EFFECTS OF A NUCLEAR POLYHEDROSIS VIRUS

OF THE WESTERN TENT CATERPILLAR ON

INDIVIDUAL PERFORMANCE AND POPULATION DYNAMICS

by

LORNE DAVID ROTHMAN

B.Sc., The University of Toronto, 1988
M.Sc., The University of Toronto, 1990

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Department of Zoology)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December 1995

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of _Zoology_

The University of British Columbia
Vancouver, Canada

Date _Dec 4 / 1995_

DE-6 (2/88)

ABSTRACT

Viral diseases are an important feature of fluctuating populations of Lepidoptera. I examined the potential for a nuclear polyhedrosis virus (NPV) to explain the changes in abundance and fecundity observed in populations of the western tent caterpillar, *Malacosoma californicum pluviale*. Fluctuating populations are characterized by prolonged declines following high density. In *M. c. pluviale*, decreases in fecundity track these declines.

While viral diseases of Lepidoptera are usually recognized for their ability to kill infected hosts, they may also reduce the fitness of individuals which survive infection. I surveyed studies in the literature which evaluated qualitative characteristics of individuals following treatment with virus. Debilitating effects of viral diseases of Lepidoptera included slower development rates, lower pupal and adult weights, reduced reproductive capacity and shorter adult longevity. These sublethal effects were observed more frequently in studies of less virulent pathogens (cytoplasmic polyhedrosis viruses or CPVs) than in studies of NPVs. Debilitating effects of both CPVs and NPVs could potentially suppress host population growth.

In the laboratory, I treated *M. c. pluviale* larvae with NPV to assess whether viral infection could potentially reduce fecundity as observed in declining field populations of the host. Weights of male and female pupae and female

fecundity were reduced in survivors of virus treatment, which suggests that NPV could reduce fecundity of field populations of the western tent caterpillar.

In small-scale field and related laboratory experiments, I examined immediate and delayed effects of NPV introduction and density on *M. c. pluviale*. In a factorial experiment, larvae at high density showed increased feeding and development rates and decreased reproductive potential. The introduction of NPV significantly increased mortality of the host, particularly at high density, and generally reduced host reproductive potential. Adults from this experiment were mated and their offspring reared in the laboratory. No treatment effects on egg viability or larval performance occurred. Treatments did have significant delayed effects on pupal weights of female offspring. However, in a correlational field study maternal fecundity was not related to pupal weights of offspring. *M. c. pluviale* introduced to host trees used in the initial factorial experiment showed no effects of previous caterpillar density on performance as predicted by host plant induction theory. However an interactive effect of previous virus introduction and density on mortality was observed owing to persistence of NPV particles in the environment. Female pupal weights were also slightly reduced in the year following NPV introduction.

I assessed the potential for NPV and density to reduce population growth by calculating net reproductive rates for treatments from the above field experiments. The introduction of NPV had a large immediate impact on population growth, particularly at high host density. Delayed effects of NPV introduction at high density were insufficient to prolong this decline, but did appreciably suppress predicted population growth. Treatment effects on individual quality (fecundity) had little effect on predicted population change.

Because delayed density dependence may lead to population instability, I concluded that viral disease may have a more destabilizing influence on tent caterpillar populations than density alone. Persistence of virus particles in the environment could contribute to, but not explain prolonged declines in field populations of *M. c. pluviale*. High density may be sufficient to initiate decreases in fecundity in these populations while viral disease may explain continued fecundity decreases during prolonged declines.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

I wish to thank my advisor Judith H. Myers for inspiring my interest in population biology, population quality and insect diseases and for her intellectual and practical support throughout my doctorate. I would also like to thank my other committee members: M. Adamson, D. Ludwig, B. Roitberg, and A. Sinclair for their insight and effective suggestions.

I thank my wife, Carolyn for her support and for comments and editorial advice on this thesis. I also thank her for encouraging me to remember the wider responsibilities of being a student of ecology.

1. GENERAL INTRODUCTION

1.1 Introduction

Viral disease commonly occurs in fluctuating populations of forest Lepidoptera and may have important effects on their dynamics. Transmission of virus is generally considered to be a density-dependent process. Viral epizootics are predicted and often observed following high larval densities (Clark 1958, Steinhaus 1958, Wellington 1962, Harcourt 1966, Stairs 1966, Doane 1970, Anderson and May 1981, Tanada and Fuxa 1987, Myers 1988, Elkinton and Leibhold 1990, Dwyer 1991, Dwyer and Elkinton 1993) and may reduce population size. An important feature of fluctuating or cyclic populations is the prolonged decline, illustrated in a time series for the western tent caterpillar, *Malacosoma californicum pluviale* (Fig. 1.1). If individuals no longer experience the immediate effects of density, why does the population continue to decline? Addressing this question involves the search for time delays in processes that act at high density. General theory also supports this approach while attempting to explain longer term dynamics. Populations are regulated by (positive) density dependent processes, while time delays in these processes may destabilize populations and lead to cyclic dynamics (Hutchinson 1948, Caswell 1972, May *et al.* 1974, Berryman *et al.* 1987, Turchin 1990). Viral disease may be involved in this prolonged decline. Some viruses are long lived and remain infectious in the environment (Clark 1955, 1956, 1958, Jacques 1969, Thomas *et al.* 1972, Podgwaite *et al.* 1979, Thompson and Scott 1979,

Murray and Elkinton 1989, Woods *et al.* 1989). This persistence can introduce time delays into pathogen-host systems (Hassell and May 1989) and models that incorporate pathogens with persistent and free living infectious stages produce cyclic dynamics (Anderson and May 1981, Hochburg 1989). Environmental persistence of virus could explain delayed recovery of host populations following epizootics at high host densities and population fluctuations observed in many lepidopteran species of north temperate forests (Myers 1988).

An additional and potentially important feature of some lepidopteran populations is a reduction in fecundity following high population densities (see Rothman and Myers, 1994 and Chapter 3). For several (four of six) populations of *M. c. pluviale,* fecundity of moths at peak density has been observed to decrease (Fig. 1.2), which suggests an immediate effect of high density on individual quality. In all recorded examples, fecundity continues to decrease as the population declines (and see Myers and Rothman 1995a). Although not often recognized, debilitating or sublethal effects of viral disease could be involved in these fecundity shifts (Myers 1993, Rothman and Myers 1994, Myers and Rothman 1995b, Rothman and Myers 1995).

Other ecological factors can have effects that span several generations and cause shifts in fecundity. High density may negatively affect individuals in

the current generation via intraspecific competition for food and space

(crowding) (Blais 1952, Miller 1957, Gruys 1970, Baltensweiler *et al.* 1977,

Capinera and Barbosa 1977, Peters and Barbosa 1977, Barbosa *et al.* 1981

Barbosa and Martinat 1987) or through rapidly induced responses in host food

plants (see Fowler and Lawton 1985, Haukioja and Neuvonen 1987, Karban

and Myers 1989, Haukioja 1990). Subsequent generations may be influenced

by delayed induced responses (DIR or induction) in host food plants

stimulated by previous defoliation (see Haukioja and Neuvonen 1987, Karban

and Myers 1989, Haukioja 1990). Delay mechanisms such as environmental

persistence of virus and DIR may be viewed as extrinsic because they operate

through the external environment. Disease and density may also have intrinsic

delayed effects on individual performance via environmentally based maternal

effects or genetic selection on variation among individuals at different densities

(Wellington 1957, 1965, Chitty 1960 and references therein, 1967, Rossiter 1991,

1992, 1994, 1995, Myers 1993, Rossiter *et al.* 1993, Ginzburg and Taneyhill

1994).

1.2 Statement of purpose and synopsis of chapters

In this thesis I will examine the potential for a nuclear polyhedrosis virus

(NPV) to explain, prolonged declines of *M. c. pluviale* populations and

observed patterns of fecundity change. Extrinsic and intrinsic effects of disease

will be considered and compared with an alternative hypothesis: density

effects *alone* are sufficient to explain observed patterns in *M. c. pluviale* populations. A literature survey on experiments involving treatment of host Lepidoptera with virus is presented in Chapter 2, to assess the generality of influences of viral diseases on host development rates and reproduction (including fecundity). In particular, debilitating effects of NPV are compared with the effects of more benign pathogens. Chapter 3 examines the potential for NPV to reduce fecundity of *M. c. pluviale* through inoculation of larvae with sublethal doses of virus. Several small-scale field and laboratory studies designed to examine the relative immediate and delayed effects of virus introduction and density on the performance of *M. c. pluviale* are presented in Chapter 4. An overall summary of important results and concluding remarks are given in Chapter 5.

1.3 Virus and tent caterpillar natural history

Nuclear polyhedrosis viruses (NPV) are members of the baculovirus or Baculoviridae family, a group of naturally occurring insect pathogens, the majority of which occur in Lepidoptera. NPVs are characterized by occlusion of virions in a protein matrix (inclusion bodies). The virions are surrounded by a lipoprotein envelope (membrane) containing one or several nucleocapsids each with a DNA core surrounded by a protein capsid (Evans and Entwistle 1987). Infection of individuals occurs through ingestion. Once in the midgut, dissolution of the polyhedral protein matrix frees the virions which then

penetrate the gut epithelial cells where replication begins. Further infection occurs by spread of virions along trachea and susceptible tissues. The hypodermis is last to be infected, but eventually the cuticle ruptures and NPV particles are released into the environment (Evans and Entwistle 1987). The principal route of horizontal transmission of NPVs is by ingestion of contaminated foliage. Virus can only be transmitted by host death and lysis. Transgenerational transmission may occur via environmental contamination (e.g. bark, tent material, soil or egg mass surfaces) or via direct transmission from females to their offspring on egg surfaces (transovum transmission). Evidence for transmission within the egg (transovarian) is scant (Entwistle and Evans 1985).

The western tent caterpillar, *Malacosoma californicum pluviale* (Dyar) (Lasiocampidae) is a periodic defoliator of deciduous trees in northern United States and Canada (Witter and Kulman 1972). *M. c. pluviale* outbreaks have been recorded in at least one site in southwestern British Columbia about every eight to 10 years (Myers 1988, 1990, 1993) (Fig. 1.3). In this area, the western tent caterpillar is characteristically found in disturbed habitats where recolonization by a major host food plant, red alder, *Alnus rubra* (Bong.) occurs (Wellington *et al.* 1975; Myers 1990). *M. c. pluviale* has one generation/year. Females lay eggs (100 to 300) in a single mass on twigs of the host plant after adult emergence and mating in midsummer (July). First instars hatch from the

eggs in April to late May, and begin construction of a communal tent by leaving silk trails as they move. Aside from forays for food, individuals remain aggregated in or on tents until the last instar, when they disperse prior to pupation in June or early July (Wellington 1957, Wellington *et al.* 1975). *M. c. pluviale* is host to over 30 species of invertebrate parasitoids while records of invertebrate and vertebrate predators are less common. The tent caterpillar genus, *Malacosoma* is host to viral, protozoan, fungal and bacterial disease (Witter and Kulman 1972). NPV is commonly observed following outbreaks of tent caterpillars (*Malacosoma* spp.) (Clark 1956, 1958, Wellington 1962, Stairs 1965, 1966, Myers 1990).
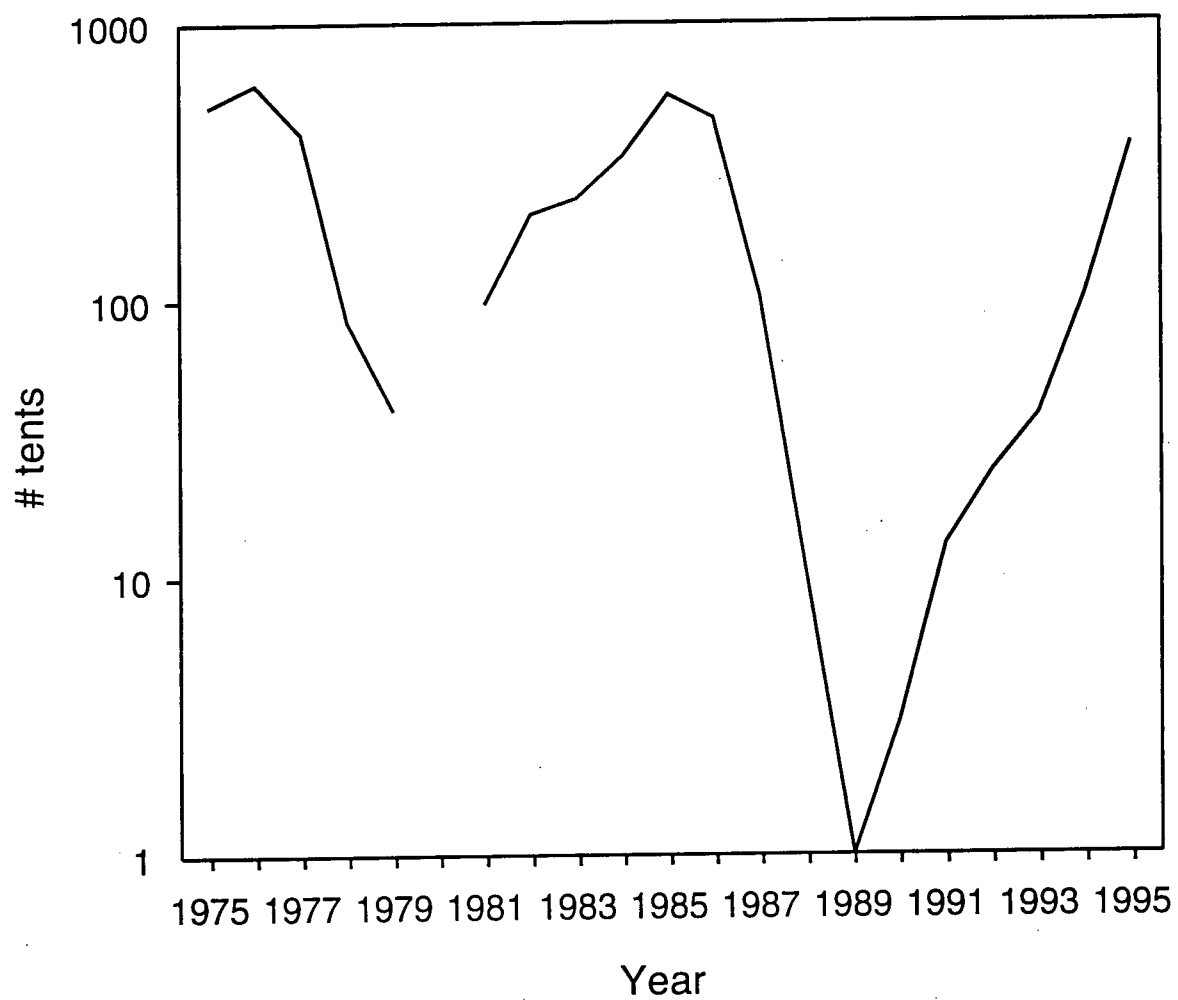
Figure 1.1. Time series of *M. c. pluviale* abundance at Mandarte Island, British Columbia. Data were collected by Dr. Judith H. Myers.
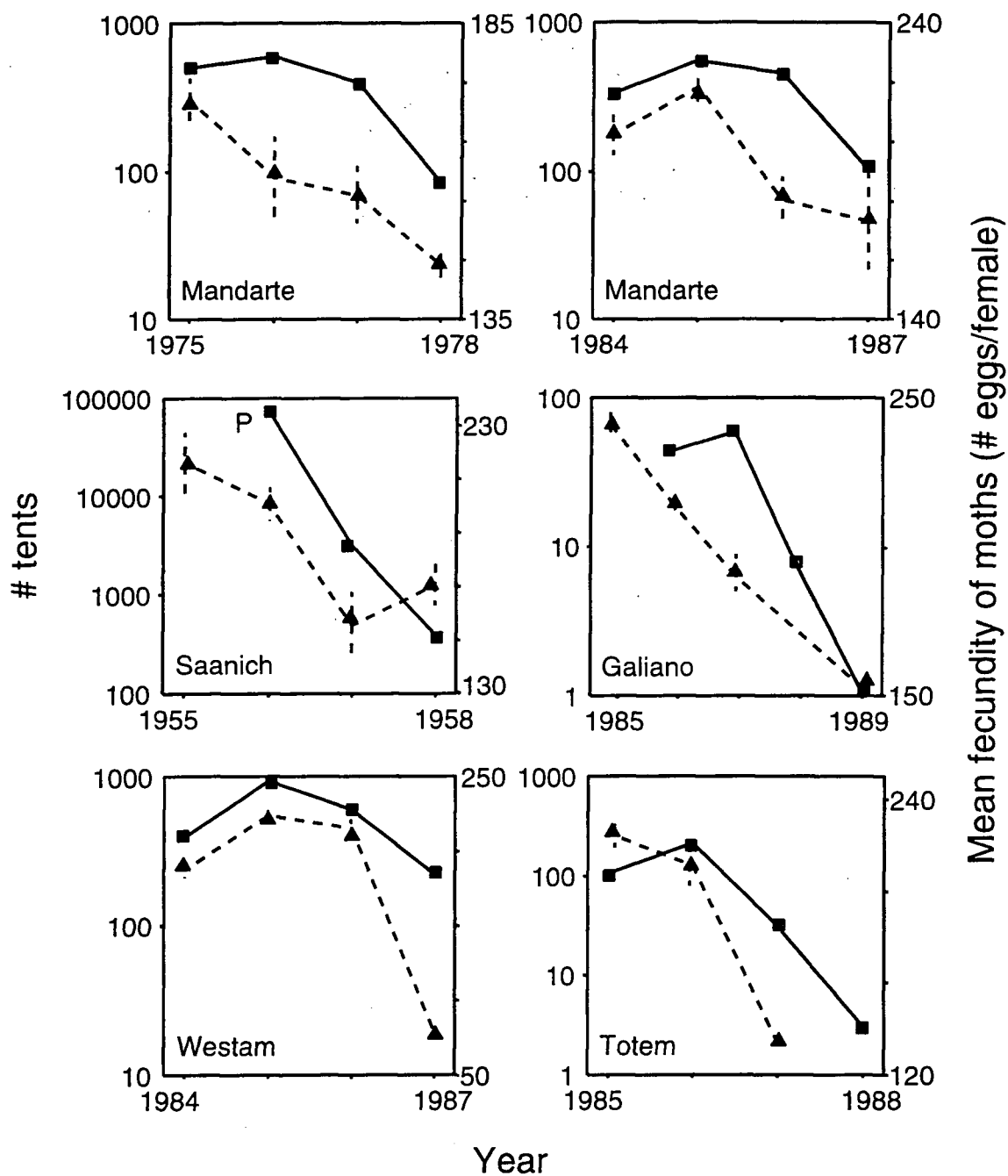
Figure 1.2. Changes in mean fecundity (dashed lines) of female moths (±S.E.) with changes in abundance (solid lines) in six populations of *M. c. pluviale*. P indicates the population peak where pre-peak abundances are not given. Data were collected by Dr. Judith H. Myers with the exception of the Saanich population (Wellington 1960).
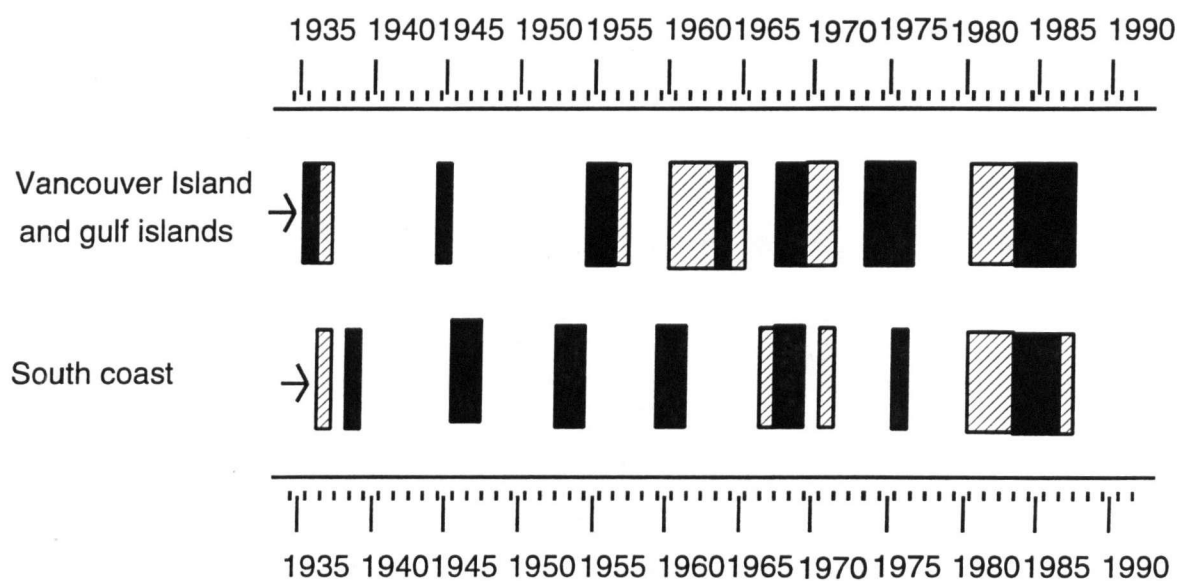
Figure 1.3. Outbreaks of *M. c. pluviale* in southwestern British Columbia. Dark bars indicate numerous larvae, extensive defoliation or infestations. Hatched bars indicate declining or increasing populations, moderate defoliation or small infestations. Arrows indicate the year when records began. South coast refers to the Lower Fraser Valley and the Sunshine Coast. Data are from the Canadian Forestry Service, Insect and Disease Survey.

2. DEBILITATING EFFECTS OF VIRAL DISEASES
ON HOST LEPIDOPTERA

2.1 Introduction

Most studies of pathogens in populations of Lepidoptera focus on mortality

and its direct causes but disease may also reduce reproductive capacity of the

host and increase host susceptibility to other mortality agents. Debilitating

effects of disease may be important in the population dynamics of

lepidopteran hosts (Anderson and May 1981, Munster-Swendsen 1991, Myers

1993, Ginzburg and Taneyhill 1994) and are often overlooked when assessing

the potential of disease to control populations of insect pests. The

baculoviruses or BVs are an important group of pathogens of Lepidoptera.

BVs are generally highly pathogenic and induce lethal infections (Entwistle and

Evans 1985). Because of this, BVs such as nuclear polyhedrosis viruses or

NPVs (Baculoviridae: Subgroup A), and to a lesser extent, granulosis viruses or

GVs (Baculoviridae: Subgroup B), have received considerable attention as

candidates for biological control and bioinsecticides. Transmission of NPV is

generally dependent on the death of the host and thus NPVs are not expected

to affect individual quality. Infected individuals should die, and caterpillars

which are exposed to virus but avoid infection should be uninfluenced by the

virus. By contrast, less pathogenic diseases such as cytoplasmic polyhedrosis

viruses [CPV (Reoviridae)] may be transmitted without death of the host,

horizontally through faecal material and vertically through transovum or

transovarian routes. Debilitating effects are therefore expected to be more common following exposure to CPVs than to NPVs because death is not necessary for transmission (Myers and Rothman 1995b).

There is evidence that NPVs can affect host quality despite their general pathogenicity. Reduced reproductive potential has been reported following NPV treatment in laboratory studies (Rothman and Myers 1994 and references therein). Further, NPV epizootics and reductions in host fecundity may be observed following high densities of the host in populations of Lepidoptera (Myers 1988, see references in Rothman and Myers 1994). In this review I survey the literature, to examine debilitating effects of viral disease on host Lepidoptera, and more specifically to examine the importance of these effects for pathogenic diseases such as NPVs as compared to more benign CPVs.

2.2 Methods

Survey of literature

Only experiments that involved measurement of debilitating effects were included in the survey. One study by Klein and Podoler (1978) reporting reduced fecundity in *Spodoptera littoralis* was not included, as no debilitating effects were quantified. The following information was gathered from each study: host species, host instar at time of inoculation, inoculation method, pathogen dose, survival, debilitating effects examined, and observed either by

using statistics or when trends were emphasized by the author(s), magnitude of effects, and presence of pathogen (inclusion bodies) in pupae or adults following treatment. Only trials where some individuals survived treatment were considered. The magnitude of debilitating effects, when available, were calculated as: $100 \times [1-(\text{mean value in treated sample/mean value in controls})]$, and scored as small (<15%), moderate (15-30%) or large (>30%).

Frequency and magnitude of debilitating effects

The frequency of debilitating effects was examined for studies involving NPVs and CPVs. Frequency (%) was calculated by: $100 \times (\text{\# of times effect was observed/total number of trials})$. For example, if two studies examined fecundity in one species, and each study examined 3 doses for each of 2 instars, the denominator for the calculation of frequency would be 12 for that species. Frequency data were summarized graphically by combining specific traits into general categories (development rate, weight, adult longevity and reproduction). For example, if fecundity reduction was observed in 4 of 12 trials while fertility reduction was observed in 8 of 12 trials for a given species, the frequency of effects on reproduction would be 12 of 24 or 50%. Each host species was given equal weighting when calculating mean frequencies across all species, regardless of the number of studies or trials within each species. Magnitude of effects was also included in the summary of frequency data.

Net reproductive rates

For each host species, I calculated net reproductive rates or the number of times a population could multiply per generation (R_o) (Southwood 1991) for each trial (dose, instar, study) for control and treated samples. Net reproductive rate is calculated as follows:

$$R_o = \Sigma lx.mx \qquad \text{(Equation 2.1)}$$

where lx is the proportion of females alive during a given age interval (x) and mx is the age specific fertility or the number of living females born/female in each age interval. Because Lepidoptera reproduce as adults only, Equation 2.1 is simplified to: $R_o = l.m$. For all calculations, sex ratios were assumed to be 50:50, and mortality values given for the pupal stage were assumed to indicate apparent mortality (Southwood 1991) unless otherwise indicated. For example, a control sample with 40% survival to adult emergence, mean fecundity of 100, and mean percentage viable eggs of 80 would result in a calculated net reproductive rate of .4x(100/2)x.8 or 16 (fecundity is halved to estimate the number of new females). Percent reduction in R_o due to treatment with pathogens was calculated by: 100x[1-(R_o treated/R_o control)]. Treatment effects were examined in three ways to assess percent reduction in R_o: (1) due to mortality (m), (2) mortality and debilitating effects (m+d) and (3) debilitating effects alone (d). R_o (m) was calculated based on survival in the treated group and values for 'qualitative' traits (e.g. fecundity, egg viability, mating success etc.) observed in the controls. R_o (m+d) was calculated based on both survival

and values for qualitative traits observed in the treated sample. R_o (d) was calculated using control survival and values for qualitative traits observed in the treated group. For example, if treatment with a pathogen resulted in 20% survival, a fecundity of 50 and 60% viable eggs, then using values for the control example above, $R_o(m) = .2 \times (100/2) \times .8 = 8$, $R_o(m+d) = .2 \times (50/2) \times .6 = 3$, and $R_o(d) = .4 \times (50/2) \times .6 = 6$. Percent reduction in potential growth for the three examples would then be 50, 81 and 63%. Mean percent reduction in R_o was calculated within each species across all doses, instars and studies (equal weighting given to each trial). Similarly, mean percent reduction in R_o was calculated across all species per pathogen, giving equal weighting to each species. In studies where mean fecundity was not examined, mean weight of female pupae or mean pupal weight (unsexed) was used in calculations as pupal weight and fecundity are correlated in Lepidoptera. Although this does not yield a true R_o value, it allows one to calculate percentage reduction in R_o when comparing treated and control samples.

