THE POTENTIAL ROLE OF BIOTIC MECHANISMS IN BACULOVIRUS DISPERSAL

by

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ABSTRACT

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The potential role of biotic mechanisms in baculovirus dispersal

The role of biotic mechanisms in baculovirus (BV) dispersal was investigated using three Lepidopteran pests; Plodia interpunctella (Hűbner), Ephestia cautella Walker and Mamestra brassicae L. and their respective BVs. The first two, pyralid moths, are pests of stored products, and the third is a pest of brassicas.

Detailed host biology and virus mortality studies were undertaken to provide background information for the main investigation of virus dispersal using P.interpunctella.

BV infection increased larval activity and thus BV dispersal in all three species until the disease at an advanced stage caused sluggish behaviour and mortality. However, larva to larva virus transmission was limited, especially as the integuments of diseased pylarids did not rupture and cannibalism was rare when a suitable food supply was available.

P.interpunctella adults which received a sub-lethal BV dose in the larval stages appeared normal but the proportion of eggs oviposited, the viability of the eggs and the survival of the progeny were reduced. However, no BV was detected in the adult stage. Transovum transmission occurred if the adults were externally contaminated with BV.

Scavangers, predators and adult parasitoids which fed on diseased prey voided viable BV in their faeces. This contaminated the larval medium and resulted in larval infection. The predators tested readily fed on diseased prey but the parasitoids were less able to compete with BV for hosts, Bracon hebetor Say preferentially avoided diseased hosts. There was little evidence to indicate that BV can be mechanically vectored between host larvae on the ovipositor of a parasitoid or the mouthparts of a predator. The relative potential of the host and other biotic mechanisms to cause BV dispersal is discussed.

By wisdom the Lord laid the earth's foundations,
by understanding he set the heavens in place;
by his knowledge the deeps were divided,
and the clouds let drop the dew.

Proverbs 3:19-20

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INTRODUCTION

The subject of insect viruses has recently been extensively reviewed by Tweeten *et al*. (1981), Payne (1982), Hunter *et al*. (1984) and Entwistle and Evans (1985). At least 1270 insect host-virus associations have been described and Entwistle and Evans (1985) supposed that the majority of insect species will be found to have virus diseases.

The Baculoviridae is the only family of viruses thought to be exclusive to arthropods, particularly insects, to which their potential as a means of control has been frequently recorded. The World Health Organisation (WHO, 1973) have therefore recommended that the development of insect pathogenic viruses should be concentrated on baculoviruses (BVs).

BVs are circular double-stranded DNA viruses with rod-shaped virus particles (VPs). The family is divided into three groups based on structural properties, although a fourth group has been proposed. The nuclear polyhedrosis viruses (NPVs) have many VPs embedded in a crystalline matrix of protein called polyhedrin. The VPs consist of nucleocapsids enveloped either singly (SNPV) or in multiples (MNPV). The granulosis viruses (GVs), the second group, have one, rarely two, VPs embedded in the rod-shaped inclusion body of crystalline protein, known as granulin. The third group, is typified by the BV of *Oryctes rhinoceros* (L.) which does not have an inclusion body. The fourth group consisting of non-occluded viruses has been found associated with the calyx fluid of parasitoid Hymenoptera (Stoltz, 1982). A GV and two NPVs were used in the present studies and this introduction will concentrate on occluded BVs infecting Lepidoptera.

The most commonly recorded BV infections result from ingestion of virus in the larval stages. The sequence of virus replication has

been described by Granados (1980), Granados and Lawler (1981), Kelly (1982), and Vaughn and Dougherty (1985). In occluded BVs solubilization of the inclusion body to release VPs occurs in the alkaline environment of the midgut. The VPs lose their envelopes upon entering the microvilli of the columnar cells. The nucleocapsids migrate to the nucleus where they release DNA and infection is initiated. The nucleus increases greatly in size, host cell synthesis is diminished and virogenic stroma is produced within the nucleus. Nucleocapsids are synthesized and develop a *de novo* envelope. The nuclear envelope and later the cell membrane ruptures. Secondary replication of virus occurs and eventually inclusion bodies are formed. In Hymenoptera the entire cycle of virus replication takes place in the midgut cells, while in Lepidoptera it occurs principally in the fatbody, but the haemocytes, hypodermis, tracheal matrix, muscles, nerve ganglia and pericardial cells may also be affected (Entwistle and Evans, 1985). The massive production of inclusion bodies, cell lysis and tissue destruction generally proves fatal after several days (Payne, 1982).

BVs are relatively specific. Infection by GVs is limited to the order Lepidoptera and in most cases is species specific (Ignoffo, 1968). Both Hymenoptera and Lepidoptera are susceptible to NPVs. The specificity of NPVs ranges from those that infect a single species such as *Gilpinia hercyniae* (Htg.) NPV (Cunningham and Entwistle, 1981) to those that infect many species over several families such as *Autographa californica* Speyer NPV (Vail and Jay, 1973). Susceptibility of larvae to BV infections decreases with age, which is linked to increasing body weight (Evans, 1981; Mardan and Harein, 1984).

Viral epizootics are characterized by an increased level of disease relative to the normal incidence within the population. The

study of an epizootic involves spatial and temporal components which are influenced by the ecology of both the disease and the host. Andrewartha (1961) defined ecology as "the scientific study of the distribution and abundance of organisms". Consequently, BV epizootiology involves the study of the distribution and abundance of the virus and host as they affect the transmission and persistence of the virus.

Natural epizootics act in a delayed density - dependent manner and usually require several host generations to develop. Evans (1986) identified two sources of virus inoculum; primary and secondary. Primary inoculum survives the period when no susceptible hosts are available and forms the initial sources of infection for the next generation. The quantity available is dependant upon the host infection and density of the previous generation and the rate of virus inactivation, while the utilization of this inoculum reflects the density, distribution, behaviour and feeding rates of the host larvae of the current generation (Payne, 1982; Evans, 1986). Virus replicates in larvae that succumb to infection from primary inoculum resulting in release of secondary inoculum when the larvae die . Large quantities of localized inoculum are thereby made available which may be sufficient to cause infection in older more resistent larval stages. Host distribution and abundance are therefore important factors in determining the efficiency of the cycling of virus.

BVs need to be able to persist during periods when hosts are not available. The presence of an inclusion body enables virus-infectivity to be protected for long periods outside of its host, even so, reduction in the amount of infective virus on leaf surfaces is mainly because of inactivation rather than physical loss (Payne, 1982). Vertical transmission from generation to generation

has been reviewed by Evans and Harrap (1982). Transovum transmission, when the egg is externally contaminated by virus has been documented by several workers (eg. Martignoni and Milstead, 1962; Hamm and Young, 1974). Reports of transovarial transmission, that is of virus within the egg, are scant and less convincing. David (1978) considered that there is no unequivocal evidence that BVs can be transmitted transovarially.

Virus persistence in secondary hosts as defined by Evans and Harrap (1982), "includes any other host, passive or active, which provides a means of protection for the virus while it is outside its primary host". They identified three types of secondary hosts. In the first the virus replicates and, more significantly, is transmitted back to the original host. Tanada and Omi (1974) demonstrated that several alfalfa pests in California were susceptible to the same GV and NPV and thereby the virus was more able to persist in the insect ecosystem. The second type of secondary host ingests virus which after passage in the gut is voided, still viable, into the environment. This is a relatively short-term persistence mechanism and predators have been studied in this context as agents of virus dispersal. Birds (Entwistle et al., 1977a, b), mammals (Lautenschlager and Podgwaite, 1979) and insects (Vago et al., 1966) have been shown to void infective BVs following feeding on virus infected insects. The final type of secondary host involves surface contamination particularly by parasitoids. For example Apanteles melanoscelus (Ratzeburg) can transmit NPV between hosts during oviposition (Raimo et al., 1977).

The two main abiotic agencies for virus persistence are on plants and in the soil. Persistence on plants is interrupted on agricultural crops by harvest and is therefore short lived. Long term persistence is offered on permanent crops such as conifers. Entwistle and Adams

(1977) demonstrated that viable NPV of G.hercyniae was retained on spruce foliage between larval generations. Persistence of inoculum depends upon not only environmental variables, particularly ultra-violet light, but also plant surface effects, temperature and pH (Payne, 1982; Evans, 1986). The GV of Plodia interpunctella (Hübner) remained viable in the protected environment of stored grain for up to a year (Kinsinger and McGaughey, 1976), while Pieris GV on leaf surfaces was largely inactivated after several days (Payne, 1982).

Most insect viruses will alternatively accumulate in the soil which is the primary reservoir of insect viruses (Hostetter and Bell, 1985). However, Evans (1986) pointed out that retention was variable and he considered that a high host density was necessary to encounter the limited quantity of virus cycled back to the host plants.

During a virus epizootic the number of susceptible larvae at an epicentre of disease will decline as more larvae succumb to infection. The virus, therefore, must disperse to exploit the host population. Hostetter and Bell (1985) have recently reviewed natural agencies of BV dispersal. Abiotic factors such as wind and rain can increase virus dispersal on a limited scale. Predators and parasitoids have been briefly discussed in the context of virus persistence and have been frequently implicated in the spread of BVs (Entwistle, 1982; Kaya, 1982). Similarly adult insects with their capacity for dispersal can introduce inoculum to new epicentres (Cunningham and Entwistle, 1981). Larval movement may be important in the localized dispersal of virus, especially as infected larvae frequently display an enhanced level of locomotor activity (Lewis, 1970; Evans and Allaway, 1983).

Entwistle et al. (1983) analysed the pattern of G.hercyniae NPV dispersal and compared it with other examples. Primary dispersal near the epicentre gradually evolves into a wave form but as further

epicentres are generated by discontinuous spread, the patterns of dispersal become more confused.

The release of inoculum by infected larvae is dependent upon the site of replication. NPV infections of Hymenoptera are localised in the gut and virus is gradually released in the faeces, while for Lepidopteran larvae the gut is the site for initial replication only. Release of inoculum for Lepidopteran larvae occurs principally at mortality, particularly if the body wall ruptures producing a large localised reservoir of virus (Payne, 1982). However, the body wall of some Lepidopteran species does not rupture as a consequence of BV disease (Hunter *et al.*, 1972), which is likely to limit the availability of inoculum. However, exploitation of virus would be increased if larvae were attracted to virus killed cadavers as is reported for *Mamestra brassicae* L. (Evans, 1986).

There is a good background of information on abiotic persistence and dispersal of BVs; biotic mechanisms of spread have also been cited but the evidence tends to be circumstantial. The intention of this study was to investigate more fully the biotic factors which could influence virus dispersal both within and between insect populations.

The work was concentrated on the *P.interpunctella*/GV interaction. The host, because of its small size and habits as a stored product pest can be easily manipulated, consequently large populations can be reared in comparatively small jars within the laboratory. The work with *P.interpunctella* and its corresponding GV was compared with dispersal studies on two other host/BV interactions. *Ephestia cautella* Walker, and its NPV were chosen as the host is closely related to *P.interpunctella* and the NPV would provide a useful comparison to the GV. The Cabbage Moth, *M.brassicae*

and its NPV were also used. It was considered that the habitat of a cabbage plot would provide an interesting contrast to the protected ecosystem of the stored product moths.

Populations of the pyralids, P.interpunctella and E.cautella exist as overlapping generations, especially in heated warehouses. Although natural epizootics of BV infections in either species have not been cited in the literature, a GV has been successfully tested as a microbial insecticidal spray against P.interpunctella (McGaughey, 1975; Hunter et al., 1973, 1977, 1979). Younger larvae were found to be most susceptible to infection (Mardon and Harein, 1984).

The parasitoids, Nemeritus canescens (Gravenhorst) and Bracon hebetor Say have been used in tests to evaluate the control of stored product pests (Press et al., 1974, 1977, 1982). All three species effectively suppressed populations of the pyralid moths. The predator preferentially predated as the eggs and younger larvae while the parasitoids preferred the older instars (Press et al., 1982).

The presence of a range of development stages of the pyralid moths would therefore increase the probability of maintaining a complex of entomophagous insects and microbial diseases within an ecosystem. The existence of such a complex would result in foraging entomophages contacting both healthy and diseased prey and consequently they could be implicated in the dispersal of the disease, particularly to young larvae of successive generations.

M.brassicae normally has only one generation per year, although there may be a partial second. This coupled with the harvesting of the habitat restricts the potential of recycling of BV. Although ichneumoids, braconids, chalcids and tachnids have been recorded as parasitoids of M.brassicae (Jones and Jones, 1974), no parasitoids were isolated from larvae during this study and the main predators appeared to be birds, small mammals and carabid beetles.

Laboratory reared populations are likely to differ genetically from their wild relations. This is because many of the normal selection pressures such as, predators, parasitoids, disease and adverse physical conditions have been removed. These are replaced with artificial selection pressures, in particular the need for a uniform generation period. This difference between wild and laboratory reared populations is likely to be less for stored product moths than for the Cabbage Moth, as rearing of the former in the laboratory more closely approximates to the wild environment than is true for the latter. Also because laboratory reared populations have no immigrations, no new genetic information can be introduced. Caution must therefore be used when applying information obtained from laboratory based experiments to the wild situation.

CHAPTER 1 Materials and Methods

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1. Insect rearing and handling

I. Plodia interpunctella (Hübner) and Ephestia cautella Walker (Lepidoptera: Pyralidae)

E.cautella is also known by its older name of Cadra cautella.

Both pyralid moth stocks were supplied by P. Cogan of Slough
.tory, England. The stocks were maintained at 26°C, 70% relative
ty and 16 hour daylength. The choice of daylength was arbitrary
.d to be greater than 14 hours to avoid onset of diapause. They
kept in glass sweet jars, which were laid on their sides to
ıse the surface area of the three cm depth of diet. The larval
ıg diet consisted of 10 parts (by weight) of Froment (stabilized
Germ, John H. Heron Limited), one part (by volume) of dried
'ṡ yeast and two parts (by volume) of glycerol, thoroughly

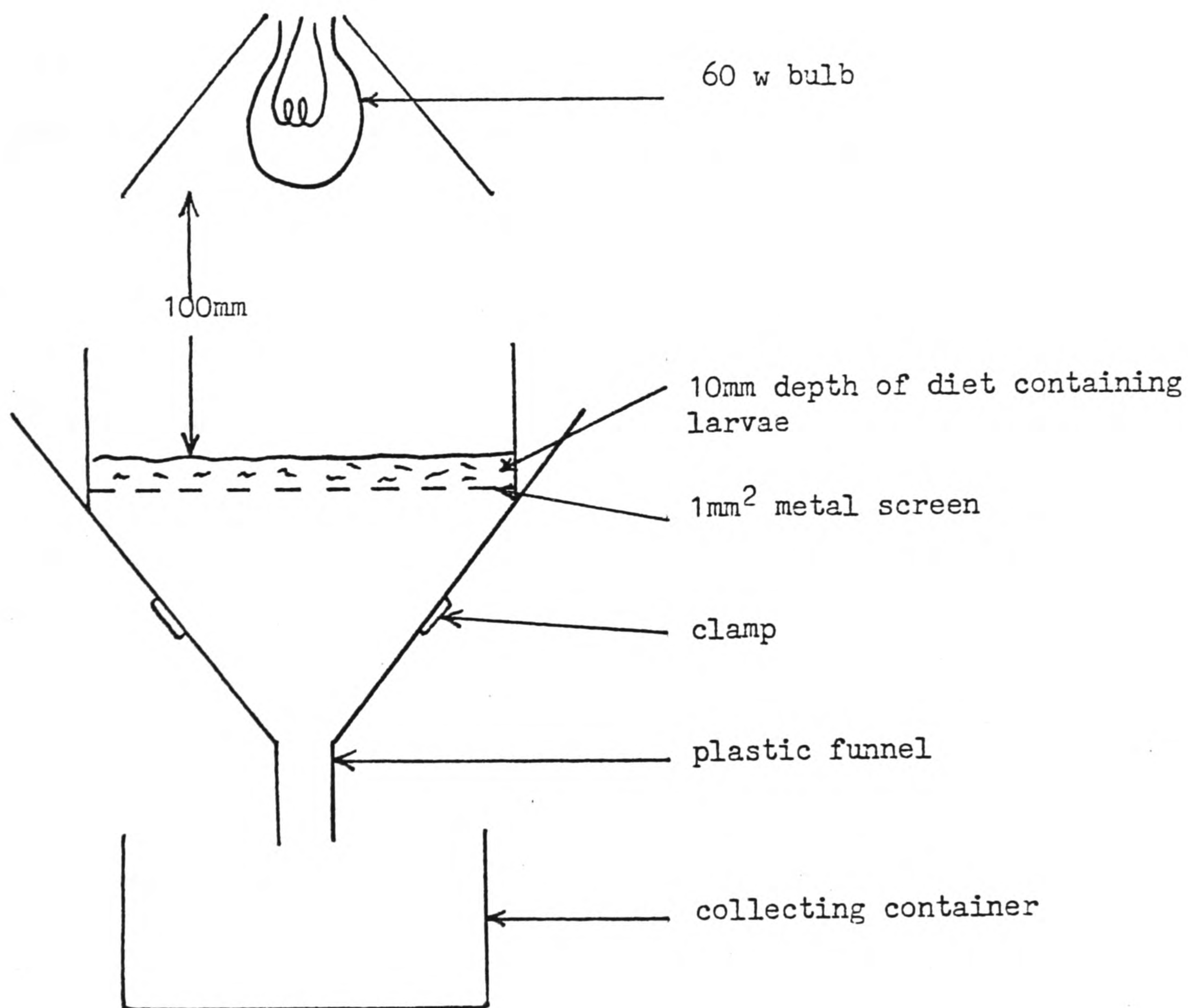
new stock was started by adding surface sterilized eggs
ited over a 48 hour period to a sterilized sweet jar containing
diet. The opening of the jar was covered with tissue paper and
, which was secured with an elastic band.

e eggs were obtained by confining adults over a sieve similar to
thod described by Bell and Walker (1973). The adult scales were
d from the eggs by gently shaking under an extraction fan. The
were surface sterilized for 20 minutes in 5% formaldehyde
on containing one drop of Triton X-100 per 100ml and rinsed at
three times in deionized water. Adults were handled after
dioxide (CO_2) anaesthetising.

en first instar larvae were required for experiments, surface
ized eggs were kept without diet. After hatch the larvae were
gently transferred to the experiment using fine camel-hair paint
brushes. Older larvae required for experiments were separated from
the larval diet by use of a heat source (see Figure 1). The heat from

the lamp caused the larvae to move down through the diet and sieve, to be collected on the bottom of the sieve and in the funnel. They were gently brushed off this surface down the funnel, and into collecting containers. The larvae were kept for short periods (up to four hours) at 4°C to reduce cannibalism before they were selected for use. Individual larvae were handled by use of soft forceps.

Figure 1. Apparatus used to extract moth larvae from diet



II. Nemeritus canescens Gravehorst (Hymenoptera: Ichneumonidae)

N.canescens is also known by the name Venturia canescens.

N.canescens stocks were obtained from M. Amphlett of the Zoology Department of Oxford University, England.

The stocks were maintained at approximately 24°C and a 16 hour daylength. A new stock was started by introducing about eight freshly emerged adult parasitoids into a plastic box (210 x 105 x 80mm) containing P.interpunctella larvae in larval diet. A polypot containing a 10% solution of honey with a wick protruding from the polypot lid was placed into a new stock for the first four days. Third, fourth or fifth instar larvae were supplied as hosts.

N.canescens adults were handled by first cooling (-20°C) for several minutes to slow down movement, after which they were easily caught in disposable universal bottles.

III. Bracon hebetor Say (Hymenoptera: Braconidae)

B.hebetor is occasionally allocated to the genus of Microbracon or Habrobracon.

B.hebetor stocks were supplied by J.W. Press (Stored-Product Insect Research and Development Laboratory, Georgia, U.S.A.).

The stocks were maintained at approximately 24°C and a 16 hour daylength. The B.hebetor were reared by placing at least 100 late instar E.cautella or P.interpunctella in a small jam jar, then adding 20 pairs of adult parasitoids to each jar. The jars were covered with several layers of tissue paper and a square of industrial nylon, and secured with elastic bands.

The adults were cold anaesthetised before transferring by use of a pooter.

IV. Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae)

X.flavipes stocks were supplied by J.W. Press (Stored-Product Insect Research and Development Laboratory, Georgia, U.S.A.).

The stocks were maintained at 26° C, 70% relative humidity and a 16 hour daylength. X.flavipes were reared in 90mm diameter cages made from two sections of acrylic tubing sealed with industrial nylon material at one end then joined in the middle with masking tape. About two or three times weekly liberal numbers of either E.cautella or P.interpunctella eggs, which had been frozen to kill, were added. Adults were removed after about seven days when a sufficient number of X.flavipes eggs were present. When pyralid eggs were in short supply, the diet of X.flavipes adults and nymphs was supplemented with P.interpunctella larvae which had been frozen to kill.

Nymphs and adults were handled by use of soft forceps.

V. Mamestra brassicae L. (Lepidoptera: Noctuidae)

Larvae were supplied as necessary for experiments by R. Warner, from the Insectary stocks of NERC Institute of Virology, Oxford, England. The larvae were maintained at 22° C on semi-synthetic diet (Hunter et al. 1984). This diet was routinely used by the Insectary for rearing M.brassicae. Eggs were routinely surface sterilized in 10% formaldehyde solution for 25 minutes.

First instar larvae were handled using camel hair brushes and soft forceps were used for older larvae.

2. Aspectic techniques

All experiments and rearing of insects were conducted using sterile materials and instruments. Camel hair brushes and plastic items were sterilized by submerging overnight in 3% formaldehyde solution. Soda glassware used in insect rearing was soaked overnight in 10% choloros solution, which, beside sterilizing the glassware also dissolved the larval silk. Soft forceps used for handling insects were sterilized between operations by heating to red-heat, plunging into absolute alcohol and flaming. After use they were further sterilized by placing in boiling water for 30 minutes. Disposable plastic containers were used for all bioassays and much of the general work, and autoclaved glassware for storage of triturated virus suspensions and reagents.

Particular care was taken to prevent introduction of virus infection in the insect stocks, which were routinely inspected for evidence of disease. Smears of insects suspected of pathogen infection were examined under the light or electron microscope.

3. Virus counts

The protein content of a purified virus suspension was estimated by use of Bio-Rad Protein Assay Reagent.

Polyhedral and granule counts were performed by a dry film method (Wigley, 1980a).

4. Microscopical techniques

Larval smears for qualitative observation of polyhedra were made on glass microscope slides and stained as described by Wigley (1980b).

5. Other methods

Further materials and methods are described in the relevant chapters.

CHAPTER 2 Biology of *Plodia interpunctella*

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INTRODUCTION

The Indian Meal Moth, Plodia interpunctella Hb (Lepidoptera: Pyralidae) is a native of Europe but its range is now cosmopolitan. It is regarded as the major species of grain infesting moth. It is frequently found in many stores, mills, warehouses, groceries and other places where grain, grain products and different food stuffs are stored (Abdel-Rahman, 1971). Richards and Thomson (1932) listed 83 different kinds of food on which the larvae are reported to feed. In England, there are extremely few records of its maintaining itself out of doors (Richards and Thomson, 1932).

There is some debate in the literature concerning the number of larval instars. McGaughey (1978) considers that there are four, Silhacek and Miller (1972) that there are five and Richards and Thomson (1932) record that the number of moults is variable, between four and seven.

P.interpunctella diapauses under normal conditions. Bell (1977) linked the onset of larval diapause to a mean critical photoperiod of about 13.25 hours while temperature was of lesser importance. During diapause, the larvae have low metabolic activity and do not wander. Tzanakakis (1959) referred to a facultative diapause in the cocoon, which according to Williams (1964) can result from feeding on certain foods. High larval density can induce a type of diapause at 30°C. The high temperature prevents normal diapause; the larvae continue to wander and pupation is delayed (Tsuji, 1959).

P.interpunctella according to Bell (1975) does not complete development at 15°C and Abdel-Rahman (1971) reports that development is possible at 35°C, while 36°C was lethal to pupae and higher temperatures caused some failure in mating and reduced fecundity (Arbogast, 1981). From his studies Abdel-Rahman (1971) concluded that survival to the adult stage was highest at 30°C. However, the most

rapid development occurred at 35° C although the heaviest adults were formed at 20° C and fecundity was highest at 25° C. Drier conditions at any temperature increased larval mortality.

Takahashi (1957a) reported that an increase in initial egg density caused a decrease in the head capsule size of adults and a decrease in their survival. Snyman (1949), in a similar experiment, demonstrated that these small adults were able to reproduce; the size of the female was correlated with the average number of eggs in the ovaries. However, higher densities caused metabolic heating of the environment and as a result development proceeded faster. When food was in short supply, pupae and larvae preparing for moult were cannibalised (Bell, 1976); this did not occur when food was present except that a few cadavers were consumed (Burges and Hurst, 1977).

Survival and development in a constant food volume were improved by a greater cross sectional area (Bell, 1976). Similarly, Williams (1964) discovered that larvae preferred bags stacked flat to those stacked upright. The larvae tended to occur in the upper parts of the sacks, spun cocoons in the necks of the bags and remained in the existing sites without showing any indication of migration, except when there was competition for pupation sites.

The mandibular gland secretion of E.kuehniella, Z. which was also isolated from larvae of P.interpunctella and E.cautella functions as a pheromone (Barrer, 1976, Corbet, 1973). It influences the population density through its effects on the temporal and spatial patterns of pupation (Corbet, 1971) and oviposition (Corbet, 1973). The amount of pheromone deposited is a measure of the intensity of crowding, it encourages emigration from a crowded situation and delays pupation (Corbet, 1971). The greatest number of eggs were laid in areas with an intermediate concentration of the larval pheromone (Corbet, 1973).

Male and female P.interpunctella adults produce sex pheromones

(McLaughlin, 1982; Mankin _et al._, 1983) and both are capable of multiple matings (Brower, 1975). Females normally mate within 24 hours of emergence (Lum, 1983); few eggs are laid within the first 24 hours after mating, a peak oviposition occurs in the second day and most eggs are oviposited by the third day (Lum and Flaherty, 1969). Ramsey and Farley (1978) found wide variation in the number of eggs per female common in the laboratory strains, which according to Snyman (1949) is influenced by the density of the adult population. Lum and Flaherty (1970) suggested that oviposition was controlled largely by the physiological state of the males and the light conditions. The number of eggs deposited according to Abdel-Rahman (1971) was highly correlated to the numbers of eggs produced. Gravid females are attracted by water (Chow _et al._, 1977) and oviposition is usually stimulated by the presence of rearing medium, although no more eggs were laid on rearing medium than in the absence of larval food (Mullen and Arbogast, 1977). Arbogast and Mullen (1978) found that eggs were not distributed randomly but aggregated,which was especially marked when small numbers of adults were present.

The above summary of some of the literature concerning _P.interpunctella_ shows that extensive work has been carried out on the biology of this moth. The studies provided in this chapter were undertaken to provide further useful background information required for the study of the virus-host interaction presented in later chapters.

EXPERIMENTS

1. Surface sterilization of eggs

Method

A method of surface sterilization of eggs was needed to reduce the danger of pathogen infection among the insect stocks.

Eggs were collected from adult moths as described previously. Twelve batches of 2mg of eggs were weighed and counted in plastic disposable bottles. The eggs in the control treatments were not experimentally surface contaminated, while three drops of crude virus suspension was applied to each of the egg batches of the virus contaminated treatments. Crude virus suspension consisted of five virus-killed fifth instar larvae triturated in 1ml of phosphate-buffered saline with a few drops of Tween (PBST).

Both control and virus-contaminated egg batches were subjected to one of six treatments.

There were two treatments using formalin solution at 5% or 10% concentration with a few drops of Triton X added. After 20 minutes treatment in formalin the eggs were washed with three changes of deionized water.

There were two treatments using a pretreatment of one minute in 70% ethanol before two or five minutes in 1% hyamine solution. After treatment, the eggs were washed as previously described.

The fifth treatment consisted of a water wash only and in the final treatment, a control, the eggs were left dry.

Each solution was removed by pipetting before the addition of the next. After the final rinse as much water as possible was removed. Filter paper was secured over the opening of the bottle and the bottles were shaken to disperse the eggs before storing on

their sides at 26° C. One day later the surface moisture had evaporated, the filter paper was dampened and lids were secured.

The treatments were checked regularly for hatch. The larvae which emerged were counted and transferred to polypots of diet, where they were checked regularly for virus infection.

Results

There was an average of 112.7 ± 2.6 eggs in a 2mg batch. The weight of 1000 eggs was estimated to be 17.7mg.

The results for the hatch and incidence of infection are shown in Table 1.* An examination of the proportion hatched in the control egg batches indicates that all treatments affect the viability of the eggs. Virus-infection was observed in the larvae from all the virus contaminated egg treatments except those treated with formalin.

There was a lower hatch observed in those treatments which exhibited an incidence of virus infection than in the corresponding controls, but when these were compared by use of a paired t-test the difference was shown not to be significant.

It was decided to use the 5% formalin treatment for routine surface sterilization of eggs.

* Much of the data expressed as proportions or percentages in this thesis have been transformed using the angular transformation. This transformation was developed for binomial proportions and increases the variance of proportions near 0 or 1. This validates the use of statistical methods developed for the normal distribution (Snedecor and Cochram, 1967).

Table 1. Efficiency of treatments for surface sterilizing eggs

a. Control

Treatment	Number of eggs	Number hatched	Proportion hatched*	Incidence of infection
10% Formalin	98	48	44.4	-
5% Formalin	118	43	37.1	-
5 mins Hyamine	99	29	32.8	-
2 mins Hyamine	107	63	50.1	-
Water	112	72	53.3	-
No treatment	113	82	58.4	-

b. Virus contaminated eggs

Treatment	Number of eggs	Number hatched	Proportion hatched*	Incidence of infection
10% Formalin	116	69	50.5	-
5% Formalin	121	64	46.7	-
5 mins Hyamine	120	32	31.1	+
2 mins Hyamine	108	15	21.9	+
Water	111	49	41.6	+
No treatment	129	39	33.4	+

- no infection

+ virus infection

* expressed as the angular transformation $[\sin^{-1} (\text{proportion})^{1/2}]$

2. Rearing medium for larvae

Method

The rearing medium consisted of Froment, dried brewer's yeast and glycerol. The ingredients were thoroughly mixed together according to the proportions given in Table 2. Two grams of each diet, except that with the highest glycerol ratio of which three grams was used, was weighed into separate polypots. There were three replicates for each treatment. Twelve third instar larvae were placed into each polypot.

The treatments were kept at 26° C and inspected regularly for adult emergence.

Table 2. The ratio of ingredients for the different treatments of larval diet

Glycerol	Dried Yeast	Froment
0	1	10
0.5	1	10
1	1	10
2	1	10
4	1	10

Results

The emergence of adults is recorded in Table 3. No adults emerged from the rearing medium without glycerol. Peak emergence occurred in the diet with two parts glycerol and emergence was low in diet with four parts glycerol. The proportion of adults emerged for these two treatments was compared using a chi-squared

Table 3. Adult emergence from larvae reared on diets of different glycerol content

Proportion of glycerol	Replicate	Day of treatment 21	25	27	29	32	34	36	39	41	43	Total emerged	Proportion* emerged for each treatment	Average day of emergence
0	I											0	0	
	II											0		
	III											0		
1/2	I				1		2	3	4		1	11		
	II					1	1	4	2			8	61.9	35.4
	III			1	1	2	2	2	1			9		
1	I				1		3	2	3			9		
	II		2		2	1	3	1				9	61.9	33.5
	III				1	2	5	1		1		10		
2	I		7		1	2	1					11		
	II		3	1	5	1						10	65.9	28.2
	III		2	1	2	3	1					9		
4	I			1	2	1	1					5		
	II		2	1	3							6	43.4	28.0
	III	1	1	2	1	1						6		

* proportion expressed as the angular transformation

test. The difference was significant at 99% probability (χ^2_1 = 10.36, p= 0.01). It was concluded that the high glycerol content of the diet reduced the probability of successful development to the adult stage.

The average day of emergence was inversely related to the glycerol content of the diet. A difference of seven days was observed in the average day of adult emergence between larvae reared in diet containing a half and four parts of glycerol.

There was no significant difference in the survival on the three diets containing intermediate levels of glycerol (χ^2_2 = 0.35). However, it was concluded that diet containing a ratio of two parts of glycerol was the most suitable larval rearing medium because of the short developmental period.

3. Effect of larval density on survival

Method

Two grams of Froment diet were weighed into each of 18 polypots (30ml disposable plastic pot with lid). Neonate larvae were counted into each to achieve a density of 1, 5, 10, 20, 30 or 50 per polypot, depending upon the treatment. There were three replicates for each treatment.

The experiment was kept at 26°C, 70% relative humidity and a 16 hour daylength. Adult emergence was recorded.

Results

The number of adults which emerged from each initial density is recorded in Table 4. All three larvae kept at a density of one per polypot survived to the adult stage. There was little difference in survival between the treaments with densities of 5, 10 or 20 larvae per polypot but at higher densities the survival rate decreased rapidly.

The maximum density for optimum survival is estimated to be between 20 and 30 larvae per 2g of Froment diet in a polypot.

Table 4. Effect of larval density on the emergence of adults

Initial larval density	Adult emergence replicate 1	2	3	Average emergence	% survival
1	1	1	1	1.0	100.0
5	4	4	4	4.0	80.0
10	9	11	5	8.3	83.3
20	18	17	16	17.0	85.0
30	21	22	19	20.7	68.9
50	28	26	30	28.0	56.0

Table 5. Effect of type of diet on larval dispersal

Type of diet		Numbers of larvae caught in moats — Days after removal of lid: 2	4	6	8	10	12	14	16	18	Total
Froment diet	1					2	10	2	2		16
	2					3	13	2			18
	3			1		3	14	2		4	24
"Toppings" diet	1			1	1	10	1	1			14
	2				1	6	4	1			12
	3				2	4	5		2	1	14
semi-synthetic	1	22	3								25
diet	2	20	3	2							25
	3	22	2								24

4. The effect of type of diet on larval dispersal

Method

Three types of larval diet were made. One was the standard Froment diet, a second used the same recipe but autoclaved wheat toppings were substituted for Froment. The third used semi-synthetic diet as described previously. About two grams of the two wheat diets were placed into three polypots for each diet. The semi-synthetic diet had a higher water content; about four grams of it were used per polypot.

Twenty-five third instar larvae were counted into each polypot. The lids were secured to allow the larvae to settle before being given the opportunity to disperse. Each polypot was positioned centrally on a large petri-dish base (85mm diameter) containing glycerol. When the lids were removed the following day, any larva which left its polypot would become trapped in the surrounding glycerol moat. Any larva caught in the moat was removed, counted and its instar noted.

Results

The numbers of larvae caught in the moats throughout the course of the experiment are shown in Table 5. The larvae quickly dispersed from the polypots containing semi-synthetic diet. Close inspection of the data revealed that most larvae left within the first day. The data suggest that one larva remained on the semi-synthetic diet, however, inspection of the diet at the end of the experiment revealed that this was not true. It is postulated that either it was cannibalised or it managed to escape. The treatments using wheat-based diet both had pupae and prepupae in the diet at the end of the experiment.

Only one larva which migrated from the semi-synthetic diet

was fifth instar, whilst all the larvae from the wheat-toppings diet and all but one of the larvae from the Froment diet, were fifth instar. This suggests that on suitable diets only fifth instar larvae normally disperse, which is characteristic of larvae searching for pupation sites.

A chi-squared comparison was performed on the proportion of larvae which dispersed from the two wheat-based diets. The difference was significant with 99% confidence (χ^2_1 = 9.54, p=0.01). It is concluded that more larvae dispersed from Froment diet than from the drier wheat 'toppings' diet.

5. Larval instars

I. Head capsule width

Method

Larvae were removed from cultures of different ages and over several generations. Using a binocular microscope with a graticule the measurement across the widest part of the head capsule (HCW) was recorded for each larva. Where larval movement prevented an accurate measurement the larvae were placed in the refrigerator for a few minutes to slow any activity or killed by freezing before measuring. A few larvae were measured every two days throughout their development to ensure that no instar was ignored.

Although no accurate records were kept of the culture density, a note was made if larvae came from a culture where the density had seemed to be particularly high.

Results

The results are presented in Table 6. When the larvae did not come from excessively crowded cultures five distinct HCWs were observed. The ratio of the width of one head capsule to the next is nearly the same for all five stages and therefore agrees with

Dyar's Law (Dyar, 1890), the regression equation is given under Table 6a.

It is therefore concluded that in uncrowded cultures there are five larval instars.

When the larvae originated from crowded cultures the ranges for each HCW were much broader and some overlap occurred. There appeared to be in some cases extra instars, which occurred between the normal third and fourth, and between the fourth and fifth instars of the uncrowded cultures. A small number of larvae that fitted in the extra instar E4 were reared separately and after the next ecdysis fitted into the normal fourth instar category. This suggests that E4 is an extra instar which may occur in crowded cultures. However, when a small number of larvae of the E5 category were reared separately, they did not undergo ecdysis to the normal fifth instar but formed pupae and eventually emerged as small adults that were capable of reproduction. This suggests that E5 may be an alternative instar to the normal fifth instar that may occur in crowded cultures.

The relationship between the numbers of instars

can be described by a logarithmic function. The equation is given under Table 6b.

Table 6. Head capsule width of the instars of *P.interpunctella* larvae

a. Larval population not crowded

Instar	Number Measured	HCW (mm) Range	Mean	Standard Error
1	39	0.15-0.18	0.17	0.013
2	64	0.26-0.28	0.26	0.008
3	37	0.36-0.49	0.42	0.029
4	28	0.62-0.69	0.64	0.027
5	33	0.92-1.05	0.96	0.034

$y = -0.003 + 0.06x$ $p = 0.01$

$r^2 = 0.977$

b. Larval population crowded

Instar	Number Measured	HCW (mm) Range	Mean	Standard Error
1	55	0.15-0.20	0.17	0.011
2	70	0.23-0.31	0.26	0.010
3	150	0.33-0.49	0.40	0.018
E4	100	0.50-0.57	0.53	0.016
4	236	0.58-0.75	0.64	0.018
E5	49	0.76-0.85	0.80	0.026
5	123	0.86-1.05	0.93	0.022

$y = 0.03 + 0.04x$

$r^2 = 0.995$ $p = 0.001$

$y = \log(HCW + 1)$ $x = \text{instar}$

II Larval developmental times

Method

Eggs were collected over a 24 hour period, surface sterilized and incubated in a petri-dish at 26° C. They were examined daily for hatch. The larvae were transferred in batches of ten to polypots containing either Froment diet or semi-synthetic diet. They were incubated at 26° C and examined daily for their stage of development.

Results

The results are presented in Table 7. Larval development progressed more rapidly on semi-synthetic diet. However, once the larvae had reached their fifth instar only a few formed pupae even after 14 days as fifth instar. Most remained as larvae for much longer than expected while others died before pupation. In a separate experiment the success of pupation was increased if there was only one larva per polypot.

Considerable overlap in larval instars occurred on Froment diet, even though all originated from eggs oviposited on the same day. Successful development to adult was greater in larvae reared on Froment diet, where peak emergence of adults was 50 days after oviposition.

Table 7. Length of the life history of P.interpunctella at 26° C

	Days during which each stage may be present	
Stage	Froment diet	Semi-synthetic diet
Egg	0-5	0-5
1st instar	4-11	4-8
2nd	11-15	8-11
3rd	15-22	11-15
4th	20-32	13-18
5th	26-40	17-
Pupa	35-48	28-
Adult emergence	47-56	35->56

III Weights of larvae

Larvae of known age which had been reared at 26°C in uncrowded cultures were weighed and their instar determined by their head capsule width.

The average weights according to instar and age of the larvae are recorded in Table 8. The relationship between age and weight can be described by the logarithmic equation.

$$\text{Log}_{10}\ (\text{mean weight} + 1) = 0.04 + 0.06\ (\text{age in days})$$

$$r^2 = 0.92 \quad p = 0.001$$

In a further experiment larvae were reared on either Froment diet or semi-synthetic diet. The weights of the fifth instar larvae obtained are given in Table 9. After about day 23 on semi-synthetic diet, the larvae ceased feeding but continued to be active until about day 32. Final instar larvae which have ceased feeding are referred to as mature larvae in this thesis. It was more difficult to identify the end of diet consumption when Froment diet was used.

Male mature larvae could be easily recognized by their dark testes which were visible through the integument; they were generally lighter in weight than mature female larvae. Both male and female mature larvae gradually lost weight during their period of activity after ceasing to feed.

Table 8. Average weights of larvae reared on Froment diet at 26° C

Instar	Age (days)	Number weighed	Mean weight (x 10^{-4}g)
2	10	10	1.2
	11	10	1.5
	14	10	2.0
3	13	10	4.0
	14	20	5.2
	15	20	7.2
	16	20	7.5
4	20	25	20.0
	28	4	27.2
5	24	5	32.6
	26	11	40.5
	29	7	60.1
	33	16	71.9
	35	10	96.0
	37	8	144.1
		♂ 4	125.2
		♀ 4	163.0

Table 9. Weights of mature larvae

a. Froment diet

Age (days)	Number	Mean weight (x 10^{-4}g)	Standard deviation
32	9	85.6	24.8
34	10	124.5	29.9
36	5 ♀	143.4	12.3
	4 ♂	128.5	13.0
39	5 ♀	135.8	21.8
	2 ♂	100.5	23.3
41	2 ♀	135.5	2.12
	2 ♂	83.0	19.8

b. Reared on semi-synthetic diet

Age (Days)	Sex and Number	Mean weight (x 10^{-4}g)	Standard deviation
20	4 ♂	172.2	15.35
	6 ♀	194.8	44.1
22	6 ♂	152.0	5.97
	5 ♀	221.4	19.97
24*	6 ♂	142.7	15.66
	5 ♀	186.0	16.31
26	4 ♂	129.0	8.04
	3 ♀	159.7	18.82
28	5 ♂	130.8	7.95
	4 ♀	156.5	26.46

* very little feeding after this age

6. Adult moths

I The effect of rearing medium during the larval stage on adults of the next generation

Method

Neonate larvae were placed in polypots containing either Froment diet or semi-synthetic diet. When adults emerged, they were weighed and paired with an adult of the opposite sex reared on the same type of diet. Some of the pairs were provided daily with water by means of damp tissue, others received only one opportunity to drink while the rest received no water. The pairs were kept in 30ml Universal bottles with no food. Any eggs oviposited were collected and counted every one to three days until the death of the adults.

Results

There was a wide variation in the number of eggs oviposited per female. The average number of eggs per female for each treatment is tabulated in Table 10. Although it appeared that fewer eggs were oviposited by females which were denied access to water or reared on semi-synthetic diet, the variance of the sample means was so large that statistical tests proved the difference to be insignificant.

The 95% confidence intervals using the student t values for the average weights of the adults are given in Table 11. There was no overlap between the four confidence intervals. It can therefore be concluded that female adults were heavier than male adults reared on the same diet. Also significantly heavier adults were produced from larvae on Froment diet than on semi-synthetic diet.

When regression analysis was used to compare the weight of female adults with the number of eggs oviposited, no correlation was obtained ($r^2 = 0.19$).

Table 10. The number of eggs oviposited by adults reared on different diets

a. Larvae reared on Froment diet

	Number of females tested	Average number of eggs oviposited ± standard deviation
No water	5	85.6 ± 90.5
Water supplied once	13	130.8 ± 94.5
Daily water supply	5	109.0 ± 100.8
Total	23	116.3 ± 92.5

b. Larvae reared on semi-synthetic diet

	Number of females tested	Average number of eggs oviposited ± standard deviation
No water	4	55.0 ± 53.8
Water supplied once	4	77.2 ± 120.7
Daily water supply	3	86.0 ± 76.6
Total	11	71.5 ± 81.2

Table 11. 95% confidence intervals for weights of adults reared on different diets

	Diet	
	Froment	Semi-synthetic
Female	111.0 - 123.4	98.1 - 108.9
Male	67.8 - 75.6	56.0 - 66.4

II Relationship between head capsule width and fecundity

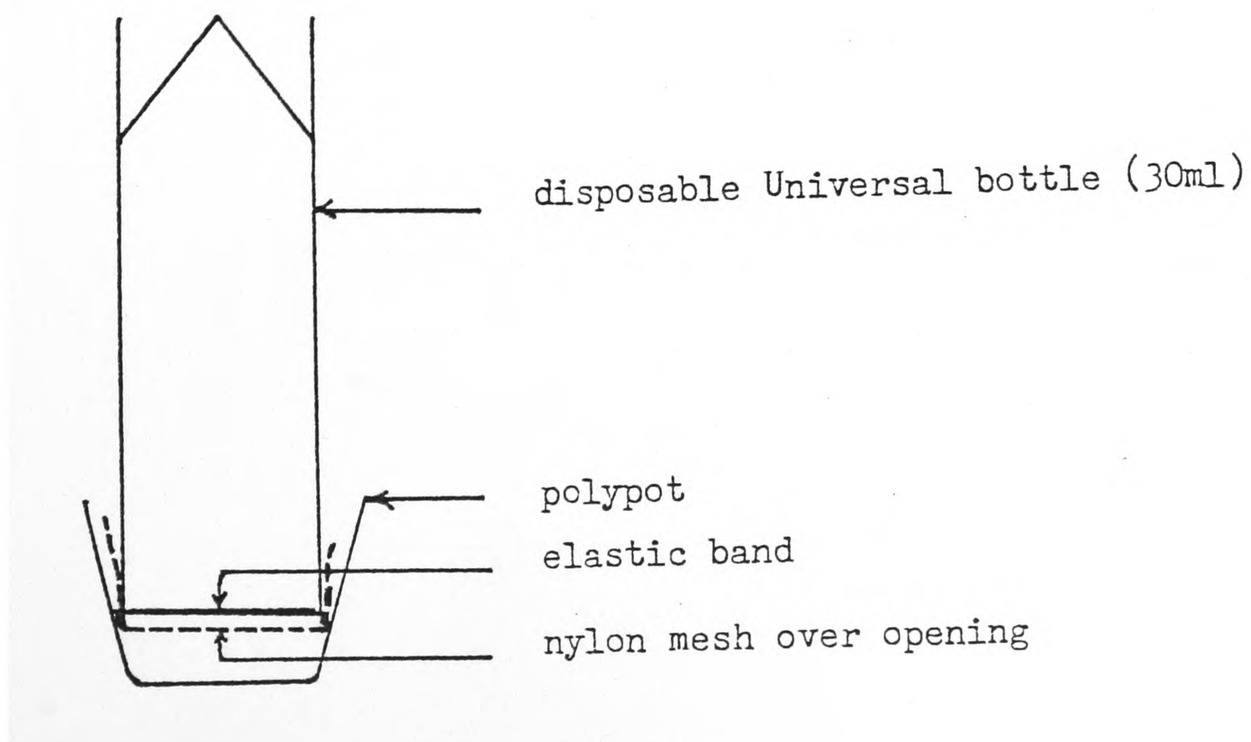
Method

Recently emerged adults from larvae reared on Froment diet were put into 21 male-female pairs. Each pair was placed in a disposable Universal bottle. A square of nylon mesh (Tygan) was secured over the opening with an elastic band and the bottle was inverted over a polypot as shown in Figure 1. No food or water was provided.

Most of the eggs oviposited fell through the mesh into the polypot. Any eggs stuck to the mesh or to the sides of the bottle were dislodged by use of a fine paint brush. The eggs were collected and counted daily. The collected eggs were kept in polypots, without diet but with damp tissue under the lid to prevent desiccation. The eggs were incubated at 26° C and the number of larvae that hatched were counted.

At the end of the experiment head capsule widths of the dead females were measured and their abdomens dissected so that the number of eggs remaining within the ovaries could be counted.

Figure 1. Diagram of oviposition bottles

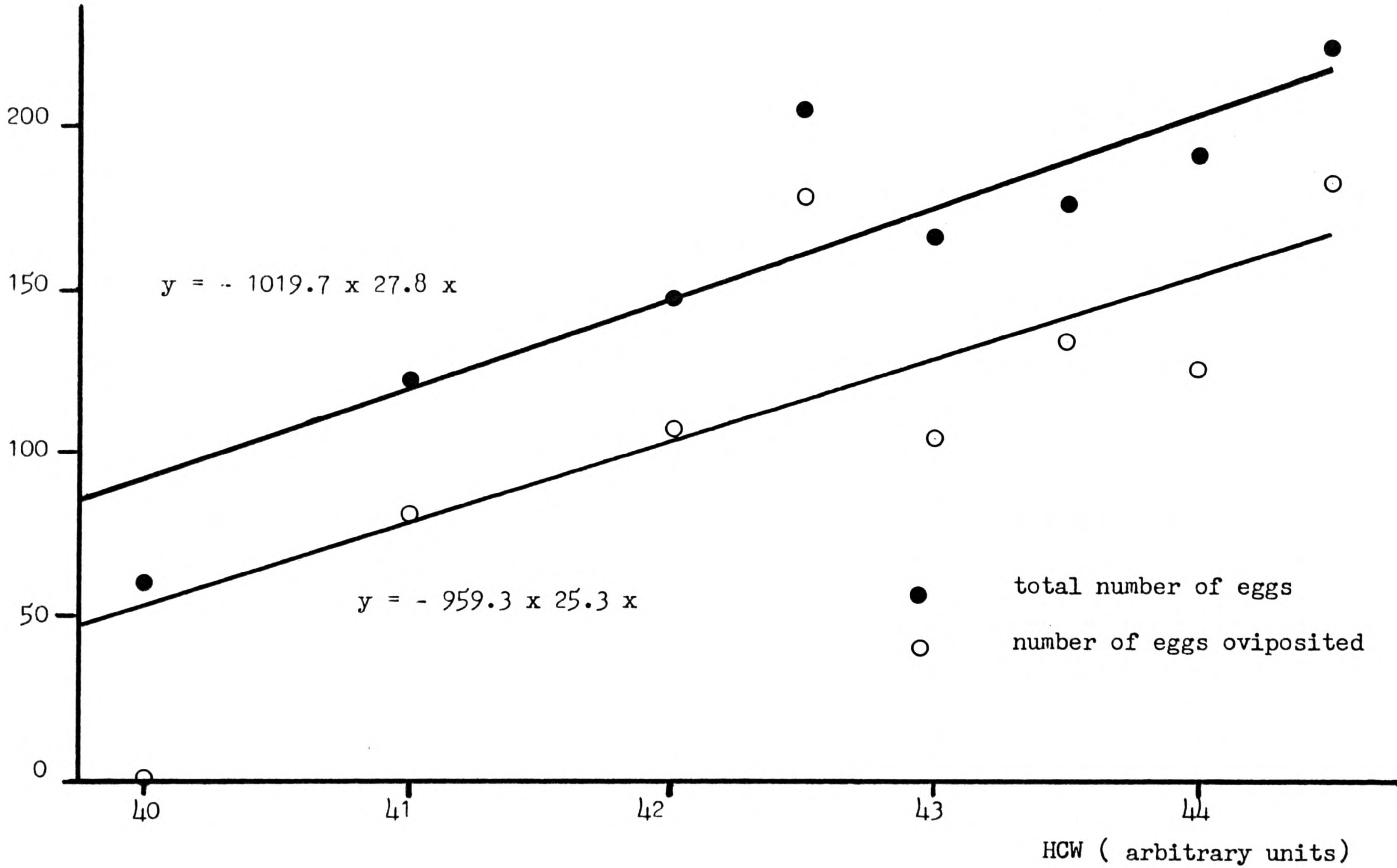


Results

Two females oviposited their full complement of eggs, whilst three did not produce any eggs having the full complement within their ovaries. Most females therefore only oviposited a proportion of their eggs, which on average was 71% of the total. Larvae hatched from 53% of those oviposited.

Figure 2 plots the averages of the total egg complement and the number oviposited per female for each head capsule width. Linear regression analysis was used to compare head capsule width with these two variables. The correlation was significant in both cases. However, a poorer correlation ($r^2 = 0.23$; $p = 0.05$) was obtained between the head capsule width and the number of eggs oviposited than between the HCW and the total egg complement ($r^2 = 0.48$; $p = 0.001$).

Figure 2 The fecundity of females with different head capsule widths



III Dispersion of oviposition and the effect of adult density

Method

Cages with a one metre square base and 600mm height were used. Mature larvae reared on Froment diet were separated according to sex and placed in groups of five in polypots with one gram of Froment diet. They were kept at 26°C and virgin adults were collected after emergence.

Sixteen petri-dish bases (85mm diameter) were placed at regular intervals on the base of the cage. Half of the dishes contained a layer of Froment diet and were spaced alternately with empty dishes. A sheet of plastic was secured over the top of the cage and the virgin adults were released from one corner. There were three treatments, using 5, 25 or 50 male-female adult pairs and two replicates of each.

The cages were kept at approximately 26°C and a 16 hour daylength. After seven days, when most of the adults were dead, the petri-dishes were removed. The number of eggs present in each "empty" dish was counted. The diet in the other dishes was transferred to 300ml tubs and more diet was provided as necessary. The tubs were kept at 26°C and the eventual adult emergence was recorded.

III Results

Tables 12 and 13 record the results of this experiment. The two tables are not directly comparable as Table 13 records the number of eggs in the "empty" dishes, while Table 12 counts the progeny by means of the resulting adults. The number of progeny recorded in Table 12 is probably less than the number of eggs oviposited, as some of the eggs may not have been viable and mortality factors will possibly have acted upon the larvae and pupae. It was impossible to

count the eggs hidden in the diet dishes. The average number of progeny per female was lower than expected, two factors that may have contributed to this are the method of counting and females laying eggs "loose" in the cage between experimental dishes.

Despite the difference in recording, the total number of progeny in the "empty" and diet dishes was compared for each treatment using a paired t-test. The test showed with 95% confidence that the difference was significant (95% confidence interval: 83.2-635.8). It is concluded that females preferentially oviposit in larval diet. If the progeny had been counted using the same method for both sets of dishes this difference would probably have been even greater.

Proportionally more eggs per female were oviposited in "empty" dishes as the density of moths increased but this trend was not observed in the diet dishes. When there were five adult females per cage more than five dishes contained progeny, therefore females must be able to lay more than one batch of eggs. Closer inspection of the data revealed that the number of progeny per dish for a density of five adult females ranged from one to 65, although less than half of the dishes with progeny had more than 11. The higher numbers may have consisted of more than one egg batch. An increase in the density of adult pairs resulted in an increase in the number of dishes containing larger numbers of progeny. However, it is not clear as to whether this was because several egg batches were being oviposited in the same dish or because females were laying larger egg batches.

Table 12. Emergence of adults from eggs oviposited in dishes containing diet

Number	Progeny			Frequency of dishes with indicated number of progeny					
female moths	Total	mean per female	mean per dish	0	1-10	11-50	51-100	101-200	>200
5	105	21.0	13.1	0	4	4	0	0	0
5	94	18.8	11.8	2	4	1	1	0	0
25	540	21.6	67.5	0	0	2	6	0	0
25	210	8.4	26.2	0	2	5	1	0	0
50	775	15.5	96.9	0	0	2	4	1	1
50	744	14.9	93.0	0	1	0	3	4	0

Table 13. Oviposition in "empty" dishes

Number of	Number of Eggs			Frequency of dishes with indicated number of eggs					
female moths	Total	mean per female	mean per dish	0	1-10	11-50	51-100	101-200	>200
5	0	0	0	8	0	0	0	0	0
5	0	0	0	8	0	0	0	0	0
25	8	.3	1	3	5	0	0	0	0
25	4	.2	.5	6	2	0	0	0	0
50	40	.8	5	6	1	1	0	0	0
50	268	5.4	33.5	1	2	3	1	1	0

IV Dispersion of oviposition and the effect of "conditioned" diet

Method

Ten sandwich boxes with dimensions 270 x 200mm were used. Ten small petri-dshes (35mm diameter) were regularly spaced on the base of each box. In five of the boxes all the dishes contained freshly made Froment diet. The other five boxes had freshly made Froment diet only in eight of the dishes, the other two dishes contained "conditioned" diet. These two dishes were in different positions in each box. "Conditioned" diet had been used for the rearing of larvae and contained frass and silk. The larvae were removed from the diet before its use.

Virgin adults were obtained by the same method as in the previous experiment. One male/female pair was released in each box. The boxes were kept at approximately 26°C with a 16 hour daylength.

Eight days after release the adults were removed. The diet in each dish was transferred to a polypot and more diet was added as necessary in order to rear any progeny. The resulting adults were counted.

Results

The dispersion of the progeny over all 10 dishes for each treatment is give in Table 14. The average number of progeny per female was 153, and therefore much higher than that recorded in the last experiment. No eggs were observed in the sandwich boxes, it was concluded that all oviposition occurred on the diet. No dish contained more than 76 progeny while 87% of the dishes contained between 1 and 50 progeny. All the females oviposited in at least eight different dishes. These results show that females can oviposit several batches of eggs.

A comparison is made in Table 15 of the average progeny per

treatment found in fresh and conditioned diet. The chi-squared value was highly significant. It is concluded that female moths preferentially laid their eggs on conditioned diet.

Table 14. Emergence of adults from eggs oviposited in dishes using 1 female per treatment

Treatment		Number of progeny Total	mean/dish	Frequency of dishes with indicated densities of progeny 0	1-10	11-50	51-100
All 10	1	93	9.3	1	5	4	0
dishes	2	124	12.4	1	6	3	0
identical	3	196	19.6	0	3	6	1
	4	157	15.7	1	5	1	2
	5	153	15.3	1	2	7	0
Average		144.6	14.5	1	4.2	4.2	0.6
	6	189	18.9	0	6	3	1
2 dishes	7	119	11.9	2	6	1	1
containing	8	198	19.8	0	5	5	0
conditioned	9	191	19.1	0	4	5	1
diet	10	110	11.0	0	6	4	0
Average		161.4	16.1	0.4	5.4	3.6	0.4

Table 15. A comparison of the average number of progeny in freshly made diet and conditioned diet

		Conditioned diet (2 dishes)	Fresh diet (8 dishes)	Total
Average total number of progeny	observed	54.6	106.8	161.4
	expected	32.3	129.1	161.4
	χ^2_1	15.4	3.9	χ^2_1=19.2

DISCUSSION

Rearing medium

The development period of P.interpunctella was shorter on diet with a higher moisture content, although maximum survival was achieved on intermediate diet. Abdel-Rahman et al. (1968) reported a negative correlation between percentage survival and development period which is in agreement with the data presented. The diet most suitable for both survival and minimum development period had two parts of glycerol to ten parts of Froment and one part of dried yeast (v,w,w). This is the same proportion as in the diet used by Bell (1975). A maximum of about 20 larvae per two grams of Froment diet was concluded to be necessary to maintain maximum survival.

Semi-synthetic diet was favourable for a short larval development period. However, the mature larvae frequently did not form pupae and wandered for weeks which was often accompanied by mortality. This failure to form pupae was not so prevalent with a density of only one larva per polypot. It is possible that the larvae found the moist conditions of semi-synthetic diet unsuitable for pupation, the humidity being greater with higher densities of larvae. In further work, not presented here, larvae reared on semi-synthetic diet were able to pupate successfully when transferred to Froment diet. It is perhaps because of this longer wandering period by mature larvae, that adults from larvae reared on semi-synthetic diet were lighter in weight than those reared on Froment diet.

The type of diet affected the behaviour of the larvae.There was a rapid and complete dispersal by immature larvae from semi-synthetic diet, whereas dispersal of young larvae from the other diets tested was uncommon. However, final instar larvae dispersed from all diets, probably in search of pupation sites which is as observed by

Takahashi (1955) for E.cautella. It is not clear as to whether the relatively few larvae leaving the wheat "toppings" diet compared with the Froment diet was because of mortality in the former or because it was more suitable for pupation, being a drier medium.

The type of diet influenced development period, larval survival and behaviour, and the weight of the resultant adults. It is therefore considered essential that the type of diet used in experiments, especially for behavioural work should be stipulated. Most experiments using P.interpunctella presented in this thesis used Froment diet in a ratio of ten parts to two glycerol and one part dried yeast. However, it proved difficult to always find larvae in this diet, so when behaviour was not considered important and if it was only necessary to rear larvae in order to diagnose virus infection, semi-synthetic diet was used.

Larval instars

The work presented revealed that P.interpunctella in the cultures used normally exhibited five instars although up to seven instars occurred when conditions were altered. The head capsule width of larvae in these successive stages followed a regular geometrical progression which was in agreement with Dyar's (1890) Law. McGaughey (1978) recorded P.interpunctella as having only four instars; his measurements for the head capsule widths of the larvae were not in agreement with those presented in this work. Silhacek and Miller (1972) reported five larval instars and their weights of larvae were in agreement with those presented here. Richards and Thompson (1932) described four to seven larval moults irrespective of moth sex and this variability was found even when the larvae were kept under identical conditions.

Mature larvae were observed to cease feeding and decline in

weight before pupation. It is suggested that if, because of unsuitable conditions, the period between cessation of feeding and pupation is extended, then the weight loss would be increased. Silhacek and Miller (1972) found that about a quarter of the weight of mature larvae was lost before pupation.

Even when larvae were kept under identical optimal conditions there was a wide variation in the age at which larvae underwent ecdysis from one instar to the next. Larval development was faster when the larvae were reared on semi-synthetic diet than when they were reared on Froment diet but pupation was more variable which may have been a result of a type of facultative diapause similar to that described by Tsuji (1959) and Williams (1964), especially as this delayed pupation was more pronounced at higher densities.

Adults

The weight of a thousand eggs was 17.7mg which is in agreement with Bell's (1976) value of 18mg and Strong et al's (1968) value of 18.5mg, although distinctly lighter than the weight of 25mg reported by Silhacek and Miller (1972). Adult females were significantly heavier than males which also is borne out by the literature (Abdel-Rahman, 1971; Silhacek and Miller, 1972). The rearing medium of the larvae influenced the weight of the adult.

There was a wide variation in the number of eggs oviposited per female, which has previously been reported (Synman, 1949; Ramsey and Farley, 1978; Abdel-Rahman, 1971). Although no correlation was found between adult female weight and the number of eggs oviposited there was a positive correlation between the female head capsule width and the fecundity. This difference can be partially explained by the greater accuracy in counting the total number of eggs rather than just those oviposited. Other workers have also found a correlation between female size and fecundity (Abdel-Rahman, 1971; Synman, 1949; Steele, 1970; Takahashi, 1956a).

Availability of water did not have a significant effect on fecundity. The females oviposited on larval diet in preference to empty dishes. Mullen and Arbogast (1977) had previously shown that the presence of food had a significant effect on oviposition. It was further shown that more eggs were laid on conditioned diet, that is diet in which larvae had been reared. This was probably because of attraction of the females to pheromones left by the larvae (Corbet, 1973). Unlike the results of Snyman (1949), the density of adults did not affect the number of eggs oviposited per female.

