Integration of Nosema locustae with chemical insecticides and entomopoxvirus for control of grasshoppers
by Gerald Louis Mussgnug

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Abstract:
The effects in grasshoppers of the protozoan, Nosema locustae, and formulations of N. locustae with the insecticides, malathion and carbaryl, or with an entomovirus, the grasshopper inclusion body virus (GIBV), were investigated during 1976, 1977, and 1978. A standard bioassay technique was devised for testing materials in the laboratory and mortality and incidence of infection were determined for three species of grasshoppers: Melanoplus sanguinipes, Melanoplus bivittatus, and Melanoplus differentialis. Plots 4 hectares in size were used to evaluate bait formulations against rangeland grasshoppers in Montana.

A continuous flow augering system for formulation and a modified seed spreader for application of bran bait were developed. N. locustae primarily infects the fat body of the grasshopper and results in reduced vigor and eventually death. The virulence of N locustae varied between strains, species, and subfamilies. A logarithmic relationship between the dosage of N. locustae used for initiating infection and the mortality and incidence of infection was demonstrated. Between 2.5 and 7.4 billion spores per hectare on 1.68 to 3.36 kilograms of bran were considered to be an efficient level of inoculum to apply for significant control of grasshoppers on rangeland. Applications when grasshoppers were in the third-instar of development resulted in better control than later applications. Once initiated, the infection spread through the population and caused reductions in density of 30 percent with 30 percent of the survivors showing infection within 6 weeks post-application. Infection by N_. Iocustae may increase the toxicity of malathion when fed orally to some species of grasshoppers in the laboratory but evidence of a stress phenomenon induced by the pathogen could not be demonstrated in field tests. Midseason spraying with ULV malathion to prevent oviposition might not disrupt an epizootic of N_. locustae initiated earlier in the season. Third-instar grasshoppers fed oral doses of malathion or carbaryl died within 24 hours while GIBV and N. locustae acted slower. Combinations of N. locustae with malathion, carbaryl, or GIBV were non-antagonistic and additive in action in laboratory assays. Formulations of malathion-N. locustae on bran rapidly reduced densities of grasshoppers and produced infection in the residual population. Reductions in density of 25-50 percent occurred within 3-5 days after malathion was applied as a 0.4 percent bran bait either alone or in combination with N_. locustae. Grasshopper populations treated with formulations of malathion-N. locustae displayed lower infection rates than when the pathogen was applied by itself. Slowing the spread of infection by simultaneously applying malathion with N. locustae was considered only temporary. The benefits of using an integrated approach for managing grasshopper populations on rangeland are discussed.

INTEGRATION OF <u>Nosema locustae</u> WITH CHEMICAL
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FOR CONTROL OF GRASSHOPPERS

by

GERALD LOUIS MUSSGNUG

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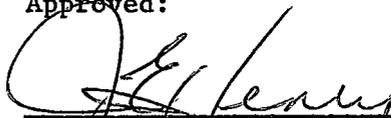of the requirements for the degree

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Approved:

Chairperson, Graduate Committee

Head, Major Department

Graduate Dean

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ABSTRACT

The effects in grasshoppers of the protozoan, Nosema locustae, and formulations of N. locustae with the insecticides, malathion and carbaryl, or with an entomovirus, the grasshopper inclusion body virus (GIBV), were investigated during 1976, 1977, and 1978. A standard bioassay technique was devised for testing materials in the laboratory and mortality and incidence of infection were determined for three species of grasshoppers: Melanoplus sanguinipes, Melanoplus bivittatus, and Melanoplus differentialis. Plots 4 hectares in size were used to evaluate bait formulations against rangeland grasshoppers in Montana. A continuous flow augering system for formulation and a modified seed spreader for application of bran bait were developed. N. locustae primarily infects the fat body of the grasshopper and results in reduced vigor and eventually death. The virulence of N. locustae varied between strains, species, and subfamilies. A logarithmic relationship between the dosage of N. locustae used for initiating infection and the mortality and incidence of infection was demonstrated. Between 2.5 and 7.4 billion spores per hectare on 1.68 to 3.36 kilograms of bran were considered to be an efficient level of inoculum to apply for significant control of grasshoppers on rangeland. Applications when grasshoppers were in the third-instar of development resulted in better control than later applications. Once initiated, the infection spread through the population and caused reductions in density of 30 percent with 30 percent of the survivors showing infection within 6 weeks post-application. Infection by N. locustae may increase the toxicity of malathion when fed orally to some species of grasshoppers in the laboratory but evidence of a stress phenomenon induced by the pathogen could not be demonstrated in field tests. Mid-season spraying with ULV malathion to prevent oviposition might not disrupt an epizootic of N. locustae initiated earlier in the season. Third-instar grasshoppers fed oral doses of malathion or carbaryl died within 24 hours while GIBV and N. locustae acted slower. Combinations of N. locustae with malathion, carbaryl, or GIBV were non-antagonistic and additive in action in laboratory assays. Formulations of malathion-N. locustae on bran rapidly reduced densities of grasshoppers and produced infection in the residual population. Reductions in density of 25-50 percent occurred within 3-5 days after malathion was applied as a 0.4 percent bran bait either alone or in combination with N. locustae. Grasshopper populations treated with formulations of malathion-N. locustae displayed lower infection rates than when the pathogen was applied by itself. Slowing the spread of infection by simultaneously applying malathion with N. locustae was considered only temporary. The benefits of using an integrated approach for managing grasshopper populations on rangeland are discussed.

INTRODUCTION

Grasshopper populations have historically been the most important

insect pest of rangeland in the western United States. Although over

100 species are found in these areas, only about 15 percent are

considered to be of economic importance since they compete with cattle

for available forage or they damage cultivated crops (Hewitt 1977).

Parker (1937) listed the five most important economic species as

Melanoplus mexicanus (Saussure) (M. sanguinipes = M. mexicanus), M.

bivittatus (Say), M. differentialis (Thomas), M. femur-rubrum (DeGeer),

and Camnula pellucida (Scudder). Anderson and Wright (1952) showed

that the amount of damage to vegetation by grasshoppers cannot be

predicted by abundance only but that damage is related to the grass-

hopper species composition and the vegetation present. Mulkern et al.

(1969) demonstrated that grasshoppers have specific food plant

preferences and utilize range resources selectively.

In 1877 congress appropriated funds for the creation of the United

States Entomological Commission to investigate the Rocky Mountain

grasshopper, Melanoplus spretus (Walsh.) (Blickenstaff et al. 1974).

Since that time control measures have changed considerably. Arsenical

baits were used in 1936 but were replaced by the chlorinated hydro-

carbons, toxaphene and chlordane, in the late forties. These were

replaced later by aldrin and dieldrin in the fifties and presently

malathion and carbaryl are recommended. Malathion, the chemical used

most frequently, is applied in ultra low volume (ULV) formulation of

8 oz. AI/acre (585 ml/hectare) in large-scale federally sponsored programs of grasshopper control on rangelands. An assessment of the effectiveness of such programs for long-term control of grasshoppers, however, failed to demonstrate protection beyond the year of application (Blickenstaff et al. 1974).