2.3 Results

Frequency and magnitude of debilitating effects

Results of the literature survey are given in Tables 2.1 to 2.3. The majority of studies show that mortality from disease is accompanied by qualitative changes in the surviving hosts (Tables 2.2 and 2.3). Pathogen treatment can reduce adult longevity, mating and oviposition success, fertility, weight,

fecundity and egg viability and rate of development, although cases of increased development rates and pupal size have been observed. Magnoler (1974b) reported a decrease in the duration of the pupal stage following treatment of *Lymantria dispar* with CPV, while Magnoler (1974a) reported a small increase in male pupal weight following NPV treatment of the same host species.

The frequency and magnitude of debilitating effects were generally greater for CPV than NPV treated hosts, although frequency of effects on reproduction approached 50% for NPV treated hosts (Figure 2.1). A Mann-Whitney U test showed a significantly greater frequency of cases of reduced weight (pupal and/or adult) in CPV than NPV treated hosts (p<0.010, one tailed test, n=8(CPV) and 11 (NPV)). No other comparisons yielded significant differences in frequencies although all tests had limited power due to small sample sizes. In general, weight and variables directly influencing reproduction were examined more frequently than developmental rates or adult longevity in species treated with NPVs and CPVs (Figure 2.1).

Testing for viral infection
Surviving pupae or adults were surveyed for inclusion bodies in a greater proportion of studies using CPVs (10/14 or 71%) than of those using NPVs (5/19 or 26%). A greater proportion of these tests confirmed the presence of

inclusion bodies in at least some trials for CPVs (9/10 or 90%) than for NPVs (2/5 or 40%). In the remaining studies of CPVs, pupae or adults were not tested, but some evidence for infection was reported. In their study of *Pectinophora gossypiella*, Ignoffo and Adams (1966) inoculated larvae using CPV obtained from previously infected adults. Bullock *et al.* (1970) tested adults but did not report the results although a sample of larvae were tested and found to contain virus. Mohamed *et al.* (1989) screened a sample of larvae only and reported 96% infection. Bird (1969) observed 100% infection in treated *Choristoneura fumiferana* larvae, but in a separate experiment involving debilitating effects of virus did not establish the presence of infection in survivors. In the remaining studies of NPVs, Young (1990) looked for inclusion bodies but did not report the results. Amongst studies of granulosis virus, GV, Melamed-Madjar and Raccah (1979) tested for and confirmed infection in 60% of the pupae, but no test for infection was carried out by Sait *et al.* (1995).

Disease effects on net reproductive rates

In most trials examined in this survey, net reproductive rates (R_o) were reduced when debilitating effects were included in my calculations compared to R_o based on mortality alone (110/126 or 87% of all trials for all pathogens groups). This effect is illustrated for NPV and CPV treated hosts in Figure 2.2. In the remaining 13%, increases in R_o may be attributed to chance events

although selection for more vigorous individuals following treatment with virus is a plausible mechanism. Considering NPVs and CPVs, for which there was sufficient replication (i.e. number of species tested), the difference between mortality effects and combined mortality and debilitating effects on R_o (i.e. % reduction in $R_o(m+d)$-% reduction in $R_o(m)$) was calculated for each host species. Nonparametric paired tests on these data showed that debilitating effects significantly reduced R_o beyond the effects of mortality alone (NPV; P<.010, n=13, CPV; P=.010, n=7). Note that sample size is only seven for tests on CPV because mortality was not given for three of the host species treated with this pathogen. Surprisingly, the magnitude of reduction in R_o was similar for species treated with NPV and CPV, when debilitating effects were considered in addition to mortality (i.e. (m+d)-m) (Fig. 2.2) (mean difference: NPV; 22 ± 7%SE, n=13, CPV; 19 ± 6%SE, n=7) or alone (d) (NPV; 34 ± 8%S.E., n=13, CPV; 32 ± 10%S.E., n=10). For hosts treated with GVs, EPV and small RNA viruses, reductions in R_o from debilitating effects in addition to mortality were 11 ± 6% (n=2 species) 25% (n=1), and 5% (n=1) respectively.

Instar dependence

To examine instar dependence, percent reduction in R_o due to debilitating effects alone (d) are plotted against larval instar at the time of inoculation with NPVs and CPVs regardless of host species (Fig. 2.3). Inoculations prior to hatch (i.e. virus treatment of eggs, progeny of virus treated parents) until

larvae were two days old, were scored as first instar. Studies involving older larvae (>2 days) which did not give larval instar were excluded. If more than one trial was performed per instar within a study (i.e. using different doses), percent reduction in R_o was calculated using a mean R_o across doses. NPV treated hosts showed increased debilitating effects at later instars, while this trend was not evident for CPV treated hosts (Fig. 2.3). Based on linear regression, larval instar explained 27 and .1% of the variation in reduction of R_o for NPVs (n=25) and CPVs (n=15), respectively (significance levels are not reported as data are not independent). Similarly, if we consider reductions in weight of female or unsexed pupae and measures directly affecting reproduction (i.e. m,o,of,f,v; see Table 2.1), and score the presence of these effects in at least one dose per instar as 1, debilitating effects were more frequent in late instars, IV-VI (8/12 or 67%) than in early instars, I-III (3/13 or 23%) for NPV treated hosts. This trend was not evident for CPV treated hosts (instars I-III; 11/12 or 92%, instars IV-VI; 5/5 or 100%).

Dose dependence

To examine dose dependence, the mean percent reduction in R_o from debilitating effects alone was calculated for the low(est) and high(est) dose from each instar and study within each species (equal weighting for all trials). Nonparametric paired tests on these data showed no consistent or significant effect of dose. The trend was toward greater debilitating effects at higher

doses of NPV. The mean difference between reduction in R_o at high and low

doses was $11\pm7\%$S.E. (n=7). This was not the case for CPV treated hosts (mean

difference=$-4\pm6\%$S.E., n=4).

2.4 Discussion

Frequency and magnitude of debilitating effects

As predicted, debilitating effects are more common and more severe amongst

CPV than NPV treated hosts (Fig. 2.1) but are commonly observed among

studies of NPVs. One potential problem with this result is the greater

probability of publication of studies showing positive results. However,

debilitating effects are rarely the sole focus of reported studies, and multiple

studies showing negative results have been published (see *Lymantria dispar* and

Trichoplusia ni, Table 2.2). Further, a differential effect of this bias between

NPV and CPV is not expected. Even so, a bias against studies showing

negative results might still be expected overall and should be considered when

interpreting frequency of effects within a pathogen group.

Reductions in net reproductive rates

Examination of the frequency and magnitude of debilitating effects alone

(Tables 2.2-2.3, Fig. 2.1) can not readily be related to potential growth of host

populations. Statistically significant changes in individual quality do not

necessarily imply biological significance, and consideration of each type of

debilitating effect independently (e.g. fecundity, percent hatch, percent oviposition etc.) can underestimate effects of pathogens. The use of net reproductive rates (R_o) provides a method by which a suite of individual characteristics can be considered in concert, and related to potential growth of populations. Further, the use of R_o allows one to consider debilitating effects in the context of mortality caused by the disease. This is the most meaningful approach since debilitating effects will be more important to the population dynamics of the host if the associated percent mortality of the host population is low. For example, a 50% reduction in reproductive capacity in a population experiencing 90% mortality from disease will only reduce population growth by a further 5%. If the same population experiences 10% mortality, debilitating effects will reduce population growth by a further 45%. Across published studies on NPV, calculations of R_o that include debilitating effects (m+d) led to significantly greater reductions in net reproductive rate when compared to calculations based on mortality alone (m). Inclusion of debilitating effects, on average, reduces potential population growth by a further 22% in hosts treated with NPV.

Surprisingly, the additional reduction in R_o caused by debilitating effects (m+d)-m) was similar for CPV and NPV treated hosts (Fig. 2.2) despite greater magnitude and frequency of effects following CPV treatment (Fig. 2.1). There are several reasons for this result. First, calculation of R_o does not incorporate

changes in development rate or adult longevity. Delayed development may influence population natality by increasing the individual's exposure and thus susceptibility to mortality agents. Rates of population growth may also be reduced by increased generation time for host species that do not have a fixed number of generations per year. However, without further study it is difficult to quantify effects of development rates on population growth. Longevity of the reproductive stage (adult) will also affect reproductive output. Individuals of species that oviposit only once during adult life may not survive long enough to mate and successfully lay eggs. Individuals of species that lay eggs throughout adult life may show reduced total reproductive output due to reduced lifespan. Although these factors may contribute to the reduced fecundity and egg viability reported in many studies, the effects of reduced adult lifespan are again difficult to quantify directly.

Second, although pupal weight and fecundity are often highly correlated, using pupal weights to calculate R_0 may underestimate effects of treatment with pathogens. In studies that examine pupal weight and fecundity, the magnitude of treatment effects on fecundity are often greater than on female pupal weight, or pupal weight of both sexes combined (e.g. see Geier and Oswald 1977, Rothman and Myers 1994). All studies finding debilitating effects and examining both pupal (or adult) weights and fecundity reported greater magnitude of effect on fecundity for both NPVs (6/6) and CPVs (3/3).

Therefore, disease may directly affect host reproductive organs, eggs, or processes involved in conversion of energy stores to eggs (Rothman and Myers 1994) in addition to the indirect effect of disease on fecundity via reduced weight (pupal or adult). Studies of CPVs examined fecundity less frequently, and in some cases did not quantify a reduction in fecundity (and so were not used for estimates of population growth) (e.g. Neilson 1965, Ignoffo and Adams 1966). This made it impossible to assess the potential effects of fecundity reduction on population growth. Six of 14 studies involving CPVs (43%) examined and quantified changes in mean fecundity compared to 13 of 19 (68%) studies involving NPVs.

Finally, examination of the reproductive characteristics of adults, in general, was less frequent among host species when larvae were treated with CPVs than with NPVs (Fig. 2.1). Further, reproductive characteristics other than fecundity were more frequently examined and quantified following NPV treatment (12/19 or 63% of studies) than CPVs treatment (3/14 or 21% of studies) (and see Tables 2.1-2.3). Examination of measures such as mating and oviposition success and particularly egg viability allow greater potential for observing reductions in net reproductive rates. In order to fully quantify potential effects of pathogens on host populations, it is therefore important to examine effects on adults such as mating success, oviposition success and particularly fecundity and percent hatch of offspring.

Mechanisms of debilitating effects

Sublethal infection is the most likely mechanism for debilitating effects of viral disease reported in this survey, particularly among the more benign CPVs. Sublethal effects may be due to the diversion of host energy reserves to support or combat the pathogen (Sikorowski and Thompson 1979, Wiygul and Sikorowski 1978, 1991), disruption of oocyte development (Neilson 1965) or hormonal changes induced by the pathogen (O'Reilly and Miller 1989, Burand and Park 1992, Park *et al.* 1993). For CPVs, infection is limited to particular tissues rather than being spread through the whole body as occurs with NPV. For example, CPV replicates in the epithelial cells lining the midgut but new cells continue to differentiate (Evans and Entwistle 1987). Although the efficiency of digestion is reduced with infection, death of caterpillars usually only occurs with infection at a very young stage, or if cross infected with another pathogen such as bacteria (Sikorowski and Lawrence 1994).

Sublethal effects remain a contentious issue particularly with respect to more pathogenic baculoviruses such as NPV. Sait *et al.* (1994) have criticized previous studies which used the contaminated diet method for host inoculation, the most common method of inoculation in the survey (Tables 2.2 and 2.3). By allowing individuals to feed on contaminated diet for extended periods, smaller and more slowly developing individuals may survive by ingesting the pathogen more slowly than more vigorous individuals feeding at

faster rates (Sait *et al.* 1994). Selection rather than sublethal infection could be a cause of reported changes in host quality. In their study of sublethal GV infection of *Plodia interpunctella*, Sait *et al.* (1994) observed GV effects on development rate and host reproductive capacity using a modified droplet technique where individuals ingest virus within a fixed and short time period (1-2 hours).

Regardless of the dosing technique, sublethal effects can only be absolutely confirmed by the examination of survivors for the presence of virus inclusion bodies or viral DNA or RNA. Thus the evidence for true sublethal effects is scant for NPVs but good for CPVs infecting Lepidoptera. This could reflect a difference in emphasis of research on the two virus groups. Because NPVs have received more attention as potential biological control agents, the emphasis may be on establishing the presence and magnitude of virus effects on field populations rather than the specific mechanisms causing these effects. This could also explain why the data on the impact of debilitating effects is more complete for studies of NPVs than CPVs. Confirmation of sublethal infection becomes important when considering the potential for vertical transmission of virus from parents to offspring within eggs or on egg surfaces. How important sublethal infection and vertical transmission may be to subsequent host generations will depend on the strength of other routes of virus transmission between generations such as via environmental

contamination. Examination of this issue is beyond the scope of this study.

Disease effects on host males

Pathogens could in some host species influence population growth through their effects on male survivors. The influence of reduced male weight on reproduction is not well understood but small males may have reduced capacity to inseminate females (Haukioja and Neuvonen 1985). Matings with virus treated males and untreated females can also lead to reduced reproductive output. Santiago-Alvarez and Vargas Osuna (1988) observed reduced egg fertility following matings with NPV treated *Spodoptera littoralis* males and suggested higher production of empty spermatophores or introduction of improperly oriented spermatophores as possible explanations for this result. Similarly, Kellen and Hoffmann (1983) observed reductions in the proportion of females laying fertile eggs following matings with *Amyelois transitella* males treated with small RNA virus, while Sait *et al.* (1994) observed reductions in the number of eggs laid and percent hatch following mating with GV treated *Plodia interpunctella* males. Tanada and Tanabe (1964) found no effect of CPV treatment on fecundity of *Pseudaletia unipuncta* when both males and females had been inoculated (Table 2.3), but observed a trend toward reduced number of eggs laid when treated males were mated with untreated females. Melamed-Madjar and Raccah (1979) found no effect of GV treated males on oviposition of fertile eggs in *Sesamia nonagrioides.*

Instar dependence

Results of the literature survey suggest that debilitating effects following NPV treatment are more likely to be observed late in larval development (e.g. Figure 2.3). This trend is not evident for CPV treated hosts. Last instar larvae should be the starting point for studies on debilitating effects of NPVs. Because NPVs are more pathogenic than CPVs, individuals infected earlier in development should succumb to disease more frequently. NPV epizootics are often characterized by heavy mortality of late instars, and the release of large amounts of inoculum into the environment due to within generation dynamics of pathogen transmission (Woods and Elkinton 1987, Dwyer and Elkinton 1993). Exposure of larvae to this inoculum prior to pupation could result in partial infection, as viral replication may be suppressed at pupation (Watanabe 1987). This could result in reduced individual vigour and reserves for egg provisioning without killing the host.

Of course, this examination of instar dependence may be confounded by host taxonomy as no distinction between species was made in Figure 2.3, nor in the calculations of frequency of effects in early and late instars. For example, the absence of debilitating effects of NPV observed in *T. ni* may be because such effects do not occur in this species, or because only early instars were examined. To examine instar dependence more rigorously, studies examining a range of instars are required. In the study by Sait *et al.* (1994), instar-

dependent increase in development time was observed in *P. interpunctella*, following GV treatment, but there was no instar effects on reproductive output (Table 2.3). Vargas Osuna and Santiago-Alvarez (1988) observed reduced egg viability of *S. littoralis* individuals treated with NPV in instars IV-VI but not in instar III. Young and Yearian (1982) observed reductions in egg viability and fecundity following NPV treatment of *Pseudoplusia includens* at instar VI, not at instars IV and V (Table 2.2).

Dose dependence

Strong evidence for dose dependent debilitating effects among NPV or CPV infected hosts was not found in this study. However, unpublished examples of dose related influence of NPV on pupal weight have occurred for *L. dispar* (Myers and Malakar, unpublished) and *M. c. pluviale* (Kukan, unpublished). In *L. dispar*, Shapiro and Robertson (1987) reported a decrease in egg mass weight with increasing doses of NPV while in the same species, Magnoler (1974b) observed a general increase in pupal weights with increasing doses following CPV treatment. Sait *et al.* (1994) reported decreases in percent hatch, egg production, female pupal weight, and increases in development time with increasing dose of GV in at least one of five instars of *P. interpunctella* tested. Dose dependence was not examined in any other studies reporting debilitating effects of disease.

Conclusions

Debilitating effects in host Lepidoptera following pathogen treatment occur primarily with less pathogenic diseases, but may also be common with NPVs. Debilitating effects following exposure to NPV and CPV reduced the net reproductive rate of host samples. Effects of disease on host reproduction and development should be considered in the construction of models and collection and analyses of field data (e.g. life table studies) aimed at understanding the population dynamics of Lepidoptera, and the evaluation of the potential of viruses for biological control. The importance of viruses may be greatly underestimated by considering mortality alone.

Table 2.1. Explanation of symbols used in Tables 2.2 and 2.3.

Development rate

dt	Total development time (inoculation to adult emergence).
dl,dlm,dlf, dlmf	Duration of larval stage (unsexed), males, females. As above, both sexes.
dp,dpm,dpf dpmf	Duration of pupal stage (unsexed), males, females. As above, both sexes.

Weight

wp,wpm,wpf wpmf	Pupal weight (unsexed), males, females. As above, both sexes.
wa,wam,waf wamf	Adult weight (unsexed), males, females. As above both sexes.
we	Weight of egg mass.

Adult longevity

la,lam,laf lamf	Adult longevity (unsexed), males, females. As above, both sexes.

Reproduction

m	Percent mated females.
o	Percent ovipositing females.
of	Percent females ovipositing fertile eggs.
f	Fecundity or egg production.
v	Percent viable eggs or percent hatch.

Test for presence of inclusion bodies (infection) in pupal or adult tissue

ni	No test performed.
i-	Test performed - infection not found.
i?	Test performed - results not given.
i*	Test performed - infection confirmed.

Table 2.2. Summary of effects of NPV treatment on host Lepidoptera. For effects on survivors, disease causes reduced development rate, weight, adult longevity and reproduction unless otherwise indicated (by superscript ^{neg}). Symbols listed under "effects on survivors examined" are for all doses listed. Symbols in parentheses under "effects on survivors observed" indicate that no statistical tests were performed. Symbols in parentheses following species names indicate test for infection and results. Magnitude of effects are indicated by: S-small (<15%), M-moderate (15-30%) and L - (>30%). See Table 2.1 for explanation of all symbols.

Host	Inoculation method	Dose (#IB)	Instar/age[a]	Effect(s) on survivors Examined	Effect(s) on survivors Observed	Reference
Lymantria dispar (i*)	diet	.69,6.9	I	waf	-	Doane (1967)
		.69,6.9	II		-	
		.69,6.9/mm²	III		-	
(ni)	diet	25-5000 (5 doses) 2.5x10⁴/larva	III	dlmf,wpmf	- dlm-S,wpm-S^{neg}	Magnoler (1974a)
(i*)	diet	500-5x10⁴ /larva	II	wpmf,m,we	(we-S-M)	Shapiro and Robertson (1987)[bc]
(i-)	droplet feeding	2500/larva	IV	wpmf,f	-	Murray *et al.* (1991)
Stilpnotia (*Leucoma*) *salicis* (ni)	field spray	2x10⁹/tree	?	dlmf,wpmf, f,v	(dlm-M,dlf-L),wpm-L, wpf-M,(f-L,v-L)	Nef (1971)
Choristoneura fumiferana (ni)	field spray	1x10¹¹/acre	IV	v	(v-L)	Morris *et al.* (1974)

Table 2.2 cont.

Host	Inoculation method	Dose (#IB)	Instar/ age[a]	Effect(s) on survivors Examined	Observed	Reference
Epiphyas postvittana (ni)	diet	1600/ 25 larvae	10	dt,wp,wa, f,v	wa-S,f-L	Geier and Oswald (1977)
Malacosoma neustria (ni)	diet	3-3000 (4 doses) 3-3000/larva (4 doses)	III IV	wpmf	- -	Magnoler (1975)
M. californicum pluviale (ni)	leaf disc	4000 4000/larva	V	wpmf,f	wpf-S,f-S wpm-S,wpf-S,f-M	Rothman and Myers (1994)
Trichoplusia ni (i-)	diet	1708- 1.708×10^5 /larva (3 doses)	I	dl,wpmf la,f,v	-	Vail and Hall (1969)[d]
(ni)	diet	5×10^4- 1.5×10^6/ml (7 doses)	II	wp	-	Ignoffo (1964)
Heliothis zea (ni)	diet	.14,.92 $4.8/mm^2$	III	dp,lamf,f,v	- f-L	Luttrell *et al.* (1982)
(ni)	diet	$3-292/mm^2$ (7 doses)	II- III	wp	-	Ignoffo (1965)

Table 2.2 cont.

Host	Inoculation method	Dose (#IB)	Instar/age[a]	Effect(s) on survivors Examined	Observed	Reference
Pseudoplusia includens (ni)	diet	2000-5x10^4 (3 doses)	IV	wpmf,lamf f,v	-	Young and Yearian (1982)
		3500-1x10^5 (3 doses)	V		-	
		8600,8.6x10^4, 8.6x10^5/mm^2	VI		- f-L,v-L	
Spodoptera littoralis (ni)	leaf disc	8x10^3	III	dl,dp,la	-	Vargas Osuna and Santiago-Alvarez (1988)[e]
		1.6x10^4	IV	f,v	v-L	
		8x10^4	V		v-L	
		4x10^5/larva	VI		v-L	
(ni)	leaf disc	7.8x10$^{5-6}$	III	dp,wp,lamf, o,f,v	(dp-M,o-L,f-M)	Abul-Nasr, Ammar and Abul-Ela (1979)[f]
		1.2x10$^{6-7}$	III	dp,wp,lamf,	(wp-S,laf-M,f-M)	
		1.2x10$^{6-7}$ /larva	V	f,v	(wp-S,f-L)	
S. ornithogalli (i?)	diet	10^7/larva	IV	dpmf, lamf,f,v	f-L	Young (1990)
S. frugiperda (ni)	diet	87,142/mm^2	2	wp,f,v	-	Perelle and Harper (1986)

Table 2.2 cont.

Host	Inoculation method	Dose (#IB)	Instar/ age[a]	Effect(s) on survivors Examined	Observed	Reference
Mythimna (*Pseudaletia*) *separata* (ni)	diet	LC25 LC50	8	wpmf,wamf, dl,dp,lamf, f,v	both doses: wpmf-S,wamf-S, dl-S,dp-S,lamf-S-M,f-L	Patil *et al.* (1989)
			progeny		both doses: wpmf-S,wamf-S, dl-S,dp-S,lamf-S-M,f-L	

[a] Instar or age denoted by roman or arabic numerals respectively.

[b] R_0 based on midpoints of mortality classes.

[c] R_0 based on larval survival only. (No information on adult emergence given).

[d] R_0 based on mean of experiment with and without formalin.

[e] Similar results given for 24-36 hour old fourth and fifth instars.

[f] Fecundity estimates incorporate adult longevity.

[g] Author measured pupal size rather than pupal weight.

[h] Vail and Gough (1970) also report effects on fecundity but results are not given in a form amenable to this analyses.

[i] R_0 based on mean larval survival for doses within each instar.

Table 2.3. Summary of effects of CPV, GV, EPV and small RNA viral treatment on host Lepidoptera. Columns, symbols and footnotes are as in Table 2.2.

Host	Inoculation method	Dose (#IB)	Instar/age[a]	Effect(s) on survivors Examined	Effect(s) on survivors Observed	Reference
CPV						
Lymantria dispar (i*)	diet	$4\text{-}4\times10^7$ (8 doses)	III	wpmf dlmf,dpmf	all doses: dlm-L,dlf-L, dpmf-S^{neg},wpmf-L	Magnoler (1974b)
		$4\text{-}4\times10^7$/larva (8 doses)	IV		all doses: dlmf-L,dpm-S-M^{neg} dpf-0-S, wpmf-L	
Choristoneura fumiferana (ni)	sprayed diet sprayed foliage	200- 2×10^6/larva (8 doses)	II III IV	wpmf,dlmf	(dlm-L, dlf-M, wpmf-M) (dlmf-M, wpmf-S) (wpmf-S)	Bird (1969)[c,g]
Trichoplusia ni (i*)	diet	.5-10 (4 doses) 1 10 100 1000/mm²	I IV	dt,wpmf	(wpmf-S) (wpmf-M,dt) (wpm-S,wpf-M,dt) (wpm-M,wpf-L,dt) (wpmf-M,dt)	Vail, Hall and Gough (1969)[h]
Heliothis virescens (i*)	egg mass immersion	10^4/ml	egg	dl,dp,wp, la,f	dl-M,wp-M, la-M,f-L	Simmons and Sikorowski (1973)
(i*)	egg mass immersion	6.6×10^6/ml	egg	dl,lamf,f	(dl-L),f-L	Sikorowski and Thompson (1979)
(ni)	diet	8×10^5/larva	III	wpmf,f,v	wpm-M,f-L	Mohamed *et al.* (1989)

Table 2.3 cont.

Host	Inoculation method	Dose (#IB)	Instar/age[a]	Effect(s) on survivors Examined	Effect(s) on survivors Observed	Reference
H. zea (ni)	leaf disc	2×10^4/larva	5	dt,wp,wa	dt-S,wa-S	Bong and Sikorowski (1991)
Pseudaletia unipuncta (i*)	dipped leaves	"high conc."	II-III	lamf,f	-	Tanada and Tanabe (1964)
Mamestra brassicae (i*)	leaf disc	137/larva	III	lamf,f,v	v-M	Maleki-Milani (1970)[c]
Pectinophora gossypiella (ni)	diet	73.6-7356.3/mm^2 (3 doses)	I	dl,dt,wp, la,f,v	all doses: (dl-M-L,dt-S-M f,v,la),wp-S-M	Ignoffo and Adams (1966)
(i?)	diet	100/mm^2	I	lamf,wpmf, m,f,v	wpmf-M,lamf-L, (f-L)	Bullock *et al.* (1970)
(i*)	diet	1000 10^4 10^5 10^6/ml	I	dlmf,dpmf, wpmf	wpmf-M dlmf-L,wpmf-M dlmf-L,wpmf-M dlmf-L,wpmf-M	Bell and Kanavel (1976)[c]

Table 2.3 cont.