Arbogast and Mullen (1978) performed tests on the spatial distribution of eggs by ovipositing moths. They estimated the number of eggs by counting the larvae in a late stage of their development, for they similarly argued that finding eggs in the larval medium was very difficult and would probably have resulted in a greater error. They used a much larger area for their tests and dependent on the size of the room, used 69 or 169 petri-dishes. The use of many more dishes than in this study more clearly showed the aggregated nature of oviposition. Common to both studies is the observation that at low adult densities most dishes containing eggs had a maximum of 50 progeny and only at the highest moth densities were dishes containing more than 200 progeny obtained. In one trial by Arbogast and Mullen (1978), a single ovipositing female distributed eggs in no less than 35 dishes, 24 of which had less than six progeny. It is concluded that females can oviposit their eggs in many small batches, rather than either delivering all their eggs in one large batch or randomly distributing their eggs in all the available, suitable sites.

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INTRODUCTION

The granulosis virus of *Plodia interpunctella* was first described by Arnott and Smith (1968a). They published a study on the ultrastructure and development of the virus in the cells of the host insect. Later Arnott and Smith (1968b) described abnormal capsules of the virus which were found during their previous study. Tweeten *et al.* (1981) and Consigli *et al.* (1983) have more recently published review articles which describe in more detail the sequence of replication of granulosis virus within the host.

Arnott and Smith (1968a) identified the fat body as the primary site of infection, although virus was also found in the tracheae. According to Hunter *et al.* (1972) the epidermis and muscle sheath are also affected although neither group of workers found any evidence of liquefaction of body contents. The production of large amounts of occluded virus caused the fat body and haemocoel in infected *P.interpunctella* to become milky white (Consigli *et al.*, 1983). Arnott and Smith (1968a) were able to gauge the stage of infection by the degree of "whiteness". The larvae died as a result of the disease.

Treatment of corn and wheat with *P.interpunctella* GV under typical storage conditions (McGaughey, 1975; Kinsinger and McGaughey, 1976), or application of the virus to processed products, such as almonds and raisins (Hunter *et al.*, 1973, 1977, 1979), resulted in a substantial reduction of feeding damage by *P.interpunctella*. The treatment of the top 100mm was almost as effective as treating the whole grain mass in control of the larvae; similarly mortality was as great when only 10% of the kernels were treated (McGaughey, 1975).

Hunter (1970) documented virus-mortality studies with neonate larvae and later Hunter and Hoffmann (1973) compared the susceptibility of two strains of *P.interpunctella* larvae. Mardan and Harein (1984) published a study giving lethal concentrations of virus for fifty

percent mortality (LC_{50}) for all five instars; the older instars were least susceptible to infection.

Some insect larvae are known to exhibit a change in behaviour during virus infection (Smirnoff, 1965). The enhanced activity of NPV-infected *M.brassicae* larvae was purported by Evans and Allaway (1983) to be a major factor in the spread of disease in their cabbage plot trials.

Cannibalism of infected larvae would increase the probability of virus transmission throughout the larval population. *P.interpunctella* larvae are not cannibalistic while food is present, although they will feed on larval cadavers (Burges and Hurst, 1977). *M.brassicae* larvae are attracted to virus-killed cadavers (Evans, 1986) but conversely insect remains acted as phagodetterent in lyophilized preparations of *Lymantria dispar* L. NPV (Capinera *et al*. 1976).

Vertical transmission of virus via the adult to its progency is arguably the most efficient means of virus passage to newly hatched larvae. Transovarial transmission of BV by incorporation of virus in the egg has not been substantiated. Nevertheless several cases of transovum tranmission by external contamination of the egg have been documented. This mechanism is especially associated with viruses when the main site of replication is in the gut. These are limited to sawflies for the occluded BVs and Rhinoceros beetles for the non-occluded BVs (reviewed by Evans, 1986). Cytoplasmic Polyhedrosis Viruses (CPV, Reovirus) similarly infect the epithelial cells of the midgut. *Heliothis virescens* F. adults developing from larvae infected with a CPV produced CPV-infected progeny, however, the offspring were healthy if the eggs were surface sterilized (Sikorowski *et al*. 1973).

Examples of successful BV transmission via the adult stage after lepidopteran larvae have received a sublethal infection do exist. They inclde the GV of *Sesamia nonagrioides* Lef. (Melamed-Madjar and

Raccah, 1979) and the NPV of Heliothis zea Boddie (Thompson, 1959). More frequently BV transmission via the adult has been demonstrated after feeding BV to the adult (Hamm and Young, 1974; Vail and Hall, 1969b) or after external contamination of the adult by virus (Martignoni and Milstead, 1962; Elmore and Howland, 1964; Tatchell, 1981).

The effects of sublethal virus infection tend to be deleterious in the adult stage. S.nonagrioides adults which survived GV infection as larvae appeared to be normal, but the oviposition and hatching rate were reduced and the sex ratio was male biased (Melamed-Madjar and Raccah, 1979). The NPV of H.zea produced a reduced fecundity relative to normal females (Luttrell et al. 1982).

The aim of this chapter was to investigate the potential for host mediated virus dispersal. In order to fulfil this aim, it was necessary to undertake mortality studies to estimate the probability of infection of susceptible larvae and the timing of their death. Infected larvae are the main sources of secondary inoculum, therefore it was necessary to investigate the factors which influenced their dispersal in order to indicate the extent of virus spread. Similarly virus inoculum spread by infected larvae can only be effective if some method of larval virus transmission can be demonstrated. Adults potentially have the capacity for dispersing virus over larger distances. Therefore, the effect on the adult of sublethally dosing the larvae with virus and the possibility of transovum virus transmission were studied.

EXPERIMENTS

A. Mortality studies

Method

Dosage-mortality responses of larvae were tested using the following basic procedure.

A series of usually five dilutions were made from a purified virus suspension of known concentration. A virus free control contained deionized water only. An aliquot of 100µl of each dilution was spread evenly over a layer of semi-synthetic diet in the base of the polypot (three polypots for each dilution). The polypots were left under an extraction fan until the excess surface moisture evaporated.

Larvae hatched from eggs laid within a 48 hour period were selected at an appropriate age. The larvae selected for a treatment were all of the same instar, had dark head capsules and as far as could be judged by the naked eye were all of the same size. A sample was weighed, the number of larvae used per polypot varied depending upon the instar and availabilities of suitable larvae; usually 25 larvae per polypot were used for treatments using first, second or third instar. Fifteen larvae per polypot were used for fourth instar and 12 larvae per polypot for fifth instar larvae.

The selected larvae were counted into the treated polypots and a lid which allowed ventilation was secured. After a few days the larvae were transferred to individual polypots of semi-synthetic diet. The timing of this transfer depended upon the size of larvae to avoid handling very small easily damaged larvae. Treatments using fifth instar larvae were transferred after two days, fourth and third instar after approximately three days, second instar after five days and first instar after approximately seven days.

The larvae throughout the experiment were incubated at 26°C. They were checked regularly for virus death or pupation. Virus infection was easily recognized by the dense white colour of the diseased

larvae. Death was assumed when no movement occurred even after disturbance. The number of deaths, the timing and instar at mortality was recorded. The cadavers in some treatments were weighed.

Results

Very few larvae died without obvious symptoms of virus infection. These deaths all occurred within the first few days post infection, before the transfer to individual polypots. The number of larvae in each test was taken to be the number transferred to individual polypots; in this way no mortality was observed in the controls and all larval deaths exhibited virus symptoms. The three replicates for each dilution were summed to give a total for each.

1. Lethal concentration of virus

The data for the mortality at each virus concentration for each treatment were tested using probit analysis. The analysis provides values for the LC_{50} and the slope of log dose/probit mortality linear regression. These values, along with the initial larval weights are recorded in Table 1. The slope values were all between 1.0 and 2.0 except for the first instar which were exceptionally high.

The relationship between the initial weight and the LC_{50} is plotted using logarithmic values in Figure 1 (a). The regression curve was highly significant, demonstrating that the increase in LC_{50} can be at least partially attributed to the increase in weight. Heavier and therefore older larvae were less susceptible to virus infection.

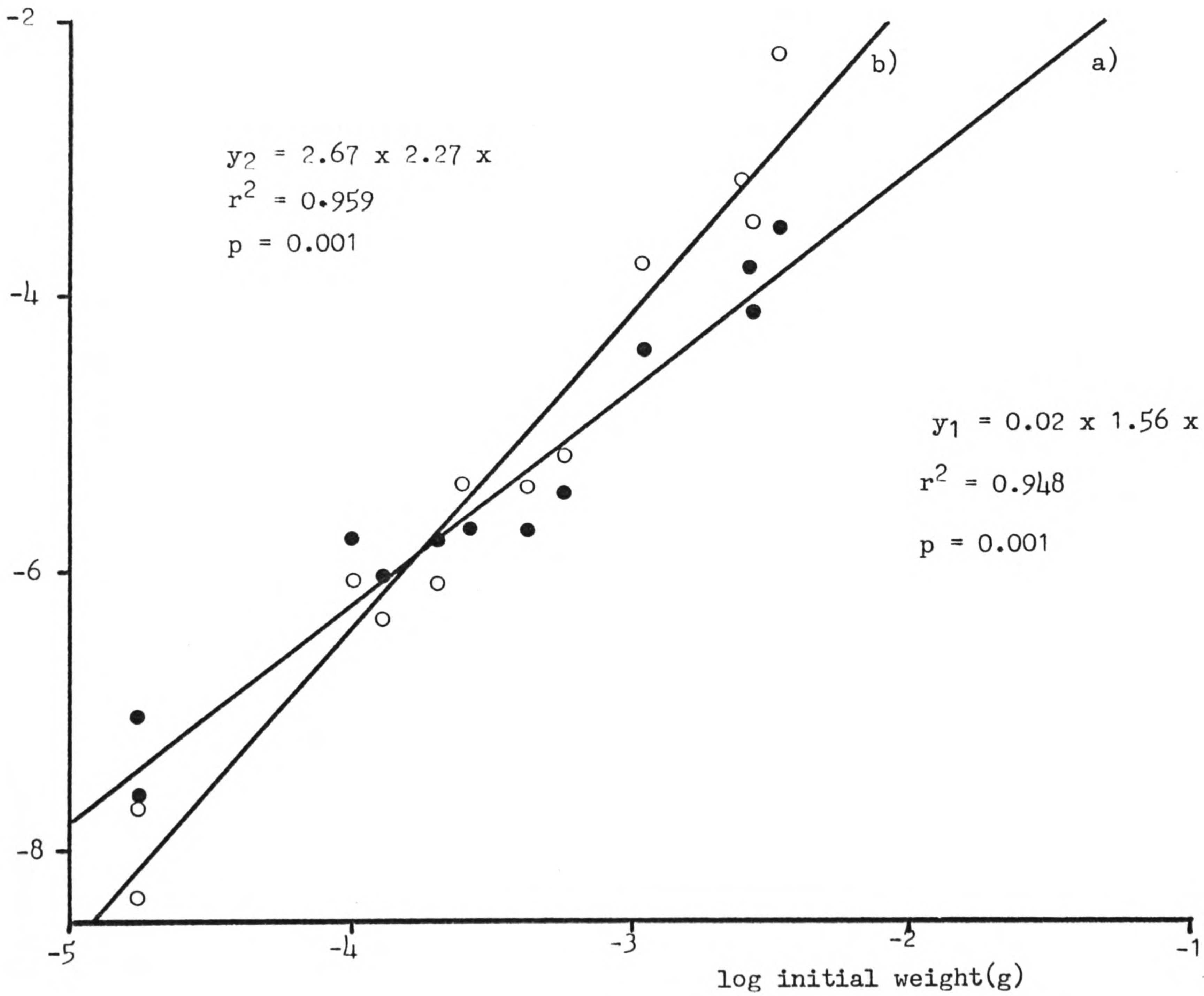
However, as the larvae were exposed to a virus concentration per unit area of diet, the actual dose received was unknown and would obviously be related to the amount of diet consumed. No values were available for the food consumption of P.interpunctella, however, Tatchell (1981b) has published figures on the mean food consumption of

Table 1. LC_{50} data for P.interpunctella and its GV

Instar		Initial wt (g)	Log wt	LC_{50}*	Log LC_{50}	Slope
1	a)	0.17×10^{-4}	-4.77	9.19×10^{-8}	-7.04	3.0
	b)	0.17×10^{-4}	-4.77	2.17×10^{-8}	-7.66	8.5
2	a)	1.0×10^{-4}	-4.00	1.71×10^{-6}	-5.77	1.5
	b)	1.25×10^{-4}	-3.90	8.87×10^{-7}	-6.05	1.7
	c)	2.0×10^{-4}	-3.70	1.67×10^{-6}	-5.78	1.9
3	a)	2.5×10^{-4}	-3.60	2.0×10^{-6}	-5.70	1.9
	b)	4.2×10^{-4}	-3.38	1.90×10^{-6}	-5.72	1.4
	c)	5.8×10^{-4}	-3.24	3.40×10^{-6}	-5.47	1.6
4	a)	11.2×10^{-4}	-2.95	4.30×10^{-5}	-4.37	1.7
	b)	26.8×10^{-4}	-2.57	1.63×10^{-4}	-3.79	1.6
	c)	28.0×10^{-4}	-2.55	8.17×10^{-5}	-4.09	1.2
5		36.6×10^{-4}	-2.45	3.24×10^{-4}	-3.49	1.4

* LC_{50} value expressed as mg of viral protein per cm^2 of semi-synthetic diet (1mg of purified P.interpunctella GV was equivalent to 1.6×10^{10} granules)

Figure 1 Relationship between LC_{50} of *P.interpunctella* larvae and their weight at infection



Pieris rapae (L.) larval instars. The food consumption per instar was assumed to be correlated to the weight increase per instar, which was calculated from earlier studies. The proportional weight increase per P.interpunctella larval instar was compared with Tatchell's (1981b) data on food consumption in Table 2. There was no significant difference in the two sets of data, however, data on the weight increase per P.rapae larval instar were not available which would have allowed a more realistic comparison. Nevertheless, the comparison supports the hypothesis that weight gain is a realistic estimate of food consumption.

Earlier studies on P.interpunctella provided values for the mean duration of each larval instar and duration of feeding of fifth instar larvae on semi-synthetic diet at 26° C. These are included in parenthesis in Table 2, the values for P.rapae were for larvae kept at 25° C (Tatchell, 1981b). The weight increase per instar divided by the duration of feeding of that instar gave values for the mean weight increase per day for each instar. These values multiplied by the corresponding LC_{50} values transform the LC_{50} data so that they at least partially take account of the relative virus dose each instar would receive because of differential feeding rates. The logarithm of those transformed data was plotted against the logarithm of the initial weight in Figure 1(b).

The regression curve using the transformed LC_{50} data was highly significant which further supports the assumption that this is a realistic transformation. The slope of the equation was greater, which implies that younger instars received comparatively less virus and the older instar comparatively more virus than the untransformed data would indicate. It can therefore be concluded that differential feeding rates partially compensated for the decreased susceptibility to virus infection of older larvae.

Table 2. A comparison of the proportional weight increase of P.interpunctella larval instars with the corresponding food consumption of P.rapae

Instar	P.interpunctella % of total weight increase per instar		P.rapae* % of total consumption per instar		χ^2
1	0.6	(4)	0.6	(3)	
2	1.0	(3)	1.1	(2)	0.03 (instars 1–3)
3	6.0	(4)	5.2	(2)	
4	14.9	(5)	22.2	(2)	1.42
5	77.5	(6)	70.9	(4)	0.29
Total	100.0	(22)	100.0	(13)	χ^2_2=1.76^{NS}

* Data from Tatchell (1981b)

Figures in parenthesis indicate ratio of duration of feeding instar

2. Timing of mortality

Table 3 presents data only for virus concentrations nearest the LC_{50} value. It gives both the percentage of cadavers of each larval instar and the overall mean larval developmental stage at mortality. The time for 50 percent mortality of infected larvae (LT_{50}) is also given, by adapting the formulae provided by Biever and Hostetter (1971).

$$LT_{50} = a + \frac{e(c-b)}{d}$$

a= number of days from initiation of experiment until the reading made just before the 50% value was reached

b= total number of larvae dead at the reading just before the 50% value was reached

c= 50% of the total number tested

d= number of larvae dying in the period during which the 50% mortality was reached

e= number of days between mortality counts

The mean LT_{50} when second or third instar larvae ingested virus was about 14 days, with 95 percent confidence intervals of 12.9 and 15.6 days. The mean value for first instar larvae was significantly higher at 16.1 days, but one of the two individual values was only 14.9 days. The LT_{50} values for fourth and fifth instar larvae at 26.9 and 30.0 days respectively were significantly greater than those for second and third instar larvae.

Larvae were usually able to successfully complete at least one ecdysis after infection. The peak of mortality frequently occurred during the next larval instar after infection, although this was not always predictable and the peak mortality occasionally occurred later. Infected fifth instar larvae died during that larval stadium; however, pupae were not examined for infection. The mortality of the larvae infected in the first instar was spread more widely over the larval development stages than expected, mortality occurred in all stages, with a peak between the third and fourth instars.

One of the experiments using third instar larvae with an initial weight of 2.5×10^{-4}g will be examined in more detail in order to illustrate some of the trends shown in the data. An increase in the virus concentration ingested by the larvae resulted in a decrease in the mean larval development stage achieved. The relationship between these two variables is plotted in Figure 2; the regression equation was significant at the 95% level. This trend was reflected in a decrease in the LT_{50} values from 18.0 to 12.4 days when the virus concentration was increased, except for an abnormally low LT_{50} value (15.5 days) at the lowest dose.

Larvae which suffered mortality as third instar had a shorter LT_{50} than fourth instar larvae, which in turn, on average died sooner than fifth instar larvae. This difference was greater when a range of virus concentrations were used, however, even when the data from just

one virus concentration were selected the difference was still apparent, especially between the fourth and fifth instars. For example, at a virus concentration of 1.9×10^{-5}mg of virus per cm^2 the LT_{50} values for third, fourth and fifth instar larvae were 13.0, 13.8 and 16.8 days respectively.

Third and fourth instar larvae infected during the third instar died before 20 days post infection. The timing of mortality of fifth instar larvae is portrayed in Figure 3. The larvae exhibited a bimodal response to virus infection with peaks of mortality at approximately 16 and 34 days post infection. This bimodal response was exhibited at all virus concentrations tested.

The time until fifty percent pupation of control larvae (15.2 days) was very similar to the corresponding time for larvae which received sub-lethal virus doses as third-instar larvae (15.8 days). Pupation was complete by 25 days which is about the same time period during which most of their infected counterparts suffered mortality. However, as already explained, some fifth instar larvae died much later and thereby displayed an extension of the normal larval period. Most infected larvae were observed to spend longer than the control larvae at certain development stages, usually the instar in which mortality occurred.

Figure 2 The relationship between mean instar at mortality and virus concentration fed to third instar larvae

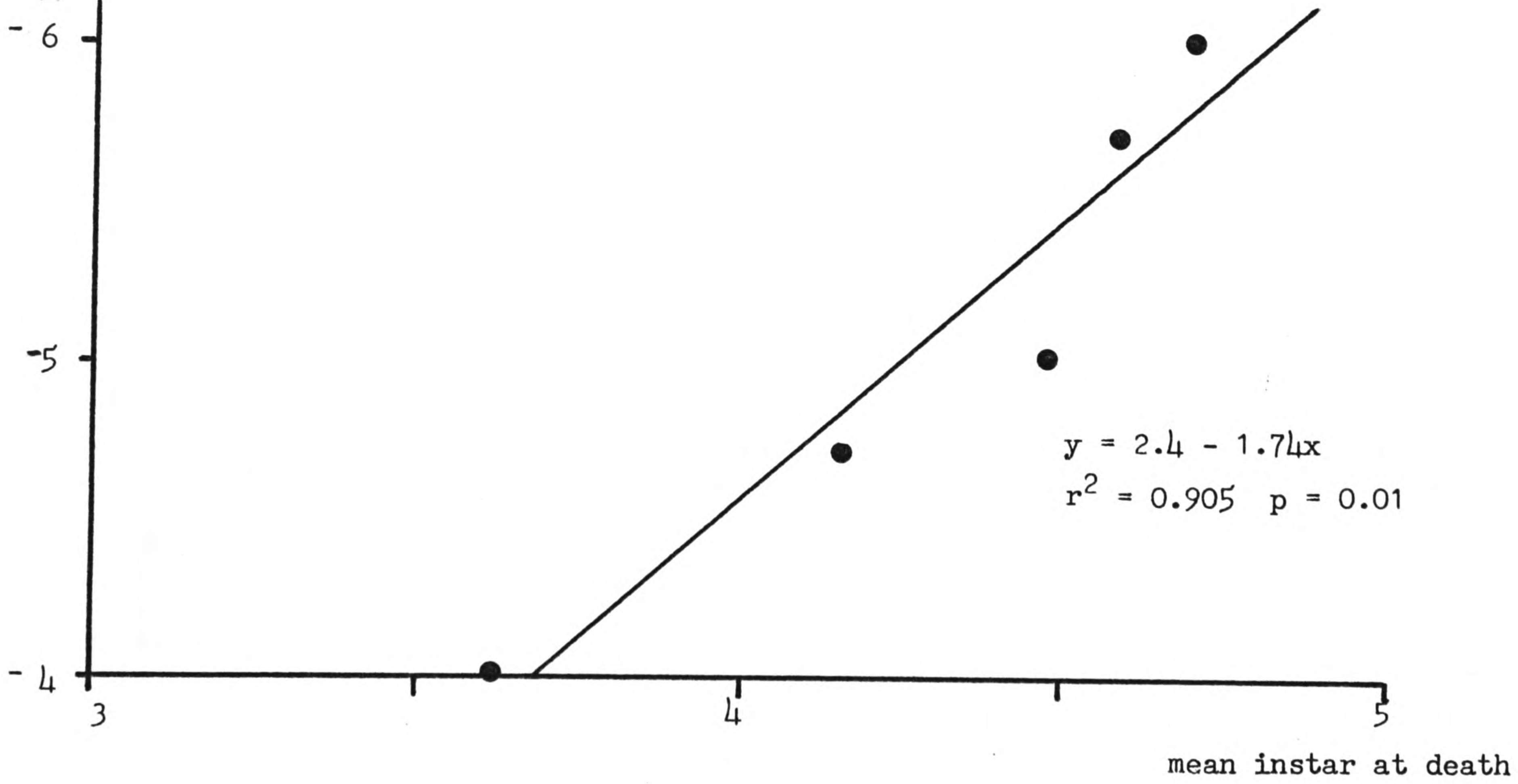


Figure 3 Timing of mortality of fifth instar larvae infected during the third instar

cumulative frequency of virus-killed fifth instar larvae

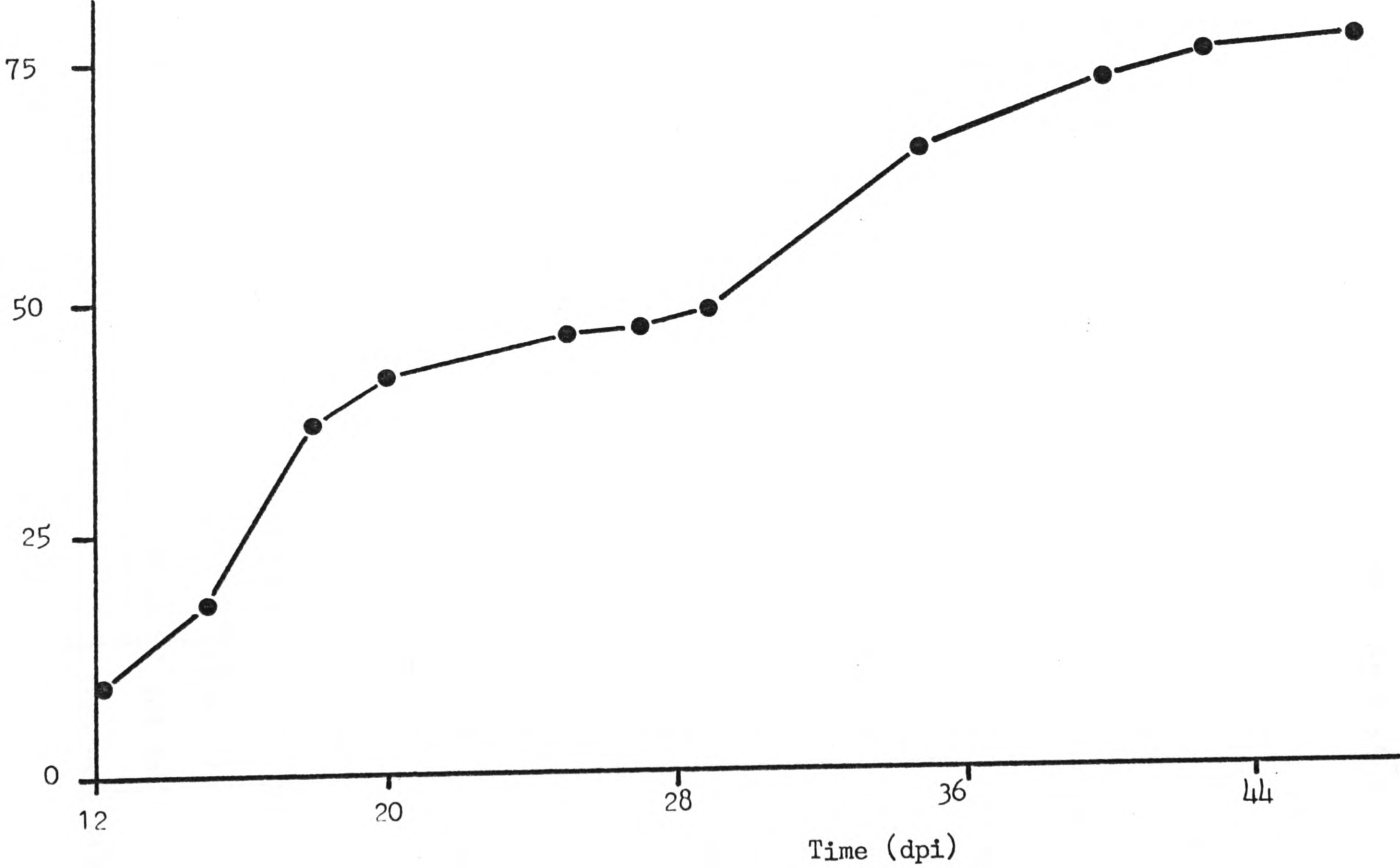


Table 3. The percentage of larvae suffering mortality in each instar and the LT_{50} values for the different treatments

Instar	Initial weight ($\times 10^{-4}$g)	Larval instar at death (%) 1	2	3	4	5	Mean	LT_{50} (days)
1 a)	0.17	3.6	10.7	35.7	39.3	10.7	3.4	14.9
b)	0.17	0.0	25.0	41.7	28.0	13.7	3.3	17.2
Mean	0.17	1.8	17.8	38.7	28.0	13.7	3.3	16.1
2 a)	1.00		0.0	61.7	36.2	2.2	3.4	12.8
b)	1.25		4.3	55.9	30.1	9.7	3.4	15.7
c)	2.0		0.0	28.9	51.1	20.0	3.9	14.7
Mean	1.4		1.4	48.8	39.1	10.6	3.6	14.4
3 a)	2.5			1.2	47.1	51.8	4.5	15.6
b)	4.2			10.0	80.0	10.0	4.0	13.5
c)	5.8			0.0	79.7	20.3	4.3	13.2
Mean	4.2			3.7	68.9	27.4	4.3	14.2
4 a)	11.2				0.0	100.0	5.0	22.8
b)	26.8				0.0	100.0	5.0	25.0
c)	28.0				0.0	100.0	5.0	33.0
Mean	22.0				0.0	100.0	5.0	26.9
5	36.6					100.0	5.0	30.0

3. Weights of infected cadavers

Table 4 summarises body weight data for virus infected cadavers resulting from dosage at a concentration close to the LC_{50} value. Little difference in weight was found within instars for the various treatments or for the mean weight of all the larvae for each treatment. It was concluded that when the virus concentration was close to the LC_{50} value, the initial weight of the larvae had little

influence on the weights of the cadavers. Fifth instar larvae cadavers were significantly heavier than fourth instar cadavers, which in turn were heavier than third instar cadavers.

The mean weight of cadavers when third instar larvae with an initial weight of 2.5×10^{-4}g were infected will be considered in more detail. Larvae which died later were on average heavier than those that died earlier, reflecting the increasing proportion of older instars. The relationship between virus concentration and cadaver weight is plotted in Figure 4. The regression equation was significant and it was concluded that an increase in virus concentration caused a decrease in mean cadaver weight. Close inspection of the data revealed that this relationship was attributed to a greater proportion of younger instars dying at higher virus concentrations. Both of these results are therefore related to the LT_{50}.

When fourth instar larvae with a mean weight of 28.0×10^{-4}g were infected all the larvae died in the fifth instar. The virus concentration did not significantly influence the cadaver weight but the time of death was of interest. The time of death showed a bimodal distribution which was reflected in the mean cadaver weight (Figure 5). There was no significant difference in the weights of larvae which died during the first part of the bimodal distribution, ie. up to day 20. However, they were significantly heavier than those which died later.

Inspection of the larvae during the experiment revealed that most of the larvae stopped feeding before any of the virus deaths. All the larvae exhibited typical virus symptoms after the first peak of mortality; many appeared to dehydrate and were immobile and some were capable only of moving their mandibles when prodded.

Figure 4 The relationship between mean cadaver weight and virus concentration fed to third instar larvae

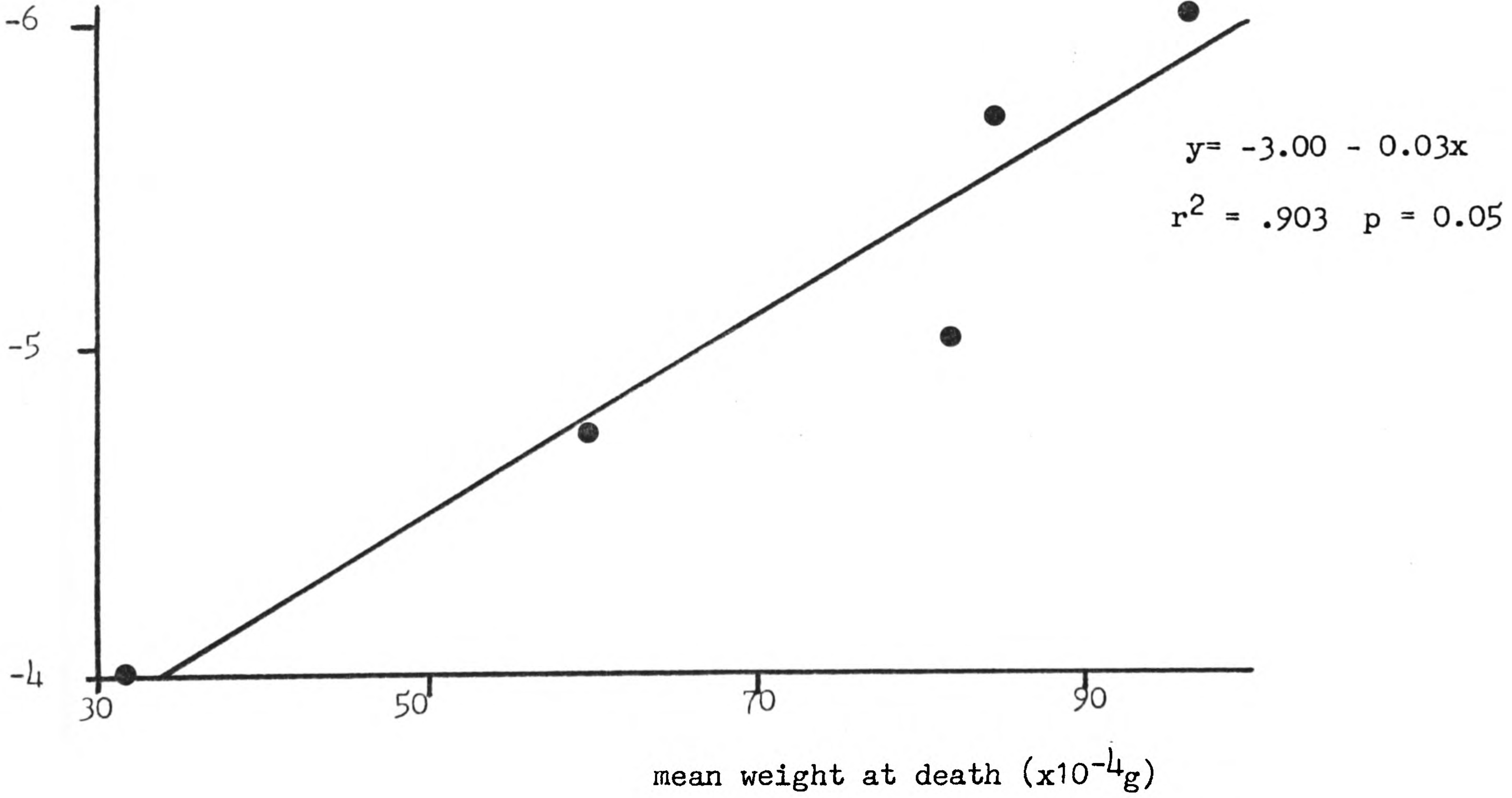


Figure 5 The weights of fifth instar cadavers after GV infection in the fourth instar.

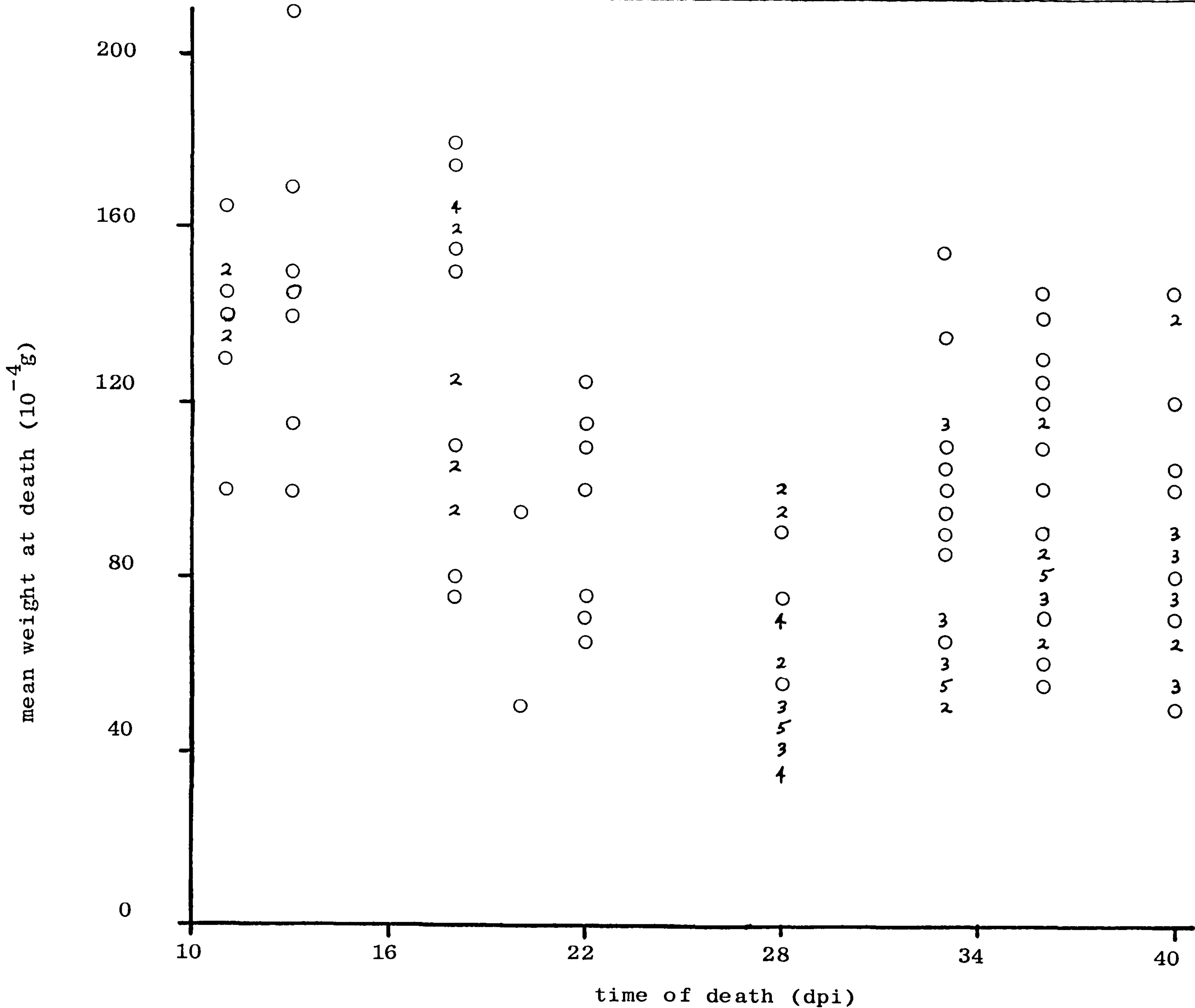


Table 4. Weights of virus-killed larvae after receiving a virus concentration near their LC_{50} value

Treatment (initial instar)	Initial weight ($x10^{-4}$g) (mean)	95% confidence intervals of larval instars at death 3	4	5	Mean weight at death (x 10^{-4}g)
2	1.25	11.7	43.5-54.1	62.9-172.3	35.7-72.5
3 a)	2.5	-	31.0-45.6	94.0-133.9	64.3-104.4
b)	4.2	23.0	38.7-49.1	-5.1-212.5	43.9-62.9
c)	5.8	-	49.4-56.0	90.3-128.2	58.2-75.8
4 a)	11.2		-	11.6-116.4	11.6-116.4
b)	28.0		-	68.2-96.5	68.2-96.5

Number per treatment = 40

B. Dispersal of healthy and infected larvae

This study was designed to identify the factors which influence larval dispersal and therefore potential spread of virus. Some insects are known to exhibit an enhanced activity when virus-infected (Smirnoff, 1965; Evans and Allaway, 1983), which would act to increase virus spread. It was not known if virus infected P.interpunctella larvae displayed such a change in behaviour.

Two experimental designs were utilized to test larval dispersal, a discontinuous and continuous diet system. The discontinuous diet system consisted of food islands surrounded by moats in which dispersing larvae would be caught. This system was artificially simple as it did not allow reentry to the food island and it was

assumed that neither the edge of the diet nor the presence of the moat differentially affected the dispersal of larvae. However, its advantages were that it was reproducible, simple to operate and daily measurements could be taken without disturbing the habitat.

The continuous diet system involved destructive sampling of the habitat and searching for the dead larvae. Its disadvantages were that it was time-consuming and prone to error as it was difficult to find the larvae in the diet. However, the main advantage of this method was that it gave a measure of the dispersion of larvae with time.

1. Using islands and moats

A series of experiments were performed using islands of diet in which larvae were placed. Larvae which dispersed from the islands were trapped in the surrounding moat, from which they were collected on a daily basis. This simple experimental design made it possible to compare the influences of virus infection, age, density and environmental factors on the tendency of larvae to disperse from a food source.

Basic experimental design

Larvae hatched from eggs oviposited within a 48 hour period, were selected for an appropriate age. Half of the larvae were placed into 300ml tubs of semi-synthetic diet on the surface of which virus had been spread, the other half of the larvae were placed into similar but virus-free tubs. The larvae were incubated at 26°C for 24 hours before evenly sized larvae were selected from both the control and virus-treated tubs for use in the experiment.

Two grams of freshly made Froment diet were weighed into 35mm diameter petri-dish bases. Twenty five selected larvae were placed into each petri-dish and the lids replaced. Each was positioned centrally in a 90mm diameter petri-dish base containing about 12ml of

glycerol. The small petri-dish lids were removed after about 4 hours, when it was assumed that the larvae would have settled after handling. The larvae were then free to leave the diet in the small petri-dish to become trapped in the surrounding moat. Usually there were three replicates for each treatment.

The experiment was kept at 26° C and the moats checked daily for larvae, until either the emergence of adults or no more larvae were active. The data on the number of larvae caught in the moats were combined for all replicates of each treatment for analysis.

a) A comparison of the dispersal of control and virus treated larvae

Method

Third instar larvae with a mean weight of 6.5×10^{-4}g were selected. There were two treatments; a virus-free control and virus-treated larvae. Each treatment consisted of four replicates.

Results

Figure 6 portrays the number of immature and mature larvae caught in the moats on successive days for both treatments. The dispersal of mature larvae occurred mainly between 12 and 16 days post infection. Dispersal of immature larvae took place throughout the period of larval activity; by the end of 19 days virus infected larvae were either dead or sluggish in their movements. Mature larvae did not exhibit external signs of virus infection.

Examination of Figure 6 reveals that control larvae showed little tendency to disperse during the immature stages but a large proportion of mature larvae readily dispersed. In contrast, virus-treated immature larvae readily dispersed throughout the larval period.

Table 5 tabulates the total numbers of larvae caught in the moats and by subtraction those which remained in the islands. A comparison of the two treatments using the chi-squared test showed no significant difference. The mature larvae were deducted from the number migrated

to give data for the immature larvae only (Table 6). The chi-squared comparison revealed with 99% confidence that more virus-treated immature larvae dispersed than control larvae.

Table 5. A comparison of the total dispersal of "virus-treated" and control larvae

	Number of larvae		
	Migratory	Static	Total
Control	51	49	100
"Infected"	50	50	100
Total	101	99	200

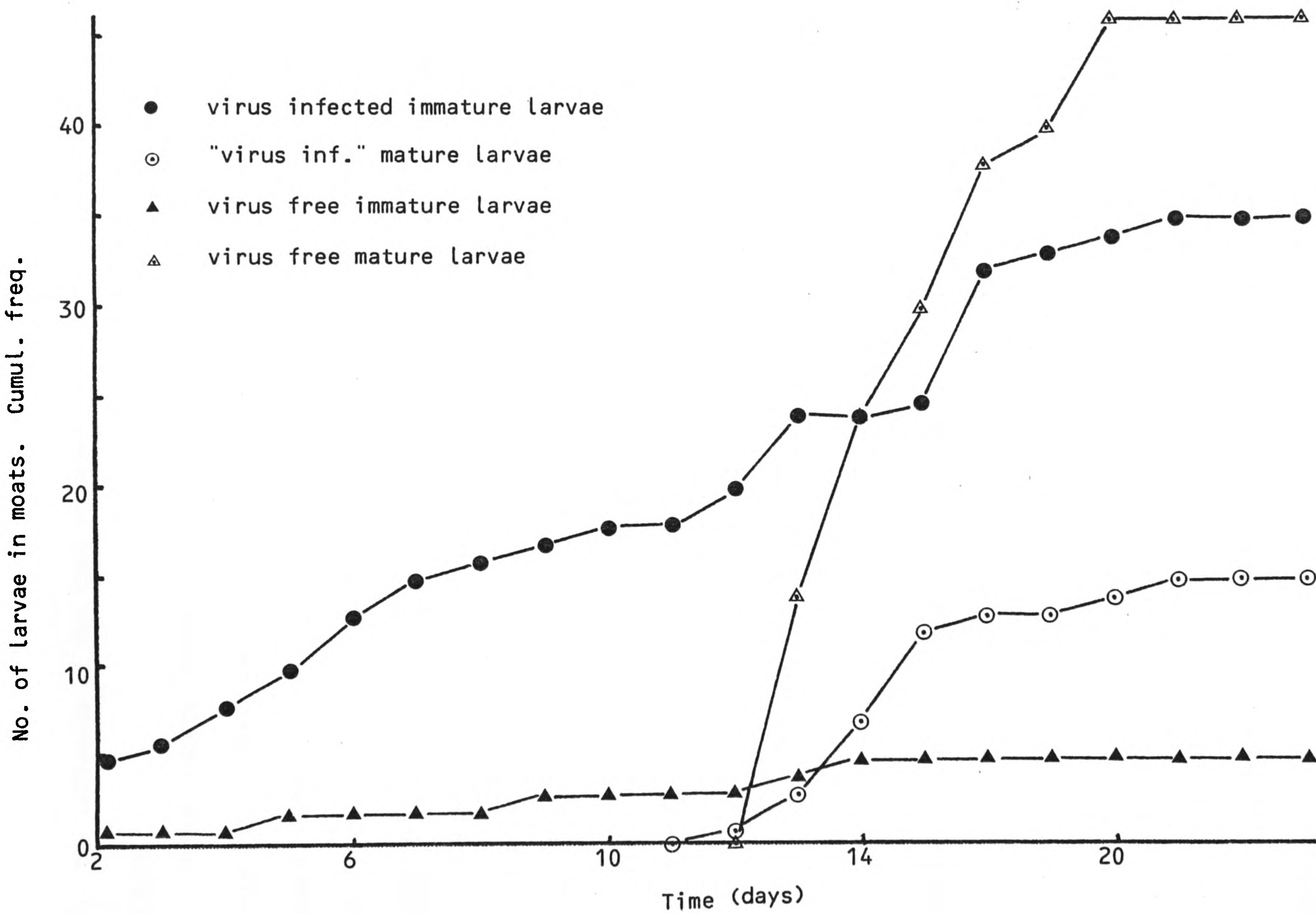
$\chi^2_1 = 0.02^{NS}$

Table 6. A comparison of the dispersal of virus-treated and control immature larvae

	Number of larvae		
	Migratory	Static	Total
Control	5	95	100
"Infected"	35	65	100
Total	40	160	200

$\chi^2_1 = 28.13 \quad p = 0.001$

Figure 6 Dispersal of larvae from food islands



b) The influence of the type of moats

Method

Third instar larvae with a mean weight of 6.0×10^{-4}g were selected. There were three treatments using different types of moats; namely glycerol, light paraffin oil and water containing a drop of Triton "X". Each treatment consisted of six replicates.

Results

The dispersal of immature virus-treated larvae for each treatment throughout the experiment is shown in Figure 7. A chi-squared comparison of the proportions of migratory immature virus-treated larvae indicates that the differences could be attributed to chance (χ_2^2=0.11^{NS}). It is concluded that the three types of moats tested did not differentially affect the dispersal of virus-treated immature larvae.

c) The influence of the diet texture

Method

Third instar larvae with a mean weight of 6.0×10^{-4}g were selected. There were two treatments using different textures of diet; a coarse diet using Froment and fine diet using wheat meal flour as the main constituent. The diets were made up in the usual ratio of ten parts wheat feed to two parts glycerol and one part dried yeast. Each treatment consisted of six replicates.

Results

Figure 8 presents the dispersal of immature virus-treated larvae from the two treatments throughout the course of the experiment. A chi-squared comparison of the proportions of these migratory larvae suggests that any difference between the treatments could be

Figure 7 The effect of the type of moat on the dispersal of infected larvae

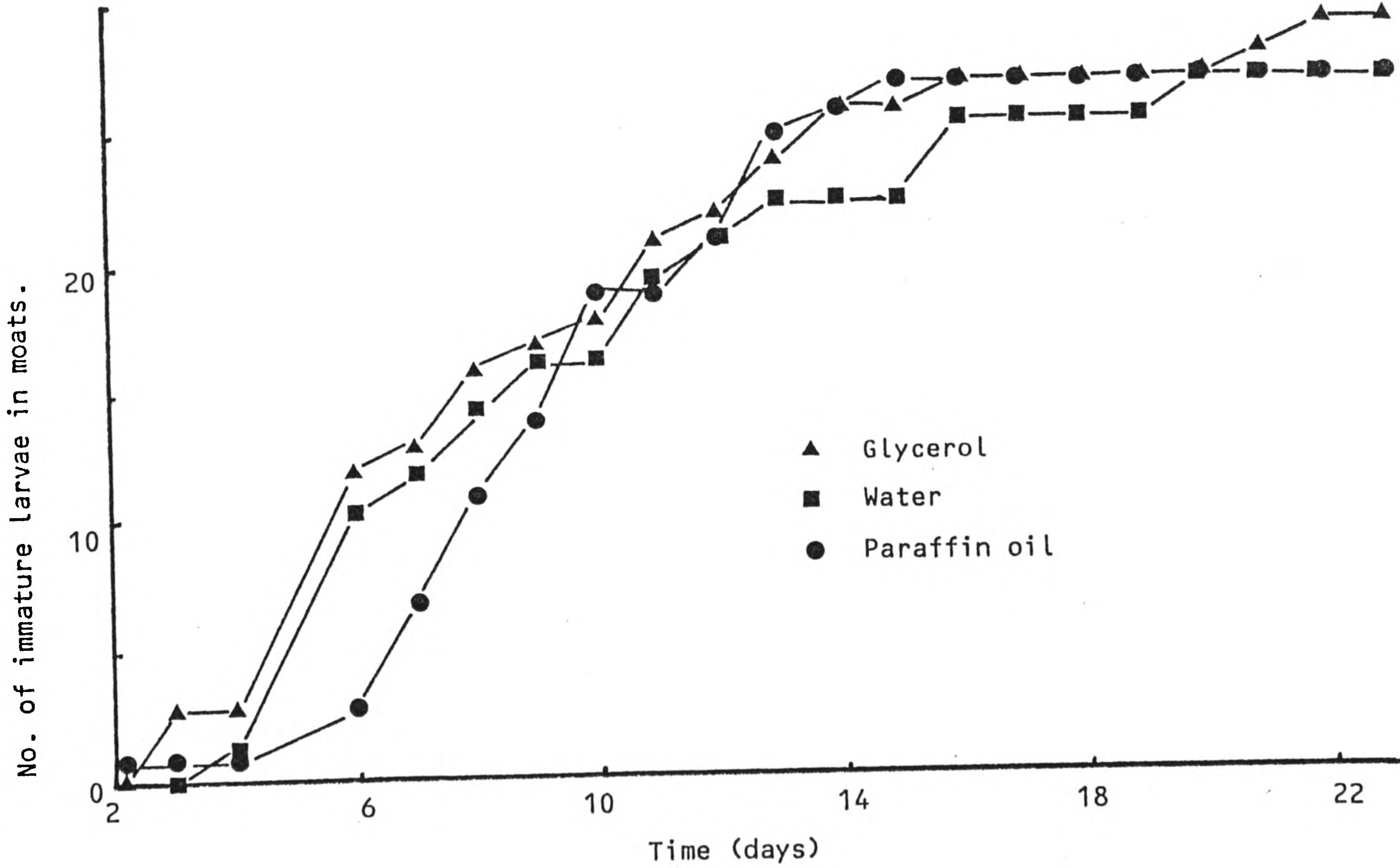
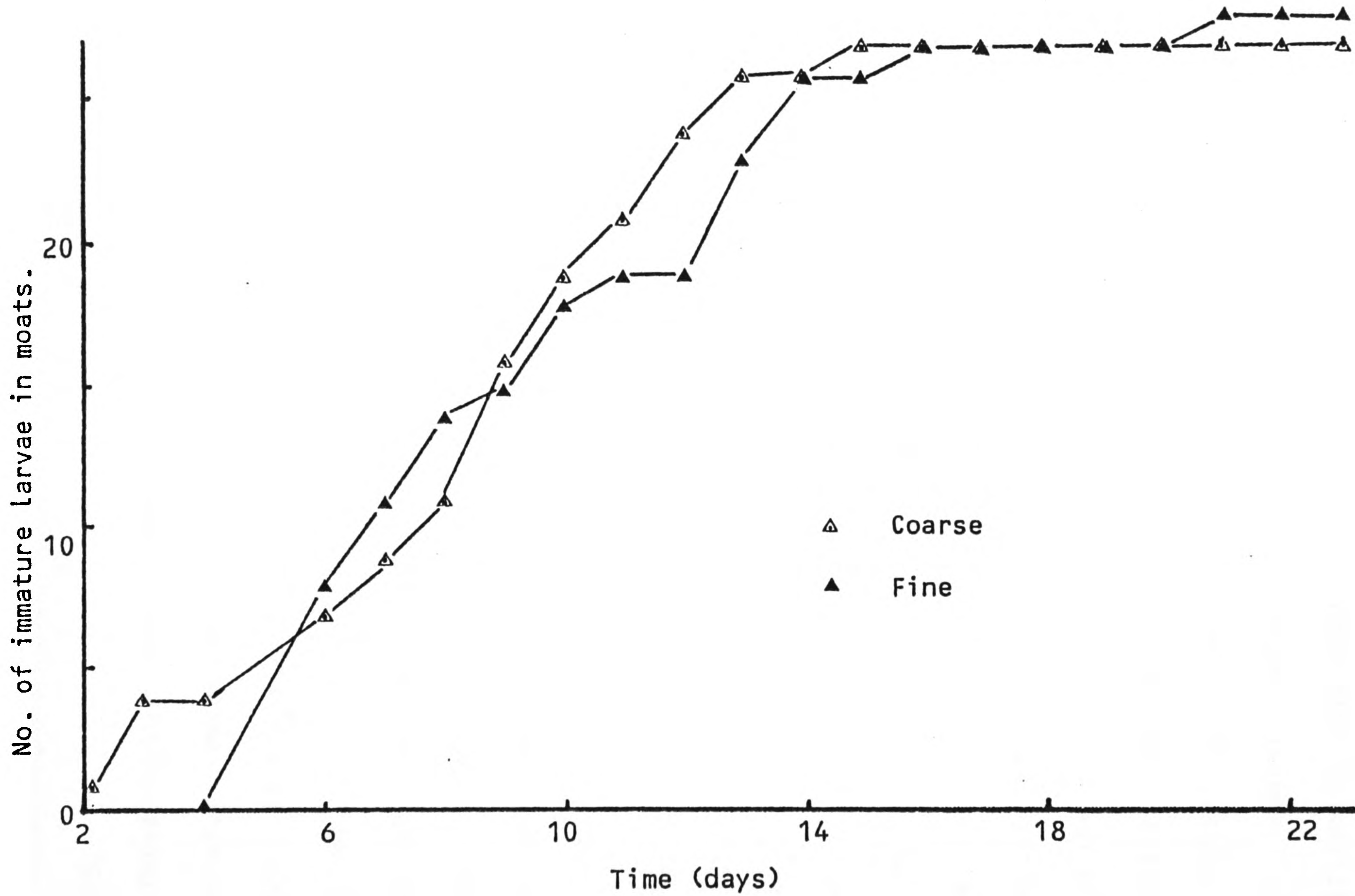


Figure 8 The effect of diet texture on the dispersal of infected larvae



attributed to chance (χ_1^2=0.09^{NS}). It is concluded that texture of diet does not influence the tendency of immature virus-treated larvae to disperse, at least for the types tested.

d) The influence of glycerol content of diet

Method

Third instar larvae with a mean weight of 3.9 x 10^{-4}g were selected. There were five treatments with different ratios of glycerol in the diet for both control and virus treated larvae. The ratio of glycerol to ten parts of Froment diet and one part dried yeast was none, half, one, two or four parts. The treatment with the highest ratio of glycerol used three grams of diet rather than the usual two grams per 35mm diameter petri-dish. There were three replicates for each treatment.

Results

The dispersal of immature larvae from the diets is presented in Figure 9 for control larvae and Figure 10 for virus treated larvae. Dispersal of control larvae from the four diets containing glycerol was very similar and occurred only at a low level, a chi-squared comparison was not significant (χ_3^2=4.02). In contrast a large proportion of control larvae migrated from the glycerol-free diet within the first few days. A chi-squared comparison of the proportion of control immature larvae which dispersed from all five treatments was highly significant (χ_4^2=157.72, p=0.001). It is concluded that healthy immature larvae readily migrate from diet without glycerol but significantly less migrate from diet containing glycerol within the range tested.

The dispersal of virus treated larvae was most rapid on glycerol free diet, although the total numbers dispersing on all diets except that with the highest glycerol content was very similar (Figure 10).

<u>Figure 9</u> <u>The effect of diet glycerol content on the dispersal of control larvae</u>

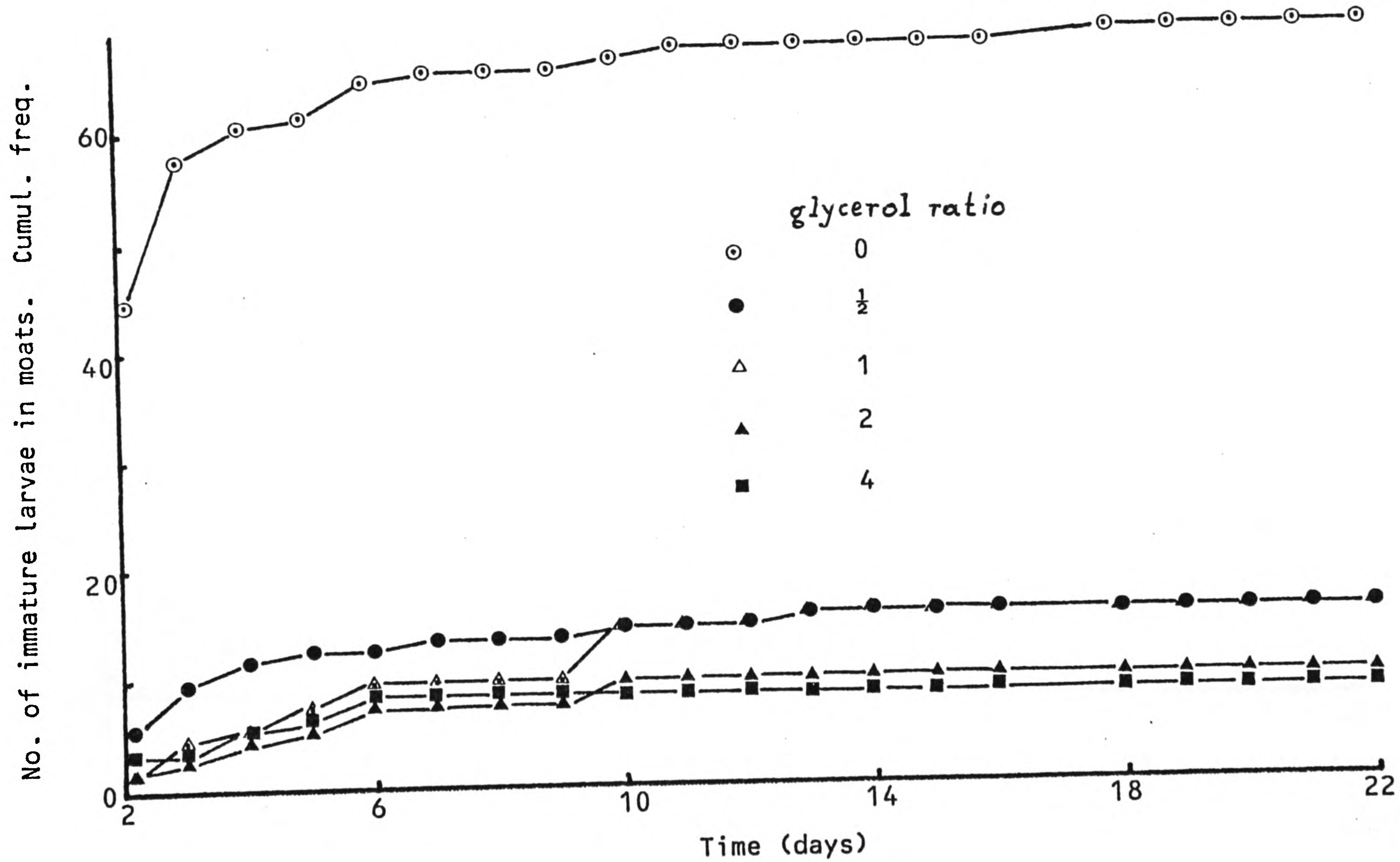
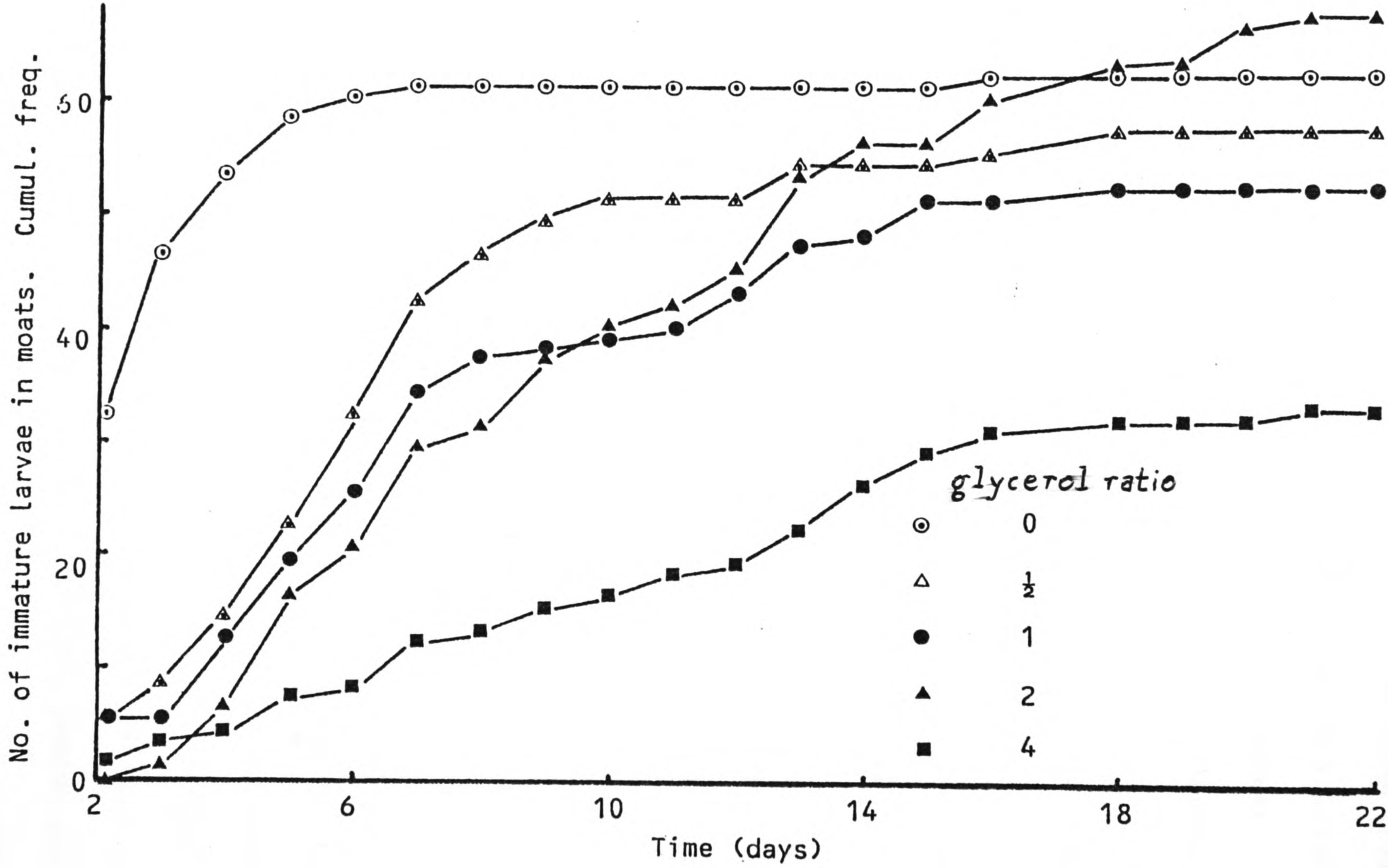


Figure 10 The effect of diet glycerol content on the dispersal of virus treated larvae



A chi-squared comparison for all five diets showed that the differences in proportions of larvae migrating from the different diets was significant (χ^2_4=49.31, p=0.001). The difference could be mostly attributed to the high glycerol diet, from which fewer larvae dispersed.

