mass. In 1985, the number of gypsy moth larvae hatching from egg masses within Plot 1 was calculated by multiplying the number of egg masses per ha by the number of larvae emerging per egg-mass. The estimate of egg-mass density was based on counts of egg masses within 10 m diameter circles surrounding 169 sampling points in a 13 X 13 grid (25 m between points). The number of larvae per egg-mass was estimated by first measuring the length and width of two egg masses at each point (n = 338 egg masses). I then used regressions of number of larvae hatching on length times width of egg masses (developed by A.M. Liebhold) to determine the number of larvae hatching per egg-mass. This regression subsumes the two variables of eggs per cm^2 of egg-mass area and percentage viability.

Timing of Hatch of Egg Masses. In 1985, I monitored egg masses in the field at 15 sampling points selected at random from the 13 X 13 grid. Prior to hatch, I tagged up to four egg masses in each 10 m diameter circle (two on overstory

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trees and two on the ground). I did not always find two egg masses in each category, and in all I monitored 44 egg masses. From 30 April to 15 May 1985 I checked each egg mass daily in the late morning and recorded the presence or absence of gypsy moth larvae. Most hatch occurs in the early morning, and newly emerged larvae spend from 1 to 49 hours on the surface of the egg mass (McManus 1973).

Density of Gypsy Moth Larvae and Pupae. On three sampling occasions, 2 X 5 m quadrats were established at two of the 40 sampling points in each subplot. I counted all the larvae and pupae within each quadrat by thoroughly searching the leaf litter, understory vegetation, and overstory trees. Because trees on Cape Cod are relatively short (generally < 10 m) I was able to sample the tops of trees above the quadrats using ladders and pole pruners to collect foliage. This sampling technique was extremely time consuming, and I was only able to estimate densities on three occasions: 4-5 June (instar 3), 24-25 June (instar 5), and 17-18 July (pupae).

Rate of Parasitism of Laboratory-Reared Trap-Host Larvae. Recruitment of parasitoids to their immature stage was estimated by deploying unparasitized, gypsy moth larvae as trap-hosts. These larvae were placed in the field for short periods of time, recovered, and reared to determine the proportion that had become parasitized. Trap-host larvae were reared from egg masses collected one week prior to hatch from a moderately high density population on Cape Cod, Massachusetts (2357 egg masses per ha). Egg masses were soaked in a 10% solution of formalin to reduce levels of viable nuclear polyhedrosis virus (NPV) on the surface of the eggs (Bell et al. 1981), were rinsed with water for 1 h, and were allowed to hatch in 26 cages (1.0 X 1.0 X 1.2 m). The cages were located outdoors and were covered with fine netting (mesh size = 0.5 mm) to prevent parasitoid attack prior to release in the field. To assess the

effectiveness of the netting in excluding parasitoids, five larvae were randomly selected from each cage once per week (n = 130), placed individually in 30 ml cups containing artificial diet (Bell et al. 1981), and monitored daily for parasitoid emergence.

Trap-host larvae were fed black oak, Quercus velutina Lam., leaves collected from areas adjacent to the study plot. Branches of foliage were soaked for 1 h in a 4% solution of sodium hypochlorite to eliminate viable NPV polyinclusion bodies and were then rinsed with water. The foliage was placed in 4 l jars of water with foam stoppers (to prevent water loss and drowning of larvae) and was replaced with fresh foliage every 2-3 days. Larvae were transferred with camel's-hair brushes to new foliage at each change.

Trap-host larvae were marked by removal of one proleg (a different proleg for each release occasion). This mark was easily recognized in the field, was not lost when the larvae molted, and did not significantly effect survivorship or mobility of the larvae (Weseloh 1985a). Larvae to be marked were held in Petri dishes over crushed ice for 15-30 min until immobilized, and a proleg was removed with surgical scissors. Larvae were replaced over ice for another 10 min to allow the wound to heal. Marked larvae were then placed in a separate cage for at least 3 days prior to release to allow recovery from surgery.

Larvae were released every week at two sampling points in each subplot (new sampling points were used each week). In 1984, 50 trap-host larvae were released on each of the two oak trees nearest to the sampling points (n = 1800 larvae per week). In 1985, 75 larvae were released on a single oak tree at each sampling point (n = 1350 larvae per week). For each weekly release, larvae were divided into three groups of equal size and were released on three successive days. Larvae were transported to the release site in 360 ml paper cups which were stapled to the trunks of the oaks at 1.5 m. Because gypsy

moth larvae in low density populations feed at night, releases were made between 16:00 h and 18:00 h so that larvae would climb up the trees and begin to feed, reducing dispersal away from the release trees.

Trap-host larvae were recaptured 4-5 days following release by searching the entire understory and litter beneath the crown of each release tree and the canopies of the trees themselves. The larvae were placed individually in 30 ml cups containing artificial diet (Bell et al. 1981), were reared in an outdoor insectary, and were checked daily for mortality. Emerging parasitoids were keyed to species (Simons et al. 1979). Because of the staggered release schedule and the resulting staggered recapture, there were larvae in the field on almost every day during each weekly interval.

Parasitism, Molting, and Death Rates of Field Collected Larvae and Pupae. In 1985, I collected weekly samples of 40 naturally occurring gypsy moth larvae and pupae at the same time I was recovering trap-hosts on the 18 sampling trees (n = 720 per week). In 1984, I collected 60 gypsy moth larvae and pupae at each of the 18 sampling points (n = 1080 per week). Gypsy moth larvae and pupae were collected from all parts of the habitat (forest canopy, understory vegetation, and leaf litter), were placed individually in 30 ml cups containing artificial diet, and were reared in an outdoor, screened insectary. In 1984, gypsy moth larvae and pupae from a sample were checked daily for mortality or emergence of adult gypsy moths until the next sample was collected. In 1985, larvae and pupae were checked daily until death or adult eclosion so that I was able to calculate percentage parasitism for each sample, as well as the timing of parasitoid emergence. If a parasitoid emerged, the parasitoid puparium or cocoon was keyed to species (Simons et al. 1979). Dead gypsy moth larvae and pupae from which no parasitoids emerged were dissected, and if they contained an unemerged, immature parasitoid, they were considered

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parasitized. Unparasitized, dead gypsy moths were checked for the presence of the polyinclusion bodies of NPV, using a phase contrast microscope at 1000X magnification.

Estimating Mortality from Predation

Predation rates of gypsy moth larvae in the field were estimated indirectly by combining data on changes in gypsy moth density and data on rates of mortality from parasitoids and disease observed in samples of field-collected gypsy moth larvae and pupae reared in the laboratory. The difference between mortality rates observed in the field (i.e. changes in density) and mortality rates observed in rearing is termed residual mortality and includes losses due to predation as well as other causes such as weather.

Comparison of Trap-Hosts and Naturally Occurring Hosts

Female C. melanoscela are more successful in attacking early instars (1-3) than later instars (4-6) because the setae and defensive movements of larger larvae make parasitoid oviposition attempts less successful (Weseloh 1976). To determine if larvae used as trap-hosts were equivalent to field larvae in terms of body size, I weighed 20 trap-host larvae and 20 naturally occurring larvae on each sampling occasion. Because the data were not normally distributed, weights of trap-host and naturally occurring larvae on each collection date were compared using a Wilcoxon Rank Sum Test (PROC NPAR1WAY, SAS Inst. 1987). I also compared the proportions of trap-hosts and naturally occurring hosts that were in the third or younger instars using a Chi-square test (PROC FREQ, SAS Inst. 1987).

An important behavior of late-stage gypsy moths that affects parasitism by P. silvestris is the daily migration from nighttime feeding sites in the forest canopy to daytime resting sites under bark flaps or in the litter (ODell & Godwin 1979). To test whether trap-host larvae exhibited migration patterns similar to naturally

occurring larvae, I isolated two 6 m tall oak trees in the forest outside of Plot 1 in 1986. A 4 X 4 m barrier of 20 cm high aluminum flashing was established around each tree. Sticky material (Tack Trap) was placed on the top of the inside of the flashing to prevent larvae from escaping. A 24.5 cm wide band of burlap was wrapped around the trunk of each tree at 1.5 m above the ground.

On 18 June 1986 I released 50 fifth instar gypsy moths reared on foliage in the screen cages (I will call them trap-hosts) and 50 naturally occurring fifth instars (collected from Plot 1) on each tree. The trap-host larvae were marked with a dot of white acrylic paint on the dorsum of the abdomen, and naturally occurring gypsy moth larvae were marked with light red paint. I observed these larvae on 20 June, 21 June, and 26 June by searching the entire area of each enclosure including the litter, under burlap bands, and the canopy of the trees (using step-ladders). I recorded the height above the ground for each larva. Larvae on the ground were considered to be at 0 m. I compared the mean height of trap-hosts versus naturally occurring larvae using the Wilcoxon Sign Rank Test (PROC NPAR1WAY, SAS Inst. 1987).

Calculations of Recruitment and Advancement

Host Recruitment. The number of hosts recruited to the susceptible (larval) stage (HR) could be measured directly because gypsy moths hatch over a relatively short period of time, and hatch is completed prior to the onset of significant larval mortality or parasitoid oviposition. The estimate of larval density, determined by quadrat sampling, was nearly three times higher than the estimate based on the number of larvae hatching within the plot, indicating a large amount of immigration of first instars (the dispersal stage of the gypsy moth). I therefore used the number based on quadrat sampling as the best estimate of the total number of gypsy moths susceptible to parasitism in Plot 1.

The timing of host recruitment was based on observations of hatching of egg masses in the field. The numbers of egg masses hatching on each day were summed over the entire period of egg hatch to give the total number of egg-mass hatch days. The number of larvae emerging on day i (L_i) was calculated as:

$$L_i = \frac{NumH_i}{\text{egg-mass days}} * TotL$$

where $NumH_i$ is the number of egg masses hatching of day (i), TotL is the total number of gypsy moths recruited to the larval stage in Plot 1, and egg-mass days are as defined above.

Parasitoid Recruitment. Recruitment of parasitoids during a given week (PR_i) was calculated as:

$$PR_i = PTH_i * \{[(GMP_i * PL_i * (1-HI_i)) + (GMP_{i+1} * PL_{i+1} * (1-HI_{i+1}))]/2\}$$

where PTH_i is the proportion of trap-hosts parasitized during week i, GMP_i is the number of gypsy moths per ha present at the beginning of week (i), PL_i is the proportion of gypsy moths that are still in the larval stage at the beginning of week (i), and HI_i is the proportion of hosts infected by disease or parasitoids at the beginning of week (i). The proportion of trap-hosts that would have been parasitized in a week (PTH_i) was calculated as:

$$PTH_i = 1 - ((1-PTH_s)^{1/d})^7$$

where PTH_s is the proportion of trap-hosts in sample (i) that were parasitized and d is the number of days that trap-hosts were exposed to parasitism in the field. The term PL_i was included because only larvae are susceptible to attack by C. melanoscela and P. silvestris. The term 1-HI was used to denote the proportion of gypsy moth larvae that did not already contain parasitoids or

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disease and were thus available for attack. Because hosts were pupating and dying during some of the weeks when parasitism was occurring, the number of hosts available for attack during the week beginning at time i was taken to be the average of the number of healthy larvae at times (i) and (i + 1).

Host and Parasitoid Advancement. Although both host and parasitoid advancement (i.e. losses from molting or death) by definition must include deaths from predation, actual interval-specific predation rates were not measured. Advancement rates as observed in laboratory rearing of field collected larvae and pupae includes all factors except predation. For parasitoids this included parasitoid emergence from parasitized hosts or deaths in rearing. Advancement of hosts included deaths and molts of all hosts not parasitized by the species of parasitoid of interest. For hosts that were not parasitized by C. melanoscela, advancement was due to death from disease, emergence of other parasitoid species, or pupation. Because immature P. silvestris can emerge from gypsy moth pupae, advancement of hosts not parasitized by P. silvestris resulted from death due to disease, emergence of other parasitoid species, or emergence of gypsy moth adults from pupae.

Because the temperatures inside the screened insectary differed somewhat from those in the field, and because I was interested in estimating the timing of emergence of hosts and parasitoids under natural conditions, I only monitored emergence of hosts and parasitoids for 1 week after the gypsy moths were brought to the insectary. I calculated the proportion of individuals in sample i that died or emerged prior to each subsequent sample (i + 1). I then monitored emergence from gypsy moths in sample (i + 1) and so on for (i) = 1 to 10.

The number of hosts not parasitized by a particular species advancing during a given week (HA_i) was calculated as:

$$HA_i = PHA_i * H_i$$

where PHA_i is the proportion of nonparasitized hosts in sample (i) that advance prior to sample (i + 1) and H_i is the number of nonparasitized hosts per ha at the beginning of week (i).

The number of parasitoids advancing during a given week (PA_i) was similarly calculated as:

$$PA_i = PPA_i * PH_i$$

where PPA_i is the proportion of parasitized hosts collected in sample (i) from which parasitoids emerged prior to (i + 1) and PH_i is the number of parasitized hosts per ha at the beginning of week (i). PH_i was calculated by multiplying the number of gypsy moths per ha by the proportion of gypsy moths in sample (i) that was parasitized by the species of interest.

Methods of Calculating Percentage Parasitism

Peak Sample Percentage Parasitism. Gypsy moths that had been collected in the field were kept in the insectary until they died or emerged as adults. Sample Percentage Parasitism (SPP) due to a particular species was calculated as:

$$SPP = HP_i / HT_i$$

where HP_i is the total number of parasitized hosts in sample i over the whole rearing period and HT_i is the total number of hosts collected for sample i. Values of sample percentage parasitism were graphed and the peak value was determined.

Pooled Percentage Parasitism. Pooled Percentage Parasitism (PPP) was calculated as:

$$PPP = PH / TH$$

where TH is the total number of hosts collected over the entire season and PH is the number of these hosts from which a parasitoid emerged or was found following dissection to contain a parasitoid. Because C. melanoscela attacks

and emerges only from larvae, TH was the total number of larvae collected, whereas pupae were also included in the TH value for calculations of pooled percentage parasitism by P. silvestris.

Southwood & Jepson Graphical Method. The Southwood & Jepson Graphical Method was used to estimate the number of parasitoid attacks on gypsy moth larvae. The number of parasitized hosts per ha on each sample occasion (PH$_i$) was plotted against accumulated degree-days in the field. To calculate the number of individuals entering the immature stage of the parasitoid, the area under the curve through these points was divided by the number of degree-days required for complete development of the immature stage of the parasitoid. Percentage parasitism was then calculated by dividing the number of parasitized hosts by the total number of hosts initially susceptible to parasitism (HR).

The accumulation of degree-days in the field was estimated using hourly average temperature data obtained with a thermistor probe in a standard weather station located in an open field near the field plots and recorded on a CR21 data logger (Campbell Scientific, Logan, Utah).

Cotesia melanoscela requires 182 degree-days to emerge from hosts feeding on oak foliage, with a developmental threshold of 7°C (Chapter 2). The development of P. silvestris is influenced by temperature, but this parasitoid does not develop under a strict degree-day regime. Immature P. silvestris responds to the developmental stage of the host and only complete development as the host nears pupation (T. M. ODell unpublished). To estimate the number of degree days required for development of P. silvestris, therefore, I estimated the number of degree-days observed in the field from mean oviposition into trap-hosts to mean emergence from naturally occurring hosts. This was found to be 160 degree-days. The developmental threshold for the

gypsy moth, $7^{\circ}C$ (Casagrande et al. 1987), was used for this calculation. When the developmental rate of C. melanoscela was estimated in a similar manner, the result was 188 degree-days, which is close to the number of degree-days based on the laboratory data.

The Recruitment Method. The total number of each parasitoid species recruited (PR) was calculated by summing weekly recruitment over the entire season as follows:

$$PR = \sum_{i=1}^{n} PR_i$$

where PR_i is the number of parasitoids per ha recruited during week (i) and n is the total number of sample intervals (10 weeks). Percentage parasitism was calculated by dividing PR by HR, an independent measure of total gypsy moths entering the larval stage.

Time-Specific Death-Rate Analysis. The proportion of gypsy moths collected on week (i) that died from each agent prior to week (i + 1) was calculated for each weekly interval. Mortality from each agent during a given week (k_{ij}) was expressed as:

$$k_{ij} = -\log_{10}(1 - m_{ij})$$

where m_{ij} is the marginal probability of hosts dying from agent (j) during week (i). Total mortality due to a given agent (K_j) was calculated by summing the weekly k-values as follows:

$$K_j = \sum_{i=1}^{n} k_{ij}$$

for i = 1 to 10. Percentage parasitism by each species during the period of sampling was then calculated as $1 - 1/10^{K_j}$.

To calculate the marginal rates of mortality (m_{ij}), based on the actual proportion observed to die in rearing, I used equations 12 and 13 of Royama (1981), which assume that attack by each species is independent of other species. Solutions to these equations are derived in Elkinton et al. (1990b) and are as follows:

$$m_{ij} = (b - (b^2 - 4c * v_{ij})^{1/2} / 2c$$

$$m_{ij'} = v_{ij'} / (1 - (c * m_{ij})$$

where

$$b = c(v_{ij} + v_{ij'}) + 1 - v_{ij'}$$

m_{ij} = the marginal probability of attack by species j during week i,

$m_{ij'}$ = the marginal probability of attack by species j'

v_{ij} = the proportion of hosts in rearing that died from species j in week i,

$v_{ij'}$ = the proportion of hosts in rearing that died from species j' during week i,

and

c = the proportion of hosts attacked by both j and j' that produce species (j).

This methodology was extended from a system of two contemporaneous species to more than two species by calculating the marginal rate of mortality due to the species of interest (m_{ij}) versus the combined mortality from all other agents ($m_{ij'}$). I assumed that if a host was attacked by both species (j) and at least one other agent, 50% would produce species (j) (c = 0.5).

This method produces a small error that is proportional to the fraction of hosts attacked by three or more contemporaneous agents. In most populations, this fraction and the resulting error are quite small (Elkinton et al. 1990b). The rationale behind the use of this method is described in a series of papers (Elkinton et al. 1990a,b).

Calculating 95% Confidence Intervals of Compound Parameters

Some of the parameters I measured (i.e. PR, HA, PA, and the number of parasitoids recruited using the Southwood & Jepson Graphical Method) were calculated by multiplying several random variables. Confidence limits associated with these parameters were therefore calculated using a bootstrap approach (Buonaccorsi & Liebhold 1988, Efron & Tibshirani 1985). I randomly sampled the raw data with replacement 1000 times and calculated an estimate of the parameter at each iteration. A frequency distribution of estimates was thus generated, and the mean and 95% confidence interval were determined.

Results

Of the 1,300 larvae collected from the rearing cages, only 1 produced an immature C. melanoscela, and P. silvestris did not emerge from any of these larvae. This indicates that I was successful in producing unparasitized trap-hosts for release. I was also quite successful in recapturing trap-host larvae, recovering from 22 to 50% of the larvae released in a given week in 1985. The proportions of larvae that were third instar or less on a given sample occasion were not significantly different between trap-hosts and naturally occurring hosts (Table 4.1). For all but the first sample, however, the mean weight of trap-hosts was consistently lower than that of naturally occurring hosts (Table 4.1), although these differences were not significant. The comparison of heights of daytime resting locations revealed that naturally occurring fifth instars rested at significantly lower levels than fifth instars reared in cages (Table 4.2).

The periods of hatch and dispersal of gypsy moths occurred prior to the onset of parasitism by the second generation of C. melanoscela (Fig. 4.1A). On 4 June I estimated from quadrat samples that there were 109,200 gypsy moth

Table 4.1. Comparisons of the proportion of larvae in the third instar or younger and weights of trap-hosts and naturally occurring (wild) hosts collected on five occasions on Cape Cod, Massachusetts in 1985. (N = 20 on each date).