Host	Inoculation method	Dose (#IB)	Instar/age[a]	Effect(s) on survivors Examined	Observed	Reference
Alsophila pometaria (i*)	sprayed foliage	?	late	wpmf,f,v	wpm-M,wpf-L (f,v)	Neilson (1965)
Nymphalis antiopa (i*)	sprayed foliage	?	late	wp,f,v	wp-M,(f,v)	Neilson (1965)
GV						
Plodia interpunctella (ni)	droplet (treated females only)	$2.12 \times 10^{1-3}$ $6.3 \times 10^{1-3}$ $2.98 \times 10^{3-5}$ $4.83 \times 10^{4-6}$ $6.36 \times 10^{4-6}$ /larva	I II III IV V	dt,wp,la,f,v	- f-M,v-S f-M,v-S dt-S,v-S dt-S,f-M,v-S	Sait et al. (1994)[c,i]
Sesamia nonagrioides (i*)	diet	1.7×10^{7}/ml of diet	7-21	dp,of,f,v	(of)	Melamed-Madjar and Raccah (1979)
Small RNA viruses						
Amyelois transitella (ni)	diet	5ng virus/g of diet	III -IV	wpmf,of,f,v	wpmf-L, (of-L),f-L	Kellen and Hoffmann (1983)
EPV						
Choristoneura fumiferana (ni)	field spray	7.6×10^{10}/ acre	IV	v	(v-L)	Morris et al. (1974)
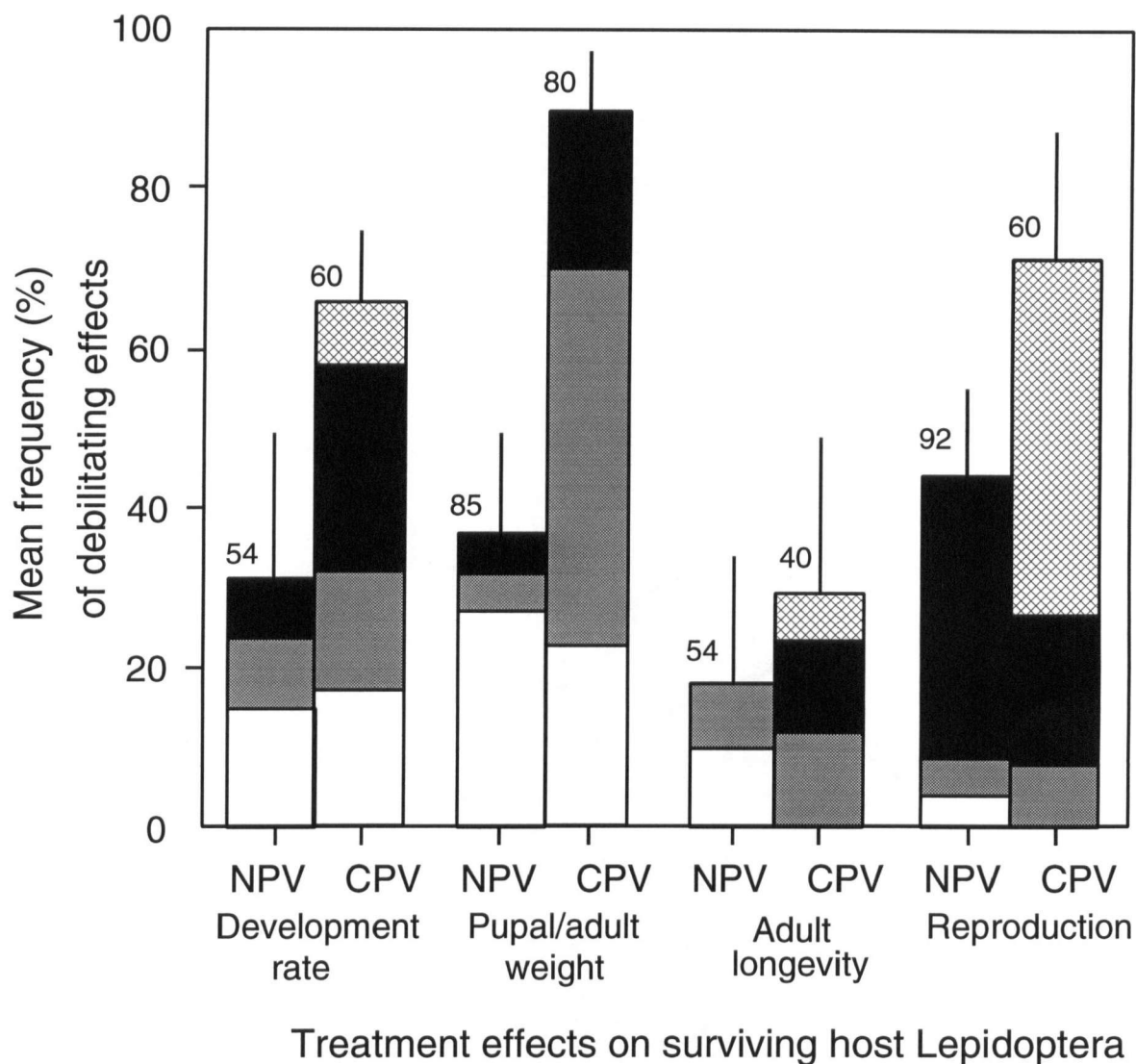
Figure 2.1. Mean frequency (%) of debilitating effects/host species (+SE) in survivors of NPV and CPV treated Lepidoptera. Magnitude of effects are: small (<15%) - unshaded bars; moderate (15-30%) - shaded bars; large (>30%) - black bars; magnitude unknown - hatched bars. Numbers above bars indicate percentage of species in survey for which development rate, weight, adult longevity and reproduction were examined in at least one study. See Table 2.1 for explanation of specific variables measured for these general categories (x-axis headings).
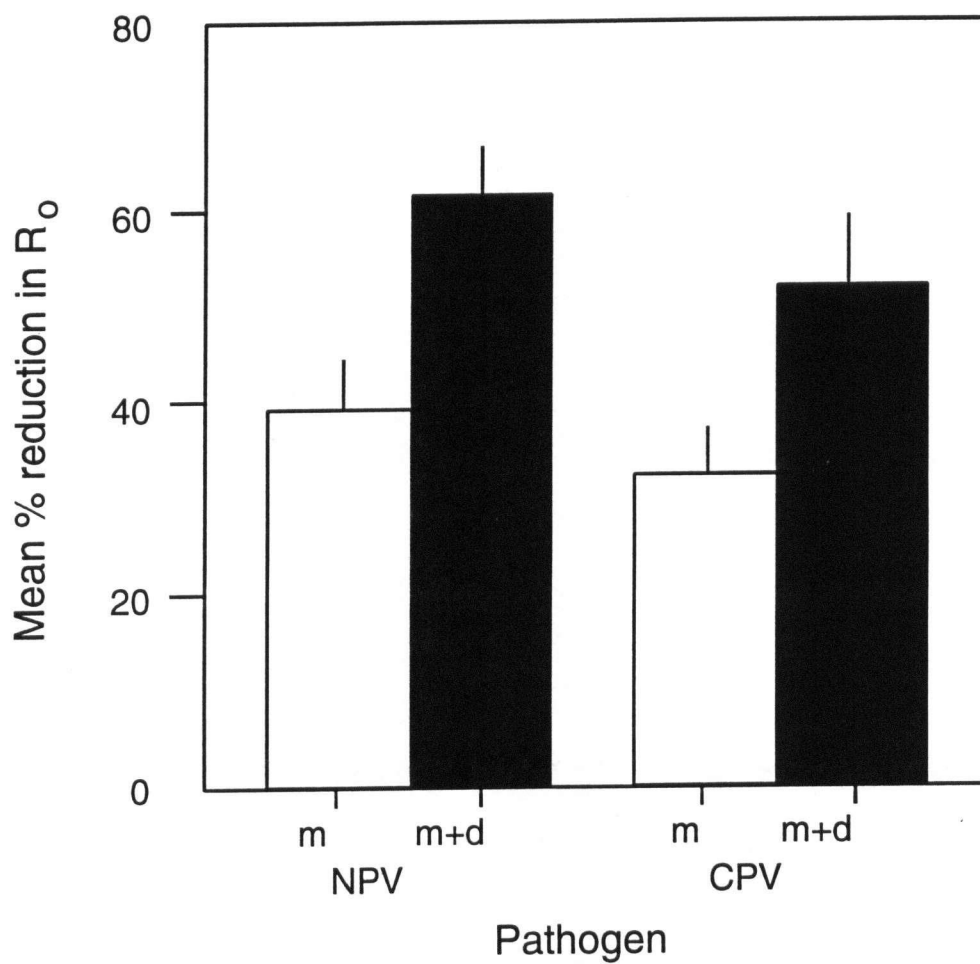
Figure 2.2. Mean percent reduction in net reproductive rate (R_o) (+SE) due to mortality alone (m) and mortality and debilitating effects (m+d) following treatment of host samples with NPVs and CPVs.
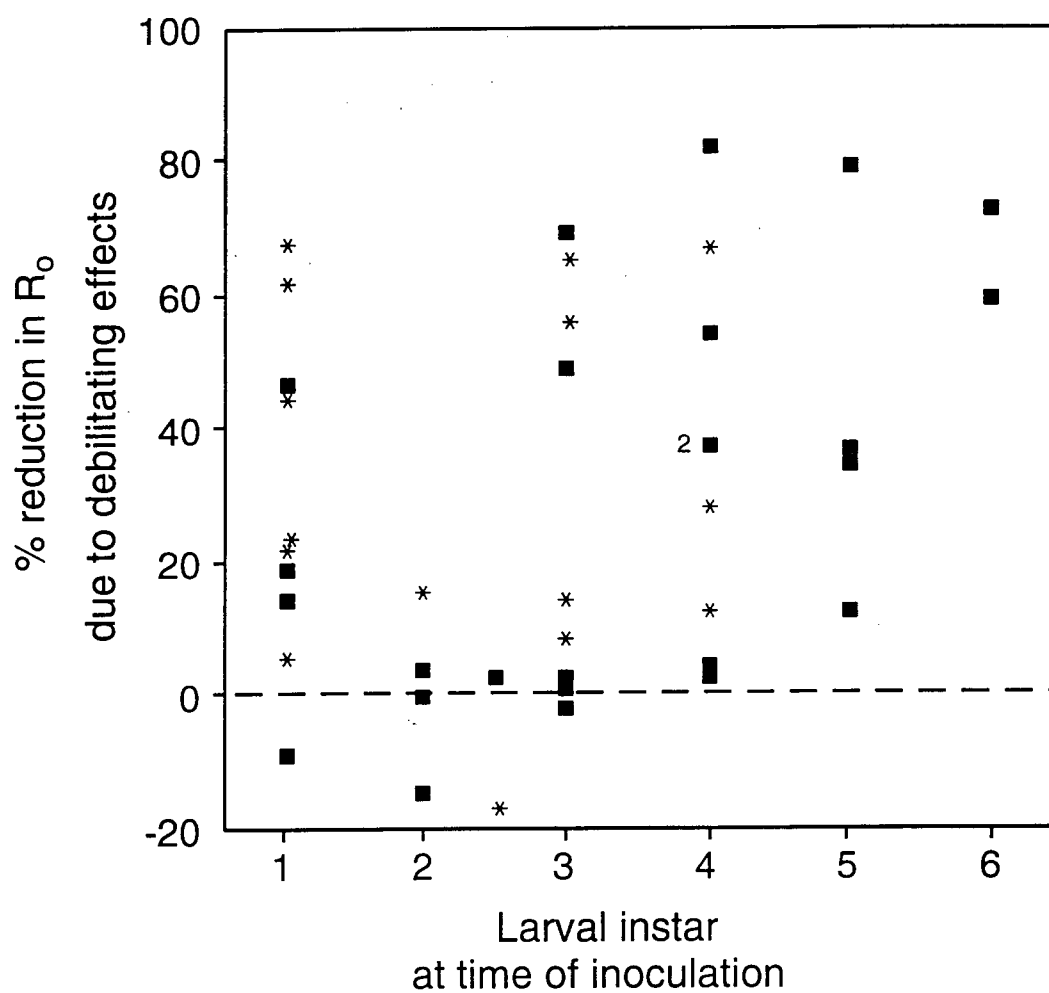
Figure 2.3. Effects of larval instar on percent reduction in net reproductive rate
(R_o) due to debilitating effects alone in survivors of NPV (squares) and CPV
(asterisks) treated hosts.

3. INFLUENCE OF VIRUS TREATMENT ON TENT CATERPILLAR REPRODUCTION IN THE LABORATORY

3.1 Introduction

Changes in fecundity are an important but often overlooked aspect of population dynamics. Reduced fecundity or pupal weight, or both, at or following high densities is commonly reported among herbivorous Lepidoptera (e.g. Hodson 1941, Miller 1963, Embree 1965, Klomp 1966, Foltz *et al.* 1972, see Baltensweiler *et al.* 1977, Mason *et al.* 1977, Campbell 1978, Dempster and Lakhani 1979, Barbour 1988, Myers 1988, 1990, Munster-Swendsen 1991) and may be a contributing factor to population declines.

In the past decade, perhaps the most common explanation for reduced fecundity among lepidopterans has been induction of defensive responses by the host food plant. Feeding by herbivorous Lepidoptera may induce changes in foliage composition that are detrimental to the herbivore in the current (rapidly induced resistance) or subsequent (delayed induced resistance) generation(s) (for reviews see Fowler and Lawton 1985, Karban and Myers 1989, Haukioja 1990). Induced plant responses have been observed in many but not all studies and often the effect is slight (Fowler and Lawton 1985). Furthermore, induced responses are sometimes associated with increases in reproductive potential of herbivores (Williams and Myers 1984, Roland and Myers 1987). Variability in results of induction experiments suggest that

alternative hypotheses should be explored.

Disease might explain reduced fecundity in lepidopteran populations. Reduced pupal weight or fecundity, or both, in hosts has been observed following treatment with protozoans (e.g. Gaugler and Brooks 1975, Bauer and Nordin 1989, Sajap and Lewis 1992) and several virus groups (e.g. Simmons and Sikorowski 1973, Magnoler 1974, Mohamed *et al.* 1989 [cytoplasmic polyhedrosis virus], Geier and Oswald 1977, Young and Yearian 1982, Young 1990 [nuclear polyhedrosis virus], Kellen and Hoffmann 1983 [small RNA virus]). However, with NPV sublethal effects are not always found (Vail and Hall 1969, Magnoler 1975, Perelle and Harper 1986, Murray *et al.* 1991). Variation in results could be associated with time of treatment with inoculation close to pupation being more likely to cause sublethal than lethal effects.

NPV infection commonly occurs at high densities of tent caterpillars, *Malacosoma* spp. (Clark 1956, 1958, Wellington 1962, Stairs 1965) and fecundity is often lower in declining populations (Myers 1990). In this chapter I examine whether exposure of caterpillars to NPV in the laboratory can reduce the reproductive potential of *M. c. pluviale*. This study is the first step toward understanding the possible role of NPV in reducing the fecundity of declining populations of the western tent caterpillar.

3.2 Methods

Extraction and identification of NPV

NPV was originally extracted from diseased *M. c. pluviale* larvae collected in Vancouver, British Columbia in 1986. NPV solution was prepared using a discontinuous sucrose gradient (see Kalmakoff and Wigley 1980) by Judith Myers. For these experiments, NPV was obtained from lab-reared *M. c. pluviale* caterpillars that died following inoculation and infection with virus. Cadavers were ground in distilled water and filtered through cheesecloth. The filtrate was centrifuged at 1,000 rpm for five minutes. The pellet was resuspended in distilled water and recentrifuged at 4,000 rpm (15 minutes) three times. The final pellet was resuspended in distilled water for treatment of larvae. Buffalo Black stained samples of virus in solution with albumen were prepared to estimate NPV concentration (see Kalmakoff and Wigley 1980) and to check for contaminants. A sample of stained (Buffalo Black) larval smears were prepared from individuals that had died following treatment with NPV in these experiments and examined for the presence of NPV.

Tent caterpillar rearing and inoculation

In 1992, late third and fourth instar caterpillars were collected in the city of Vancouver and Cypress Provincial Park, British Columbia between 20 April to 25 May 1992. Individuals were reared indoors in a naturally lit room at room temperature (17 to 28°C). Photoperiod and humidity were not controlled.

Third and fourth instars were reared in groups of five in 150 ml plastic cups

and individually at fifth instar in 60 ml plastic cups. Larvae were assigned

randomly to control and treatment groups at an approximate ratio of 1:1.5.

Family origin was not recorded and family effects due to genetic relatedness or

common environmental experience were not included in subsequent analyses

for the 1992 experiment.

Larvae were treated with NPV at fifth instar within 24 hours of moulting. Five

microliters of NPV solution was applied to an alder leaf disk at a concentration

of 8×10^5 PIBs/ml and fed to larvae. Leaf disks for control larvae received

distilled water only. Control and treated individuals that did not eat the entire

disk within 24 hours were discarded. Caterpillars were fed alder leaves every

second day until pupation. Leaves were collected from alders on the

University of British Columbia campus from areas with no recent history of

tent caterpillar infestation. Individuals were weighed within 48 hours of

pupation, and female moths were dissected to determine the number of eggs

per female.

In the autumn of 1992, *M. c. pluviale* egg masses were collected from an

outbreak population in Strathcona Provincial Park on Vancouver Island, British

Columbia. Twelve egg masses were soaked in 3% sodium hypochlorite until

the spumaline dissolved and then rinsed in running water. Egg masses were

taped to newly invading alder trees at a disturbed site near the University of
British Columbia campus on 19 May and colonies of fourth instars were
collected on 17 June 1993. Only eight of the initial 12 egg masses successfully
developed into fourth instar colonies. Individuals from each colony were
reared together in 1 L cardboard cups in an outdoor insectary. Foliage was
collected as in 1992 and changed every other day. Unlike the experiment in
1992 (above), family groups were maintained and recorded. Individuals from
each family were assigned to control and treatment groups at a ratio of 1:2 as
they reached fifth instar and reared in 60 ml plastic cups. NPV dosage and
method of treatment were the same as in the 1992 experiment.

Statistical analyses

Differences between pupal weights of control and treated individuals and
fecundity of adults were compared using t-tests for the 1992 data. The 1993
data were analyzed using a mixed model analysis of variance (ANOVA) with
virus and family effects being fixed and random respectively (PROC GLM,
SAS Institute 1983). Regressions between pupal weight and fecundity for
control and treated females were calculated for the 1992 and 1993 data and
analysis of covariance (ANCOVA) was used to examine the effect of virus on
fecundity with variability due to pupal weight removed (PROC GLM, SAS
Institute 1983). To examine whether selection for reduced fecundity could be
mediated by NPV, the correlation (Pearson r) between percent mortality from

virus per family (treated) and mean fecundity per family (control) was calculated.

3.3 Results

Pupal weight and fecundity in 1992

Survival to adult was generally poor in this study largely because of bacterial disease; survival of controls from fifth instar to adult was 45 and 53% in 1992 and 1993 respectively. Percent host mortality from NPV treatment, calculated using Abbott's formula (Kalmakoff and Wigley 1980), was 64 and 30% in 1992 and 1993 respectively. In 1992, female and male pupae were significantly heavier and adult females more fecund in the control group than in the treated group (Table 3.1). Data on pupal weights in Table 1 include individuals that pupated but failed to emerge as adults. Considering only pupae that successfully completed development does not change these results, although the difference between control and treated groups is not as strong ($p<.025$ and $p<.05$ for females and males respectively). Exposure of larvae to NPV caused a 17% reduction in fecundity.

Pupal weight and fecundity in 1993

Results of the mixed model Anova showed significant effects of virus ($P<.01$) and family ($P<.001$) on female pupal weight in 1993 (Appendix A). The effects of virus and family are illustrated in Figure 3.1. In all but one family, mean

pupal weights were lower in the treated group than in the control group. The interaction term was not significant. For males, only family had a significant effect on pupal weight (P<.001), as the effect of virus was not consistent across families (Fig. 3.2, Appendix A). There was no significant interaction between virus and family. These results are based on data that included pupal weights of individuals not surviving to adult. Omission of these pupal weights does not alter the strength of family effects (same P values as above) though the effect of virus on female pupal weight becomes weaker (P<.05). Results of ANOVA on fecundity showed significant effects of virus (P<.01) and family (P<.001). All families showed reduced fecundity in treated females compared to control females (Fig. 3.3). Based on least squares means (mean of family means) and raw means (i.e. (total # eggs laid/treatment)/(total # of ovipositing females/treatment)), treated females showed a 17% (from 153.2 to 127.2) and a 7.4% (from 165.5 to 153.2) decrease in fecundity respectively. The correlation between mean fecundity per family (control) and percent virus mortality per family (treated) was negative but not significant (P>.10).

Pupal weight - fecundity relationships

Regressions between pupal weight and fecundity for control and treated groups were significant in both 1992 and 1993 (Figs. 3.4 and 3.5) although the fits are much tighter for 1993 data. ANCOVA results from 1992 showed a significant effect of virus (P<.025) on fecundity when variability due to pupal

weight was removed (i.e. the y-intercept of the treated group regression line was significantly lower than that of the control group regression line). No significant interaction between pupal weight and virus was present (i.e. the slopes of the two regression lines were not different) (Fig. 3.4, Appendix B). In 1993, neither slopes nor intercepts were significantly different when control and treated regression lines were compared (Fig. 3.5, Appendix B). Therefore, combining the data from control and treated groups in 1993 gives the following regression equation: $y=.368x+7.525$ with an r^2 of .88 (d.f.=57).

3.4 Discussion

Pupal weight and fecundity reductions

Treatment of *M. c. pluviale* larvae with NPV at fifth instar can reduce host reproductive potential. It also appears that greater lethality of virus dosage was associated with a more severe effect. Percent reduction in fecundity was greater in 1992 (when comparing raw means) and reduced male pupal weight was significant for 1992 only. However, pupal weight and fecundity were both greater for control and treated individuals in 1992 than in 1993 (perhaps caused by rearing conditions or intrinsic qualitative variation between source populations). Larger potential size may have allowed more scope for reductions in pupal weight and fecundity in 1992.

Inclusion of family effects in the ANOVA allowed for a more powerful test of virus effects in 1993 and also avoided the confounding effect of variation in

relative family contribution to control and treated samples. This potential confounding condition would be most severe in experiments using field collected individuals with gregarious habits, where siblings share common environmental experiences and similar inherited characteristics. However, family effects could also be important in experiments using laboratory strains of host insects.

The importance of female pupal weight as an indication of reproductive potential is evident from the correlations between pupal weight and fecundity (Figs. 3.4, 3.5). The relevance of male pupal weight to reproductive success is less well understood. Haukioja and Neuvonen (1985) observed reduced reproductive performance in small (<50 mg) males of *Epirrita autumnata*, though no correlation between male pupal weight and fertilization success was observed for larger males.

Mechanisms of fecundity reduction

Sublethal effects are the most probable cause of reduced reproductive potential but this was not confirmed by testing adults for the presence of virus. Sublethal effects may be the result of diversion of host energy from metabolism and growth to combat or support the pathogen (e.g. Bong and Sikorowski 1991). Reduced fatty acids (Sikorowski and Thompson 1979) and increased oxygen uptake (Wiygul and Sikorowski 1978, 1991) have been

observed in some Lepidoptera following viral infection. There is also evidence that effects of virus are under hormonal control (O'Reilly and Miller 1989). Results of the ANCOVA in 1992 suggest that virus could directly reduce egg production (Fig. 3.4). Reduced pupal weight does not fully explain lower fecundity of treated females. Fecundity is still lower when variation due to pupal weight is removed. NPV could be affecting host reproductive organs or the processes involved in conversion of energy stores to eggs. This pattern was not evident in the 1993 data (Fig. 3.5) suggesting that this added effect of virus may only be present with more lethal dosages.

Declines in fecundity of western tent caterpillar could also be due to selection following an NPV epizootic at high host densities (see Myers 1990). Smaller, less fecund individuals could be less susceptible to NPV than larger individuals which would result in indirect selection for reduced fecundity. However, the relationship between mean fecundity per family and percent virus mortality per family was negative and not significant. The trend was toward selection for larger, not smaller individuals.

Implications for field populations

The results of this study suggest that NPV may play a role in observed reductions in fecundity following outbreaks of the western tent caterpillar. In this species, viral disease is commonly associated with declining populations.

Fecundity reduction and population declines have been observed regardless of defoliation levels, type of food plant or infestation age (Myers 1990). Persistence of NPV in the environment following an epizootic may explain continued low fecundity for several generations following peak population densities in the field (Myers 1993). This explanation is tentative as laboratory rearing represents stressed conditions for the western tent caterpillar. Survival in these experiments was poor and fecundities of control moths in 1993 was low compared with those in wild populations. Furthermore, there may have been interactive effects of viral and bacterial infection on host reproduction. Field studies under more natural conditions will add further insight into the relative importance of viruses in reducing fecundity in populations of Lepidoptera.

