THE INFLUENCE OF FOOD PLANT CONSTITUENTS ON GROWTH AND MORTALITY OF THE MIGRATORY GRASSHOPPER *MELANOPLUS SANGUINIPES* (FAB.)(ORTHOPTERA:ACRIDIDAE) INOCULATED WITH *NOSEMA LOCUSTAE* CANNING (MICROSPORIDIA:NOSEMATIDAE).

A Thesis

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by

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ABSTRACT

The effects on survival, development and adult weights of Melanoplus sanguinipes of diets composed of leaves of twenty-one plant species were investigated. The effects of six secondary plant chemicals were tested by adding each to dandelion leaf meal.

All the Cruciferous leaf meals were good diets. Of the Compositae, dandelion meal was a good diet whereas ox-eye daisy meal was toxic. The secondary plant chemicals added to dandelion leaf meal had no effect on developmental time or adult weights at the concentrations tested.

Poor leaf meal diets reduced survival time of grasshoppers inoculated with Nosema locustae. Digitonin, coumarin and dicoumarol also decreased survival time of inoculated grasshoppers.

Therefore grasshoppers were more susceptible to the pathogen when they ingested toxic chemicals. The stress of nutritional deficiency may also have been involved in the poorer leaf meal diets.

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INTRODUCTION

The nutritional value of food plants is important in the growth, development and survival of insects and is important in determining their degree to susceptibility to disease.

House (1966) suggests that the principles of nutrition in insect health are as important as nutrition in the health of humans. Various authors have demonstrated that food plants and food plant constituents have a measurable effect on the behavior, development, survival and fecundity of insects. (Harley and Thorsteinson 1967; Pfadt 1949; Pickford 1962; Nayar 1964).

Although nurtition is important in the susceptibility of insects to disease, information on the precise role of nutrition is generally lacking. Steinhaus (1954) states, "There exists extremely little reliable information relating to how nutrition affects the resistance and susceptibility of individual insects or small groups of insects to disease, let alone large populations".

This study was undertaken to explore the effects of various food plants and some of their constituents on the growth and mortality of a grasshopper, Melanoplus sanguinipes (Fabricius).

Literature Review

The Pathogen

Life Cycle

The Microsporidian Nosema locustae was first described by Canning (1953). It was isolated from the fat bodies of the African migratory locust, Locusta migratoria migratoriodes R.& F.

Schizogony: Shortly after the Microsporidian spore is ingested by the host, the polar filament is extruded, penetrating the gut wall, allowing the sporoplasm to escape into the haemocoele as an amoebula. The fat cells are the only tissue to be infected. The earliest schizont is a small spherical body, uninucleate, measuring 2.5$\mu$to 3.5μ . The schizont grows and undergoes nuclear division giving rise, first to a binucleate body and then a quadrinucleate body. Asexual reproduction continues inside the host cell until food reserves are exhausted .

Sporogony: The uninucleate products of schizogony (sporonts) become oval, the nucleus of each divides once and a thick resistant wall is formed, giving rise to a single spore. Spores are obtained from the fat tissues of the host, and the size varies, 4 to 6.5μ in length and 2.5 to 3.5μ in width. (Canning 1962). (Fig. 1).

Canning (1962) reported that biological races of the parasite exist which have differing infectivities in Locusta migratoria migratorioides, Cammula pellucida, Melanoplus sanguinipes and Melanoplus bivittatus.

A Microsporidian, Nosema acridophagus was isolated from Schistocerca americana by Henry (1967). It was also found to be infectious for M. sanguinipes, M. bivittatus and M. differentialis. Comparative morphological and pathological characteristic are

presented by the above author, to differentiate between the two Nosema species. Henry (1969) indicated that Nosema locustae is a pathogen of the fat bodies, pericardial tissues and neural tissues of grasshoppers. He also reported that infectivity of spores obtained from different host species, did not vary to the extent of indicating biological races of the disease.

The Role of food plants in the nutrition of Melanoplus sanguinipes.

Field Observations

Mulkern, Toczek and Brusven (1964) analyzed the crop content of six hundred individuals of M. sanguinipes and identified 46 different species of plants present. On this basis it was classified as a mixed feeder. Pfadt (1949) reported that where dense populations of M. mexicanus (=sanguinipes) were present the principle food plant was dandelion. The chief host plants in most cases were forbs, but not all forbs were eaten and in most cases native grasses were not fed upon. The grasshopper usually selected a particular forb out of several growing in the habitat. He concluded that the grasshopper will feed on a number of plants, but has definite food preferences.

Alfalfa is considered as one of the principal food plants of the migratory grasshopper throughout the United States and other parts of the world. (Mulkern, Anderson and Brusven 1962). Grasshoppers in alfalfa fields ingest primarily alfalfa, but also other plants. Scharff (1954) reported that M. mexicanus (=sanguinipes) when the habitat permits, generally chooses plants favorable to its growth and vitality, but may devour other species of plants in the absence of its favorites. Some grasshoppers (Melanoplus differentialis) will feed entirely on dried-up plants,

even in the presence of fresh plants (Kaufmann 1968).

Laboratory Studies

Effects of food plants on development, fecundity and survival of grasshoppers was studied by Shotwell (1930), Pfadt (1949), Brett (1947), Smith, Handford and Chefurka (1952), Smith (1959), Pickford (1959 & 1962) and Barnes (1965).

The above studies reveal, (a) that there is a definite correlation between food and development and mortality, (b) that there is little correlation between plant families and mortality, (c) that there is a highly significant positive correlation between plants which are preferred and plants which produce high survival (preference was judged by the amount of feeding on various plants, a larger amount of the preferred plant being eaten), (d) and that food plant species are correlated with fecundity and the size of egg pods.

Particular elements in food plants are also known to affect the development of grasshoppers. (Smith and Northcott 1951; Smith 1960).

The influence of plant chemicals on the feeding behavior, development and survival of grasshoppers has been investigated by Tauber (1959), Nayar (1963a & 1963b), Thorsteinson and Nayar (1963) and Harley and Thorsteinson (1966). It can be concluded that plant chemicals have a definite effect on the grasshopper, and possibly determine the degree to which they feed on the plants.

The possible role of plants in insect resistance or susceptibility to disease.

In order to examine the role of plants in insect resistance or conversely the susceptibility of insects to disease, it is

necessary to explore the field of insect nutrition and the nutritional value of food plants. The nutritional value of food plants is important in the growth, development and survival of insects and may be important in determining their susceptibility to disease.

Garber (1956) in his "nutrition-inhibition hypothesis" states "that the environment of the host directly affects the fate of an invading parasite (pathogen)". A nutrition environment may be inadequate to support or allow the multiplication of parasites or an inhibitor environment may suppress the development of the parasite, or prevent it from surviving in the host at all.

Host Nutrition - Pathogen Relationship

In order to hypothesize that host nutrition has an effect on an invading pathogen it is necessary to establish some relationship between the two.

An interesting relationship between host nutrition and resistance to disease was demonstrated by Schneider (1967). In this case working with mice and the bacterium Salmonella typhimurium, he discovered a highly potent resistance factor in some natural foodstuffs, which was termed "pacifarin".

McLaughlin (1965) working with the boll weevil, Anthonomus grandis Boheman, and its associated pathogen, a protozoan Mattesia grandis McLaughlin, found that the physiological state of the adipose tissue affected pathogen development. A similar relationship was reported by Dutky (1963) in the case of insects and the "milky disease". The nutritional state of the larvae at the time of infection and the amount of food available during the course of the disease has an effect on the development of the pathogen.

House and Barlow (1961) demonstrated that the diet of the host affects the development of a parasite. Mature dipterous host larvae, Agria affinins (Fall.), were reared on different chemically defined diets and were parasitized by the braconid Aphaereta pallipes (Sat). The effects of different diets resulted in different degrees of mortality and emergence of the parasitoid.

Silverman and Levinson (1954) working on the lipid requirements of the larvae of the housefly, Musca vicina (Macq.) showed that the larvae on diets which lacked a source of sterol, were unable to resist infection from pathogenic bacteria.

Effects of Food Plants

There are two possible effects of plant constituents on the resistance or susceptibility of insects to disease. These are: 1. The plants contain antimicrobial substances which suppress or prevent the development of the pathogen.

2. The plants provide a stress factor which induces an increase in the pathogen due to the weakening of the host.

Antimicrobial Substances

The use of plants as drugs in controlling disease is not a new endeavor. This practice dates back to about 4000 B.C. The scientific study of plants to determine antimicrobial material is however, comparatively new. Obsorn (1943) tested approximately 2300 species of plants belonging to 166 families against Staphylococcus aureus and Escherichia coli and found that 63 genera contained substances which inhibited the growth of one or both of the test organisms. He found that the substance may be distributed throughout the plant or restricted to certain parts, also drying of some plants causes a loss of inhibitory power. In other cases there is no loss. Nickell (1959)

reviewed the literature on the study of antimicrobial substances in plants, covering several hundred species from 157 families. Of these he reported 1262 species contained antimicrobial agents effective against both gram-negative and gram-positive bacteria, viruses, fungi and yeasts.

Kushner and Harvey (1960 & 1962) performed *in vitro* tests with extracts of 18 species of forest tree foliage against the following insect pathogens - *Bacillus cereus*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa* and *Serrtia marcescens*. The extracts were very effective in preventing the growth of *Bacillus cereus* and *Bacillus thuringiensis* but were less effective against non-sporeformers tested. The greatest amount of inhibitory substance was produced in some species of coniferous foliage. It is interesting to note that the antibacterial substances were also present in the gut contents of larvae that had eaten the foliage.

Smirnoff and Hutchison (1965) tested the foliage from each of 74 species of plants on the development of *Bacillus thuringiensis* var. *thuringiensis* Berliner and found that some of the extracts from the foliage completely inhibited or delayed the normal growth of the bacteria.

Chiang and Holdaway (1960) found a higher infection of the protozoa *Perezia pyrastae Paillot*, among corn borers that matured on the susceptible varieties of corn than those on resistant varieties.

Smirnoff (1967) tested the phytocidal effects of certain plant juices upon the relationship between the Microsporidia *Nosema cerasivoranus* and its host *Archips cerasivoranus*. Certain plant juices prolonged the life of the infected larval

and pupal stages and in some cases promoted the formation of twice as many pupae.

Stress Factors

Steinhaus (1960) defined stress "as a state manifested by a syndrome, or bodily changes, caused by some force, condition or circumstance in or on an insect or on one of its physiological or anatomical systems". He goes on to say "a stressor may also be thought of as any stimulus, or sucession of stimuli that tends to disrupt the homeostasis of an animal".

Pimentel and Shapiro (1962) defined stress "as the abnormal effect of any environmental factor on the insect or pathogen and their relationship". They suggest that if the host is stressed and the pathogen is not, the disease incidence increases. Conversely if the pathogen is stressed and the host is not, the disease incidence tends to remain normal. A stress factor may involve a number of environmental conditions one of which is food supply.

Feeding snapdragons to Calophasia lunula larvae, in place of their normal food, toadflax, decreased the survival of larvae infected with a benign cytoplasmic disease (Bucher and Harris, 1968).

Vago (1951) reported that 70 percent of the silkworms fed on leaves of Maclura aurantiaca Nutt., succumbed to a polyhedrosis. Steinhaus (1958), who conducted a similar experiment using the foliage of Maclura ponifera (Raf.) and a Hungarian strain of silkworm, reported that not a single case of polyhedrosis appeared. David and Gardiner (1965) reported that the incidence of granulosis death among susceptible and resistant Pieris brassicae (linnaeus) larvae was increased

when the susceptible larvae were fed unfamilar food. Larvae of Peridroma and Junonia fed on plants they do not usually accept failed to induce the appearance of virus infection. Larvae died from lack of adequate nutrition or from causes other than virus (Steinhaus and Dineen 1960).

Pimentel and Shapiro (1962) found that the larvae of Galleria mellonella (Linnaeus) were more susceptibile to polyhedrosis when maintained on a high protein diet. Tanada (1956) in experiments with Pseudaletia unipuncta reported that there was no marked increase in susceptibility when the armyworm was raised on Napier grass, Kikuyu grass or corn.

An examination of the literature reveals that a number of factors may increase or decrease an insect's susceptibility to a pathogen, but the mechanisms by which this occurs are little understood.

Materials and Methods

In this study various plants that were known to occur in the food range of this grasshopper and plants that contained particular chemicals were tested. Individual plant chemicals were incorporated into the dandelion diet, which was chosen as a standard. The test chemicals and concentration at which each was investigated are listed in Appendix II. The plants used in this investigation are listed in Appendix I.

Insect

A non-diapause strain of Melanoplus sanguinipes (Fabr.) was utilized in all tests (Riegert 1961). The eggs produced in the laboratory were incubated at 30°C. to induce hatching. Any surplus eggs were mixed with moist sand and stored at 40°F. until needed.

Nymphs were reared on lettuce and bran until the third instar was reached, this instar being used in all tests.

Pathogen

A micosporidian disease, Nosema locustae Canning, was used as the pathogen.

Cages

The cages were constructed from plastic pill vials, length 9 cm. and diameter 6 cm. Round holes were punched in the lids, which were lined with cheese cloth to allow for adequate ventilation. A disposable Pasteur pipette, sealed at the narrow end, was filled with distilled water and a piece of absorbent cotton was placed in the open end as a wick, from which the insects received water. The pipette was placed in a small hole in the plastic lid and pushed part way down into the cage (Fig. 2). A wooden applicator stick was

placed in each cage to provide a surface on which the nymphs could molt.

Preparation of food material

Plants were collected in Manitoba or Saskatchewan, washed in distilled water and dehydrated at a temperature of 40±5°C., until the plants were crisp (6 to 10 hrs.). The dry material was crushed to a powder in a Torsion electric mortar grinder. Food pellets were produced by compressing the slightly moistened powdered plants with a glass rod and tubing. The food pellets were prepared 2 to 3 weeks in advance and held at -28°C. until used. (Fig. 3).

Preparation of test chemicals

The test chemicals were incorporated into the powdered dandelion and treated as for the preparation of food material.

Preparation of the pathogen

Spores of Nosema locustae were harvested from insects inoculated in the laboratory. These were purified by differential centrifugation, then counted using an AO Spencer bright-line hemacytometer.

Tests

Grasshoppers which had molted into the third instar (within 24 hrs.) were sexed, placed individually in small shell vials and starved for 24 hours. After the period of starvation a treated lettuce disc about 3 mm. in diameter was placed in the shell vial with the grasshopper.

One group of lettuce discs were treated with a small drop of water (5 microliters) containing 5×10^5 N. locustae spores (Henry 1966). The other group of lettuce discs were treated with 5 microliters of distilled water. Both groups were allowed to dry before being fed to the grasshoppers. (Fig. 4).

Shortly after the lettuce discs were eaten, the nymphs were removed from the shell vials and placed individually in the vial cages. Food pellets were weighed (dry weight basis) and one placed with each grasshopper.

All experiments were conducted at a temperature of 26.7°±1°C., and a relative humidity of approximately 65 per cent. Constant illumination was provided by a 25 watt incandescent lamp (100 c.p.) placed approximately one foot above a 5 sq. ft. surface on which the cages were placed.

The insects were examined daily for mortality and to ensure that water was present. Records were kept for the weight of the food pellet at each molt, the number of days in each stadium, mortality, adult weight (within 24 hrs of molting) and the number of spores in each infected grasshopper.

Diagnosis of dead nymphs and living adults was performed by examining prepared smears under a phase microscope. A spore count to estimate the level of infection was determined by using a method described by Raun, York and Brookes (1960).

Figure 1. Spores of Nosema locustae Canning. Size varies 4 to 6.5μ in length and 2.5 to 3.5μ in width. Spores indicated by arrows.

Figure 2. Cage constructed of 40 dram plastic pill vial, containing a disposable Pasteur pipette and wooden applicator stick.

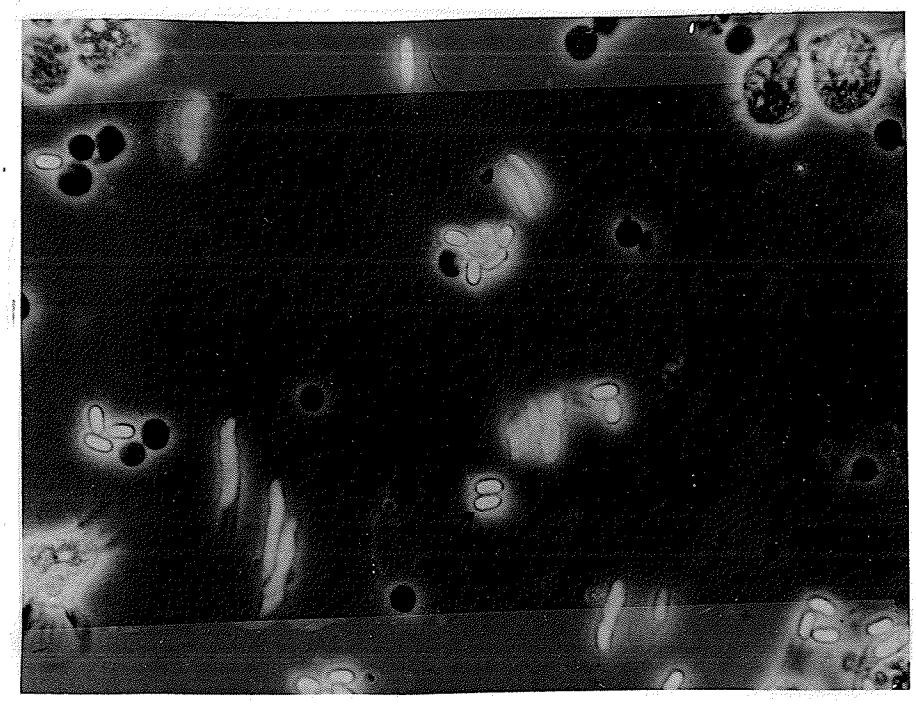
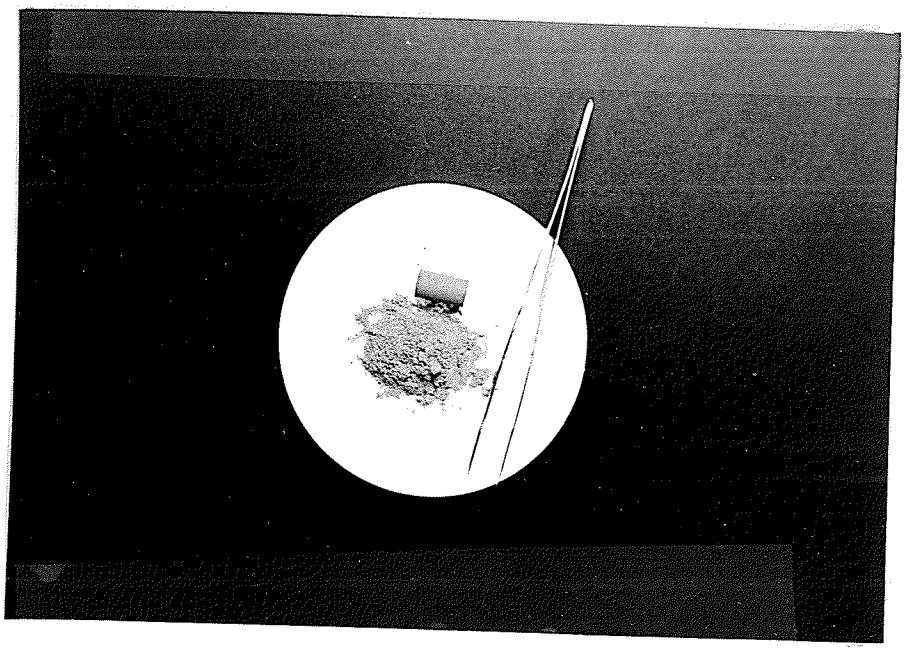
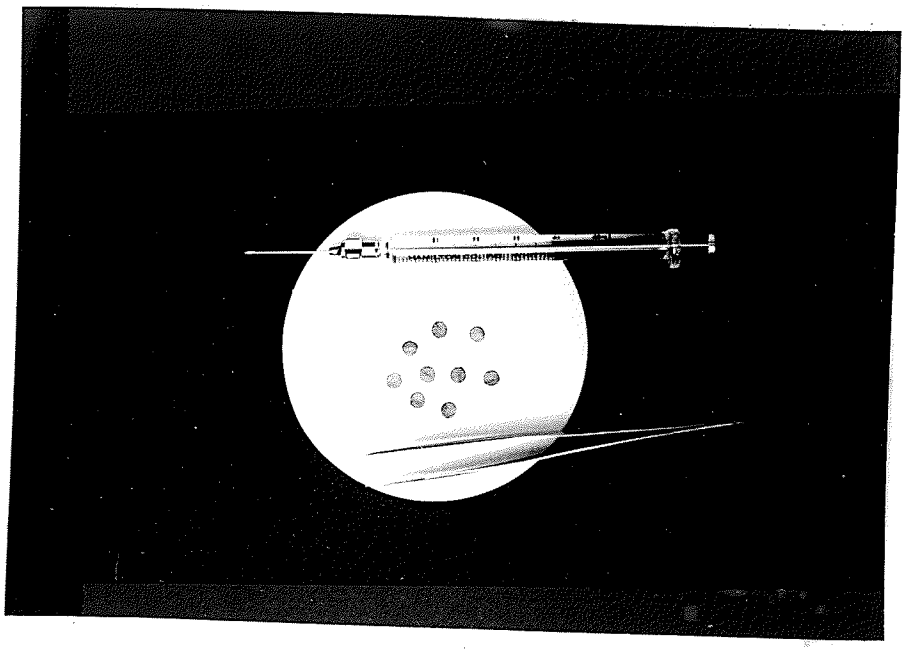


Figure 3. Powdered plant material and compressed food pellets.

Figure 4. Lettuce discs, about 3mm. in diameter and a microsyringe used to treat the lettuce discs.





Results and Discussion

Data were analysed using analysis of variance, Fisher-Yates test of significance in 2x2 contingency tables, Student t-Test and Spearman rank correlation coefficient (Baily 1959; Siegel 1956). Results of statistical tests are given in Appendix III. Interpretation of results are based on the following observations:

Uninoculated Insects

1. The greatest adult weights in the shortest time were realized with minimum mortality by insects reared on the most favourable foods. The graph indicates final weight (weight at death of nymphs or weight at time of survival to adult stage).

2. When adult weight is plotted against the number of days required to develop through instars IV and V, the performance of individual grasshoppers of both sexes reared on good foods is represented by points that occur near the upper left corner of the graph.

3. A greater dispersion of points, longer developmental time, lower adult weights and a shift of points toward the center of the graph is realized for poor foods.

4. The "food index", (adult weight in mg. divided by mg. of food consumed in fifth instar), is high for good foods and low for many of the poor foods.

5. Figures 48 and 49 indicate a relation among mean adult weight and mean developmental time with respect to plant families. Plant species are ranked according to mean adult weight and mean developmental time. A wide variation within plant families is noted.

Inoculated Insects

1. When final weight is plotted against the number of days required to develop through instars IV and V a greater dispersion

of points with lower mean final weights is realized for inoculated as compared with uninoculated insects.

2. When insects died before reaching the end of the fifth instar, the weight at death was plotted in lieu of the adult weight. These premature "final weights" occur near the lower left corner of the graph.