It is generally accepted that insect population densities fluctuate both in time and space. Any attempts to evaluate applied control must therefore also include an understanding of both the organism and the environment. As proposed by Clark et al. (1967), life systems consist of a subject population and its effective environment which interact to determine its abundance and persistence. Solomon (1949) earlier emphasized the inseparable existence of the population and its environment and showed that population numbers fluctuate within limits in such a system. The life system should then serve as the basis for considering any changes in populations of grasshoppers.

L. O. Howard (1897) was the first to recognize disease as a natural regulating factor of insect densities. Steinhaus (1954) summarized the effect of disease on insect populations and pointed out that disease may be characterized according to a number of general ecological principles. In that report, as well as in a later report (Steinhaus 1957), he pointed out the potential of utilizing pathogens for practical microbial control of insect pests.

Attempts to control grasshoppers with fungi and bacteria have been reviewed by Charles (1965). Although these pathogens are capable of initiating infections, they require specific environmental conditions and have little effect on the densities of the insect populations. Protozoan and viral pathogens generally do not require specific conditions, are more host-specific, and exhibit longer controlling activity (Henry 1970). Henry (1970) has considered these pathogens, particularly Nosema locustae Canning and the grasshopper inclusion body virus (GIBV), to have the greatest potential for use in grasshopper control since they not only produce some immediate control activity but also become established and continue as a regulatory factor for prolonged periods of time.

N. locustae was first described by Canning (1953) from the African migratory locust, Locusta migratoria migratorioides (Reiche and Fairmaire). Henry (1969) reported the host range to include at least 58 species of American grasshoppers. N. locustae infects the fat bodies thus depriving the host of energy required for growth and reproduction. Canning (1962b) reported that infection by this pathogen delays the final molt to adult and decreases the flying ability of L. migratoria. Laboratory and field studies have indicated that the microsporidan is potentially useful in controlling densities of grasshoppers (Henry 1971; Henry et al. 1973; Henry and Oma 1974b). Experimental field tests have demonstrated that applications of spores

of N. locustae on bran can result in overall reductions in density of
50 percent within four weeks of application and produce infection in
those surviving (Henry 1971; Henry et al. 1973).

GIBV was isolated from M. sanguinipes (Henry and Jutila 1966) and
later was characterized as an entomopoxvirus (Henry et al. 1969). It
replicates in the fat tissues in which inclusion bodies are formed that
contain the virions and high rates of mortality result from infection.
As with N. locustae, epizootics occur among grasshoppers and cause
noticeable reductions in density (Henry 1970).

In recent years the concept of integrated pest management has
become prominent as a means of maintaining insect densities below
economic levels (Stern et al. 1959; Smith and Van den Bosch 1967). In
its broadest sense, integrated control includes maximizing the effects
of existing natural controlling factors, monitoring pests and natural
enemies, and using pest suppression measures when needed (Train et al.
1972). Included in integrated control is the concept of combined use
of chemicals and biological agents which according to Benz (1971) can
produce interactions ranging from antagonism to synergism. Creighton
and McFadden (1974) demonstrated the integrated control of two lepidop-
terous larvae, Trichoplusa ni (Hübner), and Pieris rapae (L.), with
mixtures of the bacterium (B.t.), Bacillus thuringiensis, and
insecticide. Also Jaques (1973) and Jaques and Laing (1978) have
demonstrated the increased effectiveness of mixing B.t., nuclear

polyhedrosis virus (NPV), or granulosis virus (GV) with chemical insecticide for pest management of these insects. A series of reports (Morris 1972, 1975a b, 1977 a b c; and Morris et al. 1974, 1975) discussed the combined use of B.t. and various chemical insecticides against the spruce budworm, Choristoneura fumeriferana (Clem.). In addition, Hunter et al. (1975) and Luttrell et al. (1979) have shown the feasibility of combining entomoviruses with insecticides for better control of the Indian meal moth, Plodia interpunctella (Hübner), and the corn earworm, Heliothis zea (Boddie) respectively. McVay et al. (1977) suggested an additive action of simultaneous infections of B.t. and NPV in T. ni, whereas Tanada (1959) postulated a synergistic action in the use of NPV and GV against the army worm, Pseudaletia unipuncta (Haworth).

Based on his study of grasshopper pathogens, Henry (1970) suggested that an integrated approach using pathogens and insecticides might be effective for managing the densities of grasshoppers. This, together with the repeated success reported for the integrated control of lepidopterous insects during the past ten years, and the lack of similar information on which to base an integrated pest management scheme for rangeland grasshoppers, formed the basis for the present studies. The purpose of the studies was two-fold: (1) to extend the basis for using N. locustae for grasshopper control and, (2) to investigate the integrated uses of other control agents such as chemical

or virus with <u>N</u>. <u>locustae</u>.

METHODS AND MATERIALS

Laboratory Assays

All spores of Nosema locustae used as inoculum in the assays were produced in Melanoplus bivittatus. Eggs of M. bivittatus were collected in the fall or early spring from various locations in Montana and North Dakota and were brought to the laboratory for hatching. Fifth-instar nymphs were inoculated by feeding them for 3 days on romaine lettuce which had been sprayed with spores of N. locustae at a rate of approximately 10^7 spores/leaf. Spores were harvested 1-2 months after inoculation by homogenizing suspensions of cadavers in distilled water with a tissue grinder and then passing the homogenates through nylon organdy to remove large debris. Spores were then cleaned and concentrated by lowspeed differential centrifugation (5,000 g). Spore concentrations were quantified by direct counts with a hemocytometer and samples were stored at -10° C. The inoculum of the GIBV was produced in a non-diapause laboratory strain of Melanoplus sanguinipes obtained from Canada (Pickford & Randell 1969) and inclusions were recovered in a manner similar to that for N. locustae.

The insecticides, carbaryl (Sevin®) and malathion were diluted from technical grade stock solutions. The first material, being water based, was diluted in water and the second, being oil based, was diluted in acetone.

Three species of grasshoppers, M. sanguinipes, M. bivittatus, and Melanoplus differentialis were used in the tests. The non-diapause

strain of M. sanguinipes from Canada was used most extensively for the

assays although field collected diapausing M. sanguinipes from Montana

also were included for comparison. All M. bivittatus and M.

differentialis were collected from the field in Montana and North

Dakota and were of the diapause type. Grasshoppers were reared at

approximately 30° C under a 12-hour light-dark regimen and were fed

daily with head lettuce, wheat bran, fresh seedlings of Balbo rye,

and an agar-base diet that contained a sulphonamide, Thipyrimeth®, as a

prophylactic against possible amoebic infections (Henry & Oma 1975).

A standard bioassay procedure similar to that described by Henry

and Oma (1974 a) was chosen for the tests (Fig. 1). All inocula were

dispensed with a microinjection syringe as 5-µl drops onto 7 mm lettuce

discs. The insecticides were applied to lettuce discs as 1-µl drops

and combinations of pathogen and insecticide were mixed and applied in

6-µl drops. The grasshoppers were inoculated *per os* as third-instar

nymphs after having been starved for 24 hours. In the tests involving

the effect of N. locustae on insecticide toxicity, insecticides were

administered to fifth-instar and adult grasshoppers in similar fashion.