Table 3.1. Effect of NPV treatment on *M. c. pluviale* pupal weight and fecundity in 1992.

| | Pupal weight (mg) | | # eggs/female | |
	C	T	C	T
Female				
Mean	486.8	444.0	224.90	187.55
SE	9.6	9.4	6.25	9.08
n	57	40	42	29
	t=3.07, P=.003[*]		t=3.49, P=<.001[*]	
Male				
Mean	254.4	234.7	-	-
SE	3.0	5.3		
n	108	63		
	t=3.49, P=.<.001[*]			

C, control; T, treated.
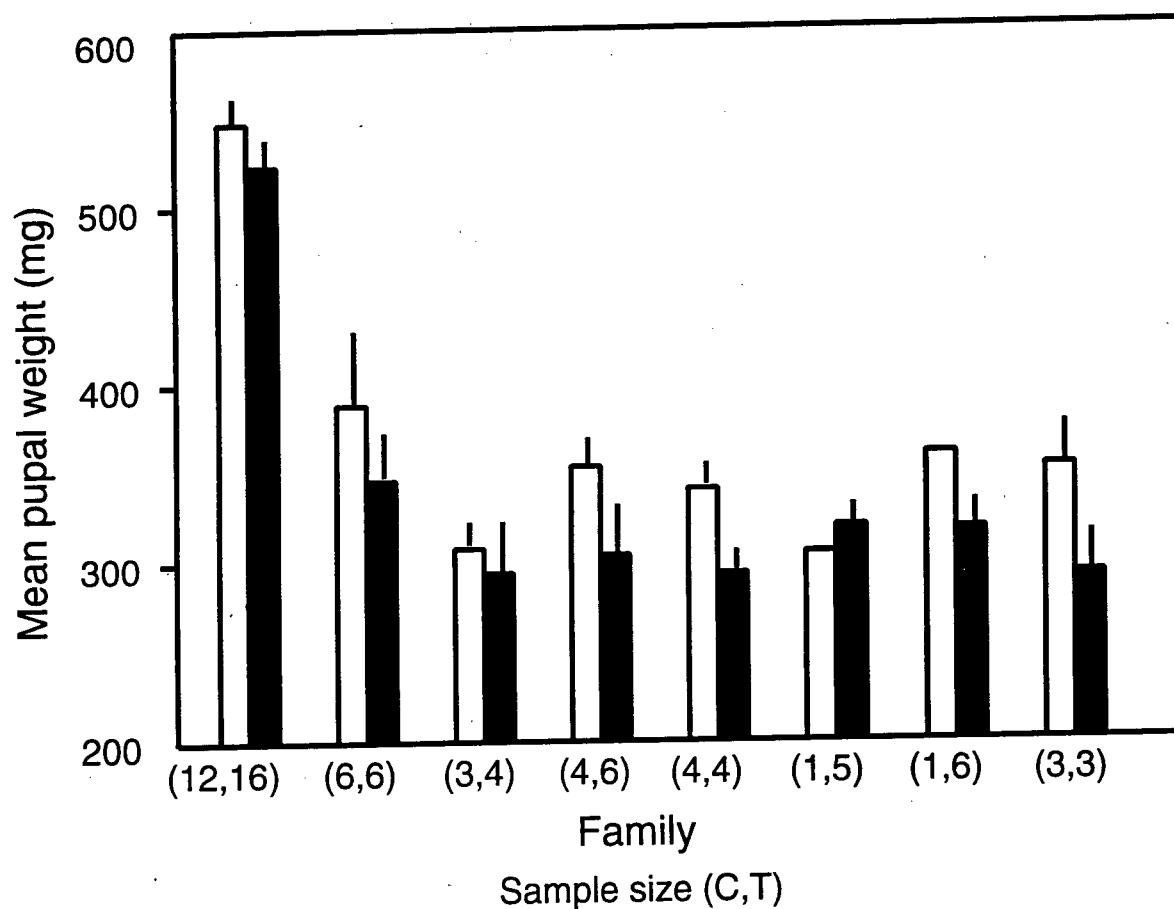
Figure. 3.1. Mean female pupal weights/family (+SE) for treated (shaded bars) and control (unshaded bars) *M. c. pluviale* in 1993. Numbers in parentheses give sample sizes for control (C) and treated (T) individuals.
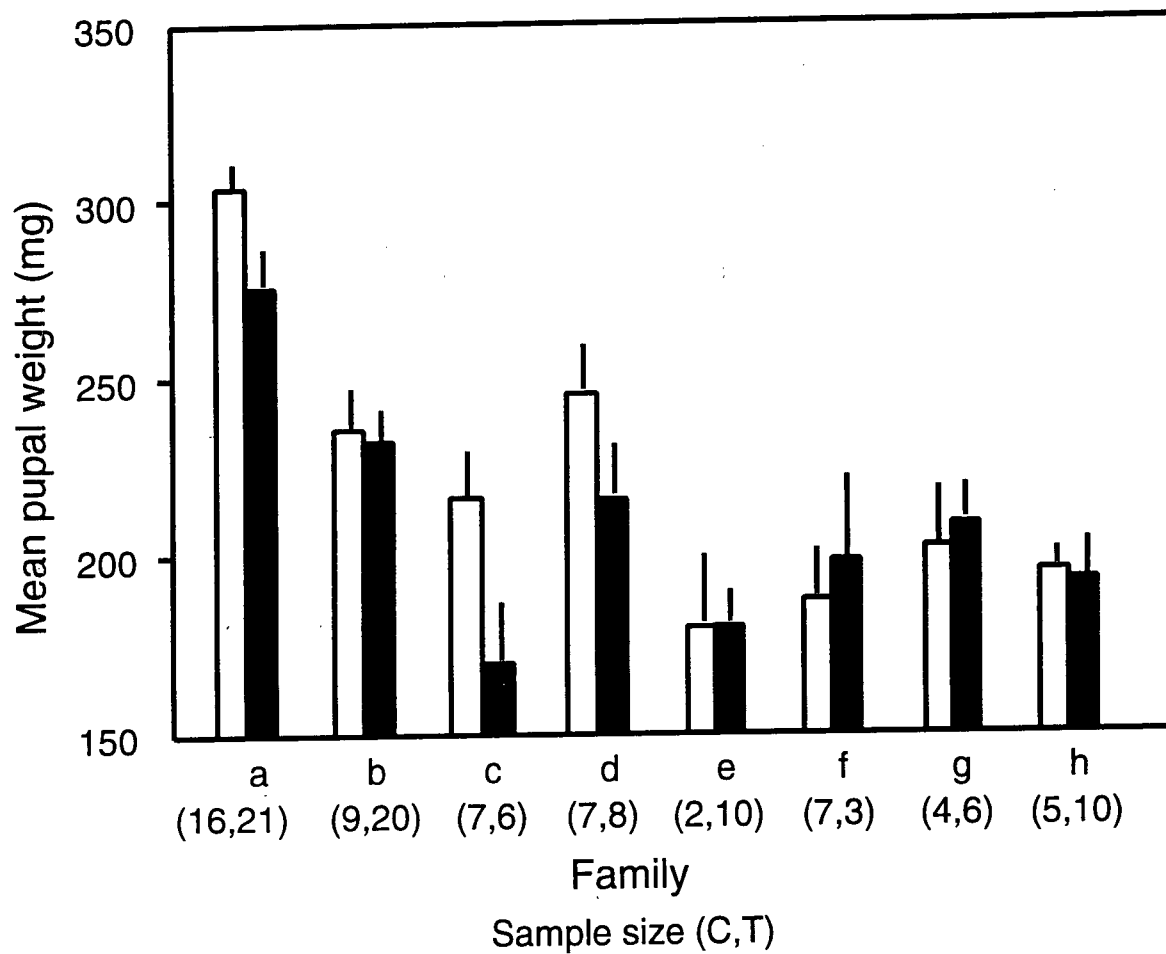
Figure. 3.2. Mean male pupal weights/family (+SE) for treated (shaded bars) and control (unshaded bars) *M. c. pluviale* in 1993. Numbers in parentheses give sample sizes for control (C) and treated (T) individuals.
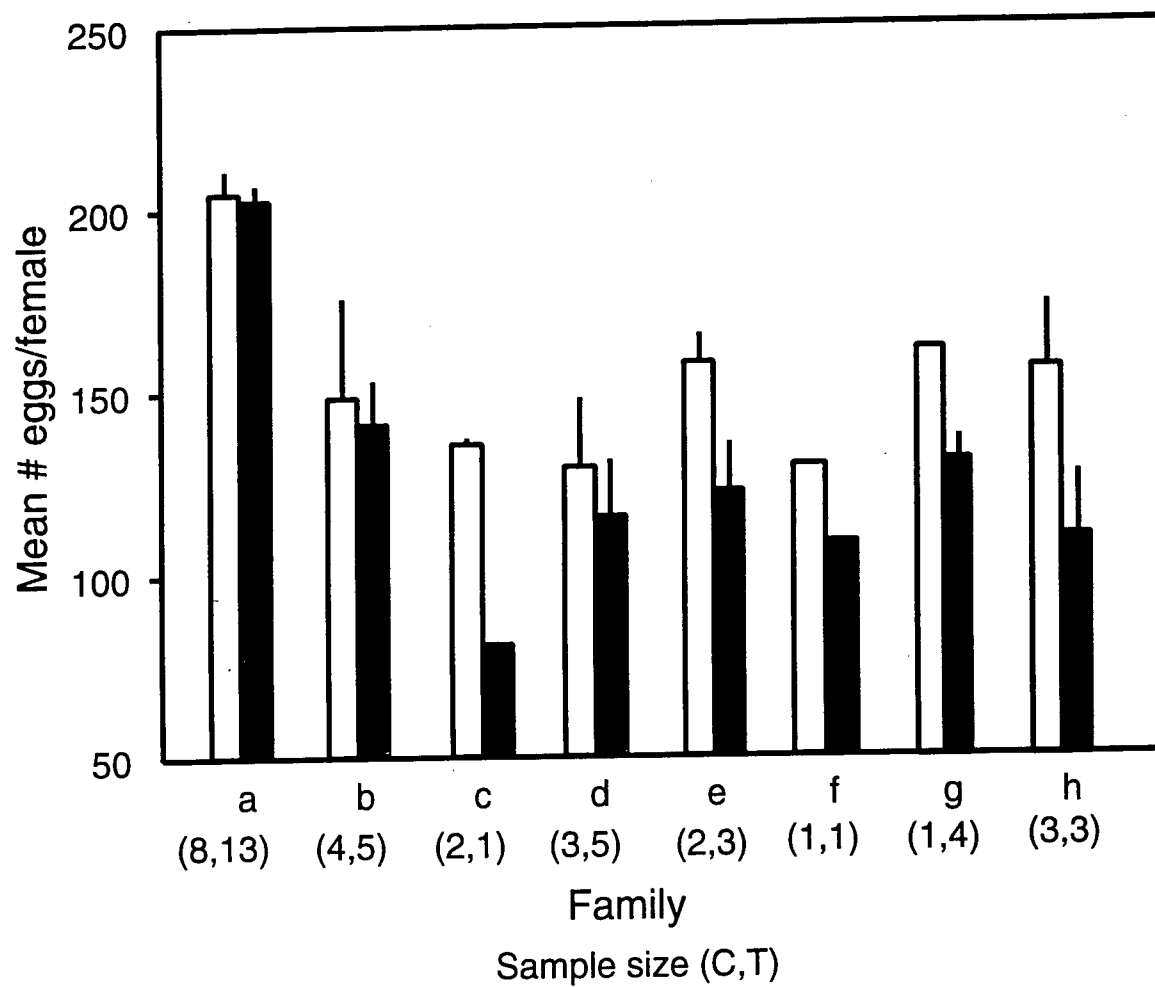
Figure. 3.3. Mean fecundity/family (+SE) for treated (shaded bars) and control (unshaded bars) *M. c. pluviale* females in 1993. Numbers in parentheses give sample sizes for control(C) and treated (T) females.
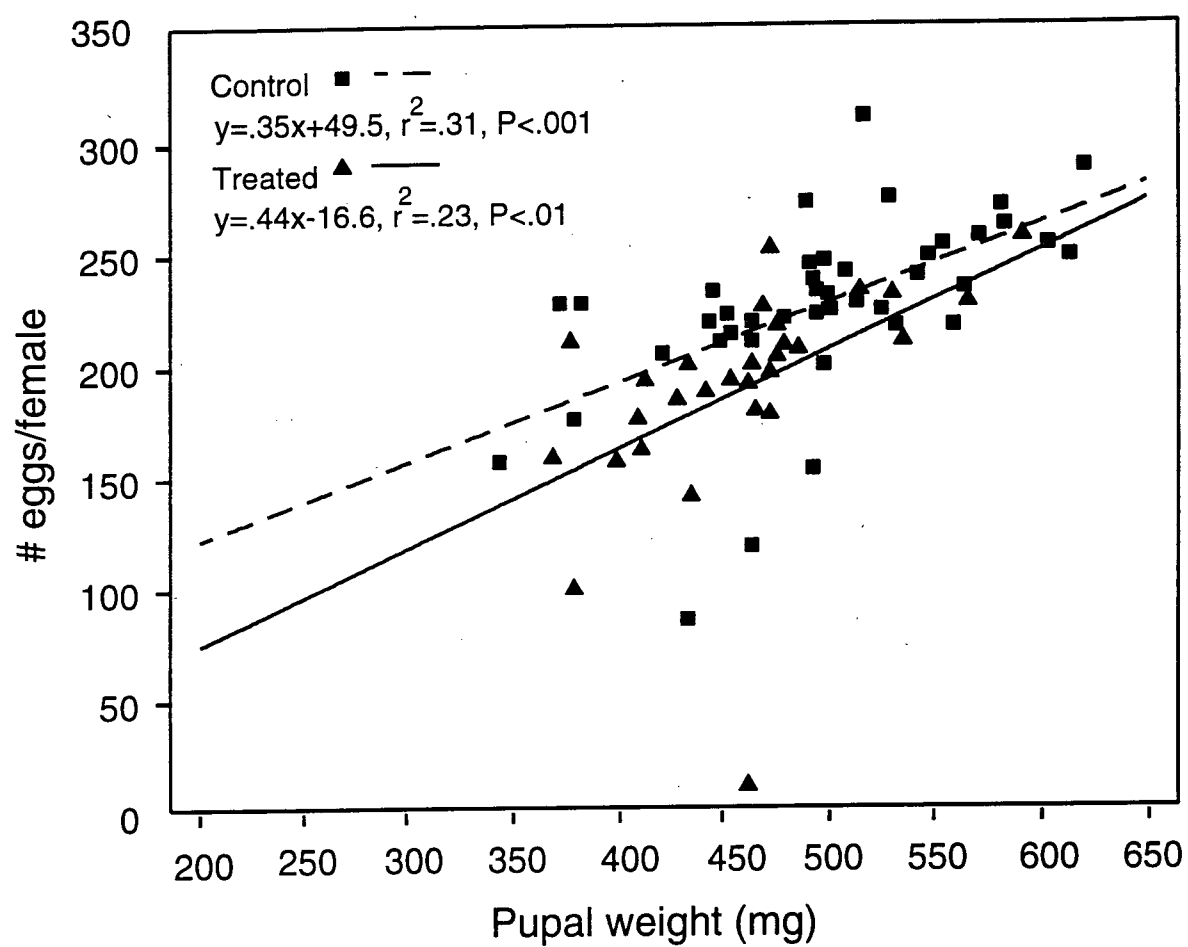
Figure. 3.4. Pupal weight - fecundity regressions (1992) for control (dashed lines and squares) and treated (solid lines and triangles) *M. c. pluviale* females.
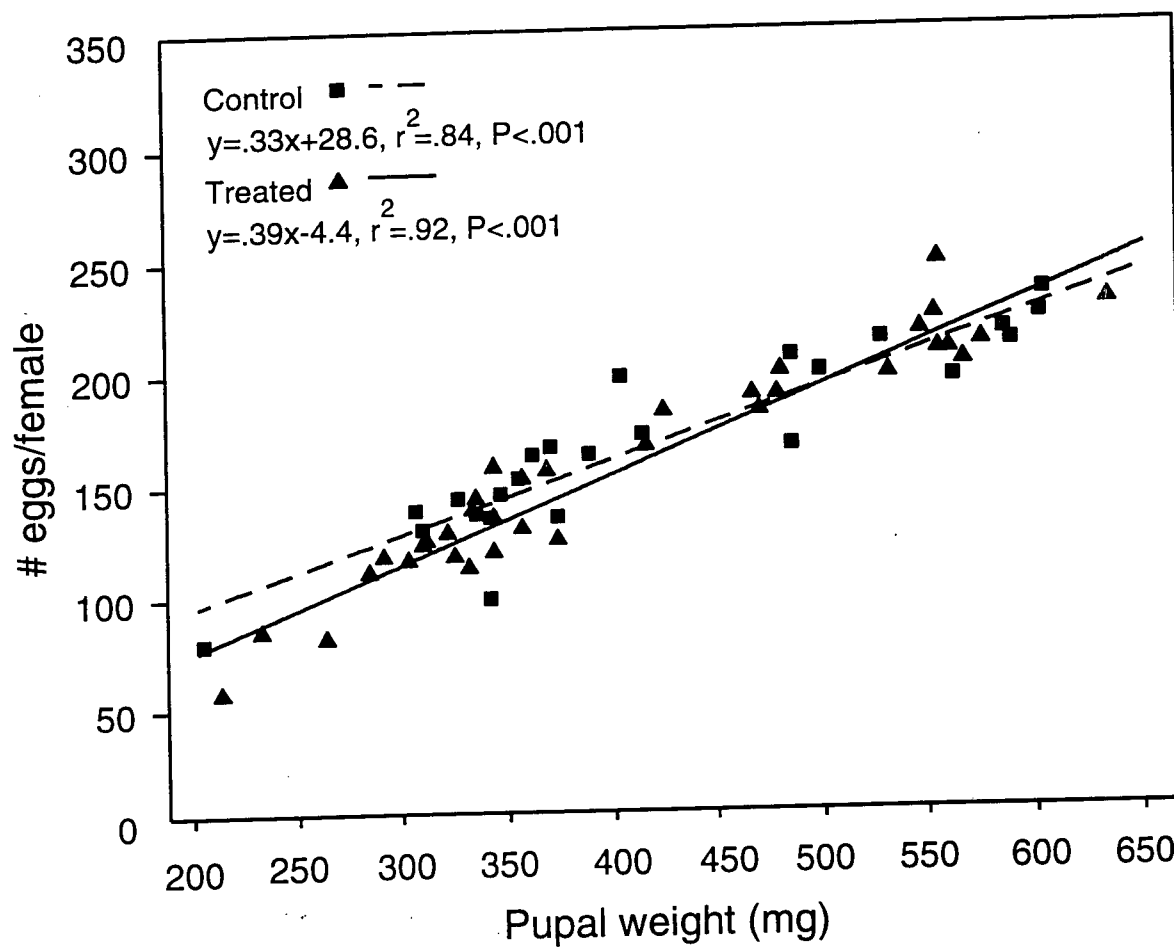
Figure. 3.5. Pupal weight - fecundity regressions (1993) for control (dashed lines and squares) and treated (solid lines and triangles) *M. c. pluviale* females.

4. IMMEDIATE AND DELAYED EFFECTS OF VIRUS INTRODUCTION AND DENSITY ON TENT CATERPILLAR PERFORMANCE

4.1 Introduction

Small-scale field experiments are an important tool for understanding mechanisms of population change. They permit the investigator to simulate conditions experienced by individuals in a population, at a scale amenable to replication and analyses of variance. Most often, experiments focus on one ecological factor with little recognition of alternate hypotheses which makes significant results difficult to interpret relative to unexplored factors that may influence populations (Karban 1993). Further, only one or a few aspects of treatment effects are usually examined (e.g. mortality) and effects on individual quality are overlooked. Finally treatment effects are rarely considered for more than one generation.

In this study I perform several small-scale field studies to assess immediate and delayed effects of viral disease and density on performance of *M. c. pluviale*. Most of this chapter deals with a factorial experiment in which tent caterpillar colonies are reared at high and low density in the presence and absence of introduced NPV. I measure effects of treatments on mortality and individual quality in the year of and the year following virus introductions and density manipulations.

High density may increase intraspecific competition for resources (food and space) resulting in increased mortality and reduced fecundity. The introduction of virus may increase mortality from disease and reduce the reproductive capacity of sublethally infected individuals. Treatments may also have delayed effects on performance. Delay mechanisms may involve extrinsic factors that operate through the external environment and intrinsic factors that influence the ability of offspring to survive and reproduce. The effects of NPV introduction and high density may carry over to subsequent generations, in the environment via persistence of free living NPV particles (Myers 1993) and delayed induced responses of host trees (or induction) (Karban and Myers 1989). Effects may carry over through the individual via disease or density-mediated maternal effects or selection (Wellington 1957, Chitty 1967, Rossiter 1991, 1995, Myers 1990, 1993). Other possible routes of virus transmission such as transovum/transovarian transmission, incidental contamination of egg mass surfaces from environmental inoculum, and latent virus are not examined.

In this study I manipulate two ecological factors thought to influence tent caterpillar populations, disease and density, so that I may compare their relative and interactive effects on performance and potential effects on population dynamics. Through 'bioassay' of the environment and mating of treatment survivors, the delayed extrinsic and intrinsic effects of these two factors are examined and compared.

4.2 Methods

Overview of design and procedure

Table 4.1. gives a summary of studies in this chapter. Experiment Ia

(immediate effects) consisted of four treatments in a 2x2 factorial design with

16 replicates per treatment. Each replicate or sampling unit consisted of an

alder tree receiving either high or low colony densities (density factor) and

either control or virus treated individuals (virus factor). The treatments are

hereafter designated as H (high colony density/no virus treated individuals

added), L (low colony density/no virus treated individuals added), VH (high

colony density/virus treated individuals added), and VL (low colony

density/virus treated individuals added). 'Composite' colonies were used for

introductions to control for strong family effects on pupal weights and

fecundity (see Chapter 3). These colonies were made by combining

individuals from three to four different egg masses for a total of 150

individuals/colony.

The experimental plot consisted of alder trees transplanted four years

previously to the Totem Experimental Field on the University of British

Columbia Campus (U.B.C.) (Vancouver, British Columbia). Each tree was

ringed with tanglefoot to provide a barrier for tent caterpillars. Individuals

could drop from trees (as occurred in fifth instars) but were unable to climb

back on. Trees were systematically assigned to treatments so that treatments

were stratified with respect to the number of buds per tree. Because bud numbers reflected environmental gradients at the study site, this sampling procedure ensured good spatial interspersion of treatment replicates. An additional experiment was also performed (Ib) to examine the effects of virus on female pupal weight by introducing virus to natural colonies at low density.

In Experiment IIa adults from pupae recovered from Experiment Ia were mated within replicates to examine intrinsic delayed effects of previous treatments. To assess the potential strength of intrinsic effects on tent caterpillar reproductive potential in the field, I also performed a correlational study examining the relationship between egg mass size and pupal weight of female offspring. In Experiment IIb (delayed extrinsic effects) the same trees from Experiment Ia were used for colony introductions with all replicates (trees) receiving low colony densities. 'Composite' colonies were used for these introductions as in Experiment Ia.

Egg masses for all studies were collected from a high density population in Strathcona Provincial Park on Vancouver Island, British Columbia in the autumn of 1992 (Experiment Ia), and from high density populations on Saturna Island, British Columbia (Experiment IIb) and in the city of Victoria, British Columbia in the autumn of 1993 (Experiment Ib and the correlational study).

Egg masses were stored over the winter at 4°C and then rinsed in a bleach solution immediately prior to each experiment. NPV for experiments was extracted from cadavers as described in Chapter 3 methods. Larval death in the field from NPV was determined by correspondence of characteristics of diseased larvae (Poinar and Thomas 1984) with a collected sample of larval cadavers containing polyhedral inclusion bodies (PIBs). The presence of PIBs was determined by microscopic examination of stained larval smears. Other causes of death were determined by microscopic examination of stained smears (for bacterial disease) and by the characteristic form and remains of individuals parasitized by hymenopteran or dipteran parasitoids.

Immediate effects: Experiment Ia

On 15 April 1993, egg masses were placed in 60 ml plastic cups maintained at 26°C with a photoperiod of 16L:8D. Upon hatching, colonies were fed every second day until second instar with alder leaves collected from the field and rinsed in a bleach solution. At second instar, composite colonies of 150 individuals were placed in 60 ml plastic cups and taped to experimental alder trees in the field. Individuals emerged from holes cut in the cup lids and began tent construction.

The number of leaf buds was counted on each experimental tree and colony density was expressed on a per bud basis. Low density trees received from 1-

2 colonies (0.4 ind/bud ± 0.02 (S.E.)) and high density trees received from 3-8 colonies (1.7 ind/bud ± 0.07 (S.E.)). High density in the experiment was comparable to densities observed during outbreaks observed on Vancouver Island, B.C. The low density treatment corresponded to low observed densities. Quantitative information on percent infection by viral disease in natural populations is lacking for the western tent caterpillar, and so a low proportion of initially treated individuals (approximately 5%) was used for virus introduction.

Virus was introduced by adding 8 virus-treated individuals to each colony. Control trees received 8 untreated individuals per colony. Several trees not used for the experiment harboured additional composite colonies to be used for these introductions. On 2 May 1993, third instar larvae were collected from these additional trees and brought into the lab. Leaf discs were treated with a lethal dose of NPV in solution (8×10^7 PIB/ml) and fed to individuals (in groups of 8) on two consecutive days (2 and 3 May one disc/day). Controls were fed leaf discs treated with distilled water. Treated and control caterpillars were returned to field colonies on 4 May 1993.

Alder trees were visited every second day to record mortality and defoliation, and to collect cocoons. Mortality was recorded until the end of the larval stage for each replicate by marking the area beside dead caterpillars with liquid

paper to avoid recounting. The liquid paper brush was soaked in bleach and then water after each marking. Caterpillars died from virus on bark, stems, foliage and on tents. Individuals rarely die of virus inside tents and so I made no attempt to open tents when recording mortality. Percent mortality values were calculated using the number of caterpillars introduced to trees at the beginning of the experiment. Defoliation was assessed by counting the number of leaves eaten per tree. This is easily determined as leaf veins are not eaten and remain visible after feeding. Defoliation was monitored until cocoons first appeared in each replicate. Cocoons were collected from 22 May to 1 June 1993. Live pupae were cut from cocoons, sexed and weighed within 48 hours of collection. The mean number of pupae recovered/tree ($\pm$S.E.) for the four treatments were as follows; H - 86.4$\pm$10.5, L - 35.9$\pm$6.3, VH - 26.9$\pm$5.5, VL - 11.7$\pm$3.7.

Immediate effects: Experiment Ib

To verify results of NPV introduction on female pupal weights at the Totem site (Experiment Ia), I performed an additional experiment involving NPV introduction on a smaller scale at a different site on the U.B.C. campus. Egg masses collected from Victoria, B.C. were placed on young alder trees (one egg mass/tree) on 3 April 1994. At third instar, 6 individuals were added to each colony. Treated colonies received NPV treated individuals (as described above) while control colonies received untreated individuals. At fifth instar, 15

individuals were collected from each colony and reared in the laboratory to pupation. Female pupae were cut from cocoons and weighed (as above).

Delayed intrinsic effects: Experiment IIa

Following collection and weighing of pupae in 1993 (Experiment Ia), emerging moths were placed for mating in 1 L cardboard containers covered with cellophane (within-replicate matings only). Subsamples of egg masses (five egg masses.five alder tree replicates^{-1}.treatment^{-1}, or 25 egg masses/treatment) were randomly selected and the number of eggs per mass was counted. On 5 April 1994, egg masses were placed in 60 ml cups in a naturally lit indoor rearing room at room temperature (17 to 28°C). Photoperiod and humidity were not controlled. Colonies were fed every other day on bleached alder leaves from a site with no recent tent caterpillar infestation. Colonies were transferred to 1 L cardboard containers at late second instar. At fourth instar, 50 individuals were randomly selected from each colony and placed in fresh 1 l cardboard containers. The remaining larvae were counted and discarded. Survival and time to fourth instar were recorded. Individuals were removed from containers when they pupated, and weighed. Families showing signs of viral disease were discarded (H-0, L-1, VH-6, VL-1) such that only inherited effects would be measured.

Delayed intrinsic effects: correlational study

Egg masses collected from a field population in Victoria, British Columbia

were used in a correlational study to determine if an association exists between

egg mass size and the pupal weight of resulting female offspring. The number

of eggs/mass was counted, and bleached masses were placed on small

individual alder trees on 3 April 1994 at a recently disturbed site near UBC

campus. At fifth instar, 15 individuals per colony were collected and reared to

pupation in the laboratory as above in 1 L cardboard containers. Female

pupae were weighed, and mean female pupal weight/colony was regressed

against the number of eggs/egg mass from which each colony had emerged.

Delayed extrinsic effects: Experiment IIb

Delayed extrinsic (tree) effects of density and NPV introduction were

examined in 1994 by introducing individuals from egg masses collected from

Saturna Island, B.C. to trees at the Totem site which had been exposed to

different treatments in 1993. The same trees from the 1993 experiment were

used for these introductions. Egg masses were removed from storage on 12

April 1994 and soaked in bleach as above. Composite colonies of late first and

early second instars (150 individuals/colony) were made and placed on alder

trees on 21 April 1994 as in 1993. To control for possible wind blown spread

of NPV between 1993 and 1994, I sprayed control alder trees with a dilute

bleach solution two weeks prior to budburst. Despite this precaution, disease

was detected in several (eight and nine) late instar larvae from two control trees with previous low density. These replicates (trees) were not included in analyses of pupal weight and development time. When analyzing mortality from all causes, the viral deaths were excluded from mortality estimates in these two control replicates (although including them did not affect the outcome of tests). Colonies were placed on alders at low densities (two/tree for a total of 128 colonies) to approximate post-outbreak densities. Because of the considerable growth of alders from 1993 to 1994, food was superabundant for all replicates and it was unnecessary to express density on a per bud basis as in the 1993 experiment. Colonies were monitored every other day for mortality until pupation. As in 1993, percent mortality was calculated using the number of caterpillars introduced to trees at the beginning of the experiment. Defoliation was not recorded. Cocoons were collected every other day and pupae were weighed within 48 hours of pupation. The mean number of pupae collected/tree for the four treatments were as follows; H - 19.8±2.0, L - 18.1±1.6, VH - 14.6±1.8, VL - 19.6±2.4.

Statistical Analyses

Data from the 2x2 factorial designs of Experiments Ia and IIb and the nested 2x2 factorial design of Experiment IIa were analyzed using ANOVA (PROC GLM, SAS institute, inc. 1983). Where assumptions of ANOVA could not be met using data transformations, nonparametric ANOVA was used (Zar 1984).

Nonparametric factorial analysis of variance was performed with unbalanced data by subjecting the ranks of the data to the GLM procedure to determine sums of squares (Zar 1984). Where possible, multiple comparison tests were used to test for significant differences between means in cases in which interaction was detected. Ryan's Q, recommended by Day and Quinn (1989), and the more conservative GT2 tests were used. In Experiments Ia and IIb ANOVAs for time to pupation and pupal weights (male and female) were run on mean values/tree based on recovered pupae, and only mean values calculated from three or more data points/tree were included in the analyses. Data from Experiments Ia and IIb (% virus mortality), and Ib were analyzed using t-tests. Data from the correlational study were analyzed using linear regression.