3. The inoculated insects often required much longer to reach a given stage of development.

Uninoculated Insects

Various criteria can be used to evaluate the effects of food plants on growth and mortality of grasshoppers. In this study, final weight, survival, developmental time and a food index were used as measures of the quality of plant diets. To facilate discussion the food plants are grouped into families.

Gramineae

The highest adult weights in this family were recorded for barley and wheat leaf meals. Mean weight of insects reared on oat meal was lower than on wheat or barley although the developmental time was similar for all three. Insects reared on orchard grass meal realized considerably lower mean adult weights and the longest developmental time for the Gramineae. The lowest food index for all diets tested occurred for orchard grass meal. This indicates that the insects had to eat more of the diet in order to obtain a given adult weight (lower food efficiency). The highest mortality was recorded for insects reared on the oat meal diet. (Figures 5, 7, 9, 11 & 47; Table 1). There was a significant difference among adult weights and developmental time for insects reared on the Gramineae diets.

Cruciferae

Adult weight, developmental time and survival were similar

for all plants tested in the Cruciferae. The highest food indexes for all plant diets tested occurred in this family, which indicates the insects ate less food in order to reach a given adult weight (higher food efficiency). The datum suggest that mustard, stinkweed and flixweed are suitable foods for this grasshopper. (Figures 13, 15, 17 & 47; Table I). There were no significant differences among the diets in respect to adult weight and developmental time.

Leguminosae

Marked differences occurred among the plants tested in this family. Highest mean adult weight and shortest mean developmental time was recorded for vetch meal. Slightly lower mean adult weight and a mean developmental time that was extended about two days longer than vetch was realized for alfalfa. A greater variation occurred among the individual grasshoppers reared on ladino clover meal. Adults of lower mean weights, longer mean developmental time and lower survival were obtained for ladino clover meal. Most insects reared on sweet clover meal did not develop beyond instar IV, most of the mortality occurring in instar III. One insect went through an incomplete fifth instar molt resulting in a very small badly deformed adult. (Figures 19, 21, 23 & 25; Table I). A chemical which occurs in sweet clover, coumarin is known to cause "sweet clover disease" in cattle.

Compositae

The lowest mean developmental time and the highest mean adult weight resulted when insects were reared on blue lettuce meal. Adults of similar weights were recorded for dandelion and yarrow meals. Dandelion, blue lettuce and yarrow appear to be suitable food plants for M. sanguinipes, based on high survival, adult weights and developmental times. (Figures 27, 29 & 31;

Tables I & II). The flowers of Pyrethrum cineriaefolium of the genus Chrysanthemum are used in the production of pyrethrum powder, an insecticide. Ox-eye daisy also belongs to the genus Chrysanthemum. There were no significant differences in adult weights and developmental times for grasshoppers reared on blue lettuce, dandelion and yarrow.

Chenopodiacea

Lamb's quarters was the only plant tested in the Chenopodiacea. Mean adult weight and developmental time are comparable to those realized for grasshoppers reared on orchard grass meal. The "food index" was higher for insects reared on lamb's quarters than orchard grass meal. Compared to good diets, lamb's quarters leaf meal resulted in lower adult weights and longer mean development time. (Figures 33 & 47; Table I).

Liliaceae

Death camas and lily of the valley meals were fatal to grasshoppers. Early mortality occurred when insects were reared on death camas and it is assumed that this plant contains substances that are toxic to M. sanguinipes. Although there was 100% mortality in grasshoppers reared on lily of the valley, survival time was longer. Either this plant contained substances that were less toxic or lacked suitable essential nutrients for continued growth of the grasshoppers. Muenscher (1951) reported that the poisonous property of death camas for animals is an alkaloid, zygadenine. He also reported that lily of the valley contains the glucosides, convallarin and canvallamarin (Table II).

Solanaceae

Cut-leaved nightshade meal was fatal to all the grasshoppers tested. Insects reared on nightshade meal realized lower final weights and shorter time to mortality than those reared on lily

of the valley. Members of the Solanaceae are known to contain alkaloidal, glucosides, for example solanine (Muenscher 1951). (Table II).

Scrophulariaceae

Foxglove meal resulted in 100% mortality of all grasshoppers tested. Results were similar to those obtained for nightshade. Foxglove contains a glucoside digitalis, which is very poisonous to animals. (Table II).

Equistaceae

Horsetail is also fatal to M. sanguinipes, all mortality occurring before the grasshoppers reach the end of instar IV. Kingsbury (1965) reported that horsetail contains the enzyme thiaminase which promotes the breakdown of thiamine (vitamin B_1). Nayar (1963) reported that without B-vitamins in a synthetic diet the nymphs of M. bivittatus survived for only about 8 days. (Table II).

Comments

The results of this study allow plants to be categorized into fairly definite groups. Leaf meals that resulted in insects similar to those reared on dandelion meal were classified as "good diets". Meals that resulted in grasshoppers of lower adult weights and longer developmental times were classified as "poor diets". Leaf meals that did not support survival to the adult stage were classified as "very poor diets". Barley, dandelion, blue lettuce, vetch, alfalfa, wheat, wild mustard, yarrow, flixweed and stinkweed were good diets. Poor diets included orchard grass, oats, lamb's quarters and ladino clover. Very poor diets included sweet clover, lily of the valley, foxglove, camas, horsetail and ox-eye daisy.

There was a significant positive correlation between adult

weights and daily food intake. The insects of higher adult weights had a larger daily food intake. This suggests that palatability in this case, affects the rate of feeding and therefore the adult weight. There was also a significant positive correlation between adult weight and developmental time; the grasshoppers on poor diets weighed less and required a longer time to develop to the adult stage. Harley (1965) using a chemically defined diet to which secondary plant chemicals were added reported that M. bivittatus adults all reached similar weights. This is not the case when M. sanguinipes is reared on various plant diets suggesting that these varied considerably in nutritional quality.

Pfadt (1949) tested nine monophytic (single plant species) diets in the laboratory and reported that alfalfa was the best food plant for growth provided that the nymphs began feeding on the plants during or after instar II. Smith, Handford and Chefurka (1952), working in Manitoba compared nine monophytic diets in cages outdoors. Dandelion, barley and wheat produced the highest survival of grasshoppers. Barnes (1965) tested nineteen monophytic diets and considering survival, rate of growth and size of grasshoppers, the best diets were desert mustard, Sisymbrium irio L; common sowthistle, Sonchus oleraceus L; and prickly lettuce, Lactuca scariola L. Wheat, mustard and dandelion were listed as preferred foods for M. bilituratus (Walker) (=sanguinipes) by Pickford (1962).

Smith (1959) fed M. bilituratus individually on wheat, western wheat grass and oats for 40 days after hatching. Survival was highest on wheat and lowest on western wheat grass but the rate of development was equal for wheat and western wheat grass

and lowest for oats. The final weight of the insects fed on oats was one third less than those fed on western wheat grass or wheat. The interesting point is that the efficiency of conversion of food to body tissue was 38% for oats, 32% for wheat and 27% for western wheat grass. This would suggest that oats are a poor food plant because M. bilituratus does not eat enough of it.

Although good foods and very poor foods may be found in the same family, the mustard family appears to contain many species suitable to the growth and survival of M. sanguinipes. In this study wild mustard, stinkweed and flixweed proved to be good food plants. Barnes (1965) reported "good to excellent" results in nymphal survival, developmental rate and adult size of M. sanguinipes when reared on desert mustard, shepherds purse and spectaclepod. Pfadt (1949) reported good results when grasshoppers were reared on tansy mustard.

Particular species within other families also provided suitable food plant diets. In this test good results were obtained for grasshoppers reared on blue lettuce of the Compositae family. Prickly lettuce, sowthistle and dandelion are also good diets (Barnes 1965). Similarly other families contain a large number of poor plant diets, such as the Chenopodiaceae. Lamb's quarters was considered a poor diet in this test. Pickford (1962) reported retarded development when grasshoppers were reared on Russian thistle and Russian pigweed. Nettleleaf goosefoot was also reported as a poor diet (Barnes 1965).

Results of these and other tests indicate that food plants have a definite effect on the growth and mortality of M. sanguinipes.

Inoculated Insects

Gramineae

Mortality of M. sanguinipes was similar for all graminaceous diets tested. The time to mortality and the instar in which the mortality occurred varied among the diets. The poor diet, orchard grass meal, resulted in a significant increase in time to mortality for female grasshoppers. Significantly more female grasshoppers died before the end of instar IV for orchard grass and oat meal. Most of the mortality for grasshoppers reared on wheat and barley meals occurred in instar V. The final weights of nymphs were highest for wheat meal and lowest for orchard grass meal. (Figures 6, 8, 10 & 12; Table I).

Cruciferae

Time to mortality was not significantly different among the Cruciferae tested; however significantly more male grasshoppers died in instar IV when reared on flixweed meal. A few inoculated grasshoppers reached the adult stage when reared on wild mustard. These individuals reaching the adult stage usually contained a large number of spores. The mean final weights varied little for grasshoppers reared on the Cruciferae. (Figures 14, 16 & 18; Table I).

Leguminosae

All nymphs fed on sweet clover meal died before reaching the end of instar III. Mortality of M. sanguinipes and time to mortality was similar for insects reared on alfalfa and vetch meals. A great variation was realized for inoculated insects reared on ladino clover meal. Some insects died before reaching the end of instar III, while mortality for others occurred in instar V. There was no significant difference in mean time to

mortality of inoculated grasshoppers reared on alfalfa, vetch or ladino clover meal. (Figures 20, 22, 24 & 26; Table I).

Compositae

Mortality and time to mortality was similar for inoculated insects reared on dandelion and blue lettuce meals. A number of inoculated insects fed on blue lettuce reached the adult stage, usually with deformities, they contained a large number of spores. All grasshoppers fed on ox-eye daisy died before reaching the end of instar III. There was not a significant difference in time to mortality for grasshoppers reared on dandelion, blue lettuce or yarrow meal. (Figures 28, 30 & 32; Table I & II).

Chenopodiacea

All inoculated insects reared on lamb's quarters meal died before reaching the end of instar V. As compared to inoculated grasshoppers reared on dandelion meal, significantly more insects died before the end of instar IV. The time to mortality was considerably shorter for insects fed on lamb's quarters meal. (Figures 34; Table I).

Lilaceae, Equisetaceae, Solanaceae and Scrophulariaceae.

Mortality was similar for both inoculated and uninoculated grasshoppers reared on plant diets of the above families. Lily of the valley and foxglove meal were the only diets in this group which produced insects with a Nosema spore count. (Table II).

Comments

The plant diets tested had a significant effect on time to mortality of M. sanguinipes. Most of the poor diets resulted in a decreased time to mortality. In most cases the inoculated female grasshoppers were able to live longer than the inoculated males. Pickford (1962) reported that female nymphs and adults have a greater propensity for survival under adverse food

condition than the males. This would also appear true for the infected females.

Uninoculated Insects Fed on Secondary Plant Substances

In these experiments grasshoppers were reared on dandelion leaf meal to which various secondary plant substances were added. The control insects were reared on untreated dandelion leaf meal.

Tigogenin and Digitonin

Tigogenin and digitonin both have a steroid configuration. The oral LD_{50} of digitonin in cats is 0.25 mg. per kg. (Stecher 1960). All grasshoppers were reared on a diet of dandelion meal. Tigogenin and digitonin incorporated into the dandelion meal at one percent dry weight, had no significant effect on mean adult weight and mean developmental time. Survival for nymphs was similar to that for the control insects. (Figures 35 & 37; Table I). These results differ from those of Harley and Thorsteinson (1967), who found that tigogenin increased survival and rate of development of Melanoplus bivittatus.

Convallarmarin

Convallarmarin, a glucoside, found in lily of the valley incorporated into the dandelion meal at one percent dry weight, did not have a significant effect on adult weight, developmental time or survival as compared to insects reared on untreated dandelion meal. Grasshoppers reared on dandelion leaf meal containing convallarmarin reached the adult stage at similar times and there was less variation in the developmental time as compared to the other test chemicals. (Figures 39; Table I).

Farnesol

Farnesol is an acyclic sesquiterpenoid, a fifteen carbon compound (Robinson 1963), Farnesol incorporated into the dandelion leaf meal at 0.5 ml. per 14g. of dandelion meal, did

not produce a significant difference in adult weights or developmental time as compared to insects reared on untreated dandelion meal. Highest mortality was recorded for grasshoppers reared on farnesol in dandelion leaf meal. (Figure 41; Table I).

Coumarin and Dicoumarol

Coumarin and dicoumarol were incorporated into the dandelion leaf meal at 0.5 percent dry weight. Two molecules of coumarin under conditions of moisture that lead to the formation of mold, combine chemically to form a single molecule of dicoumarol. Dicoumarol is used as an anticoagulant in human medicine and is also used as a component in a hemorrhagic rodenticide. When compared to grasshoppers reared on untreated dandelion meal, no significant differences occurred for adult weights or developmental times of grasshoppers reared on coumarin or dicoumarol-treated dandelion leaf meal. This suggests that the toxicity of sweet clover meal is due to a higher level of coumarin or to the presence of other toxic substances. (Figures 43 & 45; Table I).

Comments

The daily rates of food intake for all diets treated with secondary plant chemicals were similar to untreated dandelion meal. The secondary plant chemicals tested in this study did not have significant effects on the growth of M. sanguinipes. Similar results were reported by Harley and Thorsteinson (1965) when M. bivittatus was reared on a chemically defined diet to which secondary plant chemicals were added. Harley (1965) reported that although the insects reached similar adult weights, the mean daily rate of weight gain was increased for insects reared on a chemically defined diet to which tigogenin had been added. Harley also reported that digitonin at three percent per dry

weight of diet was toxic to the grasshoppers, but not so at the one percent level.

Inoculated Insects Fed on Secondary Plant Substances

In these tests M. sanguinipes inoculated with Nosema were reared on dandelion leaf meal into which various secondary plant chemicals were added. Inoculated grasshoppers reared on untreated dandelion leaf meal served as the controls.

Tigogenin and Digitonin

The time to mortality for tigogenin was not significantly different from that for the control insects. Digitonin incorporated into the dandelion meal significantly decreased the time to mortality and significantly more insects died before reaching the end of instar IV as compared to the control insects. The females survived longer that the males when reared on the digitonin diet. (Figures 36 & 38; Table I).

Convallarmarin

Results for inoculated grasshoppers reared on convallarmarin were similar to those of the control insects. There was no significant difference in time to mortality or the instar in which the mortality occurred. (Figure 40; Table I).

Farnesol

Inoculated insects reared on farnesol did not vary significantly from the control insects in respect of time to mortality. One male insect reached the adult stage. (Figure 42; Table I).

Coumarin and Dicoumarol

Time to mortality for grasshoppers reared on coumarin was not significantly different than that for the control insects; however significantly more females died before reaching the end of instar IV. The males reared on coumarin died in the fifth

instar. It is suggested that this difference was due to the age of the diet. The male grasshoppers were reared on a freshly prepared diet containing coumarin. Females were reared on the same diet about three weeks later. All insects, both males and females, reared on dicoumarol died before reaching the end of instar IV, although the time to mortality was not significantly different to inoculated insects reared on untreated dandelion leaf meal. (Figures 44 & 46; Table I).

Comments

There was considerable variation in spore counts among individual inoculated grasshoppers on the same diets and individuals on different plant diets. It is assumed that plant diets did not affect the number of spores produced. On the whole the largest mean number of spores per mg. of insect body weight occurred for grasshoppers reared on the Leguminosae. Inoculated grasshoppers reared on blue lettuce meal resulted in the largest mean number of spores of all the plant diets tested. (Table I).

The greatest number of inoculated grasshoppers reaching the adult stage was realized for grasshoppers reared on blue lettuce and wild mustard. These insects, usually with deformities, contained large numbers of spores. Canning (1962) reported similar results for the African migratory locust, Locusta migratoria migratorioides.

No spores were found in most of the nymphs that died early, this was also true for a few of the insects that died late in the tests. Wieser (1956) indicated that much of the early mortality in insects infected with a Microsporidian pathogen is caused by bacteria in the gut which enters the haemocoel and causes septicemia when the polar filament penetrates the gut wall.

Other authors have shown that plant constituents do have an effect on the number of spores produced in infected insects. Smirnoff (1967) reported that in the case of the ugly nest caterpillar, Archips cerasivoranus, infected with Microsporidia, if the host plant foliage (cherry) was treated with onion extract infection by the protozoan pathogen was considerably inhibited. Mustard extracts also reduced infection.

This study indicates that pharmacological stress may play a major role in the mortality of inoculated grasshoppers. The poor plant meal diets and also dandelion meal containing digitonin, coumarin and dicoumarol acting as stress factors which significantly decrease the time to mortality.

It is interesting to note that a few inoculated individuals on various diets surviving for a very long time, contained only a small number of spores. It would appear that the grasshopper was able by some mechanism, to inhibit the rapid development of the pathogen, producing a chronic rather than an acute infection.

Table I

Results for grasshoppers reared on various plant diets

Diet	Test	Mortality in each stadium						Proportion infected*		Mean No. Spores per mg body wt. X 10^{-4}	
		III		IV		V					
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Wheat	C										
	N					7/8	7/8	3/8	6/8	4.6	99.8
Oats	C			1/8		1/8	1/8				
	N			4/8*	3/8	4/8	3/8	5/8	4/8	21.8	79.5
Orchard grass	C										
	N			4/8*	1/8	3/8	6/8	8/8	6/8	81.1	27.1
Barley	C										
	N			1/8		7/8	7/8	7/8	6/8	98.6	131.6
Alfalfa	C										
	N				2/8	8/8	6/8	6/8	6/8	154.7	142.8
Vetch	C						1/8				
	N					8/8	8/8	7/8	6/8	75.1	122.8
Sweet clover	C	2/5	3/7	3/5*	3/7*						
	N	5/5	8/8								
Ladino clover	C	2/5					1/7				
	N	1/6	2/8	2/6	1/8	3/6	5/8	3/6	3/8	112.6	177.3
Yarrow	C					1/5					
	N				1/8	5/5	4/5	4/5	5/5	56.6	70.6

Table 1

Diet	Test	Mortality in each stadium						Proportion infected[X]		Mean No. Spores per mg body wt. X 10^{-4}	
		III ♀	III ♂	IV ♀	IV ♂	V ♀	V ♂	♀	♂	♀	♂
Blue Lettuce	C										
	N			1/8		5/8	5/8	8/8	8/8	231.4	140.3
Dandelion	C				1/8						
	N					8/8	7/8	5/8	7/8	61.3	190.1
Wild Mustard	C				1/8						
	N				1/8	6/8	6/8	8/8	7/8	35.0	2.1
Stinkweed	C					1/5					
	N				1/4	5/5	7/8	4/5	4/8	58.4	30.6
Flixweed	C			1/7							
	N				3/5*	5/5	2/5	2/5	1/5	87.6	0.6
Lamb's quarters	C				1/8						
	N	1/8	1/8		5/8*	2/8	2/8	3/8	5/8	4.4	44.4
Tigogenin	C										
	N					8/8	7/8	7/8	6/8	67.3	16.2
Digitonin	C										
	N			7/7**	8/10**		2/10	5/7	1/8	82.9	0.6
Convallar-marin	C										
	N				1/8	8/8	7/8	6/8	4/8	35.5	58.1
Coumarin	C										
	N		1/11	8/8	1/11		9/11	3/8	6/11	44.4	131.7

Table I

Diet	Test	Mortality in each stadium						Proportion infected[X]		Mean No. Spores per mg body wt. X 10^{-4}	
		III		IV		V					
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Dicoumarol	C										
	N			4/4**	4/4**			2/4	2/4	1.6	1.6
Farnesol	C					1/5	2/8				
	N			1/5	1/8	4/5	7/8	2/5	5/8	2.3	12.5

C Uninoculated Insects.
N Insects inoculated with Nosema.

Note: The proportion of insects that died before end of instar IV was compared statistically with the proportion observed for insects of the same species and sex on dandelion leaf meal.

* Probability $\leq .05$ by Fishers Exact Probability Test.
** Probability $\leq .01$ by Fishers Exact Probability Test.

X - Only insects that contained enough spores to be counted on the hemacytometer are listed as infected. Insects indicated as not infected may have contained stages of the pathogen other than spores.

Table II

Results of rearing III instar nymphs

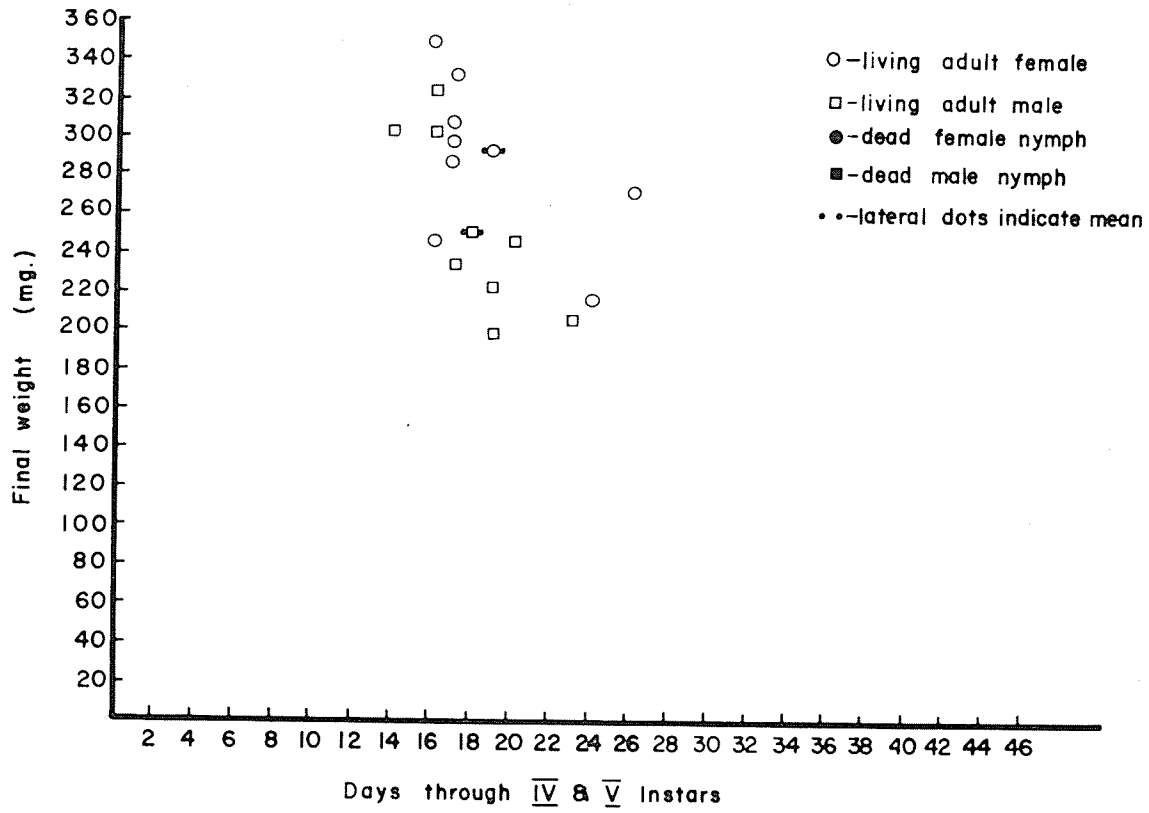
on very poor plant diets

Diet	Test	Final weight (mg.)		Mean days in each instar		Mortality		Proportion Infected	Mean spores per mg. body wt X10-5
		Mean	Median	III	IV	III	IV		
Camas [x]	C	14	15.0	7		3/3			
	N	18	18.0	5		3/3			
Ox-eye daisy[x]	C	21	22.0	3		3/3			
	N	17	15.0	4		3/3			
Nightshade[x]	C	32	20.0	21	2	2/3	1/3		
	N	20	19.0	7		3/3			
Lily of the Valley[x]	C	80	69.0	14	10	1/3	2/3		
	N	86	85.0	17	13	1/3	2/3	3/3	87.7
Foxglove[x]	C	18	18.5	25	6	3/4	1/4		
	N	18	16.0	18		4/4		1/4	0.6
Horsetail	C	45	57.0	12	13	1/9	8/9		
	N	20	18.0	12	6	7/9	2/9	4/9	0.6

x - Insects were reared at a temperature of 22±1°C.