Date	Mean weight		% < instar 3	
	trap-hosts	wild hosts	trap-hosts	wild hosts
5 June	0.040	0.040	90.2	97.3
10 June	0.080	0.086	48.0	53.6
20 June	0.164	0.190	3.2	4.8
26 June	0.249	0.271	2.5	2.5
2 July	0.391	0.417	0.0	0.0

Table 4.2. Mean height above the ground of gypsy moth larvae reared in screen cages (trap-hosts) and naturally occurring (wild) larvae released on oak trees on Cape Cod, Massachusetts.

	Mean Height	
Date	Trap-hosts	Wild-hosts
20 June	10.0 ± 0.4	5.8 ± 0.6
21 June	9.1 ± 0.5	3.1 ± 0.3
26 June	9.2 ± 0.6	4.7 ± 0.6

Figure 4.1. Timing of life-history processes of C. melanoscela and gypsy moths in 1985. (A) Hatch of gypsy moth larvae and advancement of larvae not parasitized by C. melanoscela. (B) Recruitment (oviposition) and advancement (emergence) of C. melanoscela. (C) Percentage parasitism in samples of gypsy moths from a population at Otis Air Force Base, Cape Cod, Massachusetts. Bars represent + 95% CI.

larvae per ha. Liebhold & Elkinton (1988a) recorded a similar density in this plot in 1985 by collecting frass particles. Dispersal had essentially ceased by 28 May, when 90% of all gypsy moths were second instar or greater. Recruitment of gypsy moths therefore ended prior to oviposition by the second generation of C. melanoscela, which began on ca. 4 June, and prior to oviposition by P. silvestris, which began on ca. 12 June.

The periods of recruitment (oviposition) and advancement of C. melanoscela overlapped slightly in 1985 (Fig. 4.1B) and 1984 (Fig. 4.2A). Advancement of hosts not parasitized by C. melanoscela, due to death from NPV or emergence of other parasitoid species, was quite low during the period of oviposition by C. melanoscela and peaked after most C. melanoscela immatures had emerged (Fig. 4.1A). Also, few gypsy moths were consumed by predators during this period, as evidenced by the fact that estimates of density actually increased slightly (by ca. 2,000 larvae per ha) between 4 June and 25 June.

Estimates of percentage parasitism by C. melanoscela in 1985, calculated using the five methods, are shown in Table 4.3. Values of sample percentage parasitism peaked at 7.1% on 25 June (Fig. 4.1C), about the time when the period of oviposition was ending and advancement was beginning. Using the Recruitment Method, I estimated that 25,001 (22.9%) of the 109,200 gypsy moth larvae became parasitized by C. melanoscela. The estimate of the number of hosts parasitized by C. melanoscela, using the Southwood & Jepson Graphical Method, was 8,190 (7.5%). A total of 5,400 gypsy moth larvae were collected, of which 160 (3.0%) were parasitized by C. melanoscela. The marginal rate of parasitism by C. melanoscela, using Time-Specific Death-Rate Analysis, was estimated to be 8.1%.

The periods of recruitment and advancement of P. silvestris overlapped considerably in both 1984 (Fig. 4.2B) and 1985 (Fig 4.3B). The period of
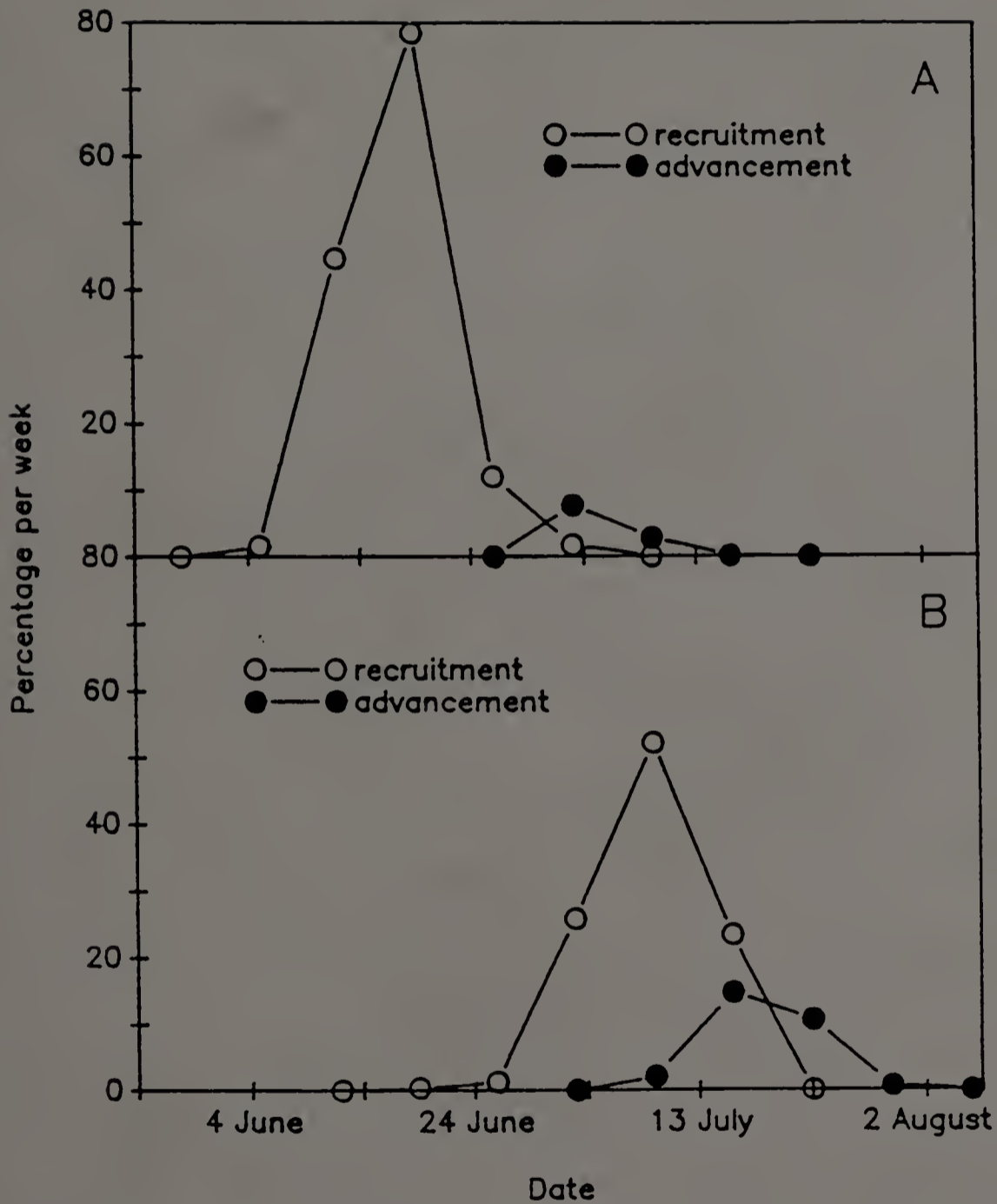
Figure 4.2. Recruitment and advancement of C. melanoscela and P. silvestris in 1984. Percentages of C. melanoscela (A) and P. silvestris (B) recruited into and advancing out of gypsy moth hosts, are for a population of gypsy moths at Otis Air Force Base, Cape Cod, Massachusetts.

advancement of hosts not parasitized by P. silvestris also overlapped with both recruitment and advancement of P. silvestris (Fig. 4.3A), and predation was a significant mortality factor at this time. Between 25 June and 17 July, mortality observed in rearing did not account for the entire amount of mortality measured in the field, and I estimate that during this period, residual mortality, presumably accounted for by predation, was 32%.

Sample Percentage Parasitism by P. silvestris peaked on 16 July (Fig. 4.3C) at 49.7%. By directly measuring recruitment I estimated that 17,784 (16.3%) hosts became parasitized by P. silvestris. The estimate of parasitoid recruitment, based on the Southwood & Jepson graphical method, was 73,710 or 67.5%. Of the 6,056 larvae and pupae I collected over the course of the season, 1,031 (17.0%) were parasitized by P. silvestris. The marginal rate of parasitism, calculated by summing weekly k-values, was 70.4%.

Discussion

I will first consider the four methods of calculating stage-specific parasitism. Although I was attempting to measure the same entity (stage-specific parasitism) in the same population, estimates of percentage parasitism varied considerably depending on the method of calculation. One would come to quite different conclusions about the contribution of these two parasitoids to mortality of the gypsy moth depending on the method used. (Table 4.3). I will explore the biases associated with the use of these four methods for measuring stage-specific parasitism by C. melanoscela and P. silvestris.

I will begin with the Recruitment method, because it is potentially the most straightforward of the four techniques. A crucial assumption of this method is that rates of parasitism of trap-hosts and naturally occurring hosts are equal.
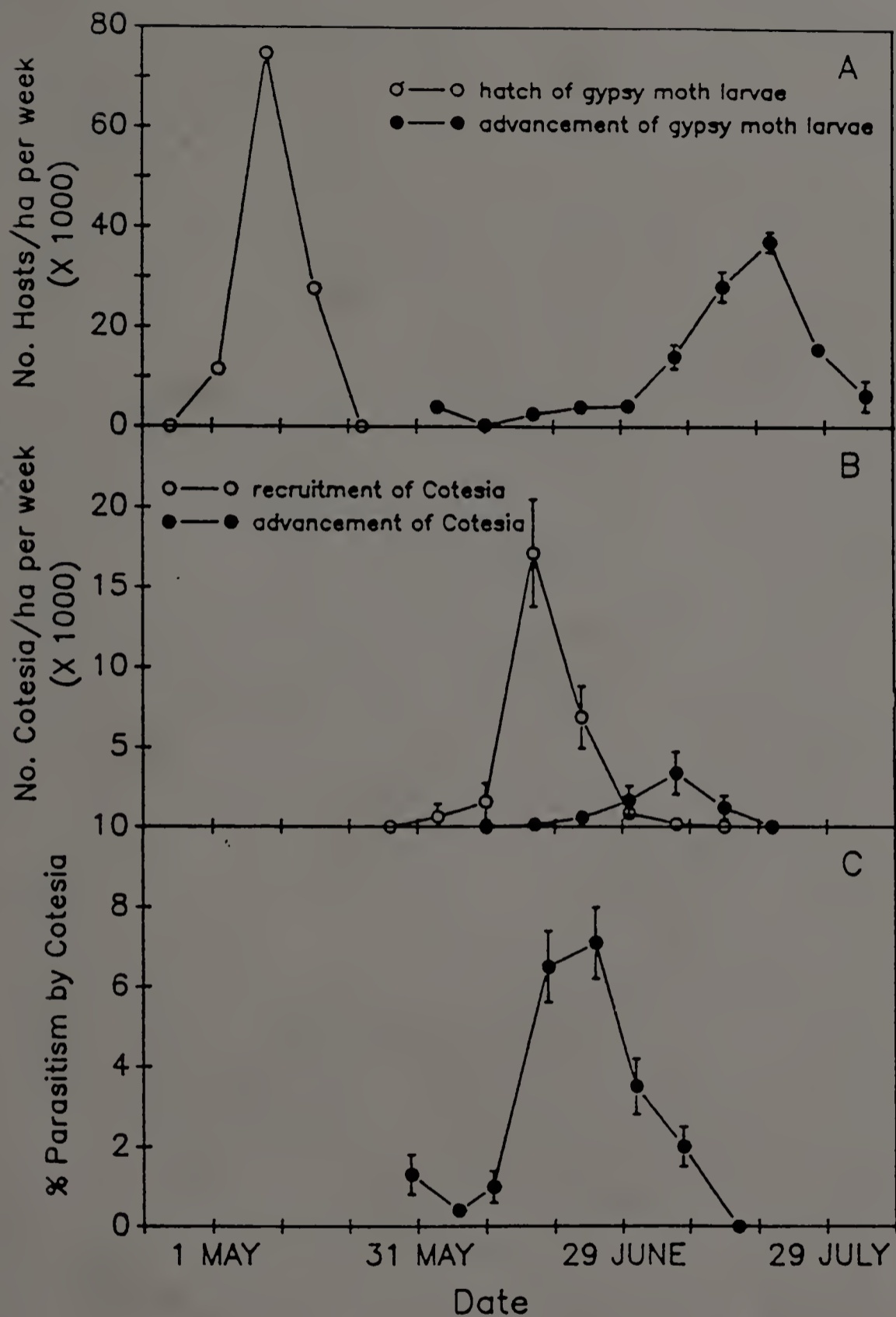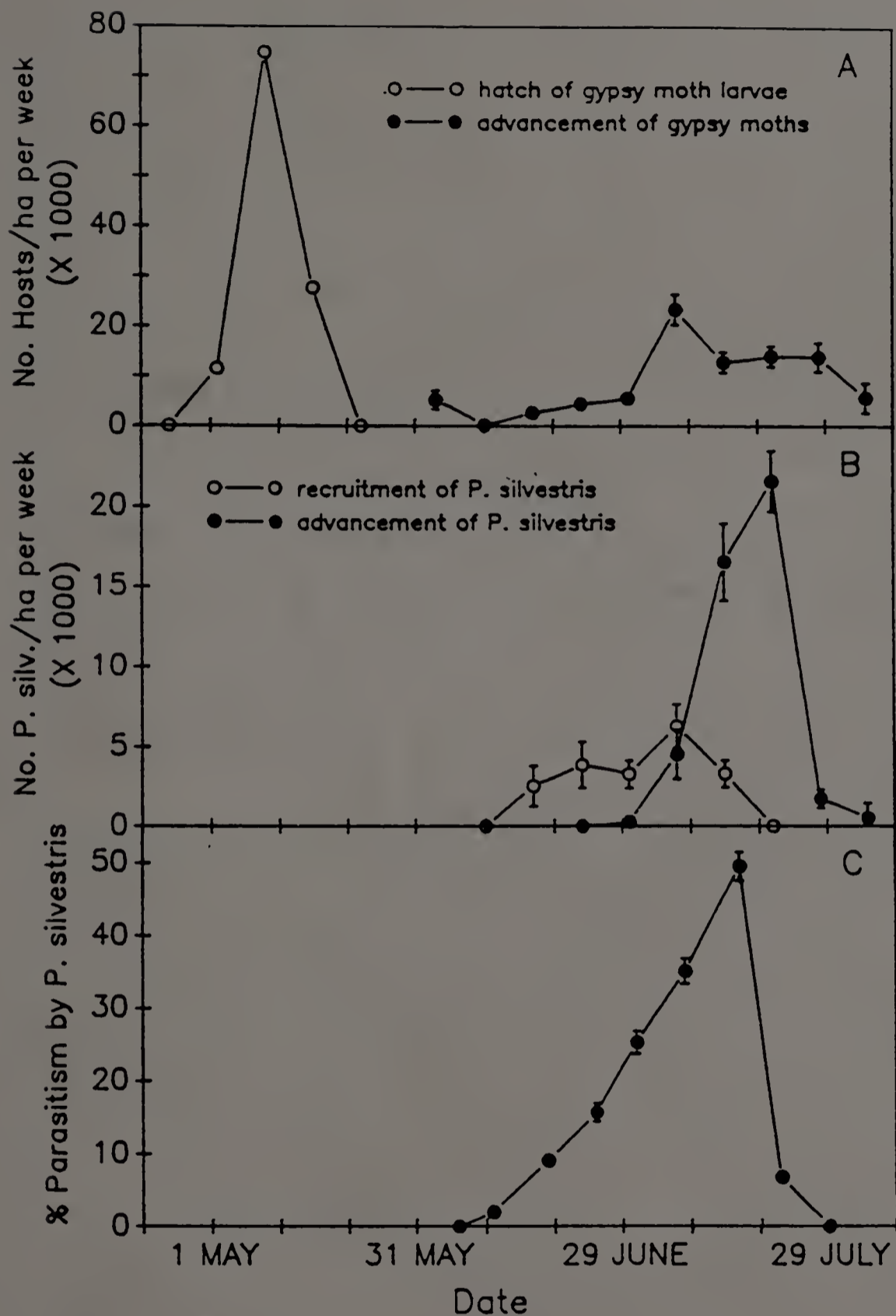
Figure 4.3. Timing of life-history processes of P. silvestris and gypsy moths in 1985. (A) Hatch of gypsy moth larvae and advancement of larvae not parasitized by P. silvestris. (B) Recruitment (oviposition) and advancement (emergence) of P. silvestris. (C) Percentage parasitism seen in samples of gypsy moths from a population at Otis Air Base, Cape Cod, Massachusetts. Bars represent ± 95% CI.

Table 4.3   Values of percentage parasitism (± 95% CI) by C. melanoscela and P. silvestris calculated using five methods. Gypsy moth larvae were collected on Otis Air Force Base, Cape Cod, Massachusetts, in 1985.

Method	C. melanoscela	% Parasitism P. silvestris
Recruitment	22.9 ± 3.9	16.8 ± 2.4
Pooled Percentage Parasitism	3.0 ± 0.5	17.0 ± 0.9
Peak Sample % Parasitism	7.1 ± 2.9	49.7 ± 4.9
Southwood & Jepson Graphical	7.5 ± 1.6	67.5 ± 3.9
Time-Specific Death-Rates	8.1 ± 0.2	70.4 ± 0.4

When adults of the second generation of C. melanoscela emerged, many gypsy moth larvae were already fourth instars, and could avoid parasitism by C. melanoscela because of their long setae and vigorous defensive movements (Weseloh 1976). Although the proportions of trap-hosts and naturally occurring hosts that were third instar or less were the same, the mean weight of trap-host larvae was consistently lower than that of naturally occurring hosts. I suspect that this difference is real and that I was unable to show a statistically significant difference because of the small sample size. I believe that due to their smaller size, a greater proportion of trap-host larvae were susceptible to parasitism by the second generation of C. melanoscela than were the naturally occurring hosts and that this accounts for the relatively high estimate of percentage parasitism using the Recruitment method.

Female P. silvestris are especially attracted to gypsy moth larvae that are moving between nighttime feeding sites in the forest canopy and daytime resting sites (ODell & Godwin 1979). I have evidence that trap-host larvae did not exhibit a typical diel migration pattern. In the enclosure experiment, most naturally occurring larvae occurred at ca. 1.5 m above the ground (the height of burlap bands). Trap-host larvae, on the other hand, remained in the canopy during the day and were probably subject to lower rates of parasitism by P. silvestris. This phenomenon could explain why the estimate of percentage parasitism by P. silvestris based on the Recruitment method was lower than that of any other method. The use of trap-hosts to assess parasitism has proven more successful for sessile organisms, such as gypsy moth pupae (Chapter 3) and Colorado potato beetle eggs (Van Driesche et al. 1990), for which host behavior is not as important.

I also calculated the number of hosts that became parasitized using the Southwood and Jepson Graphical Method. Severe biases occur with the use of

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this method if mortality of immature parasitoids is greater than 20% (Bellows et al. 1989). I have no reason to believe that mortality of C. melanoscela immatures was anything but minimal. Gypsy moths are not known to encapsulate this species and mortality of gypsy moths from predation, NPV, and other parasitoids, which would also have killed C. melanoscela, was quite low when C. melanoscela was present. The Southwood & Jepson method, coupled with the direct estimate of the number of hosts susceptible to parasitism, is therefore a relatively unbiased method for calculating stage-specific parasitism by C. melanoscela.