Magnitude of effects

The total number of female pupae recovered/treatment at the Totem field site (from Experiments Ia and IIb) and mean treatment effects on survival and fecundity (from Experiments Ia and IIa,b) were used to estimate the net reproductive rate (R_o)/treatment (Southwood 1991 and see Chapter 2) in both Year 1 (1993) and Year 2 (1994). Immediate treatment effects on fecundity (Year 1: Experiment Ia) and delayed treatment effects on female pupal weight (Experiment IIa,b), percent hatch and survival to fourth instar of offspring (Experiment IIa) were used to examine effects of individual quality on

population change. Because fecundity estimates were not available from second-year experiments examining delayed treatment effects, fecundity for this generation was estimated from the pupal weight-fecundity regression, based on combined data from control and virus treatments in the laboratory ($y=.368x+7.525$, see Chapter 3). Where treatments did not differ with respect to a given trait, the LS mean across all four treatments was used for calculation of R_o.

Of qualitative traits above, only fecundity and female pupal weight were influenced by treatments (see results). For fecundity in Year 1, Experiment Ia, and pupal weight in Year 2, Experiment IIa, R_o calculations were based on the mean value for treatment L (low density control) and the LS mean value across treatments H, VL and VH. For female pupal weight in Year 2, Experiment IIb, R_o calculations were based on the LS mean of the VL and VH treatments, and the LS mean of the L and H treatments as a trend toward a virus effect was observed ($P=.065$, see Appendix F). For calculations of R_o in Year 2, delayed intrinsic and extrinsic effects of treatments on fecundity estimates were assumed to be additive.

4.3 Results

Immediate effects

Two distinct waves of mortality occurred in both VL and VH treatments beginning after 10 May (late third instar) and 22 May (fifth instar) in 1993 (Fig. 4.1). In the first wave, per capita mortality rates were similar in the VL and VH treatments and represented death of the lab-treated larvae initially introduced to field colonies. By the second wave, colonies in the VH treatment experienced significantly greater mortality. Mortality from all causes showed a similar pattern (Fig. 4.2) with greater total mortality in the treatments receiving virus than in the controls. The interaction between virus introduction and density was evident (P=.050) (Appendix C and D). Mortality in controls was similar (H and L) and relatively slight compared with that in virus treatments (Fig. 4.2). Percent mortality in all treatments (Figs. 4.1, 4.2 and Appendix C) is likely underestimated because late instar caterpillars dispersed from trees. Ichneumonid/braconid parasitization, pentatomid predation, bacterial disease and unknown causes accounted for most mortality in controls. No virus mortality was detected, although some deaths from virus could have been counted among 'unknown causes'. All treatments showed a decrease in mortality after 30 May because most individuals had entered the prepupal or pupal stage by this time.

Total defoliation.bud^{-1}.tree^{-1} and approximate visual estimates of percent

defoliation by the beginning of the prepupal state are given in Table 4.2. Trees

harbouring high colony densities experienced the greatest defoliation

regardless of NPV introduction (H and VH treatments). Feeding rates/colony

were also greater in the high density treatments regardless of NPV

introduction. This density effect was greatest by 22 May, when cocoons were

first detected at the Totem site, but remained significant until the beginning of

the prepupal stage for each replicate (Figure 4.3). In addition, colonies at high

density developed more rapidly to the prepupal stage than did colonies at low

density (Fig. 4.4). Virus introduction had no detectable effect on development

time. Although it is possible that males develop more rapidly than females,

the effect of density on development was not likely to have been caused by

shifts in sex ratio since the proportion of male pupae ($\pm$S.E.) in the four

treatments were similar; H - 0.67 ± 0.03, L - 0.62 ± 0.03, VH - 0.67 ± 0.06, VL -

0.64 ± 0.18.

Mean pupal weights/tree are illustrated in Figures 4.5 and 4.6 for each of the

four treatments. For males, there were significant additive effects of density

and virus introduction; pupal weights were reduced in the presence of virus

and at high density (Fig. 4.5). The interaction term was not significant. For

females, there was a significant interactive effect of virus introduction and

density as pupal weights were reduced following virus introduction at low

density only. Female pupal weights were also reduced at high densities when

compared with low density controls (Fig. 4.6). Ryan's test showed significant differences between treatment L and the other three treatments, while the GT2 test showed treatment L to be different from H and VL (p<0.05). This pattern was also evident for the subsample of mated females laying egg masses (Fig. 4.7). Fecundity was reduced at high density, compared with low density controls, and virus introduction reduced fecundity at low densities only. The multiple comparison tests gave the same results as for female pupal weights (above). Female pupal weights in field colonies were also significantly reduced following the addition of individuals treated with NPV in the laboratory in Experiment Ib (Fig. 4.8).

Delayed intrinsic effects

When the subsample of egg masses from year-one matings (Fig. 4.7) were reared in the laboratory, no significant effects of previous treatments were observed on percent hatch, percent survival of offspring to fourth instar, or offspring male pupal weights (see Appendix E). Further, no treatment effects were observed on colony development time to fourth instar. No greater than four days difference was observed in time for colonies to reach fourth instar with 98% of colonies reaching this stage within a two-day period. A significant interactive effect of virus and density on pupal weights of female offspring was observed, although many families did not survive to the pupal stage largely owing to bacterial disease affecting late instars (Fig. 4.9). This

pattern was similar to that of maternal pupal weights and fecundity (Figs. 4.6 and 4.7); offspring pupae were smaller at high density (L vs. H) and in the virus treatment at low density (L vs. VL). Results of the correlational study on field collected egg masses showed no relationship between number of eggs per egg mass and mean pupal weight of female offspring (Fig. 4.10). Vertical transmission of NPV directly from parents to offspring was not examined in this experiment as all egg masses were surface sterilized to remove virus particles. Despite this, viral disease was detected during laboratory rearing with the greatest proportion of infected families resulting from parents from the VH treatment (VH - 24%, VL, L - 4%, H - 0%). These families were discarded and were not included in the above analyses.

Delayed extrinsic effects

Mortality from virus was detected in the year following NPV introduction with significantly greater mortality in colonies on trees in the previous VH treatment than on trees in the previous VL treatment (Fig. 4.11). The wave pattern was not distinct as in the previous year, although peaks were observed on 12 and 24 May 1994 (Fig. 4.11a). The effect of virus was also evident when all causes of mortality were considered (Fig. 4.12). ANOVA on total mortality showed a virus by density interaction, with greater percent mortality on trees with previously high density (Appendix F). The two multiple comparison procedures showed significant differences between the VH treatment and the

other three treatments (H, VL, L); no effect of previous density on mortality was observed in the absence of NPV. As in the previous year, percent mortality in all treatments (Figs. 4.11, 4.12 and Appendix C) is likely underestimated because of late instar dispersal from trees. Previous treatments did not affect caterpillar development time (to the prepupal stage) or male pupal weights (Appendix F). Although not significant at the 5% level, there was a trend toward reduced female pupal weights of individuals reared on trees exposed to infected caterpillars the previous year (P=.065, Appendix C and F).

Magnitude of effects

Table 4.3 gives R_o values in the year of, and year following treatment applications. Because generation time in *M. c. pluviale* is one year, R_o also represents the yearly multiplication rate (Southwood 1991):

$$R_o = N_{t+1}/N_t \qquad \text{(Equation 4.1)}$$

All treatments in both years gave unrealistically high annual multiplication rates, because R_o was based on the number of female pupae recovered from the field. Natural mortality of pupae and adults in the field and percentage of successful mating and oviposition could not be considered.

In Year 1, predicted population growth rates are lowest in the VH treatment (Table 4.3), reflecting the interactive effect of virus and density on mortality

(Fig. 4.1, Appendix C). All three treatments show suppression in predicted population growth compared with the low density control (L). Of particular interest is the reduced R_0 value in the H treatment (high density control) despite similar percent mortality in the L and H treatments (Fig. 4.2, Appendix C). This likely reflects a trend toward density dependent dispersal from host trees. In a natural field population, this might result in spatial spread of individuals and therefore reduction in density rather than reduction in abundance. NPV introduction had a large impact on R_0 in Year 1, particularly at high density. R_0 in the VH treatment was reduced by 87% compared with R_0 in the L treatment. At Mandarte Island, mean R_0 is reduced by 66% in the first year of the decline (mean $R_0=.75$ (Equation 4.1), t=1976, 1985) compared with the mean value of 2.23 (Equation 4.1) during the increase phase (t=1975, 1981-1984, 1989-1994).

In Year 2, only the previous VH treatment had any appreciable effect on predicted population growth. R_0 in the previous VH treatment was reduced by 39% compared with R_0 in the previous L treatment. At Mandarte Island, the mean R_0 was reduced by 91% and 83% in the second ($R_0=.19$, t=1977, 1986) and third years ($R_0=.39$, t=1978, 1987) of the decline compared with the mean value during the increase phase. Excluding treatment effects on fecundity does not change these general patterns in Years 1 and 2 but results in slightly smaller reductions in R_0.

4.4 Discussion

Immediate effects

The addition of NPV-treated individuals to third instar colonies of western tent caterpillar caused significant mortality. The principal route of horizontal transmission of NPV is by ingestion of contaminated foliage. Virus can only be spread after the host dies from disease and lyses, releasing NPV where it is ingested on foliage. Mortality from virus, therefore, occurs in waves, the first (after May 10) owing to death of lab-infected individuals and the second (after May 22) (Fig. 4.1) resulting from individuals that ingested NPV after its release into the environment, during or soon after the first wave of mortality. The incubation time for NPV-infected hosts was 10 to 12 days in this experiment, comparable to the incubation time of about 2 weeks observed for NPV infected larvae of gypsy moth, *Lymantria dispar* (Woods and Elkinton 1987, Dwyer and Elkinton 1993). NPV transmission was greater when NPV and host densities/tree were high as illustrated in the second wave (Fig. 4.1) and was likely due to intercolony transmission and/or increased susceptibility to disease. As the second wave occurred late in larval development, no significant effects of NPV introduction on defoliation were observed. A weak interactive effect of virus and density was also observed when mortality from all causes was examined (Fig. 4.2).

Pupal weights were reduced in individuals surviving NPV introductions,

although for females, only at low densities (Fig. 4.6). Treatment effects on fecundity (Fig. 4.7) were very similar to effects on female pupal weight, as the two are highly correlated in this species (Rothman and Myers 1994 and see Chapter 3). In males, the relevance of pupal weight to reproductive potential is not known, but may be related to reproductive performance (Haukioja and Neuvonen 1985). The absence of virus effects on female reproduction at high density could have resulted from compensatory effects of disease under conditions of resource limitation. Washburn *et al.* (1991) found that when populations of the mosquito *Aedes sierrensis* were food limited at high density, infection with a ciliate parasite *Lambornella clarki* resulted in the development of larger, more fecund females. The authors attributed this pattern to reduced host abundance following parasite infections leading to increased per capita food for survivors. Greater food availability resulted in a phenotypic shift in body size and reproductive capacity. Alternatively, the negative effects of competition for resources may have prevented any additional expression of sublethal effects on pupal size, although this is less likely; pupation of much smaller individuals is possible, at least under laboratory conditions (Figure 4.9).

The observed effects of NPV on pupal weights generally agree with finding from laboratory experiments on this species (Rothman and Myers 1994 and Chapter 3) and may have been due to sublethal infection. During the second

wave of mortality near the onset of pupation (Fig. 4.1), a large number of PIBs would have been released into the environment, and late fifth instars that ingested virus may have been able to complete larval development before succumbing to disease. However, selection via differential mortality between virus and control treatments can not be discounted.

It is possible that small individuals are less susceptible to virus (see Myers 1990, Myers and Kukan 1995). However the correlation between virus susceptibility and pupal weight must be strong to observe such an immediate effect of virus. Comparison of the distribution of female pupal weights may add insight into the mechanism of virus effects (Fig. 4.13). If pupal weight is genetically determined by multiple loci, a normal distribution of weights would be expected (Myers and Kukan 1995). If pupal weight is determined primarily by a single locus with a dominant allele, a dimorphism with large and small pupae could be expected. A more likely explanation for a bimodal distribution is sublethal disease causing a reduction in pupal size in a fraction of hosts that become infected with virus (Myers and Kukan 1995). When female pupal weights for all replicates are pooled within the VL and L treatments, the distribution of weights for VL is platykurtic (g_2=-0.78) while that of L is leptokurtic (g_2=0.46). A bimodal distribution is an extreme platykurtic distribution (Sokal and Rohlf 1981) and platykurtosis in the VL treatment may suggest a trend toward bimodality (Fig. 4.13b) resulting from

the combined distribution of female larvae not infected with NPV and sublethally infected female larvae showing reduced pupal weights.

Density did not affect mortality rates, although pupal weights and fecundity were generally reduced under crowded conditions (Figs. 4.5-4.7). This effect may have been a result of density-related changes in feeding behaviour and development as a response to intraspecific competition. Individuals at high density showed increased feeding rates and decreased development time (Figs. 4.3 and 4.4). The shift in feeding behaviour may reflect an adaptation to scramble competition in the face of limited resources whereby an individual completes development before food is depleted. In species that live in unpredictable but recurrent environments, there are adaptive advantages to genomes allowing for environmentally based expression of many phenotypes, each phenotype being advantageous under certain environmental conditions (Barbosa and Baltensweiler 1987). In *M. c. pluviale*, increased feeding rate and reduced development time could improve survival in a crowded environment at the cost of reduced pupal weight and fecundity in females. At low density, the optimum phenotype might maximize fecundity through prolonged development. Haukioja *et al.* (1988) used a similar argument to explain crowding effects on larval development and pupal weights in *Epirrita autumnata.*

Delayed intrinsic effects

Wellington (1957, 1965) and Chitty (1960, 1967) were among the first to recognize that populations could be influenced by delayed effects on offspring quality. According to Wellington (1965), the quality of western tent caterpillar individuals is determined by maternal provisioning of egg yolk reserves, with those progeny receiving greater yolk reserves being more active and vigorous than their siblings with reduced yolk. The proportion of qualitative "types" within an egg mass is determined by prior resource accumulation of the ovipositing female during the larval stage. He attempted to test this hypothesis by treating females with farnesyl methyl ether, which reduced nutrient allocation to developing eggs. Treatment led to reduced survival of offspring in the field (Wellington and Maelzer 1967).

More recently, interest in internal delay mechanisms has been stimulated by theoretical (Ginzburg and Taneyhill 1994, Rossiter 1994) and empirical (e.g. Rossiter 1991) work on maternal effects. Ginzburg and Taneyhill (1994) constructed a two-dimensional difference equation model of maternal effects that could explain periodic oscillations observed in temperate forest Lepidoptera. Rossiter (1991) observed a 'negative' maternal effect in the gypsy moth; pupal weights of offspring were higher when parents experienced greater defoliation in their diets, while their mothers had lower pupal weights and reduced fecundity. She suggested this could act to destabilize gypsy moth

populations and lead to irruptive dynamics.

I observed similar treatment effects on maternal and offspring reproductive potential (positive maternal effect, compare Figs. 4.7 and 4.9). Offspring quality was not otherwise influenced. As predicted by Wellington (1957, 1965), environmentally based maternal effects could lead to reduced offspring quality (Fig. 4.9, L vs. H) when parents experience crowded conditions. Maternal effects in these instances are likely not adaptive. Reduced reproductive potential of offspring may represent an additional cost to parents that exhibit phenotypic shifts in behaviour (Fig. 4.3), development (Fig. 4.4) and fecundity (Fig. 4.7) under crowded conditions. Results also suggest that offspring quality can be influenced by disease as well as nutritional experience of parents (L vs. VL, Fig. 4.9). Disease could mediate maternal effects by reducing egg provisioning in sublethally infected females (Myers 1993). Again, this pattern might also be explained by selection in the parental generation.

Results of laboratory rearing show that intrinsic effects of disease and density may be important in the expression of offspring traits under controlled conditions. However, this says little about the importance of such effects in the field. Owing to limited resources I was unable to rear offspring of the Totem experiment (Experiment Ia) in the field. I therefore examined the potential importance of intrinsic effects under field conditions by using natural

variation in egg masses from a high density field population. If intrinsic effects of ecological factors are strong relative to the individual's current environmental experience, one would expect to observe a relationship between parent and offspring traits in the field. However, I did not observe a correlation between parent and offspring reproductive potential using a wide range of egg mass sizes (Fig. 4.10). These results suggest that inheritance of reproductive potential (genetically or via maternal effects) is absent or weak relative to the environmental experience of offspring.

In a similar light, Wellington (1965) suggests that within-brood variability in quality could also be related to maternal effects. According to Wellington (1965) first-laid eggs in *M. c. pluviale* egg masses receive greater yolk reserves and larvae from these eggs are more active and vigorous. Myers (1978) tested for this within-brood maternal effect by releasing manipulated egg masses constructed from first-laid eggs in the field and compared colony performance with that of egg masses made of last-laid eggs. Colonies emerging from last-laid eggs were as successful as those from first-laid eggs, and introductions of colonies from first-laid eggs did not cause a population outbreak or prevent a population decline.

Delayed extrinsic effects

A great deal of attention has focused on delayed effects of host plant

defoliation on insect performance because of its potential role in generating

population cycles in herbivorous insects. Heavy defoliation during high

herbivore density may induce changes in host plant quality that affect insect

performance in subsequent generations (Benz 1974, for reviews see Karban and

Myers 1989, Haukioja 1990). In agreement with the findings of Myers and

Williams (1984, 1987) I found no significant delayed effect of density on

development time, mortality or pupal weights (Fig. 4.12, Appendix F) despite

the high level of defoliation that occurred in the high vs. low density

treatments in the previous year (Table 4.1).

By contrast, NPV introduction had delayed effects on host performance. Viral

disease was observed the year following field introductions, particularly on

trees which previously supported high densities of caterpillars (Figs. 4.11, 4.12).

A trend toward reduced reproductive potential of females was also observed

regardless of previous density (P=.065, Appendix F). PIBs released into the

environment following death of infected larvae in 1993 must have persisted on

host tree surfaces and led to reinfection and mortality in 1994. The most likely

reservoirs for virus in this experiment were bark and possibly tent material

that remained on trees. The 'wave' pattern of disease observed in 1993 was

less evident in 1994, presumably because timing of larval exposure to virus

was much more variable in the second year (Fig. 4.11).

In gypsy moth, persistence of NPV following environmental contamination of bark surfaces has been identified as an important route of transgenerational virus transmission (Woods *et al.* 1989). Woods *et al.* (1989) released neonate larvae on to sterilized, untreated and NPV-treated bark surfaces in areas in which gypsy moth infestations and NPV epizootics had been observed the previous year. Larvae were collected after a short period (15-90 min.) and reared in the laboratory on artificial diet. Percent mortality was related to the degree of NPV contamination (NPV treated > untreated> sterilized bark). Clark (1956) painted NPV suspension on to nonedible surfaces of host plants during the winter and tied egg masses of the great basin tent caterpillar, *Malacosoma californicum fragile* adjacent to these painted areas. The following summer more mortality from NPV was observed in treatment colonies than in to control colonies. My results are in general agreement with these studies outlined above and further suggest that persistence of NPV on host tree surfaces following an epizootic has a greater impact on insect performance than does host plant induction, or intrinsic delayed effects of disease or density.

Implications for population quality and population dynamics

In the western tent caterpillar, fecundity of moths tends to decrease at

population peaks (four of six populations illustrated in Figure 1.2 (Chapter 1)),

and continues to decrease as populations decline (Myers 1990 and see Fig. 1.2).

Results of this study suggest that conditions associated with density may be

involved in initiating observed decreases in fecundity, but density effects are

less likely to cause continued fecundity decreases following population decline.

Although reduced reproductive potential was observed in lab-reared offspring

of parents experiencing high density, this delayed effect may not necessarily be

expressed under more variable field conditions. By contrast, NPV is unlikely to

initially be important in reducing fecundity in high density field populations,

but could be involved in the observed delayed recovery of fecundity after tent

caterpillar outbreaks have subsided.

The results of this chapter (summarized in Table 4.4) give insight into potential

effects of density and disease on the population dynamics of tent caterpillars.

The only detectable effect of density on tent caterpillar performance in the field

was immediate. Reproductive potential was reduced at high density, but no

delayed effects of heavy defoliation were observed in the following year.

Introduction of NPV resulted in weak density-dependent mortality and had a

significant delayed effect via persistence of NPV in the environment.

Overwintered NPV resulted in significant mortality the year following NPV

introduction at high caterpillar density. Viral disease may have a destabilizing influence on tent caterpillar populations as time delays in density-dependent processes can lead to population instability (see Introduction, Chapter 1). This influence may be augmented by effects of virus on reproductive potential; reductions in female pupal weights and fecundity were observed at low density only and in the year following NPV introduction. Berryman *et al.* (1990) reached the same conclusion from simple models of the Douglas-fir tussock moth, *Orgyia pseudotsugata* and black headed budworm, *Acleris variana*; viral pathogens destabilize host population dynamics.

To this point, my conclusions have been based on the detection of statistically significant delayed effects of virus introduction. Examination of the magnitude of these effects revealed that disease from overwintered NPV could suppress population growth by about 40% (Table 4.3, Year 2, previous VH vs. previous L). This disease did not reduce survival or reproduction sufficiently to explain the continued decline at Mandarte Island where population growth is suppressed by about 90% compared with growth during the increase phase.

Table 4.1. Summary of background for studies.

Study	Egg mass source	Foliage source	Experimental conditions
Experiment Ia. Immediate effects (Year 1-1993)	- 1 high density field population (Strathcona Prov. Pk.)	- on alders at Totem site	- field reared composite colonies at 2 densities with and without virus treatment (4 treatments)
Experiment Ib. Immediate effects (1994)	- 1 high density field population (Victoria)	- on alders at U.B.C. campus	- field reared natural colonies at 1 density (1 colony/tree) with and without virus treatment
Experiment IIa. Delayed intrinsic or parental effects (Year 2-1994)	- from survivors of Experiment Ia	- alder foliage from U.B.C. campus with no history of defoliation	- no treatment applied - lab reared as intact colonies until 4th instar - 50 larva/colony reared to pupation
Experiment IIb. Delayed extrinsic or tree/ virus carry-over effects (Year 2-1994)	- 1 high density field population (Saturna Il.)	- on alders at Totem site (same trees as Exp. Ia)	- no treatment applied - field reared composite colonies at 1 density (2 colonies/tree)
Correlational study. Delayed extrinsic or parental effects (1994)	- 1 high density field population (Victoria)	- on alders at U.B.C. campus	- field reared natural colonies at 1 density (1 colony/tree) with no virus treatment

Table 4.2. Mean defoliation/tree in the four treatments based on counts of the number of leaves eaten.bud^{-1}.tree^{-1} and visual estimates.

| | | Treatment | | |
	L	VL	H	VH
Mean number of leaves eaten/bud/tree (S.E.)	.29 (.04)	.19 (.02)	1.54 (.15)	1.47 (.13)
Mean % defoliation (S.E.)	8.0 (.8)	6.8 (.8)	68.8 (4.0)	70.0 (5.1)

Table 4.3. Immediate and delayed effects of treatments on R_o values with (+F) and without (-F) effects on fecundity included. R_o values are calculated from results in Experiments Ia, IIa,b.

| | | Treatment | | | |
		L	H	VL	VH
Year 1	**-F**	14.50	7.30	4.95	2.18
	+F	14.50	6.21	4.21	1.86
Year 2	**-F**	3.26	3.59	3.12	2.17
	+F	3.26	3.40	2.87	1.99

Table 4.4. Summary of results on mortality and reproductive potential from the Totem experiments.