Figure 5. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of wheat, not inoculated with Nosema.

Figure 6. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of wheat.


Final weight (mg.)
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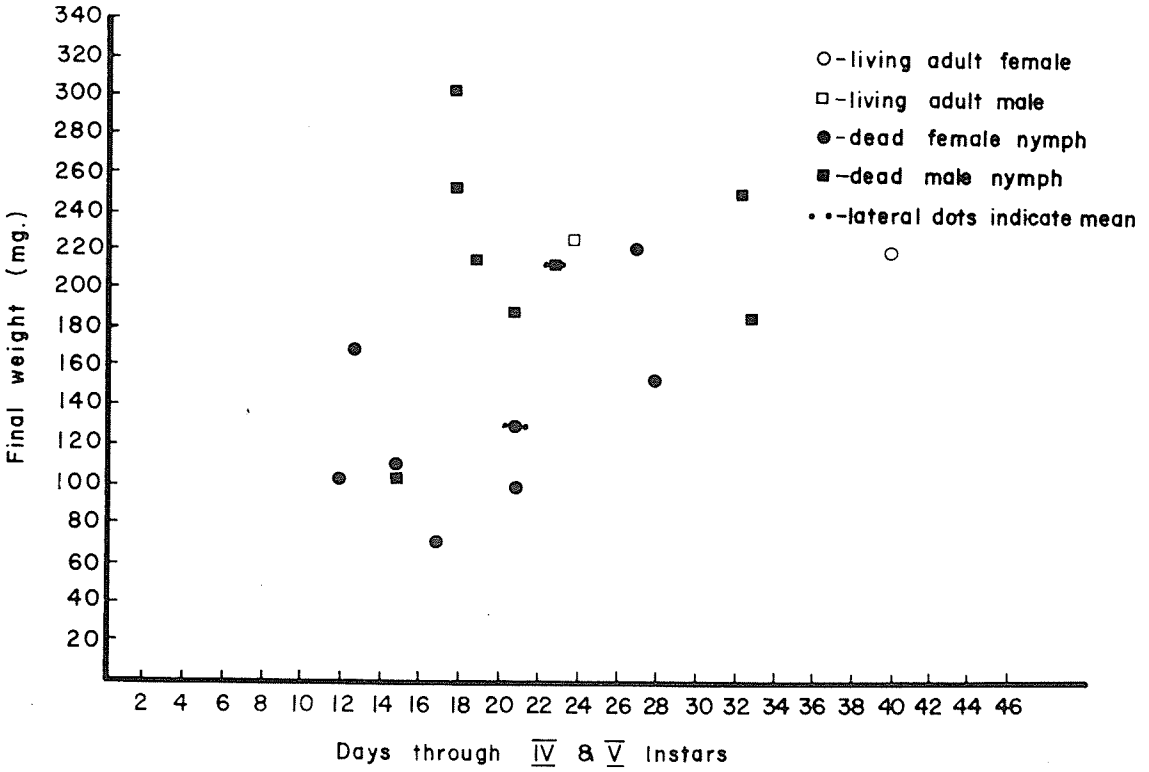
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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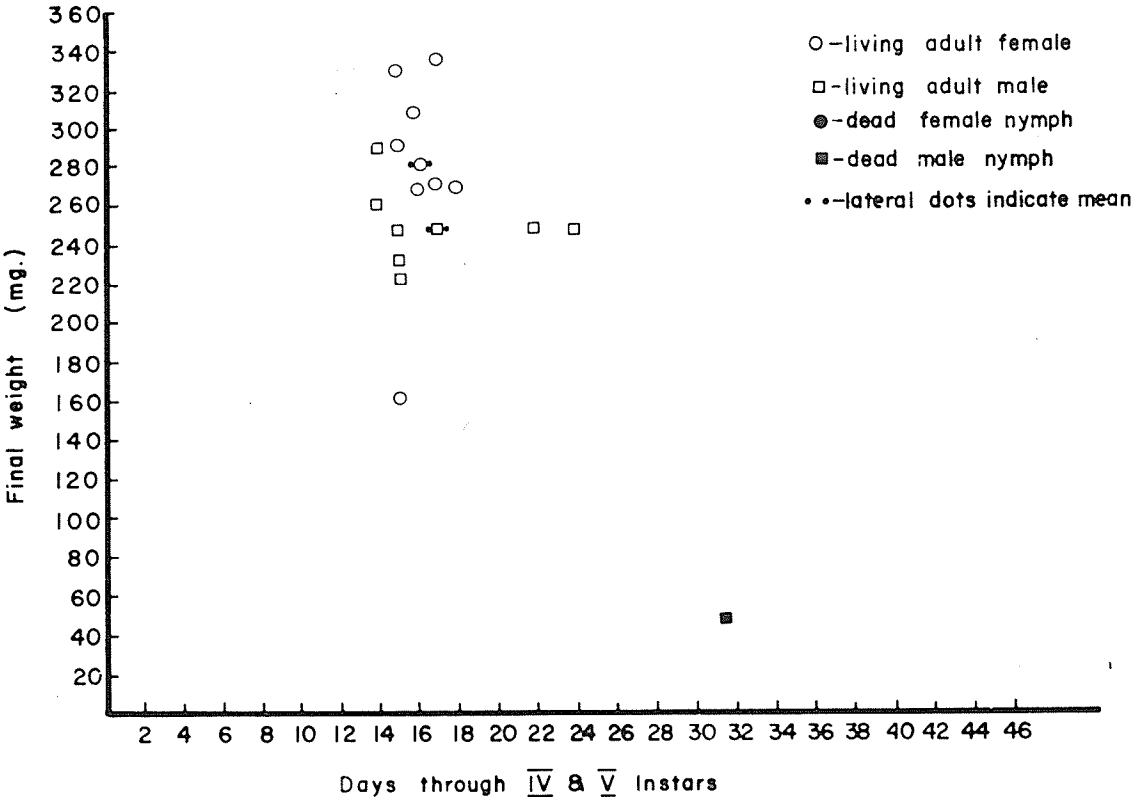
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 7. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of barley, not inoculated with Nosema.

Figure 8. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of barley.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Final weight (mg.)
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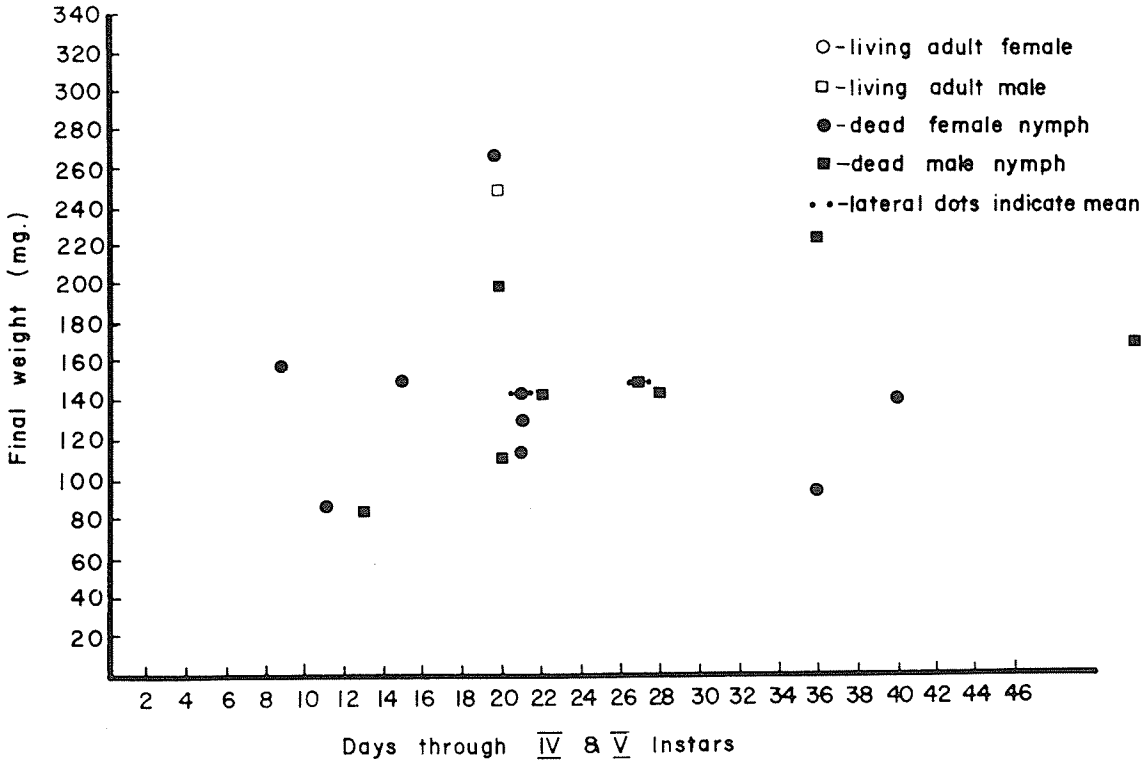
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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Final weight (mg.)
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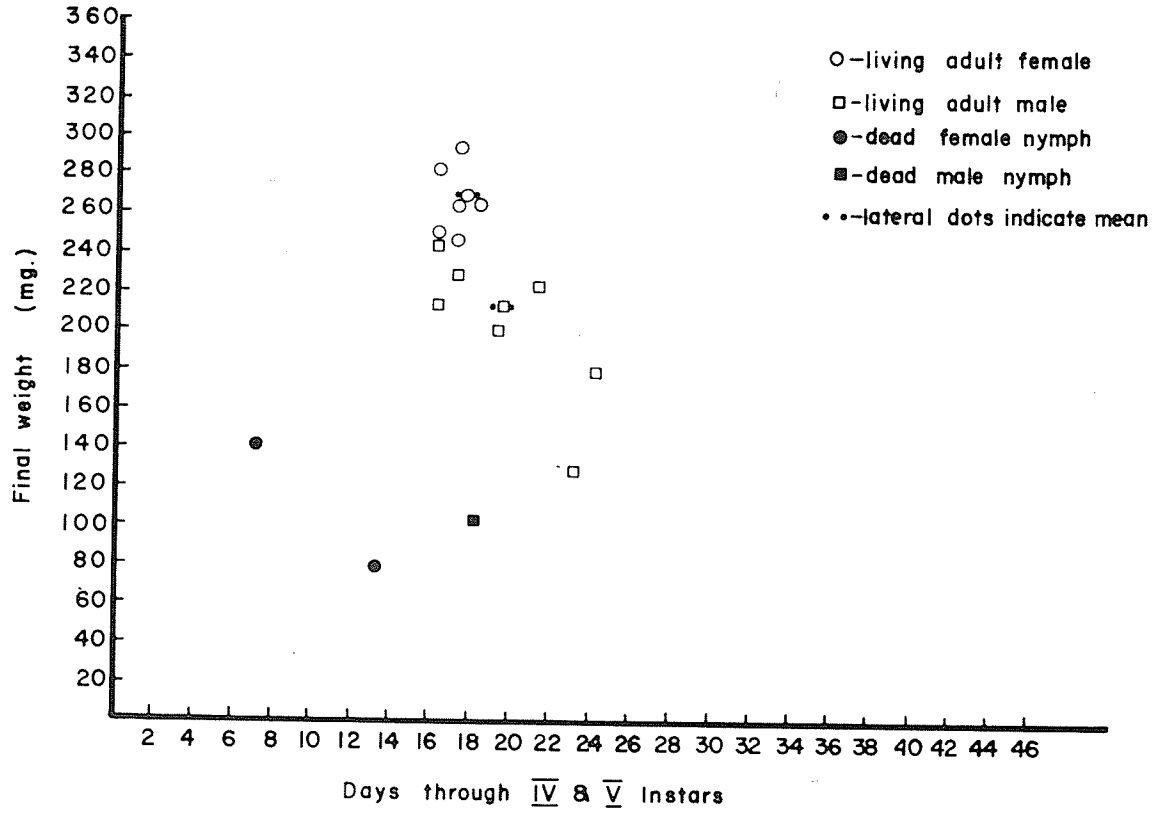
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
■51 days

Figure 9. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of oats, not inoculated with *Nosema*.

Figure 10. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with *Nosema*, and reared on a diet of oats.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Final weight (mg.)
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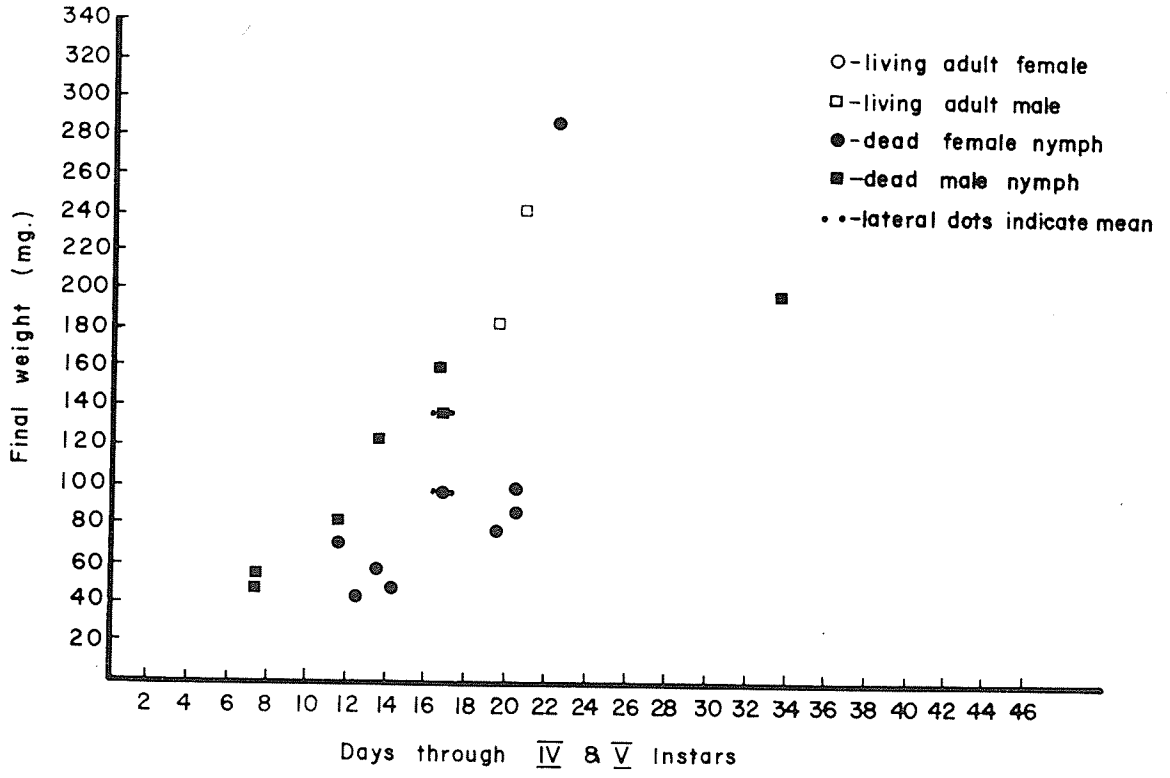
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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Final weight (mg.)
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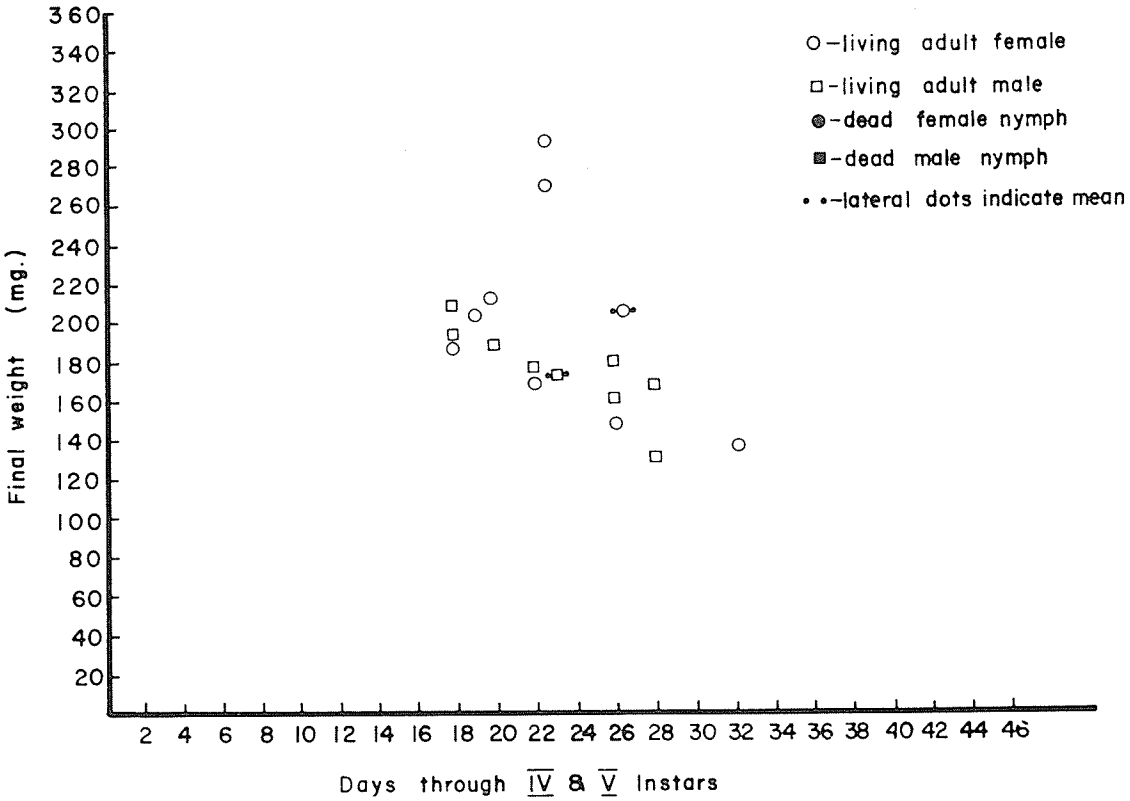
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 11. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of orchard grass, not inoculated with Nosema.

Figure 12. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of orchard grass.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Final weight (mg.)
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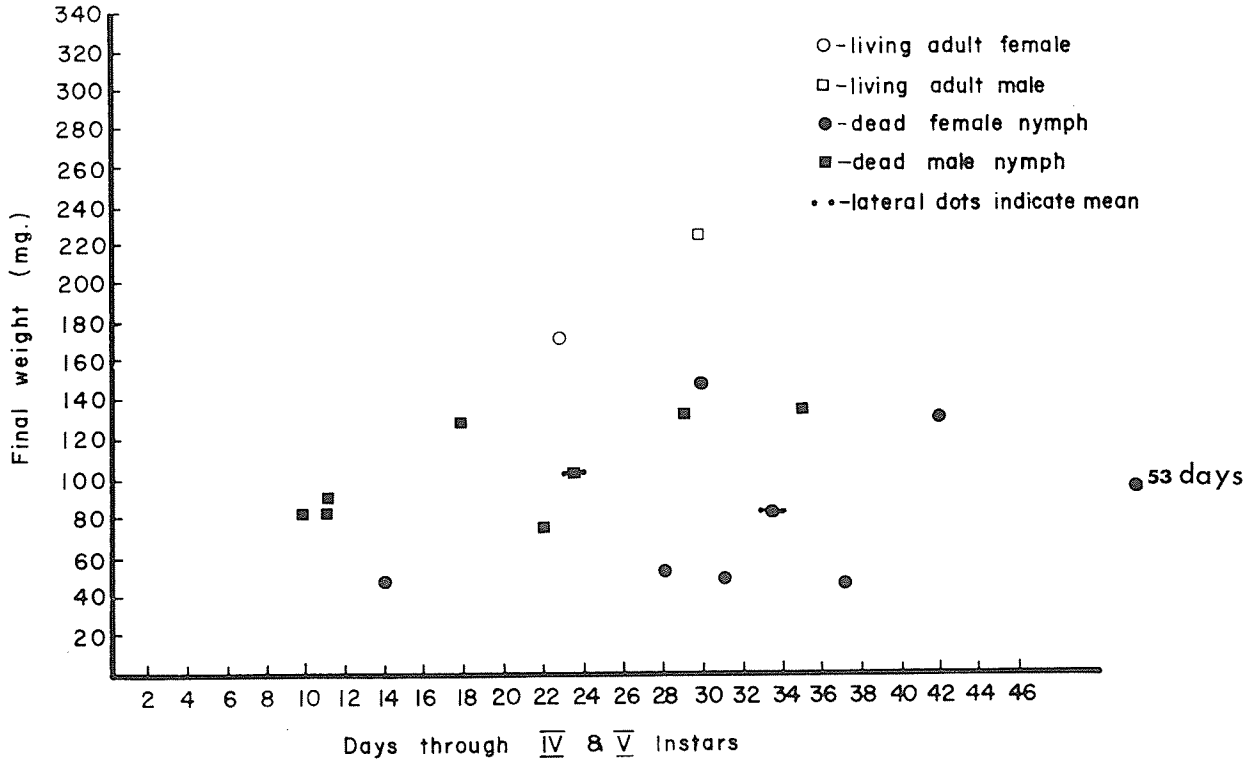
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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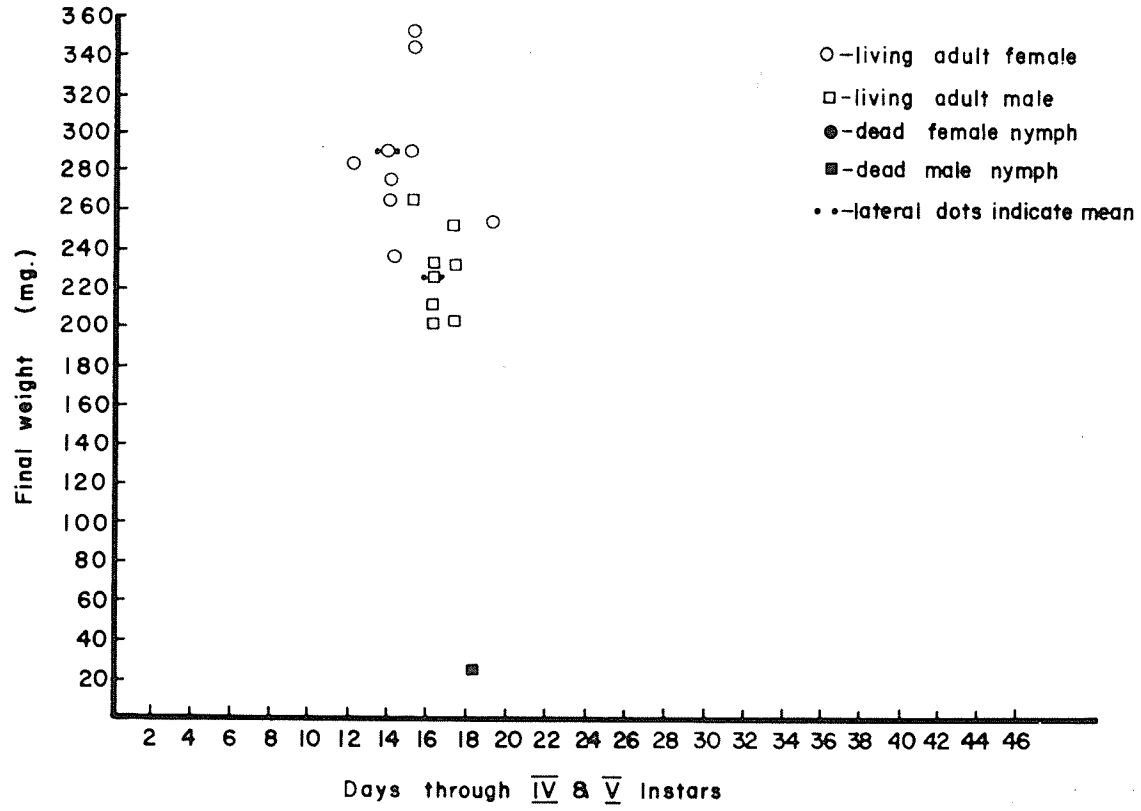
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
●53 days

Figure 13. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of wild mustard, not inoculated with Nosema.