A chi-squared comparison of the dispersal of immature larvae in glycerol free diet from both healthy and virus treated larvae showed no statistical difference (χ^2_1=2.27). However when the same comparison was made for all diets containing glycerol, the difference in dispersal of healthy and virus treated larvae was shown to be highly significant (χ^2_7=218.57, p=0.001).

e) The timing of dispersal

Method

Third instar larvae with a mean weight of 5.5×10^{-4}g were selected. There were five treatments in which larval movement was restricted to the food island for different time periods. Prevention of larval migration was achieved by retaining the petri-dish lid on the islands until the appropriate day; the periods chosen were 1, 4, 7, 10 and 13 days post-treatment. Parallel experiments were run for both control and virus treated larvae with three replicates for each.

Results

The numbers of control mature larvae which migrated in the different treatments are tabulated in Table 7. All five treatments had a similar high proportion of mature larvae migrating. When this was compared using a chi-squred test it was shown that any differences could be attributed to chance (χ^2_4=4.04). It was concluded that prevention of larval migration up to 13 days had no effect on the total migration by mature larvae. Closer inspection of the data

revealed that emigration of mature larvae was not observed before 14 days post-treatment.

The numbers of virus-treated immature larvae caught in the moats against time is shown in Figure 11. A chi-squared comparison was made between the observed proportions of migration, assuming no effects of treatments on the larval behaviour. The result was highly significant (χ^2_4=73.25, p=0.001). It is concluded that fewer larvae migrated when the period of restricted migration was increased.

Table 8 presents the data on dispersal of virus treated immature larvae so that dispersal after different time periods can be compared. In this presentation of the data, no account is taken of the proportions of larvae which remain in the diet. The mean values of all treatments give an indica tion of the number of larvae which migrate after a given period. These mean values were compared with the actual values using a chi-squared test. None of the chi-squared values were statistically significant. It is concluded that the number of virus treated immature larvae which migrated after a certain day is the same regardless of the length of the restricted period before that time.

Table 7. Dispersal of control mature larvae after release on different days post-treatment

		Number of larvae		
		Migratory	Static	Total
day post	1	45	25	70
treatment	4	45	25	70
when lids	7	41	32	73
removed	10	44	31	75
	13	53	22	75
Totals		228	135	363

Figure 11 The dispersal of virus-treated larvae after prevention of migration for different dpi

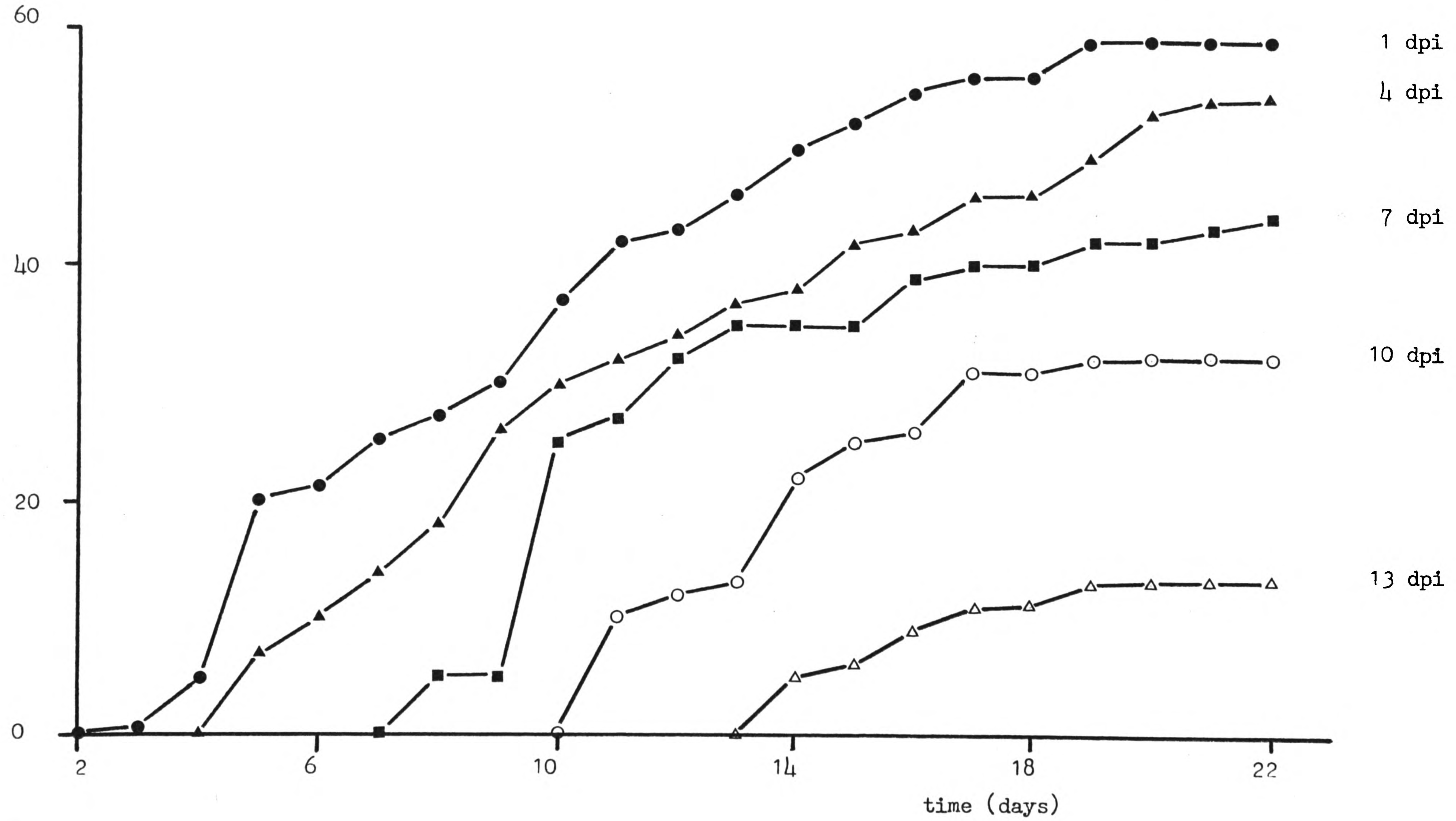


Table 8. Dispersal of virus-treated immature larvae during various periods post-infection

		Number of larvae migrating after different days post infection				
		1	4	7	10	13
	1	59	54	34	22	13
Period of	4	-	54	40	24	17
restricted	7	-	-	44	19	9
migration (days)	10	-	-	-	32	19
	13	-	-	-	-	13
Mean		59.0	54.0	39.33	24.25	14.20
χ^2		-	-	4.28^{NS}	3.82^{NS}	1.29^{NS}
				$\chi^2_9 = 9.39^{NS}$		

f) The effect of larval density

Method

Third instar larvae with a mean weight of 7.6×10^{-4}g were selected. There were five treatments in which different initial larval densities were employed for both control and virus treated larvae. The densities used were 10, 25, 50, 75 or 100 larvae per two grams of diet in a 35mm diameter petri-dish island. There were five replicates for the treatments with an initial density of ten larvae, three replicates for those with initial densities of 25, 50 or 75 larvae but only one dish for the two treatments with an initial density of 100. Throughout the duration of the experiment more diet was provided as considered necessary.

Results

Unfortunately, during the experiment B.hebetor adults escaped from a culture and parasitised some of the larvae. The islands with an initial density of 25 virus treated larvae were particularly affected, consequently this treatment was excluded from the analysis.

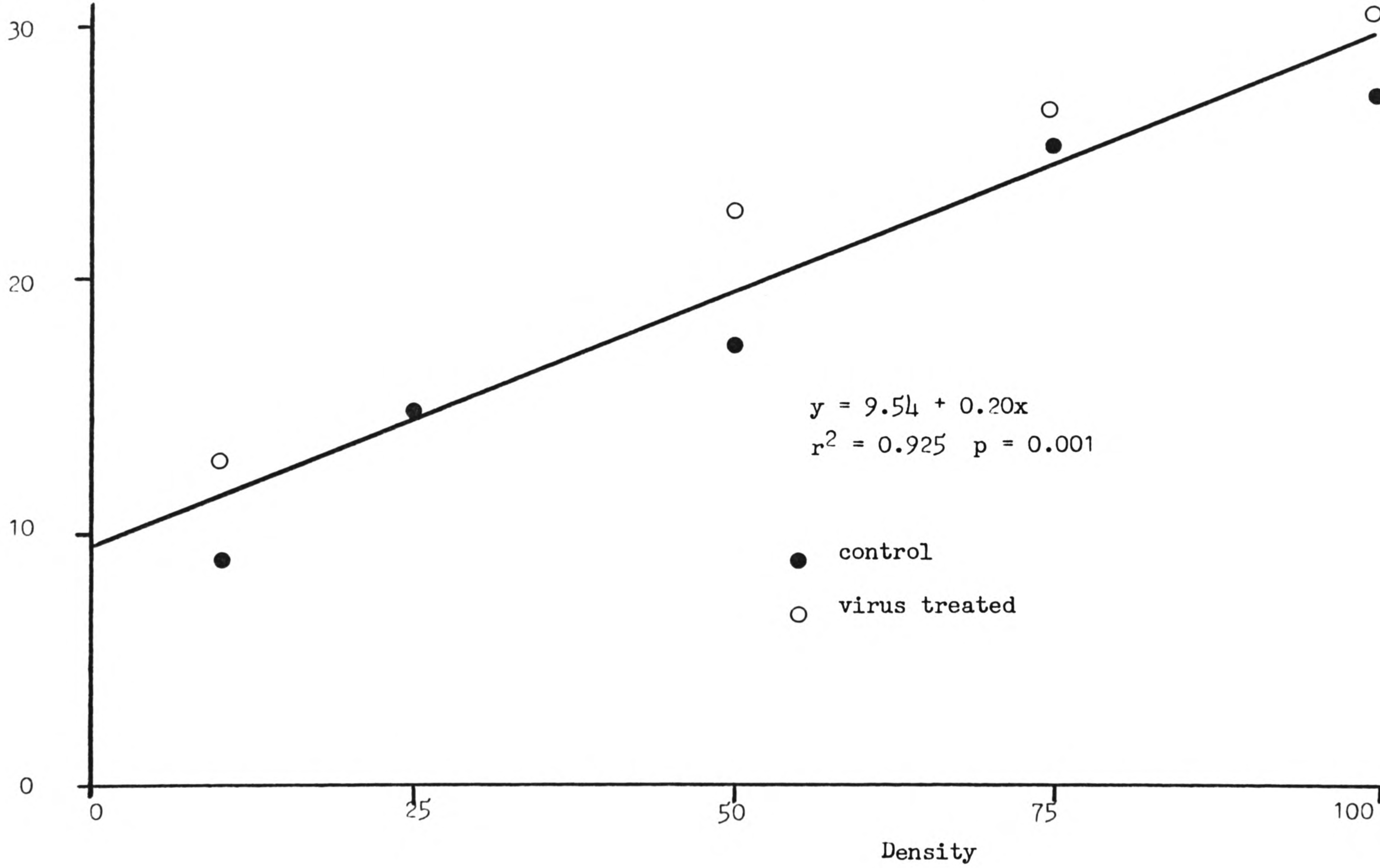
The numbers of mature control larvae which dispersed are recorded in Table 9. The data were transformed using the angular transformation before attempting to correlate the initial density with the proportion of larvae which migrated. The correlation coefficient (r^2=33.0) was not significant. It is concluded that in this experiment, the initial density did not influence the migration of mature larvae.

The proportion of immature larvae which migrated is plotted against the initial density in Figure 12. Regression equations for the control and virus treated treatments were both significant with 99% confidence. The 95% confidence intervals for the slopes of both equations were identical (0.12-0.27). The constant for the virus treated larvae (11.62) was higher than that for the control larvae (8.35), but the 95% confidence intervals overlapped. Both treatments could therefore be described by the same regression equation which is given in Figure 12. It is concluded that an increase in initial density caused a similar increase in dispersal of both healthy and virus treated immature larvae.

Table 9. Dispersal of control mature larvae from different initial densities

		Numbers of larvae			$\sin^{-1}\left(\frac{\text{Migratory}}{\text{static}}\right)^{1/2}$
		Migratory	Static	Total	
	10	34	15	49	56.4
	25	39	32	71	47.8
Initial	50	99	40	139	57.6
density	75	157	35	192	64.7
	100	60	23	83	58.2

Figure 12 The influence of density on the dispersal of immature larvae



g) The effect of virus concentration

Method

Second, third, fourth and fifth instar larvae with mean weights of 1.7×10^{-4}g, 7.4×10^{-4}g, 18.9×10^{-4}g and 54.2×10^{-4}g respectively, were selected. Four treatments of different virus concentrations were used for each larval instar tested. The larvae were kept for 24 hours at 26°C on a layer of semi-synthetic diet in a 300ml tub which had been contaminated with 300µl of the appropriate virus concentration. The four virus concentrations were , 0, 0.5, 1.0, 2.5 and 5.0% which were expressed as percentage dilutions of a stock suspension containing 1.34mg of virus protein per ml. The one day post virus treated larvae were used in dispersal experiments in the usual manner with usually three, but occasionally during larval shortages, only two replicates for each treatment.

Results

The angular transformation of the proportion of immature larvae which migrated for each treatment is given in Table 10. For each instar there was a significantly (chi-squared test) smaller proportion of control larvae which migrated compared with the virus-treated larvae. However, when the controls were excluded from the comparisons the chi-squared values were much reduced and were insignificant for the second and third instar larvae. Significance was obtained for the fifth instar larvae because of an unexpectedly high value at the intermediate virus concentration. It is concluded that virus-treated larvae of all four instars tested migrated more than healthy larvae but small increases in the virus concentration did not influence the tendency to migrate.

Chi-squared comparisons of the different instars at each virus concentration failed to reveal any trends although significant

differences were found for each treatment except the 2.5% virus concentration.

Table 10. The proportion of immature larvae (angular transformation) which migrated after treatment of different instars with a range of virus concentrations

		Virus concentration			
		0	1.0	2.5	5.0
	2	13.4	38.4	46.1	47.7
Instar	3	17.8	33.2	33.2	30.7
	4	25.6	47.7	41.6	53.1
	5	9.4	45.0	37.7	49.2

h) The influence of the size of the island on larval dispersal

Method

Second, third, fourth and fifth instar larvae were used separately in treatments using islands with diameters of 35, 85, 135 or 415mm. Each island had the same uniform depth (4mm or about one gram of diet per $5cm^2$). The experiment was repeated several times using a minimum of three sizes of islands for both control and virus-treated larvae.

Twenty-five larvae per island were used. The larvae were confined to the central 35mm diameter of each island for the first four hours, after which they were free to disperse. Each island was surrounded by a glycerol moat.

Results

A large number of zero returns were obtained for the proportion of larvae caught in the moats for both mature larvae of virus treatments and immature larvae of control treatments. Consequently, only the dispersal of mature larvae in control treatments and the dispersal of immature larvae in virus treatments were analysed. The treatment using virus-treated fifth instar larvae was not included in the analysis for the same reason. Closer inspection of the data revealed that very few of the larvae virus treated during the fifth instar exhibited symptoms of virus infection.

The following model was fitted for each instar by using a multiple regression program in GENSTAT statistical package. Correlation coefficients for the individual lines were not available using this program.

$$\sin^{-1}\left[(\text{proportion migrated})^{1/2}\right] = a + b\log_{10}\left[\text{diameter (mm)}\right]$$

The gradients of the regression equations for control mature larvae were not significantly different for different sizes of moat ($F_{3,58}$ = 0.95). The gradients for all four equations were therefore assumed to be the same with a value of -25 and a standard error of 2.8. It was concluded that the rate of dispersal of mature larvae for all four instars tested was influenced in a similar manner by the size of the island. The proportion of migratory larvae decreased with an increase in the size of the island.

The regression equations were further tested for differences in the intercepts, which were statistically significant ($F_{3,61}$ =12.9, p=0.001). The equations and the mean values of dispersal for each treatment are given in Figure 13. It was concluded that the older the larvae were when established on the island the greater the dispersal of mature larvae.

The regression equations for the dispersal of virus treated immature larvae were compared in the same manner. The gradients were not significantly different ($F_{2,48}$=1.35) with a value of -23 and a standard error of 2.2. The intercepts were significantly different ($F_{2,50}$=41.9, p=0.001) and the equations with the mean values for each

Figure 13 Dispersal of control mature larvae from islands of different diameters

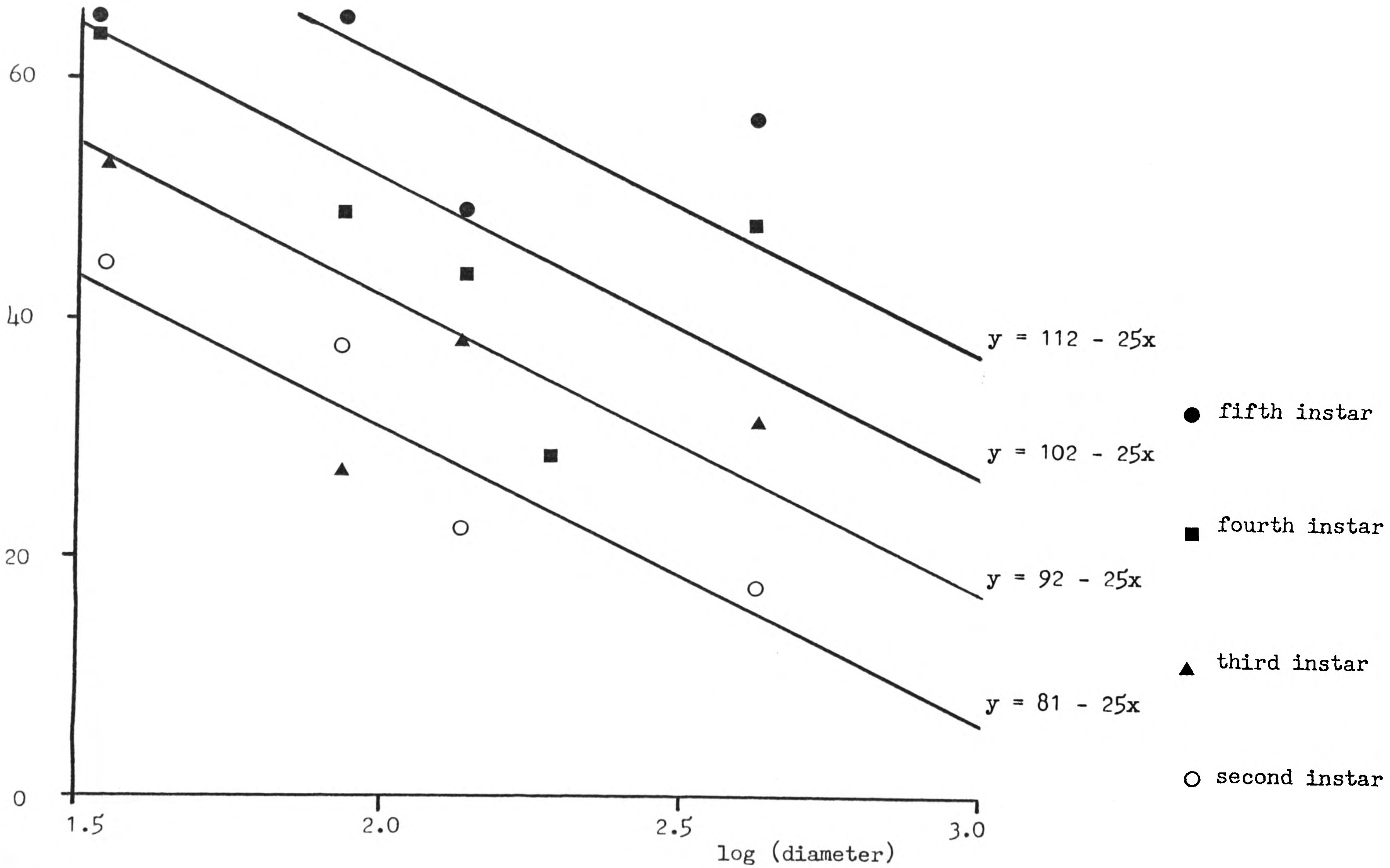
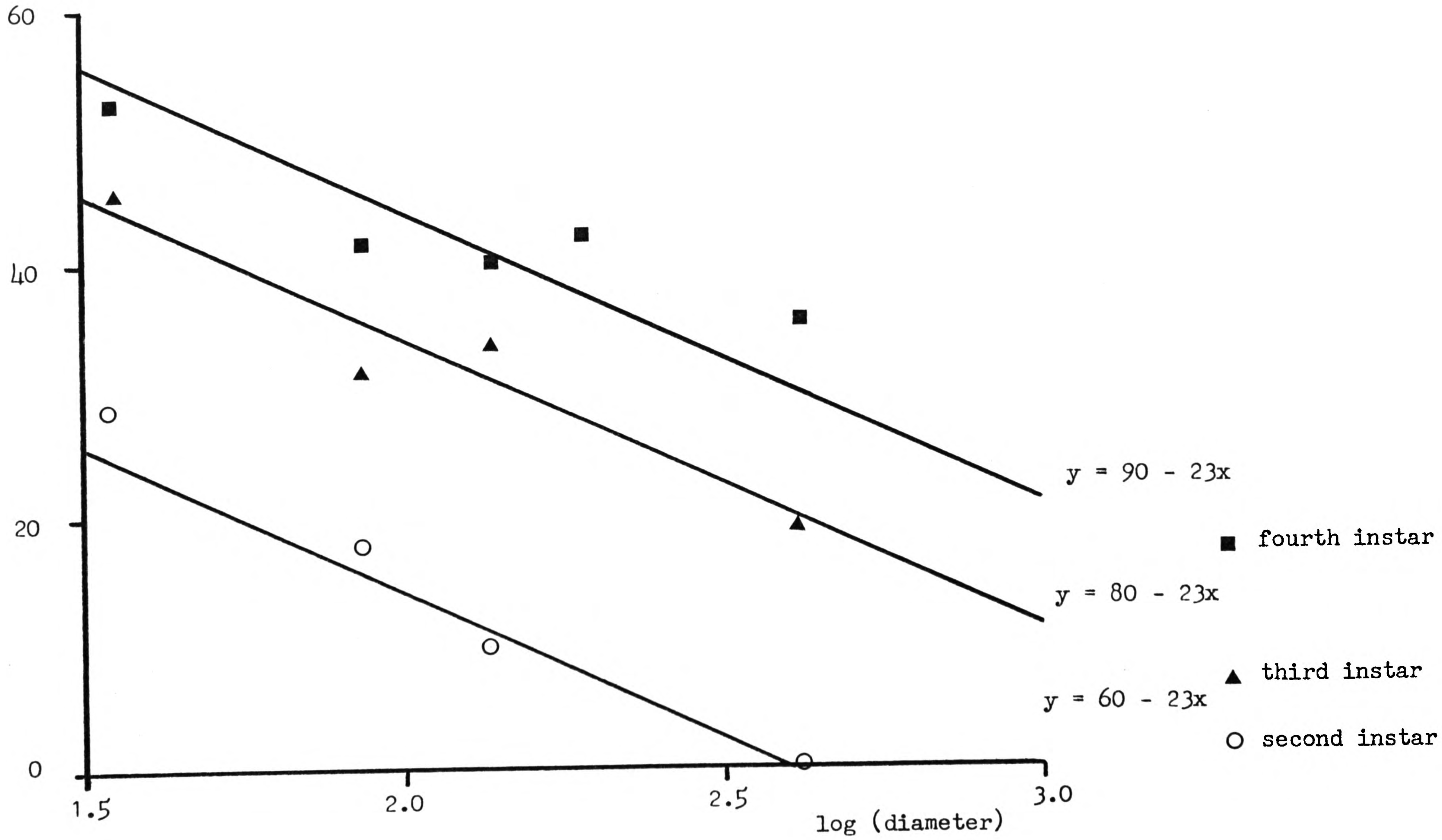


Figure 14 Dispersal of virus-treated immature larvae from islands of different diameters



treatment are given in Figure 14. It was concluded that larval age did not influence the type of migration responses of immature larvae from different sized islands. A smaller proportion of larvae migrated from larger islands and more immature larvae migrated when older virus treated larvae were introduced onto the islands.

2. Studies of dispersal using destructive sampling of experimental arenas

a) Horizontal dispersal

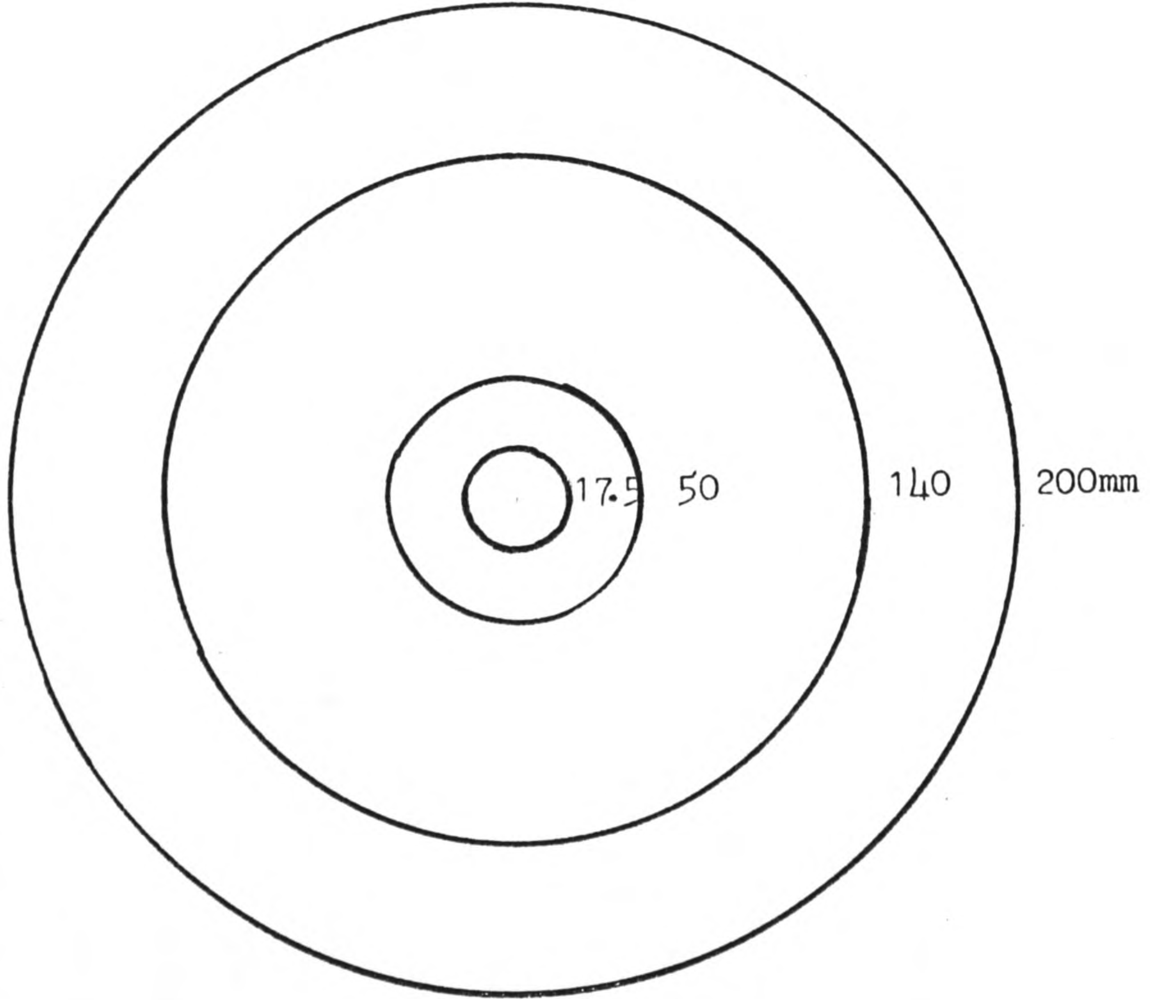
Basic experimental design

Two experiments were performed using six 400mm diameter shallow dishes. Froment diet (250g) was evenly distributed within the dish, which was placed in a large dish containing glycerol so that there was a glycerol moat surrounding the smaller dishes.

Larvae were selected as described for the previous dispersal experiments. The larvae were counted into a 35mm diameter petri-dish containing two grams of Froment diet. The petri-dish lid was removed and the base inverted over the central position of the 400mm diameter dish. After six hours the petri-dish base was removed so that the larvae were able to disperse. Three treatments used control larvae and three used virus-treated larvae. The experiment was carried out at 26° C.

At set times given in the individual methods one of each of the dishes for both treatments was selected. The diet was divided into a four concentric circles as illustrated in Figure 15. The diet from each of four sectors potentially containing control larvae was incubated and the emergence of adults was recorded. The moat was checked for presence of larvae at the time of diet removal. The diet containing virus-treated larvae was incubated for about a week before searching through the diet for the presence of larvae.

Figure 15 The positions of the four sectors used in destructive sampling



I Third instar larvae

Method

Fifty third instar larvae with a mean weight of 5.8×10^{-4}g were used in each treatment. Destructive samples were taken on 4, 7 and 11 days post virus infection.

Results

The numbers of larvae found in each sector are recorded in Table 11. Low numbers of virus treated larvae were found on day four. This error was probably due either to the difficulty in finding the small larvae or as a result of cannibalism. Although many larvae, especially those virus treated, remained in the central sector throughout the duration of the experiment, numbers of larvae dispersing increased with time. No larvae were found in the moats.

The minimum distance of each sector from the centre was used to determine the mean distance of dispersal by larvae in each dish. Regression lines correlating the distance of dispersal with time for both control and virus treated larvae are drawn in Figure 16. It can be seen that the mean dispersal of both treatments was very similar and a paired t-test showed the difference to be insignificant.

No record was made of the instar of the larvae at the time of sampling. However, from previous work it was estimated that the earliest mature larvae would not be expected until day 10 and the peak would be around days 13 and 14.

Table 11. Distribution of larvae within the diet using third instar larvae

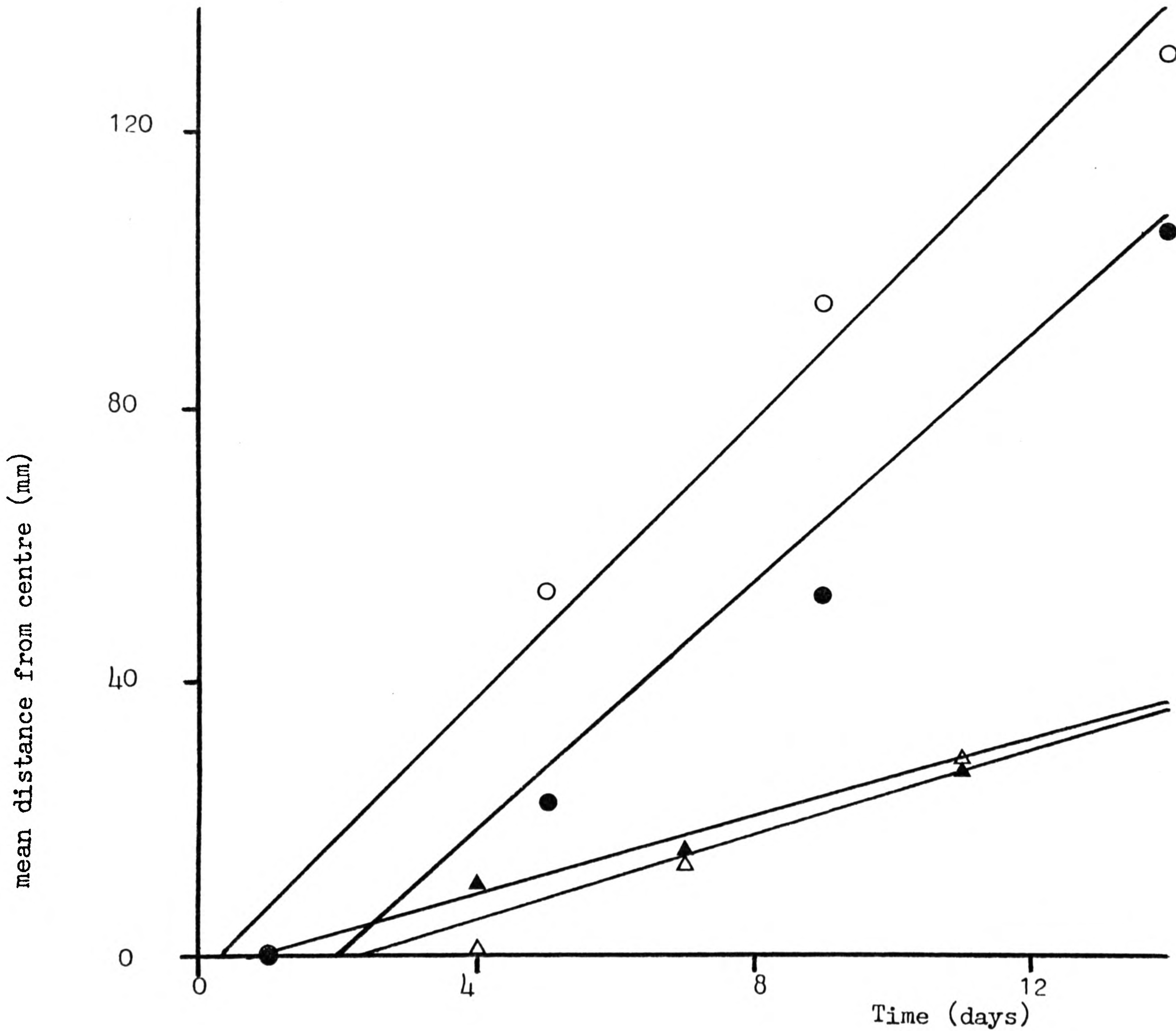
a) Control larvae

Sector	Day "Post Infection"			
	1	4	7	11
1	50	24	21	8
2		24	19	23
3		2	5	14
4		0	1	1
Total		50	46	46

b) Virus treated larvae

Sector	Day "Post Infection"			
	1	4	7	11
1	50	34	25	14
2		3	13	16
3		0	4	12
4		0	1	3
Total		37	43	45

Figure 16 Mean dispersal of larvae from the centre with time



II. Fourth instar larvae

Method

Twenty five fourth instar larvae with a mean weight of 29×10^{-4}g were used in each treatment. Destructive samples were taken on 5, 9 and 14 days post virus infection.

Results

The numbers of larvae found in each sector are recorded in Table 12. Dispersal from the central sector was most rapid for virus-treated larvae although by day 14 most of the control larvae had dispersed from the centre and many were caught in the moats. The appearance of large numbers of larvae in the moats coincided with the expected time for larval maturity. The peak of activity of mature larvae was probably about day 10.

The data were analysed in a similar manner to that described for third instar larvae. The regression equations are drawn in Figure 16. It appears from the graph that dispersal of virus-treated larvae was greater than that of control larvae, however, a paired t-test showed the difference to be insignificant.

Table 12. Distribution of larvae within the diet using fourth instar larvae

a) Control larvae

Sector	Day "Post Infection" 1	5	9	14
1	25	17	10	3
2		1	4	6
3		1	4	1
4		3	6	3
Moat		0	1	11
Total		22	25	24

b) Virus treated larvae

Sector	Day "Post Infection" 1	5	9	14
1	25	12	5	2
2		1	1	2
3		4	4	1
4		8	14	12
Moat		0	1	8
Total		25	25	25

b) Vertical dispersal

Method

Six cardboard cylinders with a 35mm internal diameter and a height of 15cm were loosely packed with 50g of Froment diet to within 12mm of the top. Fifty third instar larvae with a mean weight of 5.8×10^{-4}g were placed on the diet. Three cylinders received control larvae and the others received one day post virus infected larvae. The cylinders were covered with a fine nylon mesh to prevent escape of the larvae and were kept in an upright position at 26° C.

Thirteen days later the cylinders were sawn into four equal sections of 37.5mm height. The diet in each section was examined for the presence of larvae or pupae.

Results

All larvae and pupae from both treatments were located in the top 25mm of diet.

C Transmission of virus by larvae

1. Diet contaminated by individual larvae

Method

Fourth instar larvae with a mean weight of 13.2×10^{-4}g were placed in batches into polypots containing semi-synthetic diet. Half the polypots were surface contaminated with 2.64×10^{-3}mg of virus protein. After 24 hours 10 evenly sized control and virus treated larvae were selected and transferred to individual polypots of fresh semi-synthetic diet. Every subsequent 24 hours until death or pupation, the larvae were transferred to fresh polypots of diet. The used contaminated polypots were labelled and stored at 4°C.

For the purposes of bioassay, polypots were selected which were contaminated by three larvae which died from virus infection 14 days post infection. The polypots contaminated on days 1, 3 and 7 for control larvae and days 1, 2, 3, 5, 7, 9 and 13 for virus-infected larvae were bioassayed with 25 neonate larvae. After six days the bioassay larvae were transferred to individual polypots of fresh diet where they were inspected for death or pupation.

All larvae were kept at 26°C.

Results

Some bioassay larvae died within three days of transfer and did not exhibit virus symptoms. These were assumed to be deaths caused by handling and were not included in the data.

Table 13 records the percentage of larvae which suffered mortality attributed to virus infection. No symptoms of virus infection were observed in any of the bioassay larvae from polypots contaminated by control larvae. A large proportion of larvae suffered infection after feeding on diet contaminated by larvae one day post infection. A lower level of infection was observed in larvae on diet from two and three days post infected larvae after which no more infection was found in the bioassay larvae until they fed on pots which had contained larvae in a late stage of infection. However, at only one day prior to death the level of virus contamination was very low and only resulted in the death of one bioassay larva in the three polypots tested.

Table 13. Contamination of diet by individual larvae expressed as a percentage of virus-infected bioassay larvae

Day post infection	Initial larvae	
	Control	Infected
1	0	44.4
2	-	2.6
3	0	8.8
5	-	0.0
7	0	0.0
9	-	1.4
13	-	1.4

2. Contamination of diet by grouped infected larvae

Method

One hundred third instar larvae with a mean weight of 2.2×10^{-4}g

were placed in each of two 300ml tubs containing a layer of semi-synthetic diet. One tub was surface contaminated with 2 x 10^{-2}mg of GV.

After 24 hours the larvae were transferred to tubs without diet where they were confined for six hours before removal to tubs containing fresh diet. The larvae were subsequently transferred to fresh diet after 24 or 48 hour periods as recorded in Table 14, until 16 days post infection. Any dead larvae were discarded and the labelled tubs were stored at 4°C for bioassay.

Twenty five second instar larvae were counted into each used tub. After 24 hours these bioassay larvae were transferred to fresh tubs containing diet. Twelve days later the larvae were examined for symptoms of virus infection.

All larvae were kept at 26°C.

Results

None of the initial larvae in the control tub exhibited symptoms of virus infection after 16 days. However, in the virus-treated tub, only 52 larvae appeared to be healthy and 13 larvae displayed symptoms of virus infection, 12 of which were dead. Some dead larvae had been removed on previous days but the evidence indicates that some larvae had been cannibalised.

The incidence of virus infection in the bioassay larvae is recorded in Table 14. Some of the larvae were missing which is further evidence of cannibalism. No infection was observed in the control bioassay. Over 90 percent of the bioassay larvae in the original virus contaminated tub were virus infected. A high level of virus contamination occurred in the tub to which the virus-treated larvae were first transferred, but lower levels of virus contamination occurred in the subsequent three tubs as evidenced by infection in the

bioassay larvae. All bioassay larvae that fed on diet contaminated by larvae more than six days after infection suffered mortality.

Table 14. Contamination of diet by larvae at an initial density of 100 per 300ml tub, expressed as the percentage of virus infected bioassay larvae

Day post infection	Initial larvae	
	Control	Virus-treated
0	0	92.9
1.00-1.25	0	62.1
1.25-2.25	0	4.2
2.25-3.25	0	5.3
3.25-4.25	0	22.2
4.25-6.25	0	61.1
6.25-8.25	0	100.0
8.25-10.25	0	100.0
10.25-12.25	0	100.0
12.25-14.25	0	100.0
14.25-16.25	0	100.0

3. Cannibalism of larvae

In the absence of any other food source all larval instars were observed to cannibalise both healthy and virus-infected fifth instar larvae which had been previously killed by freezing. Similarly, healthy fourth instar larvae and fifth instar larvae were observed to cannibalise live larvae in an advanced stage of virus infection when food was absent. All larval instars resorted to cannibalism in the absence of food.

The following experiment was designed to test the role of cannibalism of infected larvae on the passage of virus to healthy larvae in the presence of a food source.

Method

Twenty five third instar larvae were placed in each of 13 polypots containing a layer of semi-synthetic diet and seven polypots containing two grams of Froment diet.

Further larvae were added to the polypots as described below and summarised in Table 15.

Control: To one polypot of each typè of diet no further larvae were introduced.

3 live L_3: Three live three day post-infected third instar larvae with a mean weight of 9 x 10^{-4}g were added to each of three polypots of each of the two types of diet.

3 dead L_3: Three dead (killed by freezing) larvae of the same batch as used in three live L_3 were added to each of the three polypots containing semi-synthetic diet.

3 live L_5: One live heavily infected fifth instar larva (mean weight 151 x 10^{-4}g) was added to each of three polypots of each of the two types of diet.

3 dead L_5: One dead (killed by freezing) larva of the same batch as used in three live L_5 was added to each of three polypots containing semi-synthetic diet.

After four days the larvae on semi-synthetic diet were transferred to individual polypots and were later checked for pupation or virus infection. The emergence of adults from larvae on Froment diet was recorded.

Table 15. The number of replicates for each treatment in the cannibalism experiment

Addition of infected larvae	Type of diet: Semi-synthetic	Froment
None (Control)	1	1
3 live L_3	3	3
3 dead L_3	3	0
3 live L_5	3	3
3 dead L_5	3	0
Total no. of pots	13	7

Results

Table 16 documents the percentage of infection of larvae on semi-synthetic diet after the addition of either infected third or fifth instar larvae to the cultures. No infection was observed in the control, it therefore can be assumed that the source of infection in the treatments was from the added larvae.

The addition of either dead or live heavily infected fifth instar larvae resulted in virus infection in all larvae on semi-synthetic diet. The addition of three day post infection third instar larvae resulted in a less dramatic infection. Despite the lack of virus infection in one replicate, the mean values indicated that virus infection was higher when dead rather than live infected third instar larvae were added.

Table 17 records the adult emergence on both diets after the addition of live infected larvae. There was no statistical difference ($\chi^2_3 = 0.44$) between the proportion of larvae that formed adults in the controls and those treatments which had received infected third instar larvae. Virus infection was observed in the treatments which had received infected third instar larvae but it was concluded that survival was not significantly reduced.

When the emergence of adults from the controls was compared with the treatments that had received infected fifth instar larvae the difference was highly significant ($\chi^2_1 = 31.05$, $p = 0.001$). Most of this significance was attributed to treatments on semi-synthetic diet where no adults emerged. The reduction in the emergence of adults where larvae were reared on Froment diet could be attributed to chance ($\chi^2_1 =$ 1.41). It is concluded that although infection was observed on both diets the addition of a heavily infected fifth instar larva to healthy third instar larvae only caused a reduction in survival when larvae were reared on semi-synthetic diet.

Table 16. Percentage of larval infection after the larval cultures on semi-synthetic diet had been seeded with dead or live infected larvae

			State of added larvae	
			Dead	Live
Stage of infection of added larvae	3 x Early (3dpi L_3)	1	0	13.1
		2	95.7	29.2
		3	28.6	20.9
		Mean	49.1	21.2
	1 x Late (10dpi L_5)	1	100.0	100.0
		2	100.0	100.0
		3	100.0	100.0
		Mean	100.0	100.0
Control			0	

Table 17. Emergence of adults from cultures seeded with infected larvae on different diets

			Type of diet	
			Semi-synthetic	Froment
Stage of infection of added larvae	3 x early (3dpi L_3)	1	20	17
		2	17	19
		3	19	17
		Mean	18.7	17.7
	1 x late (10dpi L_5)	1	0	15
		2	0	20
		3	0	9
		Mean	0	14.7
Control			19	18

4. Effect of density on the emergence of adults in treatments with and without the presence of a dead heavily infected larva

Method

Third instar larvae with a mean weight of 6.4×10^{-4}g were added to polypots containing two grams of Froment diet to give densities of 5, 10, 25, 50 and 75 larvae per pot. A heavily infected fifth instar larva which had been killed by freezing was placed in each of three polypots at each density. A further three polypots of each density were left as controls.

The larvae were kept at 26°C and the number of adults which emerged from each culture was counted.

Results

Table 18 presents the mean values of the three replicates for the emergence of adults for each treatment. A paired t-test was performed to compare the treatments with and without a dead infected larva. The 95 percent confidence interval included zero. It is therefore concluded that the presence of a fifth instar heavily infected dead larva had no influence on the number of adults which emerged. Closer inspection of data, however, showed that larvae exhibiting typical virus symptoms were found in all polypots containing the original infected larva at densities greater than 25 larvae per two grams of diet. Also the diet was exhausted and resulting adults were small at densities of over 25 larvae per two grams of diet.

The proportion of adult emergence expressed as the angular transformation is documented in Table 19. The t-test on Table 18 has already shown there to be no difference between the two treatments, the mean was therefore taken over both treatments for each density. A negative correlation was found between the proportion of adult emergence and initial density, which is given in Table 19.

Table 18. Mean emergence of adults from cultures of different densities reared in two grams of Froment diet including treatments containing a dead heavily infected fifth instar larva

		Initial density of cultures				
		5	10	25	50	75
Treatment	Control	5	9	20	21	16
	Infected	4	9.3	22	22.7	12.0
	Difference (d)	1.0	-0.3	-2.0	-1.7	4.0

$\bar{d} = 0.20 \quad s = 2.44 \quad \frac{st}{\sqrt{5}} = 3.03$

95% confidence interval includes zero

Table 19. Mean emergence of adults expressed as the angular transformation of the proportion for each density including both control and dead infected larva treatments

		Initial density of cultures				
		5	10	25	50	75
Treatment	Control	90.00	71.57	63.43	40.40	27.49
	Infected	63.43	74.66	69.73	42.36	23.58
	Mean	71.56	73.05	66.42	41.38	25.59

$y = 79.05 - 71x$ $\quad$ y = initial density

$r^2 = .97\ 3$ $\quad p = 0.01$ $\quad x = \sin^{-1}\left[(\text{proportion of emerged adults})^{1/2}\right]$

D Transmission of virus by adults

1. Adults emerging from virus-contaminated cultures

Method

Early fifth instar larvae were transferred to 300ml tubs containing semi-synthetic diet with 4×10^{-3}mg of virus spread over its surface. Froment diet was added to tubs after five days. Any adults which emerged were collected and sexed.

Twenty male-female adult pairs were transferred to individual polypots containing a small amount of Froment diet. After the death of the adults they were removed and the progeny reared. A further 10 male-female adult pairs were placed in individual 30ml Universal bottles. Any eggs oviposited were collected and transferred in batches of 30 into polypots containing Froment diet.

The polypots containing progeny were kept at 26°C and examined regularly for symptoms of virus infection in the larvae. Extra diet was provided as necessary.

Results

No infection was observed in any of the larvae reared from the eggs collected and transferred in batches of 30 onto diet. Virus infection was observed in only three (15%) of the polypots when the adults had oviposited directly onto the diet and then only at low levels.

2. Adults with direct virus contamination of abdomens

Method

Ten virgin female adults were immobilized by exposure to cold (-20°C) for several minutes. During the period of induced inactivity a crude virus suspension was repeatedly pipetted over their abdomens for 30 seconds. The crude virus suspension consisted of ten

virus-killed fifth instar larvae triturated in 1ml of deionized water. The females were placed in separate Universal bottles where they were left to recover and dry before pairing with virgin males. Seven virgin male-female pairs which were not exposed to virus served as a control.

Two control and four virus contaminated adult pairs were transferred to clean Universal bottles and eggs were collected as illustrated in Chapter 2, Fig. 1. The eggs were transferred in batches of 30 into polypots containing Froment diet.

The remaining five control and six virus contaminated adult pairs were placed directly into polypots containing a little Froment diet. One week later the adults were removed.

The polypots containing progeny were kept at 26°C and examined regularly for symptoms of virus infection. Extra diet was provided as necessary.

Results

No virus infection was diagnosed in any of the three batches of 30 eggs obtained from the controls. Sixteen batches of 30 eggs were obtained from the four virus-contaminated adult pairs. Virus infected larvae were observed in two of these batches, which both originated from the same adult pair.

No virus infection was diagnosed in the five polypots in which the control females had oviposited directly. While 100 percent mortality of the larvae, attributed to virus infection, occurred in the six polypots in which the virus contaminated females had oviposited directly.

It is concluded that females whose abdomens were virus contaminated transmitted virus infection to their progeny. A comparison of the two treatments suggests that females contaminate the diet and larvae ingest this, rather than directly from the eggs.

However, the two treatments were not directly comparable as the density of the progeny was probably much greater when eggs were oviposited directly in the diet.

3. Source of virus contamination on the adult

Method

Fourth instar larvae with a mean weight of 17 x 10^{-4}g were placed in batches of 15 into 10 polypots. The polypots contained a layer of semi-synthetic diet; six of the polypots had 6.7 x 10^{-4}mg of virus spread over the surface of the diet, the other four polypots were virus-free and provided a control.

After 24 hours the larvae were transferred to individual polypots of Froment diet. The polypots were regularly checked for emergence of adults.

The adults were sexed and transferred to empty polypots in batches of four or five of the same sex, dependent on their availability. After eight days the adults were dead and had left several meconium spots in the polypots. The adults were removed and still in their same batches were triturated with 250μl 0.1% SDS in a tissue grinder. Pupal cases from which the adults had emerged were also triturated. The meconium spots were washed with 250μl of 0.1% SDS per polypot.

The homogenates and meconium washings were used in bioassay. One hundred μl of each suspension were evenly spread over the surface of semi-synthetic diet in individual polypots. Thirty neonate larvae were placed in each polypot. After eight days the bioassay larvae were transferred to individual polypots of semi-synthetic diet. They were examined for symptoms of virus infection or pupation.

All larvae were kept at 26° C.

Results

Eighty-two percent of the initial virus-treated, fourth instar

larvae died of virus infection. There was no mortality attributable to virus in control larvae.

No virus infection symptoms were exhibited by any of the bioassay larvae.

It is thereby concluded that larvae which received a sub-lethal dose of virus in the fourth instar did not carry viable virus into the adult stage.

4. The effects on adults of the following generation of sublethal doses of virus in the larval stage.

Method

Fifth instar larvae with a mean weight of 44×10^{-4}g were placed in batches of 15 in 10 polypots. The polypots contained a layer of semi-synthetic diet. Six of the polypots had 4×10^{-3}mg of virus spread over the surface of the diet, the other four polypots were virus-free and provided a control.

After 48 hours the larvae were transferred to individual polypots of Froment diet. The polypots were regularly checked for emergence of adults. Thirteen adults developed from the 90 larvae that were virus treated. One male adult died within a short time of emergence leaving five males and seven females for the experiment.

The adults used in the experiment were weighed and put in male-female pairs in plastic Universal bottles set up as previously described for egg collection. There were five male-female pairs from control larvae and virus-treated larvae. The extra two females which had received a sublethal virus dose as larvae were paired with male adults from control larvae.

The eggs oviposited were collected, counted and the number of neonate larvae which emerged was recorded. All the larvae from adults which had received a sublethal dose and samples of the control larvae

were transferred to polypots of semi-synthetic diet on the day of hatch. Seven days post hatch the larvae were transferred to individual polypots of semi-synthetic diet, where they were examined at regular intervals for symptoms of virus infection or pupation.

After the death of the adults, the head capsule widths were measured. The females were dissected and any eggs in the ovaries were counted.

Results

Eighty six percent of the virus-treated fifth instar larvae died of virus infection, no control larvae exhibited symptoms of virus infection.

The data obtained from the adults, including the viability of the eggs oviposited are documented in Table 20. The 95 percent confidence intervals for the weights and head capsule widths are also given. The confidence intervals show no difference between the means of the control and sublethally dosed adults. It is concluded that sublethal dosing of the fifth instar larvae had no effect on the weight or size of the resultant adults.

A comparison of the proportion of eggs oviposited for the two treatments was highly significant (χ^2_1= 102.14, p= 0.001). However, when the data for the adults which remained in copulation at death were excluded most of the difference was removed, although it was still significant (χ^2_1= 5.92, p= 0.05). It is therefore concluded that sublethal dosing of the larvae reduced the proportion of eggs oviposited by the adults.

A comparison between the two treatments of the proportion of eggs laid which hatched was significant (χ^2_1= 141.53, p= 0.001). It is therefore concluded that sublethal dosing of the larvae reduced the viability of the eggs oviposited.

Table 21 records the survival of progeny. A comparison between the two treatments of the survival of the larvae during the first seven days post hatch was insignificant (χ^2_1= 2.73). However, when the comparison was made of the survival until pupation, the difference was significant at 95 percent probability (χ^2_1= 9.24, p= 0.05). It is concluded that sublethal dosing of larvae reduced the survival of progeny from hatch to pupation. It is speculated that some larvae suffered virus infection since mortality was not usually recorded until after seven days post infection; however, no symptoms of virus were observed.

No obvious symptoms of virus infection were displayed by any of the progeny. Although the above conclusions have been made, it is important to recognise that there was considerable individual variation within the treatments. Further work needs to be done to substantiate these conclusions. The data from the two mixed adult pairs indicates that the background of the male may be more important that that of the female, but this requires further investigation.

Table 20. Data for adults developed from control and sublethally dosed larvae

		Adult weight ($x10^{-4}$g)		HCW width[a]		Total egg	Eggs	%	Eggs	%
		Female	Male	Female	Male	complement	oviposited	oviposited	hatched	hatched
Control	1	127	70	44.5	43.5	124	122	98.4	72	59.0
	2	126	76	44.5	43.5	194[b]	189	97.4	152	80.4
	3	120	76	44.5	43.5	246[b]	5	2.0	0	0
	4	114	61	43.5	41.5	170	170	100.0	139	81.8
	5	132	74	45.0	43.0	181	37	20.4	0	0
Mean		123±8.6	72.4±5.4	44.4±0.7	43.0±0.7	183.0	104.6	57.1	72.6	69.4
Virus treated	1	131	72	43.5	44.5	170	75	44.1	36	48.0
	2	107	75	43.5	43.0	189[b]	11	5.8	2	18.2
	3	131	73	44.0	43.0	223[b]	0	0	0	0
	4	115	72	43.0	43.0	87	86	98.9	0	0
	5	122	75	45.0	44.0	186	112	60.2	35	31.2
Mean		121.3±8.1	73.4±1.9	43.8±0.6	43.5±0.9	171.0	56.8	33.2	14.6	25.8
Mixed	1	125	79	43.5	44.0	162	161	99.4	155	96.3
	2	118	71	44.0	42.5	219[b]	3	1.4	0	0
Mean		121.5	75.0	43.8	43.2	190.5	82.0	43.0	77.5	94.6

[a] Graticule measurement: 1 division increment ≙ 0.025mm

[b] Adult pair joined in copulation at death

Table 21. Survival of progeny from control and sublethally dosed *P. interpunctella*.

	Number of larvae used	Number of larvae at 7 days	Number of pupae	% survival until 7 days	% survival to pupal stage
Control 1	30	21	19	70.0	63.3
2	125	93	90	74.4	72.0
4	75	63	60	84.0	80.0
Mean	76.7	59.0	56.3	76.9	73.5
Virus treated 1	36	27	23	75.0	63.9
2	2	2	2	100.0	100.0
5	32	18	13	56.2	40.6
Mean	23.3	15.7	12.7	67.1	54.4
Mixed 1	117	84	81	71.8	69.2

5. The effect of a sublethal dose of virus in the larval stage on the sex ratio of surviving adults

Method

One hundred third instar larvae with a mean weight of 7×10^{-4}g were given a virus dose sufficient to cause at least 50% mortality by virus disease. A further 60 larvae which served as a control were treated in the same manner but without any virus. All larvae were individually reared on Froment diet in polypots.

Any adults which emerged were sexed and weighed.

Results

Virus infection caused 58% mortality in the virus-treated larvae. No control larvae exhibited symptoms of virus infection.

The 95% confidence intervals for the weights of adults which developed from control and virus-treated larvae are given in Table 22. The overlap of the confidence intervals obtained using the student t-test signifies that any differences between the treatments can be attributed to chance. It is concluded that a sublethal virus dose in the larval stage does not effect the weight of any surviving adults.

The proportion of males and females which emerged are compared in Table 23 using a chi-squared test. There was no statistically significant difference between the sexes. It is concluded that a sublethal dose in the larval stage does not influence the sex ratio of the surviving adults.

Table 22. 95% confidence intervals of the weights of adults developed from control and sublethally dosed larvae

	Female	Male
Control	105.1 - 115.9	50.6 - 54.4
Virus-treated	101.1 - 114.3	45.6 - 53.4

Table 23. A comparison of the sex ratio of adults developed from control and sublethally dosed larvae

	Number of adults		
	Female	Male	Total
Control	32	26	58
Virus-treated	21	18	39
Total	53	44	97

$\chi_1^2 = 0.02^{NS}$

DISCUSSION

A. Mortality studies

Lethal concentration of virus

When comparing the mortality data for the different instars the criticism could be raised that the larvae fed for different periods on the virus contaminated diet. David *et al.* (1971) compared the mortality of *P.brassicae* L. larvae which fed for either 24 or 48 hours on GV contaminated leaves. Their data revealed that neither the area of leaf consumed nor the period of feeding affected the larval mortality. Resistance to infection within 24 hours increased dramatically, this implies that all the effective dose is ingested in the first 24 hours and subsequent feeding at the same dosage does not influence mortality.

Mardan and Harein (1984) published LC_{50} data on the five larval instars of *P.interpunctella*. In order to compare the data, the LC_{50} values have been converted to the number of virus capsules per gram of diet for their data and per cm^2 for the data presented in this chapter. It is unfortunately impossible to present both sets of data in the same units, as in the present work virus was applied only to the surface layer of diet where it was most likely to be consumed by larvae which also remained on the surface. In contrast larvae burrowed into the diet used by Mardan and Harein. Mardan and Harein (1984) did not include the data on the larval weights, therefore I have used the mean weights of larvae used in this present study for both sets of data, in order to obtain regression equations, which are given below Table 24. The slopes for both sets of data were significantly greater than zero and with 95% confidence were significantly different from each other. This suggests that the two strains of *P.interpunctella* differed in their response to virus dosage. The strain used by Mardan and Harein (1984) was malathion-resistant whilst the strain used in the present studies had

Table 24. A comparison of the LC_{50} data presented by Mardan and Harein (1984) and those presented in this study

instar	Mean initial weight ($x10^{-4}$g)	Data from Mardan and Harein (1984)		Data from this study	
		LC_{50} (caps/g)	Slope	LC_{50} (caps/cm^2)	Slope
1	0.17	1.18×10^3	0.64	9.1×10^2	3.0-8.5
2	1.42	2.19×10^5	1.52	2.3×10^4	1.5-1.9
3	4.17	1.39×10^6	0.75	3.9×10^4	1.4-1.9
4	21.99	5.49×10^7	0.78	1.5×10^6	1.2-1.7
5	35.6	1.65×10^8	0.38	5.2×10^6	1.4

Regression equations	95% Confidence intervals for slope
Mardan and Harein's (1984) data	
$y = 13.6 + 2.18x$	2.16-3.00
$r^2 = 0.998$ $p = 0.001$	
Data presented in this thesis	
$y = 10.4 + 1.58x$	1.08 - 2.08
$r^2 = 0.971$ $p = 0.001$	
$x = \log(LC_{50})$	$y = \log$ (initial weight)

The slopes of the probit lines for each larval instar except the second in Mardan and Harein's (1984) data were very low, indicating a large variation in response by the larvae. This suggests that the larvae were not as homogeneous as those in the present study. The slope values obtained for first instar larvae in the present study were exceptionally high. The slope is an indication of the individual variability in susceptibility and could reflect the high degree of uniformity of neonate larvae. Sheppard and Stairs (1977) similarly found that first instar *Cydia pomonella* (L.) were much less

variable in their response to GV than fifth instar larvae. However, Hughes et al. (1984) argued that in the extreme case of complete homogeneity the slope of the probit model would be only about two therefore slopes in excess of this must result from other factors such as deviation from the assumed log. normal larval response, or virus-host interaction. If Hughes et al's (1984) exponential model assuming independent action of virus is employed, the slope is then an estimate of the proportion of virus effective in initiating infection and steeper slopes could be produced by a multi-hit relationship.

The LC_{50} values for the first instar larvae of the two P.interpunctella strains given by Hunter (1970) and Hunter and Hoffmann (1973) were higher than those presented in either this study or that by Mardan and Harein (1984). Older larvae were more resistant to infection than younger larvae, which is a conclusion supported by other workers with baculoviruses (Allen and Ignoffo, 1969; Evans, 1981, 1983).

The dosage of virus ingested by larvae is dependent on the virus concentration and the quantity of food consumed. Food consumption is related to the age and therefore to the weight of the larvae (Harper, 1973; Tatchell, 1981b). Arguably, a better indication of the response of the larvae to virus would be obtained if the LC_{50} values were multiplied by either the food consumption or weight gain per larval instar to give a relative virus dose. When the logarithm of this relative dose was plotted against the logarithm of the initial weight a steeper curve was obtained than for the logarithm of the LC_{50} alone. This indicates that the differential feeding rates of the larval instars largely compensates for the reduced susceptibility with increased age. However, even when the logarithm of the LC_{50} value per mg of larvae was regressed against the logarithm of the initial weight, the slope (0.55) was not entirely reduced to zero. This

suggests that the larval weight was not entirely responsible for increased resistance. Evans (1983) suggested that the onset of maturation resistance was linked to the hormone levels. Other factors involved may be physical changes in the gut and peritrophic membrane which would reduce the probability of initial infection of the midgut.