| YEAR	MORTALITY	REPRODUCTION
1993	**Immediate effects**	
	- virus x density interaction - greatest % mortality (from virus and all causes) in the VH treatment - no density effect	- virus x density interaction - fecundity reduced in the VL, VH and H treatments
1994	**Delayed intrinsic or parental effects**	
	- no significant effects on survival observed	- virus x density interaction - female pupal weight of offspring reduced following VL, VH and H treatment of parents
1994	**Delayed extrinsic or tree/virus carry-over effects**	
	- virus x density interaction - greatest % mortality (from virus and all causes) on trees previously receiving the VH treatment - no effect of previous density (DIR)	- trend toward virus effect - reduced female pupal weights on trees previously receiving VL and VH treatments - no effect of previous density (DIR)
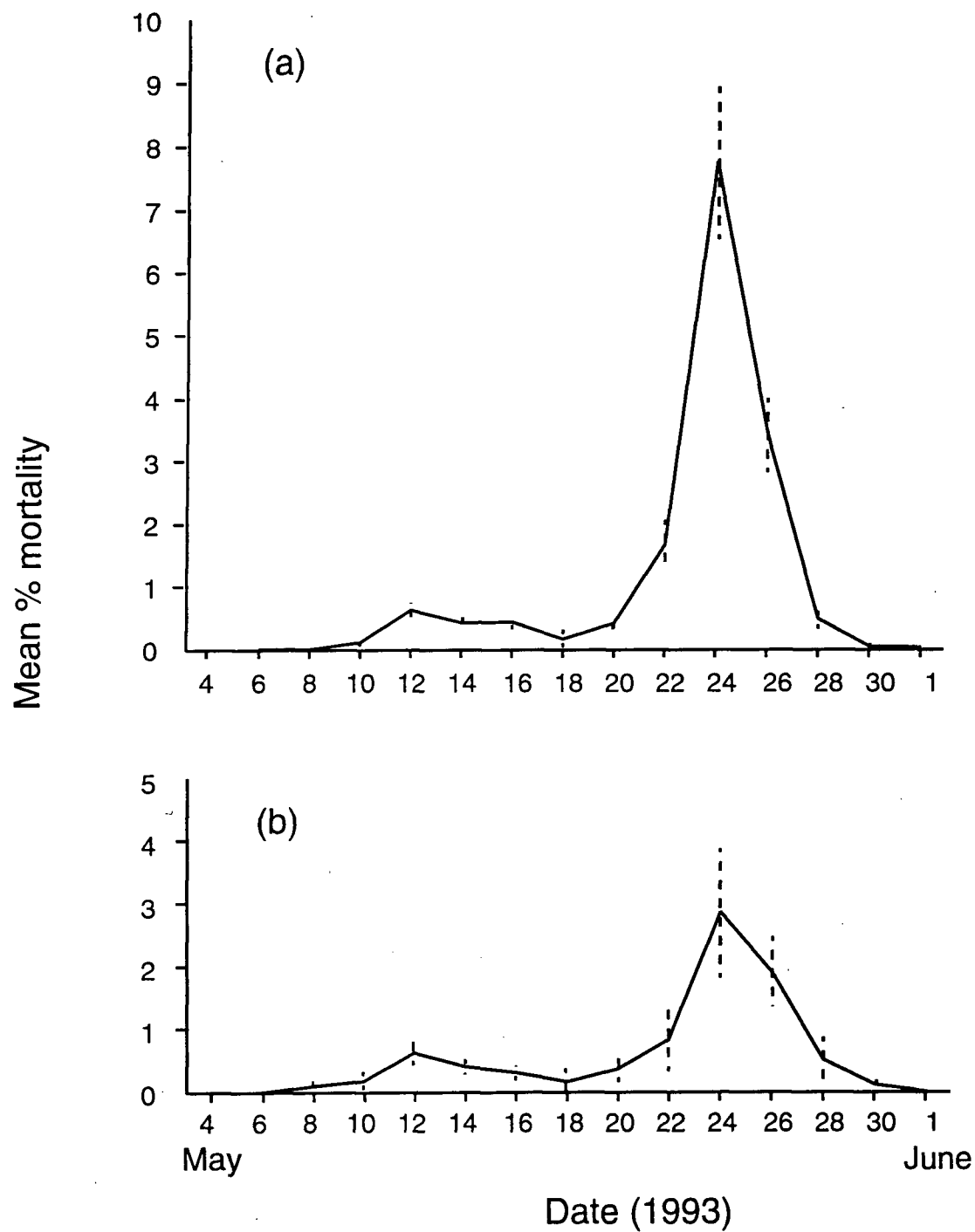
Figure 4.1. Mean % mortality from viral disease at two day intervals from third instar (May 4) till the end of the larval stage in 1993, in the (a) VH and (b) VL treatments (± S.E.) (t=3.02, n=16 trees/treatment, P=.005).
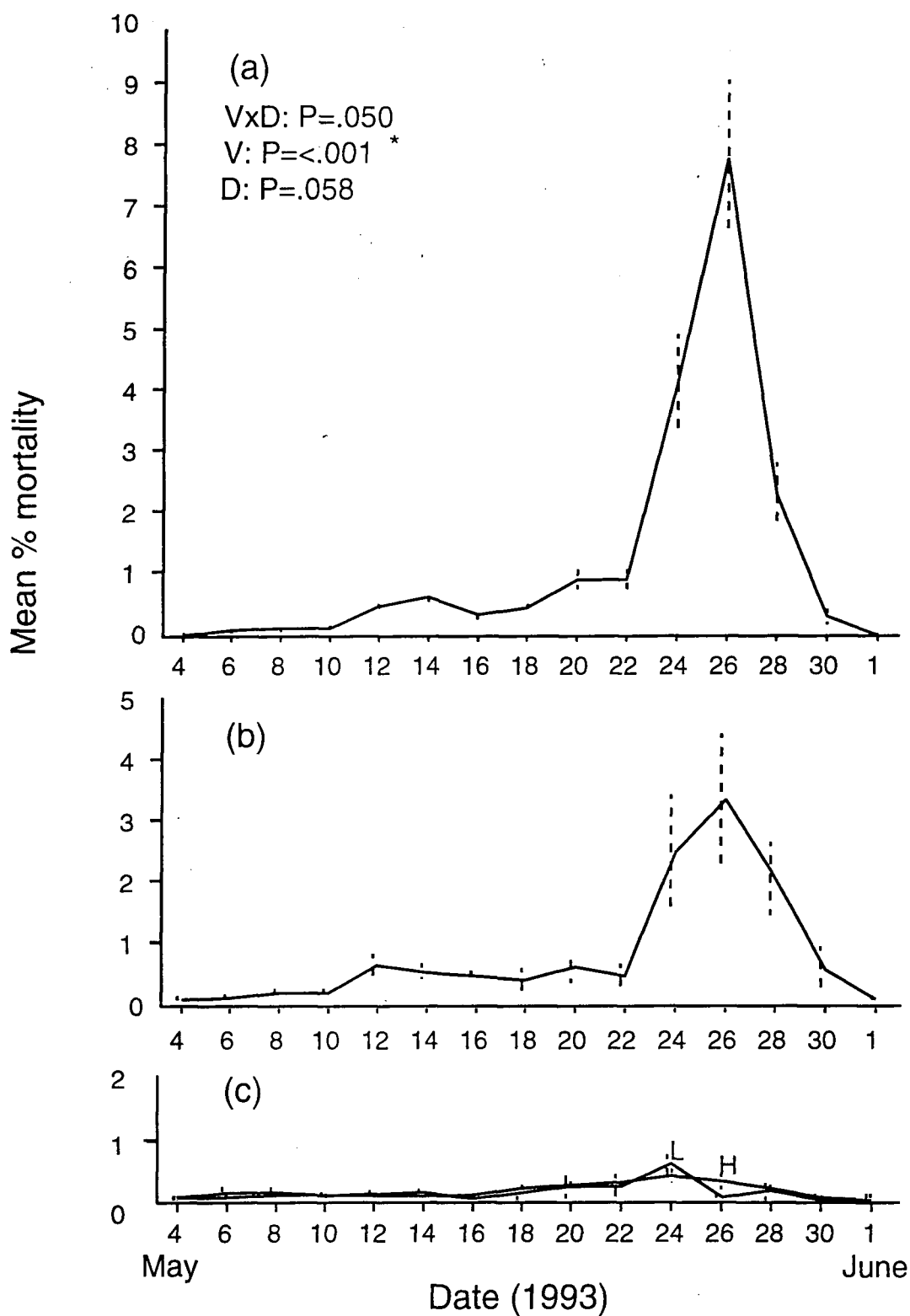
Figure 4.2. Mean % mortality (all causes) at two day intervals from third instar (May 4) till the end of the larval stage in 1993, in the (a) VH, (b) VL and (c) H and L treatments (± S.E.) (n=16 trees/treatment). P values are from ANOVA on totals.
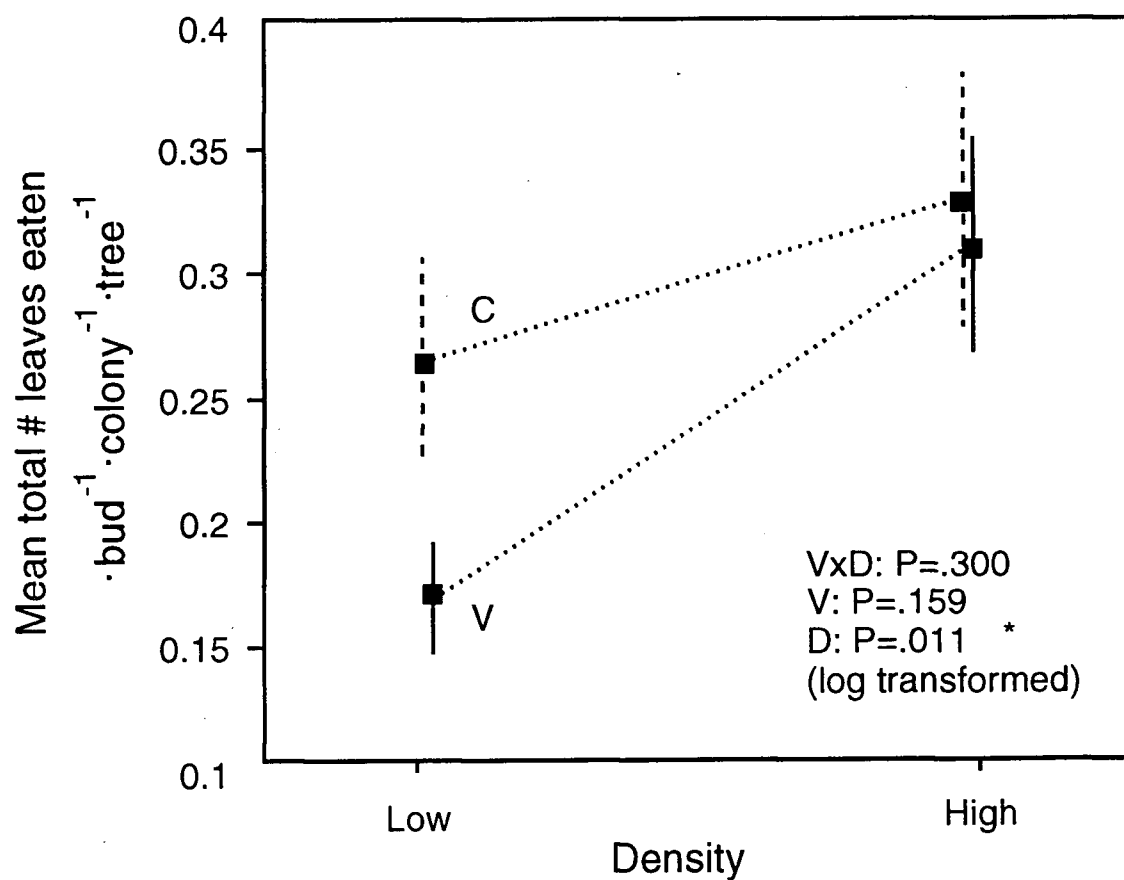
Figure 4.3. Mean defoliation.colony^{-1}.tree^{-1} by the beginning of the prepupal stage (total) in 1993, in the four treatments ($\pm$ S.E.) (C - controls, V - virus treatment, n=16 trees/treatment).
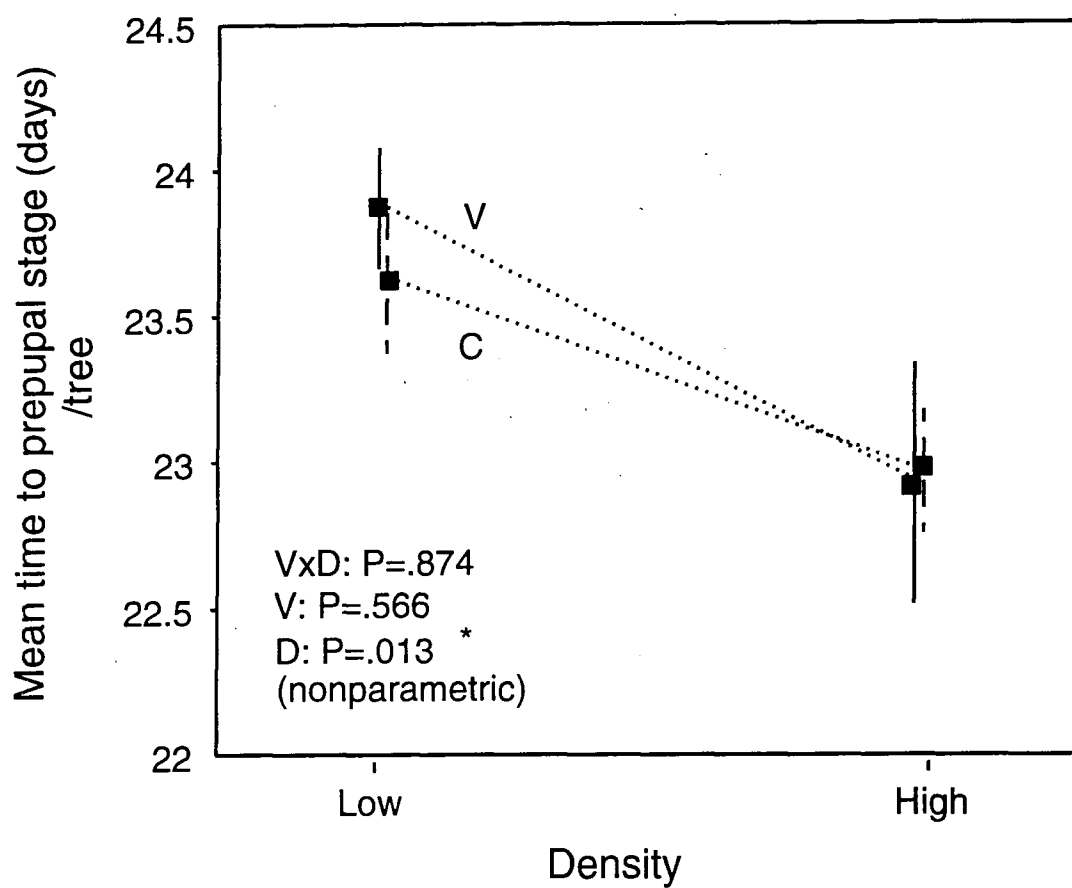
Figure 4.4. Mean time to the prepupal stage/tree from May 4 in 1993, in the four treatments (± S.E.) (C - control, V - virus treatment). The number of replicates (trees)/treatment are H,L=16, VH=15, VL=11.
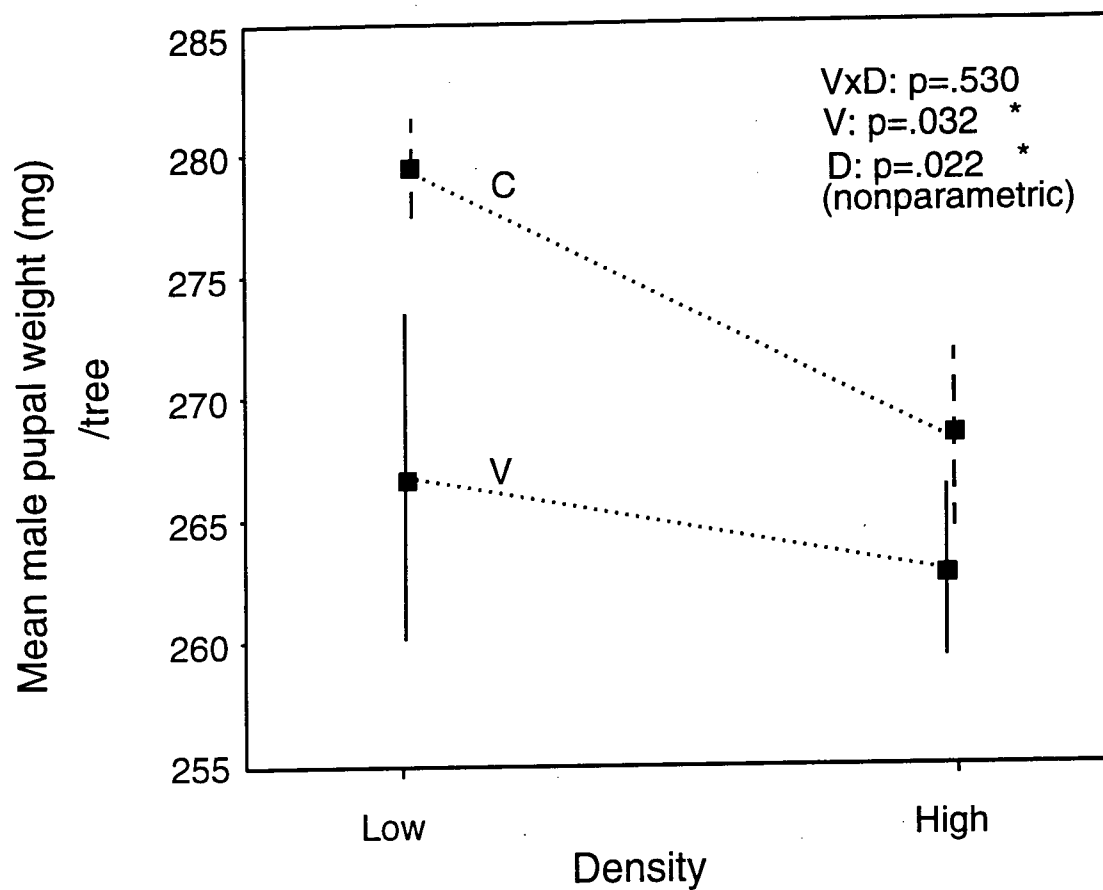
Figure 4.5. Mean male pupal weights/tree in 1993, in the four treatments (±S.E.) (C - control, V - virus treatment). The number of replicates (trees)/treatment are H,L=16, VH=15, VL=10.
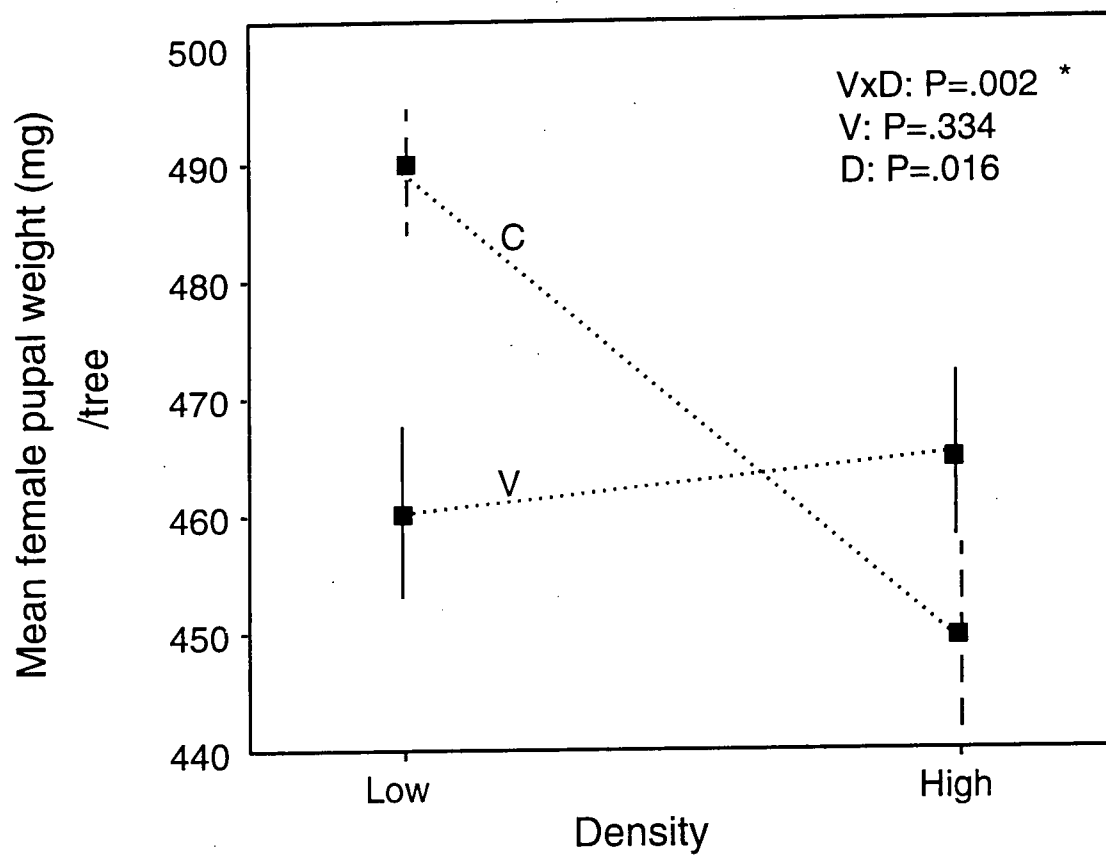
Figure 4.6. Mean female pupal weights/tree in 1993, in the four treatments (±S.E.) (C - control, V - virus treatment). The number of replicates (trees)/treatment are H=16, L=14, VH=12, VL=10.
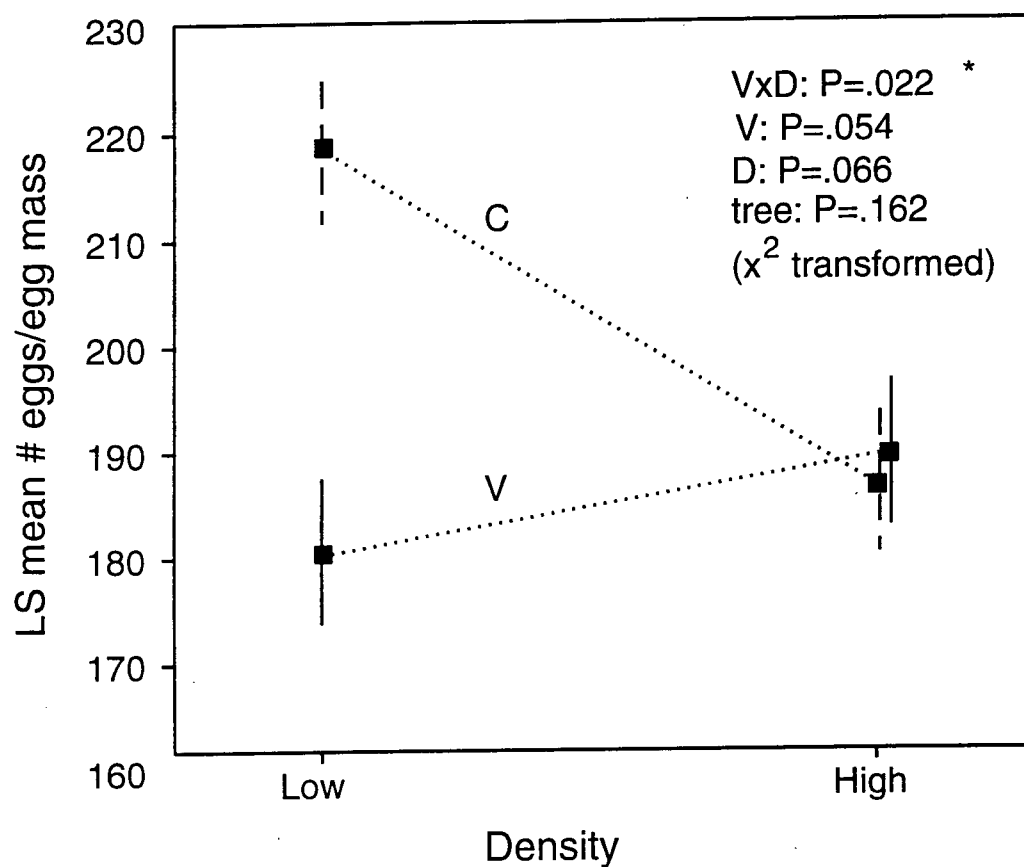
Figure 4.7. Least squares mean fecundity of females/tree in 1993 in the four treatments (± approximate S.E.) (C - control, V - virus treatment). The number of egg masses/treatment = 25 nested within 5 trees/treatment.
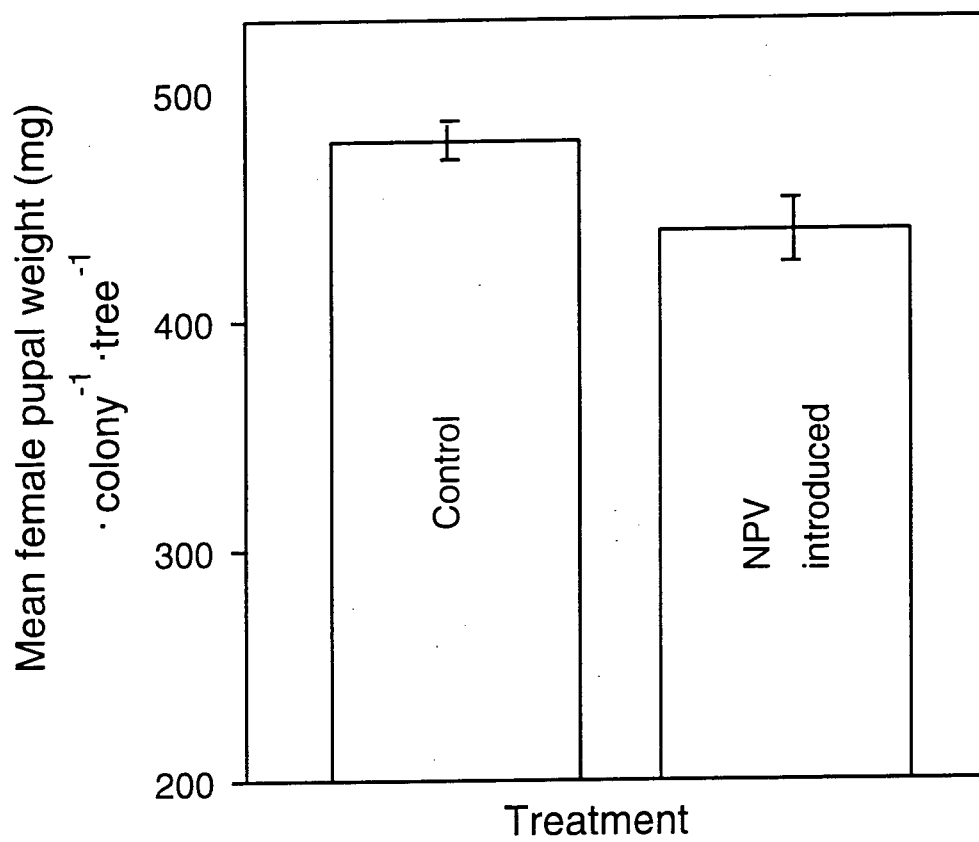
Figure 4.8. Effect of NPV introduction on mean female pupal weights/colony ($\pm$ S.E.)(n=12/treatment, t=2.7, P=.013).
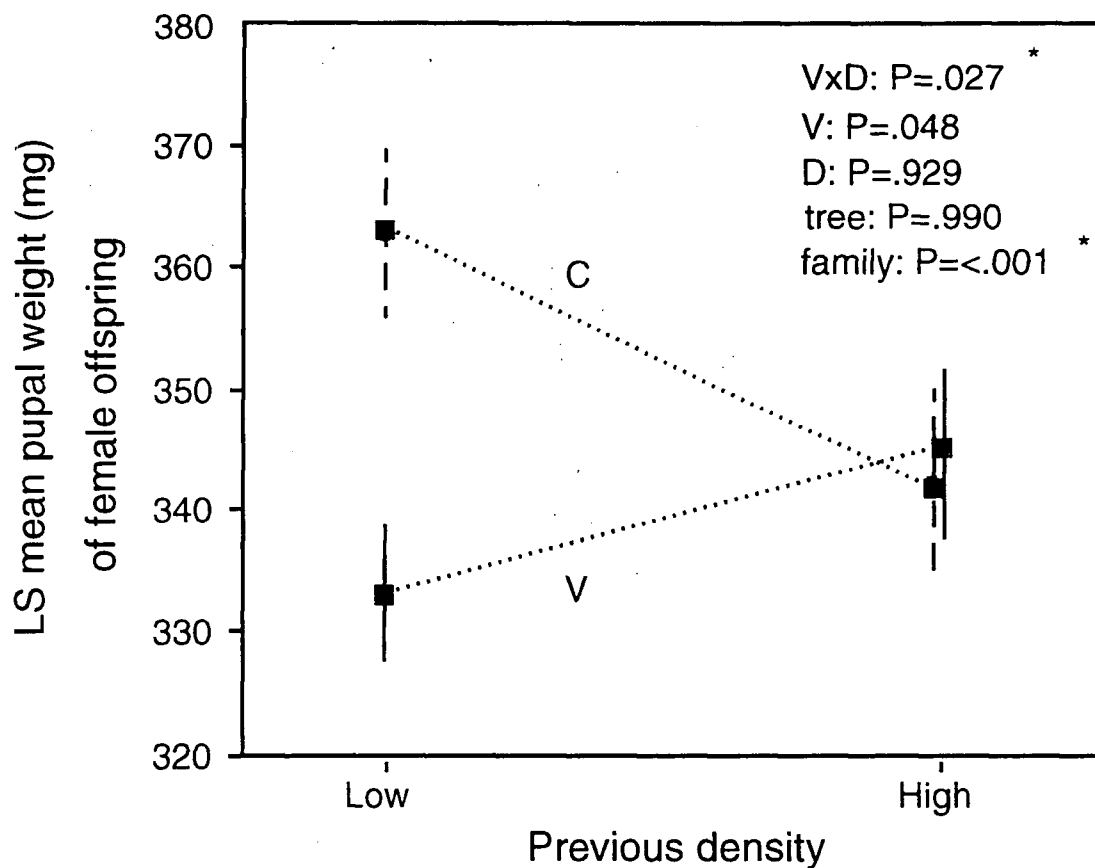
Figure 4.9. Least squares mean pupal weight of female offspring in 1994 in the four treatments (± approximate S.E.) (C - previous control, V - previous virus treatment). The number of egg masses/treatment are H=12, L=15, VH=11, VL=12.
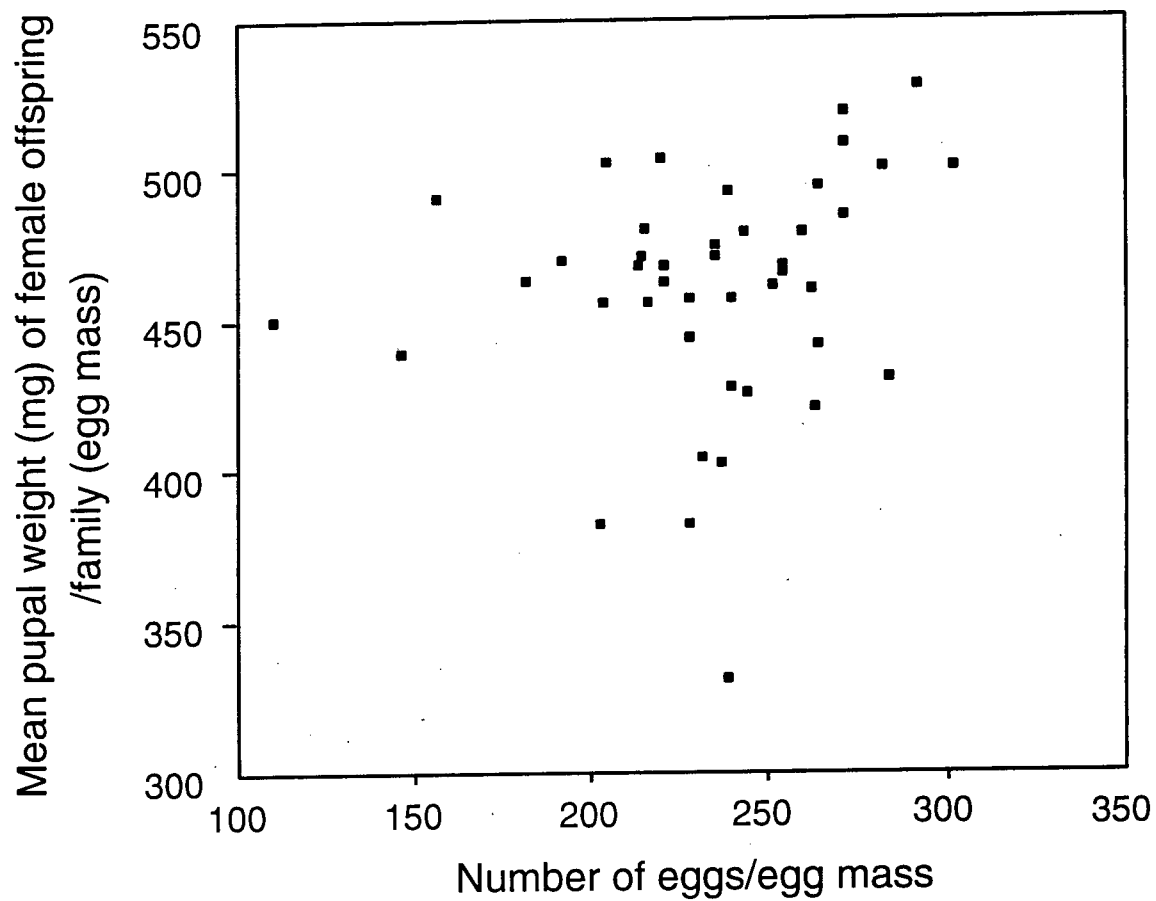
Figure 4.10. Relationship between number of eggs/egg mass and mean offspring female pupal weight/family (n=43, r^2=.035, P=.232).
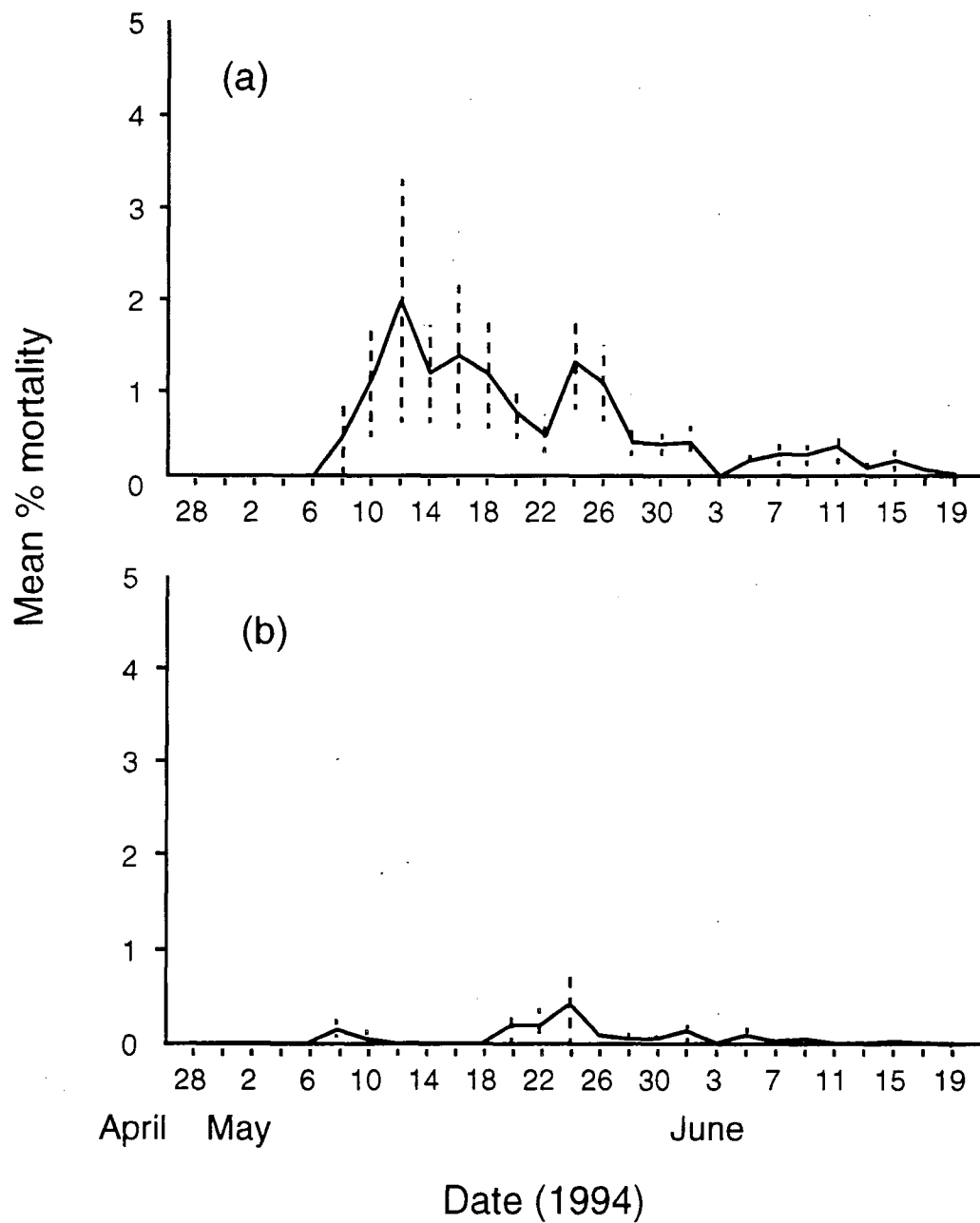
Figure 4.11. Mean % mortality from viral disease at two day intervals from second instar (April 28) till the end of the larval stage in 1994, on trees from the previous (1993) (a) VH and (b) VL treatments (± S.E.) (Mann Whitney U=47.5, n=16 trees/treatment, P=.002).
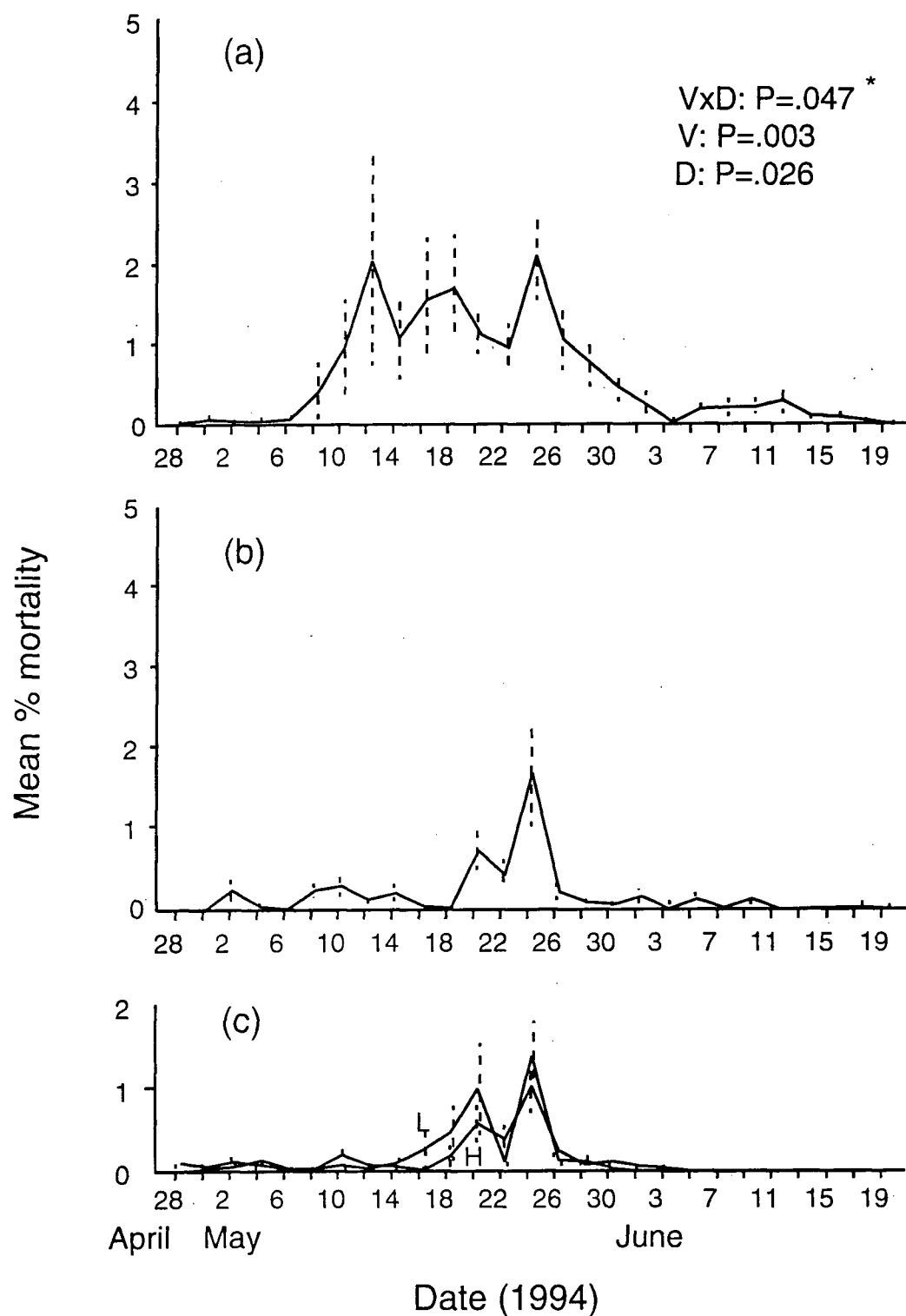
Figure 4.12. Mean % mortality (all causes) at two day intervals from second instar (April 28) till the end of the larval stage in 1994, on trees from the previous (1993) (a) VH, (b) VL and (c) H and L treatments (± S.E.) (n=16 trees/treatment). P values are from ANOVA on totals.
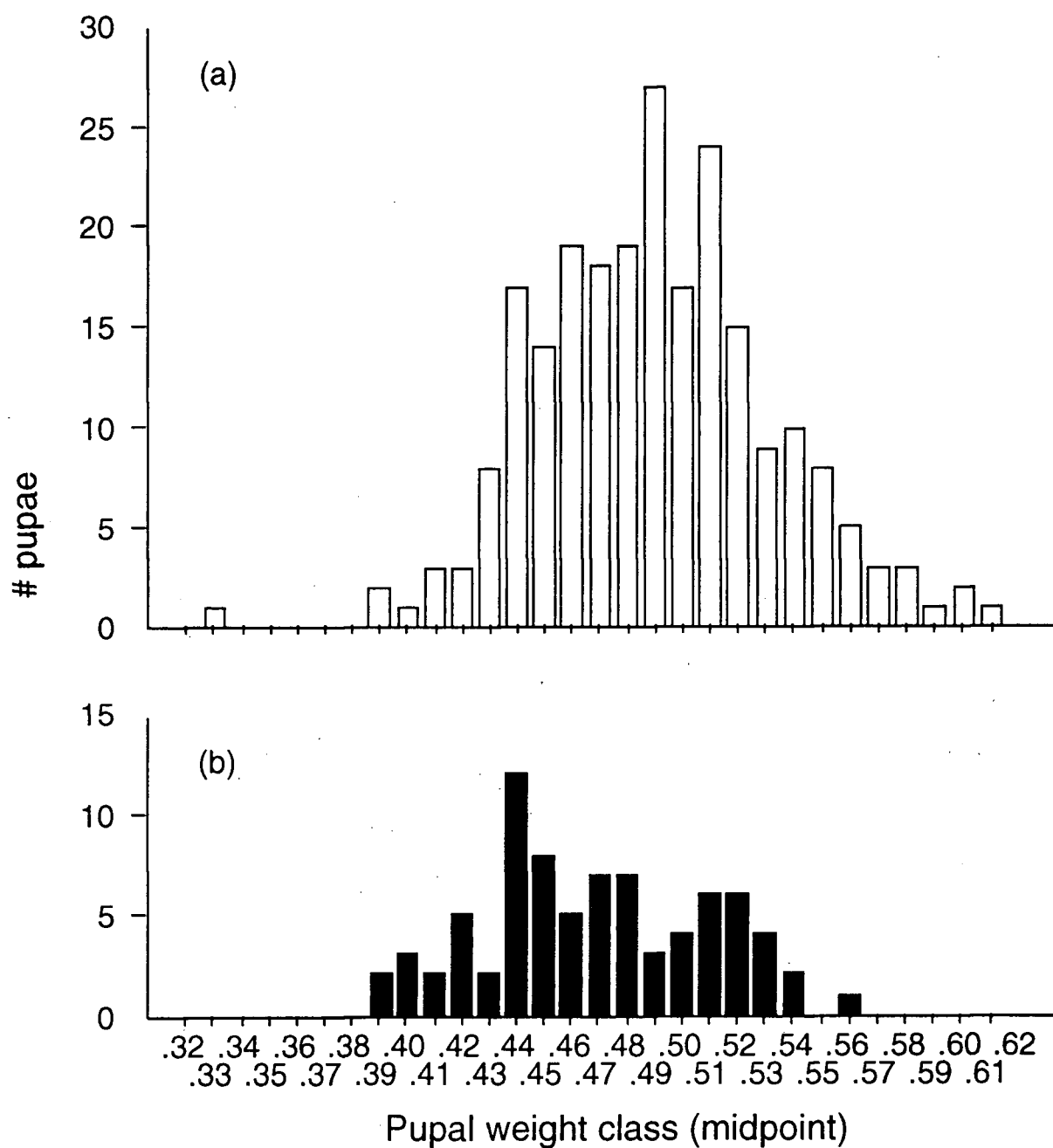
Figure 4.13. Frequency distribution of female pupal weights in the (a) L and (b) VL treatments. (Data for each treatment are pooled across all replicates).