Figure 14. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of wild mustard.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Final weight (mg.)
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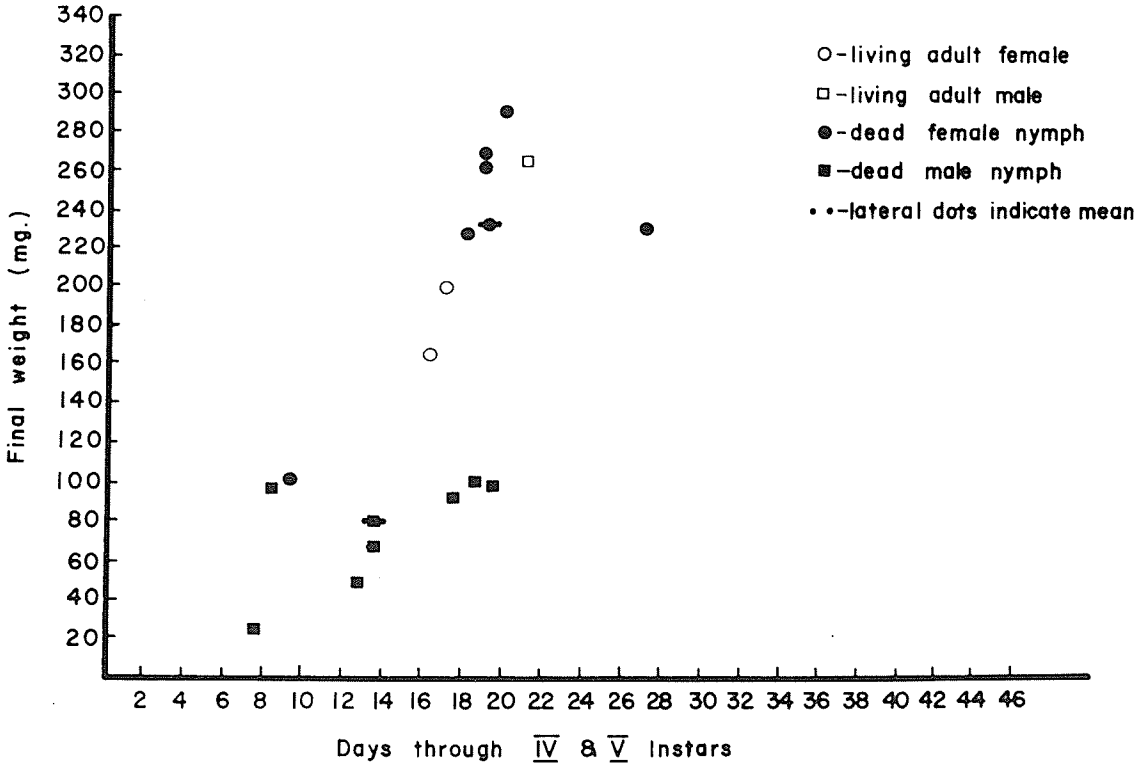
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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Final weight (mg.)
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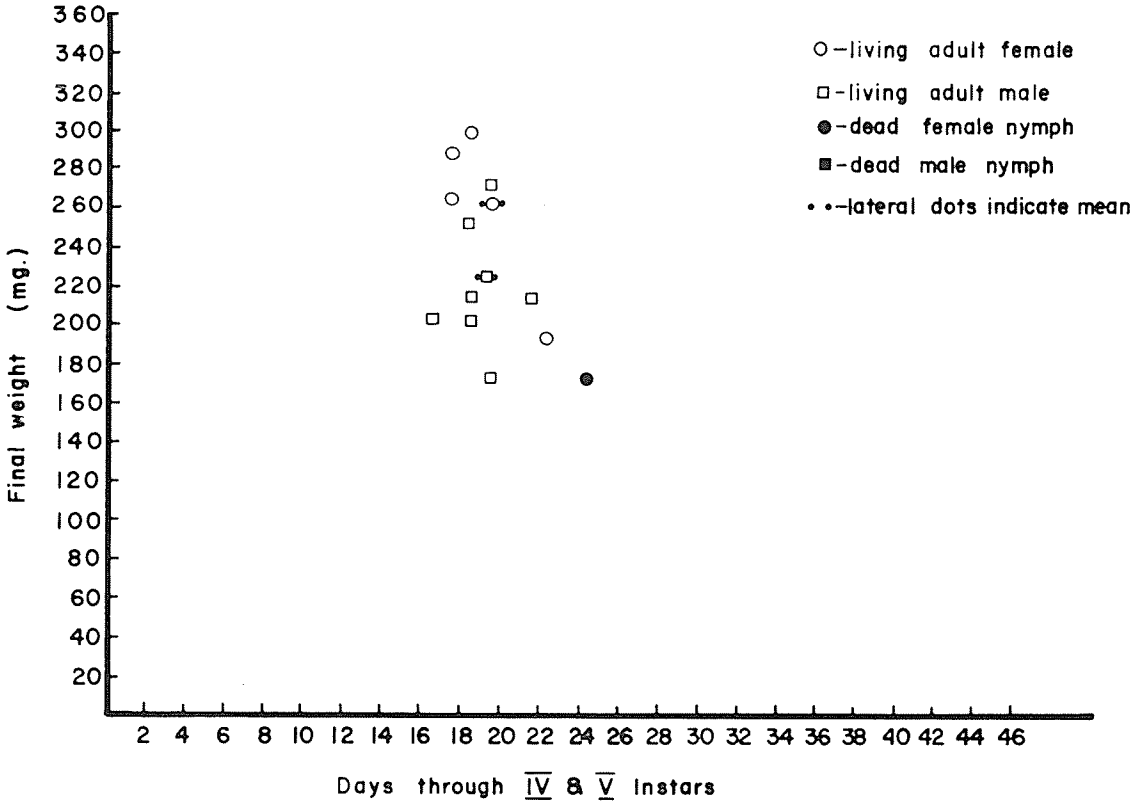
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 15. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of stinkweed, not inoculated with Nosema.

Figure 16. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of stinkweed.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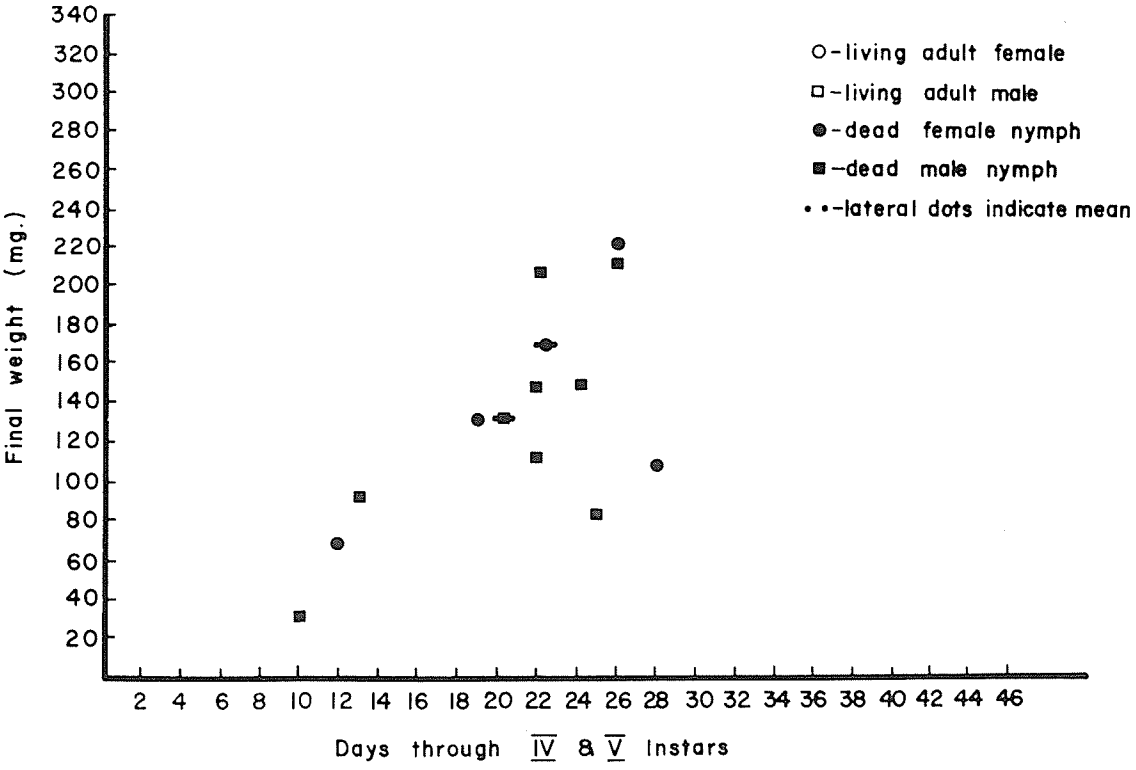
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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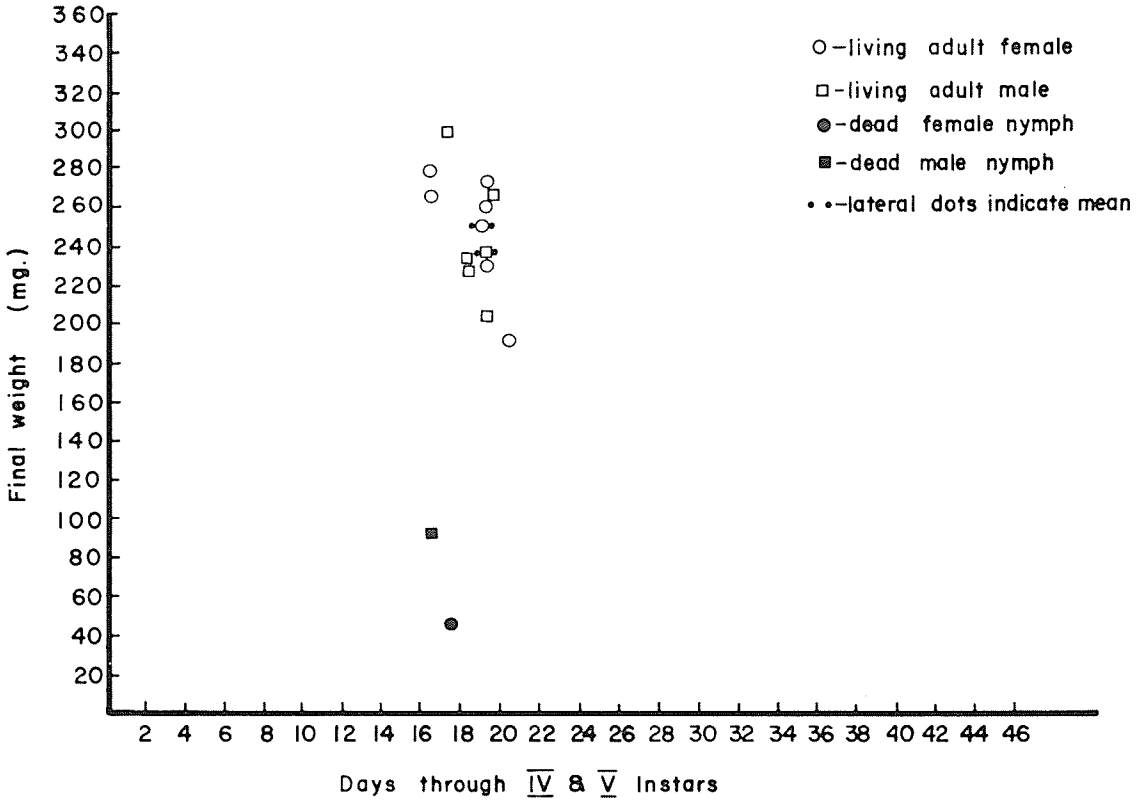
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 17. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of flixweed, not inoculated with Nosema.

Figure 18. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of flixweed.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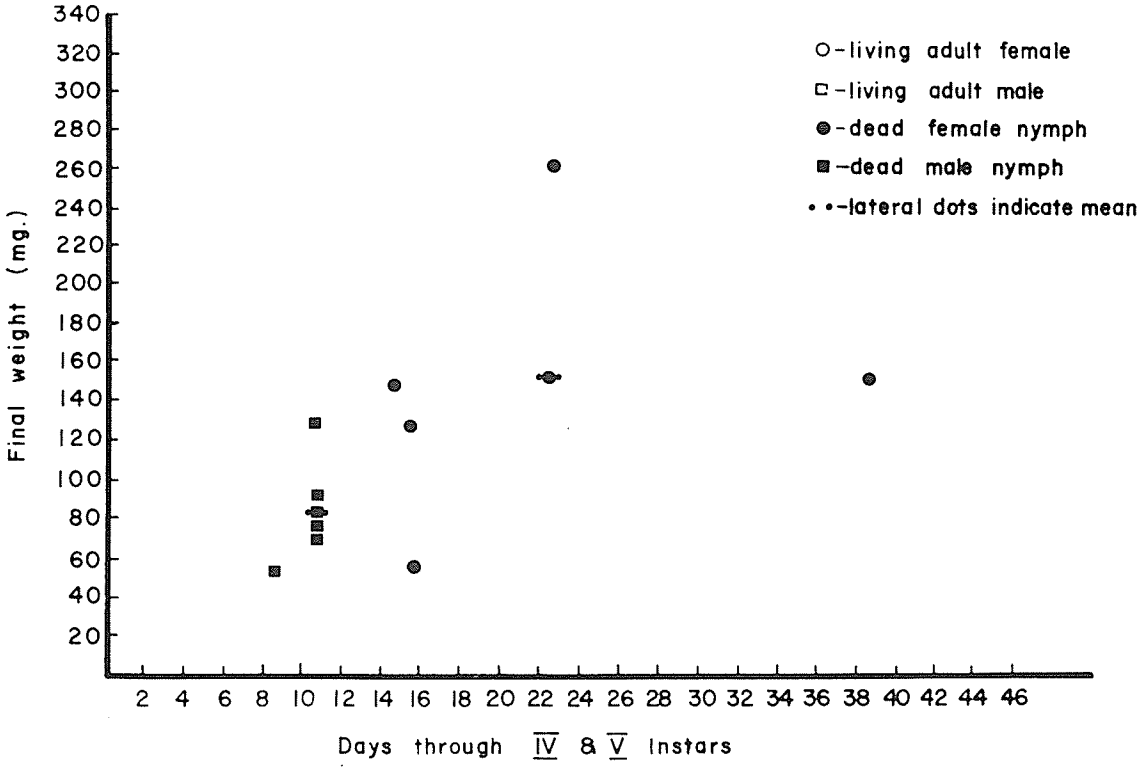
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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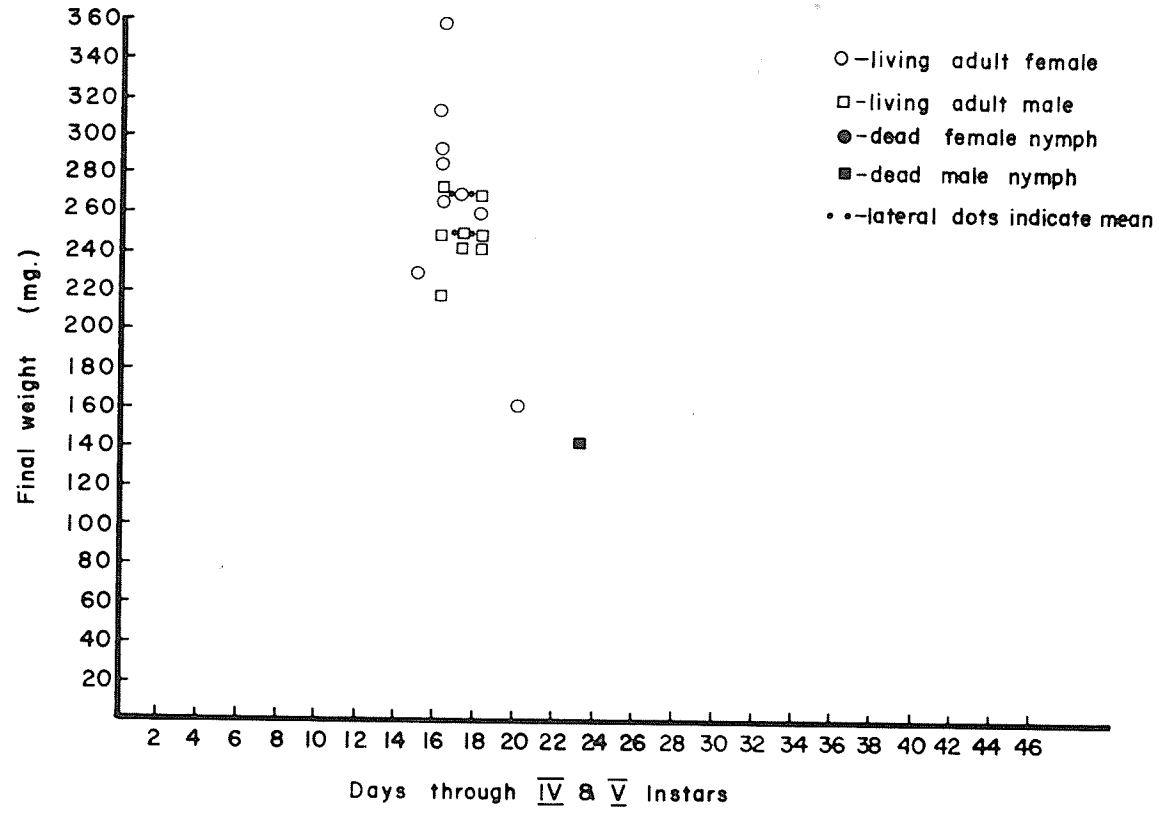
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 19. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of vetch, not inoculated with Nosema.

Figure 20. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of vetch.


Final weight (mg.)
360
340
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40
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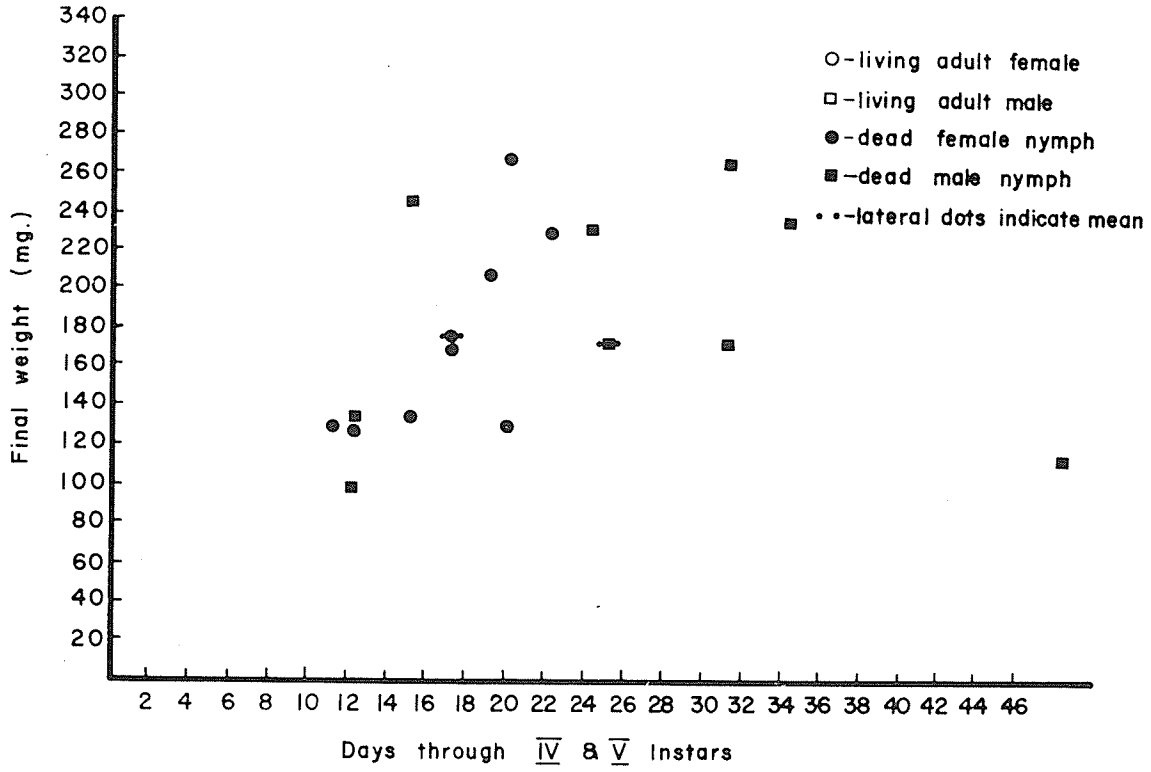
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean


Final weight (mg.)
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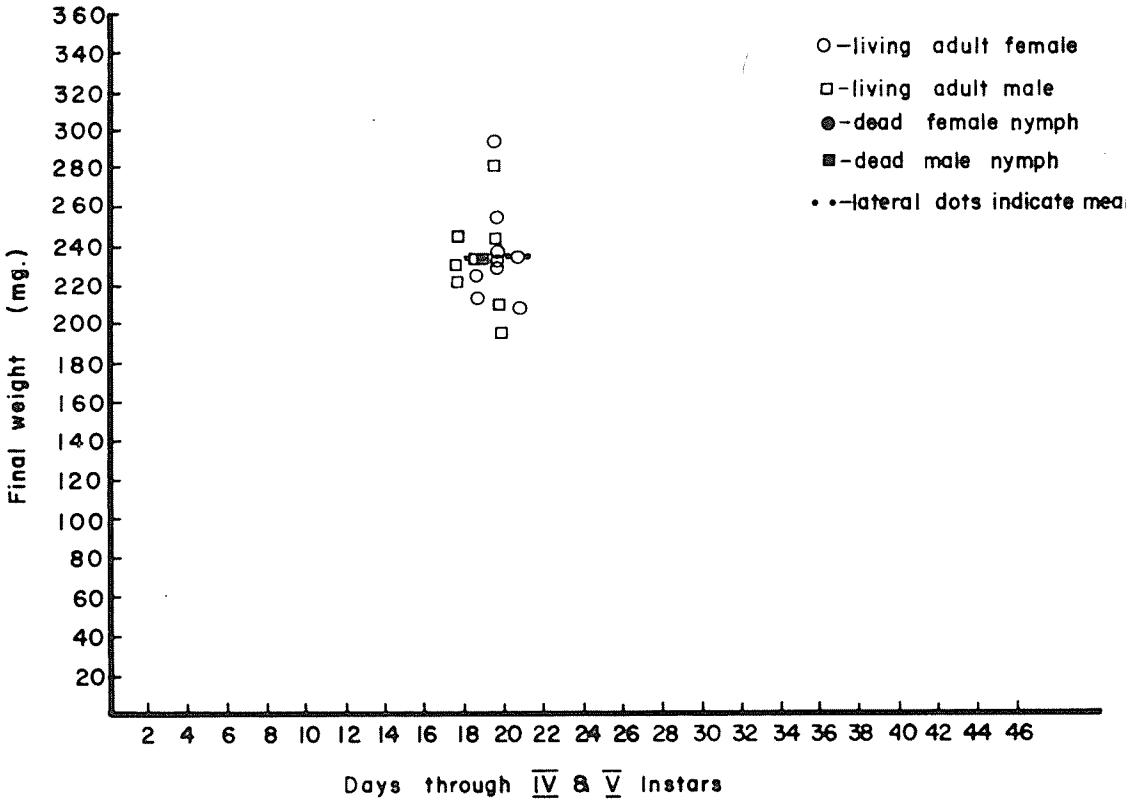
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 21. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of alfalfa, not inoculated with Nosema.