Those that failed to consume the entire disc were discarded. After

inoculation, the grasshoppers were distributed in groups of 5 per

rearing tube (17 cm long by 5 cm dia. constructed of sheet acetate with

screened ends) (Fig. 2) and were reared as described.

Fig. 1.--Materials utilized in inoculation of grasshoppers for standard
laboratory assay. (A) cork borer for cutting lettuce discs,
(B) needles, (C) syringe, (D) insecticide or pathogen, (E)
third-instar grasshopper, (F) forcep, (G) treated lettuce
discs.

Fig. 2.--Acetate tubes (17 cm long X 5 cm dia) used in rearing grass-
hoppers for standard laboratory assay.

Mortality rates were based on the number of grasshoppers that died during the post-inoculation period. Incidence of infection by the pathogen was based on the number of grasshoppers within a treatment group that exhibited either spores of N. locustae or, where applicable, inclusion bodies of GIBV. Infections were diagnosed by the presence of spores in homogenates of insects surviving 24 days post-inoculation. Samples were examined with phase microscopy at about 500 diameters magnification.

Four replicates of each treatment were included in the tests with twenty grasshoppers per replicate. Differences between treatments were assessed by an analysis of variance of arcsine square root transformations of the percentages of mortality and infeciton. Significant interaction between N. locustae and either the insecticide or the virus was interpreted as synergism or antagonism while lack of significance indicated independence or additive action. Probit analysis was used to calculate LD_{50} values.

Field Tests

Spores of Nosema locustae for dissemenation in the field were produced in the laboratory by mass inoculation of fourth and fifth-instar nymphs of Melanoplus bivittatus in cages (30 cm in dia. X 35 cm high) (Fig. 3). Grasshoppers were inoculated en masse by feeding them for 3 days on romaine lettuce that had been sprayed with 10^{7} spores/leaf. Infected adults were collected 1-2 months after inoculation, suspended

Fig. 3.--Cage (30 cm in dia X 35 cm high) used in the mass inoculation
and rearing of grasshoppers for the production of spores of
Nosema locustae for field tests.

in distilled water, and then homogenized with a hand operated wheat

mill. The homogenate was then filtered through nylon organdy to remove

the large debris and spores were concentrated by low speed differential

centrifugation (5,000 g). After spore concentrations were determined

by direct counts with a hemocytometer, the inoculum was stored at -10°

C. Malathion that was applied to bran was diluted in acetone, whereas

that which was applied as ULV sprays in the field was diluted in

diesel oil. In all tests the volume of spray was held constant (8.8

ml/kg and 585 ml/ha).

Formulation of bran treated with either N. locustae, malathion or

combinations thereof was accomplished by spraying the bran as it moved

through a continuous flow formulating system (Fig. 4). Spore suspen-

sions were diluted so that the ratio of water to wheat bran remained

constant (8.8 ml/kg bran). Hydroxymethyl cellulose (0.2% w/v) was

added as a sticker in the water-spray formulations. Treated bran was

generally stored for periods up to 2 weeks in burlap sacks.

The study areas were on rangeland at various locations where grass

was the predominant vegetation (Fig. 5). Treated bran was applied in

the field with a modified Cyclone® seed spreader (Fig. 6) that was

mounted in the back of a 4-wheel drive vehicle. Applications were

begun on June 28 in 1976 (when grasshoppers were mainly third-instar),

on July 7 in 1977 (when grasshoppers were mainly third-instar) and on

July 8 in 1978 (when grasshoppers were mainly fifth-instar). Each

Fig. 4.--The treatment cone in which bran was sprayed with malathion,
Nosema locustae or N. locustae-malathion mixtures.

Fig. 5.--Location of field tests in Montana where formulated bran was applied to plots 4 hectares in size. (1) Big Timber, (2) White Sulphur Springs, (3) Forsyth.

Fig. 6.--Bait spreader used in application of formulated bran for field
      tests.

plot was 4 hectares square and the bran was applied in 9.1 m wide

swaths. In field tests in 1976 and 1978 bran was applied at the rate

of 1.68 kg/ha, but in 1977 the rate was 3.36 kg/ha.

Sampling consisted of density determinations and collections of

grasshoppers for species composition and incidence of infection. In

some tests pre-treatment samples were taken while in other tests only

post-treatment samples were made. The density of grasshoppers was

determined by using 40 aluminum rings (each with an area of 0.1 m^2)

distributed along a diagonal transect through the middle of the plot.

Counts were made of grasshoppers within the rings the following day by

approaching each ring and flushing the insects with a pointer (a 2-m

long wand) (Onsager & Henry 1977). Sweep net samples of grasshoppers

were obtained near the center of each plot after density determinations.

The collected grasshoppers were placed in paper sacks and were frozen

immediately with dry ice. Subsequently, in the laboratory the species,

sex, and developmental stage of each grasshopper were recorded after

which each was suspended in 5 ml of distilled water and homogenized with

a tissue grinder. A sample was removed using a capillary tube and

examined at 500 diameters as a hanging drop for spores of N. locustae.

All treatments were randomized within each of 4 replications in a

randomized block design. Differences between treatments were assessed

by an analysis of variance of the density and of arcsine square root

transformations of the incidence of infection.

RESULTS

Laboratory Assays

Nosema locustae and GIBV infection in Melanoplus sanguinipes

Grasshoppers infected with Nosema locustae exhibited reduced

feeding and locomotor activity while moribund grasshoppers were almost

totally incapacitated and often exhibited tetanic convulsions. Spores

were evident at 16 days after *per os* inoculation of third-instar

nymphs. In heavily infected grasshoppers at 24 days post-inoculation

the spores of N. locustae had virtually replaced all fat tissue. The

homogenate of such individuals revealed numerous spores when samples

were examined under phase contrast microscopy (Fig. 7). Grasshoppers

infected with GIBV exhibited a slower developmental rate and less vigor

as compared to grasshoppers infected with N. locustae. Inclusion

bodies were evident at 14 days after *per os* inoculation of third-instar

nymphs. Fig. 8 shows the appearance of inclusion bodies of GIBV from

Melanoplus sanguinipes under phase contrast microscopy. The virus was

observed to infect primarily the cells of the fat body from which it

spread throughout the insect. Multiple infections were evident in M.

sanguinipes following dual inoculation with N. locustae and GIBV. Both

pathogens were evident within 21 days after inoculation (Fig. 9).

Effect of Nosema locustae in two strains of Melanoplus sanguinipes

As shown in Table 1 those grasshoppers inoculated with spores of

Nosema locustae at doses of from 10^3 and 10^6 spores per third-instar

Fig. 7. Fresh preparation under phase contrast microscopy of the
spores of <u>Nosema locustae</u> in <u>Melanoplus sanguinipes</u>. 800 X.

Fig. 8.--Fresh preparation under phase contrast microscopy of the
inclusions of the grasshopper inclusion body virus in
Melanoplus sanguinipes. 800 X.