5. CONCLUSIONS

In this thesis I evaluate the potential role of nuclear polyhedrosis virus (NPV) in changes in abundance and individual quality observed in the western tent caterpillar, *M. c. pluviale*. Results may apply more generally to species of Lepidoptera that are hosts to NPV and exhibit cyclic or irruptive population dynamics. A fundamental aspect of fluctuating populations is the prolonged decline phase, where population size continues to decrease for several generations following peaks in abundance. In *M. c. pluviale*, decreases in average fecundity of females track this population decline. Time delays in ecological factors acting at high density may be responsible for these patterns.

The literature review and laboratory experiments of Chapters 2 and 3 illustrate that NPV can significantly influence individual quality in the Lepidoptera, in ways similar to more benign viral diseases, and can potentially have an important impact on host population growth by reducing reproductive output. Further, NPV can reduce fecundity in *M. c. pluviale* and thus provides a plausible explanation for decrease in fecundity in field populations.

Chapter 4 demonstrates that both viral disease and density can influence tent caterpillar performance. However, delayed effects of high density alone via induction in the host food plant is likely absent from the tent caterpillar system. The addition of NPV at high host density is necessary to produce

delayed effects in tent caterpillar performance, which occurs via persistence of virus particles in the environment. Disease and high density in the parental generation can influence offspring reproductive potential, but other performance traits such as egg viability, survival and development rates are not affected. Further, variability in the environmental experience of offspring may be more important in phenotypic expression of reproductive potential than is inheritance of parental phenotype, genetically or via maternal effects. Results of Chapter 4 generally support those of the previous two chapters. Viral disease could explain fecundity reductions in tent caterpillar populations with the additional caveat that disease is more likely to influence fecundity during the prolonged population decline while density alone may be sufficient to initiate reductions in fecundity.

Through an examination of the magnitude of effects, I found that viral disease had a large immediate impact on potential growth of an experimental population, particularly at high density. Delayed effects of previous disease at high density could substantially reduce population growth but could not entirely explain the continued decline observed in a field population of *M. c. pluviale*. I did not examine transgenerational routes of NPV transmission associated directly with egg masses, nor possible interactions between intrinsic and extrinsic factors such as increased susceptibility to virus in offspring of parents experiencing high density or sublethal disease. Both of these factors

could intensify delayed effects of viral epizootics and deserve further study.

Reductions in fecundity did not have a large influence on potential population growth in *M. c. pluviale*. However, models attempting to explain longer term population dynamics of Lepidoptera have found virus-induced reduction in reproduction to be important in the generation of population fluctuations. In a detailed simulation model of NPV and *M. c. pluviale* population dynamics, Beukema and Myers (1995) found that fecundity reductions from 38-69% in sublethally infected females were necessary to generate cycles with a period of 8-12 years. These reductions are far greater than those observed in the laboratory experiment (17% see Chapter 3). In a more general pathogen-host model, Anderson and May (1981, page 487) observed that reductions in host fecundity from >10 to 50% could enlarge the domain of cyclic behaviour in the host population. More recently, Briggs and Godfray (1995) incorporated seasonality in a pathogen-host model and found that the additions of sublethal infections and reduced host fecundity could lead to long term oscillations in host abundance.

Nuclear polyhedrosis virus may play a role in observed patterns in tent caterpillar populations. NPV could explain continued fecundity decrease during population declines in *M. c. pluviale*. NPV is more likely to destabilize tent caterpillar populations and lead to cyclic dynamics because of its ability to

persist in the environment, than is high density alone via host plant induction. Inherited effects of previous disease and density could contribute to continued population decline but may be obscured by variability in offspring environment. Environmental persistence of NPV and reinfection of early instar larvae following an epizootic at high host density may contribute to prolonged population declines in *M. c. pluviale*.

REFERENCES

Abul-Nasr, S.E., E.D. Ammar and S.M. Abul-Ela. 1979. Effects of nuclear polyhedrosis virus on various developmental stages of the cotton leafworm *Spodoptera littoralis* (Boisd.) Journal of Applied Entomology 88:181-187.

Anderson, R.M. and R.M. May. 1981. The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society of London, Series B 291:451-525.

Baltensweiler, W., G. Benz, P. Bovey and V. Delucchi. 1977. Dynamics of larch bud moth populations. Annual Review of Entomology 22:79-100.

Barbosa, P. and W. Baltensweiler. 1987. Phenotypic plasticity and herbivore outbreaks. Pages 469-503 *in* P. Barbosa and J.C. Schultz, editors. Insect Outbreaks. Academic Press, San Diego, California.

Barbosa, P. and P. Martinat. 1987. Causes and ecological implications of egg retention in the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). Canadian Entomologist 119:765-771.

Barbosa, P., W. Cranshaw and J.A. Greenblatt. 1981. Influence of food quantity and quality on polymorphic dispersal behaviours in the gypsy moth, *Lymantria dispar*. Canadian Journal of Zoology 59:293-296.

Barbour, D.A. 1988. The pine looper in Britain and Europe. Pages 291-308 *in* A.A. Berryman, editor. Dynamics of Forest Insect Populations. Plenum, New York.

Bell, M.R. and R.F. Kanavel. 1976. Effect of dose of cytoplasmic polyhedrosis virus on infection, mortality, development rate, and larval and pupal weights of the pink bollworm. Journal of Invertebrate Pathology 28:121-126.

Benz, G. 1974. Negative ruckkoppelung durch raum- und nahrungskonkurrenz sowie zyklische veranderung der nahrungsgrundlage als regelprinzip in der populationsdynamik des grauen larchenwicklers, *Zeiraphera diniana* (Guenee) (Lep., Tortricidae). Journal of Applied Entomology 76:196-228.

Berryman, A.A., J.A. Millstein and R.R. Mason. 1990. Modelling douglas-fir tussock moth population dynamics: the case for simple theoretical models. Pages 369-380 *in* A.D. Watt, S.R. Leather, M.D. Hunter and N.A.C. Kidd, editors. Population Dynamics of Forest Insects. Intercept,

Andover.

Berryman, A.A., N. Chr. Stenseth, A.S. Isaev. 1987. Natural regulation of herbivorous forest insect populations. Oecologia 71:174-184.

Beukema, S.J. and J.H. Myers. 1995. Towards more realistic models of disease and insect population dynamics: virus-tent caterpillar interactions. Manuscript in preparation.

Bird, F.T. 1969. Infection and mortality of spruce budworm, *Choristoneura fumiferana*, and forest tent caterpillar, *Malacosoma disstria*, caused by nuclear and cytoplasmic polyhedrosis viruses. Canadian Entomologist 101:1269-1285.

Blais, J.R. 1952. The relationship of the spruce budworm (*Choristoneura fumiferana*, Clem.) to the flowering condition of balsam fir (*Abies balsamea* (L.) Mill.). Canadian Journal of Zoology 30:1-29.

Bong, C.F.J. and P.P. Sikorowski. 1991. Effects of cytoplasmic polyhedrosis virus and bacterial contamination on growth and development of the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae). Journal of Invertebrate Pathology 57:406-412.

Briggs, C.J. and H.C.J. Godfray. 1995. The dynamics of insect-pathogen interactions in seasonal environments. Theoretical Population Biology (In Press).

Bullock, H.R., E. Martinez an C.W. Stuermer, Jr. 1970 Cytoplasmic-polyhedrosis virus and development and fecundity of the pink bollworm. Journal of Invertebrate Pathology 15:109-112.

Burand, J.P. and E.J. Park. 1992. Effect of nuclear polyhedrosis virus infection on the development and pupation of gypsy moth larvae. Journal of Invertebrate Pathology 60:171-175.

Campbell, R.W. 1978. Some effects of gypsy moth density on rate of development, pupation time, and fecundity. Annals of the Entomological Society of America 71:442-448.

Capinera, J.L. and P.D. Barbosa. 1977. Influence of natural diets and larval density on gypsy moth, *Lymantria dispar* (Lepidoptera: Orgyiidae), egg mass characteristics. Canadian Entomologist 109:1313-1318.

Caswell. H. 1972. A simulation study of a time lag population model. Journal

of Theoretical Biology 34:419-439.

Chitty, D. 1960. Population processes in the vole and their relevance to general theory. Canadian Journal of Zoology 38:99-113.

Chitty, D. 1967. The natural selection of self-regulatory behaviour in animal populations. Proceeding of the Ecological Society of Australia 2:51-78.

Clark, E.C. 1955. Observations on the ecology of a polyhedrosis of the great basin tent caterpillar *Malacosoma fragilis*. Ecology 36:373-376.

Clark, E.C. 1956. Survival and transmission of a virus causing polyhedrosis in *Malacosoma fragile*. Ecology 37:728-732.

Clark, E.C. 1958. Ecology of the polyhedroses of tent caterpillars. Ecology 39: 132-139.

Day, R.W. and G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecological Monographs 59:433-463.

Dempster, J.P. and K.H. Lakhani. 1979. A population model for cinnabar moth and its food plant, ragwort. Journal of Animal Ecology 48:143-163.

Doane. C.C. 1967. Bioassay of nuclear-polyhedrosis virus against larval instars of the gypsy moth. Journal of Invertebrate Pathology 9:376-386.

Doane. C.C. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. Journal of Invertebrate Pathology 15:21-33.

Dwyer, G. 1991. The roles of density, stage, and patchiness in the transmission of an insect virus. Ecology 72:559-574.

Dwyer, G. and J.S. Elkinton. 1993. Using simple models to predict virus epizootics in gypsy moth populations. Journal of Animal Ecology 62:1-11.

Elkinton, J.S. and A.M. Liebhold. 1990. Population dynamics of gypsy moth in North America. Annual Revue of Entomology 35:571-596.

Embree, D.G. 1965. The population dynamics of the winter moth in Nova Scotia. Memoirs of the Entomological Society of Canada 46:1-57.

Entwistle, P.F. and H.F. Evans. 1985. Viral control. Pages 347-412 *in* L.I. Gilbert and G.A. Kerkut, editors. Comprehensive Insect Physiology,

Biochemistry, and Pharmacology. Pergamon, Oxford.

Evans, H.F. and P.F. Entwistle. 1987. Viral diseases. Pages 257-322 *in* J.R. Fuxa and Y. Tanada, editors. Epizootiology of Insect Diseases. John Wiley & Sons, New York.

Foltz, J.L., F.B. Knight and D.C. Allen. 1972. Numerical analysis of population fluctuations of the jack pine budworm. Annals of the Entomological Society of America 65:82-89.

Fowler, S. V. and J.H. Lawton. 1985. Rapidly induced defenses and talking trees: the devil's advocate position. American Naturalist. 126:181-195.

Geier, P.W. and L.T. Oswald. 1977. The light-brown apple moth, *Epiphyas postvittana* (Walker). Australian Journal of Ecology 2:9-29.

Ginzburg, L.R. and D.E. Taneyhill. 1994. Population cycles of forest Lepidoptera: a maternal effect hypothesis. Journal of Animal Ecology 63:79-92.

Gruys, P. 1970. Growth in *Bupalus piniarius* (Lepidoptera: Geometridae) in relation to larval population density. Research Institute for Nature Management, number 1, Centre for Agricultural Publishing and Documentation, Wageningen.

Harcourt, D.G. 1966. Major factors in survival of the immature stages of *Pieris rapae* (L.). Canadian Entomologist 98:653-662.

Hassell, M.P. and R.M. May. 1989. The population biology of host-parasite and host-parasitoid associations. Pages 319-348 *in* J. Roughgarden, R.M. May and S.A. Levin, editors. Perspectives in Ecological Theory. Princeton University Press, Princeton, New Jersey.

Haukioja, E. and S. Neuvonen. 1985. The relationship between size and reproductive potential in male and female Epirrita autumnata (Lep., Geometridae). Ecological Entomology 10: 267-270.

Haukioja, E. 1990. Induction of defenses in trees. Annual Review of Entomology 36:25-42.

Haukioja, E. and Neuvonen, S. 1985. The relationship between size and reproductive potential in male and female *Epirrita autumnata* (Lep., Geometridae). Ecological Entomology 10:267-270.

Haukioja, E. and S. Neuvonen. 1987. Insect population dynamics and induction of plant resistance: the testing of hypotheses. Pages 411-432 *in* P. Barbosa and J.R. Schultz, editors. Insect Outbreaks. Academic Press, San Diego.

Haukioja, E., E. Pakarinen, P. Niemela and L. Iso-Iivari. 1988. Crowding-triggered phenotypic responses alleviate consequences of crowding in *Epirrita autumnata* (Lep., Geometridae). Oecologia 75:549-558.

Hochburg. M.E. 1989. The potential role of pathogens in biological control. Nature 337:262-265.

Hodson, A.C. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn. in northern Minnesota. Minnesota Agricultural Experimental Station Technical Bulletin 148:1-55.

Hutchinson, G.E. 1948. Circular causal systems in ecology. Annals of the New York Academy of Science 50:221-246.