Mortality of gypsy moths (due to NPV and predation) was greater than 20% during the period when P. silvestris was present, and for this reason I would expect the Southwood & Jepson Method to underestimate percentage parasitism by this species. Perhaps an even greater confounding factor, however, is that P. silvestris does not develop in a strict degree-day fashion. Development of immatures is arrested until the host is ready to pupate (T. M. ODell unpublished) so that the offspring of individuals that oviposit several weeks before pupation accumulate many more thermal units than offspring resulting from later ovipositions. Because of these problems, it is difficult to interpret values of percentage parasitism by P. silvestris calculated using the Southwood & Jepson Graphical Method, and I do not recommend that it be used for this species.

The Pooled Percentage Parasitism and Peak Sample Percentage Parasitism methods are accurate only if the samples are collected after host and parasitoid recruitment are complete and prior to advancement of hosts and parasitoids (Van Driesche 1983). Mortalities of parasitized and nonparasitized hosts must also be equal. These requirements were essentially met for C. melanoscela for the Peak Sample Percentage Parasitism Method. Recruitment of hosts was

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complete well before the peak of percentage parasitism that occurred on 25 June, appreciable advancement of hosts did not begin until after 25 June, and the periods of parasitoid recruitment and advancement overlapped only slightly. While I would expect the peak value of sample percentage parasitism to slightly underestimate stage-specific percentage parasitism, because of the slight overlap of parasitoid recruitment and advancement, the amount of error should be small.

Researchers should be careful, however, in the use of Peak Sample Percentage Parasitism to measure parasitism by C. melanoscela in populations of gypsy moths that have been treated with Bacillus thuringiensis (Bt). Development of gypsy moth larvae that survive treatment with Bt is delayed, prolonging the availability of gypsy moths to C. melanoscela (Weseloh & Andreadis 1982). Oviposition by C. melanoscela did not end in the population I studied because female parasitoids ceased ovipositing but because hosts became too large. In a population treated with Bt, it is probable that oviposition and emergence would overlap to a greater extent, with the result that values of Peak Sample Percentage Parasitism would underestimate stage-specific parasitism.

The periods of recruitment and advancement of P. silvestris overlapped broadly with each other and also with advancement of hosts not parasitized by P. silvestris. At no time were all hosts, parasitized and nonparasitized, available to be sampled, and consequently the peak value of sample percentage parasitism is not a good measure stage-specific parasitism by this species. There was also a considerable amount of predation of gypsy moths during the period when P. silvestris was present. Although I do not know if predators consumed different proportions of parasitized and nonparasitized hosts, if they did, values of Peak Sample Percentage Parasitism would be affected.

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Values of Pooled Percentage Parasitism accurately estimate stage-specific parasitism only when levels of parasitism remain unchanged over a series of samples (Gould et al. 1989). This did not occur for the population I studied; values of percentage parasitism increasing as parasitoids oviposited and decreasing as they emerged. Pooling these samples with those collected at the time of peak percentage parasitism is equivalent to averaging percentage parasitism over the season. In general, this is a weighted average because if some samples contain more hosts they contribute more than average to the estimate of Pooled Percentage Parasitism. Pooled Percentage parasitism invariably underestimates stage-specific parasitism, and indeed percentage parasitism estimates calculated using this method were quite low for both C. melanoscela and P. silvestris.

Time-Specific Death-Rate Analysis involves calculation of the marginal probability of dying using data from rearing field-collected individuals. A few problems associated with this technique have been identified (Elkinton 1990a). Errors can occur if host recruitment or advancement overlaps with mortality, which did not occur for C. melanoscela but was a concern for P. silvestris because hosts were advancing during the period of mortality of this species. This method also assumes that simultaneous attack and competition among mortality agents occurs during a short interval (one week in this study). It does not account for individuals that are subsequently successfully attacked by another agent. Again, this was not a major problem for C. melanoscela because agents such as NPV and other parasitoids that could have subsequently attacked hosts bearing C. melanoscela take longer to develop than does C. melanoscela. It is likely that once C. melanoscela is relatively well developed, subsequent attack by NPV or P. silvestris would not prevent C. melanoscela from emerging. For P. silvestris, which delays development until

hosts near pupation, there is a large probability that a host attacked by P. silvestris during a given week would be subsequently attacked by another agent, and that this second agent would kill the host.

Concluding Remarks

Both Peak Sample Percentage Parasitism and the Southwood and Jepson Graphical Method produce relatively unbiased methods for calculating stage-specific percentage parasitism by C. melanoscela. Indeed, values of percentage parasitism calculated using these two methods were extremely similar. Time-specific Death-Rate Analysis was also robust for calculating the marginal probability of parasitism by this species and was slightly greater than values of stage-specific parasitism, as expected. None of the five methods studied were satisfactory in estimating parasitism by P. silvestris, however.

In the future, I recommend that another version of the Recruitment Method, the short-marker stage (Van Driesche 1988, Van Driesche & Bellows 1988), be used to calculate both stage-specific parasitism and the marginal rate of parasitism by P. silvestris. This technique involves identifying a sufficiently early stage of the parasitoid that, given the developmental rate of the parasitoid and accumulation of heat in the field between sample occasions, all naturally occurring hosts containing this stage must have become parasitized since the previous sample occasion. This method has the advantage over the use of laboratory-reared trap-hosts that recruitment is measured into naturally occurring hosts. For P. silvestris the obvious candidate for a short-marker stage is the egg. Eggs of P. silvestris are large and deposited conspicuously on the integuments of gypsy moths. The eggs hatch in ca. 4 days, and it is possible to distinguish eggs containing parasitoids from those that have already hatched (Prell 1915). A possible short coming of this method is that some eggs may be

infertile or be shed before the immature P. silvestris hatch. These phenomena would have to be studied before the technique could be used.

The two methods of estimating parasitism by C. melanoscela and P. silvestris most commonly used in past studies were Peak Sample Percentage Parasitism (e.g. Fuester et al. 1983, Drea & Fuester 1979, Ticehurst et al. 1978, and Tigner 1974) and Pooled Percentage Parasitism (e.g. Blumenthal et al. 1979, Reardon & Podgwaite 1976, and Barbosa et al. 1975). These methods were used without regard for the biases that affect them, and as I have shown, the use of Pooled Percentage Parasitism or the use of Peak Sample Percentage Parasitism by P. silvestris is not appropriate. One needs to be careful when conclusions of research are based on the use of these methods to estimate the impact of parasitoids on gypsy moth populations. The Southwood & Jepson Graphical Method has been used to assess recruitment of both C. melanoscela and first through third instar gypsy moths (Kolodny-Hirsch et al. 1988). The authors did not measure mortality that might bias the use of this method, but because mortality of early instar gypsy moths is usually low (Elkinton et al. 1989), this method was probably suitable.

In the calculations of stage-specific parasitism, in order to evaluate the impact of the two species separately, I had to assume that they acted entirely sequentially. This was not the case in this study. Although C. melanoscela was present earlier in the season, the two species occurred together for ca. 5 weeks. Assuming that the species acted sequentially can lead to errors in partitioning mortality for use in life-tables (Varley et al. 1973). It is my recommendation that the most unambiguous way to measure the impact of C. melanoscela and P. silvestris is to measure marginal probabilities of parasitism.

CHAPTER 5

FIELD AND LABORATORY STUDIES OF SUPERPARASITISM BY

PARASETIGENA SILVESTRIS (DIPTERA: TACHINIDAE)

Introduction

Parasetigena silvestris (Robineau-Desvoidy), a solitary endoparasitoid of

larvae of the gypsy moth, Lymantria dispar (L.), can be found throughout the

range of its host in the northeastern United States. Adults emerge and mate in

early spring, but females do not begin to oviposit until mid June, when gypsy

moths are in the fourth instar (Prell 1915, ODell & Godwin 1979). Female P.

silvestris search for hosts by flying or walking in vertical spirals around the

trunks of trees (ODell & Godwin 1979). Although active searching peaks in mid

afternoon (Weseloh 1976), when ambient temperatures are above 20°C (Prell

1915, ODell & Godwin 1979), a major stimulus for oviposition is movement of

the host larvae (Prell 1915, Weseloh 1976), which is greatest at dawn and in the

evening. At these times, larvae are in transit between nighttime feeding sites in

the forest canopy and daytime resting sites such as bark flaps and leaf litter.

Most of the large, white macrotype eggs are deposited on the integuments of

larvae during these migrations (Weseloh 1974), although P. silvestris also

attacks larvae in the daytime resting sites (ODell & Godwin 1979).

Superparasitism by P. silvestris, the deposition of more than one egg on a

single host, has frequently been reported (Prell 1915, Burgess & Crossman

1929, Weseloh 1974, 1976, ODell & Godwin 1979). Rarely, however, does

more than one P. silvestris larva survive and emerge from the host (Prell 1915,

Burgess & Crossman 1929). Pest managers often do not consider the

propensity to superparasitize to be a desirable trait for a solitary parasitoid

because it results in a wastage of eggs (Fiske 1910). Superparasitism may, however, increase the probability of parasitoid survival (and thus of host mortality) if more than one parasitoid is better able to overcome host defenses (e.g. Streams 1971, Puttler 1974, Beland & King 1976).

The inability to avoid superparasitism is also considered maladaptive from the perspective of an individual parasitoid because the probability of survival is reduced by competition among conspecifics (Salt 1961). Immature parasitoids developing in superparasitized hosts may also be smaller and less fecund (King et al. 1976). It has been suggested that there should be strong selective pressures to develop mechanisms to discriminate between parasitized and unparasitized hosts and to avoid superparasitism (Rogers 1975, van Lenteren 1981).

To study the effect of superparasitism on levels of host mortality, I measured the dispersion of parasitoid eggs on field-collected gypsy moth larvae to determine if superparasitism was the result of random oviposition or if certain hosts were more or less likely to be attacked than average. Gypsy moth larvae were also collected and reared to confirm that a single parasitoid usually emerges from superparasitized hosts. The dispersion patterns of eggs were compared between larvae collected in a populations with high and low densities of gypsy moths and between larvae collected from under burlap bands with larvae collected elsewhere in the same plot. Bands of burlap wrapped around the trunks of trees are utilized by larvae as daytime resting sites are often used as a sampling tool (e.g. Tigner et al. 1974, Reardon 1976, Bogenschutz et al. 1989). Because estimates of the impact of P. silvestris on the population dynamics of the gypsy moth are frequently based on samples collected from under burlap bands, it is important to know if the use of burlap

bands affects levels of superparasitism and ultimately biases estimates of host mortality due to P. silvestris.

To investigate the effect of superparasitism on individual parasitoids, laboratory studies were conducted to determine how increases in the number of eggs per host larva influenced the following: (1) mortality of hosts, (2) survival of parasitoid progeny, and (3) size of parasitoid progeny.

Methods and Materials

Field Studies

Field Sites and Collection Procedures. Field studies were conducted from mid June to mid July of 1986 in two 9 ha plots (300 m X 300 m) on Otis Air National Guard Base (ANGB), Cape Cod, Massachusetts. Plot 1 had a density of 694 (± 152 SE) gypsy moth egg masses per ha prior to hatch in 1986, and there were 3934 (± 402 SE) egg masses per ha in Plot 2 (based on prism-point estimates of egg-mass density - Wilson & Fontaine 1978) (J.S.E. unpublished data). Each plot was divided into nine 1 ha subplots (100 m X 100 m), which were in turn divided into 16 square sub-sub plots. Sampling points were located at the center of each sub-sub plot. Prior to sampling, three of the 16 sampling points in each subplot were selected at random. The ten trees with a diameter at breast height greater than 7 cm that were closest to the sampling points were wrapped with 24.5 cm strips of burlap. Several vertical slits were cut in each burlap band to create flaps that gypsy moth larvae could use as daytime resting sites.

Gypsy moth larvae were collected from each plot on three sampling occasions (designated Samples 1 to 3), which began on 16 June in Plot 1 and on 24 June in Plot 2. On each sampling occasion, one point with burlap bands around the trees and one point without burlap were selected at random in each

subplot. Up to eight gypsy moth larvae were collected from under each burlap band. At points without burlap, collections began at the sampling point and continued outward in concentric circles until at least 65 gypsy moth larvae were collected. All parts of the habitat including the litter, understory vegetation, boles of trees, and canopies of trees were searched. Collections were never made from the same sampling point twice.

I recorded the number of large, macrotype eggs deposited on each larva I collected. Eggs remain on the integument of the host until molts, at which time the eggs (and external evidence of parasitism) are lost. The majority of larvae were fourth instar at the time of Sample 1, fifth instar for Sample 2, and sixth instar for Sample 3. The majority of larvae, therefore, molted between samples, and I was recording mostly new ovipositions on each sampling occasion. Larvae were placed in 30 ml plastic cups containing artificial diet (Bell et al. 1981), reared in an outdoor insectary, and checked once per week until death or emergence of adult gypsy moths. The number of P. silvestris larvae emerging from each host was recorded.

Patterns of Dispersion of Parasitoid Eggs and Larvae. To determine whether the patterns of eggs laid on hosts and of parasitoid larvae emerging from hosts were random, I tested the goodness-of-fit of the sample data to a Poisson distribution using X^2 test statistics (Elliott 1983). I pooled the data from all subplots for the analysis because by the time data from single subplots were combined to get expected frequencies of greater than one, there were not enough degrees of freedom to conduct the test. I also calculated four indices of dispersion [1/k of the negative binomial, variance-to-mean ratio ($s^2/\bar{x}$), Morisita's Index (I_δ) and Green's Coefficient (C_x)] to assess the degree of aggregation or uniformity of sample objects. Values of the indices at maximum uniformity, randomness, and maximum aggregation are as follows: (1) 1/k: $-1/\bar{x}$, 0, $n-1/\bar{x}$

(2) $s^2/\bar{x}$: 0, 1, Σx (3) $I : 1-(n-1/\Sigma x-1)$, 1, n (4) C_x: $-(1/\Sigma x-1)$, 0, 1 where n =

number of hosts sampled, $\bar{x}$ = mean number of parasitoid eggs per host, and Σx

= total number of parasitoid eggs on all larvae (Elliott 1983). Sometimes the

iterative method used to solve for k of the negative binomial did not converge on

a value of k. If it was possible to estimate k, however, I tested the goodness-of-

fit of the sample data to a negative binomial distribution (Elliott 1983). For $s^2/\bar{x}$,

I_δ, and C_x, I determined whether the observed values fell within the 95%

confidence intervals of random distributions.

  Estimating Total Mortality Due to P. Silvestris. Because hosts attacked by P.

silvestris could ultimately be killed by other parasitoids or disease, and because

the incidence of these mortality factors might differ between plots or be affected

by sampling from burlap bands, I calculated the marginal probabilities (sensu

Royama 1981a) of hosts being killed by P. silvestris in the absence of other

mortality agents. The probability of surviving mortality from P. silvestris was

determined for each interval between samples and for the final sample (based

on mortality observed by rearing larvae). These values were multiplied to

determine the probability of surviving death from P. silvestris over the entire

sampling period. Total percentage mortality was calculated as 100 -

percentage survival (see Chapter 6 and Appendix 1 for a more thorough

description of this method). Within a plot, the significance of the difference in

percentage mortality between samples collected from under burlap bands and

samples collected without burlap was tested using an approximate Z-test

(Freedman et al. 1978).

Laboratory Studies

  Collection and Maintenance of Parasitoid Adults. Adult parasitoids were

collected from 28 May to 5 June 1986 near Plot 1. Sweep nets were used to

collect mating pairs to ensure that females were fertilized. Male and female

parasitoids were placed in 40 X 26 X 17 cm clear plastic cages with screening on both ends (ca. 10 pairs per cage). Honey was dabbed on the walls and ceiling of the cage, and water was provided in glass vials with cotton wicks. The cages were kept in an outdoor, screened insectary until the parasitoids were used for the experiment.

Gypsy Moth Larvae. Gypsy moth larvae were from a laboratory colony at Otis Methods Development Center, Otis ANGB, Cape Cod, Massachusetts (NJ Strain: generation 29). Larvae were reared (10 per 180 ml cup) on artificial diet (Bell et al. 1981) at 25°C, 60% RH, and 16:8 L:D until they were about to molt to the fourth or fifth instar. They were then placed individually in 30 ml plastic cups with artificial diet. Larvae were used for the experiment within 24 h of molting.

Superparasitization. The effects of superparasitism were evaluated by allowing parasitoid females to lay 1, 2, 3, or 4 eggs on a single larva. Larvae were placed individually in the cages of parasitoids and were removed after the desired number of eggs (hereafter designated egg-density) were deposited on the integument. Parasitized larvae were held in an environmental chamber at 25.0°C, 60% RH, and 16:8 L:D and were inspected daily for emergence of parasitoids. Fifty unparasitized fourth and fifth instars served as controls, were reared in the same manner as parasitized larvae, and were inspected daily for mortality or adult emergence.

The proportions of parasitized hosts that (1) produced parasitoids (2) died without producing parasitoids and (3) lived were analyzed to determine if there were significant differences depending on egg-density or the instar of the host. I used weighted-least-squares estimates of the model parameters, and the goodness-of-fit of the data to the categorical model was tested using X^2 statistics (PROC CATMOD, SAS Institute 1987). Contrasts were used to determine the significance of differences between adjacent categories of egg-

density. I also calculated the probability that a given parasitoid would survive to emerge from a host as the percentage of the total eggs laid at each egg-density that survived to emerge. The significance of differences in the probability of survival, based on host instar and egg-density, was analyzed using PROC CATMOD (SAS Institute 1987), again using contrasts to compare between adjacent categories of egg-density.

Upon emergence from the hosts, all immature parasitoids were placed individually in 30 ml cups containing 20 ml of moist leaf litter. The parasitoids formed puparia and immediately began developing into pharate adults, which entered diapause for the winter. The puparia remained undisturbed for six months and were then dissected to determine whether or not an adult had developed. Prior to dissection, the size of each puparium was determined by measuring its length (L) and diameter (D) to the nearest mm using a dissecting microscope and a ruler. The volume (V) of the puparium was then calculated as $V = L \pi (D/2)^2$. I did not weigh the puparia because in some instances desiccation had occurred. The effects of host instar and egg-density on the volume of the puparia was tested by ANOVA (PROC GLM, SAS Institute 1987). The volumes were weighted by 1/variance for the categories of instar and egg-density because the variances among these categories were not homoscedastic (Levene's Test; Milliken & Johnson 1984). Contrasts were used to compare volumes between adjacent categories of egg-density.

Results and Discussion

Field Studies

Patterns of Dispersion of Parasitoid Eggs. When Sample 1 was collected, oviposition by P. silvestris had just begun. As a result, few larvae were

parasitized, and only four of the 2157 larvae collected had more than one egg. The patterns of dispersion of eggs on larvae collected in Sample 1 were not significantly different from random, although three of the four cases tended toward a uniform distribution (Table 5.1). Only one of the patterns of dispersion in Samples 2 and 3 was significantly different from a negative binomial distribution, and all but one were significantly aggregated (Table 5.1). The aggregation of parasitoid oviposition on certain hosts suggests that superparasitism was not simply the result of random oviposition, but that certain hosts were more likely to be attacked than others.

Within a given sample and collection regime (burlap or no burlap), the degree of aggregation was higher in Plot 1, the plot with a lower density of gypsy moths, than in Plot 2 (Table 5.1). These findings support the hypotheses of Weseloh (1976) and ODell and Godwin (1979). In low density populations, most larvae are in resting locations during the day and are not apparent to parasitoid females who are attracted to larval movement. The few larvae that are exposed receive the majority of the parasitizations that occur in the early afternoon, when parasitization is at its peak, and tend to be superparasitized. These authors predicted a more even distribution of eggs in higher density populations because a greater proportion of the larvae are exposed and moving during the day due to a shift in larval behavior.