Figure 22. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of alfalfa.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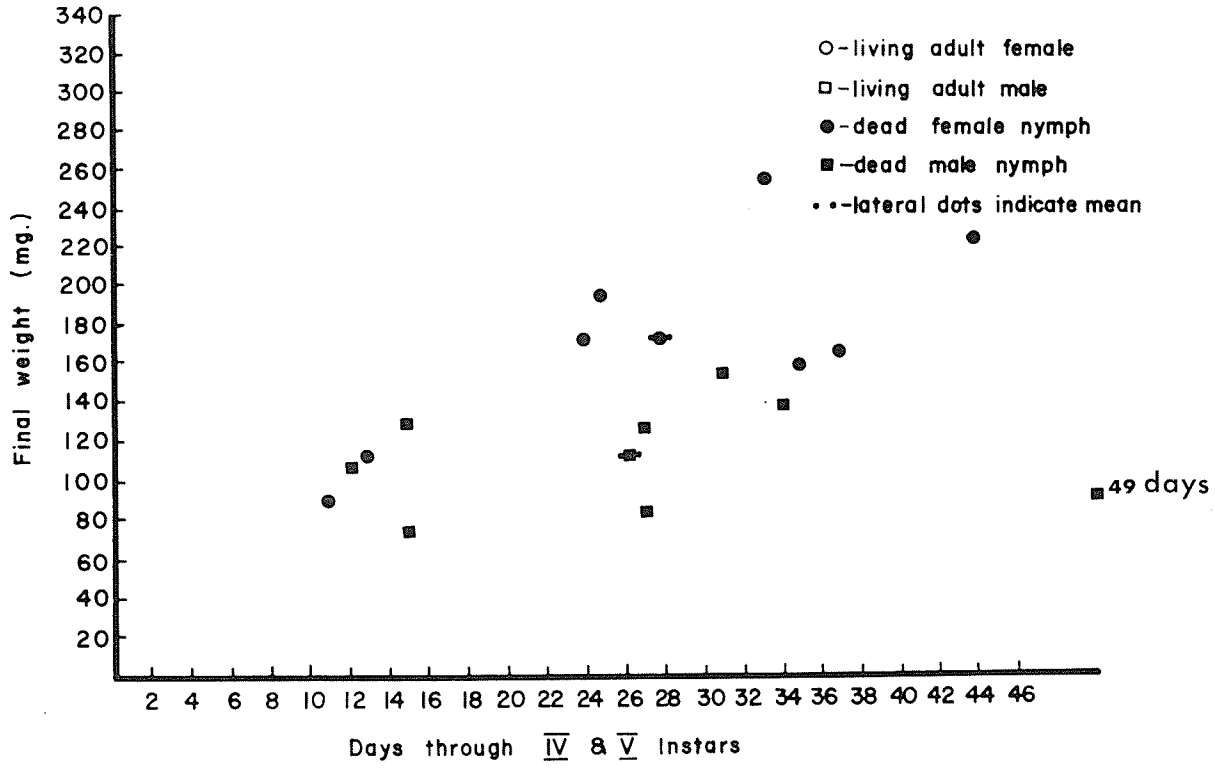
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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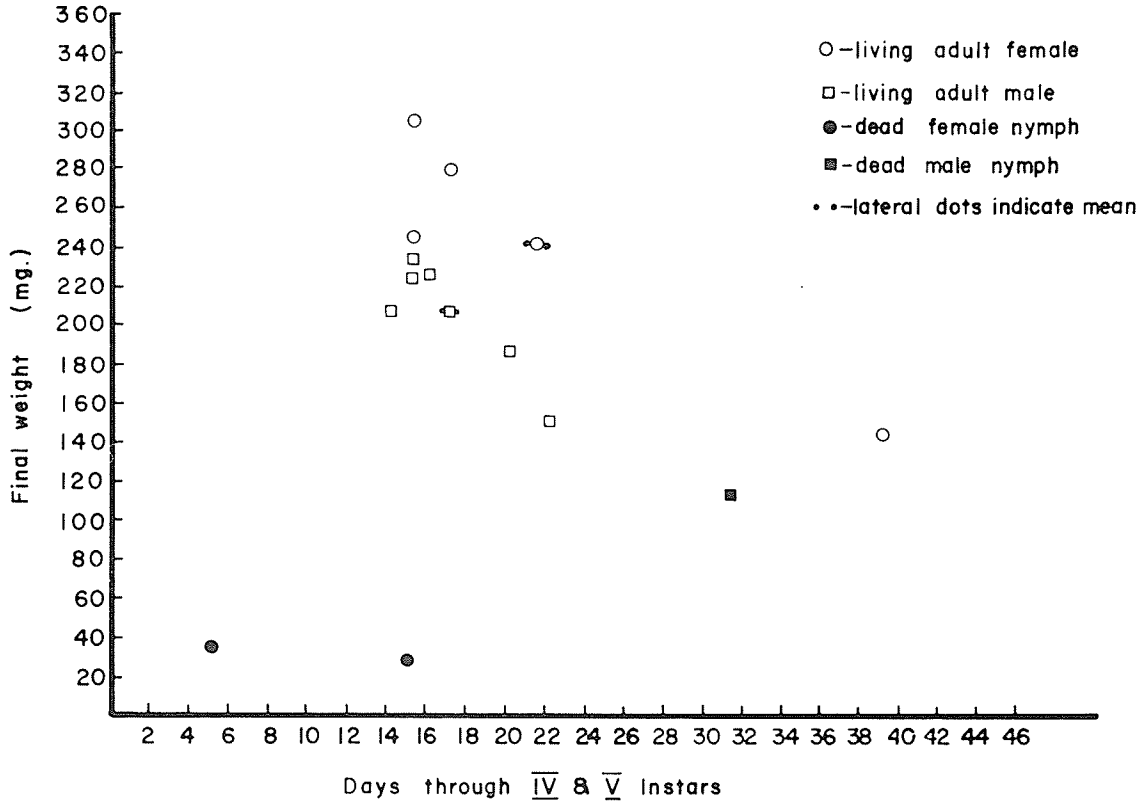
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
49 days

Figure 23. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of ladino clover, not inoculated with Nosema.

Figure 24. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of ladino clover.

Note: Individual in box died before reaching the end of instar III.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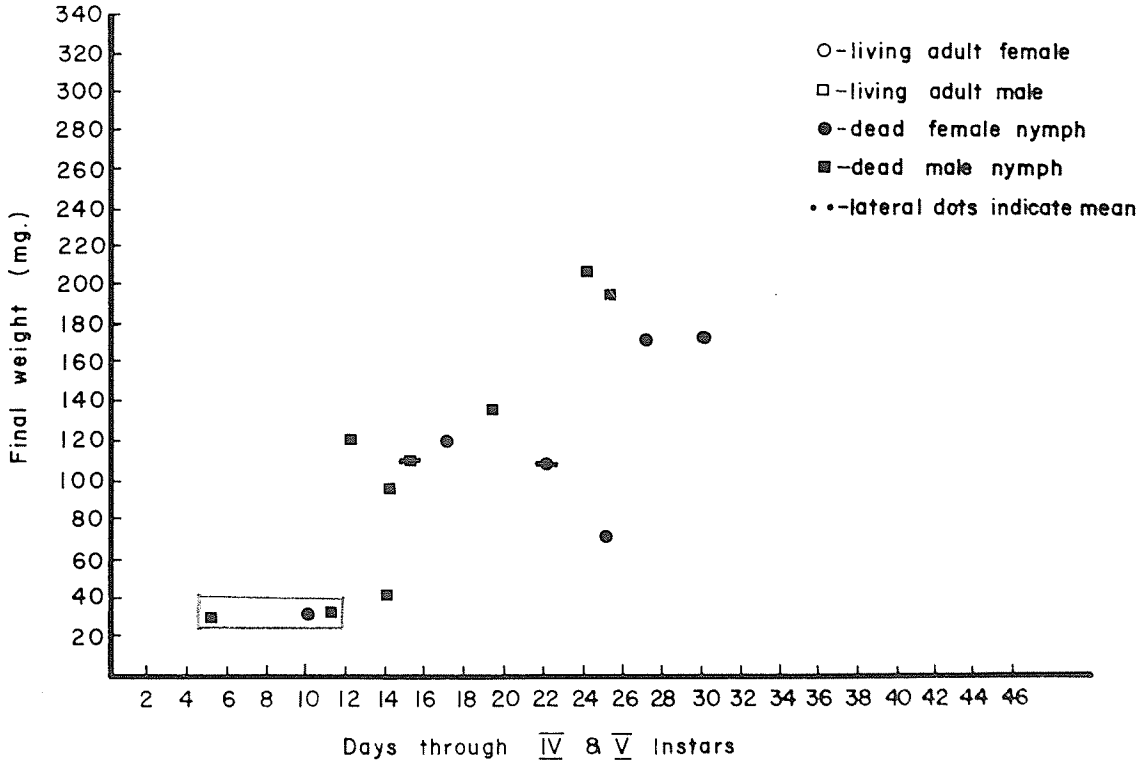
Final weight (mg.)
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars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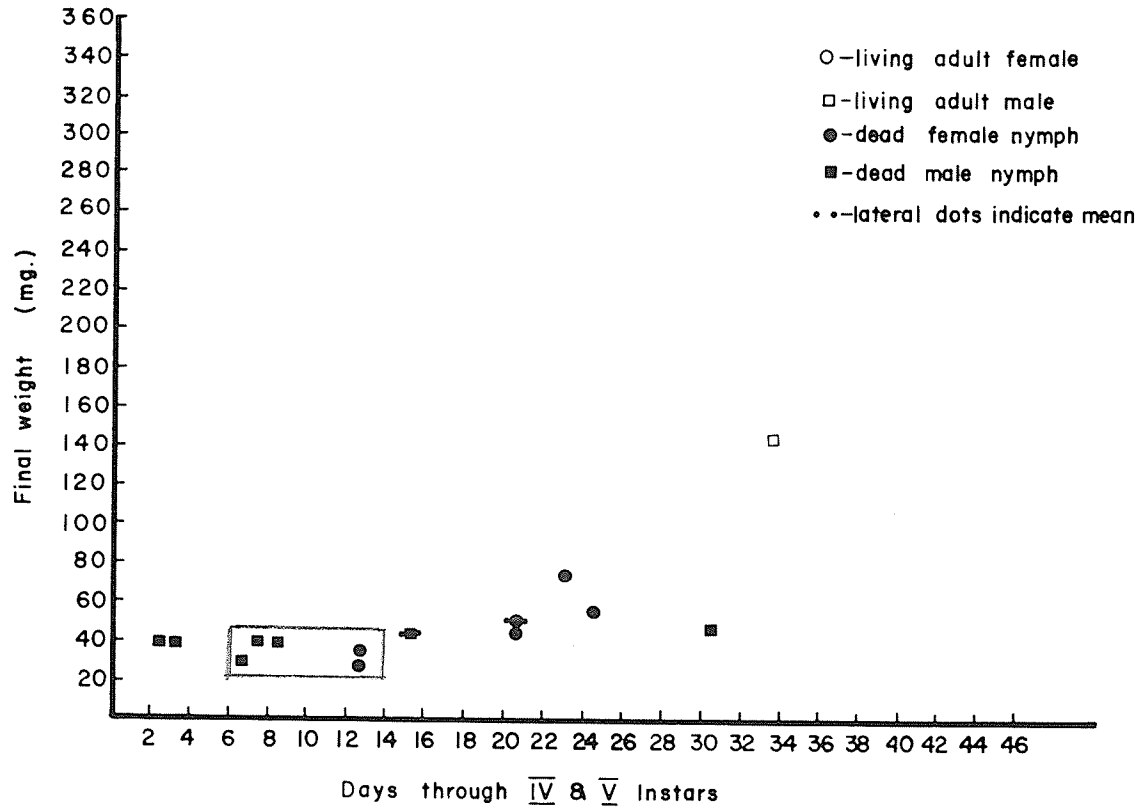
Final weight (mg.)
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars

Figure 25. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of sweet clover, not inoculated with Nosema.

Figure 26. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of sweet clover.

Note: Individuals in box died before reaching the end of instar III.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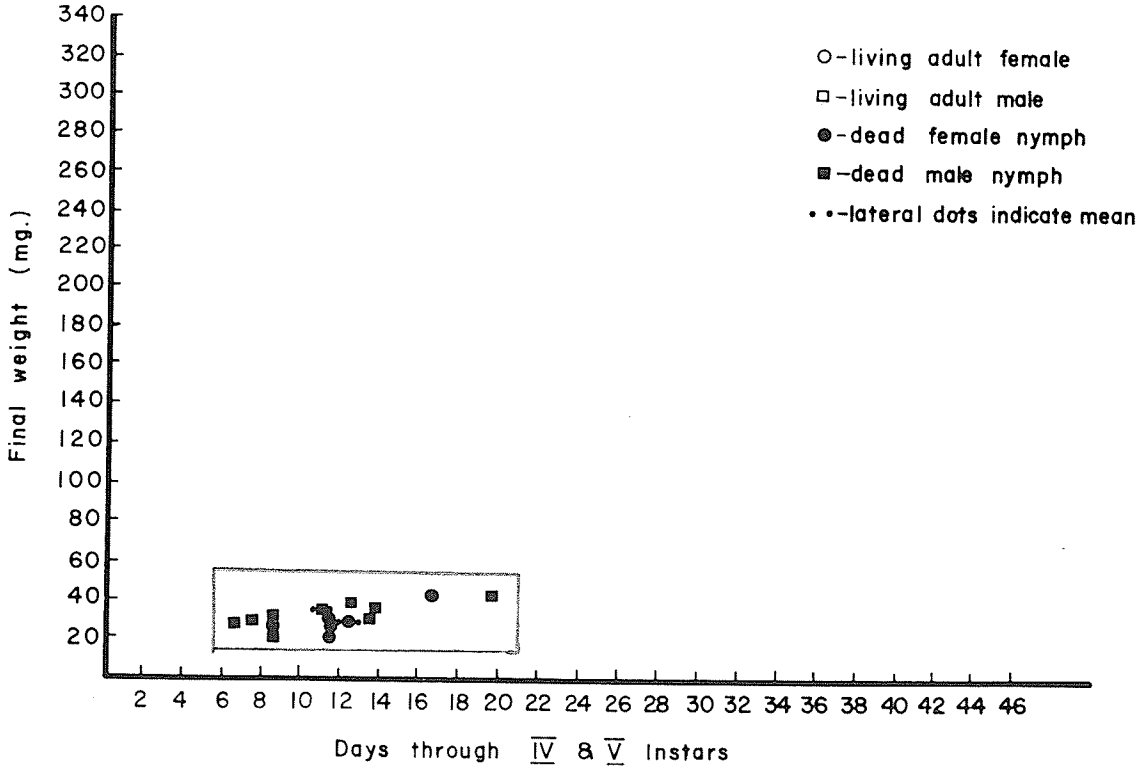
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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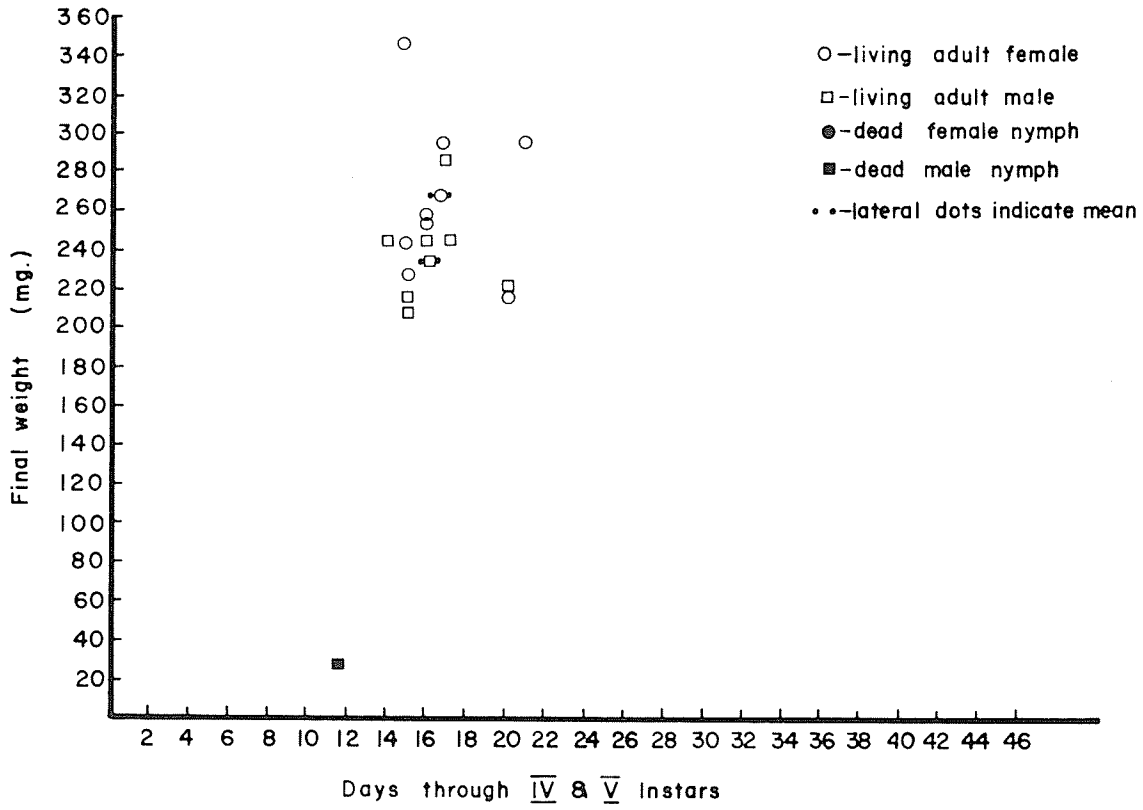
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 27. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion, not inoculated with Nosema.

Figure 28. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion.


Final weight (mg.)
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60
40
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean


Final weight (mg.)
340
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280
260
240
220
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180
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140
120
100
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60
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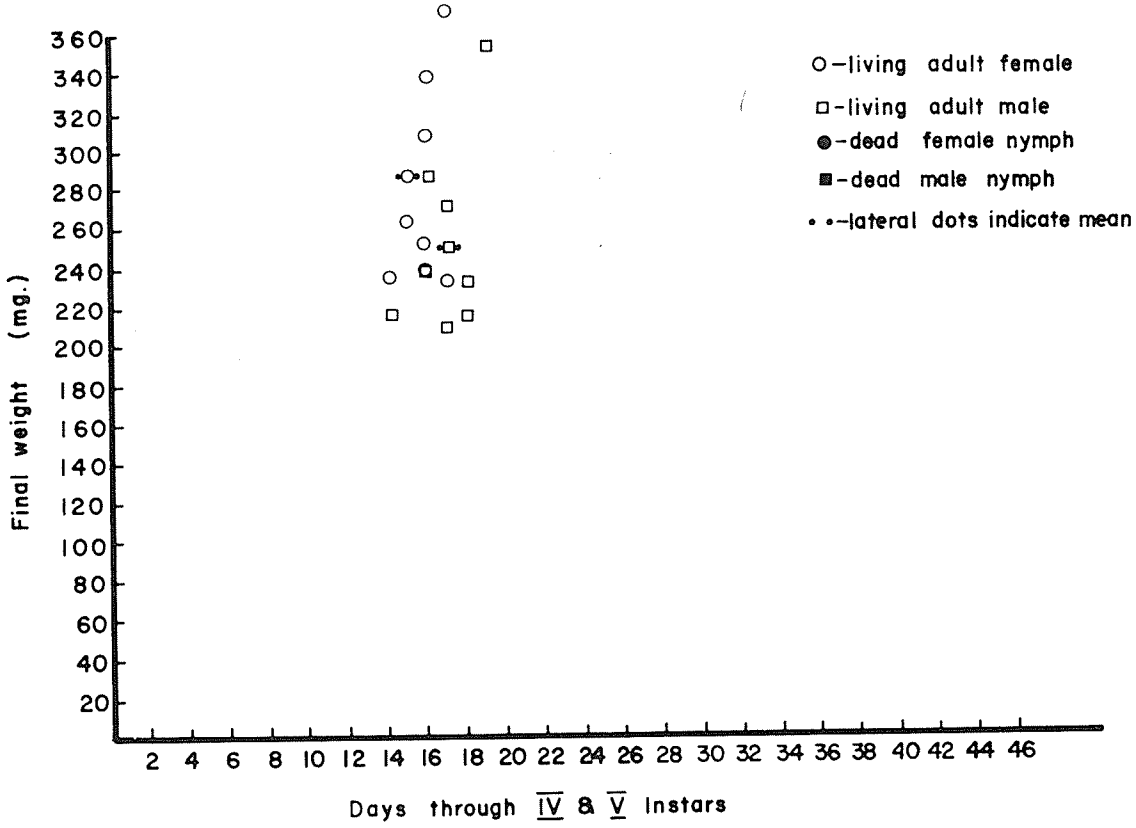
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 29. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of blue lettuce, not inoculated with Nosema.