Fig. 9.--Fresh preparation under phase contrast microscopy of the
spores of Nosema locustae (1) and the inclusions of the grass-
hopper inclusion body virus (2) from a multiply infected
Melanoplus sanguinipes. 800 X.

Table 1. Percent mortality and infection at 24 days post-inoculation of third-instar diapause and non-diapause <u>Melanoplus</u> <u>sanguinipes</u> treated with spores of <u>Nosema locustae</u> in the laboratory.

Treatment N. locustae (No. spores/ grasshopper)	% Mortality		% Infected Survivors	
	Non-diapause	Diapause	Non-diapause	Diapause
0	15.0 a	25.0 a	0.0 a	0.0 a
10^3	21.3 a	21.3 a	15.9 b	58.7 b
10^4	21.3 a	28.8 a	27.0 b	66.7 b
10^5	65.0 b	41.3 b	39.3 c	89.4 c
10^6	91.3 c	68.8 c	57.1 d	100.0 d

Percentages followed by the same letter indicate no significant difference between treatments at the 5% level of error.

exhibited higher rates of mortality than did the controls. Analysis

of variance revealed that mortality did not differ significantly

between the 10^3 and 10^4 doses, but these doses and the 10^5 and 10^6

doses were different from one another (p≤.05) and varied directly with

dosage. The non-diapause strain tended to be more susceptible than the

field-collected strain (Fig. 10). Table 2 shows that the difference in

virulence of the pathogen was significant since the 95% confidence

limits of the LD_{50} values do not overlap. The LD_{50} for third-instar

nymphs of the non-diapause strain was 0.9 X 10^5 whereas with the dia-

pause strain the LD_{50} was 5.3 X 10^5 spores. However, the percentage

infection among surviving grasshoppers of the non-diapause strain was

significantly lower than for the diapause strain (p≤.05). Regressions

calculated from the data in Table 1 show the differences in infectivity

of N. locustae between the two strains (Fig. 11). The 10^3 and 10^4

doses were not found to produce statistically different infection rates

from one another although they differed significantly from the other

doses and the controls. Analysis of the dosage-infectivity data showed

a linear relationship between the $\log_{10}$ of the initial pathogen dose

and the subsequent percent infection.

Effect of Nosema locustae on insecticide toxicity
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Tables 3 and 4 show the effect of 15 days of infection by Nosema

locustae on the toxicity of malathion among fifth-instar non-diapause

Fig. 10.--Regression of mortality vs time among a non-diapause (solid line) and a diapause (broken line) strain of <u>Melanoplus sanguinipes</u> after treatment with <u>Nosema locustae</u> at rates of 10^5 (a) and 10^6 (b) spores per third-instar grasshopper in the laboratory.

Table 2. Relative virulence of <u>Nosema locustae</u> to third-instar diapause and non-diapause <u>Melanoplus</u> <u>sanguinipes</u> at 24 days post-inoculation in the laboratory.

Treatment (Strain)	LD_{50} (No. of spores/grasshopper)	95% Confidence Limits
Non-diapause	0.9×10^5	$0.6 - 1.3 \times 10^5$
Diapause	5.3×10^5	$2.8 - 9.7 \times 10^5$

Fig. 11.--Regressions based on percent infection among survivors of a non-diapause (A) and diapause (B) strain of <u>Melanoplus sanguinipes</u> 24 days after treatment with <u>Nosema locustae</u> at rates of 10^3, 10^4, 10^5, and 10^6 spores per third-instar grasshopper in the laboratory.

Table 3. Percent mortality among fifth-instar non-diapause <u>Melanoplus</u> <u>sanguinipes</u> (uninfected and infected for 15 days with <u>Nosema</u> <u>locustae</u>) 24 hours after ingestion of malathion in the laboratory.

Treatment (µg malathion/ grasshopper)	Uninfected		Infected with N. locustae	
	No.	% Mortality	No.	% Mortality
0.0	60	0.0	60	10.0
4.5	60	36.7	60	68.3
6.0	60	51.7	60	81.7

Table 4. Oral toxicity of malathion among fifth-instar non-diapause <u>Melanoplus</u> <u>sanguinipes</u> (uninfected and infected for 15 days with <u>Nosema</u> <u>locustae</u>) and held for 24 hours at 21° C without food in the laboratory.

Treatment (Pathological state)	LD_{50} (μg malathion/ grasshopper)	Confidence Limits	
		95%	86%
Uninfected	4.31	3.18 – 5.82	3.42 – 5.42
Infected with <u>N. locustae</u> for 15 days	2.13	1.15 – 3.92	1.33 – 3.39

Melanoplus sanguinipes. Mortality was greater among infected nymphs than among uninfected nymphs. LD_{50} values showed that a higher concentration of malathion was needed to obtain a similar degree of mortality to uninfected nymphs as for those infected with the pathogen. Due to the variation in the test, differences between these LD_{50} values were not significant at the 5% level of error. However, since the 86% confidence limits did not overlap, it is possible that a disease stress effect on the toxicity of malathion may occur for this species.

.   The effect of N. locustae on the toxicity of malathion in Melanoplus differentialis, a species in which the disease is less lethal, is presented in Tables 5 and 6. Mortality due to malathion was again greater among infected grasshoppers than among uninfected adults. The difference between LD_{50} values, however, was not as great as for M. sanguinipes and did not become evident until later into the post-inoculation period (39 days after inoculation). The LD_{50} values did not differ significantly between infected and uninfected adults and the confidence limits were only separable at the 50% level of error.

Integration of Nosema locustae with insecticides: N. locustae with
    malathion using Melanoplus sanguinipes

Table 7 presents results for per os treatments of Nosema locustae, malathion, and N. locustae-malathion mixtures in the non-diapause strain of Melanoplus sanguinipes. Mortality at 1-day post-inoculation was primarily caused by treatment with malathion. Analysis of the data

Table 5.   Percent mortality among adult male <u>Melanoplus differentialis</u> (uninfected and infected for 39 days with <u>Nosema locustae</u>) 24 hours after ingestion of malathion in the laboratory.

Treatment (ug malathion/ adult)	Uninfected		Infected with N. locustae	
	No.	% Mortality	No.	% Mortality
0.0	20	0.0	20	0.0
9.0	27	14.8	27	40.7
12.0	21	38.1	18	55.6

Table 6. Oral toxicity of malathion among adult male <u>Melanoplus differentialis</u> (uninfected and infected for 39 days with <u>Nosema locustae</u>) and held for 24 hours at 21° C without food in the laboratory.