When samples collected at the same time and from the same plot were compared, the degree of aggregation of eggs for Samples 2 and 3 was higher for larvae collected from under burlap bands (Table 5.1). Gypsy moth larvae often begin the migration from the canopy to daytime resting sites as early as 03:45 h (ODell & Godwin 1979), when ambient temperature and light levels are too low for parasitoids to be active. As temperature and light levels increase, parasitoids become active and attack larvae that come down the tree later.

Table 5.1. Parameters of the distribution of eggs of P. silvestris. Gypsy moth larvae were collected from under burlap bands (B) or from trees without burlap bands (WB) on Cape Cod, Massachusetts in 1986. Values of the four indices of dispersion followed by "a" were significantly aggregated and values followed by "b" were significantly uniform (a = 0.05).

A dash indicates that the index could not be calculated.*

Sample Plot		B/WB	N	$\bar{x}$	s^2	$1/k$	$s^2/\bar{x}$	I_δ	C_x
1	1	B	681	0.05	0.04	–	0.95	0.0	-0.0015
1	1	WB	504	0.07	0.08	1.35	1.09	0.0	0.0025
1	2	B	474	0.07	0.07	-0.23	0.99	0.0	-0.0004
1	2	WB	498	0.03	0.03	–	0.97	0.0	-0.0020
2	1	B	587	0.08	0.14	11.11	1.77[a]	11.3[a]	0.0175[a]
2	1	WB	553	0.31	0.54	2.27	1.72[a]	3.3[a]	0.0042[a]
2	2	B	927	0.39	0.63	1.47	1.65[a]	2.7[a]	0.0018[a]
2	2	WB	1031	0.38	0.43	0.39	1.15[a]	1.4[a]	0.0004[a]

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Table 5.1. Continued.

Sample	Plot	B/WB	N	$\bar{x}$	s^2	$1/k$	$s^2/\bar{x}$	I_δ	C_x
3	1	B	482	0.15	0.30	5.26	1.98[a]	7.5[a]	0.0136[a]
3	1	WB	425	0.10	0.12	1.28	1.12[a]	2.1[a]	0.0027[a]
3	2	B	343	0.32	0.45	1.33	1.39[a]	2.2[a]	0.0036[a]
3	2	WB	530	0.17	0.14	-	0.86[b]	0.1[b]	-0.0016[b]

[*] k = k of the negative bionmial, $s^2/\bar{x}$ = variance to mean ratio, I_δ = Morisita's index, and C_x = Green's index.

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ODell and Godwin (1979) observed that these larvae are sometimes attacked two to three times prior to reaching artificial bark flaps. Larvae that arrive later end up on the periphery of aggregations of larvae and are also subject to higher rates of oviposition by parasitoids searching under the burlap bands. Larvae that arrive at the burlap bands later, therefore, have a greater probability of being attacked during migration and also while under the burlap bands.

In both plots, total mortality due to P. silvestris was significantly lower for larvae collected from under burlap bands ($\underline{Z}$ = 3.17, d.f. = 1, $\underline{P}$ < 0.05 and $\underline{Z}$ = 2.25, d.f. = 1, $\underline{P}$ < 0.05 for Plots 1 and 2 respectively) (Table 5.2). Lower percentage parasitism would be expected if, for some reason, larvae using burlap bands escaped attack by parasitoids; however, the number of eggs per larva was not consistantly lower for larvae collected from under burlap bands (Table 5.1). An alternative explaination is that as the degree of aggregation of parasitoid attacks increases, the same number of eggs is distributed among a smaller proportion of the hosts, resulting in lower percentage parasitism. The consistently greater aggregation of eggs on larvae collected from under burlap bands (Table 5.1) may explain the lower overall mortality of these larvae. This finding has an important practical implication. The use of burlap bands to collect larvae could result in artificially elevated levels of superparasitism and corresponding underestimates of the impact of P. silvestris. Reardon (1976) concluded that collecting larvae from burlap bands elevated rather than decreased estimates of parasitism; however, he pooled samples of larvae taken throughout the season to calculate percentage parasitism. This method does not accurately measure levels of parasitism (Gould et al. 1989).

Patterns of Dispersion of Parasitoid Larvae. My results support the contention that it is usual for only one P. silvestris larva to emerge from parasitized hosts. Two parasitoid larvae emerged from only four of the parasitized hosts collected,

Table 5.2. Total percentage mortality due to P. silvestris (± SE) for two populations of gypsy moths on Cape Cod, Massachusetts in 1986. Larvae were collected either from under burlap bands (B) or from habitats without burlap bands (WB).

Plot	B/WB	% P. silvestris mortality
1	B	33.7 ± 3.1
1	WB	52.7 ± 3.7
2	B	30.8 ± 4.2
2	WB	45.2 ± 3.5

and more than two parasitoids never emerged from these field- collected larvae. This resulted in a uniform distributions of larvae emerging from hosts (Table 5.3). The switch in the pattern of dispersion between life-stages, from aggregated in the parasitoid's egg-stage to uniform in the larval-stage, implies that the parasitoids were capable of eliminating competitors. The mechanism of competition in P. silvestris is unknown, but Prell (1915) noted that two parasitoids could survive in a single host only if they were far apart. Larvae of P. silvestris do not have large, fighting mandibles and do not move about inside of the host (Prell 1915), therefore, some sort of physiological suppression of competitors is likely. Competition in some tachinid species is thought to be the result of progressive elimination of more slowly developing parasitoids through selective starvation (Pschorn-Walcher 1971).

Laboratory Studies

Effect of Superparasitism on Host Mortality. Total mortality of gypsy moth larvae increased significantly (X^2 = 14.8; d.f. = 1; P < 0.01) with the deposition of two, rather than one, eggs per host (Figure 5.1). This phenomenon was most pronounced for instar four. The simplest explanation for the increase in host mortality with deposition of more than one egg is that, given a constant probability that a parasitoid will survive and kill its host, more ovipositions per host leads to greater levles of host mortality. I tested this hypothesis by assuming that the probability of a single parasitoid emerging from a host (PE_1) was as observed for the egg-density of one. The expected probability of a host dying from parasiotid emergence (PE_n), when more than one egg was laid, was calculated by multiplying probabilities of surviving parasitism as follows:

$$PE_n = 1 - (1-PE_1)^n$$

Table 5.3. Parameters of the distribution of emerging larvae of *P. silvestris*. Gypsy moth larvae were collected from under burlap bands (B) or trees without burlap bands (WB) on Cape Cod, Massachusetts in 1986. Indices followed by "a" showed siginficant aggregation and indices followed by "b" showed significant uniformity (a = 0.05). X^2 values followed by "c" indicate that the sample data were significantly different (a = 0.05) from a Poisson distribution as determined by a X^2 test of goodness-of-fit test. A dash indicates that it was not possible to test the goodness-of-fit to a Poisson distribution.*

Sample	Plot	B/WB	N	$\bar{x}$	s^2	X^2	d.f.	$s^2/\bar{x}$	C_x
1	1	B	700	0.05	0.05	-	-	0.95	-0.0014
1	1	WB	504	0.06	0.06	-	-	0.94	-0.0020
1	2	B	546	0.08	0.07	1.8	1	0.92	-0.0018
1	2	WB	498	0.07	0.07	1.5	1	0.93	-0.0020
2	1	B	586	0.09	0.08	2.8	1	0.91	-0.0017
2	1	WB	553	0.12	0.11	4.6^c	1	0.88^b	-0.0018^b
2	2	B	592	0.14	0.12	6.8^c	1	0.86^b	-0.0017^b
2	2	WB	507	0.18	0.15	9.8^c	1	0.82^b	-0.0020^b

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Table 5.3. Continued.

Sample	Plot	B/WB	N	$\bar{x}$	s^2	x^2	d.f.	$s^2/\bar{x}$	C_x
3	1	B	492	0.18	0.15	10.7[c]	1	0.82[b]	-0.0020[b]
3	1	WB	425	0.28	0.22	12.8[c]	2	0.79[b]	-0.0018[b]
3	2	B	391	0.27	0.20	20.9[c]	2	0.73[b]	-0.0026[b]
3	2	WB	530	0.35	0.23	52.7[c]	2	0.64[b]	-0.0019[b]

* $s^2/\bar{x}$ = variance-to-mean ratio and C_x = Green's index.
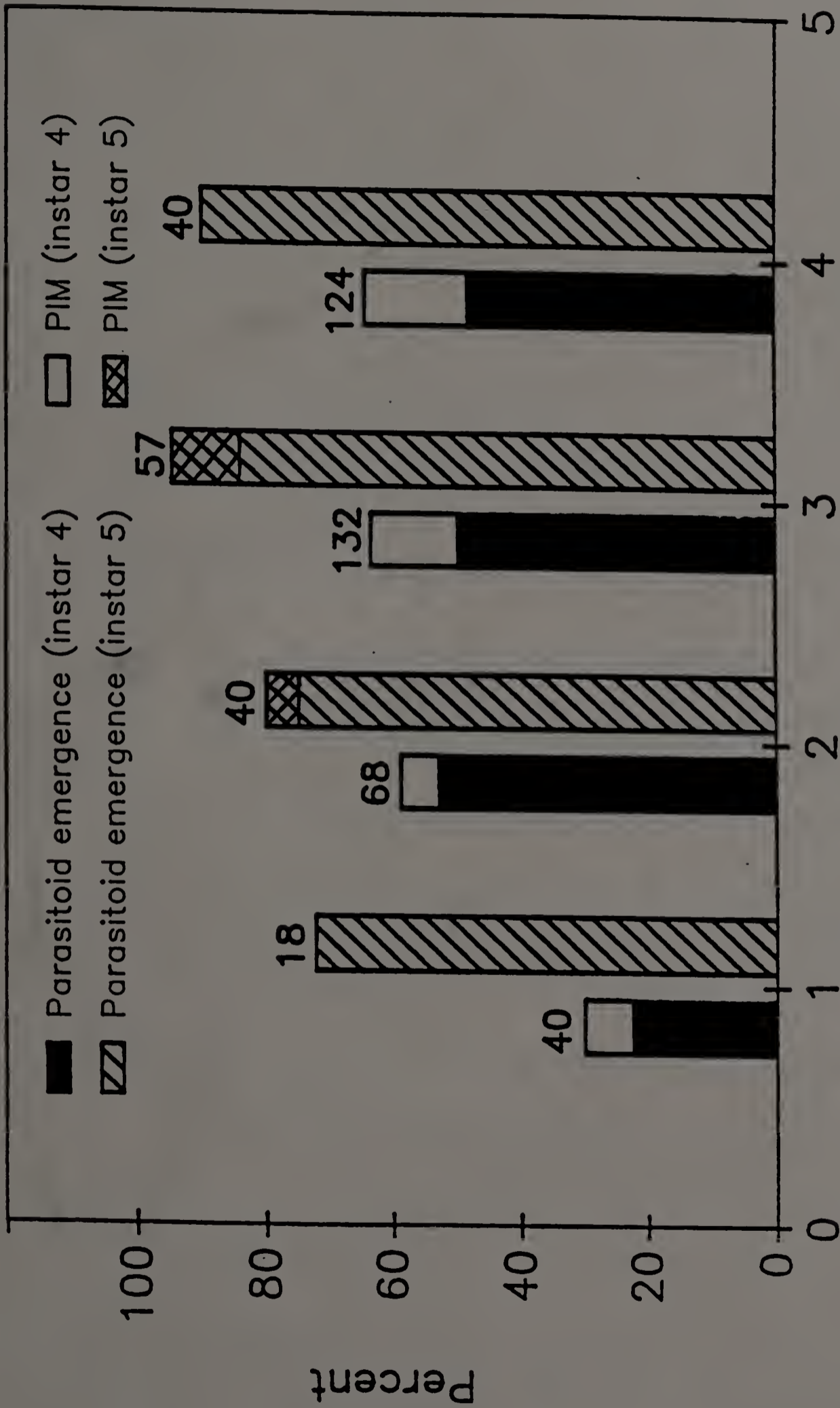
Figure 5.1. Relationship between mortality of gypsy moths and density of *P. silvestris* eggs. Mortality was due to emergence of parasitoids or parasitoid induced mortality (PIM). Numbers above the bars indicate sample size.

where n is the egg-density. The observed numbers of hosts dying from parasitoid emergence were differed significantly from this model ($\underline{X}^2$ = 5.927, d.f. = 1, $\underline{P}$ < 0.025).

Another explanation for greater host mortality with increasing egg-density is that several parasitoids are more successful in overcoming host defenses. A lower frequency of encapsulations in superparasitized hosts was reported for the tachinid, Lixophaga diatraeae (Beland & King 1976), and also for hymenopterous parasitoids (Streams 1971, Puttler 1974). If this hypothesis is correct, one would expect more superparasitized hosts to die from parasitoid emergence than would be predicted by the constant probability model. I found just the opposite; the probability of parasitoid emergence was lower than expected. This is probably attributable to the adverse effects of competition with conspecifics or to a decrease in the quality of superparasitized hosts.

No mortality occurred among unparasitized hosts, therefore mortality of parasitized hosts that did not result in emergence of a parasitoid was assumed to be parasitoid-induced-mortality (PIM). The incidence of PIM (Fig. 5.1) was lower than the values of 52.5% and 9.7% for fourth and fifth instars (respectively) found in another study using wild gypsy moth larvae and a single attack (T. ODell unpublished data). This difference could be attributed to a difference in PIM between wild and laboratory-reared hosts. PIM increases with increasing superparasitism for some species (i.e. Hughes 1975, Pawson et al. 1987). The amount of PIM caused by P. silvestris increased slightly but not significantly with increasing egg-density in fourth instars, but there was no trend for fifth instars (Fig. 5.1).

Effect of Superparasitism on Survival and Size of Parasitoids. Although parasitism by two or more individuals increased the probability that at least one parasitoid would survive to emerge from the host, superparasitism adversely

affected survival of individual parasitoid progeny. The probability that a parasitoid would survive to emerge from a host decreased as egg-density increased (Fig. 5.2). The response was the most pronounced for fifth instars. The interaction between instar and egg-density in the ANOVA model was significant ($\underline{X}^2 = 11.4$; d.f. = 1; $\underline{P} < 0.01$), therefore I analyzed at the effect of egg-density separately for each instar. There were significant differences between one and two eggs per host ($\underline{X}^2 = 3.5$; d.f. = 1; $\underline{P} < .06$) and between three and four eggs ($\underline{X}^2 = 7.08$; d.f. = 1; $\underline{P} < 0.01$) for instar five, and between two and three eggs ($\underline{X}^2 = 4.44$; d.f. = 1; $\underline{P} < 0.04$) for instar four. The probability of a parasitoid developing into an adult once it had emerged from the host ranged from 70.4% to 95.5%, but there were no significant effects on this percentage caused by host instar or egg-density.

In the laboratory, 17% of the superparasitized hosts produced more than one parasitoid larva. In contrast, very few field-collected hosts produced more than one immature P. silvestris. This difference could be due to a dissimilarity between wild and laboratory-reared hosts or to the timing of parasitoid oviposition. All eggs in the laboratory study were deposited simultaneously, while in the field, oviposition presumably occurred over an extended period. The probability of parasiotids coexisting may be greater when they are the same age. It should also be noted that in some field populations, more than one P. silvestris larva has emerged from over 10% of parasitized hosts (Ticehurst et al. 1978, J.R.G. & J. S. Elkinton unpublished data).

Superparasitism resulted in a decrease in the size of emerging parasitoid progeny. The volume of puparia produced by emerging parasitoids decreased significantly between one and two eggs per host ($\underline{X}^2 = 3.89$; d.f. = 1; $\underline{P} < 0.05$), but further reductions in volume were not significant (Fig. 5.3). The size of adult parasitoids, especially females, can affect fitness parameters such as longevity,
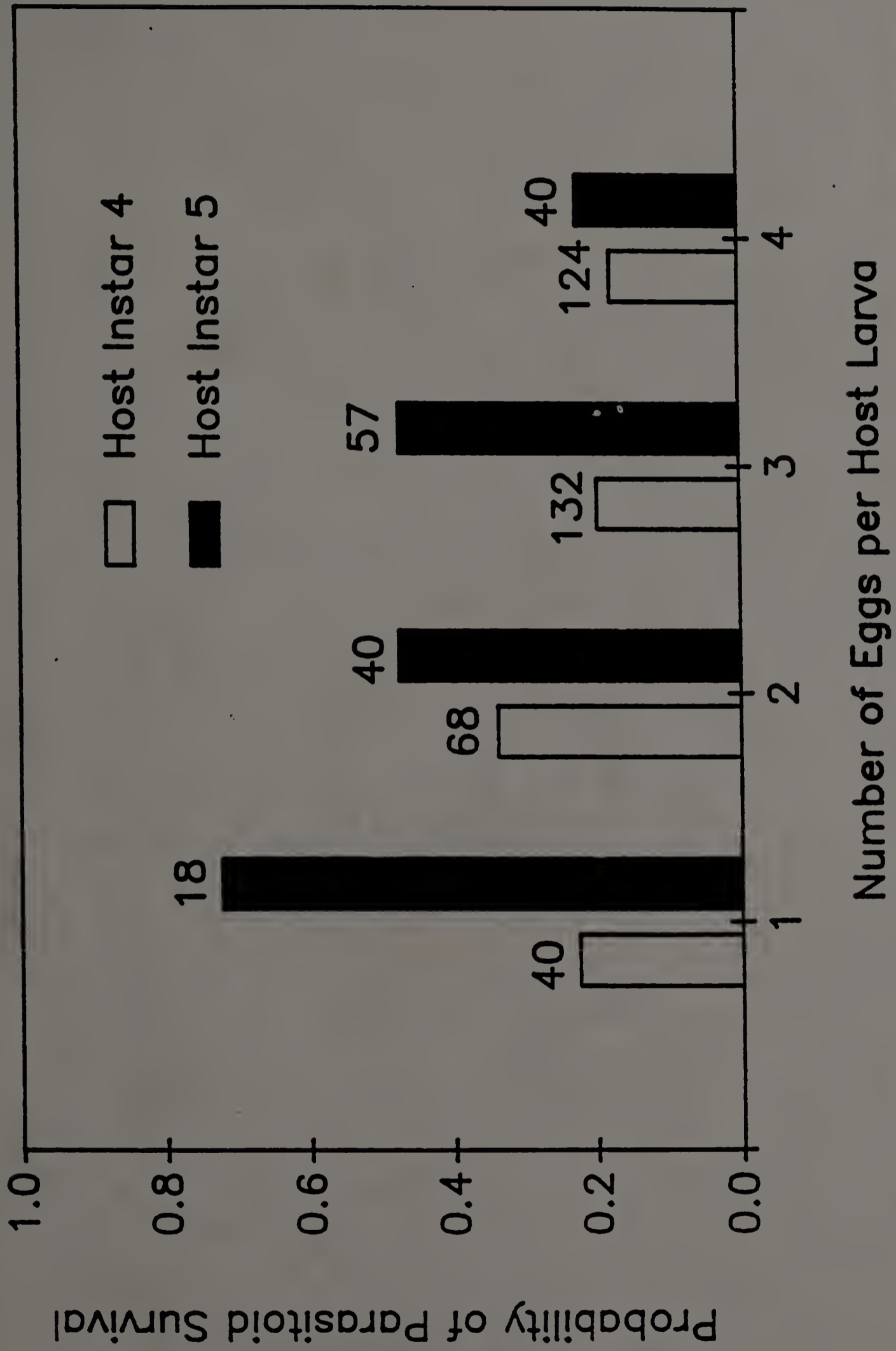
Figure 5.2. Influence of egg-density on the probability of survival of P. silvestris eggs. Numbers above the bars indicate sample size.
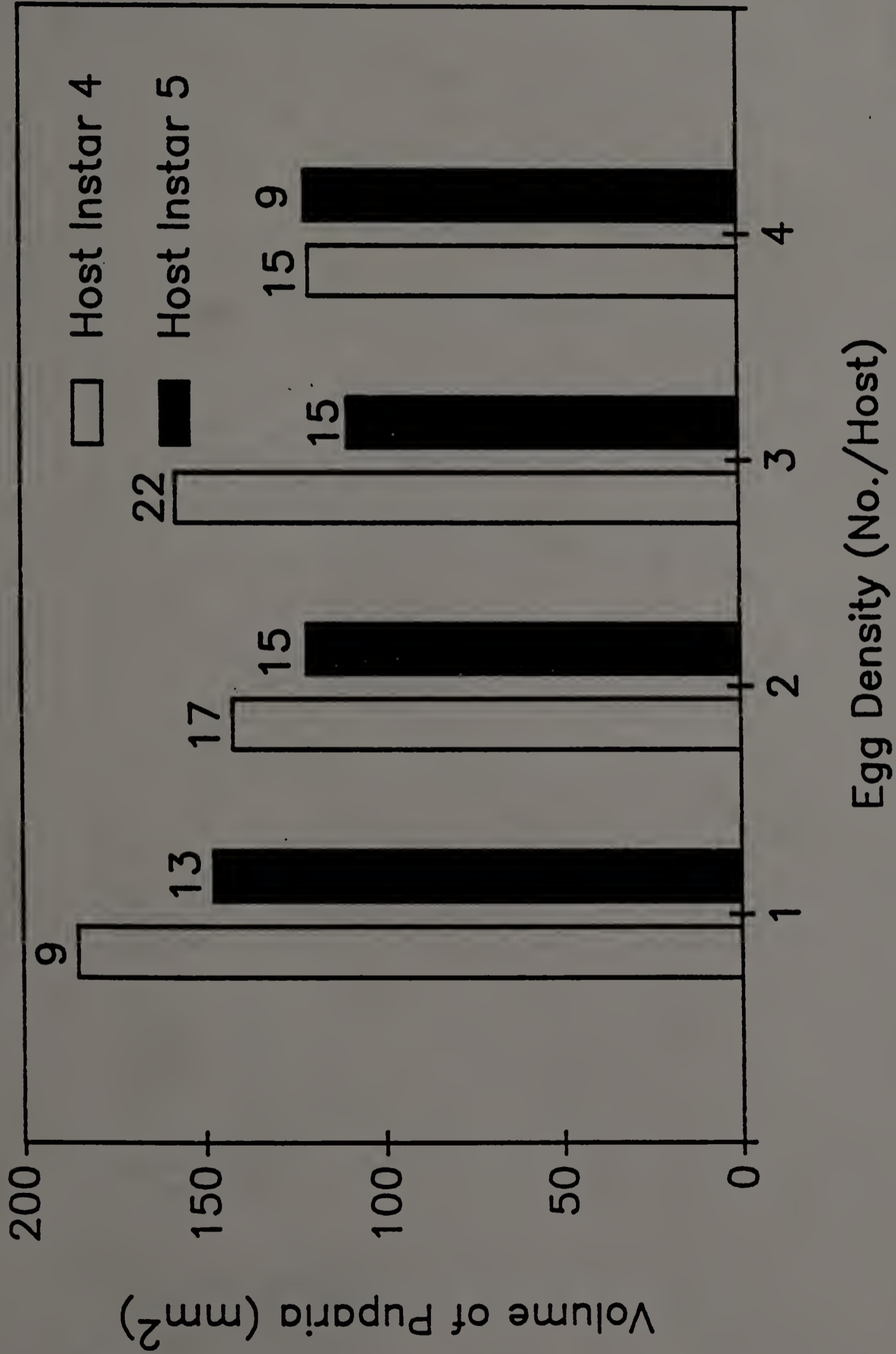
Figure 5.3 Effect of egg-density on the size of puparia produced by emerging *P. silvestris* larvae.