Figure 30. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of blue lettuce.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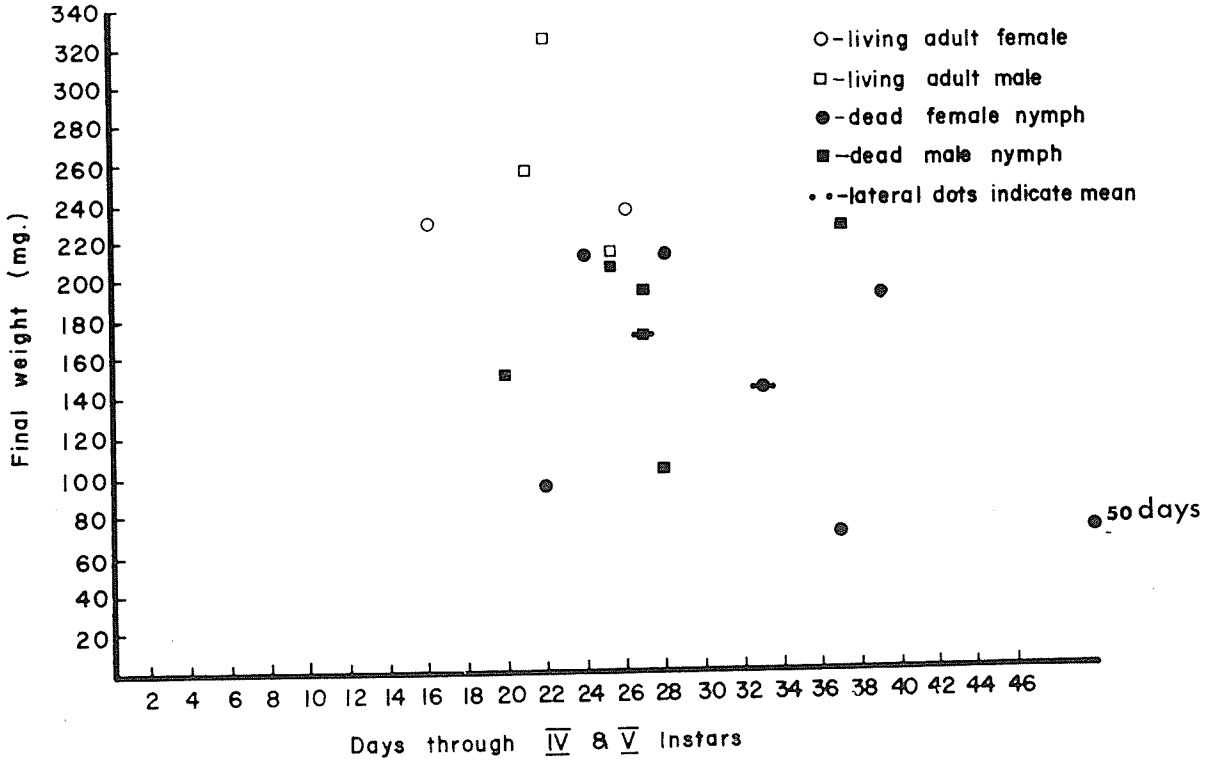
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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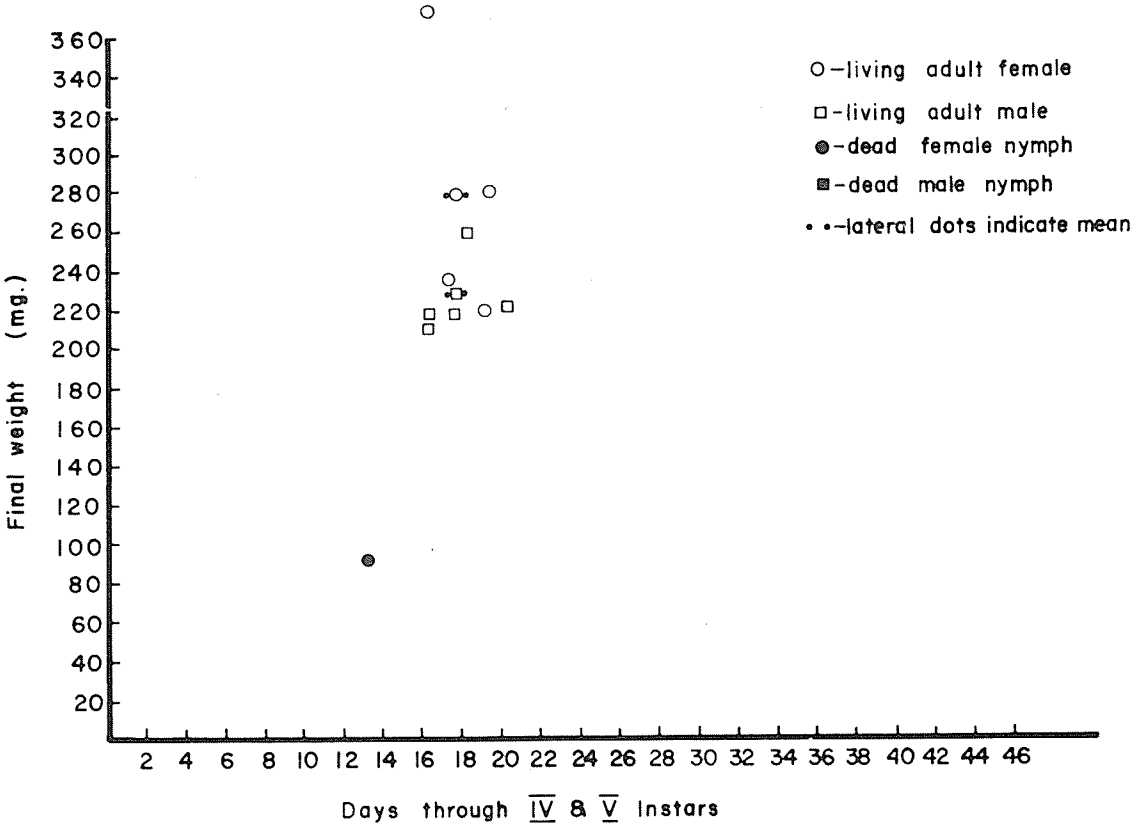
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean
50 days

Figure 31. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of yarrow, not inoculated with Nosema.

Figure 32. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of yarrow.


Final weight (mg.)
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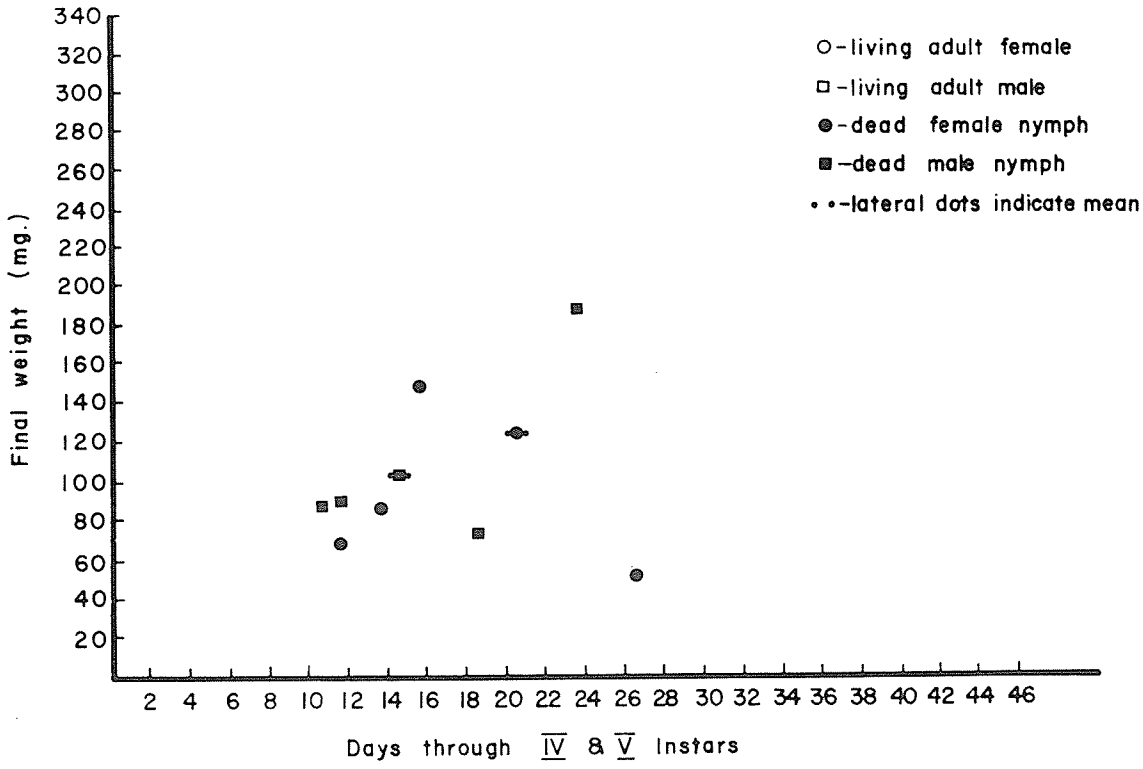
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean


Final weight (mg.)
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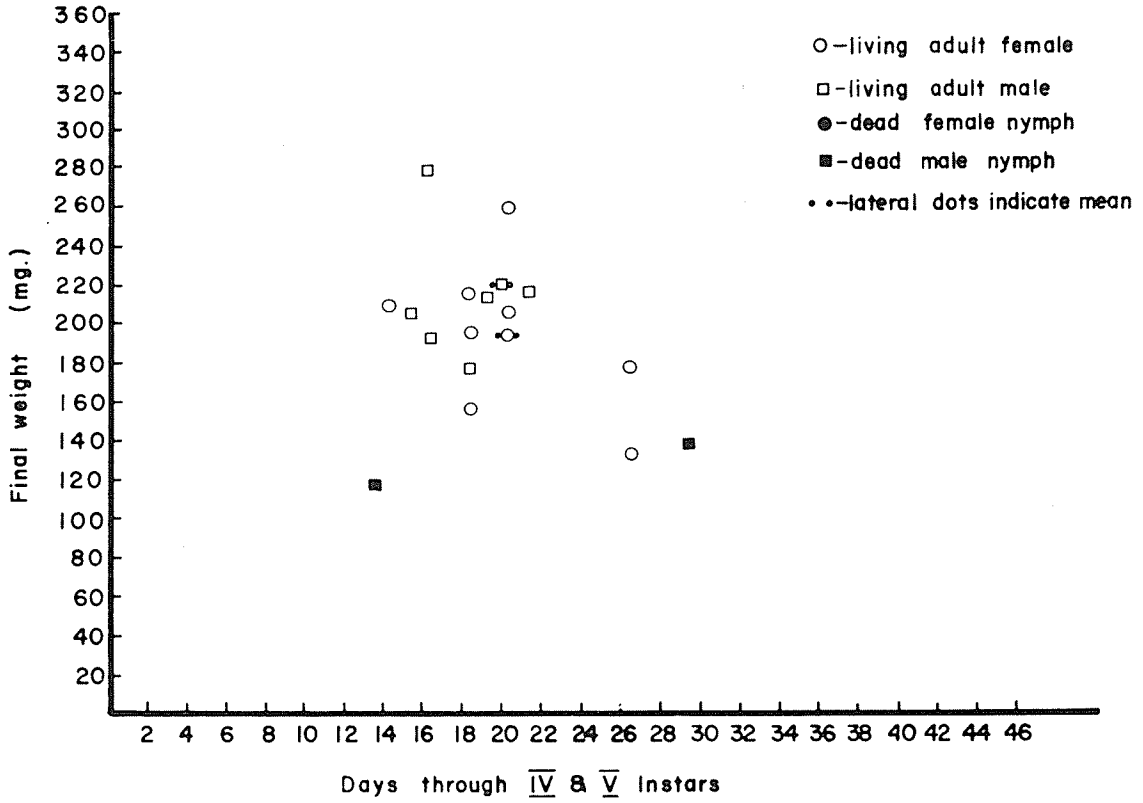
80
60
40
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 33. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of lamb's quarters, not inoculated with Nosema.

Figure 34. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of lamb's quarters.

Note: Individuals in box died before reaching the end of instar III.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40
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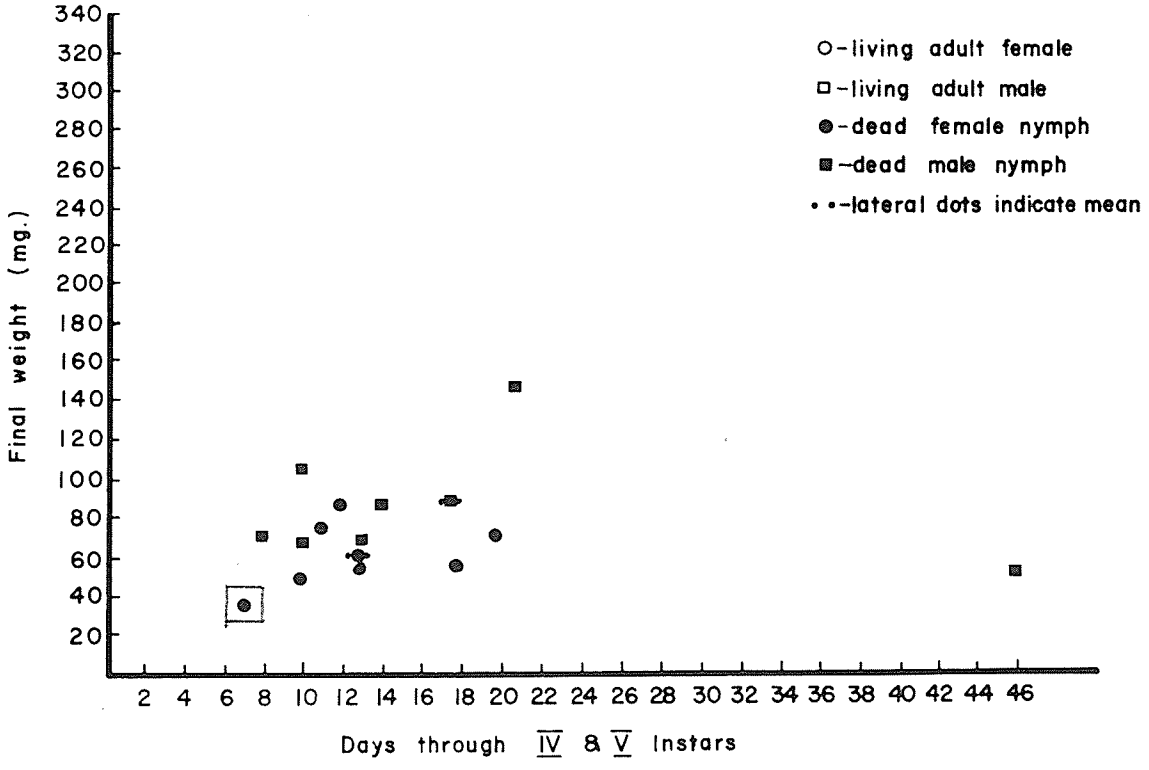
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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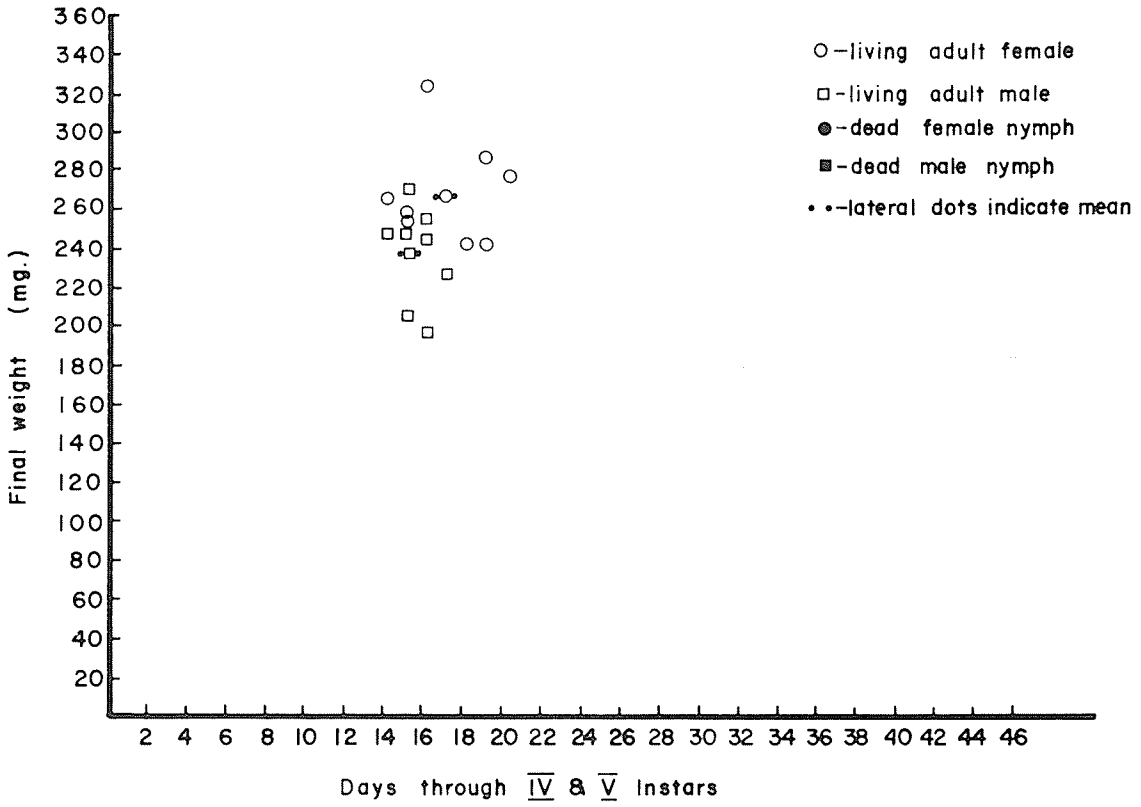
40
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 35. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and tigogenin, not inoculated with Nosema.

Figure 38. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and tigogenin.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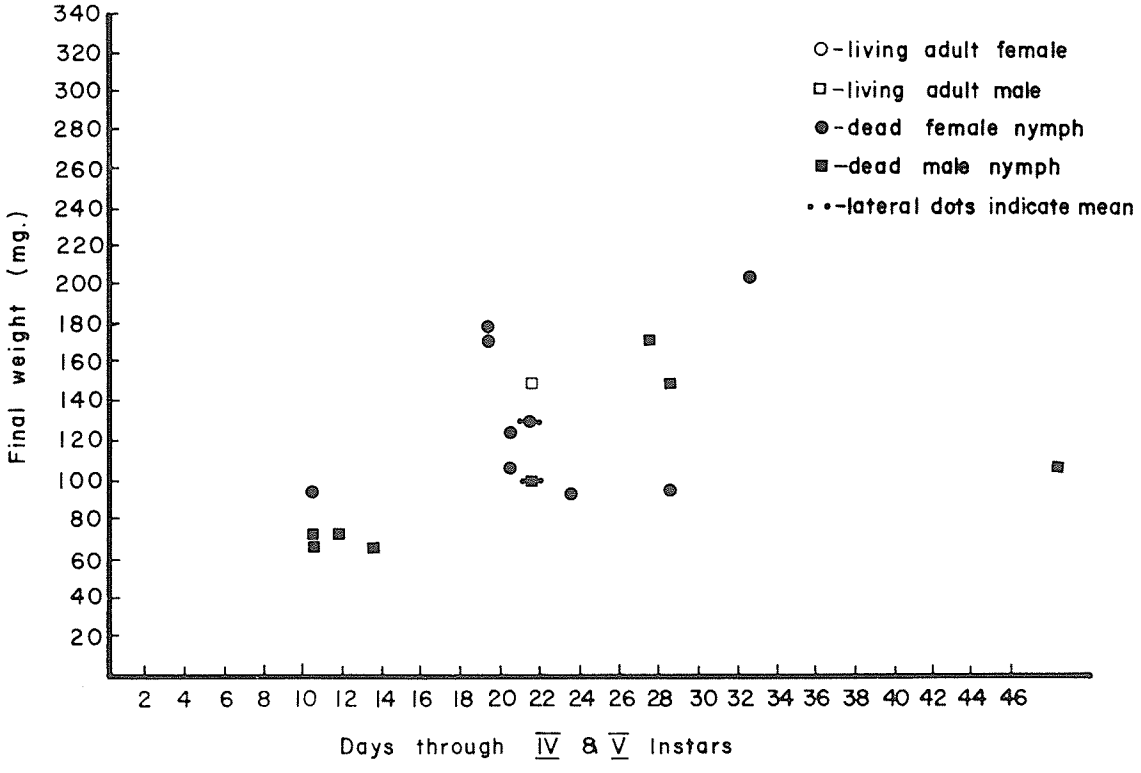
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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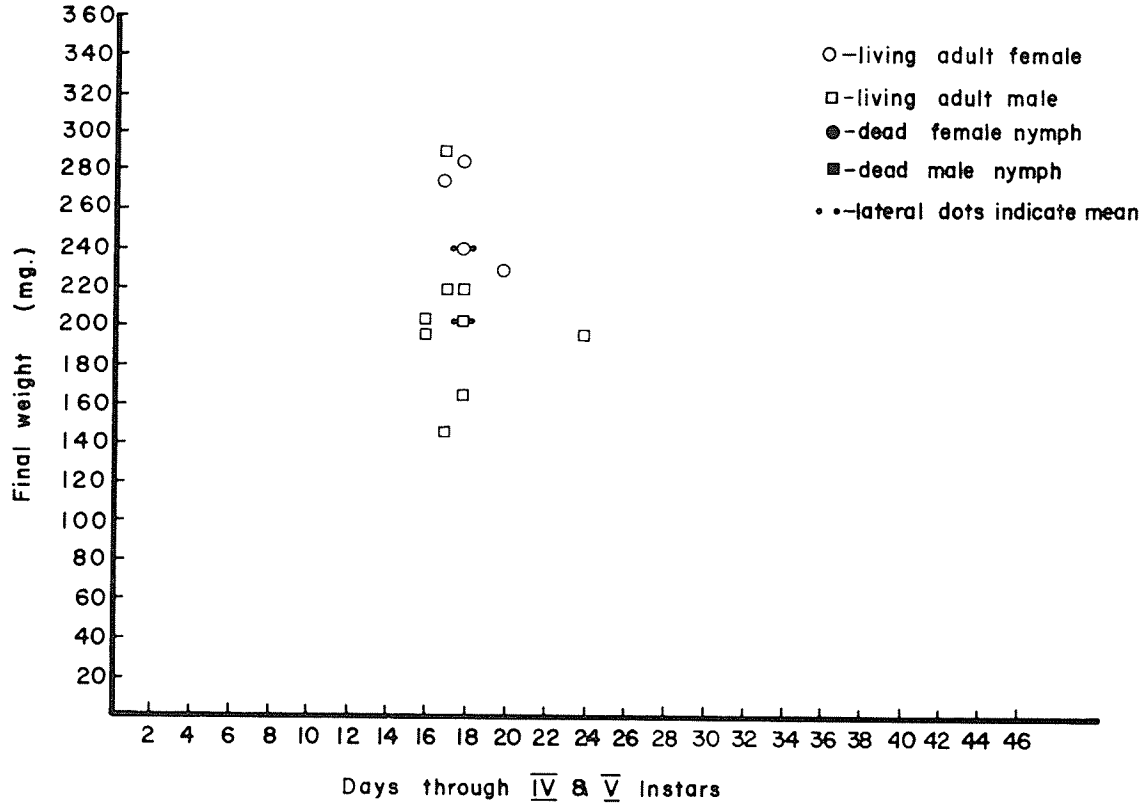
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 37. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and digitonin, not inoculated with Nosema.