Ignoffo, C.M. 1964. Bioassay Technique and pathogenicity of a nuclear-polyhedrosis virus of the cabbage looper, *Trichoplusia ni* (Hubner). Journal of Insect Pathology 6:237-245.

Ignoffo, C.M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). Journal of Invertebrate Pathology 7:315-319.

Ignoffo, C.M. and J.R. Adams. 1966. A cytoplasmic-polyhedrosis virus, *Smithiavirus pectinophorae* sp. n. of the pink bollworm, *Pectinophora gossypiella* (Saunders). Journal of Invertebrate Pathology 8:59-66.

Jacques, R.P. 1969. Leaching of nuclear polyhedrosis virus of *Trichoplusia ni* from soil. Journal of Invertebrate Pathology 13:256-263.

Kalmakoff, J. and P.J. Wigley. 1980. Purification of NPV from *Heliothis armigera* larvae and serological detection of infection. Pages 26-42 *in* J. Kalmakoff and J.F. Longworth, editors. Microbial Control of Insect Pests. New Zealand Department of Industry and Research, Bulletin #228.

Karban, R. 1993. Induced resistance and plant density of a native shrub, *Gossypium thurberi*, affect its herbivores. Ecology 74:1-8.

Karban, R. and J.H. Myers. 1989. Induced plant responses to herbivory. Annual

Review of Ecology and Systematics 20:3 31-348.

Kellen, W.R. and D.F. Hoffmann. 1983. Longevity and fecundity of adult *Amyelois transitella* (Lepidoptera: Pyralidae) infected by two small RNA viruses. Environmental Entomology 12:1542-1546.

Klein, M. and H. Podoler. 1978. Studies on the application of a nuclear polyhedrosis virus to control populations of the Egyptian cottonworm, *Spodoptera littoralis*. Journal of Invertebrate Pathology 32:244-248.

Klomp, H. 1966. The dynamics of a field population of the pine looper, *Bupalus piniarius* L. (Lep., Geom.). Advances in Ecological Research 3:207-305.

Luttrell, R.G., W.C. Yearian and S.Y. Young. 1982. Effects of Elcar (*Heliothis zea* nuclear polyhedrosis virus) treatments on *Heliothis* spp. Journal of the Georgian Entomological Society 17:211-221.

Magnoler, A. 1974a. Bioassay of nucleopolyhedrosis virus of the gypsy moth, *Porthetria dispar*. Journal of Invetebrate Pathology 23:190-196.

Magnoler, A. 1974b. Effects of a cytoplasmic polyhedrosis on larval and postlarval stages of the gypsy moth, *Porthetria dispar*. Journal of Invertebrate Pathology 23:263-274.

Magnoler, A. 1975. Bioassay of nucleopolyhedrosis virus against larval instars of *Malacosoma neustria*. Journal of Invertebrate Pathology 25:343-348.

Mason, R.R., R.C. Beckwith and H.G. Paul. 1977. Fecundity reduction during collapse of a douglas-fir tussock moth outbreak in northeast Oregon. Environmental Entomology 6:623-626.

Maleki-Milani, H. 1970. Surquelques caracteres de la virose cytoplasmizue de la noctuelle du chou, *Mamestra (Barathra) brassicae* L. [Lep. *Noctuidae*]. Entomophaga 15:315-325.

May, R.M., G.R. Conway, M.P. Hassell, T.R.E. Southwood. 1974. Time delays, density-dependence and single species oscillations. Journal of Animal Ecology 43:747-770.

Melamed-Madjar, V. and B. Raccah. 1979. The transstadial and vertical transmission of a granulosis virus from the corn borer *Sesamia nonagrioides*. Journal of Invertebrate Pathology 33:259-264.

Miller, C.A. 1957. A technique for estimating the fecundity of natural

populations of the spruce budworm. Canadian Journal of Zoology 35:1-13.

Miller, C. A. 1963. The analysis of fecundity proportion in the unsprayed area. Pages 75-87 *in* R.F. Morris, editor. The Dynamics of Epidemic Spruce Budworm Populations. Memoirs of the Entomological Society of Canada 31.

Mohamed, A.K, J.R. Yang and F.R.S. Nelson. 1989. Influence of insecticides - cytoplasmic polyhedrosis virus combinations on pupal weight and fecundity of tobacco budworm (Lepidoptera: Noctuidae). Journal of Entomological Science 24:539-544.

Morris, O.N., Armstrong, J.A., Howse, G.M. and J.C. Cunningham. 1974. A 2-year study of virus chemical insecticide combination in the integrated control of the spruce budworm, *Choristoneura fumiferana* (Tortricidae: Lepidoptera). Canadian Entomologist 106:813-824.

Munster-Swendsen, M. 1991. The effect of sublethal neogregarine infections in the spruce needleminer, *Epinotia tedella* (Lepidoptera: Tortricidae). Ecological Entomology 16:211-219.

Murray, K.D. and J.S. Elkinton. 1989. Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). Journal of Invertebrate Pathology 53:324-334.

Murray, K.D., K.S. Shields, J.P. Burand and J.S. Elkinton. 1991. The effect of gypsy moth metamorphosis on the development of nuclear polyhedrosis virus infection. Journal of Invertebrate Pathology 57:352-361.

Myers, J.H. 1978. A search for behavioral variation in first and last laid eggs of western tent caterpillar and an attempt to prevent a population decline. Canadian Journal of Zoology 56:2359-2363.

Myers, J.H. 1988. Can a general hypothesis explain population cycles of forest Lepidoptera? Advances in Ecological Research 18:179-242.

Myers, J.H. 1990. Population cycles of western tent caterpillars: Experimental introductions and synchrony of fluctuations. Ecology 71:986-995.

Myers, J.H. 1993. Population outbreaks in forest Lepidoptera. American Scientist 81:240-251.

Myers, J.H. and B. Kukan. 1995. Changes in the fecundity of tent caterpillars: A correlated character of disease resistance or sublethal effect of disease? Oecologia, in press.

Myers, J.H. and L.D. Rothman 1995a. Field experiments to study regulation of fluctuating populations. Pages 229-250 *in* N. Cappuccino and P.W. Price, editors. Population Dynamics: New Approaches and Synthesis. Academic Press, San Diego.

Myers, J.H. and L.D. Rothman. 1995b. Virulence and transmission of infectious diseases in humans and insects: evolutionary and demographic patterns. Trends in Ecology and Evolution 10:194-198.

Myers, J.H. and K.S. Williams. 1984. Does tent caterpillar attack reduce the food quality of red alder foliage? Oecologia 64:74-79.

Myers, J.H. and K.S. Williams. 1987. Lack of short or long term inducible defenses in the red alder-western tent caterpillar system. Oikos 48:73-78.

Nef, P.L. 1971. Influence de traitements insecticides chimiques et microbiens sur une population de *Stilpnotia* (=*Leucoma*) *salicis* L. et sur ses parasites. Journal of Applied Entomology 69:357-367.

Neilson, M.M. 1965. Effects of a cytoplasmic polyhedrosis on adult Lepidoptera. Journal of Invertebrate Pathology 7:306-314.

O'Reilley, D.R. and L.K. Miller. 1989. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. Science 245:1110-1112.

Park, E.J., J.P. Burand and C.M. Yin. 1993. The effect of baculovirus infection on ecdysteroid titer in gypsy moth larvae (*Lymantria dispar*). Journal of Insect Physiology 39:791-796.

Patil, U.R., C.J. Savanurmath, S.B. Mathad, P.I. Aralaguppi, and S.S. Ingalhalli. 1989. Effects of nuclear polyhedrosis virus on the growth, development and reproduction in surviving generations of the armyworm *Mythimna (Pseudaletia) separata* (Walker). Journal of Entomological Science 108:527-532.

Perelle, A.H. and J.D. Harper. 1986. An evaluation of the impact of sublethal dosages of nuclear polyhedrosis virus in larvae on pupae, adults, and adult progeny of the fall armyworm, *Spodoptera frugiperda*. Journal of Invertebrate Pathology 47:42-47.

Peters, T. M. and P. Barbosa. 1977. Influence of population density on size, fecundity, and developmental rate of insects in culture. Annual Review of Entomology 22:431-450.

Podgwaite, J.D., K.S. Shields, R.T. Zerillo and R.B. Bruen. 1979. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar*. Environmental Entomology 8:528-536.

Poinar, G.O. Jr. and G.M. Thomas. 1984. Laboratory Guide to Insect Pathogens and Parasites. Plenum Press, New York.

Roland, J. and J.H. Myers. 1987. Improved insect performance from host-plant defoliation: winter moth on oak and apple. Ecological Entomology 12:409-414.

Rossiter, M.C. 1991. Environmentally-based maternal effects: a hidden force in insect population dynamics? Oecologia 87:288-294.

Rossiter, M.C. 1992. The impact of resource variation on population quality in herbivorous insects: A critical component of population dynamics. Pages 13-42 *in* M.D. Hunter, T. Ohgushi, and P.W. Price, editors. Effects of resource distribution and animal-plant interactions. Academic Press, San Diego, California.

Rossiter, M.C. 1994. Maternal effects hypothesis of herbivore outbreak. BioScience 44:752-763.

Rossiter, M.C. 1995. Impact of life-history evolution on population dynamics: predicting the presence of maternal effects. Pages 251-275 *in* N. Cappuccino and P.W. Price, editors. Population Dynamics: New Approaches and Synthesis. Academic Press, San Diego.

Rossiter, M.C., D.L. Cox-Foster and M.A. Briggs. 1993. Initiation of maternal effects in *Lymantria dispar*: Genetic and ecological components of egg provisioning. Journal of Evolutionary Biology 6:577-589.

Rothman, L.D. and J.H. Myers. 1994. Effect of nuclear polyhedrosis virus treatment on reproductive potential of western tent caterpillar (Lepidoptera: Lasiocampidae). Environmental Entomology 23:864-869.

Rothman, L.D. and J.H. Myers. 1995. Debilitating effects of viral diseases on host Lepidoptera. Journal of Invertebrate Pathology (In Press).

Sait, S.M., M. Begon, and D.J. Thompson. 1994. The effects of a sublethal

baculovirus infection in the Indian meal moth, *Plodia interpunctella.* Journal of Animal Ecology 63:541-550.

Santiago-Alvarez, C. and E. Vargas Osuna. 1988. Reduction of reproductive capacity of *Spodoptera littoralis* males by a nuclear polyhedrosis virus (NPV). Journal of Invertebrate Pathology 52:142-146.

SAS Institute, Inc. 1983. SAS User's Guide: Statistics. Cary, North Carolina.
Shapiro, M. and J.L. Robertson. 1987. Yield and activity of gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus recovered from survivors of viral challenge. Journal of Economic Entomology 80:901-905.

Sikorowski, P.P. and A.M. Lawrence. 1994. *Heliothis* cytoplasmic polyhedrosis virus and its effect upon microbial contaminant-free *Heliothis virescens*. Journal of Invertebrate Pathology 63:56-62.

Sikorowski, P.P. and A.C. Thompson. 1979. Effects of cytoplasmic polyhedrosis virus on diapausing Heliothis virescens. Journal of Invertebrate Pathology 33:66-70.

Simmons, C.L. and P.P. Sikorowski. 1973. A laboratory study of the effects of cytoplasmic polyhedrosis virus on *Heliothis virescens* (Lepidoptera: Noctuidae). Journal of Invertebrate Pathology 22:369-371.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. W.H. Freeman and Company, New York.

Southwood, T.R.E. 1991. Ecological Methods. Chapman and Hall, London.

Stairs, G.R. 1965. Artificial initiation of virus epizootics in forest tent caterpillar populations. Canadian Entomologist 97:1059-1062.

Stairs, G.R. 1966. Transmission of virus in tent caterpillar populations. Canadian Entomologist 98:1100-1104.

Steinhaus, E.A. 1958. Crowding as a possible stress factor in insect disease. Ecology 39:503-514.

Tanada Y. and J.R. Fuxa. 1987. The pathogen population. Pages 113-158 in J.R. Fuxa and Y. Tanada, editors. Epizootiology of Insect Diseases. John Wiley, New York.

Tanada, Y. and A.M. Tanabe. 1964. Response of the adults of the armyworm,

Pseudaletia unipuncta (Haworth), to cytoplasmic-polyhedrosis-virus infection in the larval stage. Journal of Insect Pathology 6:486-490.

Thomas, D.E., C.F. Reichelderfer and A.M. Heimper. 1972. Accumulation and persistence of a nuclear-polyhedrosis virus of the cabbage looper in the field. Journal of Invertebrate Pathology 20:157-164.

Thompson, C.G. and D.W. Scott. 1979. Production and persistence of the nuclear-polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae) in the forest ecosystem. Journal of Invertebrate Pathology 33:57-65.

Turchin, P. 1990. Rarity of density dependence or population regulation with lags? Nature 344:660-663.

Vail, P.V. and D. Gough. 1970. Effects of cytoplasmic-polyhedrosis virus on adult cabbage loopers and their progeny. Journal of Invertebrate Pathology 15:397-400.

Vail, P.V. and I.M. Hall. 1969. Susceptibility of the pupa of the cabbage looper, *Trichoplusia ni*, to nuclear polyhedrosis virus. Journal of Invertebrate Pathology 14:227-236.

Vail, P.V., I.M. Hall and D. Gough. 1969. Influence of a cytoplasmic polyhedrosis on varius developmental stages of the cabbage looper. Journal of Invertebrate Pathology 14:237-244.

Vargas-Osuna, E. and C. Santiago-Alvarez. 1988. Differential response of male and female *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae) individuals to a nuclear polyhedrosis virus. Journal of Applied Entomology 105:374-378.

Washburn, J.O., D.R. Mercer and J.R. Anderson. 1991. Regulatory role of parasites: impact on host population shifts with resource availability. Science 253:185-188.

Watanabe, H. 1987. The host population. Pages 71-112 *in* J.R. Fuxa and Y. Tanada, editors. Epizootiology of Insect Diseases. John Wiley & Sons, New York.

Wellington, W.G. 1957. Individual differences as a factor in population dynamics: the development of a problem. Canadian Journal of Zoology 35:293-323.

Wellington, W.G. 1960. Qualitative changes in natural populations during changes in abundance. Canadian Journal of Zoology 38:289-314.

Wellington, W.G. 1962. Population quality and the maintenance of nuclear polyhedrosis between outbreaks of *Malacosoma pluviale* (Dyar). Journal of Insect Pathology 4:285-305.

Wellington, W.G. 1965. Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). Canadian Entomologist 97:1-14.

Wellington, W.G. and D.A. Maelzer. 1967. Effects of farnesyl methyl ether on the reproduction of the western tent caterpillar, *Malacosoma pluviale*: some physiological, ecological, and practical implications. Canadian Entomologist 99:249-263.

Wellington, W. G., P.J. Cameron, W.A. Thompson, I.B. Vertinsky, and A.S. Landsberg. 1975. A stochastic model for assessing the effects of external and internal heterogeneity on an insect population. Researches in Population Ecology 17:1-28.

Williams, K.S. and J.H. Myers. 1984. Previous herbivore attack of red alder may improve food quality for fall webworm. Oecologia 63:166-170.

Witter, J.A. and H.M. Kulman. 1972. A review of the parasites and predators of tent caterpillars (*Malacosoma* spp.) in North America. Minnesota Agriculture Experimental Station Technical Bulletin 289.

Landsberg. 1975. A stochastic model for assessing the effects of external and internal heterogeneity on an insect population. Researches in Population Ecology 17:1-28.

Wiygul, G. and P.P. Sikorowski. 1978. Oxygen uptake in tobacco budworm larvae (*Heliothis virescens*) infected with cytoplasmic polyhedrosis virus. Journal of Invertebrate Pathology 32:191-195.

Wiygul, G. and P.P. Sikorowski. 1991. Oxygen uptake in larval bollworms (*Heliothis zea*) infected with iridescent virus. Journal of Invertebrate Pathology 58:252-256.

Woods, S.A. and J.S. Elkinton. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. Journal of Invertebrate Pathology 50:151-157.

Woods, S.A., J.S. Elkinton and J.D. Podgwaite. 1989. Acquisition of nuclear polyhedrosis virus from tree stems by newly emerged gypsy moth (Lepidoptera: Lymantriidae) larvae. Environmental Entomology 18:298-301.

Young, S.Y. 1990. Effect of nuclear polyhedrosis virus infection in *Spodoptera ornithogalli* larvae on post larval stages and dissemination by adults. Journal of Invertebrate Pathology 55:69-75.

Young, S.Y. and W.C. Yearian. 1982. Nuclear polyhedrosis virus infection of *Pseudoplusia includens* (Lep.:Noctuidae) larvae: effect on post larval stages and transmission. Entomophaga 27:61-66.

Zar, J. H. 1984. Biostatistical Analysis. Second edition. Prentice-Hall, Englewood Cliffs, New Jersey.

APPENDIX A. ANOVA tables for NPV treatment and family effects in the laboratory. Type III SS used for all analyses.

Male pupal weight in 1993 (survivors to pupal stage)
(unbalanced factorial)

Source	DF	MS	Denom. MS	F	P
1) v x f	7	139245.46	141025.39	.99	.444
2) virus	1	327123.82	139596.90	2.34	.154
3) family	7	2821482.10	139596.90	20.26	<.001*
error	125	141025.39			

Error term (denominator MS) composition and DFs:
1) MS(error), DF=125
2) 0.803*MS(v f)+0.197*MS(error), DF=10.89
3) MS(v f), DF=7

Female pupal weight in 1993 (survivors to pupal stage)
(unbalanced factorial)

Source	DF	MS	Denom. MS	F	P
1) v x f	7	78839.09	289544.16	.27	.963
2) virus	1	139606.12	136523.90	10.23	.003*
3) family	7	10829630.43	78839.09	137.36	<.001*
error	68	289544.16			

Error term (denominator MS) composition and DFs:
1) MS(error), DF=68
2) 0.726*MS(v f)+0.274*MS(error), DF=33.24
3) MS(v f), DF=7

Fecundity in 1993
(unbalanced factorial)

Source	DF	MS	Denom. MS	F	P
1) v x f	7	625.80	748.97	.84	.564
2) virus	1	5851.05	664.22	8.81	.009*
3) family	7	9831.75	625.80	15.71	<.001*
error	43	748.97			

APPENDIX A cont.

Error term (denominator MS) composition and DFs:
1) MS(error), DF=68
2) 0.688*MS(v f)+0.312*MS(error), DF=15.89
3) MS(v f), DF=7

APPENDIX B. ANCOVA tables for pupal weight and NPV treatment effects on fecundity in the laboratory.

1992 test for virus x pupal weight interaction or heterogeneity of slopes (Type I SS)

Source	DF	MS	F	P
pwt	1	51891.87	35.23	<.001
virus	1	8758.03	5.95	.017
pxv	1	431.07	.29	.590
error	67	1473.15		

1992 test for virus treatment effect or heterogeneity of y-intercepts (interaction term removed)

Source	DF	MS	F	P
pwt	1	5189.87	35.60	<.001
virus	1	8758.03	6.01	.017*
error	68	1457.83		

1993 test for virus x pupal weight interaction or heterogeneity of slopes (Type I SS)

Source	DF	MS	F	P
pwt	1	100688.18	451.83	<.001
virus	1	495.25	2.22	.142
pxv	1	761.73	3.42	.070
error	55	222.85		

1993 test for virus treatment effect or heterogeneity of y-intercepts (interaction term removed)

Source	DF	MS	F	P
pwt	1	100688.18	433.13	<.001
virus	1	495.25	2.13	.150
error	56	232.47		

APPENDIX C. Means of raw data/treatment for performance traits examined in Chapter 4 (Experiments Ia, IIa,b).

Performance trait	Mean (SE)			
	L	H	VL	VH
Immediate effects				
Total mortality from NPV (%)	-	-	9.4 (1.8)	16.7 (1.9)
Total mortality from all causes (%)	2.9 (0.37)	3.0 (0.36)	12.5 (2.07)	18.8 (1.94)
Defoliation (leaves .bud^{-1}.colony^{-1}.tree^{-1})	0.26 (0.04)	0.33 (0.05)	0.17 (0.02)	0.31 (0.04)
Time to prepupal stage (days)	23.64 (0.26)	22.97 (0.21)	23.87 (0.21)	22.92 (0.42)
Pupal weight (mg) /tree - females	489.6 (5.4)	449.4 (7.6)	460.1 (7.4)	466.2 (6.6)
Pupal weight (mg) /tree - males	279.4 (2.1)	268.2 (3.5)	266.7 (6.6)	262.8 (3.5)
Fecundity (# eggs /egg mass)[a]	218.2 (8.7)	186.9 (8.7)	180.5 (8.7)	189.6 (8.7)
Delayed intrinsic effects				
Percent hatch[a]	83.4 (6.0)	89.1 (6.0)	86.6 (6.0)	85.8 (6.0)
Survival to fourth instar (%)[a]	80.6 (3.7)	82.1 (3.7)	85.9 (3.7)	86.6 (3.7)
Pupal weight (mg) female[ab]	362.7 (6.8)	344.9 (8.1)	333.0 (5.6)	348.3 (6.9)
Pupal weight (mg) males[ab]	201.1 (9.5)	186.8 (5.3)	193.8 (5.6)	198.7 (6.3)

APPENDIX C cont.

Performance trait	L	H	Mean (SE) VL	VH
Delayed extrinsic effects				
Total mortality from NPV (%)	-	-	1.4 (1.0)	12.3 (4.8)
Total mortality from all causes (%)	4.2 (0.9)	3.5 (0.6)	5.0 (1.1)	17.0 (4.4)
Time to prepupal stage (days)	45.4 (.7)	45.8 (.4)	45.1 (.7)	45.7 (.9)
Pupal weight (mg) /tree - females	499.4 (5.4)	496.4 (6.3)	483.1 (11.7)	483.0 (6.0)
Pupal weight (mg) /tree - males	275.5 (2.7)	280.4 (2.4)	275.2 (4.4)	271.1 (2.2)

[a] - Approximate standard errors calculated from MS(tree) of ANOVA.
[b] - LS means of treatment effects are given.

APPENDIX D. ANOVA tables for immediate effects. Type III SS used for all analyses.

Percent total mortality - all causes (log(arcsin) transformed)
(balanced factorial)

Source	DF	MS	F	P
v x d	1	.065	4.02	.050
virus	1	2.117	130.23	<.001*
density	1	.061	3.74	.058
error	60	.016		

Defoliation rate (log transformed)
(balanced factorial)

Source	DF	MS	F	P
v x d	1	.003	1.09	.300
virus	1	.006	2.03	.159
density	1	.019	6.86	.011*
error	60	.003		

Development rate (nonparametric)
(unbalanced factorial)

Source	DF	SS	H[a]	P
v x d	1	6.63	.02	.874
virus	1	179.12	.63	.566
density	1	1750.95	6.14	.013*
error	54	14395.76		
total	57	16252.50	MS=285.13	

Male pupal weight (nonparametric)
(unbalanced factorial)

Source	DF	SS	H[a]	P
v x d	1	148.03	.64	.530
virus	1	1238.26	5.36	.032*
density	1	1413.46	6.12	.022*
error	53	12243.57		
total	56	15428.00	MS=275.50	

APPENDIX D cont.

Female pupal weight
(unbalanced factorial)

Source	DF	MS	F	P
v x d	1	644636.29	10.37	.002[*]
virus	1	59970.21	.96	.334
density	1	391124.81	6.26	.016
error	48	62180.31		

Number of eggs per egg mass (x^2 transformed)
(balanced nested factorial)

Source	DF	MS	F	P
1) v x d	1	1210872966	6.44	.022[*]
2) virus	1	815502249	4.34	.054
3) density	1	733976464	3.90	.066
4) tree(v d)	16	188137380	1.40	.162
error	80	134082385		

Error terms:
1-3) MS(tree(v d))
4) MS(error)
[a] - Kruskal-Wallis H=source SS/total MS (see Zar 1984)

APPENDIX E. ANOVA tables for delayed intrinsic effects. Type III SS used for all analyses.

Percent hatch (arcsin transformed)
(balanced nested factorial)

Source	DF	MS	F	P
1) v x d	1	107.90	.20	.664
2) virus	1	21.20	.04	.847
3) density	1	55.77	.10	.755
4) tree(v d)	16	551.32	5.17	<.001*
error	80	106.57		

Error terms:
1-3) MS(tree(v d))
4) MS(error)

Percent survival to fourth instar (arcsin transformed)
(balanced nested factorial)

Source	DF	MS	F	P
1) v x d	1	6.49	.03	.867
2) virus	1	389.79	1.74	.205
3) density	1	89.06	.40	.537
4) tree(v d)	16	223.66	1.43	.149
error	80	156.34		

Error terms:
1-3) MS(tree(v d))
4) MS(error)

Offspring male pupal weight
(unbalanced nested factorial)

Source	DF	MS	Denom. MS	F	P
1) v x d	1	466719.51	129121.43	3.62	.072
2) virus	1	14133.08	129019.46	.11	.744
3) density	1	107993.41	129039.44	.84	.371
4) tree(v d)	16	162682.58	280318.23	.58	.877
5) family(tree)	34	277185.97	66554.24	4.17	<.001*
error	239	66554.24			

APPENDIX E cont.

Error term (denominator MS) composition and DFs:
1) 0.701*MS(tree(v d))-0.023*MS(fam(tree))+0.322*MS(error), DF=20.44
2) 0.700*MS(tree(v d))-0.023*MS(fam(tree))+0.323*MS(error), DF=20.44
3) 0.701*MS(tree(v d))-0.023*MS(fam(tree))+0.323*MS(error), DF=20.44
4) 1.015*MS(fam(tree))-0.015*MS(error), DF=33.76
5) MS(error), DF=239

Offspring female pupal weight
(unbalanced nested factorial)

Source	DF	MS	Denom. MS	F	P
1) v x d	1	1198835.22	213827.24	5.61	.027*
2) virus	1	943327.10	214199.97	4.04	.048
3) density	1	1745.81	214382.71	.01	.929
4) tree(v d)	15	219187.60	696210.14	.32	.990
5) family(tree)	31	708449.62	234799.92	3.02	<.001*
error	255	234799.92			

Error term (denominator MS) composition and DFs:
1) 0.807*MS(tree(v d))-0.018*MS(fam(tree))+0.211*MS(error), DF=21.79
2) 0.806*MS(tree(v d))-0.017*MS(fam(tree))+0.211*MS(error), DF=21.91
3) 0.807*MS(tree(v d))-0.017*MS(fam(tree))+0.211*MS(error), DF=21.89
4) 0.974*MS(fam(tree))+0.026*MS(error), DF=31.55
5) MS(error), DF=255

APPENDIX F. ANOVA tables for delayed extrinsic effects. Type III SS used for all analyses.

Percent total mortality - all causes (log(arcsin) transformed)
(balanced factorial)

Source	DF	MS	F	P
v x d	1	.210	4.11	.047*
virus	1	.493	9.63	.003
density	1	.267	5.22	.026
error	60	.051		

Development rate
(unbalanced factorial)

Source	DF	MS	F	P
v x d	1	.43	.06	.811
virus	1	3.63	.48	.491
density	1	.24	.03	.860
error	57	7.56		

Male pupal weight
(unbalanced factorial)

Source	DF	MS	F	P
v x d	1	30458.93	2.05	.158
virus	1	34838.93	2.35	.131
density	1	284.23	.02	.891
error	57	14849.33		

Female pupal weight
(unbalanced factorial)

Source	DF	MS	F	P
v x d	1	3005.63	.03	.857
virus	1	324818.68	3.56	.065
density	1	3526.10	.19	.654
error	55	91297.37		