mating success, fecundity, or searching rates (Pak & Oatman 1982, Waage & Ng 1984). King et al. (1976) reported a decrease in the weight of L. diatraeae puparia with increasing number of parasitoids per host and a corresponding decrease in egg production with decreasing weight. Prell (1915) found that there was a direct relationship between the size of the ovaries of P. silvestris females and the size of the female fly, which might translate to a reduced fecundity of the smaller progeny emerging from superparasitized hosts.

Effect of Host Instar on Host Mortality. Percentage mortality of gypsy moths was significantly higher (X^2 = 28.5; d.f. = 1; P < 0.01) when hosts were attacked in the fifth instar (Fig. 5.1). Larvae of P. silvestris emerge just prior to host pupation regardless of when oviposition occurs (T. M. ODell unpublished), therefore parasitoids remain in fourth instars longer than in fifth instars. There may be a greater likelihood of parasitoid mortality (perhaps due to host defenses), and thus of host survival, when hosts are attacked in the fourth instar.

Effect of Host Instar on Parasitoid Survival and Size. The probability of a given egg surviving to produce an immature parasitoid was significantly greater (X^2 = 25.4; d.f. = 1; P < 0.01) if the egg was laid on a fifth instar host (Fig. 5.2). This increased probability of survival was offset by the significantly smaller (X^2 = 6.04; d.f. = 1; P < 0.02) size of puparia formed by maggots emerging from fifth instars (Fig. 5.3). Because parasitoids remain in fourth instars longer, they may be able to acquire more nutrition and reach a larger size.

Concluding remarks

Superparasitism by solitary parasitoid females has been considered wasteful behavior because it decreases the probability of survival of the parasitoid's offspring. It is argued that the ability to discriminate between parasitized and unparasitized hosts, and thus to avoid superparasitism, should have a strong selective advantage (Rogers 1975, van Lenteren 1981). The question of

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whether P. silvestris has the ability to discriminate between parasitized and nonparasitized hosts has not yet been answered definitively. Weseloh (1976) concluded that P. silvestris cannot discriminate, but he compared parasitization of naturally occurring parasitized larvae with nonparasitized laboratory-reared larvae. He actually found that fewer eggs were deposited on larvae that had been previously parasitized, although the difference was not significant. Also, the fact that this species superparasitizes in both the laboratory and the field does not prove that it lacks the ability to discriminate. Recent work suggests that superparasitism may be an alternative reproductive strategy that is adaptive under certain conditions (van Alphen & Visser 1990, Hubbard et al. 1987, Waage 1986, Bakker et al. 1985, Cloutier 1984, van Alphen & Nell 1982). In past studies, superparasitism by P. silvestris ranged from quite high (>80% Bogenschutz et al. 1989) to moderate (>10% Ticehurst et al. 1978, J.R.G. & J.S.Elkinton unpublished data) to extremely low (Weseloh 1976, Tigner et al. 1974) to nonexistent (Barbosa et al. 1975). Further studies are needed to determine whether these differences depend on ecological factors or behavioral adaptations of female parasitoids.

CHAPTER 6

DENSITY-DEPENDENT SUPPRESSION OF EXPERIMENTALLY CREATED GYPSY MOTH POPULATIONS BY NATURAL ENEMIES

Introduction

In the northeastern United States the gypsy moth, Lymantria dispar L. (Lepidoptera: Lymantriidae), is one of the most damaging forest insect pests. Larvae hatch in April or May, climb to the tops of trees and often disperse on the wind by spinning down on silken threads. Following this they commence feeding and remain in the forest canopy until they molt to the fourth instar. In low to moderate density populations, late instars (4-6) undergo a daily migration from nighttime feeding sites to protected daytime resting locations under bark flaps or in the litter on the forest floor. It has been hypothesized (Campbell & Sloan 1976) that this behavior evolved in Europe in response to parasitism by tachinid flies and predation by insectivorous birds, but in North America it leads to high rates of predation by small mammals, particularly Peromyscus leucopus Raf. Larvae pupate in their resting sites and adults emerge in July. Females do not fly and they deposit their eggs in a single mass not far from the site of pupation.

In North America, densities of gypsy moths in most forest stands remain at low levels for many years and then erupt into an outbreak phase which may last for several years. The major goal of research on gypsy moth population dynamics is to identify those factors or agents responsible for maintaining populations at low densities and the mechanisms for release to outbreak levels. It has been suggested (Campbell 1976; Campbell & Sloan 1977,1978; Campbell et al. 1977) that predation by small mammals on late instars and

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pupae maintains populations at low densities and that this predation is positively density-dependent (Campbell et al. 1977). There are also eight introduced parasitoids established in North America as well as ten endemic parasitoid species that attack gypsy moths. While there have been reports of relatively high levels of parasitism by some gypsy moth parasitoids (Campbell & Podgwaite 1971; Doane 1971; Barbosa et al. 1975; Blumenthal et al. 1979; ODell & Godwin 1979; J.R.G. & J.S. Elkinton unpublished), most researchers (e.g. Campbell et al. 1977; Reardon 1976; Ticehurst et al. 1978) believe that parasitoids do not cause sufficient mortality to limit the growth of gypsy moth populations. The total amount of mortality caused by a given agent is not as important to population regulation, however, as the density-dependence of the response. The strength of the density-dependent response over a range of host densities determines whether the host population is stabilized, goes through regular cycles, or exhibits chaotic behavior (May 1986).

There is as yet no strong evidence that small mammals cause positive density-dependent mortality in populations of gypsy moths. In fact, there is some evidence (Elkinton et al. 1989) that predation by small mammals is inversely density-dependent. Some researchers have found positive density-dependent mortality due to parasitoids (Reardon 1976; Sisojevic 1977; ODell & Godwin 1979; Furuta 1982) and others have found a negative correlation between percentage parasitism and host density and/or percent defoliation (Weseloh 1973; Reardon 1976; Reardon & Podgwaite 1976; Ticehurst et al. 1978). In many of these studies, however, the methods used to determine host density or percentage parasitism were unsatisfactory (Gould et al. 1989).

Density-dependence may be difficult to detect from traditional temporal life-table studies. Natural stochastic variation may obscure underlying density-dependent processes rendering them difficult to detect (Hassell 1985, 1987),

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although Dempster and Pollard (1986) and Mountford (1988) have disputed this contention. Also, life-table studies which look at average mortality occurring in populations over several generations may not detect density-dependent responses to spatial heterogeneity among subpopulations within a generation (Hassell 1987; Hassell et al. 1987). Furthermore, when natural population densities are close to an equilibrium, there may be no direct density-dependent mortality of the host population (Murdoch & Reeve 1987). It has been suggested (Gaston & Lawton 1987; Hassell 1985, 1987; Murdoch & Reeve 1987) that a solution to these problems is to manipulate population densities (e.g. Karieva 1985; Reeve & Murdoch 1985; Furuta 1976) rather than to rely on life-table data collected from natural populations over several generations. This study was designed to manipulate the density of gypsy moth larvae to determine if natural enemies could respond to local increases in density in a density-dependent manner.

Methods and Materials

Site Description and Preliminary Counts of Egg Masses

The study was conducted in Cadwell Memorial Forest in Pelham and Belchertown, Massachusetts during 1987. I established eight 1 ha plots (100 m by 100 m) in the forest spaced at least 750 m from one another. Oaks (Quercus rubra L., Q. velutina Lam., & Q. alba L.) were the predominant overstory trees in all plots and the plots also contained Acer rubrum L. and to a lesser extent A. saccharum Marsh, Betula lenta L., B. lutea Michx., B. populifolia March, and Fraxinus americana L.

In each plot I established a ten-by-ten grid of points, with 10 m between points, to serve as a framework for various sampling regimes. Prior to density

manipulations I conducted an egg mass survey in each plot by counting all egg masses within a 7.5 m radius circle around five egg mass sample points. Mount Lincoln in Cadwell Forest has a history of outbreaks and had defoliating gypsy moth populations during an outbreak from 1979 to 1981. Following the population crash in 1981 densities remained very low and the pre-season egg mass counts revealed no egg masses in any of the plots.

Release of Larvae

Egg masses for the release experiment were collected in an area with expanding, moderately high density gypsy moth populations in Hopeville, Connecticut. These egg masses were submerged for 1 h in a 10% formalin solution to remove viable nuclear polyhedrosis virus (NPV) from the egg surface (Bell et al. 1981) and were then rinsed for 1 h with water. The eggs were divided into four groups of different sizes (two replicates per group size) to give us four densities of larvae at hatch. I weighed each group and then placed the eggs in 100 7.5 X 10 cm screen packets (200 packets for the highest density). I then sampled one of the largest packets and determined the number of larvae that emerged. The resulting value of number of larvae emerging per gram of eggs was multiplied by the weight of all eggs for each group to estimate the number of larvae at hatch. The expected numbers of larvae at hatch in the eight plots are given in Table 6.1.

On 4 May 1987 I released larvae at densities that corresponded to that expected from 174 to 4600 egg masses per ha, assuming a hatch of 250 larvae per egg mass. Egg mass densities above 2500 per ha would be expected to result in complete defoliation of a forest stand (Wilson & Talerico 1981). Prior to the release I had collected eighteen egg masses from a high density site in Hardwick, Massachusetts (20 km from Cadwell Forest). These egg masses were placed individually in 30 ml plastic cups and were kept shaded in Cadwell

Table 6.1. The estimated number of larvae ($\pm$ 95% confidence limits) released in eight plots in Cadwell Forest in 1987.

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Density Class	Plot	No. larvae/ha at hatch
1	1A	59,304 (+/- 2,206)
1	1B	43,538 (+/- 1,619)
2	2A	146,825 (+/- 5,461)
2	2B	81,821 (+/- 3,043)
3	3A	374,542 (+/- 13,930)
3	3B	296,157 (+/- 11,015)
4	4A	1,143,112 (+/- 42,516)
4	4B	1,144,325 (+/- 42,561)

Forest. Hatch was monitored daily and I timed the release to coincide with that observed from these egg masses. Oak trees in all plots were beginning to expand their leaves at this time.

To assure that gypsy moth larvae were distributed throughout each plot I released them at 100 points in the ten-by-ten grid. I deployed gypsy moth egg masses by stapling the screen packets to the trunks of the trees closest to the release points at a height of 1.5 m. Neonates emerging from the egg masses passed easily through the mesh of the screen.

Monitoring Dispersal of Larvae

To monitor dispersal of larvae away from the plots I established eight transect lines (two per cardinal direction) extending 150 m away from plots 4A and 4B (the plots with the highest density of released larvae). Transect lines were 25 m from the corners of the plots and were 50 m apart. I wrapped a 24.5 cm wide burlap band at a height of 1.5 m around every oak tree within 5 m of either side of the transect line. When larvae were in the fifth and sixth instars I took counts of the numbers of live and dead larvae under each burlap band to determine how far from the plots the larvae dispersed and if the magnitude of mortality changed with distance from the plot. I regressed both the $\log_{10}$ of the total number of larvae per m burlap and the arcsine of the square root of the proportion of dead larvae on the distance from the plot.

Monitoring Changes in Density

I monitored the change in density of gypsy moths in all plots throughout the season by taking weekly density estimates. Frass traps (Liebhold & Elkinton 1988a,b) were deployed at 48 points in each plot to estimate the densities of third through fifth instar larvae. Each week I counted the number of frass pellets collected overnight in the 50 cm diameter funnel shaped traps. I also collected twenty larvae from each of the four higher density plots and held them

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individually in 360 ml cups with oak leaves during the period of frass drop measurement. By dividing the number of pellets collected in the total area covered by the frass traps by the number of pellets produced per individual larva I was able to estimate the number of larvae per ha (see Liebhold & Elkinton, 1988a,b for a complete description of this method).

After 2 July when larval densities became so low that estimates from frass traps were no longer accurate, I measured changes in density by taking weekly counts of the number of larvae and pupae per meter of burlap band. There were four sample points per plot and the 25 trees closest to each point were wrapped with a 25.4 cm wide strip of burlap. Several vertical slits were cut in each band to create flaps which larvae used as daytime resting sites. It should be noted that it is not possible to directly convert the number of larvae under burlap bands to number per ha, and I therefore used these measures to estimate the relative drops in density occurring during the late instars. Mortality of larvae under burlap bands may differ from that experienced elsewhere in the population, but at the low densities experienced this method was the only option.

At the end of the season I estimated the final egg mass density, using the same five egg mass sample points used for the pre-season egg mass count. These points were situated at least 25 m from the nearest burlap point because burlap bands can influence pupal survival (Bess et al. 1947; Campbell et al. 1975). I recorded the location of these egg masses and returned following hatch in the spring to determine the number of larvae present in the next generation. The lengths of the egg masses were measured and the regression of Moore & Jones (1987) was used to estimate the number of eggs per mass (newly hatched larvae consume the chorion of the egg, therefore eggs from which larvae had emerged could not be counted). I then counted the number of

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unhatched or parasitized eggs in each mass and subtracted this value from the number of eggs to estimate the number of larvae.

Monitoring Larval Mortality

During the larval period I monitored mortality due to parasitoids and disease by making weekly collections of 100 larvae per plot. Four grid points were selected at random in each plot on each sample occasion. Early instars, which were mainly in the canopy, were sampled by climbing the oak tree closest to the sample point using climbing ropes. Branches were cut with pole pruners, dropped onto plastic tarps, and larvae were collected. I also collected larvae from the litter and understory vegetation around the sample point. When the larvae reached the fourth instar and descended from the canopy during the day I deployed burlap bands on the five trees closest to the randomly selected sample points on the day prior to sampling. Larvae were collected from under the burlap bands, which were then removed, as well as from the litter and the understory. On each sample occasion I also recorded the number of larvae bearing large macrotype eggs laid by Parasetigena silvestris Robineau-Desvoidy (Diptera: Tachinidae).

Larvae were placed individually in 30 ml plastic cups containing artificial diet (Bell et al. 1981) and were reared in an outdoor screen cage located approximately 200 m from one of the plots. Larvae were checked once a week until death or adult emergence and puparia or cocoons of emerging parasitoids were keyed to species (Simons et al. 1979). Dead larvae, from which no parasitoid emerged, were checked for the presence of the polyinclusion bodies of NPV using a phase contrast microscope at 1000 X magnification, and for immature parasitoids that failed to emerge. If I failed to detect polyinclusion bodies or parasitoids, I classified the mortality as "unexplained".

Because predation on released larvae could not be measured directly, I conducted additional observations to assess the potential impact of avian predators. When larvae were in the second and third instars, and were located mainly in the canopies of the trees, I attempted to determine if foliage gleaning birds were attracted to areas with high density gypsy moth populations. Four sampling points were chosen in the two plots with the highest densities. I also established a control plot 100 m from each test plot that had a similar forest composition and the same configuration of sample points, but few gypsy moths. Two experienced ornithologists recorded the numbers of each species of foliage gleaning birds that were heard or seen over an 8 min period at each sample point. Sampling was conducted between 06.00 and 09.00 hours from 29 May to 2 June with each ornithologist observing the birds in one plot and the adjacent control plot. The plots were sampled in a different order on each day.

Monitoring Pupal Mortality

During the pupal period I attempted to quantify mortality of pupae due to predators and parasitoids. In all eight plots I deployed 150 male and fifty female laboratory-reared pupae (from Otis Methods Development Center - New Jersey strain, generation 30), which were attached individually to small pieces of burlap with beeswax. Fifty of the male pupae were placed in 44 X 10 X 5 cm wire mesh cages (1.27 cm mesh) to exclude small mammals. I placed one uncaged male pupa and either an uncaged female pupa or a caged male pupa at each grid point. Pupae were placed on the ground at the base of the trees nearest to the grid point and were covered with leaf litter. Pupae were checked on each of the next three days to determine survival. The significance of the differences in the survival of pupae between plots was calculated using the SPSS 9.0 Survival procedure (Hull & Nie 1981) and the Lee-Desu D statistic (Lee & Desu 1972). After three days all surviving female pupae were returned

to the laboratory and reared in 30 ml plastic cups to determine if they had been attacked by pupal parasitoids.