Timing of mortality

The median lethal times (LT_{50}) of larvae receiving a virus concentration close to the calculated LC_{50} were similar for the three youngest instars, but longer for the fourth and fifth instars. The LT_{50} for P.brassicae and P.rapae infected with GV increased directly with larval growth (Payne et al., 1981), whilst the LT_{50} for the first four instars of M.brassicae infected with NPV were not significantly different from each other and only the fifth instar had a significantly higher value (Evans, 1981).

The mean larval development stage at mortality and the LT_{50} of larvae infected in the third instar were inversely proportional to the virus concentration. This was in agreement with the work on L.pomonella and its GV (Sheppard and Stairs, 1977), but the direct opposite of that on H.armigera and its GV (Whitlock, 1978).

The LT_{50} of larvae infected in the third instar was similar regardless of the instar in which they eventually died. It is concluded that the period until ecdysis can be prolonged by virus infection. The mean time of larval activity was not extended as the LT_{50} coincides with the median time of pupation of the control larvae. However, the mean time of larval activity was extended for larvae infected in the fourth or fifth instar.

Larvae with virus disease in the fifth instar exhibited a bimodal time-mortality response, which was not displayed by the younger instars. Payne et al. (1981) described a tendency for older Pieris larvae to respond to GV over a longer time period and Sheppard and

Stairs (1977) described a bimodal response for both first and fifth instar (the only two tested) C.pomonella to GV. Vail and Hall (1969b) observed that apparently healthy T.ni larvae formed cocoons only to leave them later and die from NPV disease. They considered that the fifth instar and prepupal stages were crisis points of infection beyond which infected larvae rarely develop. This suggests that the more susceptible larvae die earlier and the less susceptible die at this later crisis point.

The timing of pupation of apparently healthy larvae which had received a sublethal virus dose was not significantly different from that of control larvae. Whitlock (1978) also found that the larval development period of surviving H.armigera was unaffected, however, according to Mardan and Harein (1984) the development of surviving P.interpunctella larvae was prolonged.

Weights of cadavers

The weights of cadavers of different instars were significantly different from each other but there was no significant difference between the weights of larvae which died in the same instar despite variation in the instar infected and the virus concentration used. The larval weight at death was inversely related to the virus concentration and also the timing of death, because of a change in the proportion of the instars. There was no evidence to suggest that infection of older instars at a virus concentration near to their LC_{50} value resulted in heavier cadavers.

The relationship between the time post infection and the weights of cadavers which died in fifth instar showed a variable response. This was correlated to the bimodal response noted in the LT_{50} value. The larvae dying during the second part of the bimodal distribution were of two types; predominant were larvae which had been barely

mobile for several days and showed signs of dehydration and less frequent were larvae which exhibited typical virus infection symptoms very late in their development period.

The weight of larval cadavers can be used to give an indication of the yield of virus. Evans et al. (1981) working with the NPV of M.brassicae found that the variation in polyhedral production per larva was attributed to biomass change alone. Fifth instar P.interpunctella suffering mortality after a period of desiccation had on average a lower weight than those that died earlier. The weight loss was probably attributable to loss of water and energy used in metabolism and it is unlikely that it reflects a reduction in virus production. Excluding the above abnormality, in general the longer the time between infection and mortality at constant temperature, the greater the virus production per larva. Conversely the greater the virus dose per larva the lower the eventual virus production, reflecting the more rapid development of disease.

B. Larval dispersal

The simple experimental design utilizing islands and moats gave no indication of the average distance of dispersal but only the tendency of larvae to leave an area of adequate food supply for an unfavourable area. It was not intended to give realistic values of the levels of dispersal but gave a means of comparing the dispersal behaviour of healthy and infected larvae under the different treatment regimes. Although three replicates were performed for most treatments, because of the low numbers involved, it was necessary to summate the results to validate the statistical comparisons.

The emigration of healthy P.interpunctella larvae can be divided into two distinct phases. The first phase occurred during the immature larval stages and was generally at a very low level. The

second phase occurred in mature larvae, whose rate of emigration far exceeded that of immature larvae. These same phases of emigration are also exhibited by E.cautella larvae (Takahashi, 1955). The enhanced activity of mature larvae probably reflects their searching for pupation sites.

The virus treated larval populations existed as two components; a proportion which was virus infected and the rest which were apparently healthy. Consequently, the emigration of virus treated larvae included the peak of activity exhibited by healthy mature larvae. The dispersal of virus treated immature larvae occurred at a much higher rate than that of healthy immature larvae and after about five days post treatment almost entirely consisted of larvae displaying symptoms of virus disease. The most useful statistic for comparison between healthy and infected larvae was therefore the emigration of immature larvae.

The enhanced activity of virus infected larvae has been previously reported (Lewis, 1970; Evans and Allaway, 1983). What is of particular interest is that virus infected P.interpunctella larvae show this enhanced level of activity from one day post infection up to an advanced stage of infection shortly prior to death. One would expect either an incubation period to elapse until virus infects the secondary tissues before behaviour changes are exhibited or that these changes would be shown only during the initial infection of the midgut perhaps as a result of irritation or a sensation of hunger. However, in Vaughn and Dougherty's (1985) review on the replication of baculoviruses examples exist of secondary viruses being produced within 24 hours of infection and the first signs of virus replication during in vitro studies were detected as early as three hours post infection. It is thereby possible that virus induced changes are occurring in the host haemolymph by one day post infection and an incubation period would have been undetected in these studies.

Larvae at all stages of virus infection can show increased activity, but this activity was not retained. Only a few larvae emigrated on each day post infection irrespective of the proportion which had already emigrated. One possible explanation is that primary infected larvae exhibit activity only during the first few days post infection and more larvae become infected later by secondary contamination of the diet or through direct cannibalism of infected larvae. Although some larvae possibly were infected in this manner, the explanation is not satisfactory as observation revealed that as the experiment progressed most emigrating larvae displayed symptoms of an advanced stage of infection.

The virus dose was not shown to influence the activity of virus infected larvae within the limits used in the treatments. It appears that the stage of infection was of little importance in determining activity.

The relative rate of emigration of both healthy and infected immature larvae increased with an increase in the initial density. The rate of increase was small but the same for both treatments. The initial density was not shown to influence the relative emigration of mature larvae. Takahashi (1955) working with healthy E.cautella larvae reported a simlar correlation with immature larvae. However, using a wider range of densities, he found that the rate of relative emigration of mature E.cautella larvae increased with an increase in density up to an initial density of 10 larvae per g. of diet above which it diminished.

The three types of moats and the two textures of diet tested did not influence the dispersal of immature larvae. The glycerol content of the diet only had an influence at the two extremes; of no glycerol when both healthy and infected larvae rapidly emigrated and of excessive glycerol when fewer infected immature larvae emigrated.

Earlier it was shown that healthy larvae rapidly emigrated from semi-synthetic diet, although they are able to complete their larval development on it. These findings illustrate the need to standardise experimental techniques when investigating insect behaviour.

A more realistic picture of the emigration of larvae from a food island was obtained by introducing an area of diet surrounding the central 35mm core. The larger the diameter of the island, the smaller the proportion of larvae which emigrated. All larval instars used at the initiation of the experiment behaved in a similar manner, however, the older the instar introduced to the island, the greater the emigration of both immature and mature larvae. A possible explanation of why the emigration of mature larvae is influenced by larval instar is that when younger larvae are used, they have more opportunity for dispersal into the surrounding diet and thereby the mean population per unit area would be lower by the time they matured. The greater level of emigration of immature infected larvae as they aged can be partially explained by their increased size and therefore their increased speed of locomotion.

The rate of dispersal of larvae from the central 35mm diameter to the surrounding diet was also obtained by destructive sampling which was compared with the data from moat experiments. The small size and colour of healthy larvae made them very difficult to find in the diet of the samples. In order to maximize the probability of detecting larvae, the samples were incubated until adult emergence. It was therefore impossible to differentiate between the dispersal of immature and mature larvae.

The emigration with time of third and fourth instar larvae from the central 35mm diameter area for the two experimental designs of destructive sampling and moats was compared by means of multiple-contingency analysis. The chi-squared values for the

different tests are given in Table 25. The proportion of larvae which emigrated was affected by the time of sampling and the instar, which is in agreement with earlier work using moats. Similarly, it was confirmed that the combined migration of mature and immature larvae was not influenced by virus infection. However, the experimental design markedly affected the emigration of larvae and this effect differed in magnitude or direction when compared between instars. It is concluded that more larvae dispersed from the central area when surrounded by a continuous layer of diet than when surrounded by a moat. This is probably an underestimate since the level of emigration within a continuous diet system is a balance between emigration and return, the latter being prevented when emigrants are entrapped in a surrounding moat. However, the results from the analysis indicate that the experimental design did not differentially affect the behaviour of healthy and virus treated larvae. These results justify the use of moats to compare the tendency for emigration of healthy and virus infected larvae although caution should be exercised in theirinterpretation.

Both healthy and virus infected larvae remained in the top 15mm depth of diet. Takahashi (1961b) documented that the downward movement of young larvae of E.cautella depended upon their density and occurred only when contact between larvae was high. This result explains why treatment of the surface layers of bulk grain with virus was almost as effective a protectant against P.interpunctella as treatment of the whole grain bulk (McGaughey, 1975).

Table 25. Chi-squared values for comparison of the proportion of larvae which emigrated with time using third and fourth instar healthy and virus-treated larvae and two experimental designs

Effect	d.f.	χ^2	Significance
Instar	1	15.02	0.001
Virus	1	0.31	NS
Time	2	50.76	0.001
Design	1	93.26	0.001
Instar/Virus	1	0.64	NS
Instar/Time	2	0.27	NS
Instar/Design	1	26.07	0.001
Virus/Time	2	0.64	NS
Virus/Design	1	2.76	NS
Time/Design	2	3.84	NS
Instar/Virus/Time	2	2.75	NS
Instar/Virus/Design	1	9.08	0.01
Instar/Time/Design	2	4.03	NS
Virus/Time/Design	2	0.64	NS
Total	21	210.07	

C. Transmission of virus by larvae

Unlike the disease symptoms of BV infection of most lepidopteran larvae, virus killed cadavers of *P.interpunctella* do not rupture and liberate the body contents. Unless the cadaver is ruptured by some mechanical means diet is not normally contaminated by the virus within the cadaver. Cannibalism is therefore potentially the most potent means of natural spread of the disease.

It was postulated that live virus infected larvae contaminate their environment with virus. This could be achieved by virus being present in the silk, faeces or regurgitated gut contents. One day post-infection individual larvae significantly contaminated their environment with virus. This level of contamination decreased on subsequent days and by five days post-infection, none were detected. This source of contamination could have arisen from several routes. It may have been present as an external contaminant on the larvae or liberated after passage through the larval gut, either as part of the original inoculum or been produced by initial infection of the gut itself. Virus which had passed through the gut would be probably at least partially solubilized because of the highly alkaline nature of the lepidopteran gut (Tweeten *et al*. 1981). Further studies are required to determine the source and route of environmental contamination one day post-infection.

Burges and Hurst (1977) working with *Bacillus thuringiensis* infections of *P.interpunctella* concluded that larvae feeding on diseased cadavers and surviving, void bacteria in their faeces for a variable time. The related data strengthen the argument that larvae may void viable virus after a virus contaminated meal.

During the late stages of virus infection, a low level of virus contamination of the environment was detected. It was not known by what route this contamination took place but was most probably virus

that had replicated in the larvae. Although GV is known to have good persistence in the protected food environment of stored product pests (Kinsinger and McGaughey, 1976), the virus detected was unlikely to be part of the primary inoculum as the larvae had by then been transferred through several changes of fresh diet. The virus contamination was at such a low level it was unlikely to be of importance in natural populations.

When a similar experiment was performed to examine the level of environmental contamination with virus by batches of larvae, 100% mortality was obtained in the bioassay larvae after six days post-infection. This high level of contamination was attributed to the cannibalism of infected larvae. During feeding, some of the body contents of the cannibalised larvae would be liberated over the diet. A further source of infection would be the faeces of those larvae which had fed on the infected larvae.

All larval instars were observed to be cannibalistic if no food was supplied. Both healthy and virus infected cadavers were readily consumed in either the absence of food or if the food was semi-synthetic diet. Cannibalism of heavily infected larvae by younger healthy larvae was more common in cultures on semi-synthetic diet than on Froment diet. This difference could be because of a greater encounter frequency on semi-synthetic diet, for unlike the larvae on Froment diet, they remain mainly on the surface and do not build silken feeding tunnels. Burges and Hurst (1977) did not find that larvae cannibalised other live larvae when food was present.

An increase in the density of larvae per gram of Froment diet would decrease the availability of food and is therefore likely to increase the consumption of cadavers by larvae. Although the infected larval cadaver was readily consumed by larvae at higher densities, thereby initiating infection in the cultures, the number of adults

which emerged was not significantly different from the controls. It must be assumed that under these circumstances virus infection did not increase the level of larval mortality over that which normally occurred. In contrast, complete mortality had been the result when a heavily infected fifth instar larva was added to larval cultures on semi-synthetic diet.

It is concluded that unlike M.brassicae larvae (Evans, 1986), P.interpunctella larvae are not attracted to virus killed cadavers except in the absence of favourable diet.

D. Transmission of virus by adults

Adults developing from larvae that received a sublethal virus dose and were then kept individually in virus free diet did not transmit virus to their progeny. Using bioassay, virus was similarly not demonstrated to be present in the empty pupal cases, meconium or adult bodies.

When adults which had emerged from virus contaminated cultures were allowed to oviposit directly onto fresh diet, a low level of virus infection was observed in the progeny of about 15% of the adult pairs. However, when the eggs were collected and transferred to fresh diet at a lower density virus infection was not observed. This suggests that virus transmission results from adult contamination of the diet rather than the eggs. Similarly, when the abdomens of the females were artificially virus contaminated, 100% mortality of progeny was obtained when oviposition was directly into diet, but the incidence of infection was at a lower level and sporadic when the eggs were collected first.

It is concluded the virus transmission to the progeny by adults is as a result of external contamination of the adults with virus which subsequently causes external contamination of the eggs and

of more importance, the larval diet during oviposition. Under natural conditions adults are unlikely to be exposed to high levels of virus, therefore virus transmission would be at best sporadic. However, because of their capacity for flight they could be responsible for introducing virus into new habitats, especially if the old habitat contained high levels of virus contamination. In such cases, the probability of virus infection in the progeny would be fairly high as the diet would be contaminated at oviposition.

Similarly, other moths have been shown to tranmit virus to their progeny after external contamination by virus (Martignoni and Milstead, 1962; Elmore and Howland, 1964; Tatchell, 1981a).

Although virus was not demonstrated to be present in the adults, a sublethal virus dose during larval development had deleterious effects on the adult. The adults were normal in colour, size, weight and sex ratio. The fecundity was not shown to be affected although this may have been in part due to the high variability between individuals. However, the proportion of eggs oviposited, the viability of those eggs and the survival of the resulting progeny were all reduced when compared with those from adults from virus free larvae. A sublethal GV infection of S.nonagrioides larvae was shown by Melamed-Madjar and Raccah (1979) to have similar efects on the adults but sex ratio was found to be male-biased. T.ni adults were unaffected by dosing the larvae (Vail and Hall, 1969) but work with other moths after exposure of the larvae to NPV has shown the fecundity of the surviving adults to be reduced (Klein and Podoler, 1978; Geier and Oswald, 1977; Luttrell et al., 1982). Further work needs to be done with larger numbers to investigate if P.interpunctella adults have a lower fecundity after exposure of the larvae to GV.

CONCLUSION

The variability in response between P.interpunctella larvae to virus concentrations was largely attributed to differences in weight, which was partially compensated by increased food consumption of older larvae. The LT_{50} and the average weight at mortality were inversely related to the virus concentration. The fifth instar exhibited a bimodal response with regard to the timing of death and weight at death, which was partially attributed to less susceptible larvae succumbing to the effects of virus infection later but before a critical stage prior to pupation. The length of some larval development periods was extended by virus infection.

The main source of virus inoculum under natural conditions is the virus infected larvae. The live larvae released very little virus inoculum except immediately after infection, which was assumed to be part of the primary inoculum. However, cannibalism besides providing a direct route of virus transmission, increased contamination of the diet with virus. Cannibalism of infected cadavers and to a lesser extent live infected larvae, was common on unfavoured diets, but infrequent in the normal diet.

Virus infection induced behavioural changes in the larvae which would tend to increase the dispersal of the virus. The rate of dispersal was greater for older instars but was unaffected by virus dose. The texture of the diet did not influence the larval emigration but decreasing food suitability and an increased density increased the emigration of both healthy and virus infected larvae.

Adults, in contrast to larvae, potentially have the capacity of spreading virus over larger distances. Adults which were externally contaminated with virus were able to transmit virus to their progeny, predominantly through contamination of the larval diet. However, virus was not detected in the cast pupal cases, meconium or bodies of

adults which had received sublethal virus doses as larvae. The adults were normal in respect to their appearance, size, weight and sex ratio, but the proportion of eggs oviposited, the viability of those eggs and the survival of the progeny were all reduced. No virus infection was detected in their progeny.

CHAPTER 4 Interaction of Parasitoids Predators and Competitors with *P.interpunctella* and its GV

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INTRODUCTION

P.interpunctella in an infestation of stored products would normally share its habitat with other insects which will interact as parasitoids, predators or competitors. The aim of this chapter is to investigate these interactions with particular reference to the GV of *P.interpunctella*.

Two parasitoids and one predator were investigated, *Nemeritis canescens* Gravehorst (Hymenoptera: Ichneumonidae), *Bracon hebetor* Say (Hymenoptera: Braconidae) and *Xylocoris flavipes* Reuter (Hemiptera: Anthocoridae) respectively. Two competitors, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) are briefly discussed. A further competitor *E.cautella* a closely related pyralid moth is discussed in Chapter 5.

General biology

N.canescens

The biology of *N.canescens* has been described by Ahmad (1936). This parasitoid is as cosmopolitan as its pyralid moth hosts. It breeds parthenogenetically and males are rarely encountered. It is a solitary endoparasite, the eggs are laid in the body of the host larvae, in whose haemocoel the larvae remain until they are ready to pupate.

Although superparasitism can occur, only one wasp will successfully emerge from a parasitised host. Rogers (1972), described how, although wasps will probe parasitised hosts, they can detect an egg within five minutes of previous oviposition and after 30 minutes they will avoid superparasitism in about 70% of cases. The searching wasps tend to leave areas where a large proportion of the host

population is already parasitised (Rogers, 1970). However, avoidance breaks down when the host to parasitoid ratio is high or when parasitoids are deprived of hosts for long periods (Rogers, 1975). If superparasitism does occur madibulate early instar ichneumoids will attack other larvae. Older larvae have a large oxygen requirement but are less susceptible to oxygen shortage than younger larvae, which consequently suffer physiological suppression by anoxia (reviewed by Vinson and Iwantsch, 1980). Damaged larvae and eggs are quickly encapsulated by the host (Rogers, 1970). The handling time of parasitised hosts is significantly less than that required for unparasitised hosts (Cook and Hubbard, 1980).

All larval instars of the host are susceptible to parasitism. Older larvae are more easily located (Takahashi, 1959a) and parasitoid development proceeds faster in them (Takahashi, 1962). However, if the wasp is restricted to breeding on fully-grown larvae, the rate of reproduction gradually decreases and the culture is difficult to maintain over a long series of generations (Takahashi, 1962).

N.canescens explores only the surface of the diet to the depth of its ovipositor (about 0.5cm), and this determines the proportion of hosts found (Takahashi, 1959a). Takahashi (1968) found that parasitised larvae emigrated slightly more frequently than non-parasitised larvae.

Newly emerged N.canescens adults carry few mature eggs but after two to three days feeding on honey solution, they contain about 150 eggs each (Cook, 1977). Feeding on honey or sugar solution considerably increases longevity. Ahmad (1936) showed that without food the wasps lived for only three days at 27°C but when honey was provided adults lived for a mean of 32 days or 21 days in the absence or presence of hosts respectively. Ahmad's (1936) experiments also demonstrated that freshly emerged parasitoids are positively

:liotropic while after feeding they become negatively heliotropic. On ıbsequent starving they are again attracted to light. He suggested that by ıese responses wasps are able to leave a warehouse to feed on nectar from .owers. The subsequent increased longevity of the parasitoid would carry : over any temporary scarcity of suitable hosts.

N.canescens adults are attracted to regions of high host density and :nd to ignore regions with fewer hosts (Rogers, 1970). Waage (1978, 1979) :scribed the locomotory responses of the wasp to the madibular gland :cretion of P.interpunctella. A walking wasp when presented with the host ıemical exhibited a complex orthokinetic response, which involved :opping, walking at a reduced speed and probing with the ovipositor. If ıe wasp left a "patch" of the chemical, it exhibited a klinotactic :sponse which directed it back to the "patch". In this way the time spent ı the "patch" was greatly increased. The waning of this response :termined when a "patch" was abandoned. The waning was retarded by either ıcreasing the concentration of contact chemical or by oviposition. The :currence of oviposition can be determined by observation of the "cocking" :scribed by Rogers (1972).

hebetor

B.hebetor is a gregarious ectoparasite of the larvae of a number of :ored product Lepidoptera (Richards and Thompson, 1932). Its life cycle ıs been described by Benson (1972). The host larva is paralysed by the :ing of the female parasitoid. The eggs are laid on the outside of the ›st on which the emerging larvae feed until mature when they spin cocoons . or near the host remains. Fertilized eggs develop into females and .fertilized eggs develop into males. The life cycle takes between 12 and days at 25°C.

The sex ratio is directly related to the number of eggs laid per female r day. Proportionally more males are produced as the daily egg

production per female increases (Rogers, 1970). When more than about eight eggs are oviposited on a host, scramble competition occurs in the later instars. This intraspecific competition leads to size variation and mortality which affects the sex ratio, a consequence of the greater vulnerability of the females (Benson, 1973).

Benson found that female parasitoids exhibited an interference relationship, which Hagstrum and Smittle (1978) attributed to the females laying fewer eggs on hosts already having eggs. Like _N.canescens_, _B.hebetor_ females are attracted to areas of high host density and tend to ignore other areas (Rogers, 1970). The females attack mainly the older instars; wandering larvae were attacked 10 times more frequently than confined hosts (Hagstrum and Smittle, 1977).

Parasitoids often paralyse hosts without subsequent oviposition (Hagsturm and Smittle, 1977). The venoms of the genus _Bracon_ (reviewed by Vinson and Iwantsch, 1980) cause somatic muscular paralysis but the heart and intestine are largely unaffected. Paralysis is always fatal although life may continue for nearly a month (Richards and Thompson, 1932). It takes about 15 minutes from stinging to the onset of paralysis and some larvae may escape parasitism by crawling away before paralysis (Hagstrum, 1983). Paralysed larvae can remain usable for more than a parasitoid generation and thereby extend host availability (Hagstrum and Smittle, 1977). However, parasitoids prefer freshly paralysed hosts (Hagstrum and Smittle, 1978).

Females only live for six to seven days when starved and they quickly resorb mature eggs. If fed honey, the rate of resorption is slowed but when allowed to feed on host larvae, no eggs are resorbed and longevity is extended to about three weeks (Benson, 1973). The male does not feed. Hagstrum and Smittle (1977) reported that a

female may oviposit a maximum of 30 eggs in one day and is capable of laying 15 eggs per day for up to 40 days.

The long reproductive life of the female, the short generation time and the reliance upon the host as a food for both adults and progeny should make B.hebetor very responsive to manipulation of host availability. Its comparatively high reproductive power and host finding efficiency can cause the host population to fluctuate violently and result in its crash (Takahashi, 1953a).

X.flavipes

The development stages of the predaceous bug X.flavipes have been described by Arbogast et al. (1971). The adults breed sexually and the female scatters her eggs loosely on the food medium. There are five nymphal stages.

The nymphs and adults feed voraciously on a range of stored-product Coleoptera and pylarid moths (Jay et al., 1968; Le Cato and Davies, 1973). Press et al. (1974) suggested that X.flavipes has potential for controlling stored-product Coleoptera but has limited use against the Lepidoptera. The predator reduced populations of Sitotroga cerealella (Olivier) in small quantities of grain by killing the prey before they entered the grain (LeCata and Arbogast, 1979). It is however, apparently unable to attack Sitophilus oryzae (L.) which similarly feed inside grain kernals (Le Cato and Davis, 1973).

The most important factor influencing the number of prey killed per predator was the size of the prey. The predator preferred early instar larvae of larger species and late instar larvae of small species (Le Cato and Davis, 1973). Nevertheless all stages of X.flavipes will feed on eggs (Press et al., 1974), all sizes of larvae (Le Cato and Davis, 1973) and pupae (Le Cato, 1976; Le Cato and Collins, 1976) of stored-product beetles and moths.

Particle size within the food medium influenced the freedom of movement of the bug. It was able to penetrate to any depth of stock peanuts in search of prey (Press *et al*., 1979), but was not able to penetrate deeper than 12cm in wheat flour and over 90% were confined to the top 6cm (Press *et al*., 1978). Le Cato (1975) reported that although *X.flavipes* effectively suppressed populations of *T.confusum* Jacquelin du Val on whole wheat, it had no effect on wheat flour.

X.flavipes exhibits a cannibalistic behaviour which has been examined by Arbogast (1979). The nymphs and adult prey on all nymphal instars but not on eggs. Both nymphs and adults feed saprophagously on dead adults. Prey scarcity probably occurs frequently, especially in the early stages of an infestation, cannibalism would enable *X.flavipes* to survive when prey is scarce since it has little ability to withstand starvation (Arbogast *et al*. 1977).

T.castaneum and S.granarius

Jones and Jones (1974) described the biology of these Coleopteran stored-product pests. *T.castaneum*, a flour beetle occurs in a wide range of stored-product commodities, whilst *S.granarius*, the Grain Weevil attacks all types of grain but not grain products, except hard materials like macaroni.

All stages of flour beetles live loose within the commodity whilst, in contrast, the egg to pupal stage of the Grain Weevil is spent within the grain kernel; the larvae feed on the endosperm. *T.castaneum* is cold susceptible whilst *S.granarius* is cold hardy. The latter cannot establish itself in grain with a moisture content of less than 9.5%.

Neither species can fly and both feign death (thanatosis) when jarred, which is thought to be a defence reaction against predation (Prohammer and Wade, 1981). *T.castaneum* populations are enhanced in

the presence of pylarid moths, because of the former's scavanging on dead moths (Press et al., 1974).

The adults and larvae of flour beetles in high population densities cannibalise eggs and pupae (Wade, 1980). Young adults of T.castaneum emigrate sooner at higher adult densities and in the presence of large larvae (Ziegler, 1978), adults of both sexes will emigrate until no adults remain in the original habitat (Ziegler, 1977).

Interaction in a mixed culture

Press et al. (1982) found that populations of E.cautella were suppressed more effectively by either parasitoid than by X.flavipes. B.hebetor was the most effective parasitoid.

A combination of B.hebetor and X.flavipes was more effective than X. flavipes alone,but less effective than B.hebetor alone, in suppressing P.interpunctella populations. The mean number of B.hebetor progeny was reduced when X.flavipes was present, which was attributed to the latter preying on the former (Press et al., 1974).The numbers of N.canescens were suppressed by the action of B.hebetor, which was because B.hebetor was able to develop on host larvae previously parasitised by N.ca nescens; the reverse was not possible (Press et al., 1977)

X.flavipes reduced populations of T.castaneum even at low predator densities(Press et al., 1975). However, the bug is unlikely to be able to predate on S.granarius since all vulnerable stages are enclosed within grain tunnels (LeCato and Davis, 1973).

Passive carriage of Baculoviruses by predators and parasitoids

The term "passive carriage" was coined by Entwistle (1982) to denote "movement of inoculum by non-host animals which by definition are not themselves infected ". Carriage of virus can be by means of external

contamination of the mouthparts or ovipositor which have pierced an infected prey or host. Similarly virus in the lumen of the gut of a predator or parasitoid that has fed on an infected host can be considered to be physiologically external to the carrier.

N.canescens, B.hebetor, and X.flavipes all pierce P.interpunctella larvae. Some of these pierced larvae, particularly after an attack by N.canescens or X.flavipes may escape without suffering any adverse effect. Female B.hebetor and X.flavipes both feed on their prey. It is therefore conceivable that all or any of these three could carry virus from an infected to a healthy larva.

Transmission via the ovipositor of either of the above parasitoids or the mouthparts of the predator, could result in virus being directly transmitted from the haemocoel of an infected host to that of a healthy host. Infection would have to be initiated by virus particles as undissoluted BV's do not usually cause infection after injection (Entwistle 1982). Alternatively contact with a contaminated parasitoid or diet contaminated by the parasitoid or predator, could result in infection per os.

Carriage by insect predators and especially virus passage through the gut is discussed in Chapter 6.

There are several references to carriage of virus by parasitoids. Many of these involve Apanteles species, an endoparasite which is gregarious during the larval stage. Apanteles species after first ovipositing in infected larvae have been shown capable of transmitting baculovirus infections to healthy larvae of Colias philodice eurytheme Bdul (Thompson and Steinhaus; 1950), Pieris rapae L. (Kelsey; 1962 , Levin et al., 1979), Spodoptera mauritia acronyctoids (Guenée) (Laigo and Tamashiro; 1966) and Lymantria dispar L. (Raimo et al., 1977).

There is a fine balance between the success of the parasitoid and that of the virus within a single host individual. Some tachinid parasitoids seem to be able to survive host death (Bird; 1961), but parasitic Hymenoptera are only able to complete larval development if virus infected larvae do not first suffer mortality. The proportion of parasitoids is increased the longer infection is delayed after parasitism (Kelsey, 1962; Laigo and Tamashiro, 1966; Irabagon and Brooks, 1974; Levin _et al._, 1981). Some parasitoids appear to have developed mechanisms to reduce the intensity of competition with virus: _Apanteles_ species discriminated between healthy and virus infected _P.rapae_ and _L.dispar_ larvae (Kelsey, 1962; Versoi and Yendol, 1982). Some parasitoids oviposit in younger larvae that are less likely to be infected (Thompson and Steinhaus; 1950). _Hyposoter exiguae_ (Vier.) had a reduced development time in infected _Trichoplusia ni_ (Hübner) hosts and the parasitised host larvae were more resistant to NPV infection (Beegle and Oatman; 1974, 1975).

Conversely some BV infections produce toxins which inhibit the development of certain parasitoids (Kaya and Tanada; 1972, 1973).

Parasitoids which developed on virus infected hosts were frequently incapable of transmitting virus (Kelsey, 1962; Laigo and Tamashiro, 1966).
This was attributed to virus being voided from the lumen of the gut soon after emergence and before oviposition (Vail; 1981).

Virus was located in the gut of parasitoid larvae in infected hosts but not in the tissues of _Campoletis sonorensis_ (Cameroon) or _H.exiguae_ (Irabagon and Brooks, 1974; Beegle and Oatman, 1975). Surprisingly Smith and Kurczewski (1980) found NPV in the blood and fat cells of _A.melanoscelus_ (Ratz.) larvae.

EXPERIMENTS

A. *N.canescens*

Preparation of parasitoids

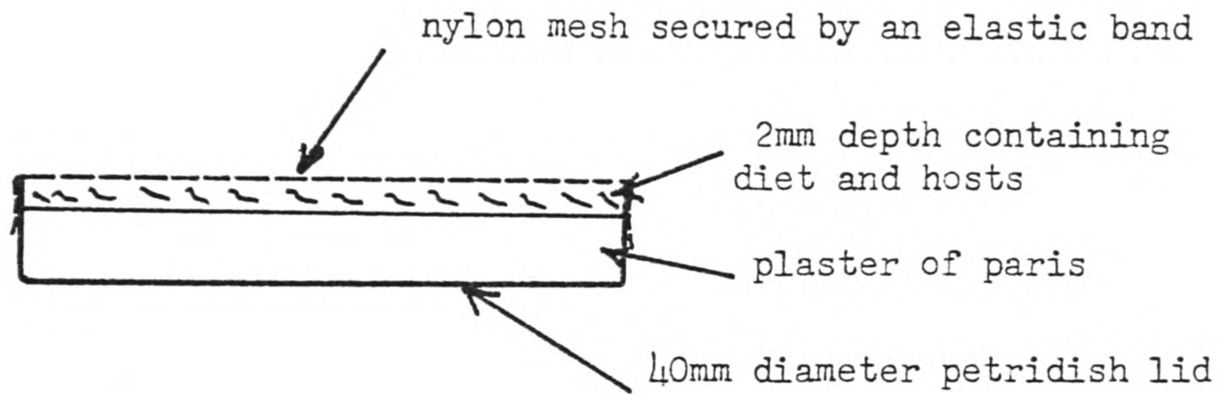
Newly emerged adult parasitoids were transferred to clear plastic containers and fed on honey solution for 48 hours without access to hosts. Immediately before an experiment, parasitoids were offered host medium containing *P.interpunctella* silk. Only those parasitoids which immediately began to probe this diet were chosen for experiments. According to Cook and Hubbard (1980) parasitoids selected in this way should have 140.6 ± 31.7 (s.d.) mature eggs in their ovarioles.

Shallow dishes used in preference studies

The dishes were similar to the arenas described by Cook and Hubbard (1980). Petri-dish lids (40mm diameter) were filled to within 2mm of the lip with plaster of Paris. The required number of evenly sized host larvae were placed in each dish and the remaining volume loosely filled with Froment diet. The opening was covered with industrial nylon, stretched tight and held in place with an elastic band (Figure 1). The hosts were thereby prevented from leaving the dish but parasitoids could easily oviposit through the mesh.

Once prepared, in some experiments, the dishes were left at 26°C for 48 hours to allow the host larvae to silk over the food medium.

Figure 1. A shallow dish used in preference studies



1. Efficiency of parasitism by N.canescens

a) Size of larvae

Method

Three sizes of larvae were used; third, fourth and fifth instar with mean weights of 5×10^{-4}g, 30×10^{-4}g and 35×10^{-4}g. One hundred evenly size larvae were placed in the bottom of a 300ml tub and 25g of Froment diet was poured over them. There were three replicates for each of the three treatments. The larvae were allowed to establish themselves for 24 hours before the addition of the parasitoids. One N.canescens adult was introduced into each tub.

The tubs were incubated at 26° C and the emergence of adults was recorded.

Results

Data on adult emergence of P.interpunctella and N.canescens are

given in Table 1. Replicate three of the treatment using fifth instar larvae gave an exceptionally low parasitism rate and is therefore excluded from the comparisons.

The proportion of host larvae which survived to the adult stage for the three treatments was significantly different (χ^2_2 = 14.42, p =0.001). It is concluded that proportionately fewer host larvae survive to the adult stage when subjected as younger larvae to parasitoids. The number of parasitoid adults from the three treatments did not differ significantly from that expected if all treatments were the same (χ^2_2 = 0.01).

It is therefore concluded that N.canescens is able to successfully parasitise the three sizes of larvae tested with equal efficiency.

Table 1. Adult emergence of host and parasitoid from cultures of different sizes of host larvae subjected to parasitism by N.canescens.

	Host adults			Parasitoid adults		
Initial host instar	L_3	L_4	L_5	L_3	L_4	L_5
Replicate 1	36	50	47	25	30	24
2	20	30	31	38	30	43
3	20	34	(78)	36	39	(3)
Total	76	114	(156)	99	99	(70)

b) Density of larvae

Method

Fourth instar P.interpunctella larvae with a mean weight of 30 x 10^{-4}g were used. Twenty-five, 50, 100, or 150 larvae were placed in 300ml tubs and 25g of Froment diet was added. There were three replicates of each treatment, except that with a density of 150 where only two were used. After 24 hours, one N. canescens adult was introduced into each tub.

The tubs were kept at 26°C and adult emergence was recorded.

Results

Emergence of P.interpunctella and N.canescens adults are recorded in Table 2. The parasitoids were not able to find and parasitise all larvae at any of the densities tested. The proportion of hosts which escaped parasitism and survived to the adult stage varied between treatments but was not correlated to the density.

The number of parasitoids which developed in each replicate when expressed as a proportion of the initial number was found to be negatively correlated to the host density (r^2 = 0.629, p = 0.01). However the number of progeny parasitoids appeared to increase with initial increases in the host density and then reached a maximum above which further host density increases had no effect.

Table 2. Adult emergence of host and parasitoid from different densities of host larvae subjected to parasitism by N.canescens

	Host adults				Parasitoid adults			
Host density	25	50	100	150	25	50	100	150
Replicate 1	7	23	50	48	13	17	30	32
2	9	24	30	38	13	17	30	35
3	5	27	34	-	17	15	39	-
Mean	7.0	24.7	38.0	43.0	14.3	16.3	33.3	33.3
proportion*	32.0	44.6	38.1	32.4	49.2	34.9	35.1	28.2

* proportion expressed as angular transformation

c) Depth of diet

Method

A hundred third instar P. interpunctella larvae with a mean weight of 7.5 x 10^{-4}g were placed in the bottom of each of nine 300ml tubs.

Twenty-five, 50 or 75g of Froment diet were poured into each tub to give a depth of one, two or three centimetres respectively. There were three replicates of each treatment. The larvae were left to establish themselves for 48 hours before the addition of parasitoids. Three parasitoids were introduced into each tub.

The tubs were kept at 26°C and the emergence of moth and parasitoid adults was recorded.

Results

The number of adults which emerged from each treatment is recorded in Table 3. The total emergence for each treatment was similar, however the proportion of host and parasitoid adults varied between the treatments. The emergence of adult P.interpunctella was positively correlated to the logarithm of the depth of the culture, while the reverse was true for N.canescens. The regression equations are given at the bottom of Table 3. It is concluded that the depth of diet affects the efficiency of parasitism by N. canescens, the greater the depth the less the efficiency.

d) Preferences of N. canescens for P. interpunctella larvae of different ages

Method

Ten evenly-sized* P. interpunctella larvae with a little Froment diet were placed in petri-dish lids with a reduced depth and covered with nylon mesh (Figure 1). Early third, late fourth, early fifth and mature fifth instar larvae with mean weights of 7×10^{-4}g, 29×10^{-4}g, 30×10^{-4}g, and 149×10^{-4}g were used.

Four dishes each containing different sized larvae were randomly positioned on the base of a plastic box (260 x 140 x 90mm). Froment diet was evenly distributed so that the diet surface was level with the top of the dishes. There were two replicates. The boxes were left for two days at room temperature before introduction of one

*evenly sized=larvae from eggs laid in the same 48 hour period, the same instar and which appeared to be the same size.

Table 3. Emergence of adults from P.interpunctella cultures in different depths of diet subject to parasitism by N.canescens

Depth of culture	Replicate	Emergence of adults: P.interpunctella	Emergence of adults: N.canescens	Total emergence
1cm	1	33	50	83
	2	31	52	83
	3	45	38	83
	Total	109	140	249
2cm	1	61	22	83
	2	69	16	85
	3	64	13	77
	Total	194	51	245
3cm	1	75	10	85
	2	75	6	81
	3	78	4	82
	Total	228	20	248

Regression equations

$Y_1 = 37.13 + 84.32X$ $\quad Y_1$ = average emergence of P.interpunctella adults

$r^2 = .992 \quad p=0.001$

$Y_2 = 45.60 - 85.42X$ $\quad Y_2$ = average emergence of N.canescens adults

$r^2 = .982 \quad p = 0.001$

$X = \log_{10}$ [depth of culture (cms)]

N.canescens adult into each box. The actions of the parasitoids were observed and recorded for the first 15 minutes after release and subsequently for the first 15 minutes of each hour for six hours.

Results

The total time spent stabbing at each dish and elsewhere by the parasitoids is recorded in Table 4. There was considerable variation between the two parasitoids but general trends do emerge. The parasitoids rarely stabbed in areas where there were no larvae and on average more time was spent stabbing in dishes containing larger larvae, especially mature larvae.

Table 4. Time spent (in minutes) stabbing dishes containing different sizes of larvae by individual N.canescens during 90 minutes observation

Initial size of larvae	Replicate 1	Replicate 2	Mean
none	.33	.00	.16
EL_3	.67	.67	.67
LL_4	.00	1.50	.75
EL_5	.00	5.75	2.88
Mature L_5	9.84	15.75	12.80
Total	10.84	23.59	17.26

e) Preferences of N. canescens for P. interpunctella larvae at different densities

Method

Third instar larvae with a mean weight of 7×10^{-4}g were counted into petri-dishes of reduced depth (Figure 1). The larval densities were 5, 10, 20 and 40 per dish. Four dishes each with a different density of larvae were randomly positioned on the base of a plastic box (260 x 140 x 90mm). There were two replicates and the experiment was conducted as described in the previous experiment.

Results

The total time spent stabbing by each parasitoid during the observation period is recorded in Table 5. Neither parasitoid was observed to probeany area which did not contain larvae. The average times indicate that N.canescens spends more time stabbing in areas of higher larval densities.

Table 5. Time spent (in minutes) by individual N.canescens stabbing dishes containing different densities of larvae during 90 minutes observation

Density of larvae	Replicate 1	Replicate 2	Mean
0	0	0	0
5	0	0.5	0.2
10	0	2.5	1.2
20	1.5	2.0	1.8
40	0	4.0	2.0
Total	1.5	9.0	5.2

2. Interaction with virus infected larvae

a) Preferences of *N. canescens* between healthy and infected host larvae.

I. Dishes of larvae with diet.

Method

Fifty third instar *P. interpunctella* larvae with a mean weight of 10 x 10^{-4}g were placed in eight 40 mm diameter petri-dish lids containing two grams of Froment diet and covered with industrial nylon. Three dishes containing healthy larvae and one containing one day post-infection (dpi) larvae were positioned on the base of a plastic box (260 x 140 x 90mm). There were two replicates. After two days at room temperature one *N. canescens* adult was released into each box. The parasitoids' behaviour was recorded for the first 15 minutes after release and for subsequent 15 minute periods at two, four and six hours after release.

Results.

The total time spent by the parasitoids stabbing dishes containing healthy and infected larvae is compared in Table 6. There was no significant difference between the results. It is concluded that *N. canescens* exhibited no preference in stabbing healthy or three dpi larvae.

Oviposition was deduced to have occurred in the last dish stabbed before cocking behaviour was observed. The number of times oviposition was observed to have occurred in healthy and infected larvae is compared in Table 7. The result was insignificant. It is concluded that *N. canescens* showed no preference for ovipositing in either healthy or three dpi larvae.

Table 6. Time spent (in minutes) by individual N.canescens stabbing dishes containing healthy or infected larvae during 60 minutes observation

	Initial state of larvae		
	Healthy	Infected	Total
Number of dishes	6	2	8
Replicate 1	12.28	4.55	16.83
2	20.27	5.40	25.67
Observed total	32.55	9.95	42.50
Expected total	31.88	10.62	42.50
χ^2	0.04	0.01	0.06^{NS}

Table 7. A comparison between the number of times oviposition was observed to occur in healthy and infected larvae during 60 minutes observation of N.canescens

	Initial state of larvae		
	Healthy	Infected	Total
Number of dishes	6	2	8
Replicate 1	2	2	4
2	8	4	12
Observed total	10	6	16
Expected total	12.00	4.00	16.00
χ^2	0.33	1.00	1.33^{NS}

II. Individually marked larvae

Healthy and 14 dpi infected fifth instar larvae were marked on the dorsal surface of the abdomen with enamel paint so each could be recognised individually. Eight healthy and eight infected larvae were released in a plastic box without any food. There were two replicates.

One *N. canescens* adult was released into each box. Observation of the larvae stabbed commenced on parasitoid release and lasted for a duration of thirty minutes.

Results

Infected larvae were less mobile than healthy larvae and tended to remain on the floor of the box, whilst healthy larvae frequently crawled on the sides and top of the container.

A comparison is made in Table 8 of the number of times healthy and infected larvae received stabs from the parasitoids. The chi-squared value is significant at the 95% level, which suggests that infected larvae are more frequently stabbed than healthy larvae. However some of the larvae, especially infected larvae, were stabbed by the parasitoids on more than one occasion. In Table 9 this is taken into account and a comparison is made between healthy and infected larvae which were stabbed or not stabbed by parasitoids. The chi-squared value was low and therefore the differences in the results could be attributed to chance. It is concluded that although proportionally no more infected larvae were attacked than were healthy larvae, infected larvae more frequently received repeated attacks by *N.canescens*. This difference was attributed to the low mobilty of infected larvae.

Table 8. The number of times healthy and infected larvae were stabbed by individual N.canescens

	State of larvae		
	Healthy	Infected	Total
Replicate 1	4	11	15
2	3	6	9
Observed total	7	17	24
Expected total	12.0	12.0	24.0

$\chi^2_1 = 4.17$ $p = 0.05$

Table 9. A comparison of the number of healthy and infected larvae which received one or more stabs for individual N.canescens

	State of larvae		
	Healthy	Infected	Total
Stabbed	6	9	15
Not stabbed	10	7	17
Total	16	16	32

$\chi^2_1 = 1.13^{NS}$

b) The influence of GV infection of the host larvae on the development of N.canescens.

Method

I. Parasitism before infection.

Individual parasitoids were offered single third instar larvae with a mean weight of 6×10^{-4}g. Larvae which were stabbed by a parasitoid that exhibited cocking behaviour shortly afterwards, showing that oviposition had occurred, were considered to be

parasitised.

Infection of parasitised larvae was achieved by keeping the larvae for one to two days on a semi-synthetic diet to which a lethal virus dose had been applied. Infected larvae were transferred to individual polypots of semi-synthetic diet.

II Infection before parasitism.

Third instar larvae with a mean weight of 6×10^{-4}g were infected by allowing to feed on diet to which a lethal dose of virus had been applied. The larvae were parasitised as described above. The parasitised larvae were transferred to individual polypots of semi-synthetic diet.

III Controls.

Third instar larvae were parasitised but not infected. Third instar larvae were infected but not parasitised.

IV Obtaining the data.

The parasitised and/or infected larvae were kept at 26°C. After 10 days any larvae which did not display visual symptoms of virus infection were excluded from the test. About 23 days after parasitism all the host larvae were dead. They were dissected and examined for the presence of parasitoid larvae, which were measured. Emergence of parasitoid adults or presence of dead pupae was recorded.

Results

All 16 of the infected but not parasitised control larvae died of virus infection and on dissection there was no evidence of parasitism. All but one of the parasitised but not infected control larvae produced parasitoid adults, the exception also suffered mortality and was found to contain a dead *N. canescens* larva.

Many of the parasitoids which developed into pupae failed to form

cocoons and the pupae developed in an exposed state. This condition was not related to the health of the host larvae and was considered to be a consequence of the type of larval diet used. Some of the adults which developed from exposed pupae died before the unfolding of the appendages.

All of the parasitoid larvae dissected from the host larvae were dead and most were melanised. A few host larvae known to have been parasitised were not found to contain parasitoid larvae. It was assumed that death of the parasitoid must have occurred either during the egg stage or during a larval stage with a length of less than 0.5mm.

The stage of development of N. canescens at mortality is recorded in Table 10 when host larvae were virus infected after parasitism and in Table 11 for infection occurred before parasitism. No parasitoids completed development when virus infection was earlier or on the same day as parasitism. On average more parasitoids were able to complete their development the longer the time interval after parasitism before infection of the host. Similarly the earlier the stage of infection relative to the timing of parasitism the younger the parasitoid stage which suffered mortality.

Table 10. Stage of development achieved by N.canescens in larvae which were GV infected after parasitism

Development stage of parasitoid	Not infected	Day of infection after parasitism 0	1	2	3	5	7
Adult	94.4	0	50.0	30.0	25.0	80.0	76.5
Pupa	0	9.1	20.0	20.0	25.0	10.0	5.9
Large larva	} 5.6*	18.2	10.0	10.0	50.0	10.0	17.6
Small larva		54.5	10.0	10.0	0	0	0
Not visible (egg)	0	18.2	10.0	30.0	0	0	0
Actual Total	18	11	10	10	12	10	17

Table 11. Stage of development achieved by N.canescens in larvae which were GV infected before parasitism

Development stage of parasitoid	Not infected	Day of parasitism after infection						
		0	1	2	3	5	7	11
Adult	94.4	0	0	0	0	0	0	0
Pupa	0	9.1	25.0	0	0	0	0	0
Large larva	} 5.6*	18.2	12.5	21.1	5.3	0	0	0
Small larva		54.5	25.0	47.4	26.3	16.7	5.0	0
Not visible (egg)		18.2	37.5	31.6	68.4	83.3	95.0	100.0
Actual Total	18	11	16	19	19	18	20	16

* not measured

Values expressed as a percentage

Large larvae > 31.5mm length > small larvae

3. The role of N. canescens in transmitting P. interpunctella GV

a) After stabbing infected larvae

Method

Early fourth instar larvae were virus infected by allowing to feed on virus contaminated semi-synthetic diet. On successive days fresh parasitoids were offered individual infected larvae then a succession of healthy fourth instar larvae. All larvae which were stabbed were transferred to individual polypots of semi-synthetic diet. They were kept at 26°C and examined regularly for virus infection or emergence of adults.

Results

No symptoms of virus infection were exhibited by any of the larvae which were stabbed by a parasitoid which had previously attacked an infected larva. The emergence of parasitoid progeny is recorded in Table

12. Two host larvae died shortly after attack by the parasitoids, it was assumed that mortality was a direct result of the wounds inflicted by the parasitoids. All other host larvae which were not consumed by parasitoid larvae completed their development.

Several of the N. canescens progeny died before completion of development. This was not apparently related to either the day post-infection of the initial larvae stabbed nor the order of the healthy larvae stabbed. It was therefore assumed that this parasitoid mortality did not signify an inapparent virus infection of the host.

Table 12. The fate of P.interpunctella larvae after stabbing by N.canescens which had previously stabbed larvae at different stages of virus infection

Fate of larvae	dpi of initial larvae							
	1	2	3	4	5	7	9	12
Host pupae	0	3	4	1	1	1	0	0
Host cadaver	0	1	0	0	0	1	0	0
Parasitoid progeny	12	10	10	3	14	13	15	7
No. tested	12	14	14	4	15	15	15	7

b) After artificially contaminating or feeding N. canescens adults with virus

Method

Fifty fourth instar larvae with a mean weight of 11×10^{-4}g were

counted into each of six 40mm petri-dish lids containing two grams of Froment diet. The dishes were covered with industrial nylon. Two dishes were placed in diagonally opposite corners in each of three plastic boxes.

There were three treatments, comprising a virus free control, a 'virus feed' treatment and a contaminated treatment. The honey solution and especially the wick was contaminated with a crude virus suspension (a homogenate of seven 10 dpi fifth instar larvae in one ml of deionized water) for the virus feed treatment. The parasitoids were confined to the area of the wick for one minute before release, during this time four of the five wasps fed from the wick.

The parasitoids for the contaminated treatment were immobilized by placing at -20°C for several minutes. Their ovipositors were swabbed with the crude virus suspension. The cold-anaesthetised contaminated adults showed a rapid recovery and were then released into the appropriate box.

Forty-eight hours after release the parasitoids were removed. The larvae and diet were transferred to polypots and kept at 26°C. The larvae were examined regularly for symptoms of virus infection.

Results

N.canescens were observed stabbing at the dishes in all three treatments. None of the larvae in any of the treatments exhibited symptoms of virus infection.

The emergence of adults is recorded in Table 13. The data are similar for the control and the contaminated treatments but the virus fed parasitoids produced fewer progeny and correspondingly more hosts survived. This difference was attributed to the observed reluctance of the parasitoids to feed on the contaminated honey solution. This would result in fewer mature eggs in their ovaries compared with the parasitoids in the other treatments. Crude homogenate was used as it is more likely to stick to the ovipositors and remain infective. However the presence of host material probably discouraged feeding.

Table 13. Adult emergence from host larvae subjected to parasitism by control, virus contaminated or virus fed N.canescens

Condition of parasitoid		Emergence of adults Host	Parasitoid	Total
Control	1	3	22	25
	2	2	25	27
Contaminated	1	4	21	25
	2	2	22	24
Virus fed	1	7	15	22
	2	14	9	23

c) Simulation of stabbing by contaminated ovipositor

Ovipositors were removed from dead N.canescens adults. The sheaths were removed and attempts were made by holding the ovipositor with fine forceps to penetrate the integument of live third instar host larvae. However, due to their flexibility the ovipositors would not penetrate the larvae.

Fine dissection pins (size A1) of a similar diameter to the ovipositors were used to simulate stabbing by N.canescens. The larvae were stabbed with a pin in one of the abdomenal posterior segments before transfer to individual polypots of semi-synthetic diet. They were incubated at 26° C and examined for early mortality, symptoms of virus infection or pupation. Early mortality was assumed to be a direct result of the wound caused by the pin.

I. Using virus suspensions

Method

Four treatments were used. No host or virus suspension was used on the pin in the control. In the other three treatments the pins

were dipped in one of the following suspensions; homogenised healthy fifth instar larvae, purified virus particles at a concentration of one mg/ml or homogenised nine dpi fifth instar larvae.

Four sizes of larvae were used with mean weights of 3×10^{-4}g, 5×10^{-4}g, 11×10^{-4}g and 23×10^{-4}g. Five larvae of each size were used for each treatment. The pin was dipped in the test suspension between stabbing each larvae. A fresh pin was used for each treatment.

Results

Early mortality occurred in only five larvae, all of these being the smallest size tested. All other larvae either successfully completed their development or exhibited symptoms of virus infection before mortality.

Virus infection occurred in only one treatment and that was when the pin was dipped in a homogenate of infected larvae. In this, virus infection was high (79%) and all four sizes of larvae were susceptible, although the larger larvae were slightly less susceptible (60%).

II. Using infected larvae

Method

There were three treatments for contaminating the pins in between stabbing healthy larvae. The treatments consisted of stabbing several third instar larvae which were healthy, one dpi or twelve dpi. Twenty-five healthy third instar larvae with a mean weight of 7×10^{-4}g were used for each treatment. A further 20 healthy fourth instar larvae with a mean weight of 34×10^{-4}g were used for the treatment using 12 dpi larvae.

Results

None of the larvae tested exhibited symptoms of virus infection

and over 80% developed into apparently healthy pupae.

4. <u>The influence of N.canescens on host dispersal</u>

When host larvae are attacked by N.canescens they typically wriggle violently. The following experiment was to investigate if attack by N.canescens increased the proportion of host larvae which left food islands.

Method

Twenty-five healthy or 25 one dpi third instar larvae with a mean weight of 8 x 10^{-4}g were counted into 35mm diameter petri-dishes containing two grams of Froment diet. The dishes were inverted and after 24 hours the bases were removed to allow the larvae to disperse. Three dishes were placed in each of eight plastic boxes (260 x 140 x 90mm), in half of the boxes the larvae were healthy and in the other half they were virus infected. A supply of honey and water was positioned in each box.

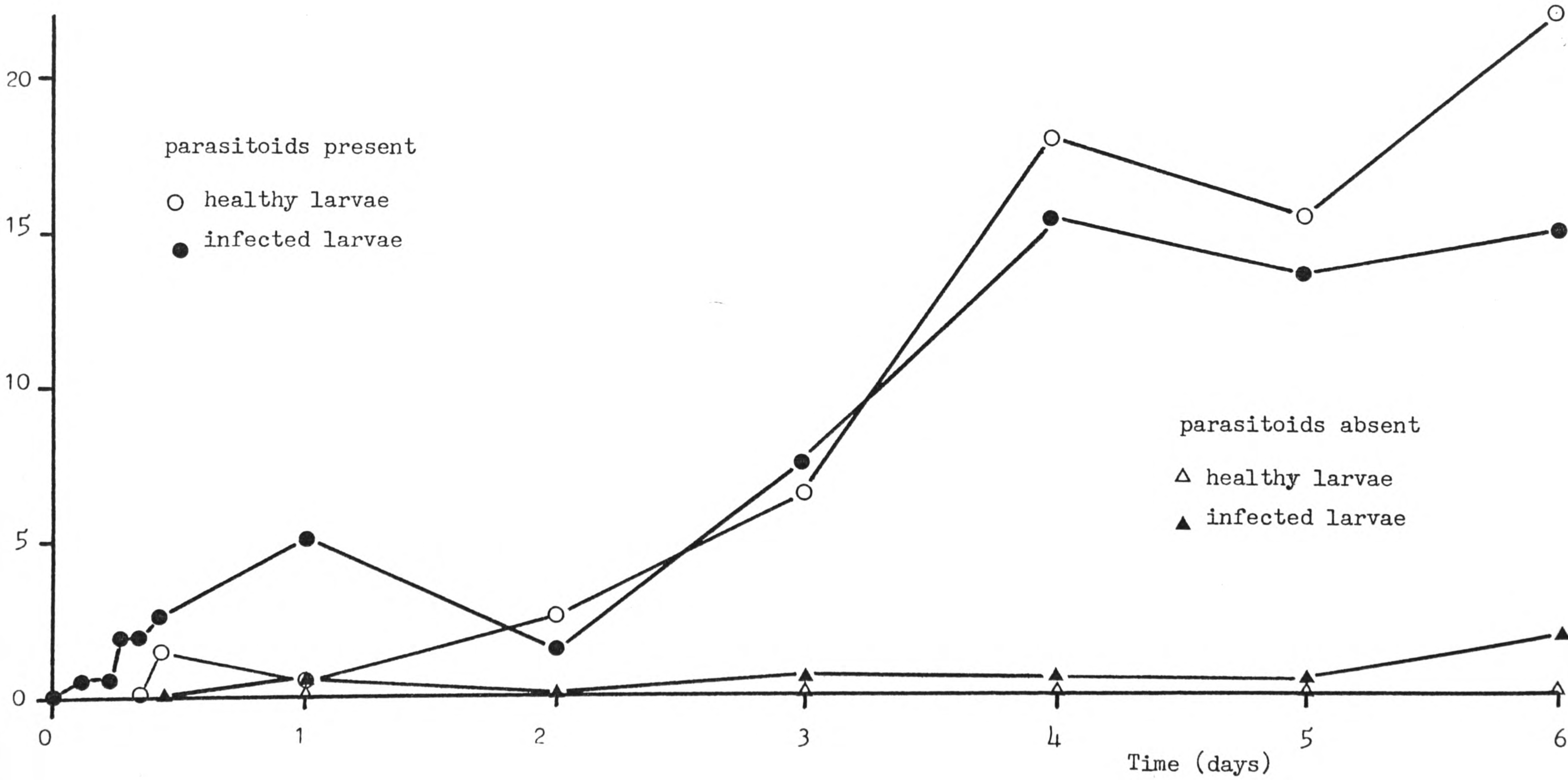
Five N.canescens adults were released in two of the boxes containing healthy larvae and in two containing infected larvae.

The number of P.interpunctella larvae which had migrated from the food islands was recorded at hourly intervals for the first six hours, two hourly intervals for the next four hours and then after every 24 hours from the time of parasitoid release for six days.

Results

The observed migration of larvae is portrayed in Figure 2. Very few infected larvae not subjected to parasitism were observed to migrate and none of the healthy larvae under the same conditions dispersed. In contrast comparatively large numbers of healthy and infected larvae subjected to parasitism dispersed. The data reveal that more infected larvae than healthy larvae dispersed within the first 24 hours after which migration for the two treatments was

Figure 2 Dispersal of P.interpunctella larvae from food islands



similar and reached a plateau about day four. A chi-squared comparison of the proportion of healthy and infected larvae which dispersed on day four when subject to parasitism was insignificant (χ^2_1 = 0.70). After this time close inspection of the dispersed larvae revealed that many had suffered mortality. This was unlikely to be due to starvation as larvae could reenter the food islands. Instead mortality was attributed to parasitoid activity; several of the larvae were observed to be repeatedly attacked.

The ability of non-parasitised larvae to reenter the food islands could also explain the low level of dispersal compared to earlier studies using glycerol moats.

It is concluded that the presence of N.canescens increased the proportion of dispersing host larvae and both healthy and infected host larvae were affected in the same manner.

B. B.hebetor

1. Efficiency of parasitism by B.hebetor

a) Size of larvae

I. Ability to parasitise different sizes of larvae

Method

Twenty-five third instar, early fifth instar or mature fifth instar P.interpunctella larvae with a mean weight of 7 x 10^{-4}g, 52 x 10^{-4}g or 134 x 10^{-4}g respectively were placed in 30ml polypots containing two grams of Froment diet. Earlier studies (page 23) have shown that 2g of Froment diet is sufficient for the development of 20-30 larvae.

One freshly emerged male-female adult pair of B.hebetor was transferred to each of three polypots for each larval size. A further three polypots for each larval size were left as parasitoid-free controls.

The polypots were kept at 26°C and the adult emergence of host and parasitoid was recorded.

Results

The emergence of host adults is recorded in Table 14. The presence of parasitoids caused a reduction in the proportion of host adults emerging from all three sizes of larvae tested. This reduction was most marked for the early fifth instar larvae but even if only the data from the other treatments are used the difference is still significant ($\chi_1^2 = 12.96$, $p = 0.001$).

Table 15 records the number of host deaths which were attributed to the activity of the parasitoids and the emergence of progeny parasitoids for the three treatments. The ratio of these two values was greatest with larger larvae although the largest number of parasitoid progeny was observed with early fifth instar larvae. When the host weight was taken into account, the efficiencies of parasitism were similar for both sizes of fifth instar larvae.

Table 14. Adult emergence of host from different sizes of host larvae in the presence or absence of B.hebetor

	Parasitoid present			Parasitoid absent		
	L_3	early L_5	mature L_5	L_3	early L_5	mature L_5
Replicate 1	3	0	15	21	23	19
2	23	0	11	21	20	19
3	19	0	17	20	22	17
Total	45	0	43	62	65	55

Table 15. A measure of the efficiency of parasitism by B.hebetor on three sizes of P.interpunctella larvae

	L_3	early L_5	mature L_5
No. of hosts killed by parasitoids (H)	17	65	12
No. of parasitoid progeny (P)	6	58	35
P/H	0.4	0.9	2.9
P/H x $\frac{1}{\text{host weight(g)}}$	504	172	218

II. Preferences of B.hebetor adults for P.interpunctella larvae of different sizes

Method

Twenty evenly sized larvae and a small amount of Froment diet were placed in each of the shallow dishes described earlier (Figure 1, page 144). Three sizes of host larvae were used; late third instar, early and mature fifth instar larvae with a mean weight of 16 x 10^{-4}g, 36 x 10^{-4}g and 94 x 10^{-4}g respectively. One dish of each larval size was positioned in a plastic box (260 x 140 x 90mm) and there were three replicates. Five male-female adult pairs of B.hebetor were released in each box.