Treatment (Pathological state)	LD_{50} (μg malathion/ grasshopper)	Confidence Limits	
		95%	50%
Uninfected	13.50	9.88 - 18.44	12.12 - 15.02
Infected with <u>N. locustae</u> for 39 days	10.78	7.89 - 14.72	9.68 - 11.99

Table 7. Percent mortality and infection among surviving non-diapause <u>Melanoplus sanguinipes</u> fed <u>Nosema locustae</u>, malathion, and <u>N. locustae</u>-malathion mixtures as third-instar and examined 1 and 24 days post-inoculation in the laboratory.

| Treatment | | % Mortality | | % Infected survivors |
N. locustae (No. spores/ grasshopper)	malathion (µg/ grasshopper)	1 day post-inoculation	24 days post-inoculation	24 days post-inoculation
0	0.0	0.0	8.8	0.0
10^3	0.0	0.0	16.3	6.0
10^5	0.0	0.0	38.8	26.5
10^7	0.0	2.5	97.5	50.0
0	0.4	17.5	25.0	0.0
10^3	0.4	22.5	37.5	16.0
10^5	0.4	25.0	46.3	14.0
10^7	0.4	36.3	100.0	–
0	0.6	41.3	50.0	0.0
10^3	0.6	46.3	52.5	10.5
10^5	0.6	37.5	58.8	27.3
10^7	0.6	62.5	100.0	–
0	0.8	55.0	61.3	0.0
10^3	0.8	72.5	75.0	5.0
10^5	0.8	67.5	78.8	11.8
10^7	0.8	77.5	100.0	–

revealed a dosage-response with significant differences (p≤.05) between

doses. The LD_{50} for malathion was determined to be 0.72 μg per third-

instar and only in combination with 10^7 spores of N. locustae did this

value change significantly (Table 8). When mixtures of malathion with

10^3 or 10^5 spores of N. locustae were fed simultaneously, slight

decreases in the LD_{50} occurred but these differences were not signif-

icant (p≥.05). Since an analysis of variance did not reveal any

significant interaction between the pathogen and the insecticide, they

apparently acted independently and their effects were additive.

Mortality between 2 and 24 days post-inoculation was attributable

to N. locustae and not malathion. Mortality of all grasshoppers

treated with N. locustae was significantly higher than for the

untreated checks (p≤.05). Mortality rates among grasshoppers treated

with 10^3 and 10^5 spores were not significantly different (p≥.05), but

were significantly lower than for those inoculated with 10^7 spores

(p≤.05). Interactions between the pathogen and insecticide were not

evident and the LD_{50} for N. locustae in this test was calculated to be

1.5 X 10^5 spores per grasshopper. Regressions based on the cumulative

percent mortality are presented in Fig. 12 and show the response of

mixtures of N. locustae and malathion in the non-diapause strain of M.

sanguinipes during the 24 days following treatment. Mortality caused

by N. locustae at the 10^5 spores dose was generally not noticeable

until 17 days post-inoculation and therefore regression lines for this

Table 8. Oral toxicity of malathion among third-instar non-diapause _Melanoplus sanguinipes_ fed _Nosema locustae_-malathion mixtures and held for 24 hours at 21° C without food in the laboratory.

Treatment N. locustae (No. spores/ grasshopper)	LD_{50} (μg malathion/ grasshopper)	95% Confidence limits
0	0.72	0.63 – 0.82
10^3	0.60	0.55 – 0.66
10^5	0.65	0.58 – 0.72
10^7	0.51	0.45 – 0.57

Fig. 12.--Regression of mortality vs time among non-diapause <u>Melanoplus</u>
<u>sanguinipes</u> fed mixtures of malathion at rates of 0.0 μg (A),
0.4 μg (B), 0.6 μg (C), and 0.8 μg (D) and <u>Nosema</u> <u>locustae</u> at
rates of 10^5 spores (broken line) and 10^7 spores (solid line)
per third-instar grasshopper in the laboratory.

dose were based only on the data after that period.

Based on examination of survivors, the percentage of infection after inoculation with 10^3 or 10^5 spores was not affected by simultaneous treatment with malathion, nor did increasing doses of malathion reduce subsequent infection. Analysis of the dosage-infection data for grasshoppers treated with N. locustae showed that infections were dosage responsive and that differences between infection rates were significant ($p \leq .05$).

Integration of Nosema locustae with insecticides: N. locustae with
malathion using Melanoplus bivittatus

As evident from Table 9, *per os* inoculations of Melanoplus bivittatus with Nosema locustae, malathion, and N. locustae-malathion mixtures resulted in a similar response to that observed at 1-day post-inoculation with Melanoplus sanguinipes. Mortality was due mainly to treatment with malathion although approximately twice as much insecticide was required to achieve a similar degree of mortality. The LD_{50} for malathion was determined to be 1.42 µg per third-instar and combination with N. locustae did not significantly change this value (Table 10). Analysis of the data revealed a dosage-response with significant differences between doses ($p \leq .05$). No interaction between the pathogen and the insecticide was evident and apparently the two agents acted independently with additive effects.

Table 9. Percent mortality and infection among surviving <u>Melanoplus bivittatus</u> fed <u>Nosema locustae</u>, malathion, and <u>N. locustae</u>-malathion mixtures as third-instar and examined 1 and 24 days post-inoculation in the laboratory.

| Treatment | | % Mortality | | % Infected survivors |
N. locustae (No. spores/ grasshopper)	malathion (µg/ grasshopper)	1 day post-inoculation	24 days post-inoculation	24 days post-inoculation
0	0.0	0.0	20.0	0.0
10^3	0.0	0.0	25.0	73.3
10^5	0.0	0.0	33.8	100.0
10^7	0.0	0.0	70.0	100.0
0	1.0	25.0	41.3	0.0
10^3	1.0	22.5	47.5	61.9
10^5	1.0	27.5	46.3	100.0
10^7	1.0	23.8	71.3	100.0
0	1.5	52.5	68.8	0.0
10^3	1.5	48.8	60.0	46.9
10^5	1.5	41.3	70.0	100.0
10^7	1.5	53.8	92.5	100.0
0	2.0	76.3	86.3	0.0
10^3	2.0	75.0	83.8	61.5
10^5	2.0	73.8	83.8	100.0
10^7	2.0	67.5	88.8	100.0

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Table 10. Oral toxicity of malathion among third-instar Melanoplus bivittatus fed Nosema locustae-malathion mixtures and held for 24 hours at 21° C without food in the laboratory.

Treatment N. locustae (No. spores/ grasshopper)	LD_{50} (μg malathion/ grasshopper)	95% Confidence limits
0	1.42	1.30 – 1.55
10^3	1.39	1.28 – 1.51
10^5	1.50	1.36 – 1.66
10^7	1.49	1.35 – 1.64

Mortality obtained between 2 and 24 days post-inoculation of this species was also caused by $\underline{N}$. $\underline{locustae}$ rather than malathion. Mortalities of grasshoppers treated with 10^3 or 10^5 spores, however, did not differ significantly from one another or from the untreated checks ($p \geq .05$). Mortality among grasshoppers inoculated with 10^7 spores was significantly higher than in the other treatments ($p \leq .05$). An analysis of variance did not reveal any interactions between the pathogen and the insecticide. The LD_{50} for $\underline{N}$. $\underline{locustae}$ was calculated to be 3.2 X 10^6 spores per grasshopper which was about 20 times greater than that recorded for the non-diapause strain of $\underline{M}$. $\underline{sanguinipes}$. Regressions based on the cumulative percent mortality (Fig. 13) show that the effect caused by $\underline{N}$. $\underline{locustae}$ in this species was less pronounced than in $\underline{M}$. $\underline{sanguinipes}$.