Analysis of Density Dependence and Calculation of K-values

To determine whether mortality occurring over the entire season and during four periods of time was density-dependent I used the method of Varley et al. (1973). I estimated the killing power, k, of a mortality agent or agents which is defined as $k=\log_{10}(N_i/S)$, where N_i is the density of the initial population and S is the density of survivors after the action of the mortality agent(s). The k-value was regressed on the logarithm of the initial density (N_i). A regression line with a slope significantly greater than zero indicated positive density-dependence, whereas inverse density-dependence was indicated by a negative slope. In this study I recorded spatial density-dependence in contrast to the temporal density-dependence measured by Varley & Gradwell (1968). The values for N_i and S used to calculate the k-values for the entire season and for four periods of time are given in Table 6.2. I added 1.0 to all final density estimates because the logarithm of zero is undefined and most plots had an estimated density of zero egg masses per ha at the end of the generation. The k-value for period 4 (which included mortality of pupae and adults, and effects of adult sex-ratio) was determined by subtracting k_1, k_2, and k_3 from K, the k-value for the entire season.

Several investigators have addressed statistical problems with detecting density-dependence by regressing k-values on $\log_{10} N_i$ (e.g. Eberhardt 1970; Benson 1973; Slade 1977; Royama 1981a,b). One problem with this technique is that when regressing $k=\log_{10}(N_i/S)$ on $\log_{10} N_i$, N_i appears in calculations of both the dependent and independent variables. A regression of this k-value on $\log_{10} N_i$ might result in a spurious positive relationship between the two variables (Atchley et al. 1976). Furthermore, the technique violates the

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Table 6.2. Parameters used to estimate the k-values of gypsy moth mortality for the entire season and for four periods of time.*

Period	Date	Stages	k-value	N_i	S
Entire Season	4 May–7 Aug	L_1– adult	K	# hatching ha^{-1}	# egg masses ha^{-1} + 1
1	4 May–4 Jun	L_1–L_3	k_1	# larvae ha^{-1} on 5 Jun	# hatching ha^{-1}
2	5 Jun–2 Jul	L_3–L_5	k_2	# m^{-1} burlap on 3 Jul	# larvae ha^{-1} on 5 Jun
3	3 Jul–16 Jul	L_5–pupae	k_3	# m^{-1} burlap on 16 Jul	# m^{-1} burlap on 3 Jul
4	17 Jul–7 Aug	pupae–adult	k_4		$K-k_1-k_2-k_3$

* L=larval instar, N_i=initial density, and S=survivors.

regression assumption that all the error resides with measurement of the dependent variable. Varley and Gradwell (1968) developed a method to verify density-dependence that solves both of these problems but their method is highly conservative (Hassell et al. 1987; Slade 1977). Other methods have been developed to overcome problems of detecting density-dependence in series of annual censuses (i.e. Bulmer 1975; Pollard et al. 1987) but this study was limited to density-dependence occurring within one generation. Following the suggestion of Hassell et al. (1987) I verified density-dependence by regressing $\log_{10} S$ on $\log_{10} N_i$ using the regression technique of Bartlett (1949), which allows regression when there is error associated with both the dependent and independent variables. Reported probability values were obtained by determining the largest confidence interval around the slope that did not overlap with unity.

In contrast to the more typical use of stage specific k-values, I calculated time-specific k-values for individual mortality agents across larval instars. I calculated weekly k-values for each parasitoid and for disease following a scheme derived from Royama (1981a). The weekly k-value for parasitoid A, k_A, can be defined as

$$k_A = -1.0 \log_{10}(1-m_A)$$

where m_A is the proportion of hosts attacked and killed by parasitoid A over a weekly interval in the absence of other simultaneous mortality agents. This value is what Royama (1981a) calls the marginal probability of mortality from a given agent. This value is greater than the proportion that are observed to die in rearings from parasitoid A, v_A, because a certain proportion of the larvae that would have died from parasitoid A died instead from other parasitoids or disease. To calculate the marginal probability for each parasitoid (m_A) I solved

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equations 12 and 13 in Royama (1981a) for the case of two simultaneous agents see Appendix for more detail).

These calculations produced estimates of k for each parasitoid and disease for each week, and these values were summed up to a value that was equal (within ca 1%) to the k-value for all parasitoids and diseases for the week. K-values for each individual mortality agent were summed across weeks to yield a total for each parasitoid or disease for each period. The difference between the total k-value for the period (from density estimates) and the k-value of parasitism and disease (from rearings) constituted a measure of residual mortality (which includes predation). Individual k-values for each mortality agent for each period were regressed on the $\log_{10}$ of the density at the beginning of the period using the technique of Bartlett (1949). If the linear regression was significant, I also looked at the significance of quadratic and cubic trends in the data using sequential sums of squares.

P. silvestris attacks middle and late instar larvae and has been shown by T.M. ODell (personal communication) to emerge just prior to host pupation. By the end of the fifth and sixth instars, when one would expect the parasitoids to emerge, host density was so low that I was unable to collect many larvae and thus directly measure mortality due to P. silvestris. I had a direct measure of the oviposition rate of this parasitoid, however, from the estimates of the number of larvae carrying macrotype eggs when larvae were still sufficiently numerous to sample. These eggs remain on the integument until a molt. During the sampling period gypsy moth larvae were advancing approximately one instar per week and I was therefore confident that the eggs seen on each sample occasion had been deposited within the preceding week. For the analysis I regressed the k-values of P. silvestris oviposition rates for each week on the

larval density prior to the sample occasion, based on frass trap density estimates, using the technique of Bartlett (1949).

Results

The densities of gypsy moths in all eight plots were reduced between the first instar and adult stages to very low levels (Fig. 6.1) and at the end of the season there were more egg masses (surviving females) per ha in the lower density release plots (Fig. 6.1a and b) than in plots with higher initial gypsy moth densities (Fig. 6.1c and d). Also, in all plots there were fewer larvae hatching in 1988 than were released in 1987, indicating that populations declined in density between generations following the release.

K-values for each plot over the entire season and the contributions to these values during periods 2 and 3 by the various mortality agents are shown in Table 6.3. The regression of K for the entire season on the $\log_{10}$ of the density at hatch showed that overall mortality was strongly density-dependent (Fig. 6.2). Positive density-dependence occurred during Periods 1 (instars 1-3) and 2 (instars 3-5) (Fig. 6.3a and b), but regressions were not significant for Periods 3 (instar 5 - pupae) and 4 (pupae - adults) (Fig. 6.3c and d). By regressing $\log_{10}$ S on $\log_{10}$ N_i I verified that the relationships observed during Periods 1 and 2 were density-dependent ($\underline{P}$ =0.004 for Period 1 and $\underline{P}$ =0.019 for Period 2).

No parasitoids emerged from larvae collected during Period 1 so the drop in density during this period was presumably due to predation and/or dispersal of first instars. I found no evidence that foliage gleaning birds, which might consume small larvae, were more abundant in plots with high gypsy moth density than in control plots with no gypsy moths (Table 6.4). In fact, more birds were observed in the control plots. Larvae did disperse from the plots, and
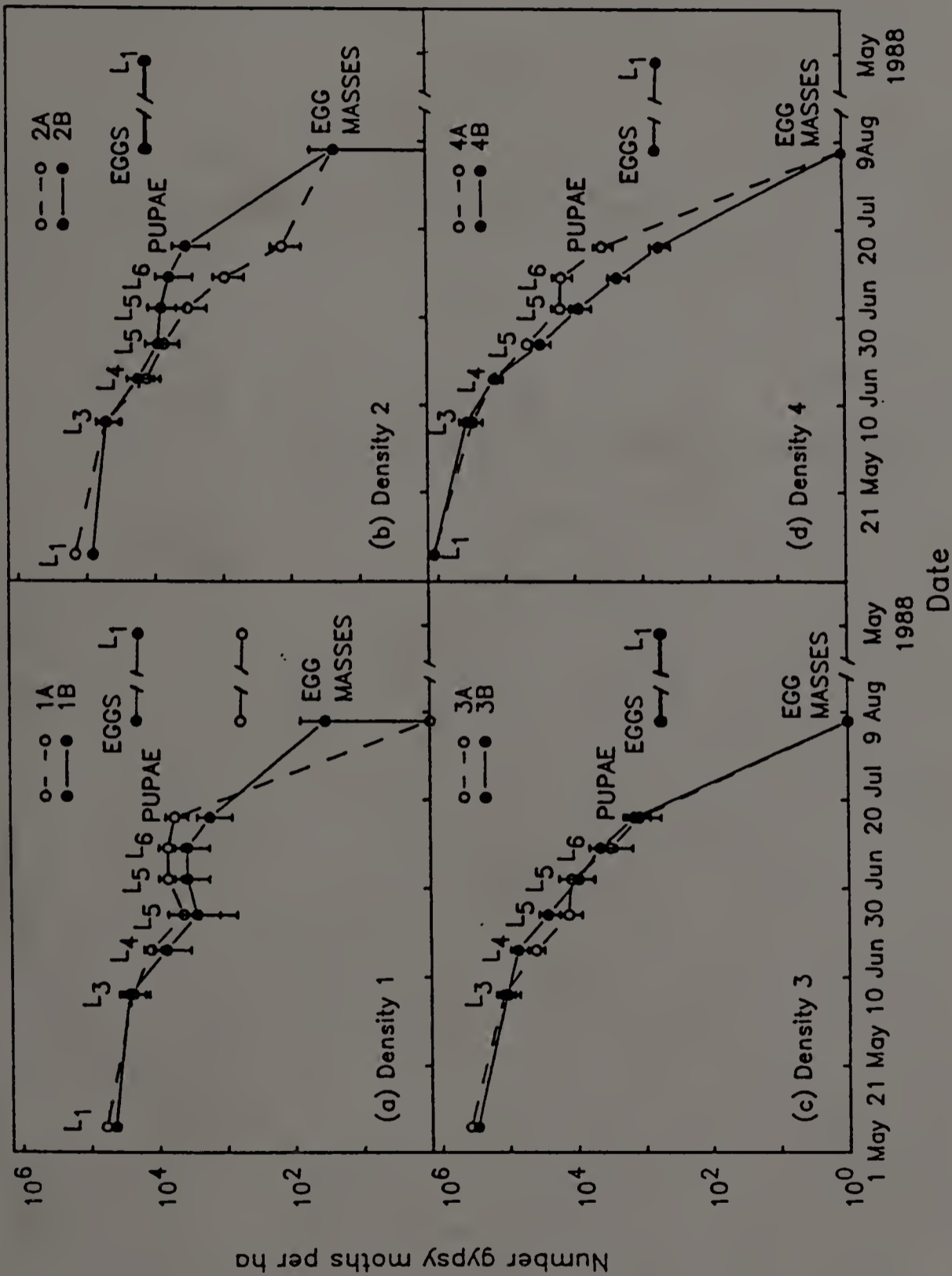
Figure 6.1. Density of gypsy moths during the summer of 1987 and in May 1988. $L1-L_6$ = Instars 1 through 6.

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Table 6.3. K-values for mortality of gypsy moths. Mortality occured over the entire season (K), from 4 May to 4 June (k_1), from 5 June to 2 July (k_2), from 3 July to 16 July (k_3), and from 17 July to 7 August (k_4) in eight plots in Cadwell Memorial Forest in 1987.*

Plot

	1A	1B	2A	2B	3A	3B	4A	4B
K	4.77	3.11	3.81	3.55	5.57	5.47	6.06	6.06
k_1	0.40	0.22	0.45	0.21	0.50	0.45	0.49	0.58
k_2	0.53	0.82	1.22	0.81	0.99	1.04	1.29	1.65
k_{comp}	0.18	0.20	0.56	0.46	0.81	0.79	0.76	0.88
k_{cote}	0.00	0.02	0.01	0.00	0.01	0.01	0.01	0.02
k_{pdis}	0.08	0.13	0.03	0.09	0.03	0.01	0.03	0.00
k_{psil}	0.01	0.02	0.01	0.00	0.01	0.01	0.01	0.00
k_{unex}	0.10	0.16	0.20	0.17	0.21	0.22	0.19	0.31
k_{resid}	0.16	0.29	0.41	0.09	-0.08	0.01	0.29	0.44

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Table 6.3. Continued.

				Plot				
	1A	1B	2A	2B	3A	3B	4A	4B
k_3	0.10	0.33	1.39	0.36	1.00	0.82	0.62	1.19
k_{comp}	0.03	0.06	0.08	0.10	0.23	0.13	0.51	0.21
k_{cote}	0.01	0.01	0.04	0.04	0.05	0.03	0.05	0.03
k_{pdis}	0.00	0.00	0.00	0.02	0.01	0.01	0.01	0.00
k_{psil}	0.07	0.05	0.02	0.03	0.04	0.04	0.10	0.01
k_{unex}	0.22	0.20	0.23	0.09	0.40	0.15	0.26	0.95
k_{resid}	-0.23	0.01	1.02	0.08	0.27	0.46	-0.31	-0.01
k_4	3.74	1.70	0.75	2.17	3.08	3.16	3.57	2.73

* comp=C. concinnata, cote=C. melanoscelus, pdis=P. disparis, psil=P. silvestris, unex=unexplained mortality observed during rearing, and resid=residual mortality not observed during rearing. $K_i = -1.0*\log_{10}(N_i/S)$ where N_i = initial density and S = number of survivors.
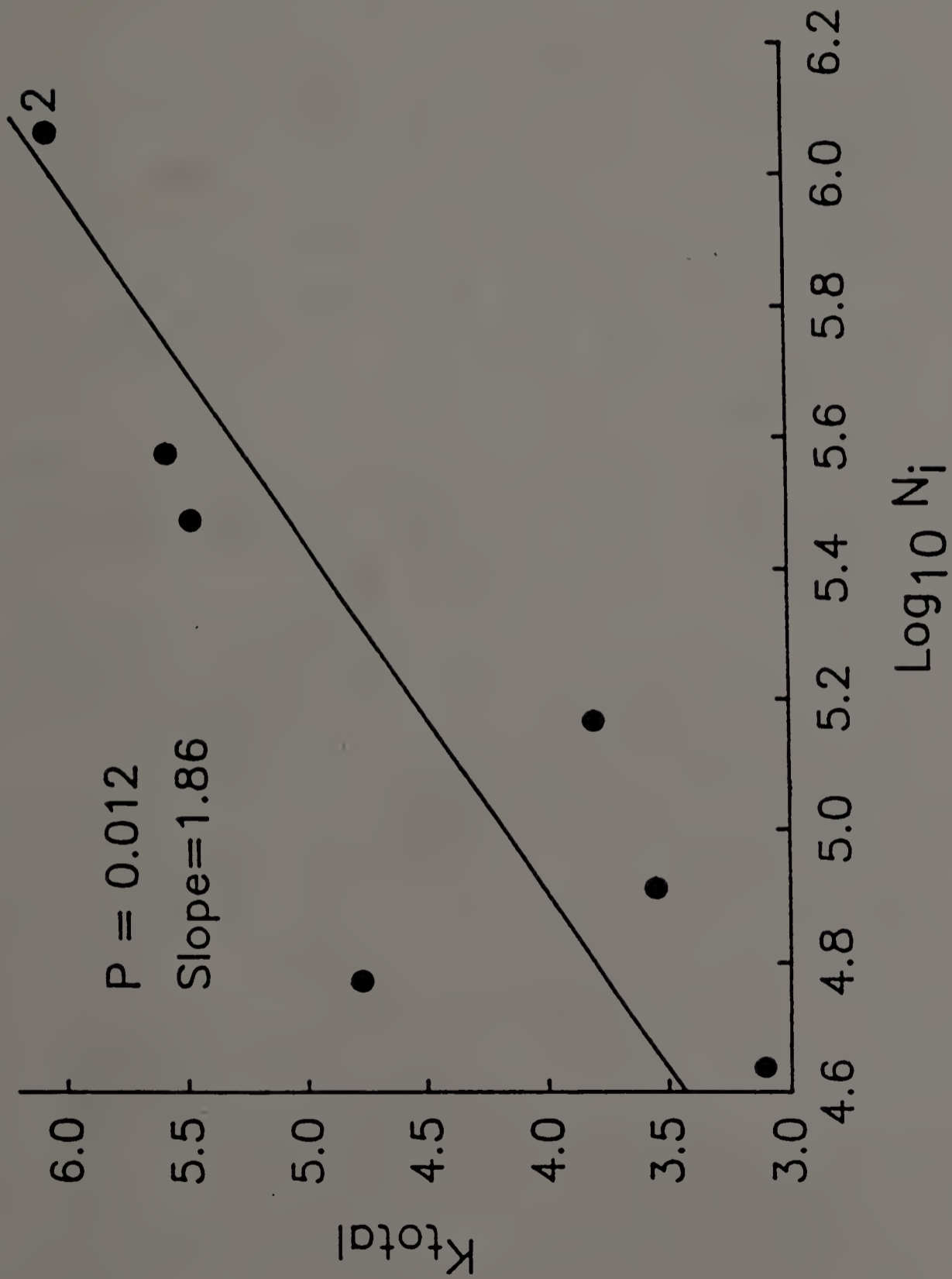
Figure 6.2. Density dependence of gypsy moth mortality occurring over the entire season. A '2' next to a data point indicates two observations.
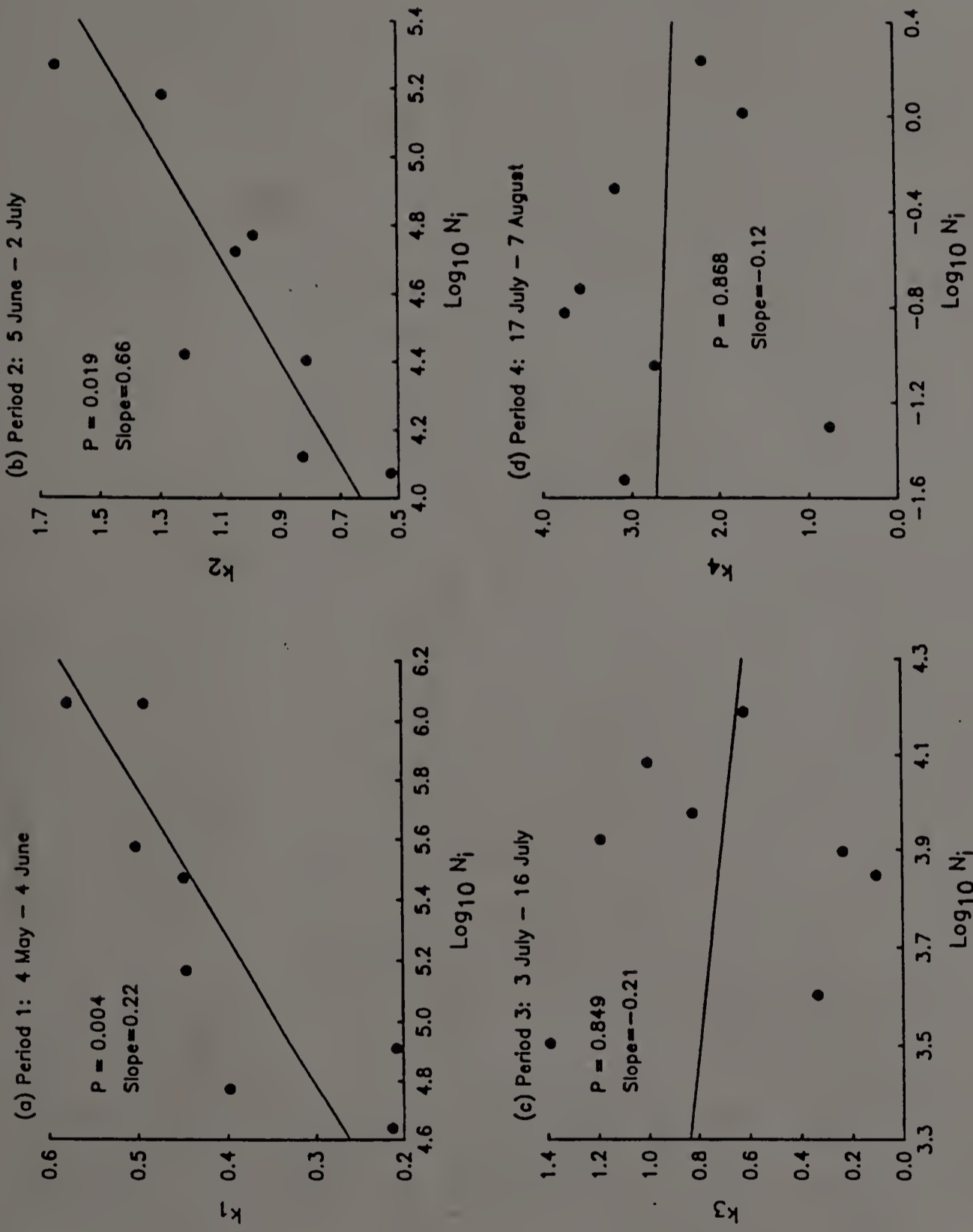
Figure 6.3. Density dependence of gypsy moth mortality during four periods of time.