Three days after release the adult parasitoids were removed. The larvae in the dishes were transferred to polypots containing Froment diet and the emergence of adults was recorded.

Results

The results are presented in Table 16. The adult emergence of the host suggests that all three sizes of larvae were attacked by the parasitoids, although larger larvae were more susceptible. A comparison for the host survival of the three treatments revealed that this difference was significant (χ^2_2= 17.09, p = 0.001). It is

concluded that B. hebetor adults preferentially attacked larger host larvae.

More progeny parasitoids completed development from larger larvae ($\chi^2_2 = 59.32$, p = 0.001). This result reflects one or more of the following: a greater number of larger larvae are attacked; larger larvae are able to support more parasitoid larvae; larger paralysed larvae are more easily found and accepted for oviposition. The first of these has already been shown to be true and at least one of the other two would be needed to account for the big difference in the ratio of parasitoid progeny to dead hosts for each larval size.

Table 16. Preference of B.hebetor adults for host larvae of different sizes

	L_3	early L_5	mature L_5
host adults	11	2	0
hosts dead (H)	49	58	60
progeny adults (P)	5	15	57
P/H	0.1	0.3	1.0
P/H x $\frac{1}{\text{weight of hosts (g)}}$	63.8	71.8	101.1

b) Depth of diet

Method

Fifty fifth instar host larvae with a mean weight of 52×10^{-4}g were placed in each of three 300ml tubs. Twenty-five, 50 or 75g of Froment diet were poured over the larvae, giving a depth of one, two or three cm. Three male-female B.hebetor adult pairs were released on the surface of the diet in each tub.

Larvae were free to move through the diet and according to Takahashi (1961b) should become evenly distributed until the mature larvae move upwards searching for pupation sites. The larvae used were not expected to reach the mature stage until 8 days later by which time adult parasitoid activity had ceased.

The tubs were kept at 26°C and the emergence of adults was

recorded.

Results

The emergence of host and parasitoid adults is presented in Table 17. There was considerable variation between replicates. A comparison of the survival of hosts for each treatment was significant (χ^2_2 = 12.15, p = 0.01), the significance was due to the greater survival of hosts on the deepest diet. It was concluded that the survival of P.interpunctella larvae subjected to attack by B.hebetor was improved in diets deeper than two cm.

However, this difference was not supported by the data on the emergence of parasitoid adults, as there was no significant difference in the data in treatments using one and three cm depths of diet (χ^2_1 = 2.71).

Table 17. Emergence of adults from host cultures in difference depths of diet subject to parasitism by B.hebetor

Depth of culture	Replicate	Emergence of adults	
		Host	Parasitoids
1cm	1	7	43
	2	3	69
	3	46	3
	Total	56	115
2cm	1	10	77
	2	13	51
	3	21	91
	Total	44	219
3cm	1	13	69
	2	42	1
	3	18	83
	Total	73	153

2. Interaction with virus infected larvae

a) Preference of B.hebetor for healthy or infected host larvae

Method

Ten fifth instar P.interpunctella larvae were placed in each of six shallow dishes as described previously (Figure 1, page 144). The larvae were either healthy or seven dpi with mean weights of 156 x 10^{-4}g or 143 x 10^{-4}g, respectively. Two dishes, one of each treatment were positioned in a plastic box (260 x 140 x 90mm) and there were three replicates. Froment diet was spread in the box so that its surface was level with the nylon on the dishes.

Three male-female pairs of B.hebetor adults were released in each box. After 24 hours the parasitoids were removed and the host larvae examined. Paralysed larvae were kept and examined for the development of B.hebetor progeny.

Control samples of five healthy and five virus infected larvae were kept separately and examined for pupation or death.

Results

The control sample of healthy larvae all developed into pupae and those in the infected sample all eventually died with typical virus symptoms. Any deaths or paralysis of larvae when first examined was therefore assumed to be a consequence of the activity of parasitoids.

Four parasitoids were observed on the dishes containing healthy larvae while only one was on those containing infected larvae. The proportion of healthy and infected hosts paralysed is compared in Table 18, the difference was found to be significant. Similarly it was found that 29 progeny parasitoids developed on healthy hosts compared to only four on infected hosts, although a further five parasitoids died as pupae on infected hosts. These results signify that B.hebetor preferentially attacked healthy rather than infected hosts, even though development was possible on both.

Table 18. A comparison of the proportion of healthy and infected host larvae paralysed by B.hebetor

		Condition of host		
		Mobile	Paralysed	Total
Initial condition	healthy	0	30	30
of host	infected	12	18	30
	Total	12	48	60

$\chi^2_1 = 15.00$, $p = 0.001$

b) Development of *B.hebetor* on infected hosts

Method

Fourth instar larvae with a mean weight of 13×10^{-4}g were selected. Half were infected by feeding virus contaminated diet, the rest were kept on virus-free diet and constituted a virus free control.

Ten healthy and 10 infected larvae were transferred to separate polypots without diet on one, three, seven or ten dpi. Three male-female *B.hebetor* pairs were introduced into each of the polypots. After 48 hours the parasitoids were removed. Paralysed host larvae were incubated and inspected for the development of progeny parasitoid pupae and adults. There were three replicates for each treatment.

Results

All host larvae were paralysed when the parasitoids were removed. None of the larvae regained mobility and all suffered mortality, although the heart in some individuals was observed beating up to seven days post paralysis.

The results were similar for all three replicates but because of the small numbers the data were summated. Table 19 records that fewer parasitoid adults developed on the smaller younger hosts than on the larger older hosts presented later in the experiment. Ninety-five percent fewer parasitoids developed on infected rather than on healthy hosts.

Table 20 compares the mortality of parasitoid pupae on healthy and infected hosts. The chi-squared test shows that the difference was significant. It is concluded that the development of *B.hebetor* is less sucessful on virus infected than on healthy host larvae.

The *B.hebetor* adults which developed on virus infected hosts were paired and released in a culture of 50 healthy third instar host

larvae in 0.5g of Froment diet for 24 hours. The parasitoids were shown to be capable of paralysing and parasitising hosts but none of the surviving host larvae exhibited symptoms of virus infection.

Table 19. Emergence of B.hebetor adults from eggs oviposited on healthy and virus-infected hosts

		Days post infection			
		1	3	7	10
host	healthy	1	4	146	176
larvae	infected	0	0	15	1

Table 20. A comparison of the mortality of B.hebetor pupae developing on healthy and virus-infected hosts

		Parasitoid pupae		
		Live	Dead	Total
Hosts	Healthy	327	2	329
	Infected	16	67	83
	Total	343	69	412

$\chi^2_1 = 305.11$, $p = 0.001$

3. Possible transmission of virus by B.hebetor

A series of experiments were performed in which B.hebetor adults were exposed to P.interpunctella larvae at various stages of virus infection before being exposed to healthy host larvae. However, when a host was stung by the parasitoid, the sting invariably proved fatal. Therefore direct virus transmission between hosts by parasitoids is unlikely and was not shown.

An alternative source of transmission is an indirect route, in which the parasitoid contaminates the host's diet with virus and

thereby provides a source of inoculum. The localised nature of the virus contamination would require a high host density for infection to be probable.

The following experiment was designed to test this hypothesis of indirect transmission.

Method

Five "control" male-female B.hebetor adult pairs were exposed to healthy fifth instar host larvae and the five "virus contaminated" pairs were exposed to seven dpi fifth instar host larvae for 24 hours. Two pairs of each treatment of parasitoids were transferred to individual polypots containing semi-synthetic diet. After 24 hours, the parasitoids were removed and the contents of the polypots were bioassayed by use of 30 neonate host larvae per polypot.

The remaining three pairs of parasitoids for each treatment were transferred to two 300ml tubs containing a thin layer of Froment diet and a high density (about 200-300) of second instar host larvae. After 24 hours the parasitoids were removed. More diet was provided as necessary and the larvae were regularly examined for visual symptoms of virus infection.

Results

None of the bioassay larvae on the semi-synthetic diet exhibited symptoms of virus infection.

No virus infected larvae were observed in the tub which had been exposed to "control" parasitoids. A low level of virus infection was observed in the larval population exposed to "virus contaminated" parasitoids. The proportion of infected larvae was not determined, however, an equally large number of larvae developed into adults from both treatments. It is therefore unlikely that indirect transmission of virus by B.hebetor would cause a significant reduction in larval numbers.

C. X.flavipes

1. Efficiency of predation by X.flavipes

a) Size of larvae

Method

Twenty-five third instar, early fifth instar or mature fifth instar prey larvae with a mean weight of 7×10^{-4}g, 52×10^{-4}g or 134×10^{-4}g were placed in a polypot containing two grams of Froment diet. This was repeated until there were six polypots for each of the three larval sizes. Five one-week old X.flavipes adults were released in three of the polypots for each larval size, the other polypots were left as predator-free controls.

The polypots were kept at 26° C and the emergence of P.interpunctella adults was recorded.

Results

The survival of prey as measured by their adult emergence is presented in Table 21. A high mortality of control larvae was observed. This was probably caused by an unexplained stress factor, such as variation in temperature or diet. It was unlikely that there was microbial disease as the cultures were checked periodically for presence of disease organisms. However, since the data were very consistent for the replicates it was still considered valid to analyse the data using a multiple chi-squared contingency test. The effect of the predator was shown to be highly significant ($\chi^2_1 = 30.24$, $p = 0.001$), similarly the prey size affected this survival ($\chi^2_2 = 27.64$, $p = 0.001$). However, when the data for third instar larvae were excluded from the comparisons, neither the influence of the predator ($\chi^2_1 = 1.52$) nor the difference in prey size ($\chi^2_1 = 0.02$) was found to be significant.

It is concluded that X.flavipes adults are effective predators of third instar P.interpunctella larvae but not fifth instar larvae.

Table 21. Survival of P.interpunctella larvae of different sizes the presence or absence of X.flavipes adults

Predator	Host size	Host survival				Host dea
		1	2	3	Total	total
Absent	L_3	21	21	20	62	13
	early L_5	23	20	22	65	10
	mature L_5	19	19	17	55	20
Present	L_3	9	1	7	17	58
	early L_5	15	20	15	50	25
	mature L_5	20	21	20	61	14

b) Depth of diet

Method

Fifty third instar larvae with a mean weight of 7 x 10^{-4}g were placed in each of three 300ml tubs. Twenty-five, 50 or 75g of Froment diet were poured over the larvae to give a depth of one, two or three cm respectively. Ten *X.flavipes* adults were released on the surface of the diet in each tub.

The tubs were kept at 26°C and the emergence of *P.interpunctella* adults was recorded.

Results

The emergence of *P.interpunctella* is recorded in Table 22. Consistently fewer adults emerged from the most shallow diet while there was little difference in adult emergence from the intermediate and deepest diets. A chi-squared comparison of the prey survival on the three depths of diet was highly significant (χ^2_2 = 71.09, p = 0.001), but the difference was mainly attributed to the data for the shallowest diet. It is therefore concluded that *X.flavipes* adults are more effective predators of *P.interpunctella* larvae on shallow diets of one cm depth than on deeper diets.

Table 22. Emergence of P.interpunctella adult cultures in different depths of diet under the predation of X.flavipes adults

		Depth of diet (cm)		
		1	2	3
Replicate	1	20	43	38
	2	24	41	35
	3	14	39	39
	Total	58	123	112

c) Density of predators

Method

Twenty third instar larvae with a mean weight of 6 x 10^{-4}g were counted into polypots containing two grams of Froment diet. There were four treatments with densities of zero, five, ten or fifteen adult *X.flavipes* per polypot. There were three replicates.

The polypots were kept at 26°C and the emergence of *P.interpunctella* adults was recorded.

Results

The summated survival of prey as estimated by the adult emergence is presented in Table 23. A chi-squared comparison revealed that the differences could not be attributed to chance. However, when the control treatment was removed from the comparison, the difference was insignificant (χ^2_2 = 0.40). It is therefore concluded that predation by *X.flavipes* significantly reduced the survival of *P.interpunctella* but varying the density of the predator in this experiment had no further effect.

Table 23. A comparison of the survival of P.interpunctella when subjected to predation by different densities of X.flavipes

		Survival of prey		
		Live	Dead	Total
Density of predator	0	51	24	75
	5	14	61	75
	10	13	62	75
	15	16	59	75
Total		94	206	300

χ^2_3 = 62.78, p = 0.001

2. Interaction of X.flavipes on the efficiency of parasitism by B.hebetor

Method

Fifty mature fifth instar P.interpunctella larvae were transferred to each of eight 300ml tubs. Twenty-five grams of Froment diet were poured into four of the tubs, the other tubs received only a thin sprinkling of diet. Four different treatments were performed in parallel in the four "diet" and "no diet" tubs. The control treatment contained no parasitoids or predators, the parasitoid treatment contained five male-female adult pairs of B.hebetor, the predator treatment contained 10 X.flavipes adults and finally the interaction treatment contained both five pairs of B.hebetor adults and 10 X.flavipes adults.

The treatments were kept at 26°C and the emergence of adults was recorded.

Results

The emergence of adult P.interpunctella is recorded in Table 24. X.flavipes in the absence of B.hebetor caused a significant reduction in the survival of P.interpunctella in both the diet (χ^2_1 = 7.53, p = 0.01) and the "no diet" treatments (χ^2_1 = 25.52, p = 0.001). B.hebetor substantially reduced the survival of hosts in all treatments (p = 0.001). Survival of P.interpunctella was less when there was "no diet", this was found to be significant with 95% confidence when the angular transformations were compared using a paired t-test. This result was attributed to the greater number of successful encounters with prey by both predator and parasitoid when there was "no diet".

Table 25 compares the emergence of B.hebetor adults in the different treatments. This was reduced in the presence of X.flavipes which was more pronounced in the absence of diet (χ^2_1 = 93.46, p = 0.001). However, the data do not reveal if the reduction in B.hebetor

progeny was caused by direct predation on the parasitoid by the predator or by competition for *P.interpunctella* hosts. Live *X.flavipes* nymphs at the end of the experiment were only found in treatments without *B.hebetor*, which suggests that their extinction in the other treatments was due to competition. Observation revealed that *X.flavipes* adults will attack *B.hebetor*-paralysed *P.interpunctella* larvae and *B.hebetor* larvae.

Table 24. Emergence of P.interpunctella from treatments subjected to predation and parasitism

		"Diet"		"No diet"	
		B.hebetor		B.hebetor	
		Present	Absent	Present	Absent
X.flavipes	Present	10	43	0	27
	Absent	3	50	0	48

Table 25. Emergence of B.hebetor adults from treatments comparing the influence of host diet and predators

		Diet		
		Present	Absent	Total
X.flavipes	Present	64	30	94
	Absent	81	165	246
	Total	145	195	340

3. Interaction with virus infected larvae

a) Preference of X.flavipes for healthy or infected P.interpunctella larvae

Method

The procedure was similar to that described for the corresponding preference studies involving B.hebetor (page 169). Ten X.flavipes adults were used in the place of three pairs of B.hebetor adults.

Results

Seven X.flavipes adults were observed on the dishes containing infected larvae whilst only four were on the dishes containing healthy larvae. Significantly more infected larvae, as recorded in Table 26, had suffered mortality than healthy larvae after 24 hours predation. These results suggest that X.flavipes adults preferentially predate on infected rather than healthy larvae.

Table 26. A comparison of the proportion of healthy and infected prey larvae killed by X.flavipes

		Final condition of prey		
		Live	Dead	Total
Initial condition of prey	Healthy	26	4	30
	Infected	18	12	30
	Total	44	16	60

$\chi^2_1 = 5.45$, $p = 0.05$

b) Development of X.flavipes on infected hosts and possible subsequent transmission of virus

Method

X.flavipes adults were kept in polypots with a sprinkling of Froment diet to allow movement on the electrostatic surface. The

predators were fed with heavily infected larvae. The adults were removed after several days and the progeny nymphs were fed on heavily infected larvae.

Three X.flavipes progeny successfully completed their development to the adult stage. These adults were tested to determine if they could transmit host virus to healthy hosts. They were first offered individual healthy third instar larvae, but these were refused. The predators were then placed into individual polypots containing 0.5g of Froment diet and 25 healthy third instar host larvae with a mean weight of 14×10^{-4}g. Controls were set up using healthy X.flavipes adults. After 24 hours the predators were removed. More diet was added as necessary to the polypots, the larvae were examined for symptoms of virus infection and emergence of adults was recorded.

Results

No virus infected larvae were observed in the control polypots, whilst many of the larvae exhibited virus infection symptoms in the polypots which had been exposed to the predators reared on infected larvae. The emergence of P.interpunctella adults from the treatments is given in Table 27. From the control data it was calculated that the exposure to X.flavipes caused a 48% reduction in the population by predation. A further 43% (or 82% of the survivors) of the population suffered mortality as a result of the virus introduced into the environment by the predators.

Table 27. Adult emergence of P.interpunctella after exposure to X.flavipes reared on healthy or virus infected hosts

		X.flavipes	
		Control	"Virus contaminated"
Replicate	1	12	0
	2	13	1
	3	14	6
	Total	39	7

4. The role of X.flavipes as a possible vector of P.interpunctella GV

There are potentially two methods by which X.flavipes can act as a vector of its host's virus. The direct method is injection of virus into a prey individual by means of virus either adhering externally to the predator's mouthparts or being present in the saliva. The mouthparts of the predator would become virus contaminated during a feed on an infected prey. Contamination of the environment with virus is the indirect method envisaged, this could be achieved by virus adhering to the exterior of the predator being brushed onto the prey's food medium or by defecation of viable virus after a feed on a virus-infected prey.

The following experiments were designed to investigate the potential of X.flavipes to transmit virus by these two methods.

a) Direct method of virus transmission

The predators were first fed virus-infected prey after which they were offered healthy prey. It was necessary to observe the predators at this stage, in order to remove any prey after the initial attack and before feeding commenced. This need for careful observation was very time-consuming, many predators refused all prey offered

individually within the time allocated, and very few attacked a succession of prey. Many of the prey suffered immobility within minutes of the initial attack by the predator which frequently led to mortality. The prey which survived a predator encounter were reared individually in polypots of semi-synthetic diet, where they were examined for virus death or pupation.

Two of the experiments undertaken are reported below.

I. Varying the stage of infection of the initial prey

Five *X.flavipes* adults were used for each treatment. The predators were allowed to feed for four days on healthy, four dpi, 11 dpi or virus-killed 20 dpi larvae, according to the treatment. The predators were then transferred individually to clean pots and offered individual healthy third instar or early fourth instar larvae. Any attacked larvae were immediately removed to individual polypots of semi-synthetic diet, which were labelled according to the initial food supply of the predator and the succession of larvae previously attacked.

Results

Most of the predators refused all larvae offered. None of the predators which had previously fed on healthy larvae could be induced to attack the larvae offered and only one predator from each of the three treatments cooperated.

The fate of the larvae which were attacked by the predators is recorded in Table 28. The small number of larvae involved, especially the low number which survived the initial consequences of the predator attack prevents the presentation of general conclusions. However, one larva exhibited typical virus infection symptoms and suffered mortality in the fifth instar. Closer inspection of the data reveals that it was the first larva attacked by that particular predator in the test, the second larva attacked did not exhibit virus symptoms and

developed into a pupa. The first larva to be attacked by both of the other predators in the test, died as a direct result of the attack.

Table 28. Fate of prey larvae after attack by predators which had previously fed on larvae at different stages of infection

		Fate of prey			
		Death attributed to:		Healthy:	Total number
		Predator	Virus	(Pupae)	of prey
Initial feed	4 dpi	5	0	2	7
of predators	11 dpi	1	0	1	2
	20 dpi	8	1	2	11

II. Varying the length of feeding on infected prey by the predators

Ten *X.flavipes* adults were used for each treatment. In the control treatment, the predators were fed on virus-free fifth instar larvae which had been killed by freezing. Virus-killed larvae were used in the other two treatments on which the predators were allowed to feed for either one or eight days, dependent on the treatment.

The predators were starved for three hours and then after transfer to individual clean containers were offered a succession of individual third or fourth instar larvae with a mean weight of 17×10^{-4}g. After stabbing by the predator, the prey were immediately removed and reared as previously described.

Results

Seven of the control and six of each of the "virus-fed" predators attacked at least one of the prey offered. Three larvae which were the first prey attacked by predators after their virus feed, survived this encounter. However, none of the prey exhibited symptoms of virus infection. The fate of the prey is recorded in Table 29.

Table 29. Fate of prey larvae after attack by predators which had previously fed on infected larvae for different lengths of time

		Fate of prey			
		Death attributed to:		Healthy:	Total No.
		predator	Virus	(pupae)	of prey
Initial feed of predators	Healthy	6	0	5	11
	Infected, 1 day	7	0	4	11
	Infected, 8 days	8	0	3	11

b) Indirect method of virus transmission

The predators were allowed to feed on virus-infected prey before release onto larval diet. The predators were removed after 24 hours and larvae were reared on the diet. The larvae were later examined for symptoms of virus infection, the only source being from the predators. However, one source of variation was that some predators oviposited in the larval diet and the subsequent progeny nymphs predated on the bioassay larvae.

The following experiments, although scanty in their data, provide

evidence to support the hypothesis that *X.flavipes* can transmit virus to their prey by an indirect method.

I. Exposure of predators to diet containing larvae

Five *X.flavipes* adults were used for each treatment. They were allowed to feed for four days on healthy, four dpi, 11 dpi or virus-killed 20 dpi larvae. The predators were then transferred, still in their groups of five to polypots containing 25 healthy third instar *P.interpunctella* larvae in a little Froment diet. The predators were removed after 24 hours. More larval diet was provided as necessary. The larvae were reared and examined for virus infection or emergence as adults. The "virus-fed" treatments contained two replicates.

Results

No virus infection was observed in the control larvae. Virus infected larvae were present in all the treatments which involved the use of "virus-fed" predators. It was, however, impossible to count the number of dead larvae in Froment diet. The number of adults which emerged from each treatment is recorded in Table 30. The data show that survival to the adult stage was, on average, 73% less in the virus treatments than in the control.

Table 30. Emergence of adult P.interpunctella after exposure as larvae to X.flavipes fed on larvae at various stages of infection

		Replicates		
		1	2	Mean
State of larvae of initial feed	healthy	14	-	14
	4 dpi	8	5	6.5
	11 dpi	3	3	3
	20 dpi	5	5	5

II. Bioassay of two types of larval diet exposed to "virus-fed" predators

Method

X.flavipes adults were allowed to feed for 24 hours on heavily infected P.interpunctella larvae. Five predators were transferred to individual polypots containing a layer of semi-synthetic diet and a further five were transferred in individual polypots containing 0.5g of Froment diet. After 24 hours the predators were removed and 30 neonate P.interpunctella larvae were counted into each polypot. When the larvae were large enough to handle, those on semi-synthetic diet were transferred to individual polypots of the same type of diet. Those in Froment diet were kept in the original polypots and more diet was provided as necessary. The larvae were reared and examined for virus infection.

Results

No evidence of virus infection was observed in the larvae reared on Froment diet. Table 31 records the proportion of larvae reared on semi-synthetic diet which were diagnosed as virus infected, which on average was 68%.

Table 31. The proportion of virus infected larvae reared on semi-synthetic diet exposed to "virus-fed" X.flavipes

Replicate number	% infected
1	93.7
2	88.9
3	0.0
4	100.0
5	57.1
Mean	67.9

P.interpunctella GV contamination on X.flavipes

Method

X.flavipes adults were allowed to feed for 24 hours on either healthy frozen-killed or virus-killed fifth instar P.interpunctella. After their feed, the predators were frozen to kill and tested to determine if virus contamintaion occurred either externally or internally.

Twenty-five X.flavipes adults with a combined weight of 125 x 10^{-4}g were used for each treatment. The predators were washed to remove any external contaminants and the washings were bioassayed to determine if virus was present. The predators were then homogenised to release any internal contaminants, the homogenate was similarly bioassayed.

The detailed procedure is given below.

1. Wash batch of 25 predators in 0.5ml of 0.1% SDS on a whirlimix for one minute.
2. Keep washings.
3. Rinse predators in three changes of 0.5ml of 0.1% SDS.
4. Homogenise in 0.5ml of 0.1% SDS using one ml tissue grinder.
5. Keep homogenate.
6. Spread 100ml of suspension (either washings or homogenate) evenly over surface area of semi-synthetic diet in polypot. Three replicates.
7. Allow excess moisture to evaporate.

*8a. Count 30 neonate P.interpunctella larvae into each polypot.

9a. Transfer larvae to individual polypots of semi-synthetic diet after 10 days or when large enough to handle.

*8b. Count 22 third instar larvae with a mean weight of 4 x 10^{-4}g into each polypot.

9b. Transfer larvae to individual polypots of semi-synthetic diet

after three days.

10. Incubate at 26°C and examine for virus death or pupation.

* Neonate larvae were initially used in the bioassay but because of the high mortality encountered, the bioassay was repeated using third instar larvae.

Results

No symptoms of virus infection were observed in either the neonate or third instar larvae which had been used to bioassay the washings and homogenate from control predators. Apart from a small number of larvae which suffered mortality which was attributed to handling, all larvae used in the bioassay of both the washings and homogenate of "virus-fed" predators suffered mortality with typical virus infection symptoms. It is therefore concluded that "virus-fed" *X.flavipes* adults carry viable *P.interpunctella* GV both externally and internally.

D. Carriage of *P.interpunctella* GV by *T.castaneum* and *S.granarius* adults

Method

Twenty-five beetle adults were placed in polypots containing 0.5g of Froment diet and 10 healthy or eight dpi fifth instar *P.interpunctella* larvae. There were six replicates for both types of beetles using both control and infected larvae. After two days the beetles were transferred to fresh polypots containing 0.5g of Froment diet or a layer of semi-synthetic diet. There were three replicates for each treatment.

The beetles were removed from the diet after 24 hours and the diet was bioassayed by use of 25 neonate *P.interpunctella* larvae per polypot. The larvae on semi-synthetic diet were transferred to

individual polypots of this diet after 10 days. The larvae on Froment diet were kept together and more diet was supplied as necessary. The incidence of infection and the emergence of adults was recorded.

Results

None of the bioassay larvae on the "virus contaminated" semi-synthetic diet survived to develop into adults. All suffered mortality as larvae, showing symptoms of virus infection. All the bioassay larvae from the weevil "virus contaminated" semi-synthetic diet suffered mortality in the first instar. Similarly 85% of the bioassay larvae on the parallel T.castaneum treatment suffered mortality during the later larval instars. None of the bioassay larvae from the control polypots of semi-synthetic diet for either of the beetle treatments exhibited symptoms of virus infection and in both treatments about 70% of the larvae produced adults.

The emergence of adults from the treatments on Froment diet is recorded in Table 32. No adults emerged from the weevil "virus contaminated" diet and a few emerged from only one of the replicates for the T.castaneum "virus contaminated" diet. The average emergence of adults from the control treatment was similar for both beetle treatments on which about 57% of the bioassay larvae developed into adults. No evidence of virus infection was observed in the control treatments.

It is concluded that both T.castaneum and S.granarius adults are able to carry significant quantities of virus from a virus infected P.interpunctella culture to previously uncontaminated diet. The evidence presented suggests that S.granarius is more effective than T.castaneum in transporting virus.

Table 32. The emergence of adults from bioassay larvae on T.castaneum and S.granarius conditioned diet

Treatment	Replicate 1	2	3	Mean
T.castaneum control	14	17	11	14.0
"virus contaminated"	0	4	0	1.3
S.granarius control	15	14	14	14.3
"virus contaminated"	0	0	0	0.0

P.interpunctella GV contamination on T.castaneum and S.granarius

Method

Twenty-five adult beetles were placed in polypots containing 0.5g of Froment diet and 10 healthy or 8 dpi fifth instar P.interpunctella larvae for two days. The beetles were then carefully removed from the diet and frozen. The two species of beetles for both treatments were washed and homogenised in batches of 25 as described for X.flavipes.

The washings and homogenates were bioassayed using neonate and third instar P.interpunctella larvae, as described for X.flavipes. There were three replicates.

Results

No symptoms of virus infection were observed in either the neonate or third instar larvae which had been used to bioassay the washings and homogenates from control beetles. Apart from a small number of larvae whose mortality was attributed to handling, all neonate larvae used in the bioassay of the washings and homogenates of "virus contaminated" beetles suffered mortality with typical virus infection symptoms.

Similarly all the third instar larvae used in the bioassay of washings and homogenates of "virus contaminated" T.castaneum suffered mortality. The number of pupae obtained from the bioassay of

S.granarius washings and homogenates using third instar larvae is recorded in Table 33.

Although all the larvae which had been used to bioassay the washings of "virus contaminated" weevils suffered mortality, a few (22%) which were used to bioassay the homogenates of the same, survived to form pupae.

It is therefore concluded that both T.castaneum and S.granarius adults can carry P.interpunctella GV both internally and externally.

Table 33. The number of third instar larvae which survived to form pupae in the bioassay of S.granarius "washings" and homogenates

	Number of pupae				Number of handling deaths	Absolute Total
	Replicate			Total		
	1	2	3			
Control						
"washings"	22	21	20	63	3	66
"homogenate"	22	22	16	60	6	66
Virus contaminated						
"washings"	0	0	0	0	7	66
"homogenate"	5	4	5	14	2	66

DISCUSSION

N.canescens

N.canescens was able to successfully parasitise all three sizes of larvae tested with equal efficiency. However, when presented with a choice, the parasitoids preferentially spent longer probing dishes with larger larvae. Takahashi (1957b) documented that N.canescens can parasitise all larval sizes of E.cautella but larger larvae are more easily encountered (Takahashi, 1957a). However, the ease of search alone is unlikely to account for the difference in the preference data, especially as a similar proportion of all three sizes of larvae were successfully parasitised when there was no choice. Larger larvae produce more silk and therefore probably more mandidular gland secretion than smaller larvae. Waage (1978) has decribed how N.canescens spends more time on "patches" with a higher concentration of host-produced chemical. The concentration of chemical is related to the size and density of host larvae which explains why parasitoids spent longer on dishes containing larger larvae or a greater density of larvae.

The number of progeny per parasitoid rises with the increase in the number of host larvae up to a certain point, after which there is a levelling off at about 33 progeny per female. Ahmad (1936) recorded a similar plateau of 30 progeny per female even at initial densities of 300 host larvae.

The depth of diet contributed significantly to the efficiency of parasitism. When the food was deep, the parasite efficiency became small. There is a hyperbolic relation between the number of progeny parasitoids and depth of diet. Takahashi (1959a) obtained a similar relationship using N.canescens and E.cautella. Observation revealed that N.canescens do not burrow into the diet, but walk on the surface probing with the ovipositor. The maximum depth the parasite can reach in this way is about 0.5cm. However, the host population is unlikely to be

evenly distributed throughout the diet as earlier work has shown that P.interpunctella tend to accumulate in the surface layers.

Unlike Apanteles species (Kelsey, 1962; Versoi and Yendol, 1982), N.canescens did not appear to distinguish between healthy and infected larvae. Heavily infected larvae received more multiple attacks than healthy larvae, but this was attributed to their lack of mobility which made a parasitoid encounter more likely. Despite this lack of discrimination by the parasitoid, the parasitoid progeny were unable to complete development in a host that was already virus-infected. The parasitoid development was able to proceed further when the time between parasitism and infection was increased. This type of competition between Hymenopteran parasites and viruses for hosts has been documented by other workers (Kelsey, 1962; Laigo and Tamashiro, 1966; Irabagon and Brooks, 1974; Levin et al., 1981).

N.canescens larvae mature about 12-14 days after oviposition at 25° C (Rogers, 1970), whilst the average time until mortality of third instar P.interpunctella larvae after GV infection was about 13 days at 26° C. As Hymenopteran endoparasitic larvae are unable to survive the death of their host (Entwistle, 1982), this explains at least in part why N.canescens progeny do not survive in hosts virus-infected prior to parasitism. However, the parasitoid progeny should have had time to complete their development in hosts which were infected at the same time or especially after parasitism. The considerable mortality of parasitoid progeny even when virus infection was as late as three days post parasitism suggests that the virus infection contributes to parasitoid mortality even before host death. This could be a result of the virus making the host less nutritionally suitable to the parasitoid or the result of a toxic factor produced by the virus. However, Kaya and Tanada (1972) found that N.canescens in an Anagasta host was not affected by toxin which was produced by a synergistic strain of a GV in

Pseudaletia unipuncta (Haworth) although the toxin was active against *Apanteles militaris* (Walsh).

Experimentation failed to provide any evidence to suggest that *N.canescens* can act as a vector of *P.interpunctella* GV. In contrast Kurstak and Vago (1967) claimed that *N.canescens* was a very efficient vector of *Galleria mellonella* (L.) Densonucleosis virus, a non-occluded single-stranded DNA virus (Parvoviridae). Parasitoids which oviposited in virus-infected hosts carried the infection to 100% of the healthy hosts presented within six hours.

Pins were used to simulate stabbing by the ovipositor in order to help ascertain if the lack of virus carriage was a consequence of the parasitoid or of the virus and host. Carriage of virus was not achieved after first stabbing infected larvae at various stages of infection or by contaminating the pin with purified virus particles. Infection only occurred in host larvae when the pin had been dipped in a crude virus preparation. However, the stickiness of this suspension meant that the pin was thickly laden and external contamination of the larvae may have resulted. Infection could thereby have been by ingestion, this is particularly feasible as larvae frequently turned to "lick their wounds" after being stabbed. Further work is required to verify if *P.interpunctella* larvae can be infected by injection.

B.hebetor

The efficiency of the venom causing paralysis and eventual death of both small and large P.interpunctella larvae was high. However, the efficiency of parasitism measured in terms of the number of progeny B.hebetor which completed development was greater on larger larvae. Close inspection of paralysed larvae revealed that small larvae were more prone to desiccation. When given a choice larger larvae were preferentially paralysed and used for successful parasitoid progeny development.

The depth of diet up to three cm did not influence the efficiency of parasitism of B.hebetor with respect to the number of parasitoid progeny. However, slightly more host larvae survived in the deepest diet. It is therefore concluded that B.hebetor adults are able to burrow into Froment diet to at least a depth of three cm in search of hosts.

B.hebetor adults preferentially paralyse and parasitise healthy rather than virus-infected host larvae. Ninety-five percent less B.hebetor progeny developed on infected rather than on healthy hosts, this was attributed in part to mortality in the immature stages, especially mortality of the pupae. However some parasitoids were able to complete development on seven dpi host larvae, which suggests that the parasitoid was able to survive virus death of the host. This was probably because B.hebetor larvae being ectoparasites may not rely on the host for a supply of oxygen. In view of the greater mortality factor, acting on B.hebetor progeny on infected larvae, it is hardly suprising that the adults avoided infected host larvae. Apanteles species have been documented as exhibiting a similar behaviour (Kelsey, 1962; Versoi and Yendol, 1982).

Parasitoids which completed their development in infected hosts were not shown to carry virus to healthy hosts. Any virus in the gut

was probably voided soon after adult emergence, which Vail (1981) found to be the case for *Voria ruralis* (Fallen) after development on NPV infected *Trichoplusia ni*. Parasitoids which had been exposed to infected larvae and then released in a culture of healthy host larvae at a high density, were found capable of introducing a low level of virus infection. It was assumed that the host larvae had ingested the virus inoculum on diet contaminated by the parasitoids. Contamination of the diet could be by mechanical transfer from the external surfaces of the parasitoid or from the faeces, as *B.hebetor* females feed on host larvae. As a host larva rarely survives an attack by *B.hebetor*, it was considered unlikely that virus infection via the ovipositor would occur.

X.flavipes

X.flavipes were not able to significantly reduce the numbers of fifth instar larvae although they were efficient predators of third instar *P.interpunctella* larvae. LeCato and Davis (1973) observed that *X.flavipes* more readily prey on the smaller stages of *P.interpunctella*, a relatively large stored-product insect species. They found that the size of the prey affected the number of prey killed and suggested that the size and thrashing movements of late-instar larvae when attacked by *X.flavipes* probably reduced the number the predator could kill.

X.flavipes was shown to be a more efficient predator on shallow diet of one cm depth than on Froment diet of two or three cm depth. Press *et al.* (1978) documented that finely particulated media prevented deep penetration by *X.flavipes*, although their data would suggest that the media used in the present experiment should have allowed the predators free access in the three cm depths used. In view of their results, the predators were probably able to penetrate

to greater depths but preferentially preyed in the surface layer of the diet.

All densities of X.flavipes significantly reduced the emergence of P.interpunctella larvae. Increasing the density of prey did not further reduce the survival of prey larvae. Five X.flavipes adults were adequate to suppress a larval population of 25 larvae in two grams of diet, and it is possible that an even lower predator density might achieve the same level of control. It would appear that the predators kill more larvae than required for food especially when the prey is in abundance. Press et al. (1975) similarly found that low predator densities of 10 pairs per bushel of peanuts were sufficient to cause highly significant suppression of T.castaneum at densities of 80 pairs per bushel.

B.hebetor was more efficient that X.flavipes in suppressing P.interpunctella populations. The combination of B.hebetor and X.flavipes was less effective than B.hebetor alone, but was more effective than X.flavipes alone. Press et al. (1974) reached the same conclusion in a similar interaction study. The adult emergence of B.hebetor was reduced when X.flavipes was present. This was attributed to competition for paralysed hosts and direct predation by X.flavipes on B.hebetor larvae, interactions which were confirmed by direct observation. The survival of P.interpunctella larvae was further reduced in treatments with predators or parasitoids when no diet was present, which suggests that the presence of diet provides hiding places for the larvae to escape attack by predators or parasitoids. As mature larvae were used, diet was not required for feeding. Hagstrum (1983) observed that susceptibility of E.cautella larvae to parasitism by B.hebetor was affected by the degree of concealment.

X.flavipes preferentially preyed on infected prey larvae.

Smirnoff (1959) reported that infected larvae characteristically showed a loss in their ability to perform defensive movements so increasing vulnerability. Infected larvae may be preferentially selected (Hostetter and Biever, 1970).

X.flavipes was able to complete development when only infected larvae were supplied as a food source. Abbas and Boucias (1984) reared the hemipteran Podisus maculiventris (Say) on NPV infected Anticarsia gemmatalis (Hűbner) without any adverse effects.

Both X.flavipes reared on infected prey and adults allowed to feed on infected prey were capable of carrying viable virus to larval diet, which was bioassayed using healthy larvae. The method of transmission was thereby established to be by contamination of the larval diet, with infection being a consequence of ingestion by the larvae of virus-contaminated diet. Infection in bioassay larvae was most noticable when the diet used was semi-synthetic rather than Froment diet. This is probably because the larvae burrow into Froment diet and produce feeding tunnels, whilst larvae on the semi-synthetic diet feed on the surface and remain mobile.

Predators fed on infected larvae were contaminated both on the external surfaces, which was at least partially removed by washing and internally, probably in the gut. A sample of the washings, or homogenates of five X.flavipes adults fed on virus-infected larvae spread over the surface of seven cm^2 of semi-synthetic diet was sufficient to cause 100% mortality in third instar bioassay larvae. The virus present was therefore in excess of the equivalent of 3.0×10^{-4}mg of GV protein (or 4.8×10^6 granules).

Several workers have reported that hemipteran predators are able to void virus in their faeces after feeding on infected prey (Abbas and Boucias, 1984; Beekman, 1980; Cooper, 1981). Beekman (1980) reported that there was no loss of viability of the virus after

passage through the gut. Biever *et al.* (1982) established that surface contaminated *P.maculiventris* can effectively transmit the NPV of *T.ni* to plant surfaces and thereby initiate virus infection in prey larvae. It is therefore suggested that *X.flavipes* transmits *P.interpunctella* GV to larval diet by both mechanical transfer of external contaminants and by voiding faeces containing virus.

Only one larva succumbed to virus infection after an attack by a predator which had previously fed on infected larvae. Further attempts to substantiate this result failed. It therefore cannot be concluded that this larva contracted the disease by injection from the predator. It is possible that the contact with the contaminated predator indirectly resulted in infection *per os*. No examples were found in the literature where hemipteran predators are known to have transmitted virus infection to prey larvae by injection.

Coleopteran competitors

T.castaneum and *S.granarius* adults both carried virus from cultures containing virus-infected *P.interpunctella* larvae to virus-free diet. The virus contamination on the beetles was both external, which could be removed by washing, and internal, probably in the gut, which was released by homogenising. The washings and homogenates of the two species of beetles after exposure to virus-infected larvae were highly infective. The virus present on the washings or homogenate of a group of five beetles was in excess of the equivalent of 5×10^6 granules. The data suggest that *S.granarius* adults carried more virus on the external surfaces than *T.castaneum* although the latter carried more virus in the gut than *S.granarius*. McGaughey *et al.* (1975) reported that the closely related stored product beetles, *S.oryzae* and *T.confusum* transferred viable *Bacillus thuringiensis* spores from treated to untreated wheat by carrying them

externally or passing them with their faeces. In these tests the weevils transferred more spores than the flour beetles and more spores washed off the former. However, the flour beetles distributed larger numbers of spores in their faeces.

It is concluded that the beetles can carry external contaminants which are present in the food media. Nevertheless, this cannot adequately explain the mechanism in the present study. The virus was introduced into the original cultures in the form of live infected larvae. *P.interpunctella* larvae do not rupture as a consequence of GV disease and earlier studies have shown that individual infected larvae contaminate their environment with virus only at a very low level. The beetles could only come into contact with significant levels of virus if the larval cadavars had been mutilated. There are two possible means by which this could have occurred; cannibalism by other larvae or scavenging on the infected larvae by the beetles themselves. Although cannibalism is known in *P.interpunctella* larvae it is unlikely at an advanced stage of infection. The most plausible mechanism therefore is that the beetles indulge in direct scavenging on infected larvae. This would result in external contamination and virus in the gut, which would explain why both the homogenates and washings were so infective. *T.castaneum* has already been reported to feed on eggs (Hagstrum and Sharp, 1975) and dead or moribund adults of *P.interpunctella* (LeCato and Flaherty, 1973).

This ability to carry virus from an infected culture and the high mobility of young *T.castaneum* adults (Ziegler, 1977) could make them a significant factor in the dissemination of virus to local healthy infestations of *P.interpunctella*.

CONCLUSION

N.canescens, B.hebetor and to a lesser extent X.flavipes can all effectively reduce the survival of P.interpunctella larvae. N.canescens and B.hebetor prefer the larger instars, whilst X.flavipes preferentially preys on the smaller instars. T.castaneum and S.granarius apparently scavenge on dead or moribund larvae.

The depth of the diet was an important factor in limiting the efficiency of the parasitoids and the predator. N.canescens only searched the surface of the diet and therefore any larvae below the reach of the ovipositor escaped parasitism. Both B.hebetor and X.flavipes burrowed into the diet, X.flavipes efficiency declined with an increased depth, but B.hebetor was able to search all depths tested.

The parasitoids and predators exhibited varying degrees of success when in competition with virus for a host. N.canescens was unable to complete development in a host that was infected before or on the same day as oviposition. B.hebetor preferentially avoided infected hosts but a small proportion of progeny were able to complete development on seven dpi hosts. X.flavipes preferentially preyed on infected prey and there was no evidence to suggest any detrimental effects to the predator.

There was no evidence to support the hypothesis of transmission of virus to host or prey by injection by either parasitoids or predator. N.canescens was not observed to act as a carrier of PiGV in any of the tests. X.flavipes and to a lesser extent B.hebetor carried virus by means of external contamination, including passage of virus through the gut after feeding on infected larvae. T.castaneum and S.granarius were shown to be effective carriers of virus externally, including in the gut.

It is therefore suggested that parasitoids are unlikely to be of any significance in the dissemination of P.interpunctella GV.

Predators and scavengers, however, have the potential of being important in the dispersal of host virus. Their high mobility could increase the probability of neighbouring infestations of *P.interpunctella* succumbing to virus infection.

CHAPTER 5 Ephestia cautella and its Nuclear Polyhedrosis Virus

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INTRODUCTION

The Almond Moth, Ephestia cautella, Walker is a pyralid moth of similar habits and distribution to that of P.interpunctella . It primarily attacks dried fruit, cereals, nuts, cocoa beans and cottonseed meal (Kurtz and Harris, 1962).

1. General biology

There are normally five larval instars (McGaughey, 1978) although Takahashi (1961a) reported that there could be more than six on larval cultures at high densities. Richards and Thompson (1932) only found four instars for the male. A generation lasts between 23 and 39 days at 27.5°C and 55% RH (Strong et al., 1968) and can be as short as four weeks in commercial warehouses (Hagstrum and Stanley, 1979). Dry food retards development, decreases the survival rate and the size of the adult moths (Takahashi, 1956b).

E.cautella like P.interpunctella breeds best in warmer climates. Both can breed successfully at 30°C, but fail to complete development at 15°C. A further similarity is that the final instar of E.cautella may enter diapause. The critical photoperiod to induce diapause at either 20 or 25°C was between 12 and 13 hours and the principal sensitive larval phase is near the time of the last moult (Bell and Bowley, 1980). Diapausing larvae remain active after non-diapausing larvae of the same generation, and can remain in diapause for over five months (Hagstrum and Sharp, 1975). Diapause can be terminated by exposure to as few as five 15 hour daylengths at 25°C (Bell and Bowley, 1980).

Takahashi (1955) described a two phase emigration of larvae; one by immature larvae which is increased with higher initial densities and the other by fully grown larvae searching for pupation sites. In a later paper, Takahashi (1961b) described the vertical movement of larvae in a container; young larvae stay in the upper layers but at

higher densities older larvae will move downwards. Survival and development rate in a constant food volume at high larval densities is improved by rearing cultures of greater cross sectional area (Takahashi, 1959c), but not by an increase in the depth of culture with a constant cross sectional area (Bell, 1976).

E.cautella is more sensitive than P.interpunctella to crowding (Bell, 1976) and when subjected to interspecific competition, P.interpunctella was dominant (Soderstrom and Lovitt, 1973). E.cautella larvae switch from an undercompensating density-dependent mortality to an overcompensating density-dependent mortality at an initial density of about 25 eggs per gram of diet. Rogers (1970) suggests that this signifies a switch to be a more cannibalistic existence. Takahashi (1953b) found the efficiency of consumption of rice bran reached a maximum at about 30 eggs per gram. Takahashi (1956b) observed that denser larval populations slowed post-embryonic development, which he attributed to cannibalism of the pupae by larvae. The mean body weights and head capsule widths of adults and the mean number of eggs per moth decreased with an increase of initial density (Takahashi, 1956a). The addition of frass to diet, had the same effect as high population density (Takahashi, 1957a).

Various workers (Takahashi, 1956a; Steele, 1970; Hagstrum and Tomblin, 1975) described a correlation between fecundity and female weight at adult emergence. Both sexes underwent multiple copulations, females up to five times and males up to 15 times, but there was no relationship between the number of copulations and fecundity (Steele, 1970). The fecundity and longevity of adults was reduced by about a half if deprived of drinking water (Hagstrum and Tomblin, 1975). Free-flying gravid females readily located and oviposited in the vicinity of small sources of diffusing grain odour (Barrer and Jay, 1980). When given a choice, no eggs were laid where there was no food

and the mean number of eggs laid at a location was proportional to the amount of food at that location (Hagstrum, 1984).

Adults exhibit rhythmic behaviour when exposed to normal daylight. The peak emergence occurs one to two hours before dusk, copulation primarily occurs at dusk and oviposition at dawn and dusk (Steele, 1970).

Grant et al. (1975) have described interspecific courtship between E.cautella and P.interpunctella. Male P.interpunctella were strongly excited by calling E.cautella females, however, the courtship behaviour was incompatable and copulation did not occur. If courtship continued to a later stage in the sequence then the female rejected the male because he released an inappropriate aphrodisiac. Courtship between E.cautella males and P.interpunctella females was rare but did lead to copulation, insemination apparently did not occur.

2. E.cautella BVs

There are two BVs that have been isolated from E.cautella; an NPV (Thompson and Redlinger, 1968) and a GV (Hunter and Hoffmann, 1970).

The NPV principally replicates in the nuclei of the hypodermis, fat body and tracheae (Thompson and Redlinger, 1968). A further study on the histopathology of infected larvae revealed polyhedra also in the blood cells, nuclei of Malpighian tubules, tissue associated with the reproductive organs and the nervous system, muscle and cell nuclei of the anterior midgut (Adams and Wilcox, 1968).

Hunter et al. (1973a) studied the effect of virus concentration on mortality of neonate larvae and also the timing of death. When neonate E.cautella and P.interpunctella larvae were exposed to 40 x 10^3 polyhedra/g of bran diet, the mortalities were 75 and 59% respectively. The sites of virus replication in P.interpunctella larvae were essentially the same as in E.cautella. However,

replication in *P.interpunctella* developed more slowly and tissue tropisms were less apparent, also the polyhedra were more often cuboid in *P.interpunctella* than in *E.cautella* (Hunter *et al.*, 1973b).

The GV of *E.cautella* attacked the cells of the fat body, tracheal matrix, epidermis and muscle sheath (Hunter and Dexel, 1970). *P.interpunctella* was moderately susceptible to this GV. The capsules of the GV in cross-infected *P.interpunctella* were generally abnormal in form (Hunter and Hoffmann, 1972). In an earlier study Hunter and Hoffman (1970) found that *E.cautella* was not susceptible to *P.interpunctella* GV.

The methods of virus transmission for pyralids has been discussed in Chapter 3. Descriptions of their predators and parasitoids and their potential as virus carriers were described in Chapter 4. The aims of this chapter were to provide a comparison for the work done on *P.interpunctella* and its GV, to investigate the interaction between these two pyralid moths and to test the cross-infectivity of their BVs.

EXPERIMENTS

Section A GENERAL

1. Life History

Method

Eggs from adult moths were collected over a two day period. The eggs were surface sterilized then incubated in a petri-dish at 26° C, 70% RH. They were examined regularly for hatch. The larvae were transferred in batches of 10 to polypots containing either Froment diet or semi-synthetic diet. The larvae were incubated at 26° C, 70% RH and examined regularly for their stage of development. The head capsule widths (HCW) of samples of the larvae were measured every two days.

Results

The HCWs of the larvae are presented in Table 1. Five distinct head capsule sizes were observed which correlated with the instar stage, the regression equation is included under Table 1.

The development times are recorded in Table 2. Larval development progressed more rapidly on semi-synthetic diet than on Froment diet. Some of the larvae remained in the fifth instar for prolonged periods, possibly exhibiting a partial diapause. Only the day on which the fifth instar larvae and subsequent developmental stages were first recorded are included in the table.

Table 1. HCWs of the instars of *E.cautella* larvae

Instar	Number measured	HCW (mm)		
		Range	Mean	Standard error
1	30	0.18-0.21	0.19	0.002
2	28	0.26-0.31	0.27	0.003
3	33	0.42-0.51	0.44	0.003
4	15	0.59-0.72	0.63	0.006
5	21	0.82-0.95	0.90	0.006

$y = 0.01 + 0.05x$ $r^2 = 0.983$ $p = 0.001$

$y = \log(HCW+1)$

$x = \text{instar}$

Table 2. Length of life history of E.cautella at 26°C on two types of diet

Stage		Age (days)	
		Froment diet	Semi-synthetic diet
Egg		0-5	0-5
larval instars	1	5-11	5-9
	2	7-22	9-11
	3	13-26	11-13
	3	20-34	13-18
	4	26-	15-
pupa		31-	26-
adult		37-	32

2. Intra and inter-specific competition between larvae

Method

Two grams of Froment diet were weighed into each polypot. There were three series of treatments; E.cautella larvae only, P.interpunctella larvae only and finally a mixed population containing equal numbers of both species. Neonate larvae were used. There were five densities of larvae for each series of treatments; 2, 4, 8, 16 and 32 larvae per polypot for each species. There were three replicates for each treatment.

The experiment was incubated at 26°C and the emergence of adults was recorded.

Results

The angular transformation of the proportion of larvae which completed development to the adult stage for each species is given in Table 3. The differences between the proportions which survived revealed that at almost every density the survival of E.cautella was less than that of P.interpunctella.

Table 3. The survival of E.cautella and P.interpunctella larvae under the pressures of intra- and inter-specific competition

Initial density of each species	Single population			Mixed population		
	E.cautella	P.interpunctella	Difference (d)	E.cautella	P.interpunctella	Difference (d)
2	54.74*	90.00	-35.26	24.09	65.91	-41.82
4	73.22	73.22	0.00	35.26	73.22	-37.96
8	52.24	57.31	-5.07	40.20	54.74	-14.54
16	40.20	61.40	-21.20	38.99	57.31	-18.32
32	40.81	57.31	-16.5	30.68	40.20	-9.52
95% CI for $\bar{d}$	-32.87 to 1.65			-42.45 to -6.41		

* values expressed as the angular transformation of the proportion of larvae which developed into adults.

A paired t-test was performed to compare the survival of E.cautella and P.interpunctella at each density for both single and mixed populations. The 95% confidence intervals for the t-test are given in Table 3. The interval included zero for the single populations but excluded zero for the mixed population. It is concluded that the survival of E.cautella larvae is not significantly less than that of P.interpunctella under the pressures of intra-specific competition, but it is significantly less under the pressure of inter-specific competition.

3. Dispersal of healthy and infected larvae

Method

Moats and islands were used to compare the dispersal of healthy and infected E.cautella larvae. The experimental design has been described for P.interpunctella larval dispersal (Page 68).

Third instar E.cautella larvae with a mean weight of 6.4×10^{-4}g were used. The control larvae were kept on virus-free semi-sythetic diet for 24 hours after their separation from the rearing medium, the virus-treated larvae were confined for the same period on NPV-contaminated semi-synthetic diet.

There were three treatments and three replicates. The control island contained 30 control larvae, the mixed islands had 15 of each of the control and virus treated larvae while the virus islands contained 30 virus treated larvae. The experiment was kept at 24°C with a 16 hour daylength. The data from the replicates were summed.

Results

Larvae were caught in the moats up to 26 dpi. The dispersal of larvae from the three treatments is recorded in Table 4. When the proportions of the total migratory population were compared for the control and virus treated larvae the difference was significant

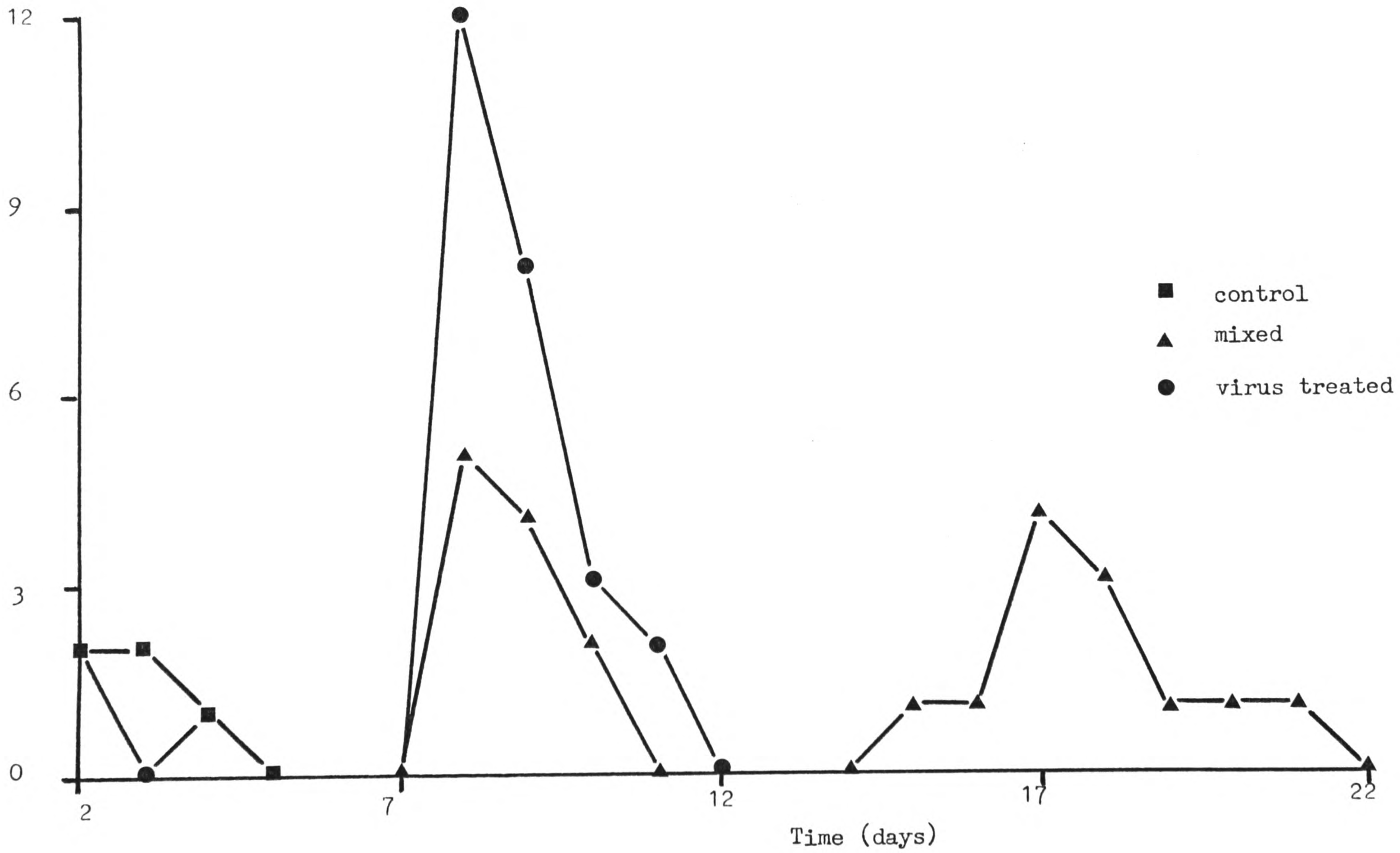
(χ^2_1 = 15.14, p = 0.001). It was concluded that more larvae dispersed from the control than the virus treatment. However, most of the larvae which dispersed from the control treatment were mature while all the larvae which dispersed from the virus treatment were immature. When the same comparison was repeated using data for only immature larvae the result was significant (χ^2_1 = 19.63, p = 0.001) and it was concluded that more immature larvae dispersed from the virus treatment than from the control.

The emigration from the mixed treatment was not intermediate to that of the other two treatments. Its very low dispersal of mature larvae and comparatively high dispersal of immature larvae resembled that of the virus treatment. A comparison of the proportion of migratory immature larvae between the mixed and virus treatments failed to reveal any significant difference (χ^2_1 = 0.43). This suggests that a large proportion of the larvae in the mixed treatment suffered virus infection.

The number of immature larvae which migrated on a daily basis is portrayed in Figure 1. There were three peaks of activity. The first and smallest peak was exhibited by both control and virus treated larvae and occurred immediately after release. The second peak, which was the most pronounced, was between seven and 12 dpi and was displayed by larvae from the mixed and virus treatments. The final and more diffuse peak occurred between 14 and 22 dpi and was displayed by larvae from the mixed treatment only.

These results suggest that healthy immature larvae normally only migrate from food islands within three days of disturbance. Infected larvae show an abnormally high rate of dispersal between 7 and 12 dpi at 24°C. The final peak which started at 14 dpi probably represents initially healthy larvae which succumbed to infection by secondary

Figure 1 The daily migration from food islands of immature *E.cautella* larvae



cycling of virus, perhaps during the abnormal activity of infected larvae seven days previously.

Table 4. Dispersal of E.cautella larvae from control, NPV-treated and mixed treatments

Treatment	Number of migratory larvae			Number of static larvae
	Immature	Mature	Total	
Control	5	49	54	36
Mixed	24	1	25	65
Virus-treated	28	0	28	62

4. A comparison of the dispersal of healthy and infected E.cautella larvae with P.interpunctella larvae

Method

The experimental procedure described for the dispersal of E.cautella larvae was repeated using third instar P.interpunctella larvae with a mean weight of 4.7 x 10^{-4}g. PiGV was used in the place of EcNPV. A direct comparison for the two experiments was then possible.

Results

The dispersal of immature and mature P.interpunctella larvae for the three treatments is given in Table 5. In a manner similar to that already described for E.cautella, the mixed treatment more closely resembled the virus treatment than the control treatment. The characteristic geater rate of dispersal of infected P.interpunctella immature larvae from food islands into surrounding moats has already been described (page 69).

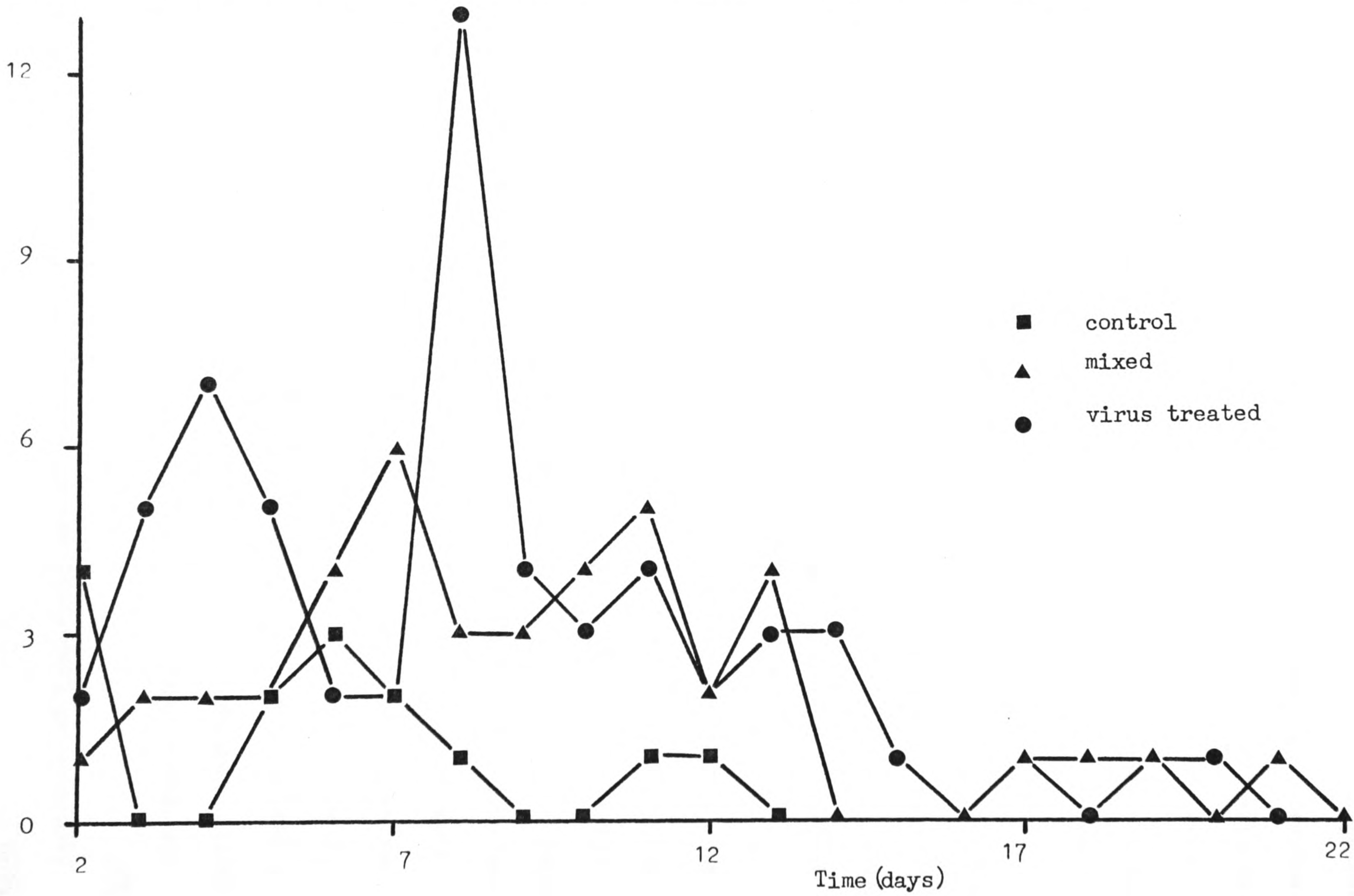
The numbers of larvae which migrated on a daily basis is portrayed in Figure 2. The three peaks of activity clearly seen for

the *E.cautella* larvae were much more diffuse for *P.interpunctella* larvae. However, for both species, the peak of activity for virus-treated larvae was on eight dpi.