Figure 38. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and digitonin.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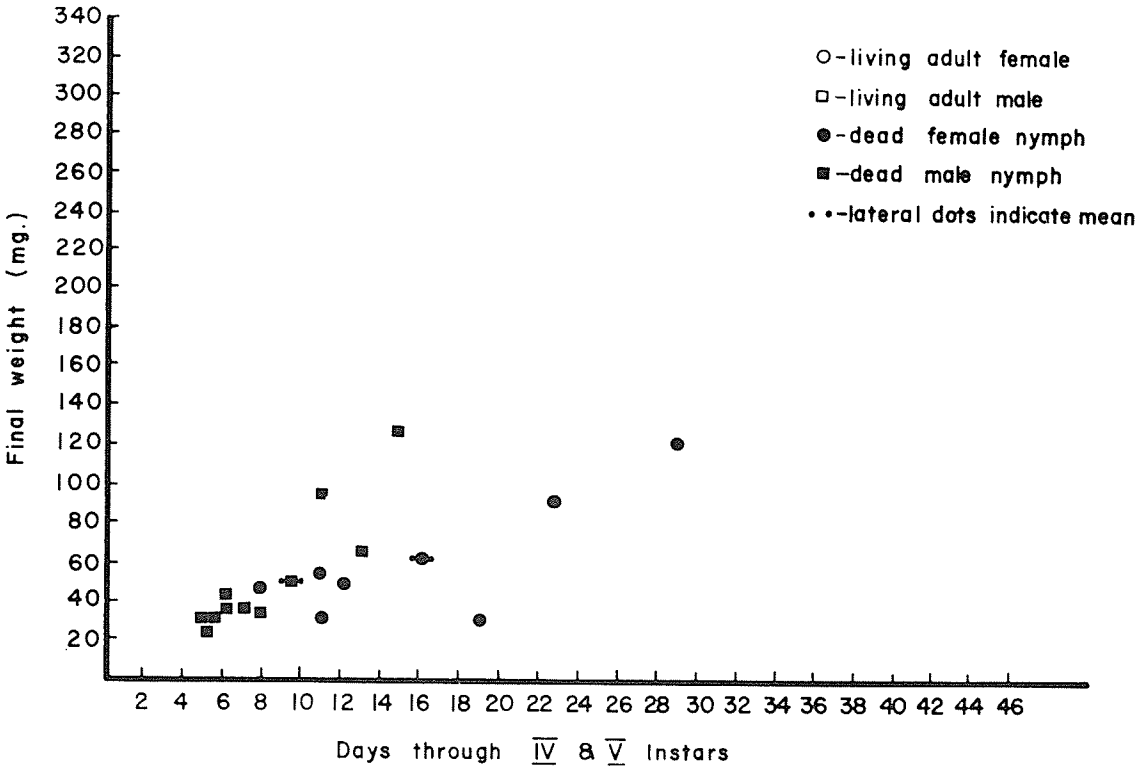
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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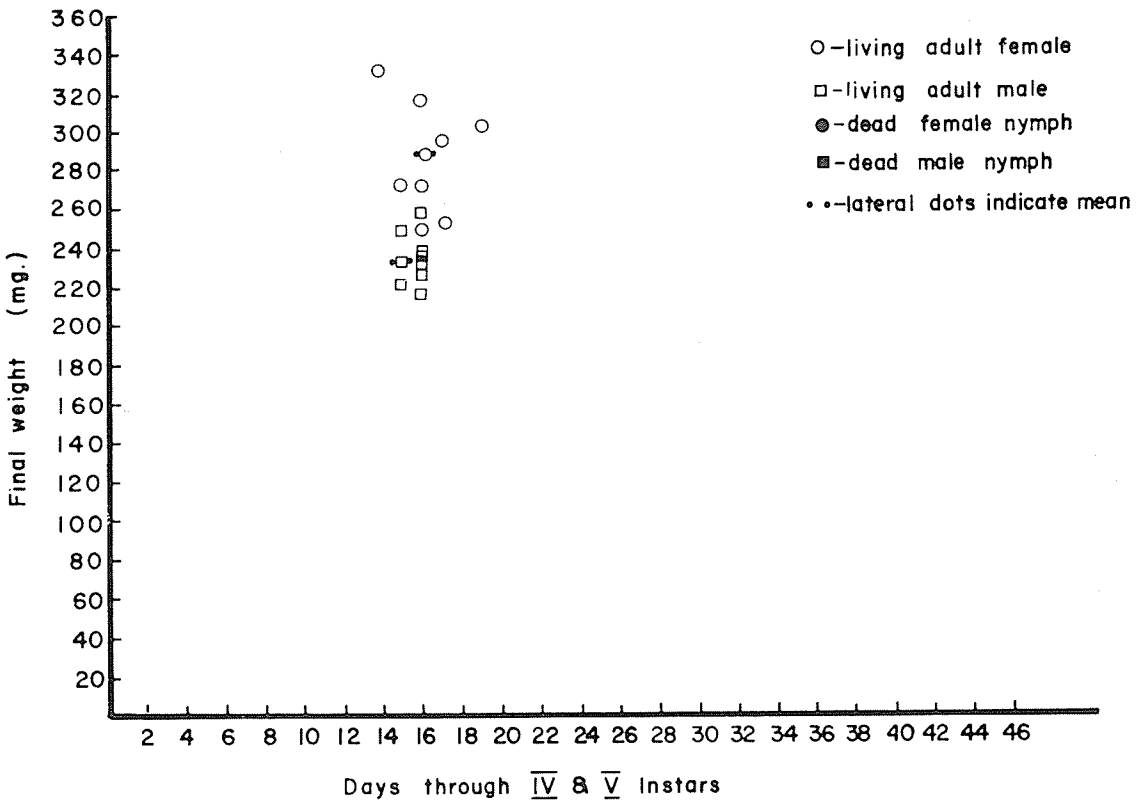
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 39. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and convallamarin, not inoculated with Nosema.

Figure 40. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and convallamarin.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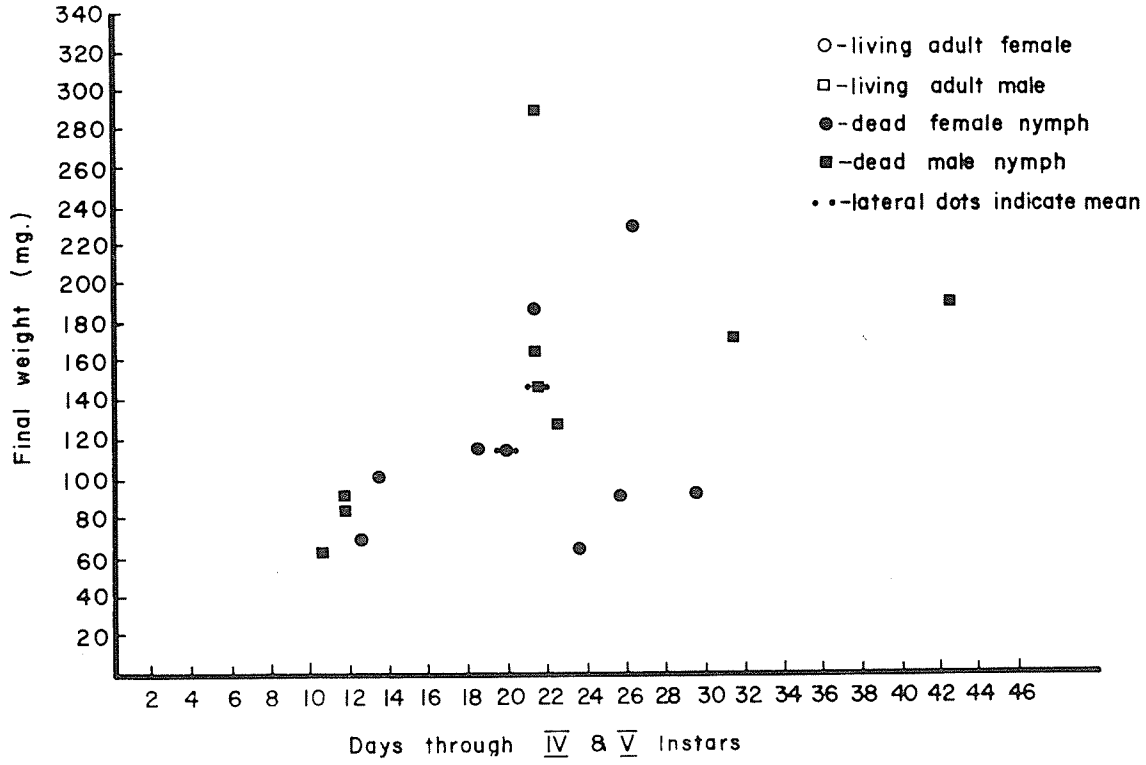
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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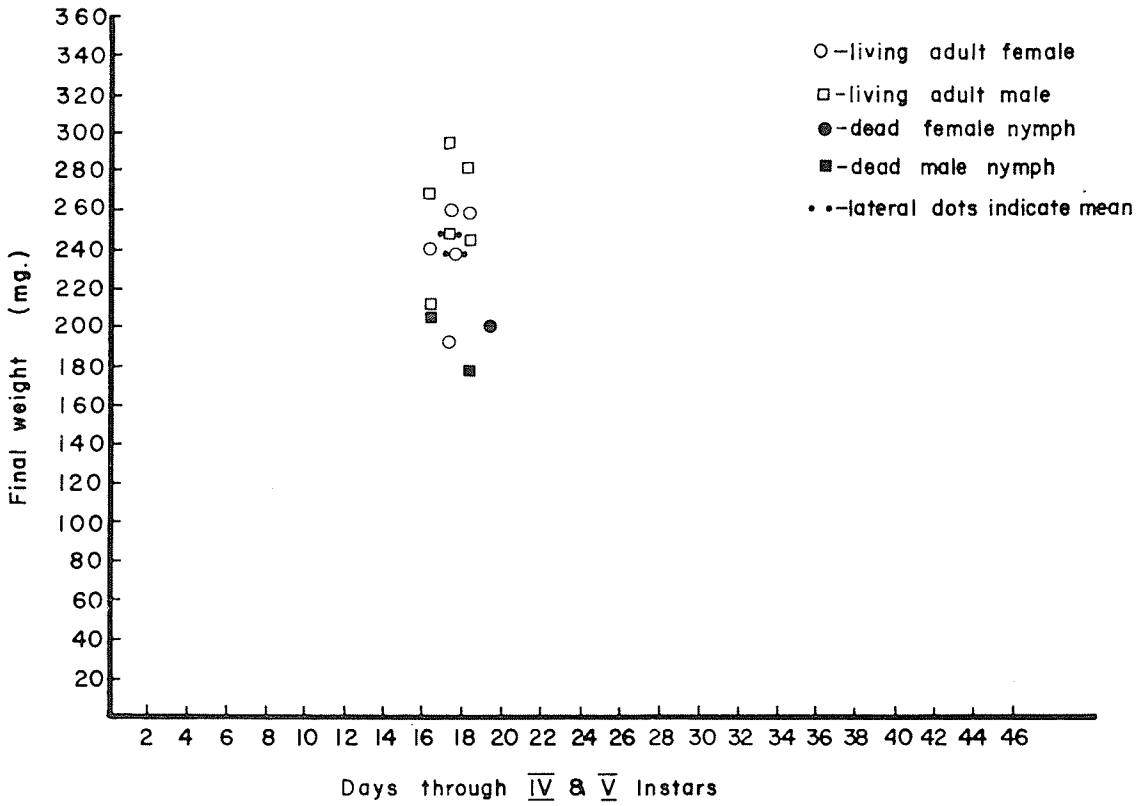
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 41. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and farnesol, not inoculated with Nosema.

Figure 42. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and farnesol.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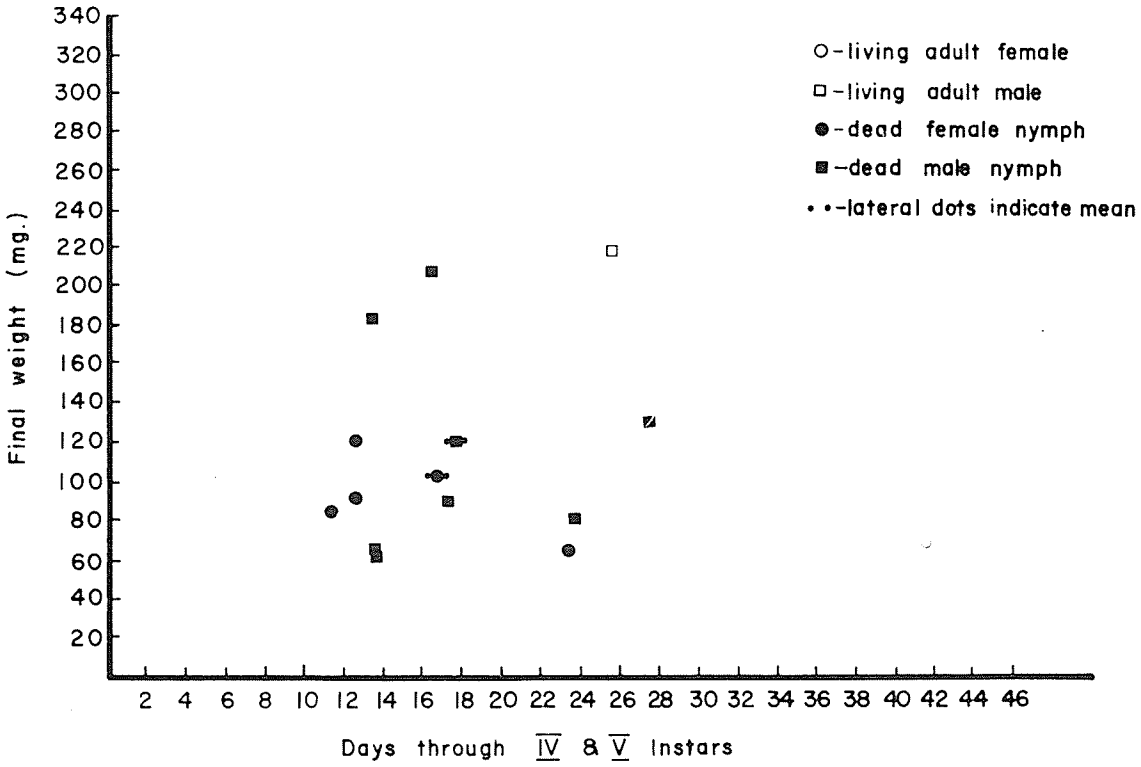
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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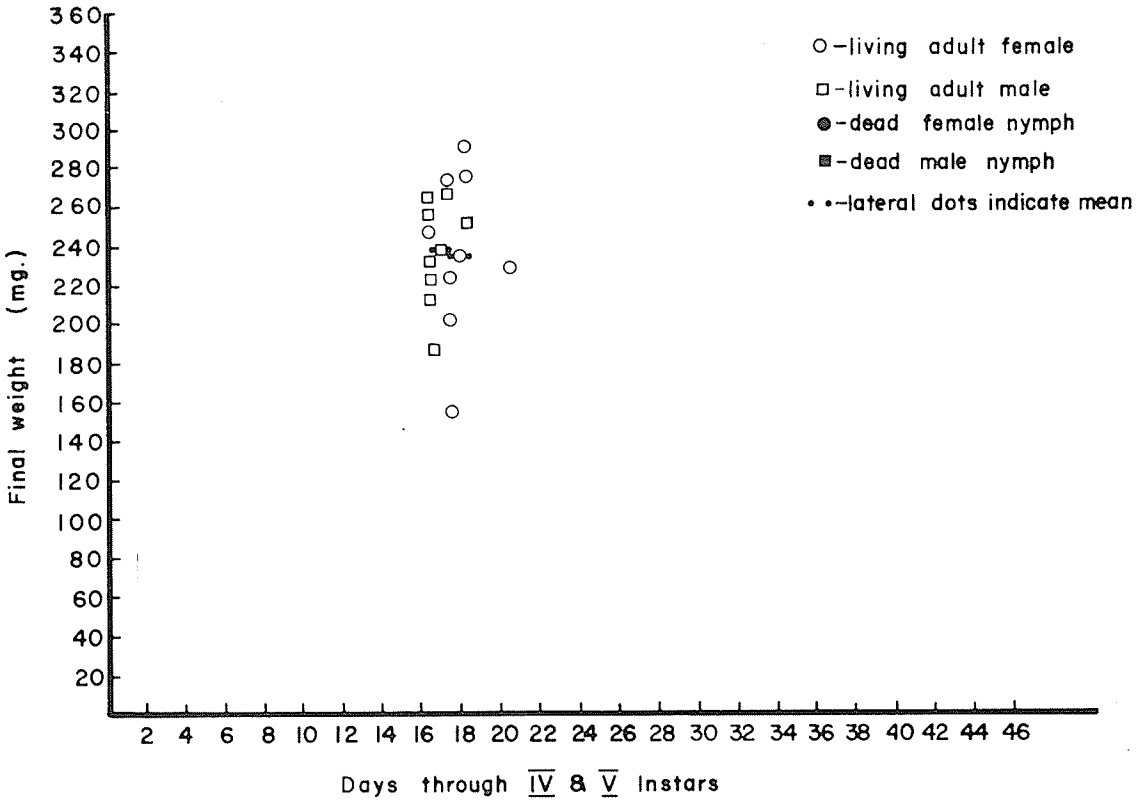
40
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
○-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 43. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and coumarin, not inoculated with Nosema.

Figure 44. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and coumarin.

Note: Individual in box died before reaching the end of instar III.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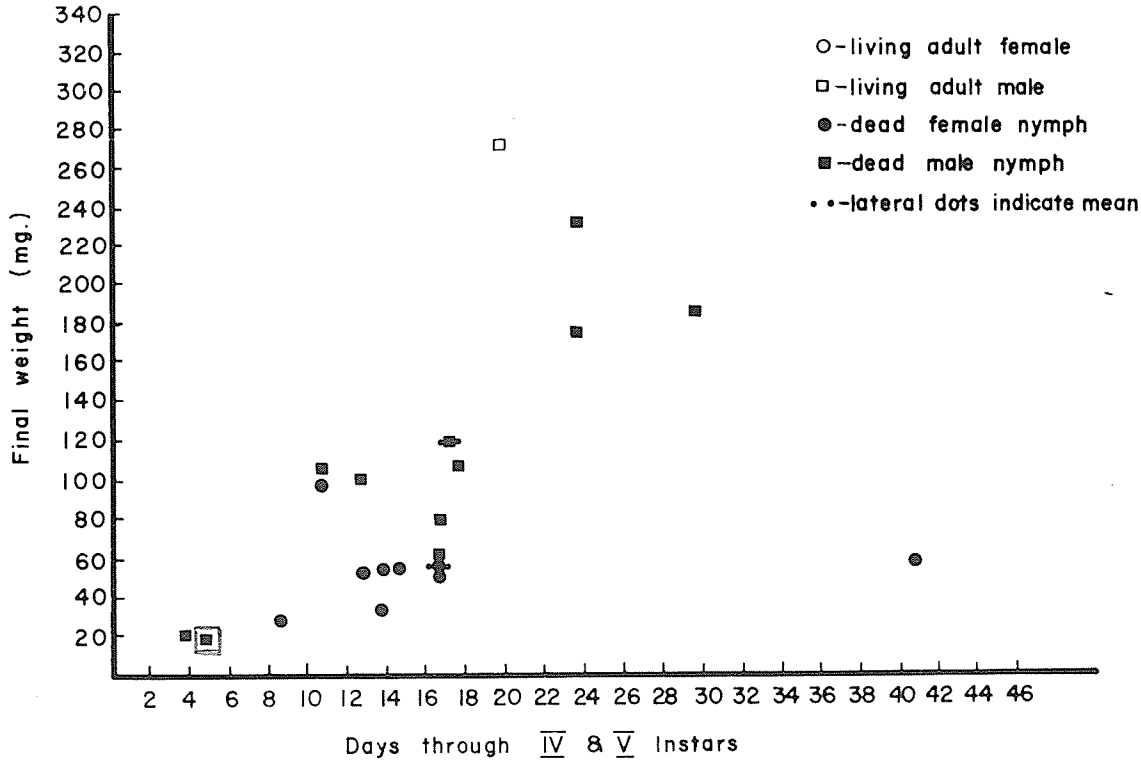
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean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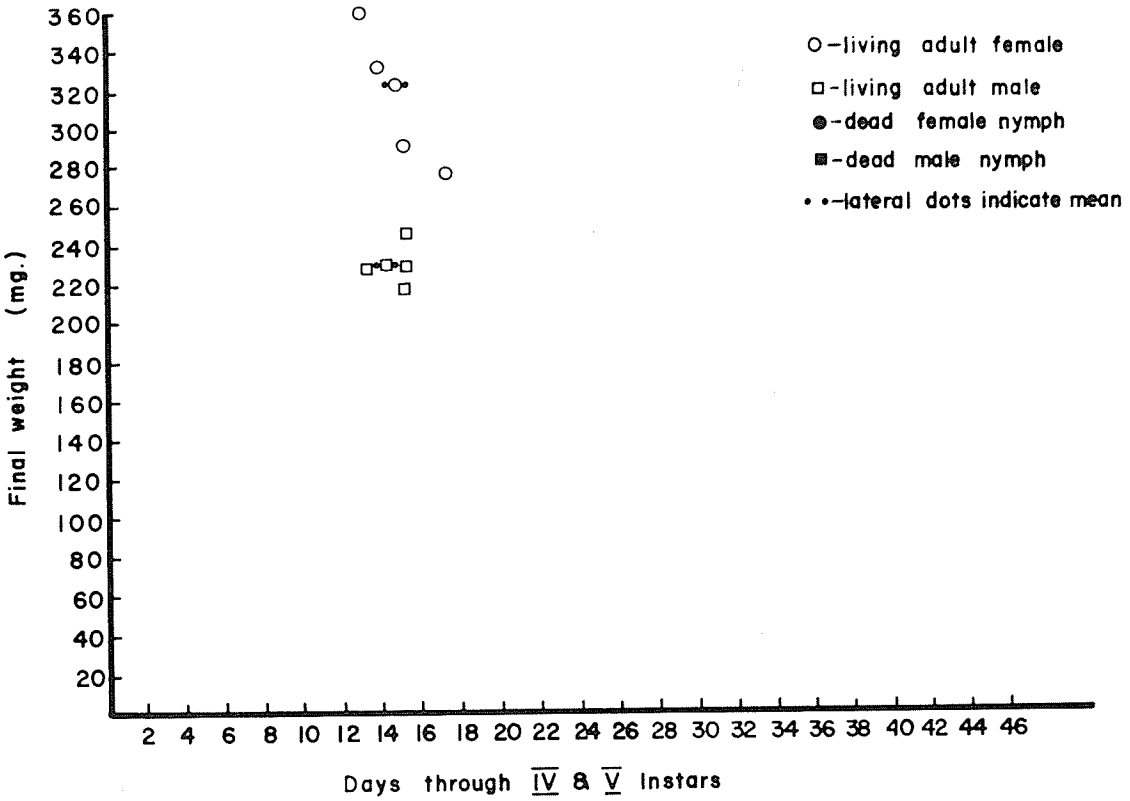
20
Final weight (mg.)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 45. The final weight and duration in days through instar IV and V of individual grasshoppers reared on a diet of dandelion and dicoumarol, not inoculated with Nosema.