Table 6.4. Number of foliage gleaning birds sighted or heard during eight minute periods. For four sample points from 29 May 1988 to 2 June 1988 in plots 4A and 4B and two control plots.

Bird Species			Plot		
Scientific Name	Common Name *	4A	4A Control	4B	4B Control
Parus bicolor	Tufted Titmouse	0	1	0	0
Dumetella carolinensis	Gray Catbird * # @	0	3	0	0
Catharus fuscescens	Veery	6	4	6	7
Catharus guttatus	Hermit Thrush #	1	0	1	2
Vireo olivaceus	Red Eyed Vireo * $ @	8	9	7	7
Mniotilta varia	Black-&-White Warbler $	1	1	5	3
Wilsonia canadensis	Canada Warbler $	2	1	0	0
Dendroica caerulescens	Black Thr. Blue Warbler $	0	1	1	6
Dendroica fusca	Blackburnian Warbler $	0	0	1	0
Dendroica castanea	Bay-breasted Warbler	0	1	0	0
Setophaga ruticilla	American Redstart $	0	2	0	0

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Table 6.4. Continued.

Piranga olivacea	Scarlet Tanager *	0	1	0	1
Seiurus aurocapillus	Ovenbird # $	7	12	14	15
Icterus galbula	Northern Oriole * @	1	0	0	0
Pheucticus ludovicianus	Rose-breasted Grosbeak	2	1	0	0
Sitta carolinensis	White-breasted Nuthatch	0	2	0	0
Bombycilla cedrorum	Cedar Waxwing	0	0	0	2
Cyanocitta cristata	Blue Jay * # @	0	0	5	0
	Total	28	39	40	43

* Species that are known to consume gypsy moth larvae * = Smith & Lautenschlager (1981),
# = JSE (unpublished data), @ = Forbush & Fernald (1896), and $ = Whelan, Holmes, & Smith (1989).

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while I did not directly measure dispersal of first instar larvae I did find fifth and sixth instar larvae under burlap bands up to 130 m from plots 4A and 4B. No gypsy moths were found under burlap bands between 130 and 150 m from the plots.

During Period 2, when the gypsy moths were mainly instars 3-5, there was high weekly mortality due to parasitoids (Fig. 6.4). Compsilura concinnata Meigen (Diptera: Tachinidae) caused the most parasitism and there was some parasitism by Cotesia melanoscela Ratzeburg (Hymenoptera: Braconidae), Phobocampe disparis Veireck (Hymenoptera: Ichneumonidae), and P. silvestris. Unexplained mortality was also high which is consistent with studies of other gypsy moth populations (Blumenthal et al. 1979, Campbell 1963, Reardon & Podgwaite 1976). Unexplained mortality could be due to trauma associated with collection and rearing but there is evidence that parasitoid induced mortality that does not result in the emergence of a parasitoid can be high (Blumenthal et al. 1979; Godwin & ODell 1984). It is possible, therefore, that the overall contribution of parasitoids to the mortality observed in the populations was greater than the observed percentage parasitism values would indicate. Unexplained mortality could also be due to pathogens such as Streptococcus faecalis Doane (Doane 1970a).

C. concinnata was the principal source of the density-dependent decline of the population during Period 2 (Fig. 6.5a). The regression of k_{comp} on $\log_{10} N_i$ was highly significant and had a relatively large positive slope. The response was nonlinear, however, and leveled off above approximately 100 000 hosts per ha. A quadratic model ($Y = -18.87 + 7.32 X - 0.68 X^2$) fit the data better than the linear model ($P=0.006$, $F=21.77$, d.f.=1,5) and is shown in Fig. 6.5a. Unexplained mortality also increased significantly with increasing density (Fig. 6.5e) which suggests that it may be related to attacks by C. concinnata. The
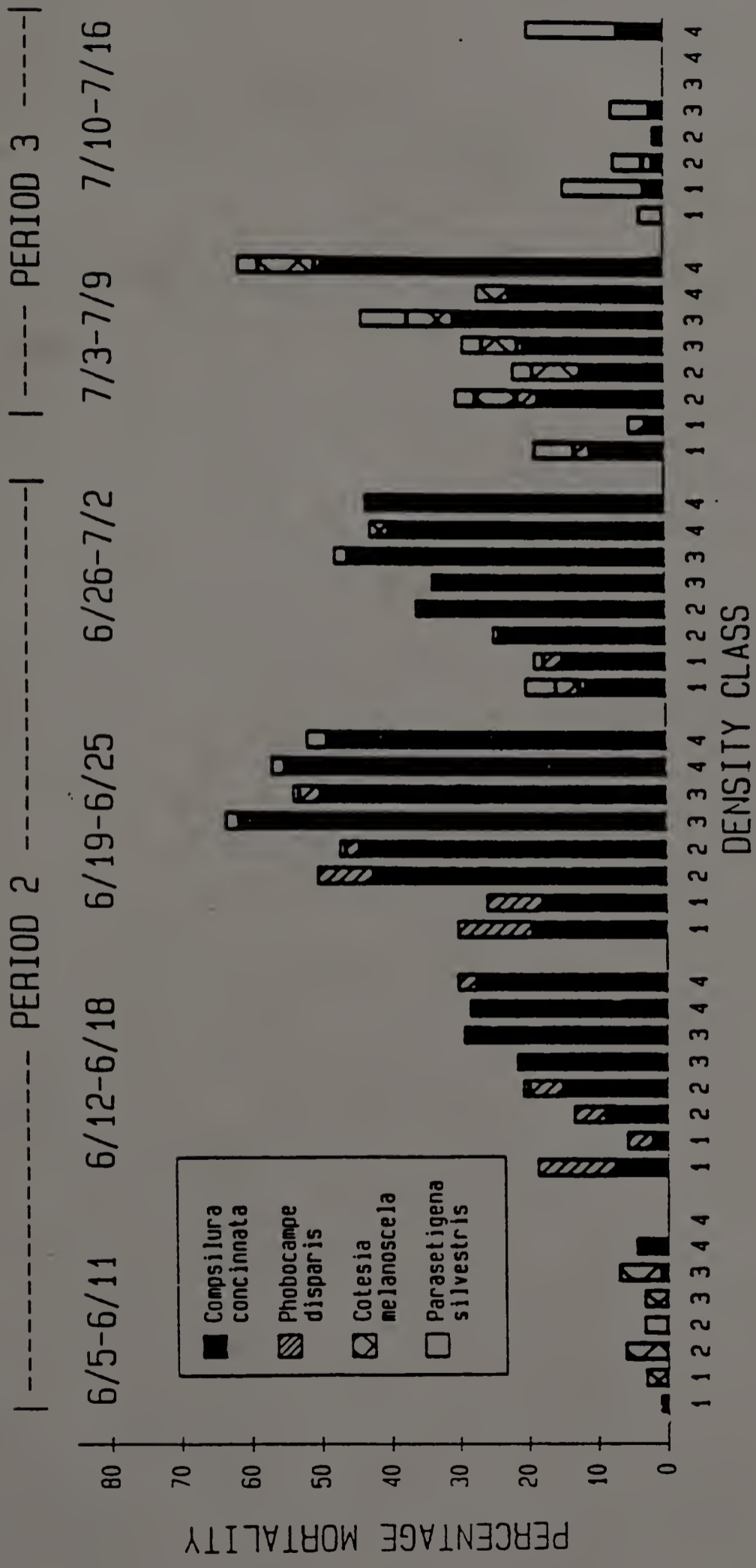
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Figure 6.4. Percentage parasitism during Periods 2 and Period 3. Mortality was observed by rearing gypsy moth larvae collected from plots at four initial densities.
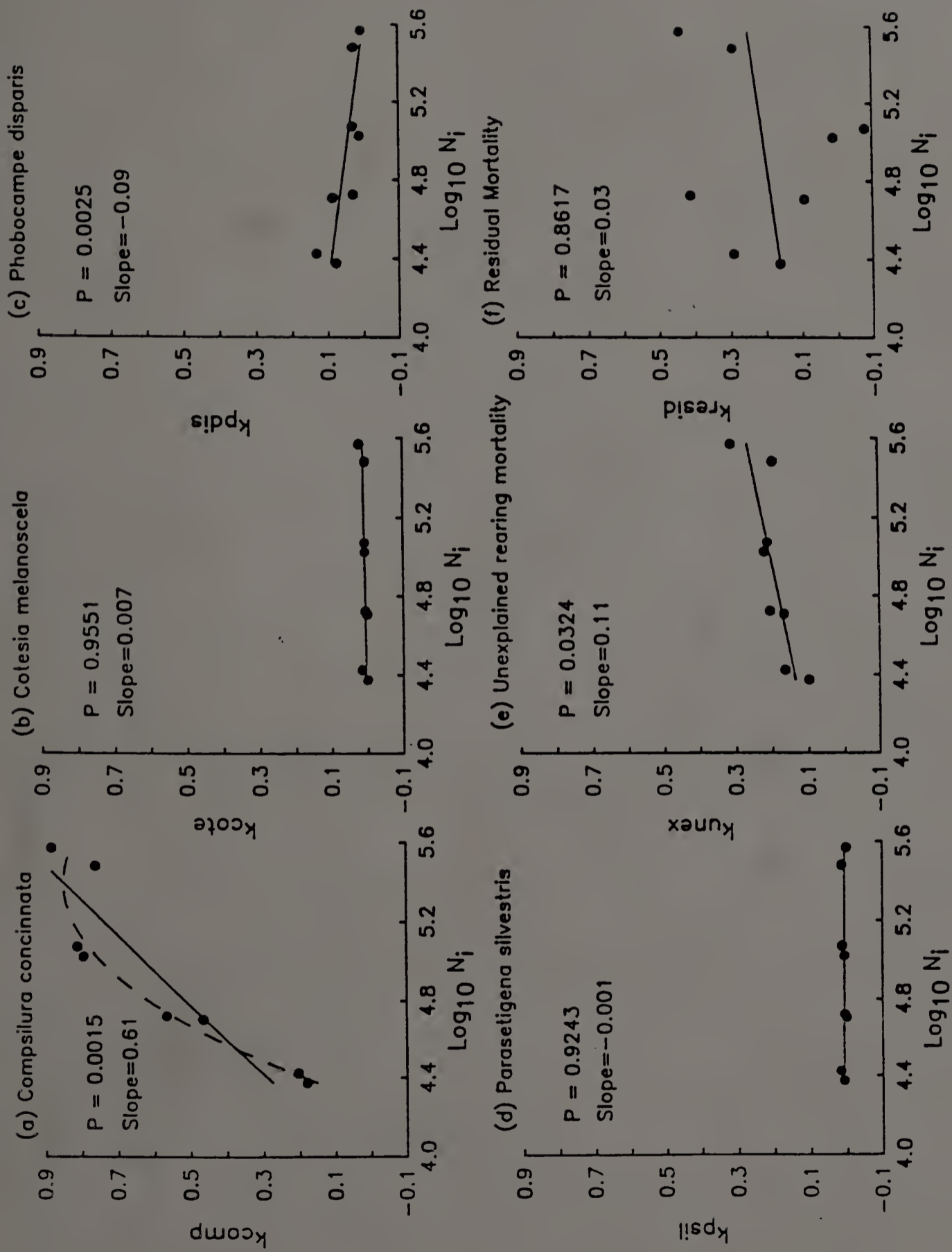
Figure 6.5. Density dependence of six mortality agents acting during Period 2. Solid lines indicate linear trends and dashed lines indicate quadratic trends (if present).

only other mortality agent for which the regression was significant was P. disparis but for this parasitoid the slope of the regression line was negative (Fig. 6.5c).

At the beginning of Period 3 when gypsy moths were in the late larval instars there was substantial parasitism (Fig. 6.4). Mortality due to C. concinnata was density-dependent (Fig. 6.6a) although the overall mortality for this period (k_3) did not show this trend (Fig. 6.3c). Again the response of C. concinnata was nonlinear with a quadratic model ($Y = 25.16 -13.60 X + 1.84 X^2$) providing a significantly better fit than the linear model ($P=0.012$, $F=14.95$, d.f.=1,5). At this range of densities the density-dependent response leveled off at the lower densities (Fig. 6.1a). None of the regressions for the other mortality agents acting during this period were significant.

The density-dependence of oviposition by P. silvestris reversed over time. On the first two sample occasions the relationship between attack rate and density was inversely density-dependent (Fig. 6.7a and b). The third sample showed no significant relationship (Fig. 6.7c) yet for the fourth sample there was a strong positive density-dependent relationship (Fig. 6.7d).

Additional evidence of density-dependent mortality during the mid and late larval instars was obtained from the transect line data. The total number of larvae under bands declined with distance from the plot, as I would expect from the limited dispersal of first and late instar larvae (Mason & McManus 1981). I also found that the proportion of dead larvae decreased significantly with distance from the plot ($P=0.020$, $F=7.37$, d.f.=1,11) indicating that mortality was higher closer to the release plots where densities were higher.