Table 5. Dispersal of *P.interpunctella* larvae from control, GV-treated and mixed treatments

	Number of migratory larvae			Number of
Treatment	Immature	Mature	Total	static larvae
Control	14	52	66	24
Mixed	42	5	47	43
Virus-treated	59	0	59	31

Figure 2 The daily migration from food islands of immature *P.interpunctella* larvae



CROSS INFECTION

Section B

1. Infection of *E.cautella* and *P.interpunctella* larvae using EcNPV

Method

The basic experimental procedure described for *P.interpunctella* mortality studies (page 53) was followed. The mean weights of the third instar *E.cautella* and *P.interpunctella* larvae selected were 9.7 x 10^{-4}g and 9.6 x 10^{-4}g respectively. The two experiments for the two species were conducted simultaneously. Fifteen larvae were placed in each polypot to which the appropriate virus concentration had been spread over the surface of the semi-synthetic diet. There were five treatments; a control with no virus, and four virus treatments of concentrations 1.4 x 10^4, 1.4 x 10^5, 1.4 x 10^6 and 1.4 x 10^7, polyhedra per polypot. The larvae were transferred to individual polypots of semi-synthetic diet after three days.

The larvae were checked for death or pupation. All dead *P.interpunctella* larvae and a sample of dead *E.cautella* larvae were smeared, stained and examined using a light microscope for the presence of polyhedra.

Results

No control larvae suffered mortality. Pupae were assumed to be healthy which is supported by the work of Thompson and Redlinger (1968). Almost all dead virus-treated larvae contained polyhedra. The examination of larval smears revealed two general trends. *E.cautella* larvae which suffered mortality contained large numbers of polyhedra. *P.interpunctella* larvae which suffered mortality had comparatively few polyhedra, which usually consisted of a few isolated nuclear groups. The polyhedra in *P.interpunctella* appeared to be smaller and frequently cuboid or more irregularly shaped than those in *E.cautella*. The polyhedra in *P.interpunctella* also appeared speckled especially under phase contrast, which suggests that they were pitted

young polyhedra. The use of triple stain confirmed that the polyhedra in both species of larvae were NPV.

The data for mortality of larvae diagnosed as virus infected at each virus concentration were tested using Probit analysis. The LC_{50} and the gradient values are given in Table 6. For fifty percent mortality third instar E.cautella larvae are seven times more susceptible to EcNPV than the same stage of P.interpunctella larvae.

The timing until fifty percent mortality of P.interpunctella larvae was far longer at 21 dpi than that recorded for E.cautella larvae at 13 dpi.

Table 6. Mortality of third instar E.cautella and P.interpunctella larvae in response to EcNPV

Species	LC_{50}*	95% CI for LC_{50}	Gradient
E.cautella	49	31-77	1.48
P.interpunctella	344	241-494	2.11

* dosage: polyhedra/mm^2 of diet

2. Infection of E.cautella larvae using PiGV

The mortality studies of P.interpunctella larvae in response to PiGV have already been recorded (page 54).

Method

The basic experimental procedure already described for P.interpunctella was followed (page 53).

Neonate E.cautella larvae were used. There were four treatments; a control with no virus and three virus treatments with concentrations of 1.34×10^{-3}, 1.34×10^{-2} and 1.34×10^{-1} mg of virus protein per polypot. there were about 25 larvae per polypot and three replicates

for each treatment. The larvae were transferred to individual polypots of semi-synthetic diet after seven days.

Results

Three larvae in the control treatment suffered mortality, none of which showed symptoms of virus infection. They were therefore excluded from the virus mortality figures. All virus treated larvae which suffered mortality exhibited virus symptoms; that is a whitish colour and sluggish behaviour.

The data were examined using Probit analysis. The medium lethal concentration was 1.4×10^{-2}mg of virus protein per polypot with a 95% confidence interval of $1.1 \times 10^{-2} - 1.7 \times 10^{-2}$mg per polypot. The slope value for the mortality response was 1.4.

A comparison with the data obtained for neonate P.interpunctella larvae revealed that PiGV is 2×10^{4} more infective to P.interpunctella than E.cautella larvae in order to achieve fifty percent mortality. The slope for P.interpunctella was much greater than the slope value obtained for E.cautella. This suggests that neonate E.cautella larvae were more variable in their response to PiGV than neonate P.interpunctella larvae, although the slope for P.interpunctella must be regarded as unusually high.

3. E.M. studies of E.cautella larvae infected with PiGV

Method

Two E.cautella larvae which were used in the cross-infection studies were examined by electron microscopy. The two larvae had been exposed to 1.34×10^{-3}mg of PiGV per polypot of semi-synthetic diet. Thirty-six dpi both larvae were in the fifth instar. They were alive but exhibited symptoms of advanced virus infection.

The larvae were killed and the gut and fat body were dissected out. The remaining integument with underlying tissues, the gut and

the fat body were separately cut into small pieces and fixed in 2% glutaldehyde.

The specimens were prepared for examination in the EM by a series of dehydration and embedding steps, before final embedding in resin. The resin blocks were trimmed and thin sections of the specimens were cut 5-7μm thick using an ultramicrotome. The sections were mounted on Formvar-coated copper grids and stained with 2% uranyl acetate.

The stained sections were examined.

Results

a) Integument and underlying tissues

Many GV granules were present, virus particles and granulin were also seen but were not as numerous. It was difficult to locate exactly which tissues were infected, but granules were found in the tissues immediately underlying the cuticle. Plate 1 shows virus particles and granules on these tissues.

b) Fat body

Exceptionally large numbers of GV granules were present but separate particles were not seen. Some of the granules appeared to be "empty", that is lacking virus particles as illustrated in Plate 2.

c) Gut

Large numbers of GV granules and isolated virus particles were found in the tissues surrounding all parts of the gut as shown in Plate 3.

4. Cross infection studies using virus-killed larval cadavers

The use of purified virus incorporated into the larval diet confirmed that the two viruses were cross infective to the two species of stored product moths used. However, since neither species when virus infected rupture, the greatest potential source of inoculum in

Plate 1. Tissues underlying the integument of E.cautella larvae infected with PiGV

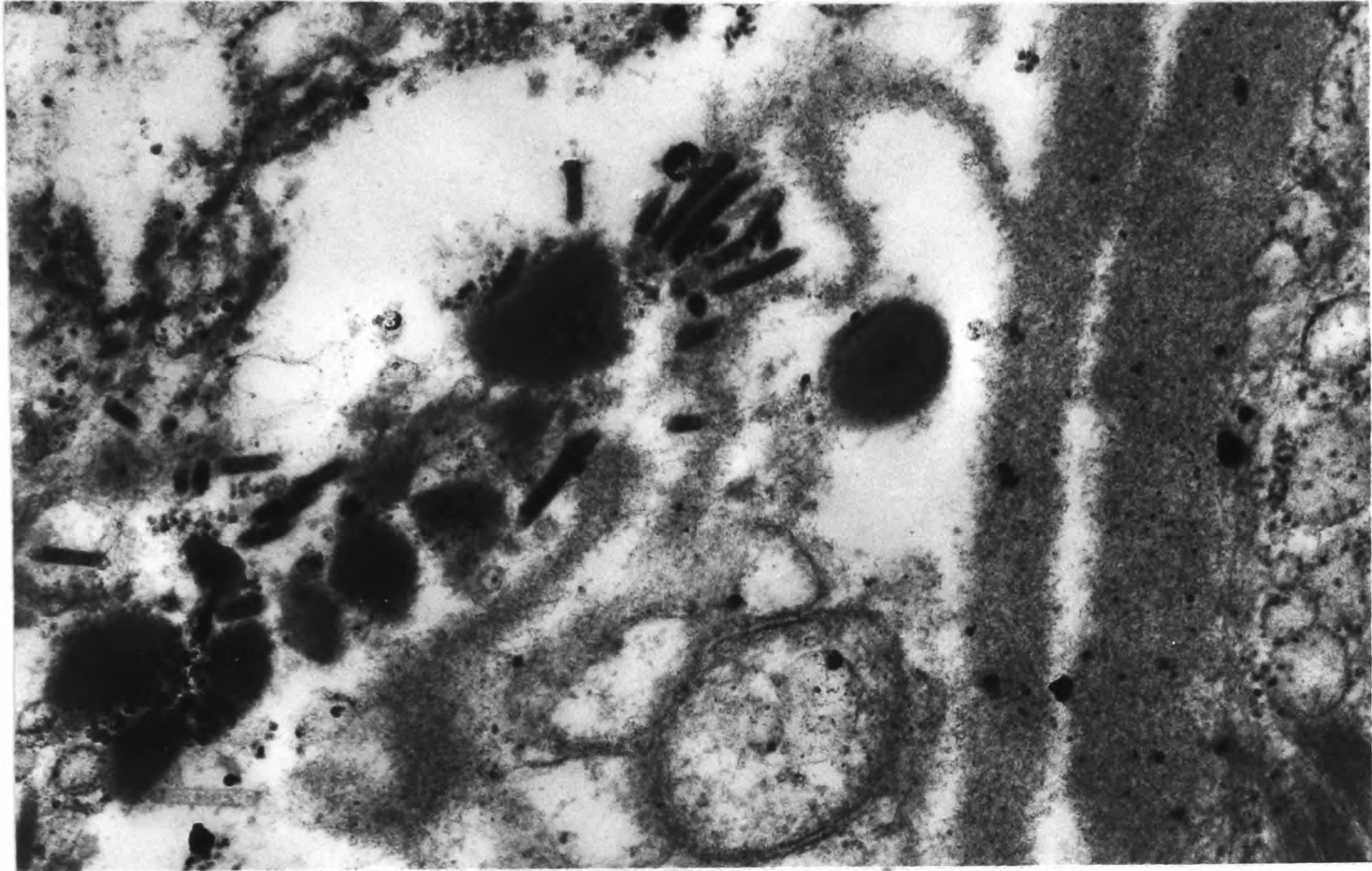
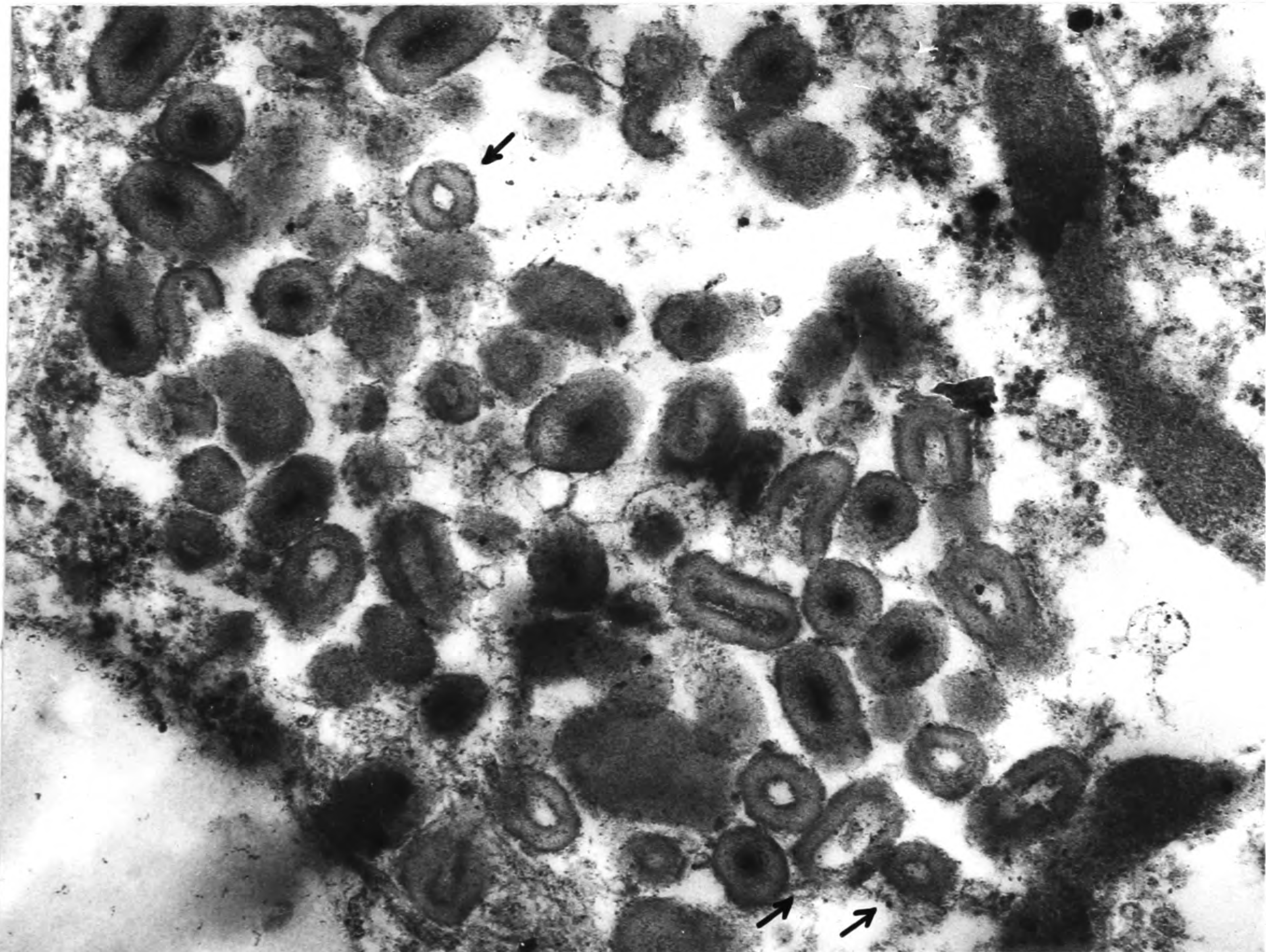
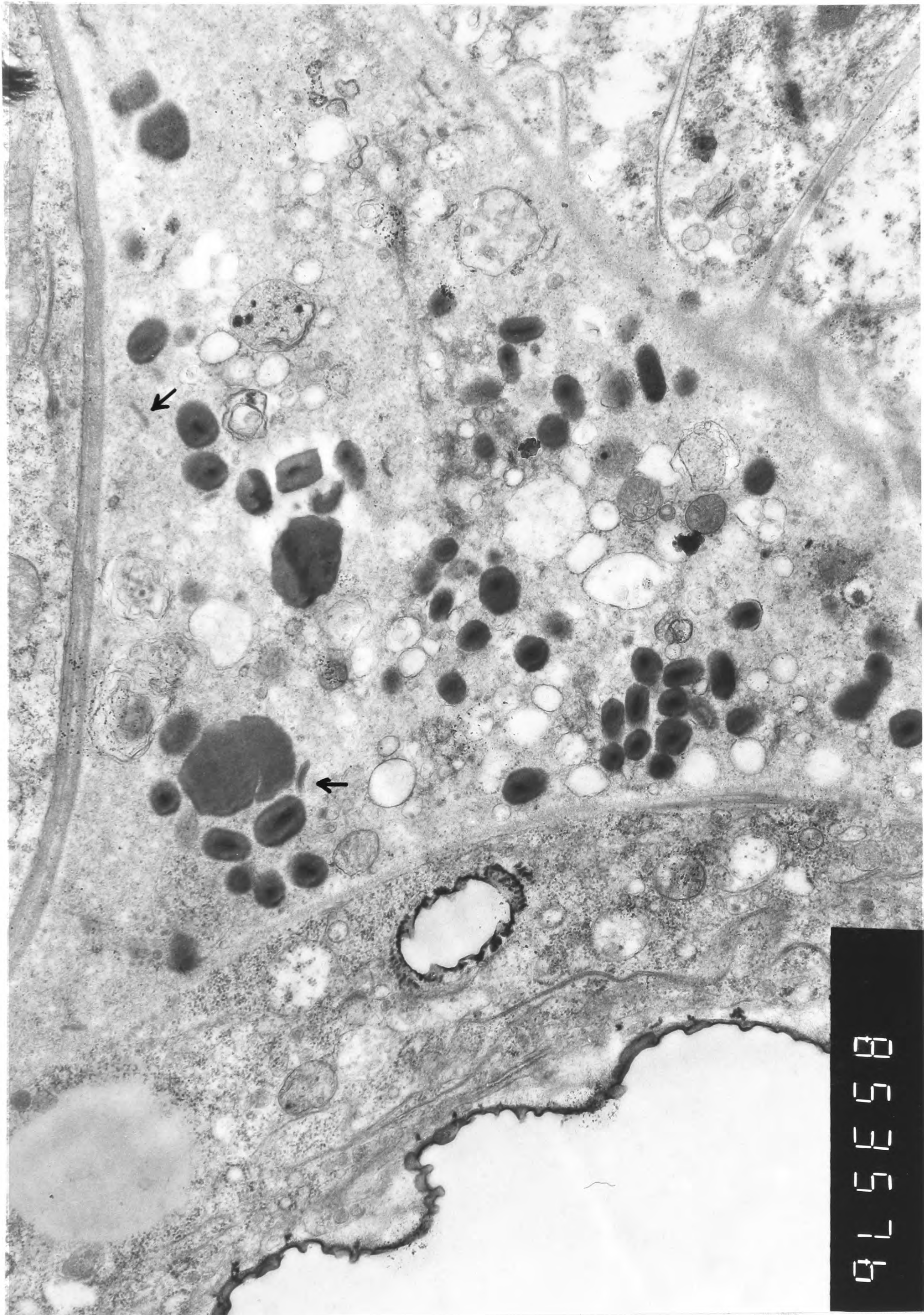


Plate 2. Fatbody of E.cautella larvae infected with PiGV



Empty granules ↗ Magnification x 50000

Plate 3. Gut tissues of E.cautella larvae infected with PiGV



Virus particle ↗

Magnification x 20000

culture are the cadavers of virus-killed larvae. Unless the cadavers are ɘchanically ruptured liberating the virus inoculum on the larvae medium, ıe healthy larvae would need to scavenge the cadavers to ingest the ıoculum.

The following experiment was designed to test the potential of larval ıdavers as sources of virus inoculum to larvae with an available food ›urce but subjected to high intra-specific competition.

ɘthod

Thirty-two E.cautella or P.interpunctella third instar larvae with a ɘan weight of 6 x 10^{-4}g were counted into each polypot containing two :ams of Froment diet. There were three treatments for each species and ıree replicates. Two healthy fifth instar larvae killed by freezing were laced in each of the control treatments. Two PiGV killed P.interpunctella ifth instar larvae were placed in each of the GV treatments and two EcNPV illed E.cautella larvae were placed in each of the NPV treatments.

The polypots were incubated at 26° C and the emergence of adults was ɘcorded.

ɘsults

The survival to the adult stage for each treatment for .interpunctella and E.cautella is recorded in Tables 7 and 8, ɘspectively.

The proportion of P.interpunctella which survived was not ignificantly reduced (χ^2_1 = 0.49), when NPV-killed cadavers were ntroduced. However, there was a highly significant reduction (χ^2_1 = 29.95, = 0.001) in the survival of P.interpunctella when GV-killed cadavers were ntroduced. It is concluded that GV-killed P.interpunctella cadavers but ot NPV-killed E.cautella cadavers were a viable source of inoculum to '.interpunctella larvae.

The proportion of E.cautella larvae which survived was not ignificantly reduced (χ^2_2 = 3.07) when either NPV-killed or GV-killed

cadavers were introduced. It is concluded that virus-killed cadavers are not a suitable source of inoculum to *E.cautella* larvae. NPV-killed cadavers of *E.cautella* were more prone to desiccation than GV-killed *P.interpunctella* cadavers.

Table 7. The survival of P.interpunctella larvae to the adult stage when virus-killed larval cadavers were introduced into the culture

Larval cadaver	Live	Dead	Total
Control	77	19	96
NPV	73	23	96
GV	40	56	96
Total	190	98	288

Table 8. The survival of E.cautella larvae to the adult stage when virus-killed larval cadavers were introduced into the culture

Larval cadaver	Live	Dead	Total
Control	54	42	96
NPV	47	49	96
GV	59	37	96
Total	160	128	288

PREDATORS AND PARASITOIDS

Section C

1. Contamination of B.hebetor and X.flavipes with NPV

Method

Adult *X.flavipes* and *B.hebetor* were allowed to feed on six dpi *E.cautella* larvae, the controls fed on healthy *E.cautella* larvae. After 48 hours the parasitoids and predators were removed and killed. The sexes of *B.hebetor* were divided. In groups of five the parasitoids and predators were crushed onto a glass slide. The smears which formed were stained with Giemsa and examined for the presence of polyhedra using a light microscope.

Results

No polyhedra were found in any of the control smears nor in that of male *B.hebetor* which had been exposed to infected larvae. Polyhedra were present in the smears from *X.flavipes* and female *B.hebetor* adults which had been exposed to infected larvae.

2. The role of predators and parasitoids in the transmission of EcNPV

a) N.canescens

Method

Individual wasps were observed stabbing three *E.cautella* larvae which were one, two, three or eight dpi. Up to four healthy third instar larvae with a mean weight of 4×10^{-4}g were then offered to the adult parasitoids. The stabbed larvae were transferred to individual polypots of semi-synthetic diet. Each treatment was repeated with at least five wasps. The controls were larvae which were stabbed by wasps which had been exposed to only healthy larvae.

The larvae were kept at 26° C. Pupation of *E.cautella* was recorded, any host larval cadavers and all parasitoid larvae or pupae were smeared and examined for the presence of NPV.

Results

Table 9 records the fate of healthy larvae stabbed by the wasps.

Successful parasitism occurred at a low level. None of the parasitoid larvae or pupae contained polyhedra. The *E.cautella* larvae which successfully formed pupae were assumed to be healthy. Only one of the dead *E.cautella* larva contained polyhedra. The one infected larva contained large quantities of NPV and was the first healthy larva stabbed by a wasp which had previously attacked three dpi larvae.

Table 9. The fate of E.cautella larvae which were stabbed by N.canescens females which previously attacked larvae at various stages of infection

Fate of larvae	Initial larvae stabbed				
	Control	1 dpi	2 dpi	3 dpi	8 dpi
Host pupae	5	13	17	20	20
Host cadaver	0	1	1	2	0
Parasitoid larvae/pupae	5	4	4	3	3
No. tested	10	18	22	25	23

b) *B.hebetor*

Method

B.hebetor adults were exposed to one, two, three or eight dpi *E.cautella* larvae for 24 hours. The controls were exposed to only healthy hosts.

The parasitoids were used in two experiments.

I. To test for carriage of virus to semi-synthetic diet

Three pairs of each treatment of *B.hebetor* were released into individual polypots of semi-synthetic diet. After two days, the parasitoids were removed and the diet was tested for the presence of virus by bioassay with 25 neonate *E.cautella* larvae per polypot. The larvae were transferred to individual polypots

after nine days. Any dead larvae were smeared and examined for polyhedra. Larvae which formed pupae were assumed to be healthy.

II. To test for carriage of virus to larvae in Froment diet

One pair of each treatment of B.hebetor was released in a polypot containing either 50 third instar E.cautella larvae with a mean weight of 4 x 10^{-4}g or 20 fourth instar E.cautella larvae with a mean weight of 21 x 10^{-4}g. A small quantity of Froment diet (about 0.5g) was placed initially in each polypot, more diet was supplied as necessary. The larvae were incubated at 26° C. Samples of dead larvae were smeared and examined for polyhedra.

Results

I. To test for carriage of virus to semi-synthetic diet

Very few of the bioassay larvae suffered mortality. None of the larval cadavers were diagnosed as infected. It is concluded that the parasitoids did not carry virus to semi-synthetic diet which could act as an inoculum to E.cautella larvae.

II. To test for carriage of virus to larvae in Froment diet

No larvae smeared from any of the treatments were found to contain polyhedra. These results suggest that B.hebetor adults do not act as effective carriers of EcNPV.

c) X.flavipes

Method

X.flavipes were allowed to feed on one, two, three or eight dpi E.cautella larvae for two days. The controls were provided with only healthy larvae as a food source.

The predators were used in the following experiments.

I. To test for carriage of virus to individual larvae

The predators were starved for six hours before being offered a

succession of individual third instar *E.cautella* larvae with a mean weight of 4×10^{-4}g. Each larva stabbed was quickly removed to an individual polypot of semi-synthetic diet. At least five predators were used for each treatment.

The larvae were kept at 26° C, they were checked for pupation or mortality. Any dead larvae were examined for the presence of polyhedra.

II. To test for carriage of virus to semi-synthetic diet

Five predators from each treatment were released into polypots containing semi-synthetic diet. After two days the predators were removed and the polypots were bioassayed as previously described for *B.hebetor*.

III. To test for carriage of virus to larvae in Froment diet

Five predators from each treatment were released for two days into polypots containing 25 healthy third instar *E.cautella* larvae with a mean weight of 4×10^{-4}g. A small quantity (about 0.5g) of Froment diet was provided initially to the larvae in each polypot, more diet was supplied as necessary. The larvae were incubated at 26° C and examined regularly for symptoms of infection. A sample of dead larvae was examined for the presence of polyhedra.

Results

I. To test for carriage of virus to individual larvae

The proportion of larvae stabbed by the predators which survived is recorded in Table 10. Most of the larvae which suffered mortality did so shortly after their encounter with a predator. All larval cadavers were examined for polyhedra, but none were found. These results suggest that *X.flavipes* adults with mouthparts contaminated after feeding on infected larvae cannot transmit EcNPV by stabbing larvae.

II. To test for carriage of virus to semi-synthetic diet

The incidence of infection of the bioassay larvae is recorded in Table 11. Virus infection was only diagnosed in bioassay larvae on diet contaminated by predators that had fed on eight dpi larvae. It is concluded that X.flavipes adults can only contaminate their environment with viable virus after feeding on prey at a late stage of infection.

III. To test for carriage of virus to larvae in Froment diet

No larvae in any of the treatments were diagnosed as infected. Many of the larvae suffered mortality probably as a result of predation. The results suggest that under the conditions of these experiments X.flavipes adults are not likely to act as effective carriers of EcNPV.

Table 10. The fate of E.cautella larvae which were stabbed by X.flavipes adults which had previously fed on larvae at various stages of infection

Fate of larvae	Initial larvae fed to predators				
	Control	1 dpi	2 dpi	3 dpi	8 dpi
Mortality	5	12	3	9	5
Formed pupae	5	8	12	10	16
No. tested	10	20	15	19	21

Table 11. Incidence of infection in bioassay larvae on semi-synthetic diet contaminated by predators which had previously fed on larvae at various stages of infection

Incidence of infection	Initial larvae fed to predators				
	Control	1 dpi	2 dpi	3 dpi	8 dpi
Positive	0	0	0	0	13
Negative	46	22	20	30	11
Total	46	22	20	30	24

DISCUSSION

Section A General

The number of larval instars was in agreement with the work of McGaughey (1978), although the HCWs did not entirely agree.

The survival of *E.cautella* at all larval densities was less than that of corresponding *P.interpunctella* cultures. Bell (1976) observed that *E.cautella* was more sensitive than *P.interpunctella* to crowding. However this cannot explain the lower rate of survival of the former at low densities. The results suggest that individual larvae of the *E.cautella* stock had a lower innate capacity for survival under the conditions employed than the corresponding *P.interpunctella* culture. Neonate larvae of *E.cautella* may be more sensitive than *P.interpunctella* to handling which could explain the discrepancy.

The dispersal of healthy *E.cautella* larvae occurred in two phases as described by Takahashi (1955): a low level of emigration from food islands of immature larvae and a comparatively high level of emigration of mature larvae, searching for a site for pupation. Infected immature *E.cautella* larvae exhibited an enhanced level of emigration compared with healthy larvae. The pattern of emigration of *E.cautella* larvae was thereby similar to that described for

P.interpunctella, although the rate of emigration of immature larvae was much less for E.cautella. The peak of activity of infected larvae for both species was about eight dpi. The peak was more discrete for E.cautella larvae (variance = 2.16) than for P.interpunctella larvae (variance = 4.20).

Mixed intra-specific cultures containing both healthy and infected larvae exhibited an enhanced level of emigration of immature larvae as compared with the controls and a corresponding decreased level of emigration of mature larvae, which suggests that most larvae in the culture succumbed to virus infection. The emigration of larvae which succumbed to infection at a later stage was typically delayed until after the usual peak of emigration of infected larvae. In E.cautella mixed cultures a second peak of emigration was observed, nine days after the first. A second peak of emigration was not obvious in P.interpunctella mixed cultures.

Section B Cross-infection studies

E.cautella and P.interpunctella were both susceptible to EcNPV. A dosage of 200 polyhedra per square millimetre of semi-synthetic diet caused 87% mortality and 23% mortality in third instar larvae of E.cautella and P.interpunctella respectively. Hunter et al. (1973a) obtained similar differential mortality figures when they fed neonate larvae of E.cautella and P.interpunctella with EcNPV on diet. It is concluded that E.cautella larvae are more susceptible than P.interpunctella larvae to EcNPV.

EcNPV did not replicate in P.interpunctella as in E.cautella and the polyhedra were more often cuboidal. These results are in agreement with those obtained by Hunter et al. (1973b) and the evidence presented by these workers supports cross-infection rather than activation of a latent NPV in P.interpunctella. They too found

that the timing of mortality was later in *P.interpunctella* larvae.

E.cautella was only slightly susceptible to PiGV. Hunter and Hoffman (1970) had been unable to infect larvae of *E.cautella* with PiGV. It is suggested that they did not use dosages high enough. Studies on the histology of diseased larvae revealed polyhedra in the fat body and tissues surrounding the gut and hypodermis which is in close agreement with the main sites of replication in *P.interpunctella* of the hypodermis, tracheae and fat body (Hunter *et al.* 1972). Some of the virus capsules in *E.cautella* appeared to be hollow. Hunter and Hoffman (1972) observed similar abnormal capsules in *P.interpunctella* larvae cross-infected with EcGV. They suggested that the abnormal development was the result of an enzyme system essential for normal DNA replication being absent in the alternate host. However, Arnott and Smith (1968a,b) reported the presence of both cuboid and hollow capsules in *P.interpunctella* larvae infected with PiGV.

Although cross-infection can occur with all three viruses known to commonly infect either of these two species of Pyralid moths, it is suggested that cross-infection is normally unlikely to be of any importance. The dosage required to infect *E.cautella* with PiGV would prove prohibitive. Neither species exhibited signs of infection after the cultures were seeded with NPV-killed *E.cautella* larvae, suggesting that neither species will scavenge these cadavers when food is present, although *P.interpunctella* larvae did scavenge GV-killed *P.interpunctella* larvae. Transmission of EcNPV between *E.cautella* larvae in the mixed dispersal experiments was therefore not by scavenging on larvae killed by virus early in the experiment. Possible sources of inoculum were by cannibalism of live infected larvae or contamination of the diet by infected larvae, possibly by presence of virus in the frass.

Section C Predators and Parasitoids

X.flavipes and *B.hebetor* adults were found to contain polyhedra after being exposed to six dpi *E.cautella* larvae. However, only *X.flavipes* carried viable virus to larval diet after feeding on infected larvae and only after feeding on heavily infected larvae. These results suggest that mechanical carriage of EcNPV by *B.hebetor* and *X.flavipes* is less efficient than their carriage of PiGV.

No *E.cautella* larvae exhibited symptoms of virus infection after attack by a predator which had previously fed on infected larvae. However, one larvae contracted NPV infection after being stabbed by a *N.canescens* adult which had previously stabbed a three dpi larvae. Three dpi larvae are likely to contain large numbers of virus particles and it is therefore possible that infection in the healthy larva was by injection of virus particles on the contaminated ovipositor. However, further work needs to be done in order to verify this result. Nevertheless, the low rate of virus transmission obtained in this study suggests that neither parasitoids nor predators are likely to be of any significance in the transmission of EcNPV.

CONCLUSION

BV infection caused an increased mobility of immature larvae of both E.cautella and P.interpunctella, which could increase the dispersal of virus. There were however, two small differences between the virus-induced mobility of the two species; proportionally fewer immature E.cautella larvae emigrated than in the parallel P.interpunctella experiments and the timing of emigration of infected E.cautella larvae was more distinct than that of P.interpunctella larvae.

E.cautella is unlikely to be effective as an alternative host to PiGV as an unrealistically high dose would be necessary to initiate infection. However, EcNPV applied to grain at a concentration appropriate to control E.cautella pests would probably achieve control of both species.

E.cautella is susceptible to attack by the same predators and parasitoids as P.interpunctella. The presence of NPV in the smears of adult X.flavipes and female B.hebetor revealed that they will feed on NPV-infected E.cautella larvae. However, B.hebetor was not effective and X.flavipes was only moderately effective in causing contamination of larval diet with viable virus after feeding on infected E.cautella larvae. X.flavipes was not shown to transmit virus by injection. However, one larva succumbed to infection after being probed by the ovipositor of N.canescens which had previously probed a three dpi E.cautella larvae. It is suggested that X.flavipes and the parastoids tested are unlikely to be important in the dissemination of EcNPV except at very high densities when an initial introduction of virus could act as a source of primary inoculum in a previously virus-free population.

CHAPTER 6 *Mamestra brassicae* and carabid beetles

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INTRODUCTION

Mamestra brassicae and its Nuclear Polyhedrosis Virus

Mamestra brassicae L. (Lepidoptera: Noctuidae) is common throughout Europe and Asia. It is a pest of a wide range of crops particularly brassicas. The damage to crops is caused by feeding of the six larval stages on the leaves and the accummulation of frass. *M.brassicae* is usually univoltine in Britain although in warm years it can exhibit a partial second generation.

The biochemical and morphological characteristics of the baculovirus of *M.brassicae* a multiply enveloped nuclear polyhedrosis virus are described in the Appendix.

Evans (1981, 1983) provided detailed information on the dosage-mortality responses of *M.brassicae* larvae which showed that larval susceptibility to virus infection decreased with larval weight. Evans and Allaway (1983) studied the dynamics of virus growth and the dispersal of larval populations of *M.brassicae* in small cabbage plots. Their aim was to quantify the role of NPV in the population dynamics of discrete populations of *M.brassicae* larvae. They showed that infected *M.brassicae* exhibited enhanced activity compared with healthy larvae. During their trials, carabid beetles were caught in pitfall traps within the cabbage plots. A few of these carabids were found to have infective polyhedra in their gut.

The aim of this present study is to investigate the potential role of carabid beetles in dispersal of *M.brassicae* NPV.

The *M.brassicae* larvae used in the following experiments were from the same culture as those described by Evans (1981). The method for individually dosing larvae with virus is described by Evans (1981).

Carabid beetles 1. General

Carabid beetles tend to form relatively stable populations. Extreme specialisation is rare among the predatory carabids and the majority of species consume a broad spectrum of prey (Thiele, 1977). They, therefore, constitute a reservoir of potential general predators. They adapt to the prey complex available and therefore, constitute a reservoir of potential general predators. Carabids have been reported as preying on many insect pests, for example, M.brassicae (Oku and Kobayashi, 1973), Operophtera brumata L. pupae (East, 1974), Erioischia brassicae Bouché eggs (Mitchell, 1963), Pieris rapae L. larvae (Dempster, 1967) and aphids (Dunning et al., 1975). The suitability of prey will depend partially on the relative size of the predator and prey. It is therefore probable that only the larger carabids will attack the older larval instars of M.brassicae (Figure 1a). Similarly, due to their greater daily food requirements, the larger carabids have more potential for carriage of large quantities of BV from diseased prey.

Several species of carabid are able to climb plants in search of prey. Dempster (1967) observed that Harpalus rufipes De Geer and Trechus quadristriatus Schrank climbed Brussel sprout plants, the former was also active on oat plants (Loughridge and Luff, 1983). Demetrias atricapillus L. readily climbed cereal plants in search of aphids (Cory, 1984). Carabus species climbed potato plants in search of slugs (Scherney, 1955) whilst Calosoma sycophanta L. was found in trees in pursuit of Lymantria dispar L. larvae (Capinera and Barbosa, 1975). Dunning et al. (1975), by use of a fluorescent powder tested the climbing ability of several species on sugar beet plants. Pterostichus melanarius Illiger was the most active climber tested while Agonum dorsale Pont., Amara familiares, Notiophilus biguttatus Fabr., T.quadristriatus and Bembidion lampros Herbst also showed climbing ability.

Baars (1979) used radioactive-labelled carabids to trace their mobility. The normal locomotor activity showed periods of small distances covered per day in random directions alternating with periods of directed movement with large distances covered per day. In an unfavourable habitat directed movement occurred more frequently and more extremely but in localized regions of abundant food, carabids

aggregate by kinesis, that is by a decreased average speed of movement and an increased rate of turning (Mitchell, 1963).

The mobility of carabids is affected by ground cover (Speight and Lawton, 1976), population density (Ericson, 1978) and species (Greenslade, 1964a). Thiele (1977) considered from a literature survey that average velocities of dispersal for most carabids were of the order of a few metres per 24 hours. However, individual carabids were able to traverse much greater distances. Baars (1979) tracked an individual P.versicolor Sturm that travelled 126 metres in one day. The maximum distance travelled by P.chalcites Say in Best et al's (1981) study was 91 metres per day, although the average was only 8.5 metres per day.

Even without flight, exchange of individuals between subpopulations and dispersal out of the habitat are common. Many species have lost the ability to fly and some have become dimorphic.

P.madidus Fabr. is able to act as a carrier of trichinous infection after feeding on trichinous carrion (Kullmann and Nawabi, 1971). The larvae and capsules of the worms are excreted undigested in the faeces. Capinera and Barbosa (1975) found that the faeces of field-collected C.sycophanta contained enough NPV to induce mortality when ingested by gypsy moth larvae. Entwistle (1982) in his review reported that passage of BV through a predator's gut has been demonstrated in the insect orders Orthoptera, Dictyoptera, Hemiptera and Coleoptera. Birds and mammals after predation on diseased prey have also been implicated in BV spread (Entwistle et al., 1977 a, b; Lautenschlager and Podgwaite, 1979). The survival of BV through gut passage seems variable and Entwistle (1982) suggested that survival was high for predatory insects but low for vertebrates. In contrast breakdown of BV inclusion bodies is rapid in the host gut which is attributed to the strongly alkaline pH of the midgut of Lepidoptera

and sawflies.

The high mobility of carabids and their potential as both predators of M.brassicae and carriers of its NPV led to the present study.

2. Pterostichus madidus, Nebria brevicollis and Harpalus rufipes

P.madidus, Nebria brevicollis Fabr. and H.rufipes are all omnivorous. P.madidus and N.brevicollis are mainly predatory (Greenslade, 1964b) while H.rufipes is mainly herbivorous (Ericson, 1978) and only consumes approximately half animal matter (Thiele, 1977). However, Dempster (1967) working with Pieris rapae (L.) on Brussel sprout plants attributed much of the larval mortality to predation by H.rufipes on the plants. P.melanaria which is closely related to P.madidus was not found active on plants but M.brassicae serum was present in their guts, Dempster (1967) assumed that predation was of larvae which strayed on the ground. Similarly P.madidus was shown to be a pupal predator of winter moth on the ground at Wytham, Oxford (East, 1974).

All three species as adults are usually nocturnal (Luff, 1978). However, although P.madidus is nocturnal in woodland it is mainly diurnal in open grassland (Williams, 1959). P.madidus and N.brevicollis are both found in woodland and grassland (Greenslade, 1964b) while H.rufipes is found mainly in open grassland (Thiele, 1977).

The three species generally breed in the autumn and the larvae overwinter (Thiele, 1977; Greenslade, 1964b). However, some adults overwinter, and breed in the spring (Luff, 1973; Loughridge and Luff, 1983; Greenslade, 1964b). P.madidus adults are predominantly active during July and August, while those of N.brevicollis are most active in autumn and H.rufipes are active from April to November. The adults of N.brevicollis emerge in May and June and, after a brief activity, pass through a summer diapause, reappearing from September onwards (Greenslade, 1964b).

EXPERIMENTS

A. The carabid population

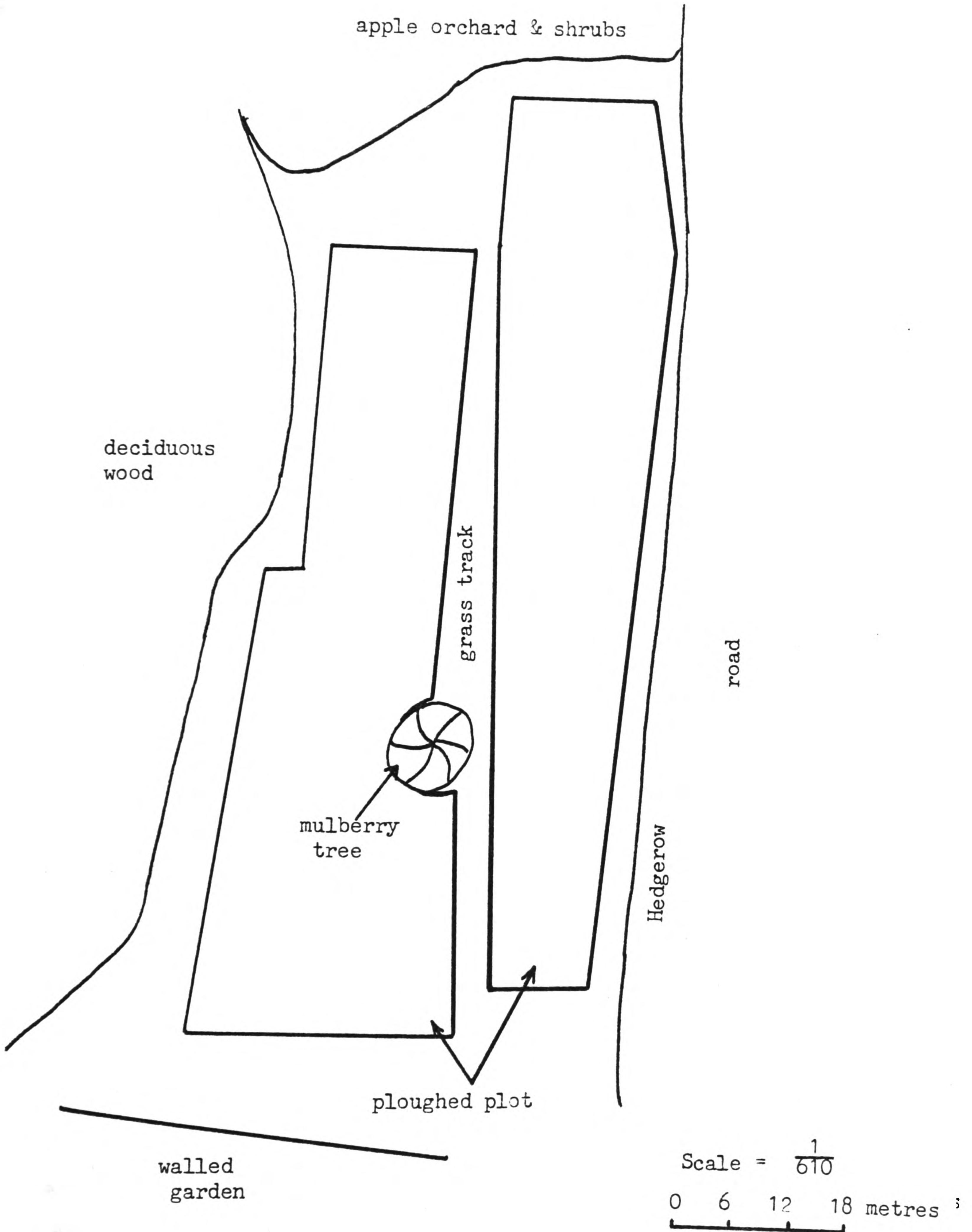
1. Materials and Methods

The field work for this topic was situated near Wytham Wood,Oxford. The study area was bordered by deciduous woodland, a small apple orchard, a mixed hedgerow alongside a narrow road and on the fourth side by a walled garden (Figure 1.). The plot itself was a ploughed field which supported a weed cover.

Pitfall traps were used extensively during the investigation. These consisted of 180ml plastic drinking cups which had several small holes punched into the base to facilitate drainage. These were sunk into the ground so that the rim was flush with ground level. The position of the traps was indicated by canes. Baits or killing solutions were not used in the traps. After the preliminary trials it was found necessary to cover the traps with a basket of 1cm diameter wire mesh to prevent predation on the captives by birds and small mammals. The use of the mesh covering did not significantly reduce the number of species of carabids caught as stipulated for the individual trials.

The traps were emptied regularly. The contents of each trap were counted and identified before either release or removal to the laboratory. Only healthy undamaged carabids were kept for further work. These were separated according to species and kept in plastic boxes containing moist compost. They were fed cooked minced beef and kept at 6°C.

Figure 1 Diagram of the plots used in the field trials



2. Preliminary examination of the carabid population (May-June, 1981)

Thirty pitfall traps were located throughout the plot. These were emptied at one to three day intervals for three weeks. The carabids caught were counted and identified. The result for the total carabid catch is listed in Table 1. Other catches included wolf spiders, millipedes and staphylinid beetles.

P.madidus, representing 35 percent of the total, was the species caught most frequently. Two other species frequently caught were *H.rufipes* and *N.brevicollis*, representing 32 and 13 percent of the total respectively.

The smaller carabids, particularly *Bembidion* and *Trechus* species, were probably under-represented in the catch. This is because they frequently became prey to the larger carabids in the trap and were occasionally able to escape through the drainage holes.

The proportions of the species caught do not necessarily represent the proportions of those species in the population. The catch in pitfall traps is dependent primarily on the population density and activity of each species (Greenslade, 1964a). Other factors which influence the catch are the ability of carabids to escape predation within the trap and groundcover.

The studies were primarily concerned with the three most abundant species caught, ie. *P.madidus*, *H.rufipes* and *N.brevicollis*.

P.madidus was present as two variants, the predominant with black legs and the other with red legs. The sexes are distinguishable easily for both *P.madidus* and *H.rufipes* by examination of the tarsi on the forelegs. In the male the tarsi were dilated whilst in the female the tarsal segments were narrower. Dissection was necessary to differentiate between the sexes of *N.brevicollis*. None of these species were observed to fly in the area studied.

Table 1. Carabids caught in pitfall traps May-June 1981

Species	Number caught	Proportion of total catch
Pterostichus madidus Fabricius	253	35.0
Harpalus rufipes DeGeer	234	32.4
Nebria brevicollis Fabricius	92	12.7
Amara species	41	5.7
Bembidion and *Trechus* species	31	4.3
Loricera pilicornis Fabricius	19	2.6
Notiophilus biguttatus Fabricius	13	1.8
Harpalus aenens Fabricius	11	1.5
Abax parallelus Pill et Mitt.	10	1.4
Pterostichus cupreus Linnaeus	9	1.2
Carabus nemoralis Müller	8	1.1
Agonum dorsale Pontoppidan	1	0.1
	722	100.0

3. Mark-recapture studies

Methods

a) P.madidus and H.rufipes June 1981

Forty-seven pitfall traps were positioned on a regular grid spacing as shown in Figure 2a, with a further five in out-lying positions of the whole plot.

Adult carabids were marked on the elytra using enamel paint. The marked carabids were kept in 300ml tubs containing minced beef and damp tissue in batches of ten at 6°C for a few hours until shortly before release.

The marked carabids were released at the central pitfall, position number 26, which was covered for 24 hours to prevent immediate recapture.

One hundred and ten H.rufipes and one day later 100 P.madidus were released. The pitfall traps were emptied daily for seven days, all marked and unmarked carabids were noted.

b) N.brevicollis October 1981

Twenty-eight pitfall traps were positioned in grid, a further 13 out-lying traps were positioned as shown in Figure 2b.

Adult carabids were marked and kept as described above.

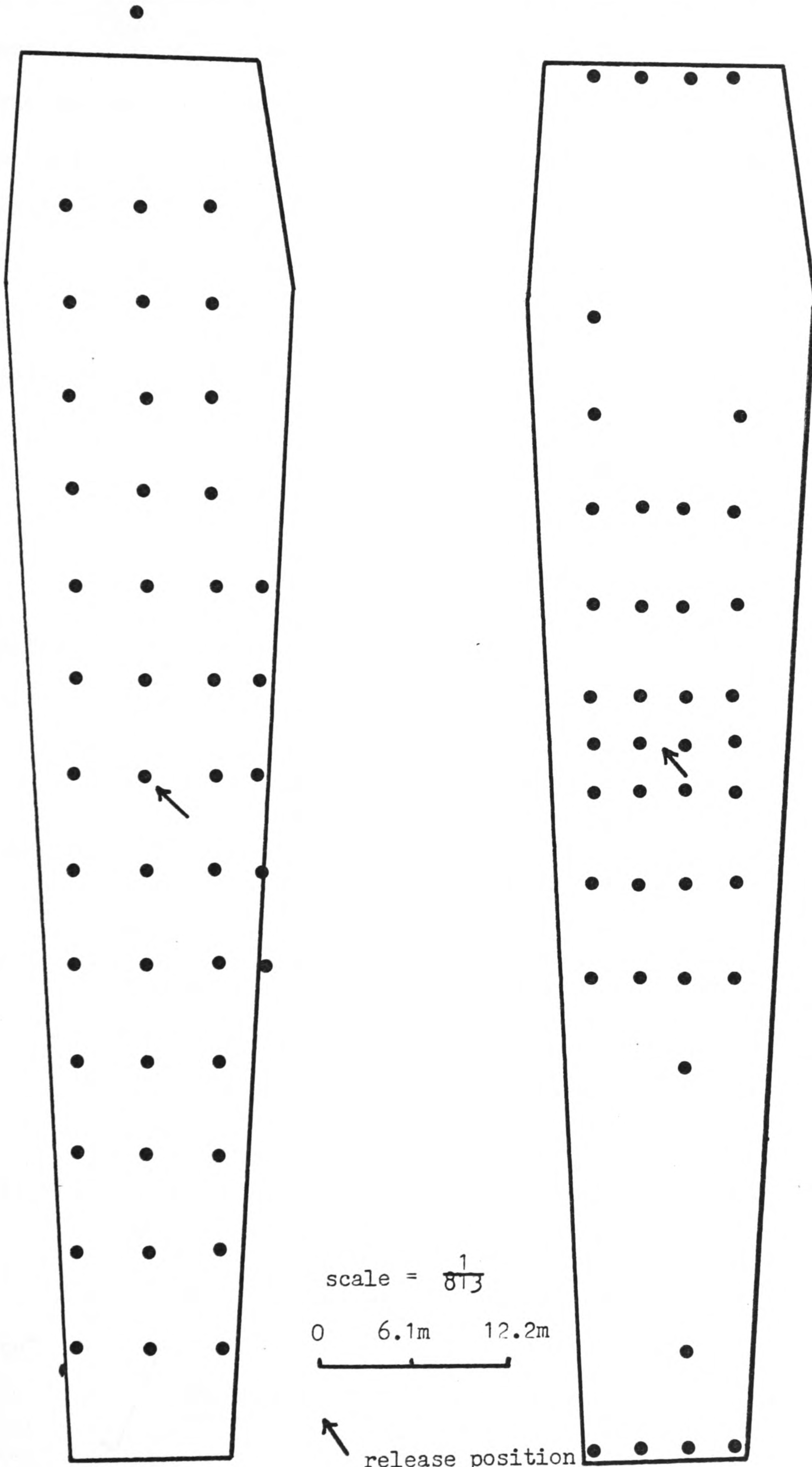
One hundred and ten marked N.brevicollis were released halfway between the central pitfalls, 14 and 15. The pitfalls were emptied at the same time every one or two days for 15 days. The captives were identified, counted and examined for marks.

Analysis

The number of captures and recaptures for each species was totalled for each day. The data were analysed by use of two different models to obtain estimates of the population density and the standard error of these estimates.

The simplest model used was Bailey's modification of the Lincoln

Figure 2 Diagram of the right-hand plot to show positions of pitfall traps used in mark-recapture studies



Index (Begon, 1979). The catch over the whole trapping period was summmated before analysis by this model, which assumes that the proportion of marked to unmarked individuals remains the same.

The second model was Jackson's positive method as described by Begon (1979), which requires several recaptures and gives an estimation of the birth-rate.

The equations are given below.

a) Bailey's modification of the Lincoln Index

$$\hat{N} = \frac{r(n+1)}{m+1} \qquad SE_{\hat{N}} \sqrt{\frac{r^2\ (n+1)\ (n-m)}{(m+1)^2\ (m+2)}}$$

b) Jackson's positive method

$$\ln\ (1-b) = \frac{\Sigma\ m_i\ (\ln q_i - \overline{\ln q})\ (i-\bar{i})}{\Sigma m_i (i-\bar{i})^2}$$

$$\ln q_o = \overline{\ln q} - \ln\ (1-b)\bar{i}$$

$$q_o = \frac{r_o}{N_o} \qquad \hat{N} = \frac{r_o}{q_o}$$

$$SE_{\ln(1-b)} = \sqrt{\frac{\Sigma m_i\ [\ln q_i - \overline{\ln q}\ -\ \ln\ (1-b)\ (i-\bar{i})^2]}{(n-2)\ \Sigma m_i\ (i-\bar{i})^2}}$$

$$SE_{\ln qo} = \sqrt{\frac{\Sigma m_i (\ln q_i - \overline{\ln q} + \bar{i}\ \ln\ (1-b))^2}{(n-1)\ \Sigma m_i}}$$

Notation

N	estimate of population size
r_i	number of marked individuals released on day i
n_i	number of individuals captured on day i
m_i	numbers of marked individuals caught on day i
b	birthrate per day in the populations
q_i	proportion of the day i sample carrying a mark
N_o	population size on day 0

Results

a) P.madidus and H.rufipes

I. Population density

The number of captures and recaptures are recorded in Table 2. The number of recaptures was very low and there were no recaptures after day three. Only the captures during the first three days were used to obtain estimates of the population density, which are presented in Table 3.

The total size of the main recapture area was 1170 square metres.

Table 2. The captures and recaptures of beetles, June 1981
H.rufipes

Day i	Release r_i	Total catch n_i	Marked catch m_i	q_i $\left(q_i = \frac{m_i}{n_i}\right)$	$\ln q_i$	
0	110					
1		39	1	0.026	-3.664	
2		57	2	0.035	-3.350	
3		54	1	0.019	-3.989	$\bar{i} = 2$
4		33	0			
5		3	0			
6		7	0			
7		0	0			

P.madidus

i	r_i	n_i	m_i	q_i	$\ln q_i$	
0	100					
1		16	1	0.062	-2.773	
2		39	7	0.179	-1.718	
3		22	1	0.045	-3.091	$\bar{i} = 2$
4		6	0			
5		5	0			
6		4	0			

Table 3. Results table for population density estimates using marked beetles

Jackson's positive method	population density (m^{-2})		birth-rate (day^{-1})	
	estimate	SE	estimate	SE
P.madidus	0.78	0.75-0.81	0.147	
H.rufipes	2.66	2.55-2.77	0.150	0.146-0.154

Bailey's modification of the Lincoln Index	population density (m^{-2})	
	estimate	SE
P.madidus	0.67	0.48-0.85
H.rufipes	2.84	1.70-3.98

II. Distance dispersed

The maximum distance dispersed as shown by recaptured beetles was the same for both species and was recorded on day two. A female H.rufipes and two male P.madidus were caught in different traps at a distance of 30.8 metres from the release point. These results gave a maximum recorded distance dispersed of 15.4 metres per day for both species.

Table 4 gives the average distance from the release point where marked beetles were caught. The recaptures in the first day were only one for each species. It is therefore impossible to obtain a value for the average distance dispersed on the first day of release. However by summing the values over the three days of recapture, it is possible to arrive at crude estimates of the average recorded distance dispersed by marked beetles from the release point per day.

The results are given below.

P. madidus 7.0 metres per day

H.rufipes 11.1 metres per day

Table 4. Average distance of recaptured beetles from the release point

P.madidus

Day after release	Number recaptured	Average distance (m)	Average distance per day
1	1	14.3	14.3
2	7	12.7	6.4
3	1	12.2	4.1
			$\bar{x}$ = 7.0

H.rufipes

Day after release	Number recaptured	Average distance (m)	Average distance per day
1	1	7.6	7.6
2	2	22.5	11.3
3	1	42.7	14.2
			$\bar{x}$ = 11.1

III. Dispersion of P.madidus and H.rufipes populations

The data from the pitfall catches can be used to test if the dispersion of the two beetle populations throughout the plot could be attributed to chance.

By ommission of one pitfall trap on each of the five rows where there were four instead of the usual three pitfall traps per row, it was possible to divide the pitfall traps into three equal sectors. These were designated the left sector which was positioned nearest the woodland, the central sector, and the right sector which was alongside the hedgerow. Summation of the catch of marked and unmarked beetles in each sector gave the results recorded in Table 5. The results were analysed using a chi-square test comparing the results with those of an evenly distributed population.

Table 5. The distribution of P.madidus and H.rufipes in the three sectors of the plot

P.madidus

		Sector 1	Sector 2	Sector 3	Total
catch	observed	43	32	24	99
	expected	33	33	33	99
χ^2		3.03	0.03	2.45	χ^2_2=5.52^{NS}

H.rufipes

		Sector 1	Sector 2	Sector 3	Total	
catch	observed	35	47	81	163	
	expected	54.33	54.33	54.33	162.99	
χ^2		6.88	0.99	13.09	χ^2_2=20.96	p = 0.001

P.madidus was caught in greater numbers nearer the woodland than alongside the hedgerow, however this was not statistically significant. It is concluded that the distribution of the population of *P.madidus* within the plot could be attributed to chance.

H.rufipes was captured in increasing numbers the nearer the sector to the hedgerow. This was statistically significant at the 99.9% level. It is concluded that the population of *H.rufipes* was unevenly distributed within the plot and showed a positive bias towards the hedgerow.

B. *N.brevicollis*

I. Population density

The results for the numbers of captures and recaptures are shown in Table 6. There were no recaptures on day 3 nor after day 5. The results for the first six days are summed to give values for two discrete time intervals of three days, this avoids any zero returns and aids analysis. The summed results are given in Table 7 and were used in analysis.

The total size of the main recapture area was 446 square metres.

The population density estimate and its standard error are given in Table 8.

Table 6. The captures and recaptures of N.brevicollis, October 1981

Day	Release (r)	Total catch (n)	Marked catch (m)
0	110	18	10
1		16	7
2		7	0
3		10	4
5		2	0
6		2	0
8		1	0
10		9	0
13		1	0

Table 7. The captures and recaptures of N.brevicollis, using three day time intervals

i	r_i	n_i	m_i	q_i	$\ln q_i$	
0	110					
1		41	17	0.415	-0.880	$\overline{\ln q} = -0.990$
2		12	4	0.333	-1.099	$\bar{i} = 1.5$

Table 8. Population density estimate and birth-rate estimate for N.brevicollis

	Population density (m^{-2}) estimate	SE	birth-rate (3 $days^{-1}$) estimate	SE
Bailey's modification of the Lincoln Index (days 1-6)	0.61	0.51-0.70	-	-
Jackson's positive method	0.48	0.46-0.50	0.20	0.11-0.27

II. Distribution of N.brevicollis in the total population

One of the capture-recapture assumptions for estimating the population density is that the marked individuals became randomly dispersed within the total population. If the released beetles had dispersed in all directions, then after dispersal they can be expected to constitute the same proportion of the total catch in each of four 90° sectors centred on the point of release.

This comparison is shown in Table 9, where differences between observed and expected marked catches can be attributed to chance. It is concluded that the marked N.brevicollis became randomly distributed within the whole population with no bias due to direction of dispersal.

Table 9. The distribution of marked N.brevicollis in the four sectors of the plot

	Sector 1	2	3	4	Total
Total catch	8	18	24	16	66
marked catch observed	2	7	4	8	21
marked catch expected	2.4	6.0	6.8	5.8	21.0
χ^2	0.07	1.67	1.15	0.83	$\chi^2_3 = 3.72^{NS}$

III. Distance dispersed

The maximum distance from the release point where a marked beetle was caught on day one was 18.8 metres. The average distance from the release point where beetles were caught on day one was 6.2 metres. Table 10 gives the values for all the days when recaptures were made, the crude value for the average recorded distance dispersed from the release point from the summation of all three values is 5.5 metres per day.

Table 10. Average distance of recapture of N.brevicollis from the release point

Day after release	Number recaptured	Average distance (m)	Average distance per day (m)
1	11	6.2	6.2
2	12	13.3	6.6
3	0	-	-
5	6	22.4	4.5
6	0	-	-
8	3	27.9	3.5
10	0	-	-
13	2	34.2	2.6
15	1	37.2	2.5
			$\bar{x}$ = 5.5m

IV. Dispersion of N.brevicollis population

The data obtained from the pitfall catches were used to test if the N.brevicollis population was distributed randomly throughout the four areas of the plot. Column one was on the side nearest the woodland and column four was alongside the hedgerow. The total catches for pitfall traps on each of the four columns is compared to the estimated

value of one quarter of the total catch, which would be expected if the distribution of beetles was random. The comparison is shown in Table 11, which shows with 99% confidence that the population was not distributed randomly. There were more N.brevicollis observed in the catches nearest the hedgerows than could be attributed to chance.