The incidence of infection among surviving $\underline{M}$. $\underline{bivittatus}$ was considerably higher than for $\underline{M}$. $\underline{sanguinipes}$. Inoculation with 10^3 spores produced infections in survivors ranging from 46.9 to 73.3 percent and all grasshoppers inoculated with 10^5 or 10^7 spores were infected. Increased doses of malathion apparently had no effect on reducing subsequent infections. Analysis of the dosage-infection data for grasshoppers treated with $\underline{N}$. $\underline{locustae}$ showed that infections were dosage responsive and that differences between infection rates were significant ($p \leq .05$).

Fig. 13.--Regression of mortality vs time among _Melanoplus bivittatus_
fed mixtures of malathion at rates of 0.0 μg (A), 1.0 μg
(B), 1.5 μg (C), and 2.0 μg (D) and _Nosema locustae_ at rates
of 10^5 spores (broken line) and 10^7 spores (solid line) per
third-instar grasshopper in the laboratory.

Table 11 shows that the infectivity of $\underline{N}$. locustae was not reduced

after 48 hours in solutions of from 0.02 to 2.0 gm/1 malathion. Also

mortality of grasshoppers infected with $\underline{N}$. locustae was not signifi-

cantly different from the untreated checks nor was mortality different

between the 1 and 48 hours storage treatments ($p \geq .05$). Examination

revealed that all surviving grasshoppers inoculated with $\underline{N}$. locustae

were infected at 24 days post-inoculation.

Integration of Nosema locustae with insecticides: N. locustae with

carbaryl using Melanoplus sanguinipes

Table 12 presents results for *per os* treatments of Nosema locustae,

carbaryl, and $\underline{N}$. locustae-carbaryl mixtures in the non-diapause strain

of Melanoplus sanguinipes. As with malathion, the mortality evident

at 1-day post-inoculation was caused primarily by the carbaryl.

Analysis of the data revealed a dosage-response with significant

differences between doses ($p \leq .05$). The calculated LD_{50} for carbaryl

was 0.47 µg per third-instar and combination with $\underline{N}$. locustae did not

significantly change this value (Table 13). No interaction between the

pathogen and the insecticide was evident, indicating that the two agents

acted independently and that their effects were additive.

Also as with malathion, the mortality observed between 2 and 24

days post-inoculation was attributable to $\underline{N}$. locustae and not to

carbaryl. Mortality among grasshoppers treated with 10^3 spores did not

differ significantly from that of the untreated checks ($p \geq .05$) but

Table 11. Percent mortality and infection among surviving _Melanoplus bivittatus_ fed _Nosema locustae_ (10^5 spores/third-instar) which had been previously stored in solutions of malathion for 1 and 48 hours and examined 24 days post-inoculation in the laboratory.

Treatment (Solution/ N. locustae)	% Mortality No. of hours spores stored			% Infected survivors No. of hours spores stored		
	1	48	1 and 48 avg.	1	48	1 and 48 avg.
Check	50.0	50.0	50.0	0.0	0.0	0.0
Water	58.3	48.3	53.3	100.0	100.0	100.0
Acetone	61.7	56.7	59.2	100.0	100.0	100.0
0.02 gm/l malathion	61.7	56.7	59.2	100.0	100.0	100.0
0.20 gm/l malathion	55.0	38.3	46.7	100.0	100.0	100.0
2.00 gm/l malathion	46.7	51.7	49.1	100.0	100.0	100.0

Table 12. Percent mortality and infection among surviving non-diapause *Melanoplus sanguinipes* fed *Nosema locustae*, carbaryl, and *N. locustae*-carbaryl mixtures as third-instar and examined 1 and 24 days post-inoculation in the laboratory.

| Treatment | | % Mortality | | % Infected survivors |
N. locustae (No. spores/ grasshopper)	carbaryl (μg/ grasshopper)	1 day post-inoculation	24 days post-inoculation	24 days post-inoculation
0	0.0	0.0	17.5	0.0
10^3	0.0	0.0	17.5	24.2
10^5	0.0	0.0	43.8	51.1
10^7	0.0	0.0	98.8	0.0
0	0.37	35.0	63.8	0.0
10^3	0.37	28.8	63.8	13.8
10^5	0.37	30.0	73.8	38.1
10^7	0.37	27.5	98.8	0.0
0	0.55	61.3	83.8	0.0
10^3	0.55	70.0	80.0	12.5
10^5	0.55	65.0	87.5	30.0
10^7	0.55	58.8	100.0	–
0	0.74	75.0	81.3	0.0
10^3	0.74	82.5	86.3	27.3
10^5	0.74	72.5	91.3	0.0
10^7	0.74	77.5	100.0	–

Table 13. Oral toxicity of carbaryl among third-instar non-diapause _Melanoplus sanguinipes_ fed _Nosema locustae_-carbaryl mixtures and held for 24 hours at 21° C without food in the laboratory.

Treatment N. locustae (No. spores/ grasshopper)	LD$_{50}$ (µg carbaryl/ grasshopper)	95% Confidence limits
0	0.47	0.42 - 0.53
10^3	0.46	0.43 - 0.50
10^5	0.48	0.44 - 0.54
10^7	0.50	0.46 - 0.54

mortality among grasshoppers inoculated with 10^5 or 10^7 spores did

differ significantly from both the untreated checks and from one

another ($p \leq .05$). Interactions between the pathogen and insecticide

were not evident and the LD_{50} value for the test was 2.3×10^5 spores

per grasshopper. Regressions based on the cumulative percent mortality

are presented in Fig. 14 and are similar to those presented in Fig. 12

for malathion in this species.

Examination of survivors revealed that the percentage of infection

after inoculation with 10^3 or 10^5 spores was not affected by simulta-

neous treatment with carbaryl, nor did increasing doses of carbaryl

reduce subsequent infection. Although infection rates for the 10^5 dose

appeared higher than for the 10^3 dose in all but the highest carbaryl

treatment, these differences between the two doses were not significant

($p \geq .05$).

Table 14 shows that the infectivity of N. locustae was not reduced

after 48 hours in solutions of from 0.02 to 2.0 gm/l carbaryl. Also

mortality of grasshoppers infected with N. locustae was found to be

significantly higher than the untreated checks ($p \leq .05$) but mortality

was not different between the 1- and 48-hour storage treatments ($p \geq .05$).