Figure 46. The final weight and duration in days through instar IV and V of individual grasshoppers, inoculated with Nosema, and reared on a diet of dandelion and dicoumarol.


Final weight (mg.)
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

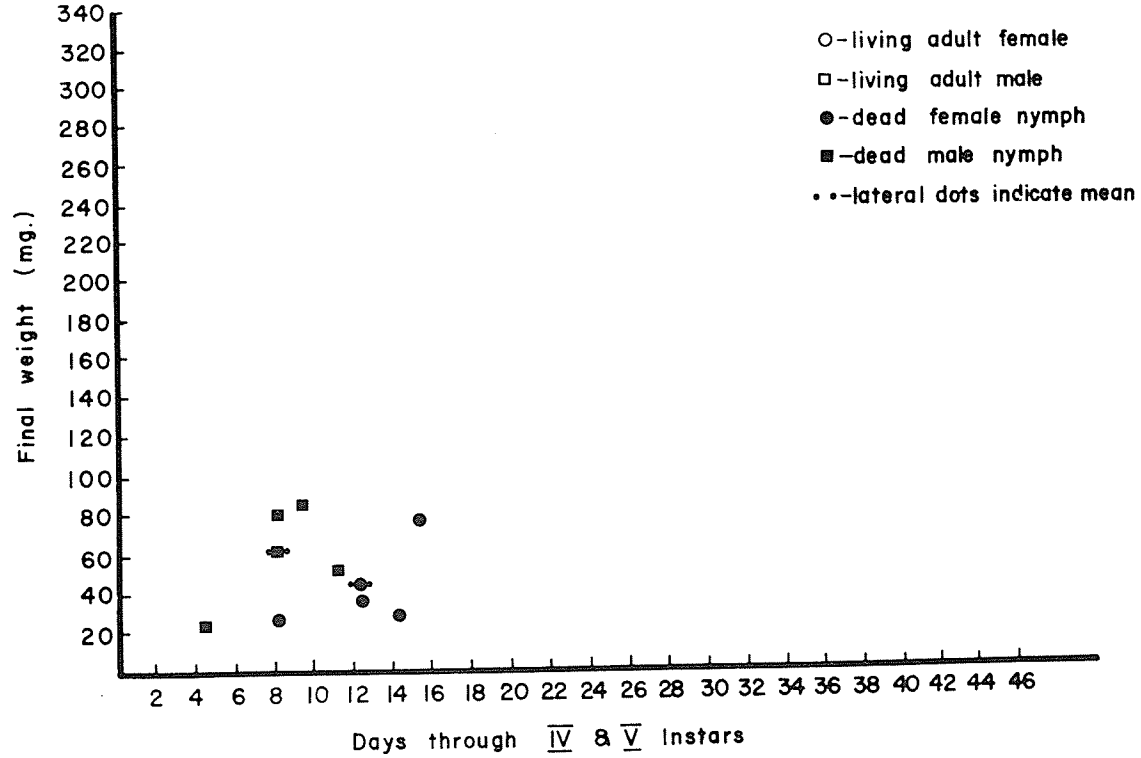

Final weight (mg.)
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Days through IV & V Instars
O-living adult female
□-living adult male
●-dead female nymph
■-dead male nymph
• •-lateral dots indicate mean

Figure 47. Comparison of effects of leaf meals of various plant species on the "food index" for mixed sexes of grasshoppers.

*Pharmacological chemicals were mixed with dandelion leaf meal.

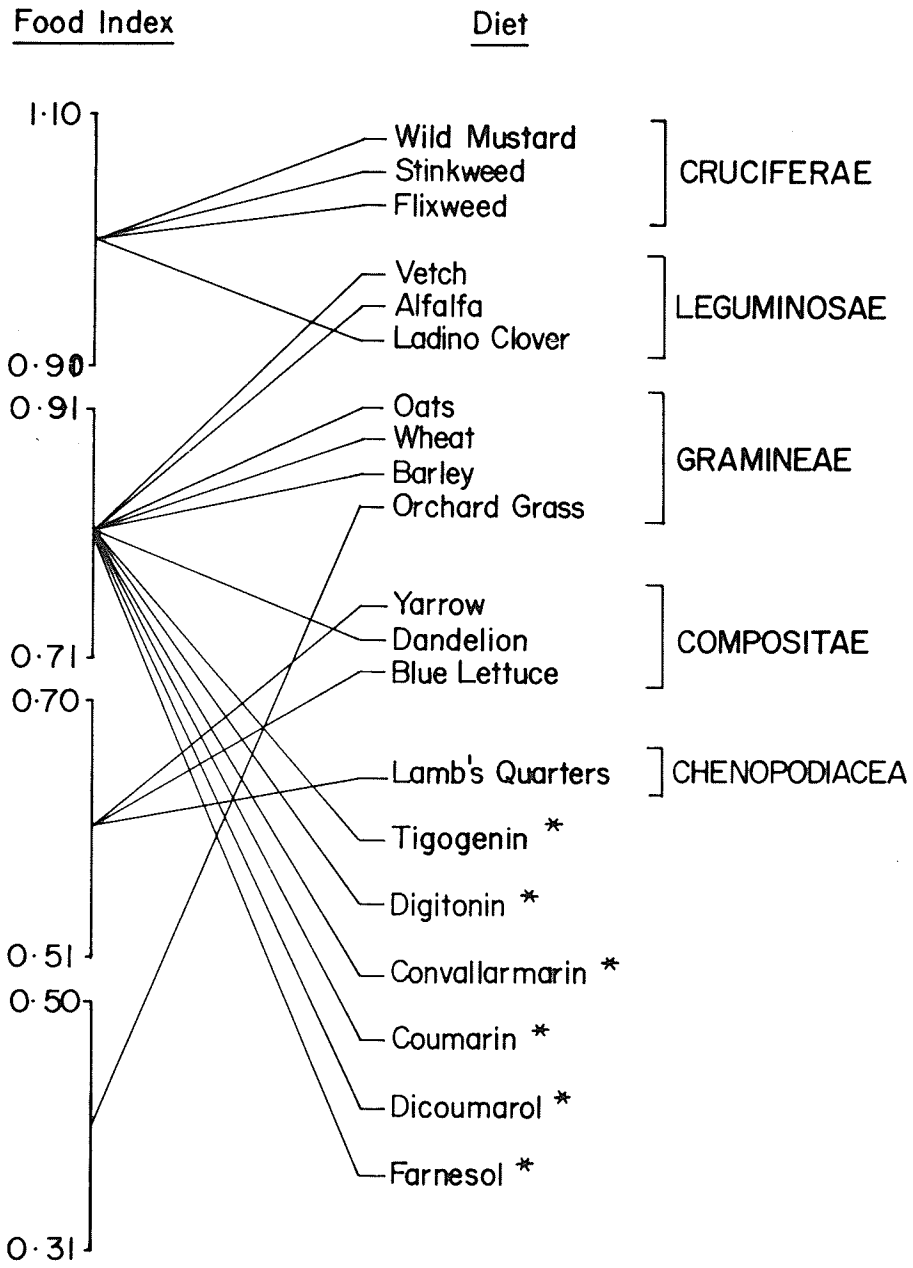

Food Index
Diet
1·10
0·90
0·91
0·71
0·70
0·51
0·50
0·31
Wild Mustard
Stinkweed
Flixweed
CRUCIFERAE
Vetch
Alfalfa
Ladino Clover
LEGUMINOSAE
Oats
Wheat
Barley
Orchard Grass
GRAMINEAE
Yarrow
Dandelion
Blue Lettuce
COMPOSITAE
Lamb's Quarters
CHENOPODIACEA
Tigogenin *
Digitonin *
Convallarmarin *
Coumarin *
Dicoumarol *
Farnesol *

Figure 48. Relation among mean adult weights and mean developmental times of female grasshoppers with respect to plant families.

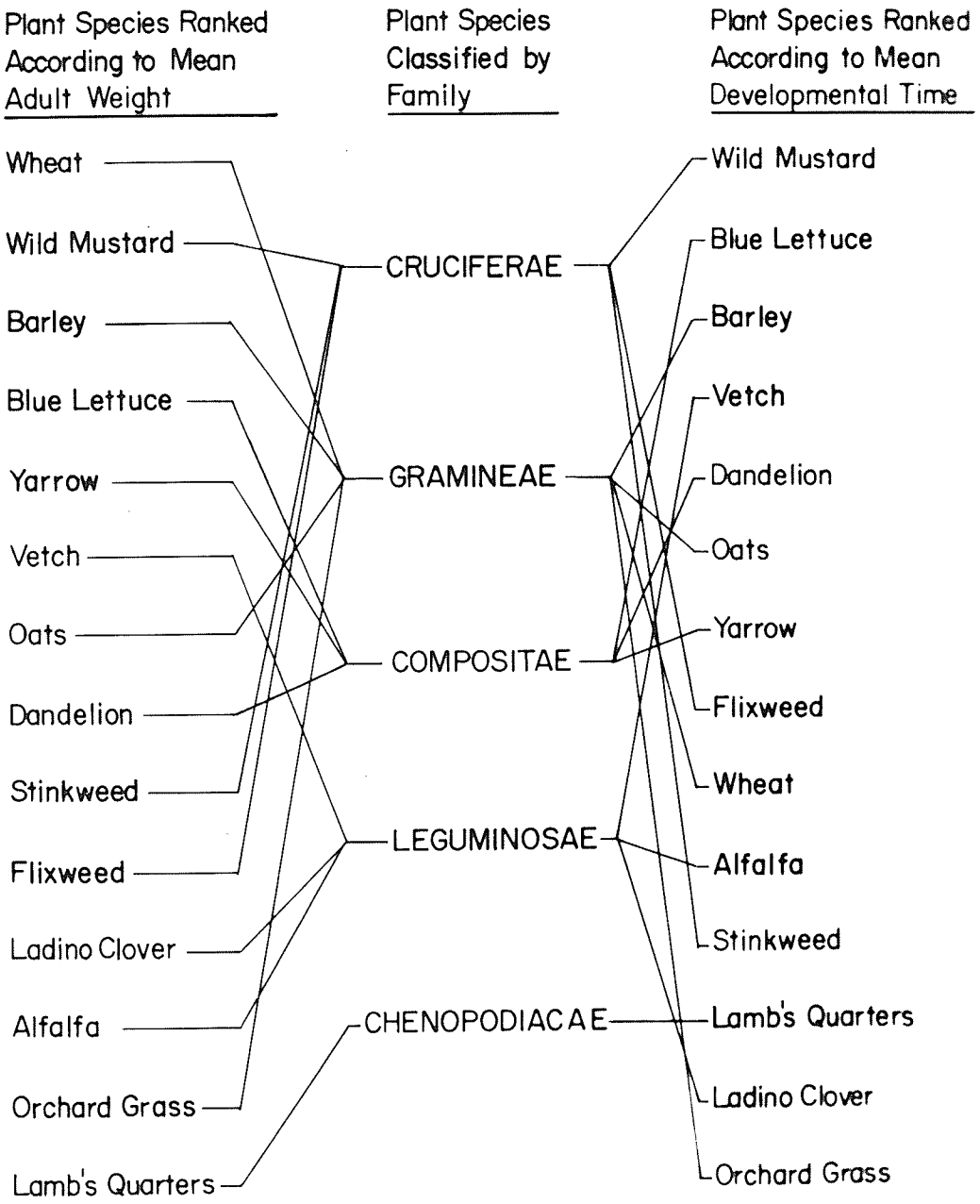
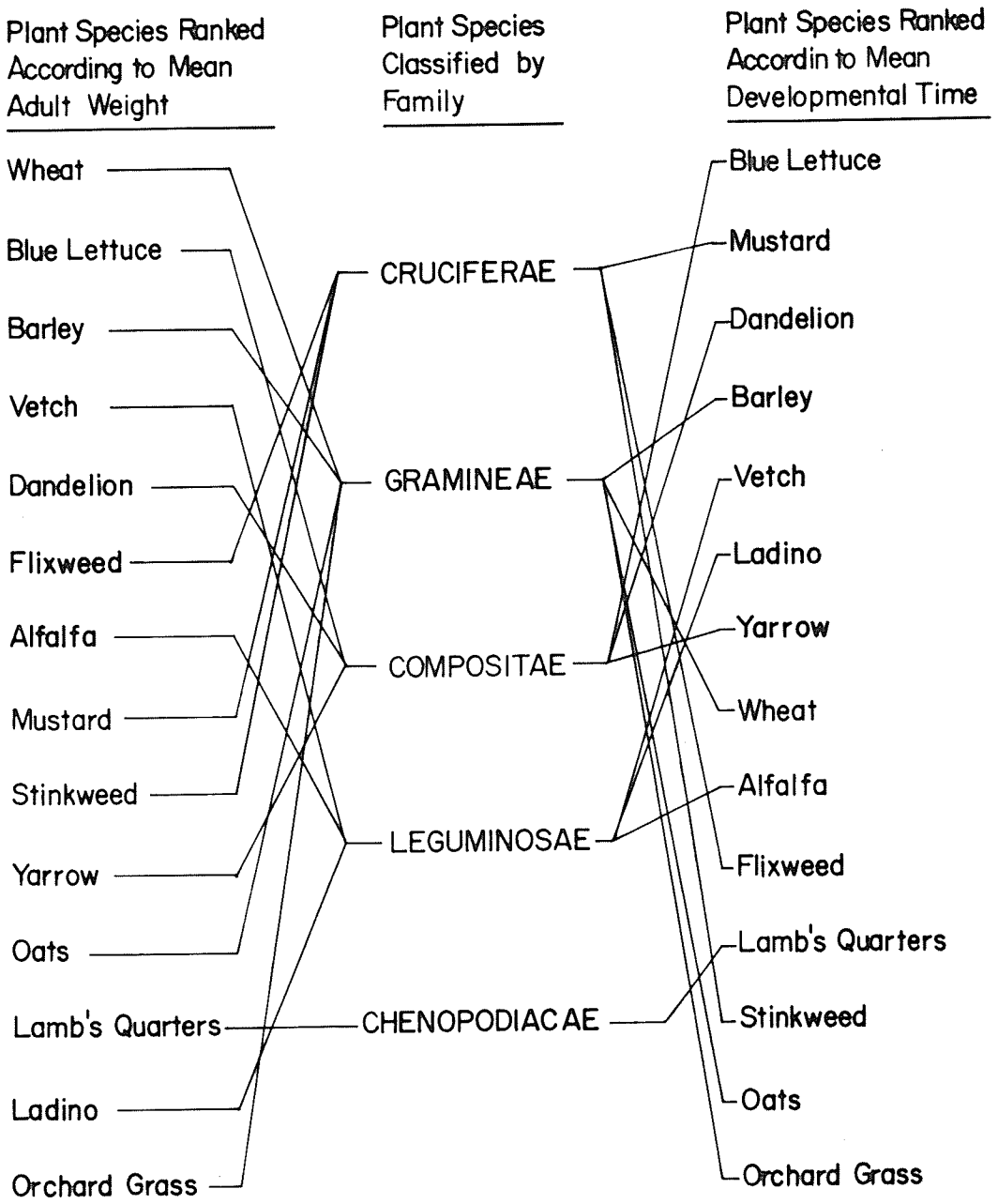

Plant Species Ranked According to Mean Adult Weight
Plant Species Classified by Family
Plant Species Ranked According to Mean Developmental Time
Wheat
Wild Mustard
Barley
Blue Lettuce
Yarrow
Vetch
Oats
Dandelion
Stinkweed
Flixweed
Ladino Clover
Alfalfa
Orchard Grass
Lamb's Quarters
CRUCIFERAE
GRAMINEAE
COMPOSITAE
LEGUMINOSAE
CHENOPODIACAE
Wild Mustard
Blue Lettuce
Barley
Vetch
Dandelion
Oats
Yarrow
Flixweed
Wheat
Alfalfa
Stinkweed
Lamb's Quarters
Ladino Clover
Orchard Grass

Figure 49. Relation among mean adult weights and mean developmental times of male grasshoppers with respect to plant families.


Plant Species Ranked According to Mean Adult Weight
Plant Species Classified by Family
Plant Species Ranked Accordin to Mean Developmental Time
Wheat
Blue Lettuce
Barley
Vetch
Dandelion
Flixweed
Alfalfa
Mustard
Stinkweed
Yarrow
Oats
Lamb's Quarters
Ladino
Orchard Grass
CRUCIFERAE
GRAMINEAE
COMPOSITAE
LEGUMINOSAE
CHENOPODIACAE
Blue Lettuce
Mustard
Dandelion
Barley
Vetch
Ladino
Yarrow
Wheat
Alfalfa
Flixweed
Lamb's Quarters
Stinkweed
Oats
Orchard Grass

Summary

Very little is known of the role of nutrition in insect resistance or susceptibility to disease. In this study the effects of various plant diets in the form of leaf meals on mortality and development of Melanoplus sanguinipes (Fab.) inoculated with Nosema locustae Canning were investigated.

Third instar nymphs were reared on compressed dehydrated leaf meal of various plants. Secondary plant chemicals incorporated into the dandelion leaf meal were also tested. Survival, developmental time, food index and final weights were recorded for individual grasshoppers of both sexes. Effects on inoculated grasshoppers and uninoculated grasshoppers reared on untreated dandelion leaf meal were compared.

Adult weights and developmental times allowed the plant diets to be grouped into fairly definite categories. Good diets included wild mustard, dandelion, barley, blue lettuce, wheat, vetch, alfalfa, yarrow, flixweed and stinkweed. Poor diets included orchard grass, oats, lamb's quarters and ladino clover. Sweet clover, lily of the valley, foxglove, death camas, horsetail and ox-eye daisy were very poor diets.

Effects on insects reared on dandelion leaf meal treated with secondary plant chemicals and on insects fed on untreated dandelion leaf meal were similar when the grasshoppers were not inoculated.

The plant diets tested had a significant effect on survival time of inoculated M. sanguinipes. Most of the poor diets increased the survival time for inoculated insects, possibly because the Nosema organism multiplies rapidly in the fat bodies. The secondary plant chemicals digitonin, coumarin and dicoumarol decreased the survival time of inoculated grasshoppers as

compared to inoculated grasshoppers reared on untreated dandelion meal suggesting an additive toxic stress.

Nosema spore counts varied considerably among individual inoculated grasshoppers reared on the same diets and individuals on different diets.

It can be concluded that nutrition does play a role in the insect's ability to survive infection by the pathogen.

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Appendix I

A list of plants used as test diets for *M. sanguinipes*

Family	Common Name	Scientific Name
Gramineae	Wheat (Manitou)	*Triticum vulgare*
	Barley (Conquest)	*Hordeum vulgare*
	Oats (Harmon)	*Avena sativa*
	Orchard grass	*Dactylis glomerata*
Leguminosae	White sweet clover	*Melilotus alba*
	Ladino clover	*Trifolium repens*
	Alfalfa	*Medicago sativa*
	Tufted vetch	*Vicia cracca* (L)
Cruciferae	Wild mustard	*Brassica Kaber* (D.C.)
	Flixweed	*Descurainia sophia* (L.) Webb
	Stinkweed	*Thlaspi arvense* L.
Chenopodiacea	Lamb's quarters	*Chenopodium album* (L.)
Compositae	Dandelion	*Taraxacum officinale* Weber
	Ox-eye daisy	*Chrysanthemum leucanthemum* L.
	Yarrow	*Achillea millefolium* L.
	Blue lettuce	*Lactuca pulchella* (Pursh) D.C.
Liliaceae	Lily of the valley	*Convallaria majalis*
	Death camas	*Zygadenus* sp.
Equisetaceae	Horsetail	*Equisetum palustre* L.
Solanaceae	Cut-leaved nightshade	*Solanum triflorum*
Scrophulariaceae	Foxglove	*Digitalis purpurea*

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1. Frankton, C. and W.H. Wright (1963).
2. Neatby, K.W. and F.J. Greaney (1947).
3. Martin, J.H. and W.H. Leonard (1959).
4. Muenscher, W.C. (1951).
5. Britton, N.L. and A. Brown (1943).

Appendix II

A list of chemicals, plants from which they have been isolated and concentrations used in tests.

Chemical	Species from which the chemical has been recorded	Concentration Used
Tigogenin	Digitalis lanata	1.00% per dry weight
Convallamarin	Convallaria majalis	1.00% per dry weight
Coumarin	Melilotus alba	0.50% per dry weight
Digitonin	Digitalis purpurea	1.00% per dry weight
Farnesol	Convallaria majalis	.5 ml. per 14 g. of diet.
Dicoumarol		0.50% per dry weight

References:

1. Muenscher, W.C. (1951).
2. Robinson, T. (1963).
3. Pammel, L.H. (1911).
4. Karrer, W. (1958).
5. Kingsbury, J.M. (1965).
6. Welch, H and H.E. Morris (1952).
7. Harley, K.L.S. and A.J. Thorsteinson (1966).
8. Kraemer, H. (1915).
9. Stevenson, T.M. and J.S. Clayton (1936).

Appendix LII

Results of statistical analysis

A. Analysis of variance of adult weights of *M. sanguinipes* reared on leaf meal of the Gramineae.

Females:

Source	df	ms	f
Individuals	5	4340	2.62
Diets	3	13365	8.46**
Error	15	1588	

Males:

Source	df	ms	f
Individuals	5	1459	2.42
Diets	3	6165	10.12**
Error	15	598	

B. Analysis of variance of developmental time of *M. sanguinipes* reared on leaf meal of the Gramineae.

Females:

Source	df	ms	f
Individuals	6	10.5	0.93
Diets	3	62.3	5.56**
Error	18	11.2	

Males:

Source	df	ms	f
Individuals	6	25.0	2.38
Diets	3	44.3	4.21*
Error	18	10.5	

C. Analysis of variance of adult weights of *M. sanguinipes* reared on leaf meal of the Compositae.

Females:

Source	df	ms	f
Individuals	3	9520	5.08*
Diets	2	244	.13
Error	7	1872	

D. Analysis of variance of time to mortality of inoculated *M. sanguinipes* reared on leaf meal of the Gramineae.

Females:

Source	df	ms	f
Individuals	7	147	1.88
Diets	3	364	4.66*
Error	21	78	

E. The "t" Test of time to mortality of M. sanguinipes reared on dandelion meal as compared to dandelion meal plus digitonin.

Females:

df	t
6	2.44*

Males:

df	t
6	3.49*

F. The "t" Test of time to mortality of M. sanguinipes reared on dandelion meal as compared to dandelion meal plus dicoumarol.

Males:

df	t
3	3.7*

G. Spearman rank correlation of adult weights with developmental time.

Females: $r_s = .77^*$

Males: $r_s = .60^*$

H. Spearman rank correlation of adult weights with daily rate of feeding.

Females: $r_s = .66^*$

Males: $r_s = .47^*$

* significant at the .05 level.
** significant at the .01 level.

Note: Only tests that proved to be significant are listed.