If the data for the catches on days one and two are ommitted from the calculations, the comparison is not significant ($\chi^2_3 = 2.24$). It is concluded that after day two the N.brevicollis population was randomly dispersed.

Table 11. The distribution of N.brevicollis in the four columns of the plot

		Columns 1	2	3	4	Total
Total catch	observed	12	14	11	29	66
	expected	16.5	16.5	16.5	16.5	66.0
χ^2		1.23	0.38	1.83	9.47	χ^2_3=12.91 p = 0.01

B. The potential of carabids to act as carriers of M.brassicae NPV

The aim of these studies was to investigate whether carabids can carry viable virus after predation on NPV-infected M.brassicae. The two possible methods of carriage are either by surface contamination or by the voiding of viable virus in the faeces.

The first experiment used three species of carabids but did not distinguish between the two methods of carriage. The second experiment, a refinement of the first, used only one species but by washing some of the carabids before and after feeding reduced the potential of surface contamination in one treatment.

1. Experiment 1. Using three species of carabids

Method

The number and species of carabids used in each treatment are recorded in Table 12. There were three treatments, in two treatments the carabids were given an initial feed of NPV-killed M.brassicae larvae and in the third treatment the carabids received minced beef. During the 15 minutes allowed for the initial feed, P.madidus and H.rufipes voraciously attacked the larvae but N.brevicollis was not observed feeding. However, all three species walked over the cadavers and became surface contaminated with larval haemolymph.

Table 12. The number of carabids in each treatment

Species	Sex	Number used
P.madidus	Male	2
	Female	3
H.rufipes	Male	2
	Female	2
N.brevicollis	(unknown)	3

After the initial feed the carabids were placed in individual wells of a sample plate which contained semi-synthetic diet suitable for Lepidopteran larvae. The carabids in one of the treatments which fed on NPV-killed larvae were provided with a daily supply of minced beef. The other carabids received no food. The wells were covered with damp tissue paper and an inverted plate, which allowed extra ventilation. The carabids were retained in the initial wells for 48 hours and were subsequently transferred to fresh identical wells every 24 hours for a total of six days. During this period the carabids were kept at 14°C. The used plates were stored at -20°C. At the end of the experiment the carabids were kept at -20°C to both kill and store.

The plates and carabids were tested for the presence of viable virus by bioassay.

Bioassay of the plates

The semi-synthetic diet in the wells was allowed to thaw and excess moisture to evaporate. Tissue and minced beef were removed. Ten neonate *M.brassicae* were counted into each well. The plates were covered with a fresh damp tissue paper and an inverted plate. After four days incubation at 22°C, the larvae were transferred to individual polypots containing semi-synthetic diet. Some larvae died before transfer, where possiblethese were examined for the presence of NPV. Any larval smears containing NPV were added to the totals for virus deaths but negative results from smears, missing larvae or larvae too dry to smear were deducted from the original total of ten larvae per well. The larvae kept individually were incubated at 22°C and were checked regularly for death or pupation. Samples of dead larvae were examined for the presence of NPV.

Bioassay of carabids

The carabids were tested for the presence of viable virus as

either a surface contaminant or in the gut.

The individual carabids were shaken vigorously for one minute in 5ml of 0.1% sodium dodecyl sulphate (SDS) solution. The carabid was removed and the suspension was centrifuged to pellet any solid contaminants. The supernatant was discarded and the pellet was resuspended in 200µl of deionized water, which was called the 'beetle washing',

The gut was extracted from each carabid and triturated in a 0.1ml tissue homogeniser using 200µl of deionized water. The suspension was poured into a small vial. The homogeniser was rinsed with a further 100µl of water which was added to the vial. The contents of the vial were known as the 'gut extract'.

A standard volume (100µl) of either 'beetle washing' or a 'gut extract' was spread evenly over the surface of semi-synthetic diet in a polypot. This was repeated for all the test suspensions using individually labelled polypots. The excess moisture was allowed to evaporate before ten neonate M.brassicae larvae were placed in each polypot. After five days the larvae were transferred to 300ml tubs containing semi-synthetic diet. The larvae were kept at 22°C and were checked regularly for death or pupation. Cadavers were examined for the presence of NPV.

Results

The results from the bioassay of the wells contaminated by carabids fed on infected larvae are recorded in Table 13. There was only one infected larva out of a total of 527 from the bioassay of the controls, that is the wells contaminated by carabids fed minced beef only. This infection was probably caused by cross contamination and the level is insignificant when compared to the level of infection obtained in the other treatments. It is concluded that carabids fed on NPV-killed larvae can contaminate their environment with viable virus. All three species and both sexes are capable of carrying virus.

Some of the carabids died within the first few days of the experiment, probably as a result of the high humidity, restricted space and the stage of the life cycle. The data from dead carabids were excluded when calculating regression lines and performing statistical tests.

Table 14 shows that viable NPV was carried by carabids as both an external contaminant and in the gut, although as expected by the end of the experiment few carabids were still contaminated.

Regression equations for the proportion of infected bioassay larvae from the wells against day of the treatment are plotted in Figure 3. The values for the first 48 hours were omitted, because the carabids were not transferred to fresh wells during this time and the bioassay larvae suffered 100% mortality. By extrapolation of the curves it is estimated that the carabids, fed daily, can act as carriers for up to 9.7 days after the initial infection feed, whilst those without a daily provision can only act as virus carriers for up to 7.1 days. This apparent difference was compared using a paired t-test (Table 15), which confirms that the differences could not be attributed to chance.

Table 13. Mean Virus infection in bioassay larvae on diet contaminated by carabids fed NPV-killed larvae

a) Carabids fed minced beef on subsequent days

	Day of contamination										Totals	
	1-2		3		4		5		6			
(I) N.brevicollis	14/14	100%	13/24	54%	4/29	14%	2/30	7%	3/28	11%	36/125	29%
P.madidus ♂	12/12	100%	19/20	95%	11/14	79%	13/16	81%	6/18	33%	61/80	76%
(II) P.madidus ♀	10/12	83%	2/24	8%	2/23	9%	2/29	7%	0/20	0%	16/114	14%
H.rufipes ♂	6/6	100%	10/11	91%	14/14	100%	11/19	58%	6/15	40%	47/65	72%
H.rufipes ♀	9/9	100%	15/19	79%	10/13	77%	12/17	71%	6/16	38%	52/74	70%
Totals	51/53	96%	59/98	60%	41/93	44%	40/111	36%	21/108	20%	212/458	46%

b) Carabids not fed on subsequent days

	Day of contamination										Totals	
	1-2		3		4		5		6			
N.brevicollis	21/21	100%	16/25	64%	7/28	25%	5/28	18%	3/26	12%	52/128	41%
(II) P.madidus ♂	8/8	100%	6/20	30%	3/16	19%	1/17	6%	5/16	31%	23/77	30%
(III) P.madidus ♀	23/23	100%	8/26	31%	7/25	28%	3/28	11%	1/30	3%	42/132	32%
H.rufipes ♂	16/16	100%	7/19	37%	7/17	41%	1/17	6%	1/17	6%	32/86	37%
H.rufipes ♀	13/13	100%	14/17	82%	6/19	32%	3/21	14%	0/19	0%	36/89	40%

Figure 3 Transmission of viable NPV by carabids of various species after a feed on infected larvae

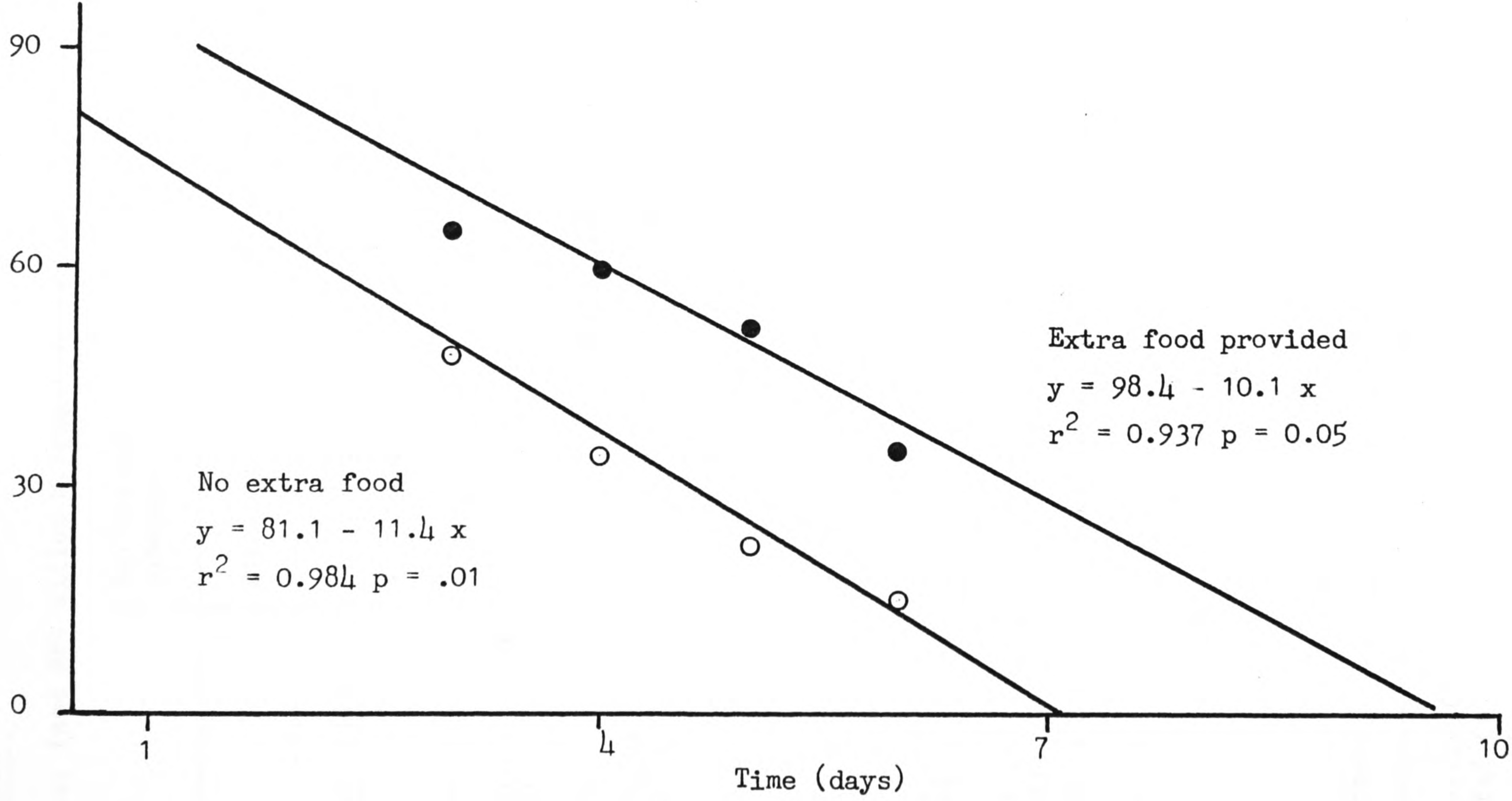


Table 14. Experiment 1. Bioassay of "beetle-washing" and "gut extracts"

Initial feed NPV killed M.brassicae larvae

		Fed minced beef on subsequent days		No food on subsequent days	
		washings	gut extracts	washings	gut extracts
N.brevicollis	1	+	-	-	-
	2	-	+	+	-
	3	-	-	-	-
P.madidus (male)	1	-	-	+	-
	2	-	-	+	+
P.madidus (female)	1	+	+	-	-
	2	+	-	-	+
	3	+	+	-	-
H.rufipes (male)	1	-	-	-	-
	2	+	+	+	-
H.rufipes (female)	1	-	-	-	-
	2	+	-	-	-

\+ Indicates where NPV infections were observed in the bioassay larvae
\- Indicates where no NPV infections were observed in the bioassay larvae

Table 15. Paired t-test comparing the effect of daily feeding on ability of carabids to act as virus carriers after an initial feed on virus-killed larvae

Treatment	Proportion infected* Days after initial feed on virus-killed larvae: 1-2	3	4	5	6
Fed daily (A)	90.00	65.47	60.00	51.53	34.57
Not fed (B)	90.00	48.27	34.54	21.93	14.48
d= A-B	0.00	17.20	25.46	29.60	20.09

$\bar{d} = 18.47$ $\quad s = 11.38$ $\quad$ 4df

$\frac{s}{\sqrt{n}}$ $t = 15.80$ at 5% level

95% confidence interval excludes zero.
(2.67 - 34.27)
(*data: $\sin^{-1}(\text{proportion})^{1/2}$)

Experiment 2. Using P.madidus only

Method

The carabids were caught in pitfall traps during the 24 hours prior to the experiment. They were collected from the traps and kept individually in moist compost at 16° C until they were used in the experiment. Five female P.madidus were used for each treatment.

The carabids were washed by submerging three times in deionized water before the initial feed which lasted an hour by which time the carabids were satiated. Half of the fed carabids (Table 16) were washed individually by shaking for 30 seconds in 5ml of 0.1% SDS solution in a polypot. After washing they were rinsed by submerging in two changes of deionized water followed by transfer into clean polypots until recovery (up to two minutes). Washed and unwashed carabids were placed in individual polypots containing semi-synthetic diet. Half were provided with minced beef as indicated in Table 16.

Every 24 hours for 10 days the carabids were transferred to identical fresh polypots and were kept at 16° C. After 10 days the carabids, which were all still healthy, were removed and frozen. Minced beef was removed from the used polypots, which were stored at 4° C. The contents of the polypots were tested for the presence of viable virus by bioassay using neonate M.brassicae larvae as described previously.

Table 16. The number of treatments for experiment 2

		Initial feed			
		minced beef		NPV killed larvae	
		washed	not washed	washed	not washed
Feeding on subsequent days	minced beef	0	0	1	1
	None	0	1	1	1

Results

The results for the bioassay of the diet in the polypots contaminated by carabids fed virus-killed are tabulated in Table 17. No carabids died and none of the control larvae were infected. It can be seen therefore that female P.madidus adults are capable of acting as carriers of M.brassicae NPV. This was true for both washed and unwashed carabids, thus carriage was probably by both surface contamination and passage of virus through the gut and subsequent defecation of viable virus.

The data were transformed using the angular transformation before computing regression lines and statistical tests. Only data after day four were used for the regression lines because of 100% mortality of bioassay larvae before this. The regression equations for contamination of the diet with viable virus against time after a virus feed for each of the four treatments are given in Table 18. The 95% confidence intervals for the constants and slopes for all four equations overlap, it is therefore concluded that the treatments did not significantly affect the transmission of viable virus by P.madidus females. A plot using the data from all four treatments is given in Figure 4. From extrapolation of the plot, defecation of viable virus takes place up to 13.8 days after a virus feed.

Table 17. Mean virus infection in bioassay larvae on diet contaminated by *P.madidus* females fed NPV-killed larvae

	Days of contamination										
Treatment	1	2	3	4	5	6	7	8	9	10	Totals
Fed daily washed	*90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	86.3±8.2	86.3±8.2	68.3±24.1	60.1±33.5	42.5±33.1	37.4±38.2	68.2±12.9
Not fed washed	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	79.7±15.1	47.9±23.7	45.2±11.5	42.5±16.0	65.7±5.4
Fed daily Not washed	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	86.3±8.2	70.7±20.0	75.8±14.2	75.7±20.7	67.2±18.4	47.6±18.7	71.0±6.9
Not fed Not washed	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	90.0±0.0	74.8±16.3	51.5±18.3	39.0±13.4	35.4±19.8	32.2±18.7	59.8±7.1

* Values expressed as $\sin^{-1}$ (proportion infected) $^{1/2}$ ± standard deviation

Figure 4 Transmission of viable NPV by *P.madidus* after an infected feed

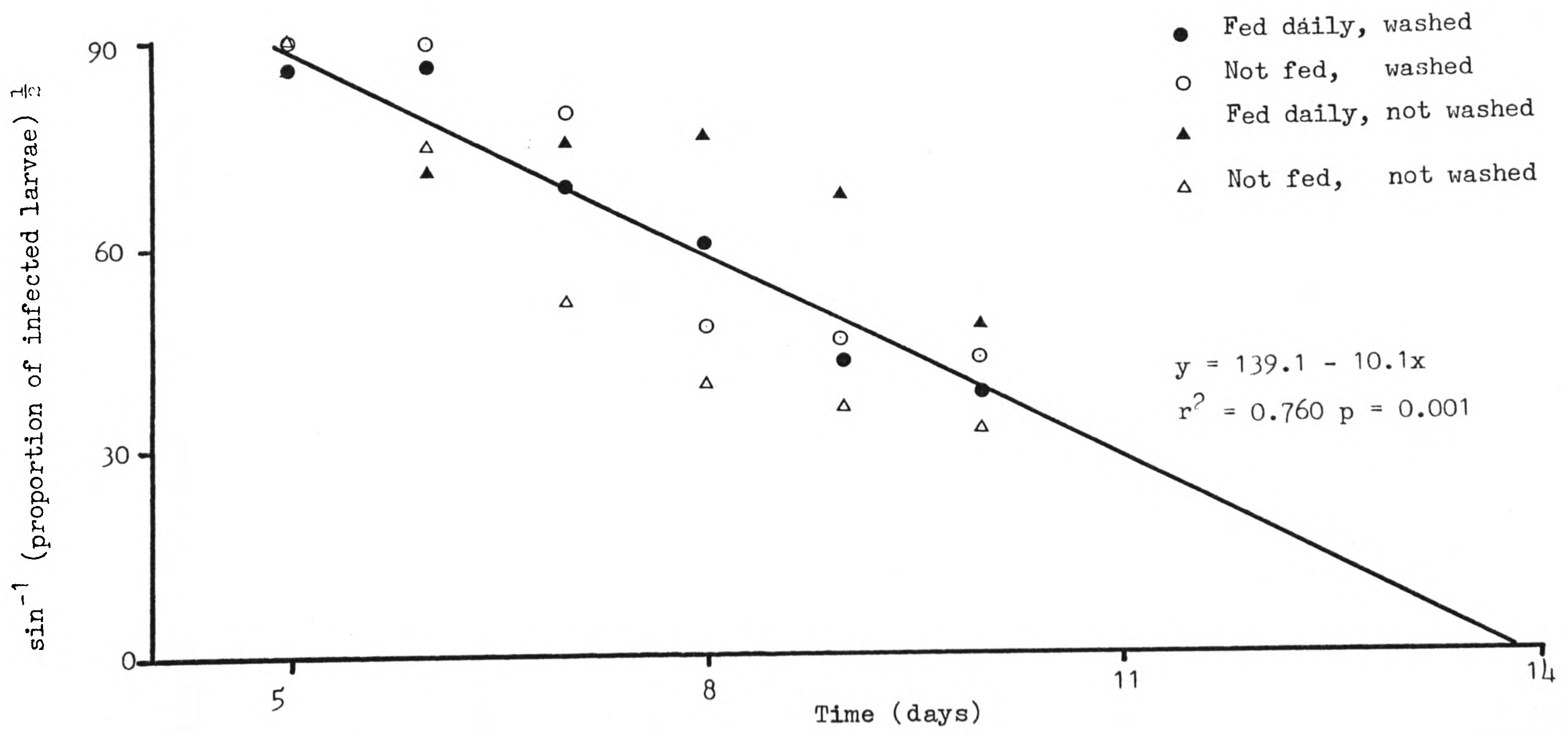


Table 18. Regression curves for Experiment 2 using data between days 5 and 10

Treatment	Equation	r^2 value	Significance	Value of X, when Y = 0
Washed, fed daily	Y=145.8 - 11.0X	0.960	p=0.001	13.3
washed, no food	Y=152.4 - 11.5X	0.878	p=0.01	13.2
not washed, fed daily	Y=114.3 - 5.8X	0.708	p=0.05	19.6
not washed, no food	Y=1.438 - 12.0X	0.905	p=0.01	12.0

$Y = \sin^{-1}$ (proportion of infected bioassay larvae)$^{1/2}$

X = day after carabids infected feed

C. Field trials in Cabbage plots

1. The use of barriers

The aim of the field work was to see if the density of carabid beetles had an influence on the dispersal of M.brassicae larvae and NPV.

One-way barriers were designed so that the beetle numbers could be affected. The barriers consisted of 23cm high strips of plastic which were supported by wooden stakes. The plastic was dug into the soil to a depth of 8cm to discourage burrowing by the beetles. A wall of plastic 15cm high was left above soil level, which was considered high enough to prevent climbing. An embankment of soil was dug up on one side flush with the top edge of the plastic (see Figure 5). This allowed beetles to run up the soil embankment and fall over the barrier, their return journey being restricted by the smooth, steep wall of plastic.

The plot where the soil was banked on the outside of the plastic was known as the 'Ingress Plot' whilst the plot with the soil embankment on the inside was the 'Egress Plot'. In order to further decrease the numbers of carabids in the egress plot pitfall traps were situated at regular intervals along the inside edge of the plot.

The efficiency of the barriers was tested using two small cabbage plots containing a grid of nine cabbages spaced 0.5m apart. The plots were covered with nylon netting to prevent predation by birds, and one-way barriers were constructed. Each plot contained two central pitfall traps which were emptied regularly to monitor carabid activity. The egress plot had a further five pitfall traps along the edge of the plot; these were emptied outside of the plot regularly but the counts from these traps were not included in the comparison between the two plots.

Ten early third instar M.brassicae larvae were placed on each cabbage to act as prey for the carabids.

After 19 days the cabbages were inspected for any remaining larvae, in order to estimate the level of predation. The catches from the central pitfall traps were tallied.

Results

No larvae were found on any of the cabbages at the end of the trial. This may have been a result of predation but more likely was due to the larvae emigrating after being unsettled by handling and the stormy weather which was prevalent during the trial.

Only one carabid beetle was caught in the central pitfall traps of the egress plot compared with 31 caught in the ingress plot. It is concluded that there was an enhanced number of carabid beetles in the ingress plot compared to the egress plot.

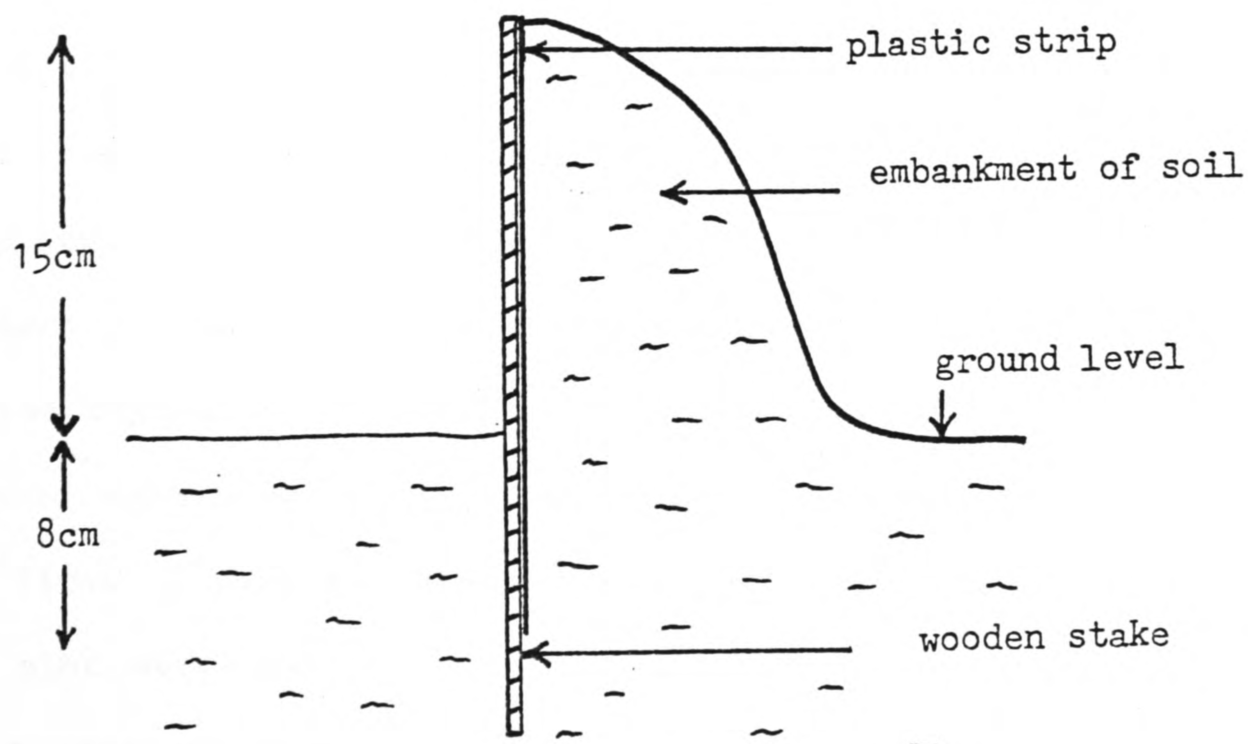


Figure 5. Diagram to show a vertical section through a one-way barrier in the region of a wooden support

2. Large Cabbage plots

Methods

Two plots were constructed consisting of 100 cabbages (var. January King) 0.5m spaced apart in a 10 by 10 matrix. The plots were covered with nylon netting and one-way barriers were constructed around the plots. Each plot had four central pitfalls which were covered with a basket of wire netting. The egress plot had an additional 25 pitfall traps dug at regular intervals along the inside edge. The plots were weeded and any larvae infesting the cabbages were removed before the start of the trial.

The experiment consisted of four parts:

a) Primary dispersal of infected *M.brassicae* larvae

b) Secondary infection of *M.brassicae* larvae

c) Bioassay of cabbage samples

d) Monitoring of carabids

a) Primary dispersal of infected larvae

Early third instar *M.brassicae* larvae with an average weight of 10.4×10^{-3}g were individually dosed with 6.39×10^{5} polyhedra (according to the method described by Evans, 1981). The larvae were kept at 22°C. Two days later 25 of these larvae were placed on each of the four central cabbages of the two plots. However, in the ingress plot cabbage F4 was used instead of F5 (F5 was a stunted cabbage with thick waxy leaves). Every second day, for the first 12 days and again on the 19th day all the cabbages in the plot were checked leaf by leaf for the presence of larvae. Great care was taken to avoid disturbing any larvae found. This provided an absolute sample of the population on the cabbage plants.

b) Secondary infection of *M.brassicae* larvae

Three weeks after the placement of the primarily infected larvae, it was assumed that they had all died. Twenty five neonate *M.brassicae* larvae were then placed in open polypots onto each of the 200 cabbages, a method which allowed the larvae to establish themselves on the cabbages with minimum disturbance. After a further 20 days each cabbage was cut down, placed in a labelled plastic bag and stored at 4°C. Within a few days all the cabbages were checked leaf by leaf. Any Lepidopteran larvae found were transferred to individual polypots containing semi-synthetic diet. The larvae were kept at 22°C and regularly inspected for death or pupation, any dead larvae were examined for NPV.

c) Bioassay of cabbage samples

Throughout the duration of part b) of this trial the weather had been very cool and wet. Consequently, at the time of collection the larvae were still very small and there was a low recovery of larvae. It was therefore decided to further bioassay the cabbages within the laboratory.

Samples were taken from each cabbage and placed in 300ml tubs with semi-synthetic diet. The samples were 2cm wide strips cut from the outer edges of the outside leaves. Twenty-five neonate larvae were placed into each tub. The tubs were covered and kept at 22°C. After five days the larvae were transferred onto fresh semi-synthetic diet. The larvae were checked regularly for death or pupation, those that died being examined for the presence of NPV.

d) Monitoring of carabid beetles

The pitfall traps were emptied regularly and the contents were identified and counted. Carabid beetles were tested for

contamination by M.brassicae NPV by placing then in a polypot containing semi-synthetic diet, which was later bioassayed. After two days at 16°C the beetles were released and the polypots were frozen before bioassay with ten neonate M.brassicae larvae.

Results

a) Primary dispersal of infected larvae

The plan views in Figures 6 and 7 of the two experimental plots show the extent of larval movement away from the central cabbages.

Figure 8 shows the reduction of larval numbers with time. As expected, the reduction was greater in the ingress plot where predation was likely to be at a higher level. However, this large reduction in the numbers of larvae in the ingress plot meant that after only a few days there were very few larvae left, which undoubtably affected later results.

The distribution of the larvae within the plots expressed as distance from the point of introduction over time is shown in Figures 9 and 10. By day 10 half of the larvae in the egress plot still remained on the central cabbages, whilst less than one quarter of the larvae in the ingress plot remained on the original cabbages. This difference in dispersal of the larvae in the two plots is shown more clearly in Figure 11, which gives the average distance of dispersal with time. It would appear that an enhanced number of carabid beetles increased the rate of dispersal of infected M.brassicae larvae.

The accumulated totals of plants with larvae present is given in Figure 12. It would appear that the decreased number of larvae in the ingress plot is matched by their increased rate of dispersal as there appears to be little difference in the rate of colonization of cabbage plots between the two plots. This rate can be described by a linear equation as shown in Figure 12.

Figure 6 PLAN VIEW OF THE DISPERSAL OF NPV-INFECTED LARVAE
1 EGRESS PLOT

A B C D E F G H I J

1 2 3 4 5 6 7 8 9 10

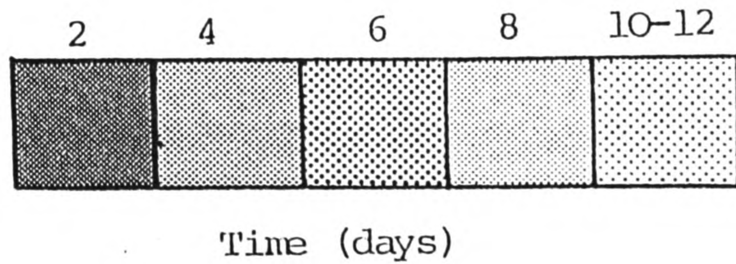


Figure 7 PLAN VIEW OF THE DISPERSAL OF NPV-INFECTED LARVAE
2 INGRESS PLOT

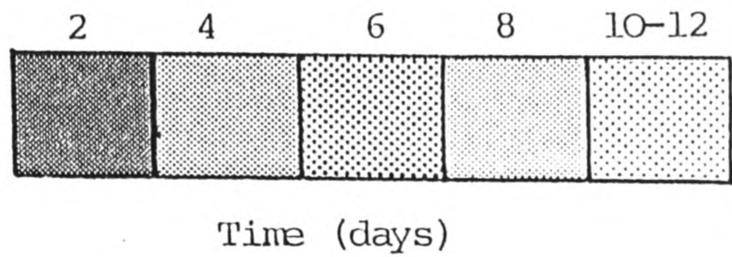


Figure 8

REDUCTION OF LARVAL NUMBERS WITH TIME AND ENHANCED CARABID NUMBERS

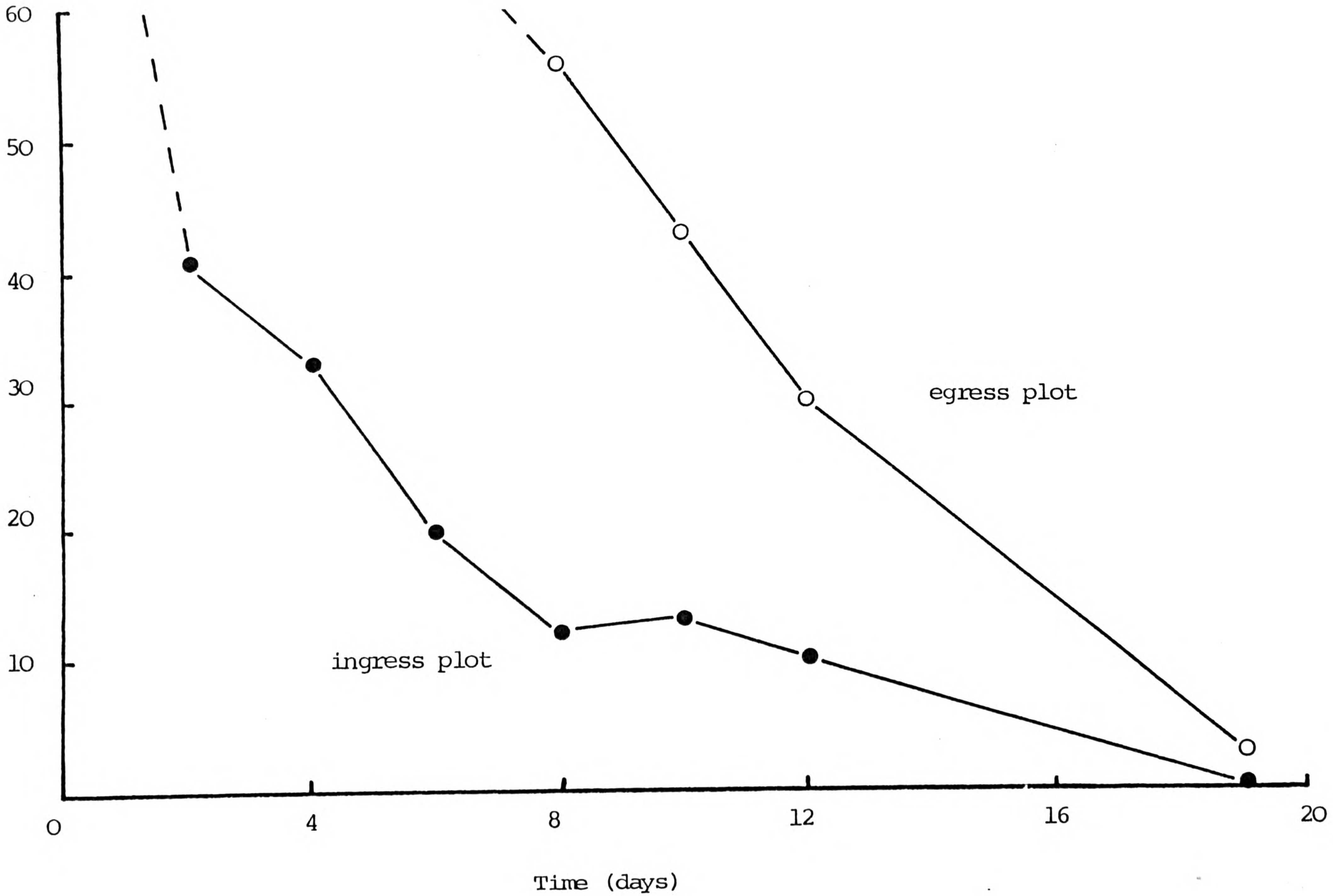
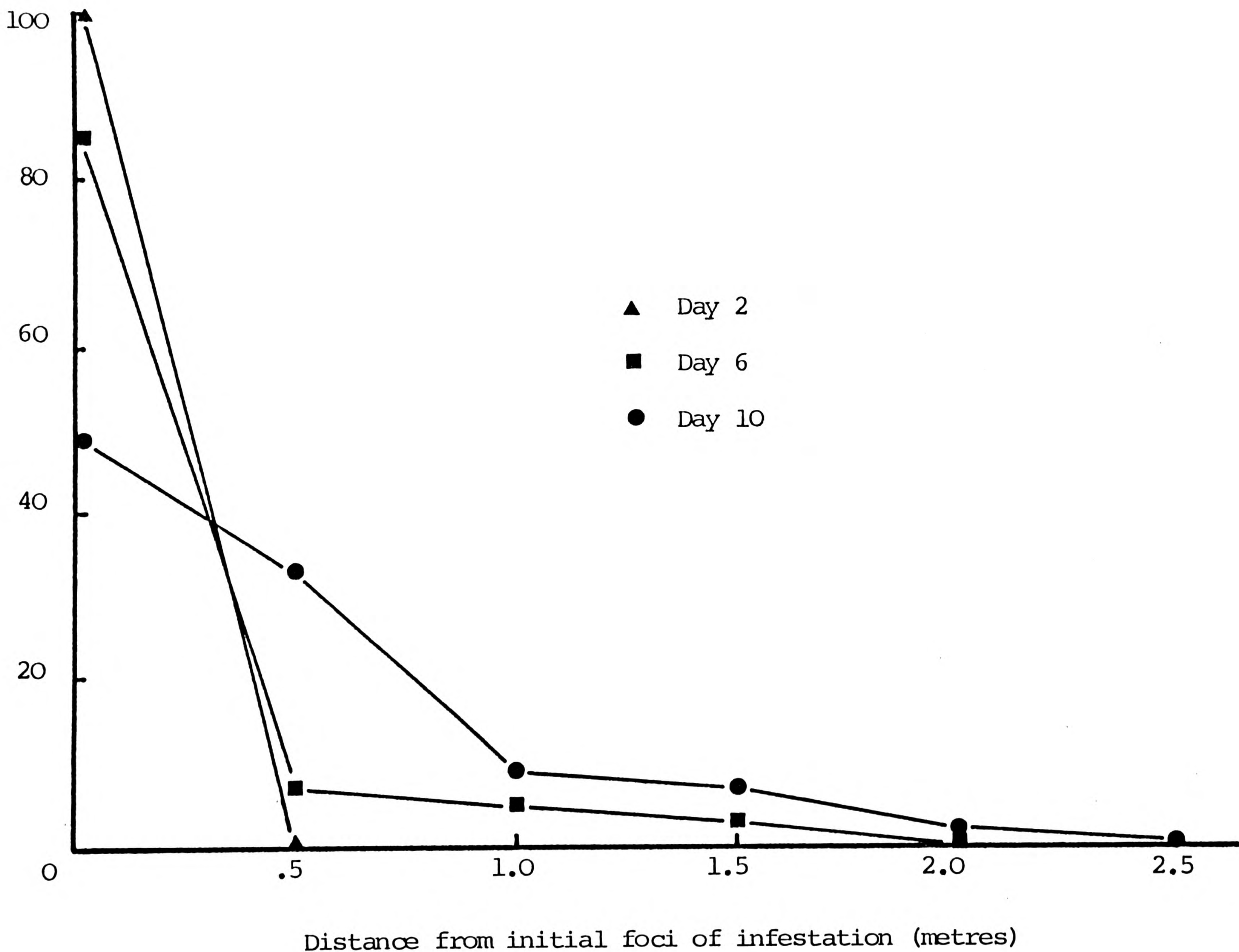


Figure 9

DISTRIBUTION OF NPV-INFECTED LARVAE
1 EGRESS PLOT



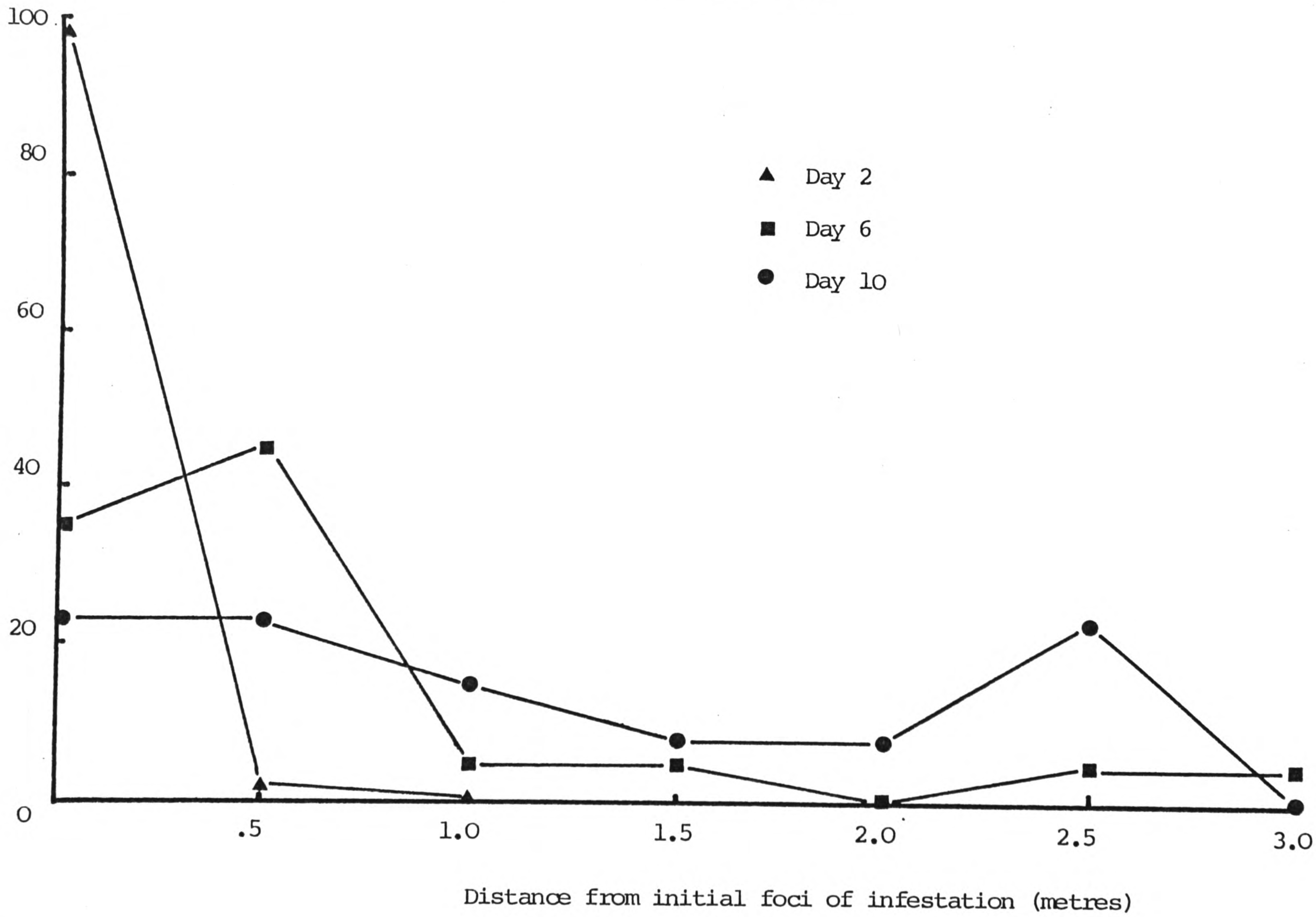


Figure 10
DISTRIBUTION OF NPV-INFECTED LARVAE
2 INGRESS PLOT
Day 2
Day 6
Day 10
% No. of larvae
100
80
60
40
20
0
.5
1.0
1.5
2.0
2.5
3.0
Distance from initial foci of infestation (metres)

Figure 11

AVERAGE DISTRIBUTION OF INFECTED LARVAE WITH TIME

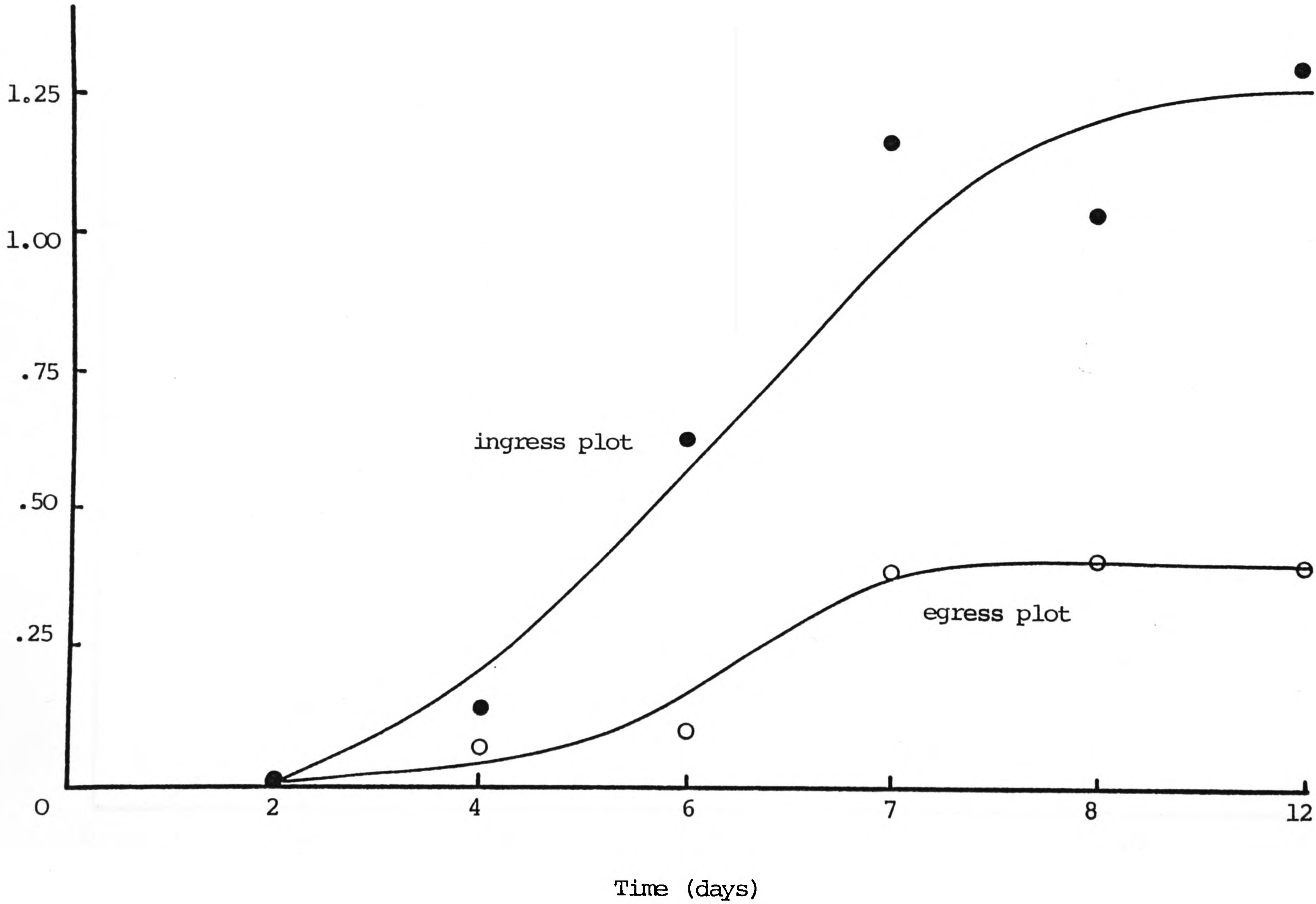
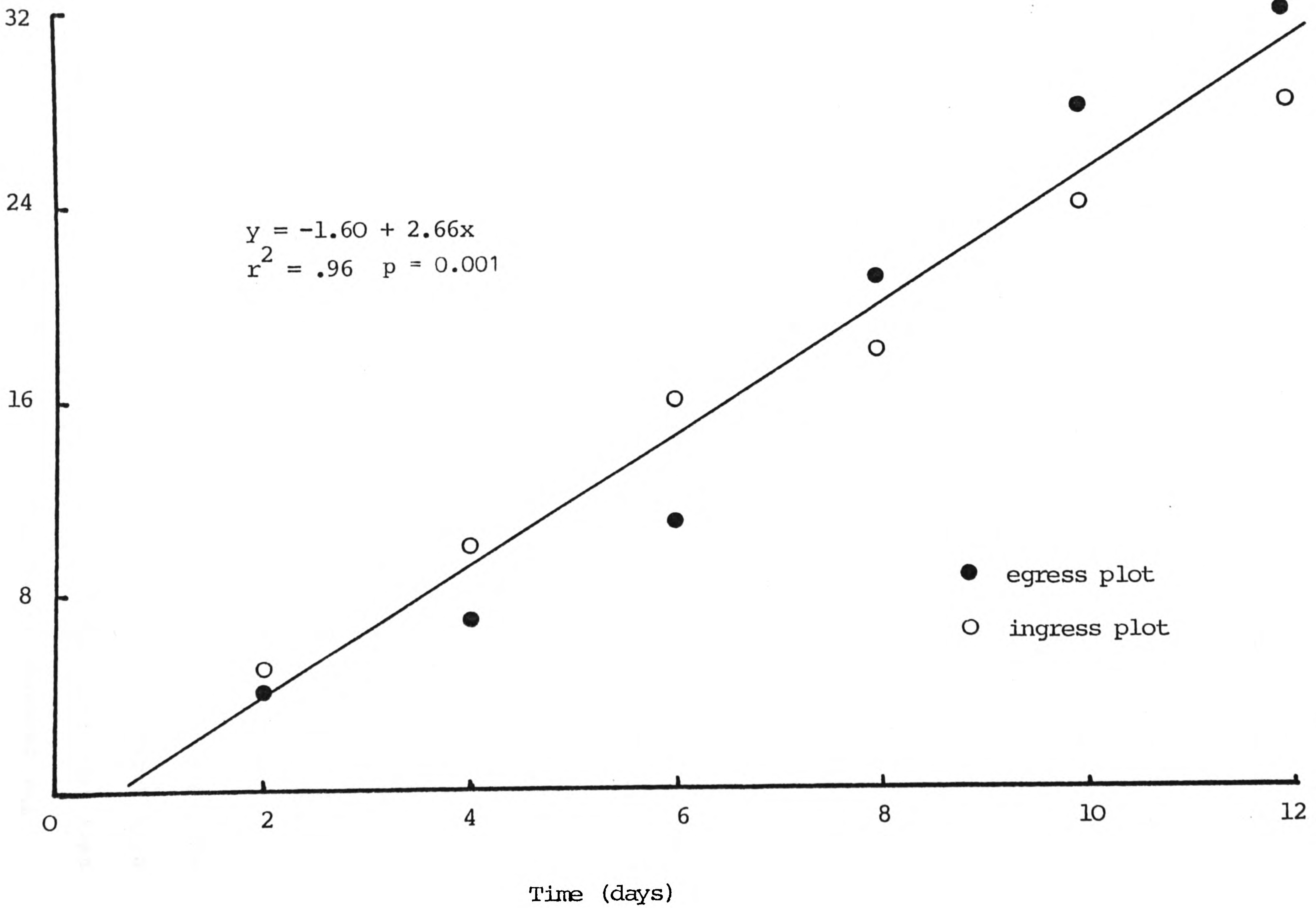


Figure 12

COLONIZATION OF CABBAGE PLANTS



b) Secondary infection of M.brassicae larvae

The recovery of bioassay larvae from the experimental plots was very low. Only 66 M.brassicae larvae were recovered from the egress plot and 31 from the ingress plot. 2500 larvae had been released in each plot. Only two of the bioassay larvae were diagnosed as being virus infected, both were from the egress plot and were found on cabbages B10 and D6.

The other two main species of Lepidoptera found on the cabbages were Noctua pronuba L. and Plutella maculipennis (Curtis). There were seven N.pronuba found in the egress plot and 17 in the ingress plot. Two of the N.pronuba larvae died of a NPV infection, which was later shown to be M.brassicae NPV (see Appendix). The infected larvae came from cabbage C9 in the egress plot and cabbage J1 in the ingress plot. There were two P.maculipennis larvae found in the egress plot and ten in the ingress plot. None of these larvae were diagnosed as being infected with NPV but four were parasitized by Apanteles species.

(N.B. Cabbages F10 and D8 in the egress plot and D6 and 14 in the ingress plot were stolen before the start of Part b).

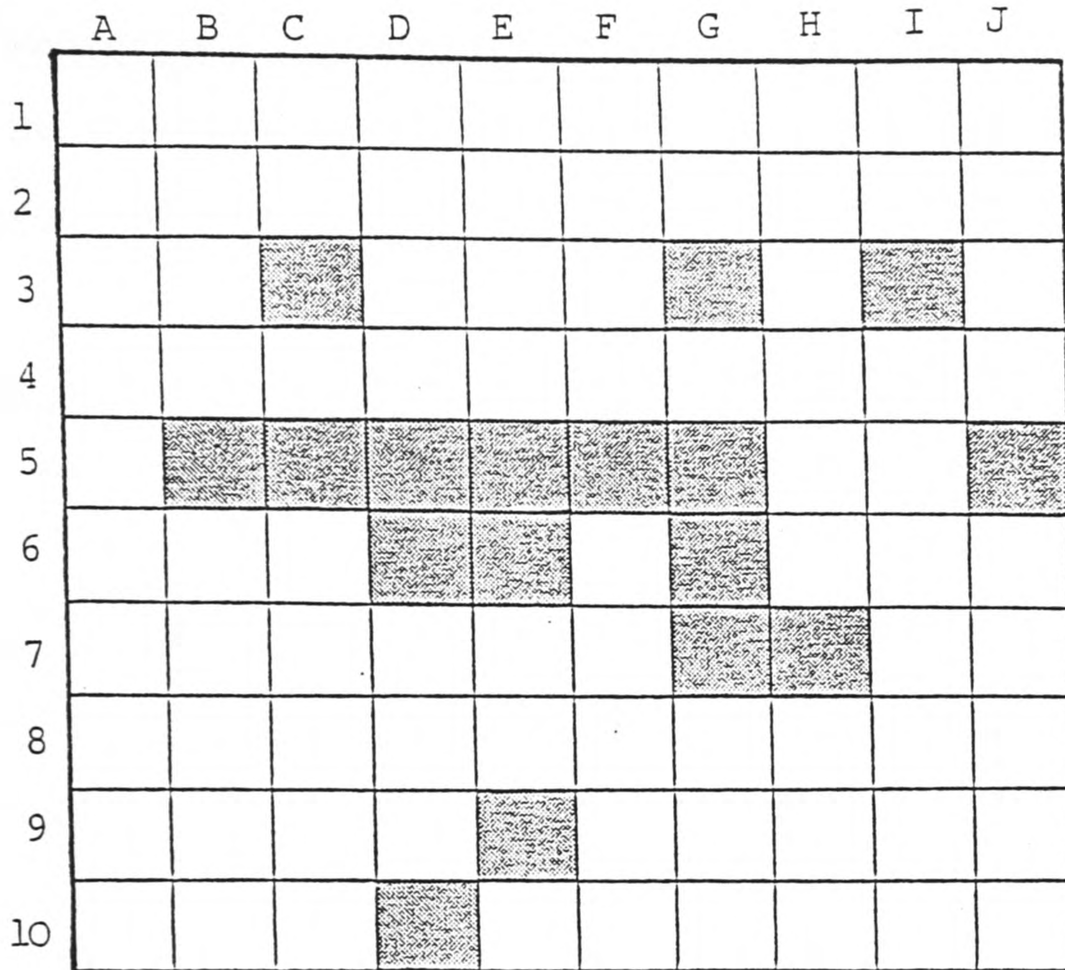
c) Bioassay of cabbage samples

The incidence of virus-infection in bioassay larvae on the cabbage samples is shown in the plan views of the plots in Figure 13. Virus was detected by bioassay on samples from 17 cabbages in the egress plot and on only eight in the ingress plot. A further two NPV contaminated cabbages diagnosed in the bioassay of the whole cabbages but not from the samples can be added to the total of NPV contaminated cabbages of the egress plot to give 19. When these values of 19 and 8 were compared to that expected if the plots had been identical, the difference was found significant at the 95% level ($\chi^2_1 = 4.48$).

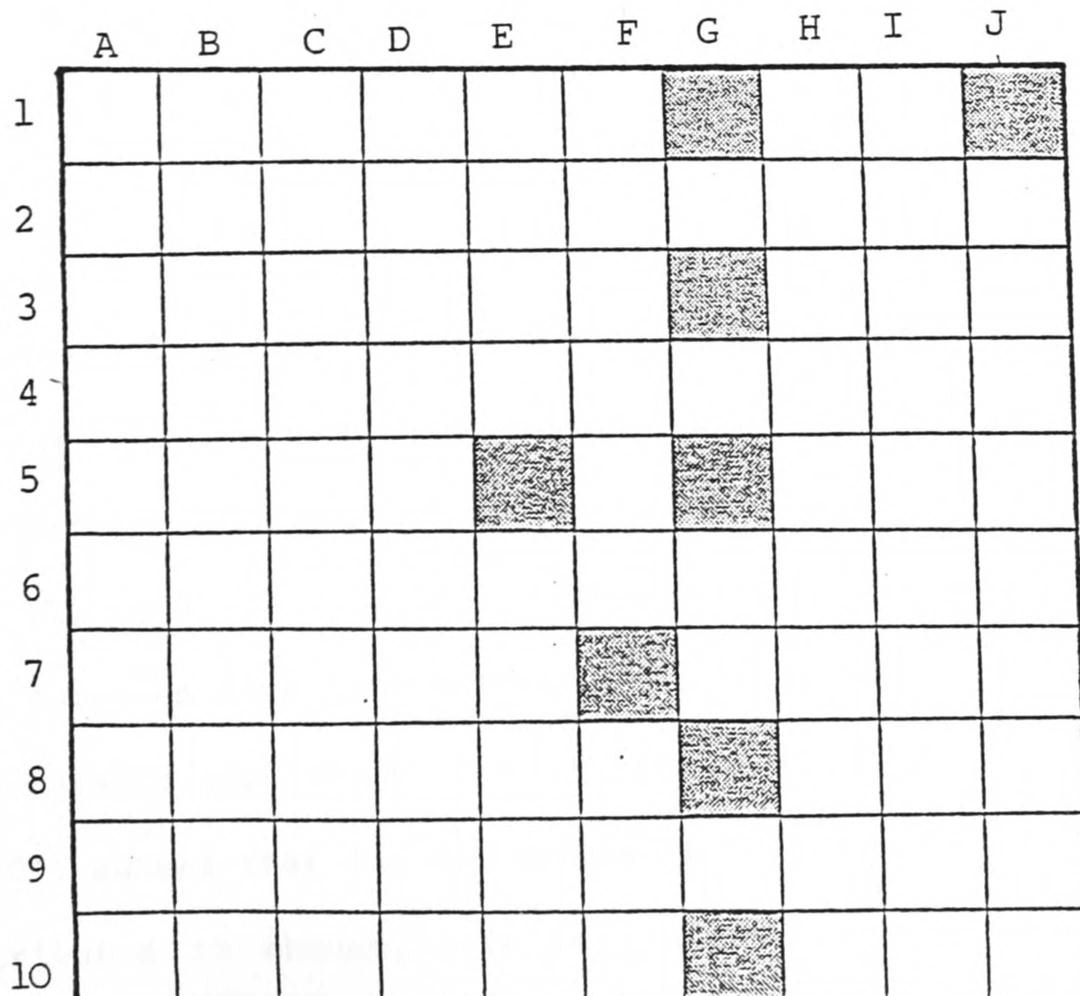
Seven of the NPV positive cabbages in the egress plot and four in

Figure 13 Distribution of secondary NPV infection

1. EGRESS PLOT



2. INGRESS PLOT



the ingress plot had appeared to have no larval cadavers from the primary phase, see Figures 6 and 7. In the ingress plots these cabbages were all on row G.

d) Monitoring of carabid beetles

The total numbers of carabid beetles caught in the central pitfall traps during Parts a) and b) of the trial are shown in Table 19.

Table 19. The numbers of carabid beetles caught in the central pitfalls of the two plots

Stage of the trial	Number of days of trapping	Number of carabids caught	
		Ingress plot	Egress plot
Part a)	19	154 adults	2 adults, 3 *P.madidus* larvae
Part b)	19	13 adults	0
Totals	38	167	2 adults, 3 larvae

The low numbers caught in Part b) when compared with Part a) can be partially explained by the much colder weather experienced during Part b). The activity of the beetles would be less at a lower temperature and therefore capture less probable. A further explanation is that the main season of *P.madidus* adults was at an end by the time Part b) commenced, whilst *N.brevicollis* still had not reached its main peak of activity, which is in autumn.

The total catches of carabid adults and larvae for the two plots was compared with that expected if the barriers had had no effect on the carabid population. The chi-squared comparison ($\chi_1^2 = 152.58$, $p = 0.001$) showed that the difference, with 99.9% confidence, could not be attributed to chance. It is concluded that there was an enhanced number of carabid beetles in the ingress plot when compared with the egress plot.

Only two of the carabids tested caused virus infection in the bioassay larvae. The first of these was a female P.madidus adult caught in the ingress plot when the primary larvae were eight days post infection. The other was a N.brevicollis adult caught in the egress plot during Part 2 of the trial when bioassay larvae were on the cabbages.

D Small Scale Trials

One trial using one square metre cages was performed at the field site at Wytham. A series of further studies were undertaken in the laboratory. These were designed to simulate the field situation but with control over the variables of weather and predation. It was hoped that by using these simplified trials it would be possible to demonstrate the potential of carabid beetles in both the predation of M.brassicae larvae and the dispersal of the virus.

The laboratory studies involved the use of tubs. These were of two sizes; water tanks of dimensions 400 by 600mm with a surface area of 0.24 square metres and packing cases 570 by 370mm with a base surface area of 0.21 square metres. A depth of 80 to 100mm of compost was poured into each tub and cabbages at about the nine leaf stage were planted directly into the compost shortly before the start of each experiment. When the treatments had been set up muslin was placed over the top of the tub and secured with tape. The tubs were kept at approximately 21°C away from direct sunlight with a 16 hour daylength.

1. To test the ability of carabid beetles to influence the incidence of infection in M.brassicae larvae when the soil is virus contaminated

Most agricultural crops are removed from the fields at the end of the season. The most probable source of virus which is going to persist from year to year is therefore in the soil. Jaques (1967)

found no appreciable loss of activity of T.ni NPV after 98 weeks in soil, however, Evans (1982) working with the NPV of M.brassicae recorded a loss of 98% during one year.

Viruses which persist in the soil from the end of one season to the start of the next season are possible reservoirs for initiation of epizootics. Investigations have demonstrated epizootics of NPV in P.rapae on cabbage (David and Gardiner, 1967; Harcourt and Cass, 1968). Young and Yearian (1979) induced epizootics in Pseudoplusia includens Walker on soybean following soil application of NPV at planting.

The aim of this investigation was to see if carabid beetles would influence the incidence of infection in M.brassicae larvae when the soil was sprayed with NPV. It was postulated that the carabids might become surface contaminated with virus from the soil and deposit this on the plants when climbing or alternatively by increasing the rate of dispersal of larvae between plants (see earlier work).

Methods

a) By use of cages

Groups of four cabbages at about the nine leaf stage were planted at the field site at Wytham. The cabbages were checked for any natural infestations which were removed and the one metre square plots were weeded. One pitfall trap was dug into the centre of each plot. The cabbages were covered by plastic bags and the soil surface was sprayed using a plant mist spray. Each square metre plot received a dose of 2.67×10^9 polyhedra in a volume of 20ml of deionized water. The virus control plot was not sprayed. One metre square cages covered in a fine nylon mesh were placed over the plots and the soil was banked up around the outside to prevent access by carabids.

Twenty-four hours after spraying virus onto the soil through an opening in the top of each cage, and removing the plastic bags on the

plants, 25 neonate M.brassicae larvae in an open polypot were wedged between the leaves of each cabbage. Various densities of carabids were dropped through the opening onto the soil. The virus control received 10 P.madidus and 15 N.brevicollis adults, the carabid control which was virus sprayed received no carabids. The remaining eight treatments all of which were virus sprayed received the number, sex and species of carabids as listed in Table 20.