Examination revealed that from 25.0 to 56.7 percent of the surviving

grasshoppers inoculated with N. locustae were infected at 24 days post-

inoculation; however, the differences in the incidence of infection

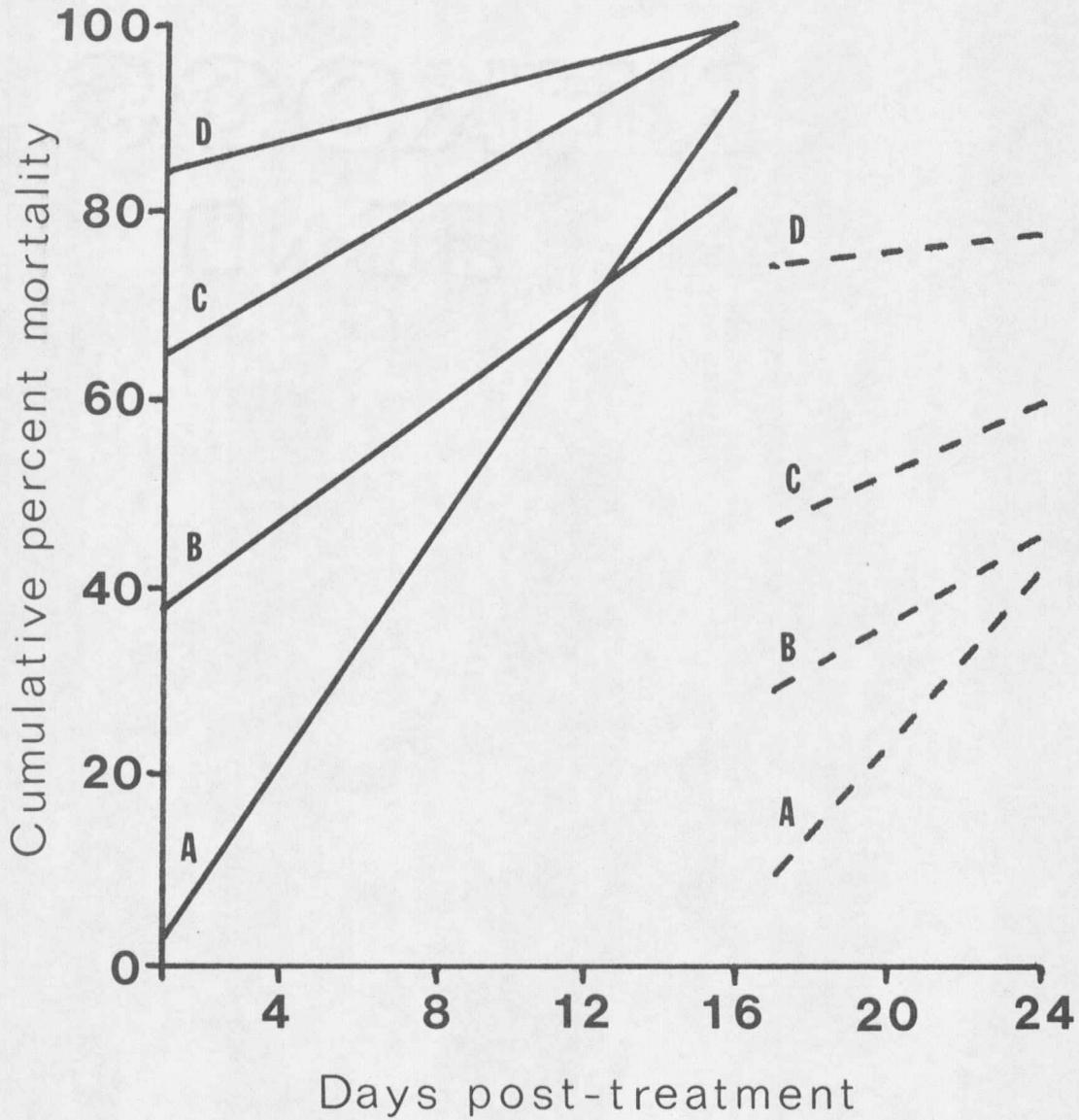between treatments of the 1- and 48-hour in carbaryl were not

Fig. 14.--Regression of mortality vs time among non-diapause <u>Melanoplus</u>
<u>sanguinipes</u> fed mixtures of carbaryl at rates of 0.0 μg (A),
0.37 μg (B), 0.55 μg (C), and 0.74 μg (D) and <u>Nosema</u> <u>locustae</u>
at rates of 10^5 spores (broken line) and 10^7 spores (solid
line) per third-instar grasshopper in the laboratory.

Table 14. Percent mortality and infection among surviving non-diapause <u>Melanoplus sanguinipes</u> fed <u>Nosema locustae</u> (10^5 spores/third-instar) which had been previously stored in solutions of carbaryl for 1 and 48 hours and examined 24 days post-inoculation in the laboratory.

Treatment (Solution/ N. locustae)	% Mortality No. of hours spores stored			% Infected survivors No. of hours spores stored		
	1	48	1 and 48 avg.	1	48	1 and 48 avg.
Check	25.0	25.0	25.0	0.0	0.0	0.0
Water	62.5	56.3	59.4	30.0	37.1	33.8
0.02 gm/1 carbaryl	55.0	52.5	53.8	25.0	50.0	37.8
0.20 gm/1 carbaryl	48.8	52.5	50.6	36.6	31.6	34.2
2.00 gm/1 carbaryl	47.5	62.5	55.0	35.7	56.7	44.4

significant ($p \geq .05$).

Integration of Nosema locustae with microbials: N. locustae with GIBV
    using Melanoplus sanguinipes

Table 15 and Fig. 15 present results for *per os* inoculations of

Nosema locustae, GIBV, and N. locustae-GIBV mixtures in the non-

diapause strain of Melanoplus sanguinipes. Since mortality did not

occur within 24 hours after inoculation as in the tests with insecti-

cides, the table includes data at 7 and 24 days post-inoculation. The

highest dose of GIBV (10^6 inclusions/third-instar) resulted in

significantly higher mortalities 7 days after inoculation than either

the control or the low dose ($p \leq .05$). The LD_{50} for GIBV at 7 days post-

inoculation was 1.17 X 10^6 inclusions per third-instar which in

combination with N. locustae did not change significantly (Table 16).

Mortality due to N. locustae was evident at 24 days post-inoculation

and the LD_{50} was calculated to be 2.4 X 10^5 spores per grasshopper.

More than 50 percent mortality occurred among grasshoppers inoculated

with 10^5 spores, which was significantly greater than the mortality

among grasshoppers inoculated with 10^3 spores or the untreated grass-

hoppers ($p \leq .05$). Interaction between N. locustae and the virus was not

significant, indicating that they acted independently with additive

effects.

Examination revealed that the percentage of infection by N.

locustae in surviving grasshoppers was not reduced by simultaneous

Table 15. Percent mortality and infection among surviving non-diapause <u>Melanoplus</u> <u>sanguinipes</u> fed <u>Nosema</u> <u>locustae</u>, GIBV, and <u>N</u>. <u>locustae</u>-GIBV mixtures as third-instar and examined 7 and 24 days post-inoculation in the laboratory.

| Treatment | | % Mortality | | % Infected survivors 24 days post-inoculation | |
<u>N. locustae</u> (No. spores/ grasshopper)	GIBV (Inclusions/ grasshopper)	7 days post-inoculation	24 days post-inoculation	<u>N. locustae</u> infection	GIBV infection
0	0	1.3	15.0	0.0	0.0
10^3	0	2.5	30.0	26.8	0.0
10^5	0	3.8	52.5	42.1	0.0
0	10^4	8.8	35.0	0.0	44.2
10^3	10^4	3.8	40.0	33.3	41.7
10^5	10^4	5.0	61.3	45.2	48.4
0	10^6	48.8	86.3	0.0	45.5
10^3	10^6	55.0	73.8	42.9	42.9
10^5	10^6	52.5	92.5	50.0	50.0

Fig. 15.—Regression of mortality vs time among non-diapause *Melanoplus sanguinipes* fed mixtures of GIBV at rates of 0 (A), 10^4 (B), and 10^6 (C) inclusions and *Nosema locustae* at a rate of 10^5 spores per third-instar grasshopper in the laboratory.