The rate of mortality during Period 4 was higher than during any other period in all but one plot (Table 6.3) but there was no evidence of an overall density-dependent reduction in pupal density. The pupal deployment experiment
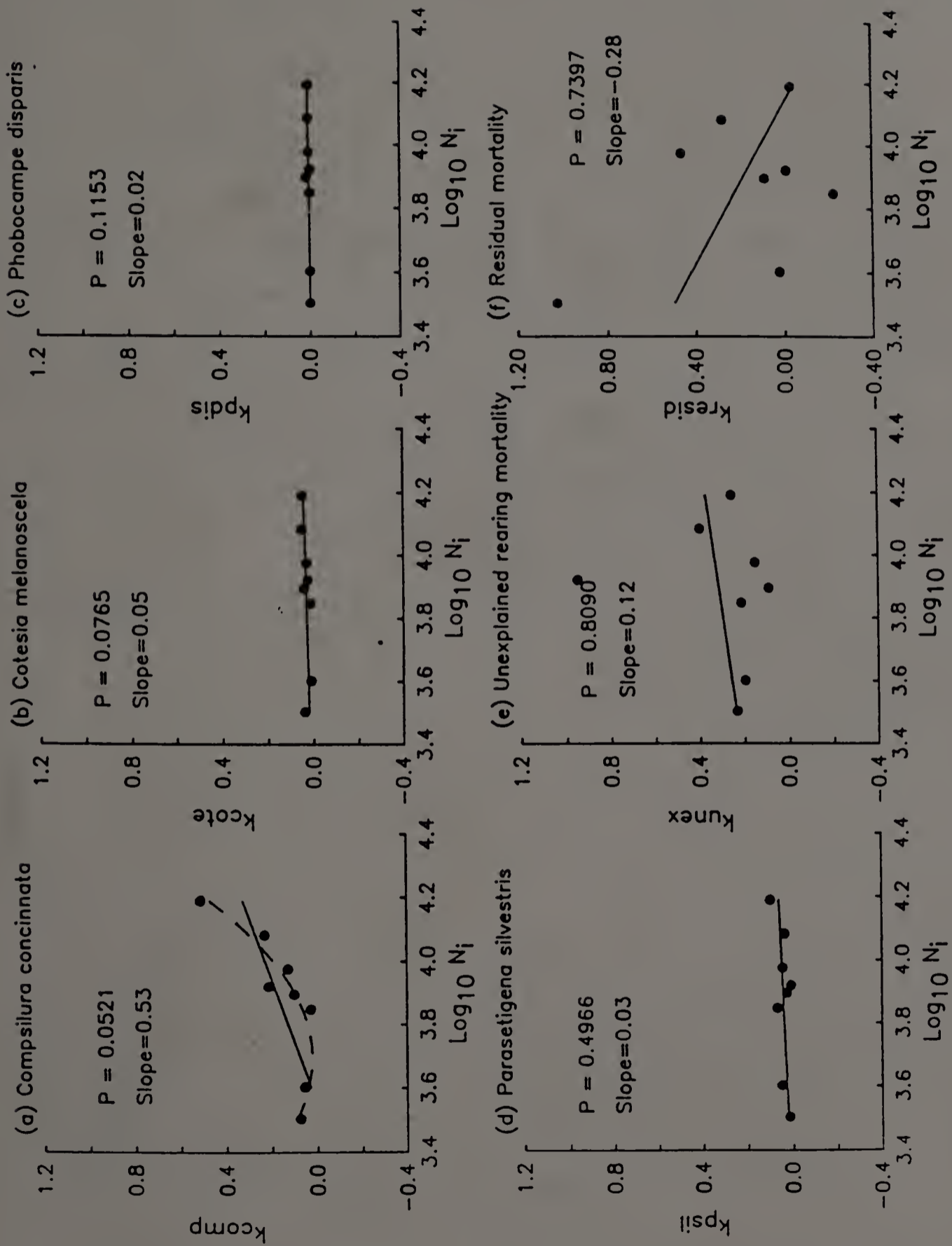
Figure 6.6. Density dependence of six mortality agents acting during Period 3. Solid lines indicate linear trends and dashed lines indicate quadratic trends (if present).
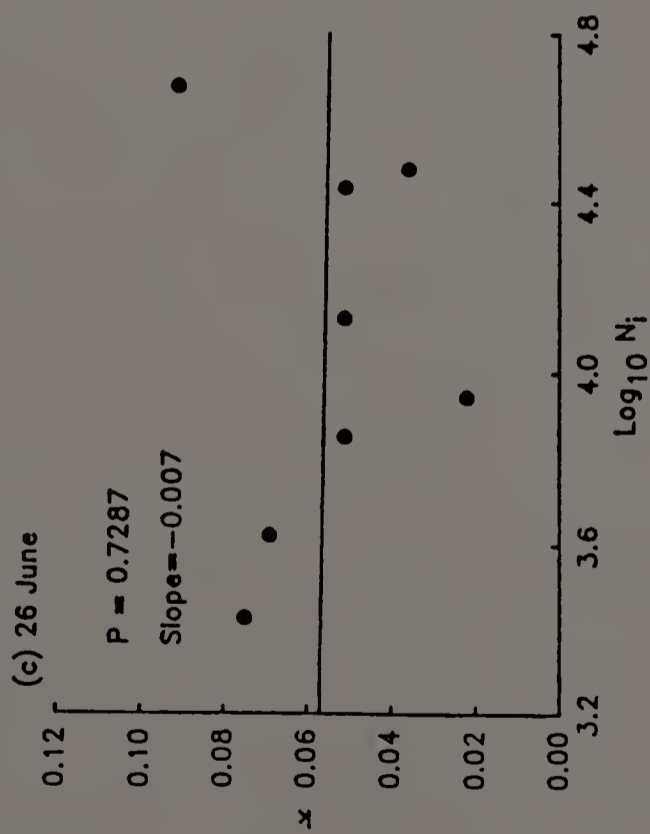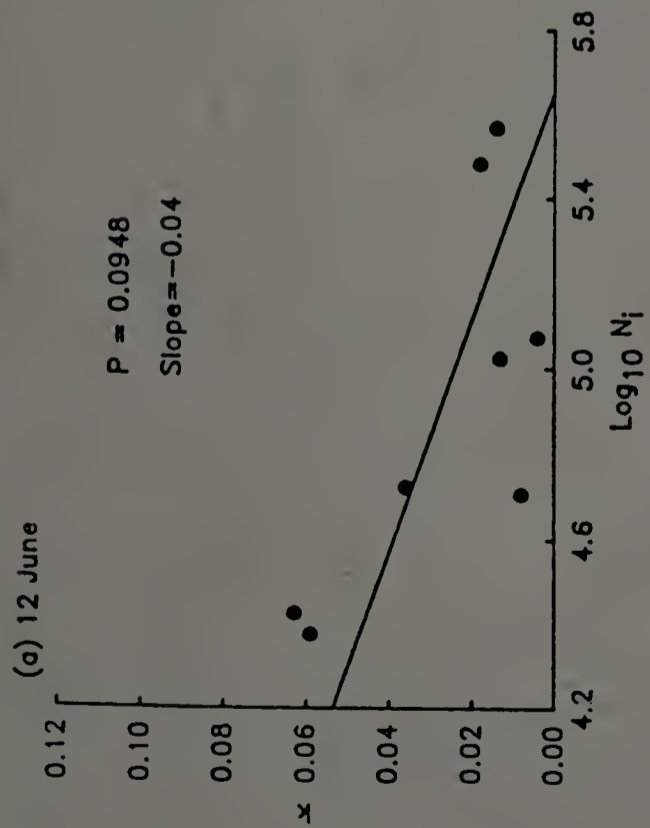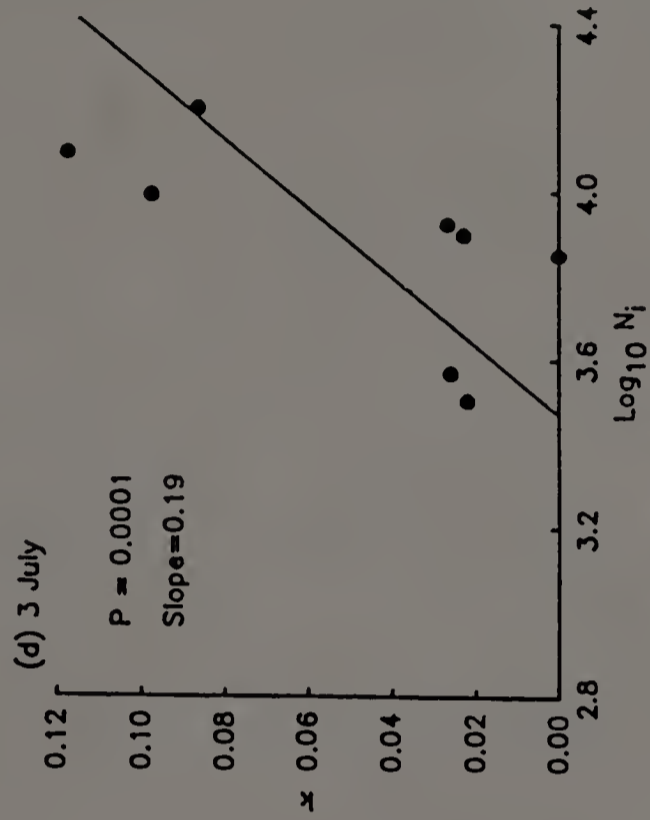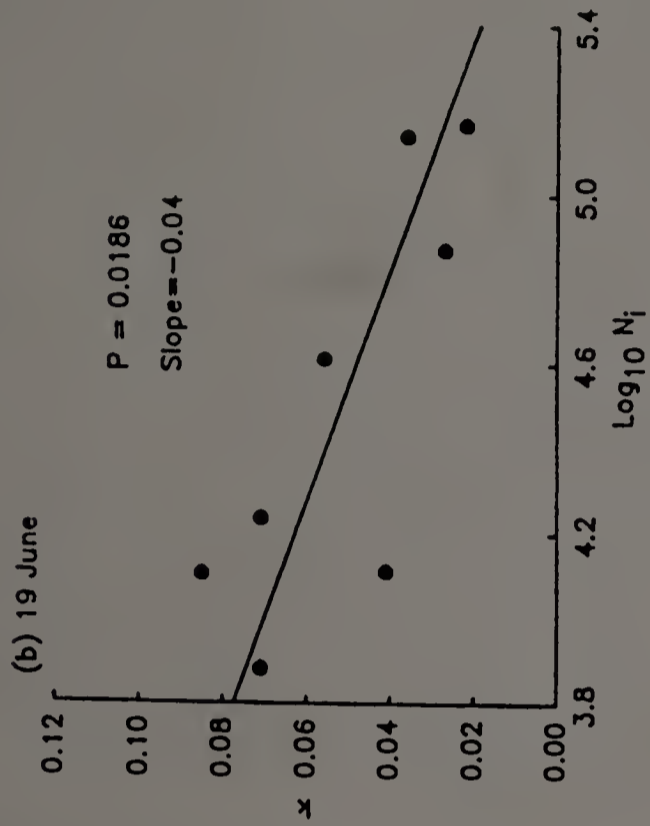
Figure 6.7. Density dependence of oviposition by _P. silvestris_ on four weekly sample occasions.

suggested that predation on pupae was inversely density-dependent (Table 6.5). The survival of both male and female pupae was greatest in Plots 1B and 2B, which were the two plots with the greatest number of gypsy moth larvae per metre of burlap band prior to the experiment. These two plots were also two of the three plots in which egg masses were found. Survival of deployed female pupae in plot 2A was not significantly greater than in the other plots, but survival of females under burlap bands was 100%. The major predators were probably small mammals because survival of male pupae inside of wire cages was high. An invertebrate, Calosoma sycophanta L. (Coleoptera: Carabidae), is also too large to go through the mesh of the wire cages. C. sycophanta has been implicated as an important predator in high density populations (Weseloh 1985b), but I saw little evidence of these predators under burlap bands (where they are often found) in any of the plots. None of the surviving pupae that I collected and reared yielded pupal parasitoids such as Brachymeria intermedia (Nees) (Hymenoptera: Chalcididae), and there were no B. intermedia emergence holes in any of the pupal cadavers collected under burlap bands.

Discussion

Contrary to what many investigators have suggested I have evidence that parasitoids may have an important impact on the population dynamics of the gypsy moth and can, in combination with predation, cause in excess of 99% mortality within a generation. I found no NPV, a common cause of mortality in high density populations (Campbell 1967; Doane 1970b), yet populations in all eight plots declined in a density-dependent fashion to quite low levels. Predators caused a high rate of mortality during the pupal stage but there was no evidence that they were responding in a positively density-dependent

Table 6.5. Survival of gypsy moths pupae in Cadwell Forest in 1987. Male (M) and female (F) pupae were deployed in the litter, and male pupae were placed in exclosures (MX).

| | Proportion Surviving 3 days * | | | Burlap Band Counts | | | | |
| | | | | No. per m.burlap | | Proportion Surviving | | Number egg masses |
Plot	M	F	MX	M	F	M	F	per ha
1A	.48bc	.38bcd	.98a	.08	.07	0.80	.50	0.0
1B	.79a	.62a	.98a	.42	.61	1.00	.32	33.9
2A	.50c	.36ce	.92a	.04	.01	0.33	1.00	22.6
2B	.77a	.59ab	.98a	.70	1.01	0.33	.49	22.6
3A	.52bc	.34ce	.98a	.01	.02	1.00	.00	0.0
3B	.49bcd	.50ac	1.00a	.45	.05	0.80	.50	0.0
4A	.40be	.27de	.90a	.14	.05	0.83	.00	0.0
4B	.28de	.34ce	1.00a	.09	.00	0.00	–	0.0

* Means followed by the same lower case letter were not significantly different at $p < 0.05$, Lee-Desu D Statistic (Lee & Desu 1972).

manner. The density-dependent mortality occurred during the early and mid instars; not during the late instar and pupal stages as would be predicted if predation on late instars and pupae was a regulating factor.

My results are not unique. Similar responses of gypsy moth parasitoids have been noted by S. Wilmot et al. (personal communication) for releases of F1 sterile larvae (progeny of partially sterilized adults) in Vermont and by Liebhold and Elkinton (1989b) for releases of both F1 sterile and feral larvae on Cape Cod, Massachusetts. Also, a number of previous research projects (M.L. McManus personal communication) have tried yet failed to create high density populations of gypsy moth larvae by releasing egg masses, indicating a strong response by natural enemies. These results suggest that the strong response of parasitoids to local increases in host density is common and consistent in many places in different years.

The drop in density from hatch to third instar was positively density-dependent, but I was unable to determine its cause. Parasitoids had not begun to appear in samples, and I found no evidence of birds aggregating in areas of high gypsy moth density. The observations were mainly on resident birds with established territories, however, and it is possible that migrant birds removed a higher proportion of larvae in the higher density plots. This period coincided with peak migration of many bird species. Furthermore, the resident birds may have foraged more intensively for gypsy moth larvae in the high density plots. Furuta (1976) found that when gypsy moth larvae were released in clumps of different densities, birds searched for prey longer in higher density clumps and the number of larvae decreased in a density-dependent fashion.

Density-dependent dispersal of first instars is another possible explanation for the disappearance of larvae. If more than one larva reaches a branch terminal there is an increased probability of dispersal (Semevsky 1971), and

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Campbell (1969) found a greater loss of first instar larvae due to dispersal in dense populations. Leonard (1970) suggested that the lower nutrition of eggs laid in high density populations resulted in an increased probability of dispersal, but this could not account for my results because all the eggs for this experiment were collected from the same population.

The polyphagous parasitoid, C. concinnata, was the major mortality agent in the plots during the mid larval stage, and it acted in a positively density-dependent fashion. This parasitoid showed a remarkable ability to locate and parasitize gypsy moths in the experimental populations. Gypsy moths were not present in the plots prior to the release, yet I measured up to 55% mortality due to this parasitoid in a single week. Beddington et al. (1978) suggested that successful natural enemies are most likely specialists, but in this study, the more specialized parasitoids, such as P. disparis and C. melanoscela, did not respond as strongly as C. concinnata to increases in pest densities. Polyphagous natural enemies can remain abundant when a particular host species has become extremely sparse or locally extinct, and when reinfestation of the host occurs they can respond quickly (Murdoch et al. 1985). This seems to be what occurred in this study. C. concinnata was present in the area and was able to respond by either migrating from surrounding areas or by switching to gypsy moths from alternate hosts within the plots.

C. concinnata has up to 4 generations per year (Culver 1919), and requires hosts other than the gypsy moth during some of these generations. The availability of alternate hosts, therefore, may be an important factor in determining parasitoid abundance from one year to the next. In an inundative release of C. concinnata in Pennsylvania, Blumenthal et al. (1979) found significantly higher percentage parasitism in release plots than in control plots in the year of release, but found no differences the following year.

Another factor that might limit the effectiveness of this parasitoid in regulating gypsy moth populations is the spatial scale on which gypsy moth populations typically increase in density. I recorded a density-dependent response to hosts in areas of one ha. If gypsy moth populations increase in density over larger areas, the pool of available parasitoids might be overwhelmed and populations could continue to increase. There is preliminary evidence, however, that C. concinnata can respond to population increase over larger areas (T.M. ODell personal communication). In a 16.7 ha plot and a 57.5 ha plot in Vermont, F1 sterile gypsy moth larvae were released at rates of 1 million and 600 000 larvae hatching per ha, respectively. At the end of the season both plots contained only 1 egg mass per ha. A major mortality agent in these plots was C. concinnata.

The possible nonlinearity of the density-dependent response of C. concinnata is also important to consider. Taken together, Fig. 6.5a and 6a imply that C. concinnata exhibits a positive density-dependent response, but only over the range of densities from approximately 6000 to 100 000 larvae per ha. Outside of this range of densities, the response may be inversely density-dependent or density independent. The ability of C. concinnata to regulate populations at extremely low densities or to suppress outbreaks once the threshold of 100 000 mid instars per ha is exceeded is, therefore, uncertain.

The response of the oligophagous parasitoid, P. silvestris, was initially inversely density-dependence but switched to positive density-dependence as the season progressed. One plausible explanation for this pattern would be that a greater number of P. silvestris females were attracted to the high density plots, but at the beginning of the season there were so many larvae in these plots that inversely density-dependent parasitism nevertheless occurred. As host densities declined, due primarily to the action of C. concinnata, the greater

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number of P. silvestris females in the higher density plots attacked a greater proportion of larvae. Greater continued recruitment of P. silvestris females to the high density plots is another possible explanation of these results.

It should be noted that the density-dependent oviposition by P. silvestris that occurred during the week of 3 July was not evident when I looked at mortality that occurred over the entire Period 3 (3-16 July). One possible explanation is that the early inverse density-dependent attack rate canceled the later direct density-dependent attack rate so that the overall effect was not density-dependent. Another explanation is that most mortality due to P. silvestris occurred during Period 4, when population densities were too low to sample for estimates of mortality. This is possible because P. silvestris emerges just prior to pupation, which in this study occurred during Period 4.

C. concinnata and P. silvestris responded to differences in the density of hosts among subpopulations within a generation in a density-dependent fashion, but whether spatially density-dependent responses can lead to temporal density-dependence and population regulation has been debated in the recent literature. It has been demonstrated (e.g. Hassell 1985; Beddington et al. 1978) that a spatially density-dependent response among subpopulations can contribute to long-term stability of simulated populations. These conclusions were based on models in which the number of parasitoids in a particular generation depended on the number of hosts parasitized in the previous generation, as might be expected for P. silvestris. The stabilizing ability of a generalist natural enemy such as C. concinnata, which has a strong spatially density-dependent response but, presumably, little generational carryover, is less clear. The model of Hassell (1985) has predicted that spatially density-dependent mortality by such agents can stabilize a population, but this occurs only if the degree of clumping of the host within subpopulations changes

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as overall density changes (Latto & Hassell 1988). I would expect such a change in the dispersion pattern of gypsy moths following the action of density-dependent natural enemies because gypsy moth females do not fly and dispersal of first instars is limited (Mason & McManus 1981).

Life-table analyses of gypsy moth population dynamics in North America have so far failed to identify regulation or temporal density-dependent mortality due to parasitoids (Dempster 1983). There is some evidence of density-dependent pupal mortality, presumably due to predation (Campbell & Sloan 1978; Campbell et al. 1975), but spatial and temporal density-dependent effects were considered together in these studies and there was little or no data on the contribution of parasitoids to overall mortality. It may be that populations are not regulated around an equilibrium density, but are instead characterized by local extinction and colonization which results in area wide stability (e.g. Murdoch et al. 1985; Nachman 1987; Morrison & Barbosa 1987; Elkinton et al. 1989). Persistence of such populations has been shown to be most likely when there is some density-dependent coupling of hosts and parasitoids, and when migration rates of the parasitoid exceed those of the host (Reeve 1988). This would be possible for gypsy moth populations because gypsy moths do not generally migrate over large distances and parasitoids can migrate further than their hosts and can respond in a density-dependent fashion.

Another reason that regulation and temporal density-dependence may not have been detected in gypsy moth populations is that few long-term studies have been conducted. It is uncertain, however, that temporal density-dependence, if it exists, would be detected by such studies. Stochastic variation (Hassell 1985, 1987) or conducting the studies on an inappropriate spatial scale (Heads & Lawton 1983; Hassell et al. 1987) may obscure density-dependent processes. Furthermore, it is extremely difficult to measure host

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density or parasitism with any degree of precision when gypsy moth densities are low. As a result, low density populations are rarely studied. If regulation is the result of rapid suppression of local increases in gypsy moth density, it is unlikely that previous studies would have detected these processes.

Further experimental manipulations of low density gypsy moth populations are needed to resolve the question of population regulation. If I discover that populations of gypsy moths at low densities are indeed regulated, it is possible that parasitoids attacking the mid instars, not pupal predators, will be the agents responsible for regulation. Campbell & Sloan (1978) theorized that gypsy moth populations are characterized by a low density threshold above which mortality by small mammal predators is inversely density-dependent and populations escape into outbreak phase. During the periods of low population density between outbreaks, the role of parasitoids such as C. concinnata and P. silvestris may be to suppress local increases in the density of gypsy moths to levels at which predation by small mammals would cause sufficient mortality to prevent population increase. Because C. concinnata is multivoltine and is dependent on the availability of alternate hosts, however, this parasitoid may not be present in large numbers in certain years. Lowered mortality from C. concinnata in a given year might result in a greater number of gypsy moth pupae than could be consumed by pupal predators, leading to an increase in reproduction by the gypsy moth. The number of host larvae the following year might then be outside the range over which the density-dependent response of C. concinnata occurs. These processes could rapidly propel the population above the thresholds of both parasitoids and predators and into an outbreak phase.

APPENDIX

CALCULATING THE MARGINAL PROBABILITY OF MORTALITY

I calculated the marginal probability of mortality due to a mortality agent in the presence of other simultaneous mortality agents (after Royama 1981b). The marginal probability can be thought of as the proportion that would have died from a given agent if none of the other agents were present. For a system with two simultaneous parasitoids (A & B) the marginal probabilities (m_A, m_B) are:

$$m_A = v_A/(1-cv_B)$$

$$m_B = ((c-1)v_A + cv_B + 1 - ((v_A-cv_A-cv_B-1)^2 - 4cv_B)^{1/2} / 2c)$$

where

   v_A = proportion of reared hosts producing parasitoid A

   v_B = proportion of reared hosts producing parasitoid B

   c = proportion of hosts that yield parasitoid B when both

      A and B attack the same individual.

   1-c = proportion of hosts that yield parasitoid A when both

      A and B attack the same individual.

These formulae assume that either parasitoid A or B, but not both, can emerge from hosts parasitized by both species. In my calculations I assumed c = 0.5 for all interactions. This value is largely unknown for interactions between gypsy moth parasitoids but for the interaction between C. concinnata and C. melanoscela, the parasitoids emerged in equal proportions from multiparasitized host larvae (Weseloh 1983).

I extended this analysis to four simultaneous parasitoids and one disease (unexplained rearing mortality) by analyzing each agent separately against all other agents combined. In other words I defined v_B as death due to all four of the other simultaneous agents. This scheme caused a small error in

partitioning the marginal probabilities for host individuals attacked by three or more agents simultaneously but this constituted an extremely small fraction of the mortality and the resulting error in the estimated k-values was less than 1%.

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