Table 20. The number of virus sprayed plots for each treatment

Species	Number of carabids		
	10	25	50
N.brevicollis	1	1	1
P.madidus (Male)	1	1	1
P.madidus (Female)	1	1	0

Every three days the central pitfall traps were emptied. The carabids were identified and counted before being put into groups of no more than three into a polypot containing semi-synthetic diet. After two hours the carabids were returned to their pens and the polypots were bioassayed for the presence of NPV by the use of ten neonate M.brassicae larvae per polypot.

Nineteen days after the addition of the larvae, the cabbages were cut off at the stem and placed into plastic bags for transfer to the laboratory. All larvae found in the cabbages were transferred to individual polypots of semi-synthetic diet and kept at 22°C. The larvae were inspected regularly for death or pupation. Dead larvae were examined for the presence of polyhedra.

Samples of the cabbages were bioassayed in the laboratory using the method described for the field trials, Part c).

Results

Very few larvae were recovered from the plants. The proportion of virus infected larvae is recorded in Table 21. The results from the bioassay of the cabbage samples is given in Table 22. There was little agreement between the two sets of data, although in both cases there was no infection in the non-sprayed controls.

The low numbers of larvae recovered from the plants irrespective of the carabid density suggests that most of the larvae migrated from the plants with only a small proportion becoming established. Migration would ensure that larvae came into contact with virus contaminated soil and would increase the probability of infection. It was unlikely that virus infection reduced the establishment of larvae on the plants, particularly as no larvae were left on the plants in the non-sprayed control.

The carabid density did not appear to affect the level of virus contamination on the cabbage samples, which was positive only in the P.madidus plots. Larval movement may have attributed to the virus contamination of the plants. The action of wind and rain may also have been involved, but their influence would have been reduced by the protection offered by the nylon mesh tops of the cages.

The small number of larvae virus infected in both cabbage bioassays renders the results inconclusive and prohibits the use of statistical tests.

The bioassay of the diet contaminated by carabids caught in the pitfall traps showed that virus was present on some of the carabids during the first five days. Cages which contained virus contaminated carabids are recorded in Table 23.

Table 21. The proportions of virus infected larvae reared from cabbage plants in cages with virus sprayed soil

	Density of carabids per m^2							
	0		10		25		50	
N.brevicollis	-		1/4	25.0%	1/1	100.0%	0/0	
P.madidus ♂	-		4/7	57.1%	0/1		3/6	50.0%
P.madidus ♀	-		0/7	0.0%	2/23	8.7%		
No carabids	1/3	33.3%						
Non-sprayed control	-		-		0/0		-	

Table 22. The proportion of virus infected larvae in the bioassay of cabbage samples

	Density of carabids per m^2							
	0		10		25		50	
N.brevicollis	-		0/70	0.0%	0/28	0.0%	0/47	0.0%
P.madidus ♂	-		3/68	4.4%	1/30	3.3%	0/74	0.0%
P.madidus ♀	-		4/49	8/2%	0/75		-	
No carabids	0/45	0.0%	-		-		-	
Non-sprayed control	-		-		0/0		-	

Table 23. The incidence of virus contaminated carabids (+) in cages where the soil was virus sprayed

	Density of carabids per m^2		
	10	25	50
N.brevicollis	-	-	+
P.madidus ♂	-	+	-
P.madidus ♀	+	+	

b) <u>By use of tubs</u>

Method

This trial was performed twice using the water tanks described earlier. Three cabbages per tub were used and 25 neonate *M.brassicae* larvae were placed on each cabbage. The concentration of virus used was 1.6 x 10^9 NPV per tub, that is about 4 x 10^9 polyhedra per square metre. The virus was sprayed onto the compost surface in a volume of 20ml using a plant spray. After the duration of the trial the larvae were collected and put into individual polypots of semi-synthetic diet. They were kept at 22°C and any deaths were examined for NPV.

In the first trial the compost was sprayed before the cabbages were planted. The cabbages were planted carefully to avoid contaminating the leaves with the compost and unnecessarily disturbing the compost surface. The larvae were placed on the cabbages half an hour before the release of the carabids. There were three treatments, one without carabids, one with 20 *N.brevicollis* adults and the other with 20 *P.madidus* adults. The larvae were collected from the cabbages after six days.

In the second trial the cabbages were planted and sealed in plastic bags before the compost surface was sprayed. The tubs were left overnight for the spray mist to settle before the plastic bags were removed from the cabbages. The larvae were placed on the plants and left for two hours to establish themselves before the release of five male and five female *P.madidus* adults in one of the tubs. The control tub had no carabids present. The larvae were collected from the cabbages after five days.

Results

The incidence of virus infection in the larvae from both experiments as a proportion of the larvae recovered from the plants is recorded in Table 24. The data from the two experiments can be summed, which, despite the differences in the density of carabids, can then be used to consider the influence of P.madidus on the incidence of larval infection.

The comparison between the proportion of infected larvae when there were no carabids and when P.madidus was present was not significant ($\chi^2_1 = 3.01$).

Table 24. The proportion of virus infected larvae recovered from cabbage plants when the soil in the tubs was virus sprayed

	Experiment 1		Experiment 2	
N.brevicollis	0/52	0.0%	-	
P.madidus	9/57	15.8%	2/38	5.3%
No carabids	2/60	3.3%	3/43	7.0%

2. A comparison of the rate of predation by P.madidus adults on healthy and infected M.brassicae larvae

In earlier studies it was observed that P.madidus voraciously attacked and consumed both healthy and heavily infected M.brassicae larvae when offered. The aim of this study was to compare the ability of P.madidus adults to find and attack healthy and infected larvae on cabbage plants. M.brassicae larvae are reported to be more active when virus infected (Evans and Allaway, 1983), an increased movement between plants would probably increase the opportunities of predation by P.madidus.

Method

Three cabbages were planted in each of four packing cases; each plant had at least two leaves touching the compost surface. Twenty M.brassicae fourth instar larvae were placed on the leaves of each plant. Two treatments had healthy larvae and the other two had one day post infected larvae. The tubs were sealed and left overnight for the larvae to establish themselves on the plants. The following morning five male and five female P.madidus adults were released in one tub containing healthy larvae and in one tub containing infected larvae. The temperature was maintained at approximately 23°C. Three days after the carabids' release, the larvae and carabids were collected and counted.

Results

All carabids were recovered alive. The numbers of larvae recovered from the four treatments are tabulated in Table 25, which represents numbers from a possible total of 60 in each case.

The proportion of larvae recovered when P.madidus was absent was

compared to that when *P.madidus* was present. The result was highly significant (χ^2_1 = 35.00, p=0.001). It is concluded that predation by *P.madidus* reduced the numbers of larvae.

The reduction in the proportion of healthy and infected larvae in the presence of *P.madidus* was compared. The test was significant (χ^2_1 = 5.65) and it was concluded with 95% confidence that *P.madidus* attacked more healthy than infected larvae.

There was no significant difference between the proportions of healthy and infected larvae recovered in the absence of *P.madidus*. The loss of larvae was probably as a result of escape from the tubs or cannabalism.

The average number of fourth instar larvae lost which was attributed to the activity of *P.madidus* was:

0.9 healthy larvae per *P.madidus* per day

0.5 infected larvae per *P.madidus* per day

Table 25. The number of larvae recovered from each of the four treatments

	Larvae	
P.madidus	healthy	Infected
Present	25	38
Absent	52	53

3. Incidence of virus infection in M.brassicae larvae when carabids were virus contaminated

Earlier work demonstrated that after feeding on virus infected larvae carabids can carry viable virus by both surface contamination and by passage through the gut. The aim of this experiment was to investigate if the resultant virus contamination of the environment by carabids could provide a source of virus inoculum to M.brassicae larvae.

Methods

a) Using N.brevicollis adults

Twenty-five neonate M.brassicae larvae were released onto each of four cabbage plants in two water tank tubs. Ten N.brevicollis adults were allowed to feed for three hours on virus killed M.brassicae larvae, before release into one of the tubs, while ten N.brevicollis adults fed on only minced beef were released in the other tub.

Seven days after the carabids' release, the larvae were collected and transferred to individual polypots of semi-synthetic diet. The larvae were kept at 22°C and examined regularly for virus infection.

b) Using P.madidus adults

Three cabbages were planted in each of three packing cases. Twenty-five neonate larvae were released onto each plant and left to establish themselves for two hours before the release of the carabids. The P.madidus adults were allowed to feed for one hour before release into the tubs. The control carabids were fed on healthy M.brassicae larvae and the virus contaminated carabids were fed on NPV-killed M.brassicae larvae. Five male and five female carabids were released in each of the control and virus-contaminated treatments designated according to the feeding of the carabids. The

third tub received no carabids and constituted a predation control.

Five days later the larvae were removed and transferred to individual polypots of semi-synthetic diet. The larvae were kept at 22°C and examined for virus infection.

Results

The numbers and condition of larvae recovered from both experiments are recorded in Table 26. The low number of virus infected larvae obtained prohibits the use of statistical tests. However, as there was no infection in the control, the result suggests that a low incidence of infection could be attributed to carriage by contaminated carabids.

Table 26. The numbers of infected and healthy larvae recovered when carabids were virus contaminated

		Numbers of larvae recovered		
		Infected	Healthy	Total
N.brevicollis	control	0	84	84
	virus contaminated	1	97	98
P.madidus	control	0	27	27
	virus contaminated	1	37	38
	No carabids	0	53	53

4. Ability of carabids to climb cabbage plants

Carabids are more likely to be both useful predators of *M.brassicae* larvae and effective carriers of the corresponding NPV if they can climb cabbage plants to search for their prey. Experiments were performed to try to determine the climbing ability of carabids. A sticky gum was used on the plants to immobilize any carabids which climbed onto them.

Methods

a) Using a single cabbage

A small upright cabbage plant with no trailing leaves was planted within a five litre glass beaker. Sticky gum was placed on the upper surface of the lower leaves and a bait of minced beef was wedged between the upper leaves. Six adults of each of *P.madidus*, *H.rufipes* and *N.brevicollis* were dropped onto the compost surface. The experiment was kept at about 20°C for seven days before inspection of the plant for carabids.

b) Using a tub

Four cabbage plants were planted in a tank so that each had at least one leaf trailing on the compost surface. Sticky gum was smeared on selected leaves. Ten *N.brevicollis* adults were released on the compost surface. After seven days the position of the carabids was noted.

c) Using cages

Two groups of four cabbage plants were planted at the field site at Wytham. Selected plants were smeared in sticky gum. The plants were covered with one square metre cages to prevent access by birds but a gap of 100mm was left at the base to allow access by carabids.

At regular intervals over a period of two weeks the plants were checked for the presence of carabids.

Results

No carabids were found on the cabbage plants in either experiment a) or c). Two *N.brevicollis* adults were stuck to the gum on the cabbage plants in experiment b). Both of these were stuck on the same leaf which was resting on the compost surface. No carabids were found higher up the plants and most were found sheltering underneath the leaves lying on the compost. During observation, carabids readily ran over leaves on the compost surface but were not seen climbing higher.

DISCUSSION

A The Carabid population

The number of carabids of a particular species caught in pitfall traps depends on both the abundance of the species and its activity (Greenslade, 1964a). Therefore factors which influence the activity of the carabids will influence the trapping. A more direct and accurate method of obtaining an estimate of the population density is by direct quadrat counts. However, that method is very time consuming especially with small species at a low population density. Despite the erroneous impression which pitfall catch data can give of carabid population size, Lesiewicz et al. (1983) considered it valuable for distribution and activity determination. However, they go on to point out that comparisons of population density or individual species' abundance as interpreted from pitfall data should be avoided because experimental design can be critical. Greenslade (1964a) did not consider that these criticisms affected the value of pitfall traps as a sampling method in mark-recapture work.

The use of mark-recapture data is based on several assumptions; these are listed by Begon (1979). One assumption is that the marks are permanent. Greenslade (1964b) found that although on N.brevicollis marks remained for four months, the marks on P.madidus were rubbed off within three weeks. During the present work some of the P.madidus in laboratory cultures rubbed away marks within a week, while the marks on H.rufipes and N.brevicollis were more permanent. It is therefore possible that the number of marked and recaptured P.madidus is artificially low because of loss of marks .

Another assumption is that being caught and handled has no effect on an individual's chance of dying or emigrating. Greenslade (1964b)

reported extensive migration of *Pterostichus* but only slight migration of *N.brevicollis*. However, even in *N.brevicollis* populations he reported that locomotor activity of marked individuals during the first 10 days after release was twice the level recorded afterwards. It was only by using data collected after a 10 day dispersal period that Greenslade (1964b) was able to show the marked *N.brevicollis* randomly distributed within the population. The data presented in this work needed a two day dispersal period for the marked *N.brevicollis* to be randomly distributed within the population (Table 11). Indeed if data were only used after a 10 day period, no recaptures would have been recorded. A similar comparison could not be made for *H.rufipes* or *P.madidus* because of the low recapture rate.

The Lincoln index assumes either that there are no births or immigrations, or that there are no deaths or emigrations. It involves only one release and one recapture. Jackson's positive method allows loss but gain is presumed constant and estimated. As the method relies on proportions captured, carabids do not need to be released between successive captures. During the studies reported here, there should have been little adult emergence and the "birth-rate" estimate should give an indication of the rate of immigration. For all three species studied this was found to be in close agreement. As recapture occurred over a comparatively short time the effects of gains and losses on the populations can be assumed to be small, this is supported by the close agreement between the two methods used for estimating the population density.

Despite the low recapture rate, which always casts doubt on the validity of tests, the population estimates obtained were in agreement with those reported by other workers in England. Manga (1972) reported values of below $1/m^2$ for *N.brevicollis*. Briggs (1965) gave the density of *H.rufipes* on arable land as $0.2-6.0/yd^2$. Thiele (1977)

gave the general rule that for middle-sized species such as Pterostichus the maximum population expected would be only a few individuals/m^2.

There may be several reasons why marked carabids were recaptured only for a comparatively short time. The first, which was probably partially true for P.madidus, was the loss of marks. Another could be the result of a smaller proportion of marked carabids in the population as captured carabids were not released; this is more likely to be true for N.brevicollis where a greater, though still a low, proportion of the marked beetles were recaptured. This would be aggravated by the immigration or birth of unmarked carabids. A further reason could be the result of death or emigration of marked carabids which could have been aggravated by the peculiar plot shape.

The average dispersal of marked carabids recorded was in approximate agreement with Thiele's (1977) general rule of a few metres per day and Best et al. (1981) averages for P.chalcites and Scarites substriatus Haldeman of 8.5 and 12.2m/day respectively. The maximum distance dispersed in one day was not unusual, although higher values may have been obtained if more out-lying pitfall traps had been used. However, the modest maximum distances of 15-19 metres/day indicate the potential for dispersal into new habitats.

Both H.rufipes and N.brevicollis were captured in greater numbers nearer the hedgerows. Greenslade (1964b), because of the selectivity of pitfall traps, suggested that differences between traps should only be accepted as real when the greater numbers are caught where movement is more restricted. These results satisfy this stipulation as movement is more restricted in very weedy ground that was found near the hedgerow. In Pollard's (1968) experiment he found that N.brevicollis occurred in greater numbers in the hedgerow than in the adjacent field. He also reported that P.madidus and H.rufipes occurred as abundantly in the field as the hedgerow, which is in

agreement with the data for P.madidus presented here but not for H.rufipes. However, Dempster (1969) found that H.rufipes was particularly dependent on ground cover and its numbers were drastically reduced in hoed compared with weedy Brussel sprout plots. It could be that as at the time of the mark-recapture work there was little ground cover in the field, H.rufipes sought refuge in the more weedy area near the hedgerow.

B The potential of carabids to act as carriers of M.brassicae NPV

The ability of predators to act as carriers of virus has been documented in the literature. An early reference is by Smirnoff (1959) of a hemipteran, Pilophorus uhleri (Knight), which was considered responsible for transferring virus disease from an infected to a healthy colony of Neodiprion swainei Middi. Polyhedra were not found in the gut of the predator so the transfer may have been by surface contamination only. Biever et al. (1982) found that surface contaminated Podisus maculiventris (Say) could transfer the NPV of Trichoplusia ni (Hübner) for at least a week and in some cases for over two weeks. Abbas and Boucias (1984) demonstrated that the same pentatomid predator was capable of excreting large amounts of infectious NPV after feeding on infected larvae. Similarly Capinera and Barbosa (1975) documented that the carabid C.sycophanta defecated polyhedra in sufficient quantities to infect gypsy moth larvae.

The data presented in this study further show that carabids are able to contaminate the environment with viable virus after a meal on virus infected larvae. The first experiment demonstrated by means of bioassay of gut homogenate and carabid washings that the virus is carried both in the gut and as an external contaminant. All three carabid species tested, transmitted virus but, because of the low number of individuals tested and the deaths of some during the course of the experiment, a statistical comparison between the three species

could not be carried out.

The second trial revealed that washing the carabids to remove surface contamination did not appreciably reduce the level of environmental contamination with virus. However, the plot of the environmentral contamination, as measured by bioassay, against time after the infection feed more closely resembled a linear relationship for washed rather than unwashed carabids. By exploration of the graph it was estimated that P.madidus female defecate viable virus for 14 days after a meal on virus infected larvae. Beekman (1980) had found no loss of infectivity of Heliothis punctigera Wallengren NPV during its passage through the gut of the predator, Nabis tasmanicus Remane. Vago et al. (1966) studied the passage of Lymantria dispar L. NPV through the gut of Ephippiger bitterensis Finot. They found the effect of gut juice insignificant and detected polyhedra 15 days after the ingestion of diseased insects. Those working with heteropteran predators have only detected polyhedra in the faeces for up to six days (Abbas and Boucias, 1984; Beekman, 1980; Cooper, 1981).

The effect of feeding the predators after a meal on virus infected larvae does not appear to have been demonstrated by other workers. The data presented in this study are, however, inconclusive.

C Field trials using cabbage plots

Ingress and egress barriers were used by Dunning et al. (1975) to regulate carabid densities to investigate the predation of aphids in sugar beet crops. Before sowing, the egress plot was sprayed with parathion to kill most existing beetles and larvae. He caught signficantly fewer carabids in the egress plot but there was little increase in numbers of carabids caught in the ingress when compared with a control plot. The results presented in this study show that the use of an insecticide spray is not necessary in order to achieve a

large difference in carabid density between ingress and egress plots. However, with carabids that have little locomotor activity, a spray might be necessary and estimation of their abundance would need to be done by some method other than pitfall trapping. A control plot was not used in the present studies as all that was needed was two plots with significantly different levels of carabid activity, which was obtained.

The start of the field trials unfortunately marked the start of a period of very stormy weather. This was probably partially responsible for the rapid depletion of larvae in both plots. However, it can be seen from Figure 8 that the reduction in larval numbers was more severe in the ingress plot, this can be attributed to the increased number of predatory carabids. The data presented in Figure 11 suggest that an enhanced number of carabids caused an increase in the average daily larval dispersal. An alternative explanation could be that the carabids preferentially predated on larvae on the central cabbages where they occurred at higher densities. This would require further investigation. The accumulated number of plants colonized as the experiment progressed followed a linear relationship rather than the logarithmic form, described by Evans and Allaway (1983). However, this linear relationship cannot be expected to hold true beyond day 12 of the experiment as by that time most of the larvae were sluggish and at the point of death. This view is upheld by the levelling of the slopes in Figure 11.

The bioassay of the cabbages revealed a significant difference between the number of virus contaminated plants from the two plots. The egress plot had more contaminated cabbages than could be attributed to chance. This is probably a direct result of predation of larvae by carabids in the ingress plot and thereby removal of sources of virus from the cabbage plants. There were some cabbages in

the plots which were virus contaminated but no larval activity had been recorded on them, this is in agreement with the results of Evans and Allaway (1983). However, there is no evidence to suggest that this virus contamination was a result of carabid activity as more unaccounted for contaminated cabbage plants were in the egress rather than in the ingress plot, although this difference was insignificant.

Only two carabids collected from the experimental plots were found to be virus contaminated. This suggests that at least these two carabids had been feeding on virus infected larvae, however, it is unknown if the encounter with the prey occurred on the soil or on the cabbage plants. Moreover, it is suggested that if gut extractions of the carabids had been tested, the results would have been more reliable. It is probable that some of the carabids did not defecate during the two hours whilst held in the polypots which were later bioassayed. Evans and Allaway (1983) found polyhedra in the gut from a few of the carabids caught during their field trials.

D Small scale trials

Although carabids became surface contaminated with virus after the soil was virus sprayed, the data presented in this study did not suggest that they were instrumental in increasing virus infection in larvae on cabbage plants. However, there was evidence that cabbage plants in plots with an increased density of P.madidus became more contaminated with virus than those with no added carabids. The data are therefore inconclusive concerning the role of carabids when the soil is virus contaminated.

Experiments showed that P.madidus adults reduced the number of fourth instar M.brassicae larvae on cabbage plants. However, it was

not shown conclusively if the predation occurred on the soil or on the plants. More larvae became prey in the healthy treatment than in the infected treatment which is surprising as infected larvae are known to have an increased locomotor activity (Evans and Allaway, 1983) which is likely to increase contact with predators on the soil surface.

A small proportion of M.brassicae larvae on cabbage plants become virus infected when the only source of virus was from carabids. However, this probably would only be demonstrated in the field by using artificially high numbers of carabids and M.brassicae larvae.

The ability of carabids to climb plants was not demonstrated. This however could be due to poor experimental design as carabids may have avoided the sticky gum. If the method used by Dunning et al. (1975) had been employed, the results may have been different. According to Dempster (1967) H.rufipes is active both on the ground and on plants.

CONCLUSION

The ubiquitous and mobile characteristics of carabids combined with their ability to act as carriers of NPV gives them the potential to be instrumental in the dispersal of a pathogenic virus of prey species. However, the studies presented indicate that carabids are more important in reducing the number of prey by direct predation.

Nevertheless their capacity for virus dispersal between pest populations remains unclear. This is most likely when carabids disperse from a region when the pest population has collapsed due to a virus epizootic. If they enter an area with the same prey species but disease free, any viral contamination of the habitat by the carabids could constitute an epicentre for the initiation of an epizootic, especially if there is a high density of susceptible insects.

The data presented suggest that virus infected M.brassicae larvae can only disperse a maximum of five metres before virus-induced sluggish behaviour and mortality prevents further dispersal. Similarly, Evans and Allaway (1983) captured virus infected larvae in pitfall traps 4.5 metres from their release point, however, no estimation was made of the likely maximum dispersal.

Carabids, in contrast to larvae, have a much greater potential for spread of virus over a large area. P.madidus females voided viable virus up to 14 days after feeding on virus-infected prey. Even by use of the modest maximum daily distance of dispersal of 15.4 metres documented in this study, it is estimated that P.madidus could carry virus for 200 metres. However, the value for dispersal is probably an underestimate, especially as Baars (1979) and Best et al. (1981) recorded the maximum daily dispersal for Pterostichus species as 126 and 91 metres respectively. These values would give a potential for virus dispersal by carabids in excess of one kilometre.

Hostetter and Biever (1970) recorded that English sparrows actively search cabbage plants for insects. *T.ni* larvae in advanced stages of NPV infection, lost their natural defence mechanisms and became easy prey. The faeces from birds in the cabbage field contained large numbers of viable polyhedra. Hostetter and Biever (1970) considered it conceivable that sparrows could disseminate virus and cause natural epizootics of NPV disease in *T.ni* populations. It is therefore probable that *M.brassicae* NPV is dispersed in the same manner. Entwistle *et al.* (1977a) and Lautenschlager and Podgwaite (1979) have described several bird species which passed viable NPV in their faeces. The peak quantity of virus is detected in the faeces within an hour, although virus may be passed for seven days (Entwistle *et al.*, 1978). Entwistle *et al.* (1977b) gave evidence to suggest that birds may carry NPV for at least six km. Birds are therefore likely to be more important than carabids in dispersing NPV over long distances.

The evidence presented in this chapter points to the involvement of carabids in both predation of *M.brassicae* larvae and BV dispersal. Nevertheless, there were no replications of the trials using the large cabbage plots and much of the evidence produced from them was circumstantial. Therefore, further studies are needed to clarify the role of carabids and other predators in dispersing virus between susceptible populations. The ability to climb plants and their preference for predation on healthy or infected prey also warrants further investigation.

DISCUSSION

The survival of BVs is dependent on the ingestion of inoculum by susceptible larvae. Particular problems are associated with this, especially in reference to secondary inoculum. *P.interpunctella* larvae did not release significant quantities of secondary inoculum throughout the course of virus disease. A small amount of virus was passed onto the diet within the first few days, but this was probably part of the primary inoculum. Even at death the integument of diseased *P.interpunctella* and *E.cautella* larvae did not rupture. Therefore the diet would not become contaminated unless some other agency acted to rupture the cadavers. On the other hand NPV-killed larval cadavers of *M.brassicae*, in accordance with the classical symptoms of BV disease, ruptured and the liquified contents were released.

The most likely means by which larvae of *P.interpunctella* and *E.cautella* would encounter secondary inoculum would therefore seem to be by cannibalism. The evidence presented here, shows that cannibalism is infrequent when an abundance of preferred food is present. However, when food is in short supply or is sub-standard, cannibalism by *P.interpunctella* larvae occurs readily, even so the evidence suggests that *E.cautella* larvae rarely cannibalise cadavers. The larval transmission of *M.brassicae* NPV is undoubtably improved by the attraction of larvae to freshly dead NPV-infected cadavers (Evans, 1986).

A further comparison could be made between *M.brassicae* and the pyralid moths concerning the position of larval mortality. NPV diseased *M.brassicae* larvae typically die on the outer leaves of cabbage plants, in this position the liberated body contents are likely to be further dispersed over the leaves by the action of rain. Young, highly susceptible larvae feed mainly on the outer leaves before moving to the heart of the plant (Evans and Allaway, 1983). The behaviour of diseased larvae therefore promotes the probability of

virus transmission. Healthy larvae, of both P.interpunctella and E.cautella typically remain within silken feeding tunnels until the time immediately prior to pupation. This behaviour reduces the chances of larval encounter. BV-infected larvae exhibited an increased tendency to disperse and many died on the diet surface, although some died in their silken tunnels. Larval encounter with diseased cadavers is limited except at high densities when immature larval dispersal is enhanced, also exhaustion of the food source increases the probability of cannibalism.

BV-infection enhanced the locomotor behaviour of all three species studied, which will increase the spread of disease on a local scale. Adults with their increased powers of dispersal through flight would be able to spread virus inoculum over a wider area if they are capable of transmitting virus to their progeny. Studies on virus transmission by adults was limited to the P.interpunctella/GV interaction. The data presented failed to provide any evidence to support the hypothesis of transovarial transmission of BVs. Furthermore, the evidence suggested that larvae which receive a sub-lethal dose do not carry viable virus into the adult stage. Significant numbers of M.brassicae larvae infected late on in their development produced infected pupae, but similarly no adults were found to be virus infected (Evans, 1983).

P.interpunctella adults which were externally contaminated with virus were able to transmit it to their progeny but this was circumstantially linked to contamination of the larval diet rather than contamination of the eggs. Natural virus contamination of adults is unlikely to be high and therefore any sources of inoculum deposited are probably at a very low concentration. However, young larvae are very susceptible to infection and the tendency of the females to lay eggs in batches will increase the probability of virus encounters,

particularly as they preferentially oviposit on conditioned diet. Their tendency to oviposit several batches of eggs could further increase the virus distribution.

Sub-lethal doses given to P.interpunctella larvae were detrimental to the emerging adults. Their appearance, weight and sex ratio were normal, but the proportion of eggs oviposited, the viability of the eggs and the survival of the progeny were all reduced.

BVs can be carried passively by parasitoids and predators (Kaya, 1982; Enwistle, 1982). However, the present study provided little if any evidence to support the assumption that BVs can be mechanically vectored on the ovipositor of a parasitoid or on the mouthparts of a predator. The literature on this topic is not strong (Entwistle, 1982) and in view of the work presented, it is suggested that the ability of parasitoids and predators to act as BV vectors by direct inoculation into the host's haemocoel is peculiar to certain host-virus interactions and may be uncommon.

Nevertheless, parasitoids, predators and general opportunists which feed on infected prey were shown to disperse viable BV by external contamination and by voiding BV in their faeces. Predators may therefore be important in dispersing BVs. Insect predators generally have a greater mobility than their prey and therefore a higher potential for distributing host virus over a greater area. Birds, with an even greater capacity for dispersal, have been implicated as important in the dispersal of BVs of forest pests (Entwistle et al., 1977a, b; Lautenschlager and Podwaite, 1979) and agricultural pests (Elmore and Howland, 1964; Hostetter and Biever, 1970; Reed, 1971). Small mammals such as rats and mice, are more likely to be important in the context of stored products. Lautenschlager and Podwaite (1979) showed that predatory mammals

passed significant quantities of polyhedra in their faeces and suggested that mice and shrews may be important in transporting Lymantria dispar L. NPV. A high host density or increased host movement, as a response to an unsuitable or exhausted food source, would increase the probability of host contact with voided virus.

Parasitoids and predators in general, may be expected to act in competition with the virus. The predators tested were able to feed on infected prey and probably some of the virus would be lost from the host's environment by the activities of the predator. The parasitoids were less able to compete with the virus. N.canescens was unable to complete development in diseased hosts, whilst only a small proportion of B.hebetor progeny were able to do so. B.hebetor adults preferentially avoided diseased hosts.

The availability of alternative hosts would potentially increase the reproductive capacity and persistence of the BVs, particularly if the alternate host extends the period of host availability. Recycling of BV back to the original host would be probable if both hosts share the same food sources. N.pronuba was found to be an alternative host for M.brassicae NPV, and E.cautella and P.interpunctella were susceptible to each other's homologous NPV or GV. However, the cross-transmission for the pyralid moths, particularly E.cautella, required such artificially high doses that cross-infection is probably rare. The cutworm, N.pronuba in the M.brassicae/NPV interaction field trials was shown to be an alternative host on cabbages, the young larvae overwinter on the soil and could therefore introduce BV onto brassicas in the spring.

Persistence of BVs is very different in the two environments studied. Jaques (1975) documented that on leaves the BVs of two other cabbage pests were almost entirely inactivated after ten days, which compounded with harvest of the plants prevents long-term

persistence at the host feeding site, although virus persists well in the soil (Evans and Harrap, 1982). P.interpunctella GV, in contrast, can persist for up to 12 months on stored grain (Kinsinger and McGaughey, 1976). Storage times of commodities can be extremely variable but even after removal, pockets of pests may remain in inaccessible crevices. BVs may consequently persist in warehouses for considerable periods and particularly in heated warehouses there could be many host generations to exploit this inoculum, which could result in the development of epizootics.

The requirement of a large, well distributed population to utilize virus inoculum is seen as fundamental for the initiation of an epizootic. However, because of the low level of damage tolerated on cabbage crops and many stored products it is improbable that the development of natural epizootics over several pest generations would ever be commercially acceptable. Although the present work was not pursued for commercial considerations but as an investigation into baculovirus dispersal, the implications of poor control using biotic dispersal may provide some insight into future control strategies. In order to further this investigation it would be necessary to use larger areas and several generations so that the actual dispersal of host and virus can be mapped and the progression of epizootics analysed.

Although this work is of limited use within the context of control many of the lessons learnt could be applied to crops which can tolerate high levels of damage, such as grassland or forestry. The study on virus dispersal by parasitoids and predators requires furtherinvestigation especially as there are few definitive studies on parasitoid-aided virus dispersal and these are conflicting. Autodissemination of virus by adult moths remains a possibility which invites further study.

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APPENDIX Biochemical comparisons of the viruses

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DISCUSSION

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INTRODUCTION

The BVs utilized in ecological studies in this thesis were compared using gel electrophoresis of both the structural polypeptides and the fragments of DNA after restriction endonuclease digestion (REN). The methods have been reviewed by Harrap and Payne (1979).

Purified Plodia interpunctella (Hübner) GV (PiGV) was obtained from K.A. Tweeten (Kansas, USA) and the source of Ephestia cautella Walker NPV (EcNPV) was from dead larvae supplied by D.F. Hoffmann (Fresno, USA). The F2 generation of both viruses was used for biochemical analysis. The F3 generation of the German isolate of Mamestra brassicae L NPV (MbNPV) from A. Gróner (Darmstadt, W. Germany) was used in the field work and for polypeptide analysis, however, a French isolate was used for DNA analysis.

Two isolates of NPV were obtained from two individual Noctua pronuba L. larvae which succumbed to NPV disease and were collected from an experimental cabbage plot in which NPV-infected M.brassicae larvae had been previously released. The NPV liberated from the diseased N.pronuba cadavers was fed on diet to healthy M.brassicae larvae. The larvae succumbed to NPV infection, the progeny virus of which was used in biochemical analysis to compare with the homologous MbNPV. The two N.pronuba NPV (NpNPV) isolates were designated J1 and C9 after the cabbage positions from which the original larvae were collected.

METHODS

Purification of inclusion bodies (IBs), virus particles (VPs) and nucleocapsids was carried out as described by Harrap et al. (1977). Samples were disrupted with sodium dodecyl sulphate (SDS) and mercapthoethanol to release the structural polypeptides as described by Brown et al. (1977) before being used in polyacrylamide gel

electrophoresis (PAGE). The gels were 10-30% linear gradient or 12% uniform discontinuous SDS-polyacrylamide slab gels using the discontinuous buffer system of Laemmli (1970). The polypeptides were located by staining with Kenacid R.

MbNPV and the two NpNPV isolates were treated to extract the DNA using the method described by Archard and Mackett (1979). The DNA of PiGV and EcNPV was extracted using the method described by Payne (1974). The purified viral DNAs were digested with restriction enzymes and the fragments were resolved by electrophoresis in 1.0% horizontal agarose slab gels. The gel was stained with ethidium bromide and photographed under ultraviolet light.

RESULTS

1. PAGE

The positions of the resolved VP polypeptides of PiGV, MbNPV and the two NpNPV isolates are shown in Figure 1. The profiles of the polypeptides obtained from EcNPV VPs and polyhedra are shown in Figure 2 where PiGV is included for comparison on the 12% gel.

The molecular weights of the polypeptides were calculated from 12% gels, those obtained for PiGV and EcNPV are given in Table 1, while those for MbNPV and NpNPV are given in Tables 2 and 3.

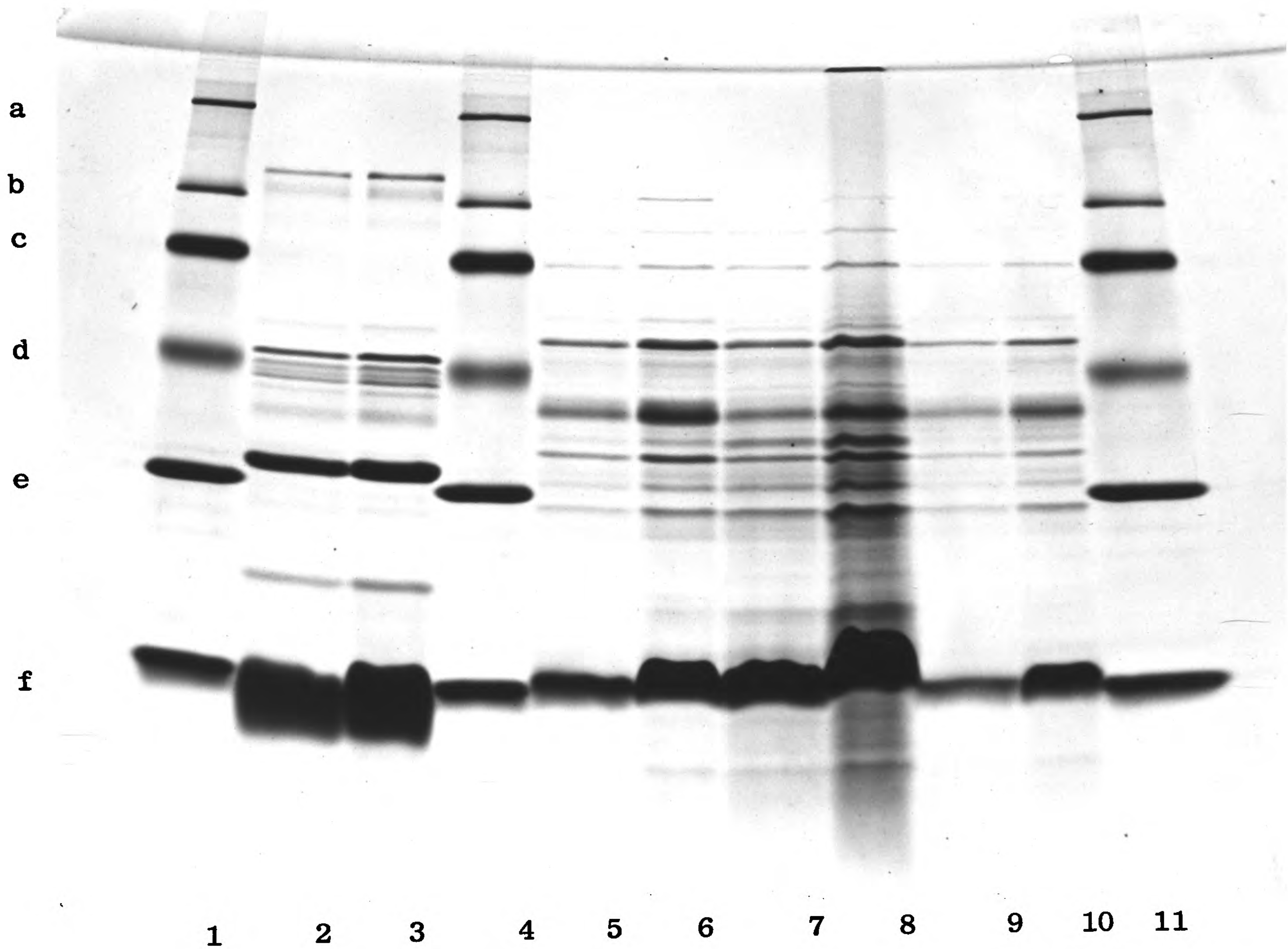
2. REN analysis by agarose gel electrophoresis

Figure 3 is a photograph of a gel containing the cleavage products of PiGV and EcNPV DNA after digestion by three different RENs. The mean molecular weights of the viral fragments are given in Tables 4 and 5.

The DNA of C9, J1 and a French isolate of MbNPV were digested by Hind III and the fragments run on the gel shown in Figure 4. The molecular weights of the fragments were estimated and are recorded in Table 6.

Figure 1.

SDS PAGE of polypeptides of VPs of PiGV J1, C9 and MbNPV on a 10-30% linear gradient discontinuous slab gel



Tracks 1, 4, 11 standard molecular weight markers (molecular weights in parenthesis)

a - myosin (200,000)

b - phosphorylase b (92,500)

c - bovine serum albumin (69,000)

d - ovalbumin (46,000)

e - carbonic anhydrase (30,000)

f - lysozyme (14,300)

Tracks 2,3 PiGV

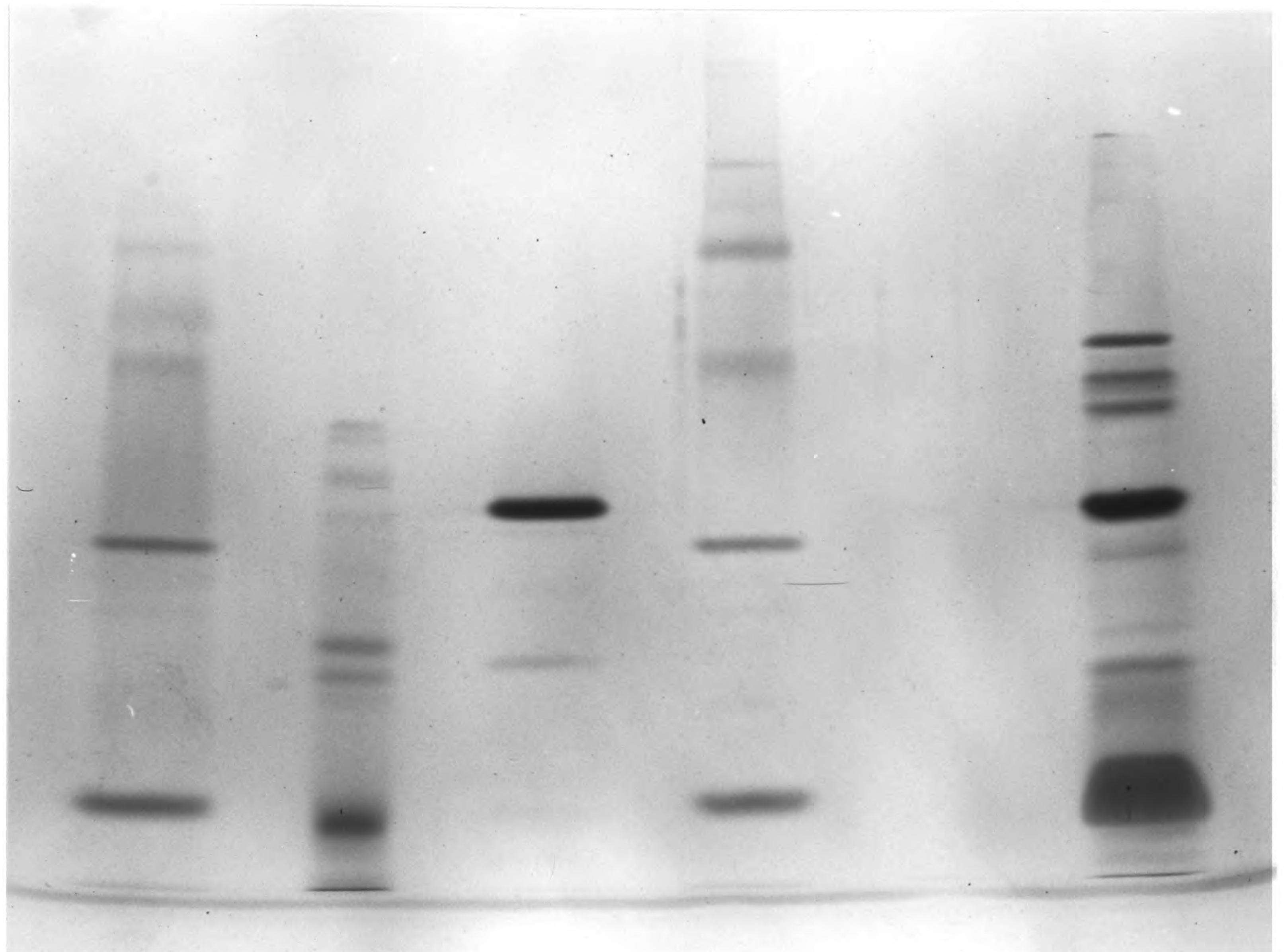
Tracks 5,6 J1

Tracks 7,8 C9

Tracks 9,10 MbNPV

Figure 2

SDS-PAGE of polypeptides of VPs of EcNPV and PiGV and polyhedra of EcNPV



Tracks 1,4 - standard molecular weight markers

Track 2 - EcNPV VPs

Track 3 - EcNPV polyhedra

Track 5 - PiGV VPs

Table 1. Estimated molecular weights of the structural polypeptides of PiGV and EcNPV

PiGV			EcNPV	
Granules[c]	Virus particles[a]	Nuclecapsids[b]	Virus particles[c]	Polyhedra[c]
32700	111800 (±6500)		39100 (±200)	32300 (±1000)
	94400 *		37700 (±200)	
	78300 *		35100 (±200)	
	57900 *		32500 (±100)	
	49200 (±1500)	48000 (±100)	29000 *	
	44000 (±900)		23900 (±200)	
	41900 (±400)	42500 (±400)	21900 (±400)	
	39500 (±400)	39500 (±400)	20400 (±300)	
	32300 (±500)	32400 (±700)	13600 (±100)	
	29400 (±600)	30500 (±500)		
	24600 (±800)			
	21400 (±1100)			
	19200 (±600)			
	14000 (±500)	13600 (±100)		

a mean from 5 gels

b mean from 3 gels

c mean from 2 gels

* only one value

Table 2. Estimated molecular weights of the structural polypeptides [a] of MbNPV and NpNPV VPs

J1	C9	Mb
97200 ± 2800	94400 (b)	101400 (b)
77100 ± 2200	76100 (b)	81200 (b)
67600 ± 1000	66800 (b)	68400 ± 800
65400 ± 800	64900 (b)	66400 ± 1400
55800 ± 1000	55800 ± 1700	55700 ± 1200
51200 ± 1000	50300 ± 1700	51300 ± 1100
47800 ± 800	47000 ± 500	48900 ± 1400
45300 ± 700	44300 ± 500	45800 ± 1000
43400 (b)	43400	42600 ± 700
41600 ± 1000	40700 ± 400	39900 ± 400
38900 ± 300	39000 ± 400	38700 (b)
37500 ± 500	37300 ± 400	36900 ± 300
35700 ± 300		35000 ± 100
34200 ± 1300	34500 ± 1700	33000 (b)
30700 ± 300	30600 ± 300	31300 ± 400
29200 ± 200	29000 ± 700	29800 ± 300
27000 ± 400	27300 (b)	27400 ± 100
25500 ± 300	25500 ± 300	25500 (b)
15700 ± 600	15700 ± 400	15800 ± 500

(a) mean molecular weight and standard deviation calculated from three gels.

(b) value obtained from only one gel

Table 3. Estimated molecular weights of the structural polypeptides of isolates of MbNPV and NpNPV nucleocapsids

J1 (a)	C9 (a)	Mb (b)
	51600 ± 1000	52300 ± 1000
	44800 ± 200	42200 ± 100
41000 ± 800	40200 ± 800	40200 ± 700
39400 ± 600	38000 ± 1100	38100 ± 900
	32600 ± 2000	
	30700 ± 100	30700 (c)
	29000 ± 100	29000 (c)
	14600 ± 100	14800 ± 200

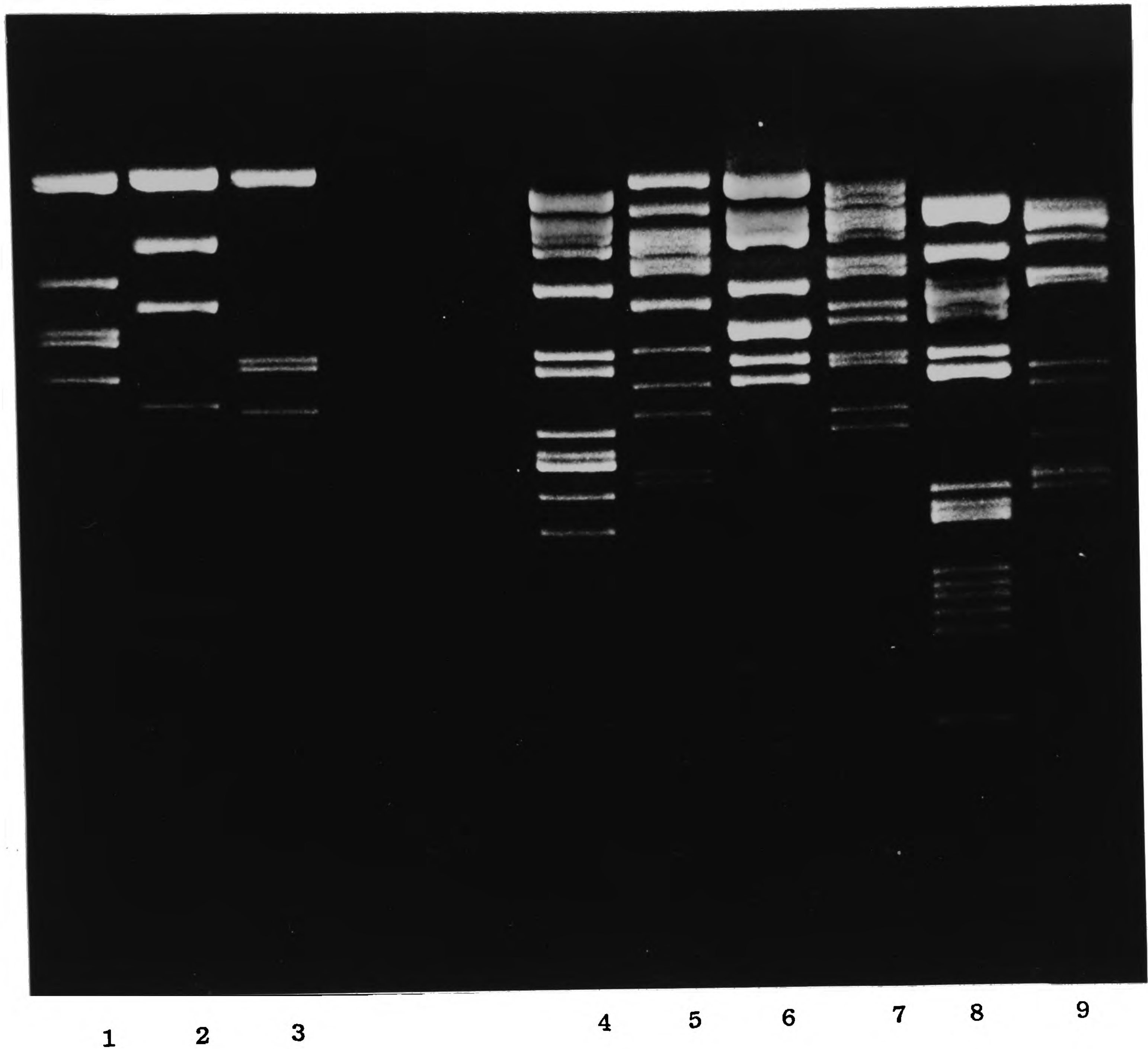
(a) mean molecular weights and standard deviations calculated from 2 gels.

(b) mean molecular weights and standard deviations calculated from 3 gels.

(c) Value obtained from only one gel.

Figure 3.

1.0% (w/v) agarose gel electrophoresis of PiGV and EcNPV DNA REN digests



Track 1 EcoRI digests of λ DNA

Track 2 Hind III digest of λ DNA

Track 3 EcoRI and Hind III double digest of λ DNA

Tracks 4, 6, 8 PiGV

Tracks 5, 7, 9 EcNPV

Digested by:

Tracks 4, 5 Bgl II

Tracks 6, 7 Hind III

Tracks 8, 9 EcoRI

Table 4. Estimated molecular weights of PiGV DNA REN fragments

Molecular weights(x 10^6)

Hind III	Bgl II	EcoRI
10.6	8.9	7.7
7.2	6.9	5.2
6.4	6.3	4.4 (2)
4.5	5.5	3.94
3.67	5.0	3.35
3.28	3.45	3.10
3.07	3.25	2.14
1.20	2.61	1.87 (3)
0.66	2.38	1.64
0.59	2.34	1.59
0.25	2.13	1.55
	1.92	1.47
	0.69	1.37
		1.10
		1.06
		0.96
		0.69
		0.61
		0.43
		0.41
		0.34
		0.31
41.4	51.4	53.4

Table 5. Estimated molecular weights of EcNPV DNA REN fragments

Molecular weights (x 10^6)

Hind III (a)	Bgl II (b)	EcoRI (a)
11.8	13.8	7.1 (2)
9.4 (2)[c]	8.3	5.7
7.5	6.3	4.6 (2)
6.7	6.0	3.20
6.1	5.6	3.01
5.1	5.3	2.56
4.9	5.1	2.26
3.95 (2)	4.3	2.15
3.33 (2)	3.55	1.90
2.78	3.00	1.81
2.60	2.73	1.69
1.10	2.30	1.11
0.96	2.24	1.02
	1.54	0.91
	1.45	0.87
	1.17	0.70
	0.82	0.61
		0.49
		0.45
82.7	73.6	53.8

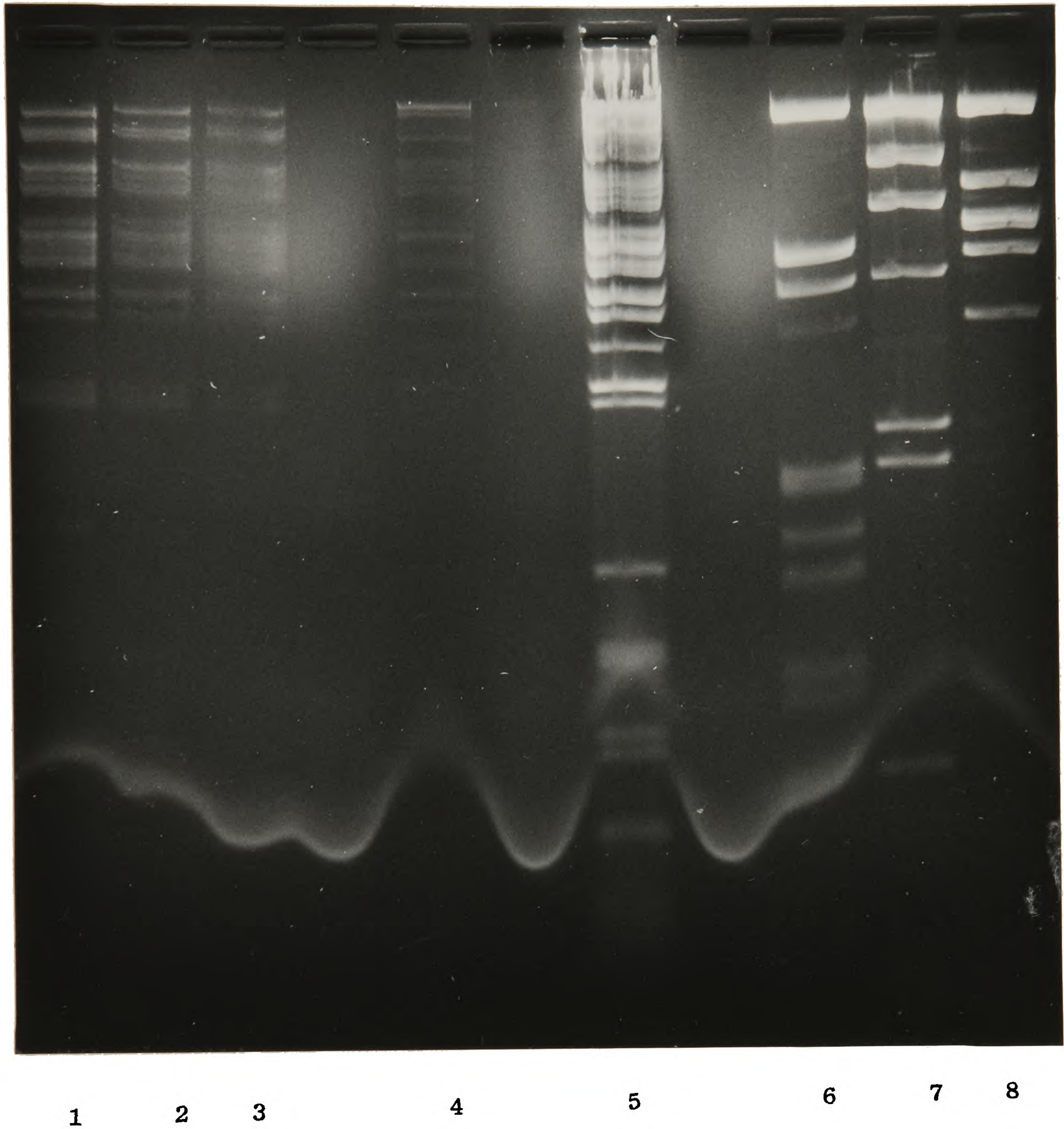
(a) mean from 3 gels

(b) mean from 2 gels

c values in parenthesis indicates possible double fragments

Figure 4.

1.0% (w/v) agarose gel electrophoresis of the French isolate of MbNPV C9 and J1 DNA REN digests using Hind III



Tracks 1, 2, 3 MbNPV, French isolate

Track 4 C9

Track 5 J1

Tracks 6, 7, 8 λ DNA digested with Hind III and EcoRI, Hind III, EcoRI respectively

Table 6. Molecular weights of MbNPV, J1 and C9 DNA isolates digested by Hind III

Molecular weights (x 10^6)

J1	C9	MbNPV
17.4	17.4	14.0
9.5	12.8	9.5
8.3	8.3	8.3
6.7	6.8	6.0
5.5	5.5	5.5
4.8	4.9	5.0
4.6		4.9
4.3		
3.79	4.0	4.0
3.51	3.72	3.79
3.32	3.32	3.32
3.09		
2.75	2.81	2.87
2.54	2.54	2.60
2.20	2.20	
1.87	1.89	1.89
1.76	1.79	1.81
0.96		1.30
0.74		
0.47		
0.42		
0.28		
88.8	78.0	74.8

DISCUSSION

1. P.interpunctella GV

The gel profiles of the structural polypeptides and the DNA fragments of PiGV were clearly distinguishable from the corresponding profiles of the other BVs tested.

Generally when using 12% acrylamide gels 14 polypeptides were resolved from the VPs and six from the nucleocapsids. All the polypeptides found in the nucleocapsids had molecular weights similar to polypeptides in the VPs. The remaining eight polypeptides were probably associated with the envelope which was removed from the VPs to release the nucleocapsids.

Two or three more VP polypeptides were resolved when a gradient gel was used. Tweeten et al. (1980a) had similarly been able to resolve more polypeptides by using gradient gels, which is attributed to their greater resolving power compared with uniform concentration gels. Tweeten et al. (1980a) were however, able to resolve one more VP polypeptide and two more nucleocapsid polypeptides than presented in this report. This is probably because of the greater range of concentrations of gels used by those workers.

The profiles of the polypeptides from the VPs and nucleocapsids on discontinuous gels obtained in this work are similar to those presented by Tweeten et al. (1980a). However, the actual molecular weight estimates were different, but these estimates are subject to variation dependent upon the exact method used.

The fragments generated from PiGV DNA and EcNPV DNA by digestion with EcoRI, Hind III or Bgl II differed markedly in number and size. Similarly there was a large degree of genetic diversity revealed when the fragment profile from the digestion of

viral DNA by Hind III was compared for PiGV with MbNPV or the isolates of NpNPV.

The patterns of the fragment profiles obtained from PiGV DNA were virtually identical to those presented by Tweeten et al. (1980b) for the same virus and RENs. However, the actual molecular weight estimates of the fragments showed marked variation when compared to those given by Tweeten et al. (1980b). Like those found for the structural polypeptides, it is thought that the molecular weight estimates are sensitive to the methods used. The similarity between the profiles of PiGV DNA presented in this work and those presented by Tweeten et al. (1980b) strongly suggests that both are the same virus.

The molecular weight for the PiGV genome of 49×10^6 estimated in this study is much lower than the 70×10^6 provided by Tweeten et al. (1980b). The value is probably artificially low as some of the bands may have contained more than one fragment, densitometer tracings of the gels may have enabled this error to be remedied.

2. E.cautella NPV

The gel profiles of both the structural polypeptides and the DNA fragments of EcNPV were clearly distinct from the corresponding profiles of the other viruses tested.

Only nine polypeptide bands were obtained from the VPs, these ranged in molecular weight from 13600 to 39100. The polypeptides of the nucleocapsids were not isolated because of a shortage of material. VPs normally consist of 12 to 18 polypeptides (Tweeten et al., 1980a). The low number resolved could indicate that the gels were underloaded.

Characteristic profiles of EcNPV DNA cleaved by three RENs

were presented. The genome size was estimated to be 70×10^6 by summing the molecular weights of the REN fragments.

3. M.brassicae NPV and N.pronuba NPV isolates

A comparison of the homologous MbNPV, J1 and C9 failed to reveal any differences in the profiles of the structural polypeptides run on a gradient gel. It was tentatively suggested that the two NpNPV isolates, J1 and C9 were MbNPV. However, when the molecular weights of the various polypeptide bands were estimated from uniform 12% gels, there was evidence to suggest slight differences between the three isolates. The two highest molecular weight polypeptides for MbNPV were heavier than the corresponding polypeptides for the NpNPV isolates, however the small number of replicates makes this result uncertain. Perhaps of more significance is the polypeptide with a molecular weight of about 35000 which was present in MbNPV and J1 but absent in C9.

It was concluded from the polypeptide analysis that the three viruses were closely related but it was unclear as to whether all three isolates were the same. It was therefore considered worthwhile to compare digestion fragments of the viral DNAs as this would produce a more definitive result. However, because the DNA produced did not readily go into solution only one digest could be performed on the NpNPV isolates and these were of unknown concentration. For the same reason the German isolate of MbNPV DNA was unsuitable for use and a French isolate which had previously been prepared and stored was used at three different concentrations to provide a comparison. The fragment profiles of the three isolates were very similar despite the differences in loading. However, there were few differences in number and position of the fragment bands. Some of these differences would

probably have been eliminated if the German rather than the French isolate of MbNPV had been used. Brown et al. (1981) have previously demonstrated that these two isolates of MbNPV have slight differences in the migration rates of a few of the DNA fragments after REN digest. There were a few differences between the two NpNPV DNA fragment profiles but further work would need to be performed to show whether these differences were real.

The results from both the polypeptide analysis and REN digests of the DNAs suggests that all three isolates were closely related. The results support the hypothesis that the virus which infected the two N.pronuba larvae originated from the NPV-infected M.brassicae released in the cabbage plots earlier in the trial.

The profiles of the structural polypeptides of MbNPV VPs and nucleocapsids had the same pattern on discontinuous 12% SDS-acrylamide slab gels as those published by Kelly and Brown (1980). However, the actual estimated molecular weights revealed variations but these could be attributed to the differences in the methods employed. Kelly and Brown (1980) used 10% SDS-acrylamide cylindrical gels which means that molecular weight markers must be run on separate gels.

The profile of the DNA fragments of the French isolate of MbNPV digested by Hind III was essentially the same as the corresponding profile of the Netherlands strain of MbNPV analysed by Vlak and Gróner (1980). Brown et al. (1981) in their comparative studies of MbNPV demonstrated that the Dutch isolate was the same as the French isolate, whilst the Oxford and German isolates were another variant. The profiles of C9 and J1 DNA fragments more closely resembled the corresponding profile of the German isolate than the Dutch isolate published by Vlak and Gróner (1980). However one notable difference was an additional high

molecular weight fragment (13 x 10^6 daltons) present in C9 and possibly also in J1.

The molecular weight of MbNPV genome estimated by taking the mean of the three isolates of the summed molecular weights of the DNA fragments was 80.5 x 10^6 daltons. This value compares well with the value of 78.6 x 10^6 obtained by Kelly and Brown (1980) using the DNA fragments produced by the same REN.The same workers pointed out that the summed molecular weights of REN fragments were lower than the estimates of total genome size (1.2 x 10^6) by other methods.