Table 16. Oral toxicity of GIBV among non-diapause _Melanoplus sanguinipes_ 7 days after third-instars were inoculated with _Nosema locustae_-GIBV mixtures in the laboratory.

Treatment N. locustae (No. spores/ grasshopper)	LD$_{50}$ (No. inclusions/ grasshopper)	95% Confidence limits
0	1.17 X 10^6	0.45 - 3.02 X 10^6
10^3	0.83 X 10^6	0.49 - 1.40 X 10^6
10^5	0.97 X 10^6	0.55 - 1.69 X 10^6

treatment with GIBV. The data indicated slightly, but not significantly (p≥.05), increased infection by N. locustae when inoculated simulta- neously with the virus. An analysis of the dosage-infection data showed that differences in percent infection between the doses were not significant for either pathogen (p≥.05). Many of the grasshoppers were infected with both pathogens.

Field Tests

Dosage response of Nosema locustae: Plots at Big Timber, 1976

The density of all grasshoppers 4 and 6 weeks after application of bran treated with Nosema locustae is presented in Table 17. Densities varied between 4.2 and 9.7 grasshoppers per m^2 for the two sampling periods and increased significantly during the two weeks (p≤.05). The percentage increase in density, however, was found to be less on those plots treated with N. locustae and this difference was attributed to the effects of the pathogen on the population. The average of the two samples shows that those plots treated with the highest dose of spores (4.0×10^{10}/ha) exhibited the lowest densities ($5.2/m^2$). When compared to the check plots, the treated plots showed between 0 and 33 percent reduction in the number of grasshoppers at 6 weeks after application. Due to the variability between replications, however, only the densities in plots treated with the highest and lowest doses of N. locustae differed significantly from one another (p≤.05).

Table 17. Density of grasshoppers in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with Nosema locustae.[a]

Treatment (No. spores/ha)	4 week No./m^2	6 week No./m^2	4 and 6 week avg. No./m^2
0	5.0	9.0	6.9
6.2 X 10^8	6.7	9.7	8.2
2.5 X 10^9	4.2	7.5	5.8
1.0 X 10^{10}	6.7	6.0	6.3
4.0 X 10^{10}	4.5	6.0	5.2

[a]Average based on 4 replications.

Examination of survivors collected from the treated plots at 4 and
6 weeks revealed that the incidence of infection for all species of
grasshoppers (Table 18) and for _Melanoplus sanguinipes_ (Table 19)
increased as the season progressed. Proportionally the largest
increases in infection were noted for the lowest rates of application
(6.2 X 10^8/ha) although the highest dose (4.0 X 10^{10}/ha) increased from
14.9 to 21.1 percent for all species and from 22.2 to 31.6 percent for
M. sanguinipes. All plots treated with _N. locustae_ were significantly
different from the controls (p$\leq$.05) and the low incidence of infection
observed among grasshoppers from the control plots showed that migration
of grasshoppers was minimal. The lowest dose (6.2 X 10^8/ha) was
significantly different (p$\leq$.05) from the other three doses although
these higher doses were not separable statistically (p$\geq$.05). Regres-
sions based on the percent infection among all species and among _M._
sanguinipes alone showed a dosage-response at 4 and 6 weeks (Fig. 16).

Dosage response of _Nosema locustae_: Plots at Forsyth, 1978
_____

Although the same treatments were applied in 1978, only a 6-week
post-application sample was taken. Applications were made when the
grasshoppers were in a later stage of development than in the previous
field test and therefore the effects of _Nosema locustae_ were less
prominent. Only 12.1 percent of the _Melanoplus sanguinipes_ were third-
instar at the time of application, while 28.6, 50.5, and 8.8 percent

Table 18. Incidence of infection among grasshoppers in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with <u>Nosema locustae</u>.[a]

Treatment (No. spores/ha)	4 week		6 week		4 and 6 week	
	No. examined	% infection	No. examined	% infection	No. examined	% infection
0	360	0.6	241	0.0	601	0.3
6.2×10^8	321	3.1	308	6.8	629	4.9
2.5×10^9	286	7.3	268	15.7	554	11.4
1.0×10^{10}	250	9.2	205	16.1	455	12.3
4.0×10^{10}	377	14.9	322	21.1	699	17.7

[a]Average based on 4 replications.

Table 19. Incidence of infection among <u>Melanoplus</u> <u>sanguinipes</u> in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with <u>Nosema</u> <u>locustae</u>.[a]

Treatment (No. spores/ha)	4 week		6 week		4 and 6 week	
	No. examined	% infection	No. examined	% infection	No. examined	% infection
0	142	0.0	71	0.0	213	0.0
6.2×10^8	131	3.8	110	10.9	241	7.1
2.5×10^9	84	10.7	88	22.7	172	16.9
1.0×10^{10}	114	14.0	83	27.7	197	19.8
4.0×10^{10}	144	22.2	133	31.6	277	26.7

[a]Average based on 4 replications.

Fig. 16.--Regressions based on percent infection among surviving grass-
hoppers collected from plots at Big Timber (1976) 4 and 6
weeks post-application of bran treated with Nosema locustae
at 4 doses. (A) Melanoplus sanguinipes, (B) all species.

were in the fourth, fifth, and adult stages of development respectively.

Table 20 shows the density of all grasshoppers 6 weeks post-application

of bran treated with $\underline{N}$. $\underline{locustae}$. Densities ranged from 6.8 to 10.1

grasshoppers per m^2 with the highest numbers occurring on the check

plots. Although between 11 and 32 percent fewer grasshoppers could be

found in the plots treated with $\underline{N}$. $\underline{locustae}$ 6 weeks after application,

variability between plots masked any significant differences between

dosages.

The incidence of infection among survivors for all species of

grasshoppers and for $\underline{M}$. $\underline{sanguinipes}$ is presented in Table 21. A

smaller percentage of grasshoppers was found to be infected in these

tests compared to those at Big Timber and this was attributed primarily

to the differences in the stage of development of the grasshoppers when

applications were made. The highest dose of $\underline{N}$. $\underline{locustae}$ (4.0×10^{10}/

ha) produced infections in only 6.7 and 17.4 percent of all species and

$\underline{M}$. $\underline{sanguinipes}$ respectively. Although the differences in infection

rates between treatments were not significantly different ($p \geq .05$),

regressions based on the percent infection among all species and among

$\underline{M}$. $\underline{sanguinipes}$ alone showed a dosage response at 6 weeks (Fig. 17).

This indicated, as in the previous field test, that the incidence of

infection by $\underline{N}$. $\underline{locustae}$ increased linearly with the $\log_{10}$ of the

initial dose applied.

Table 20. Density of grasshoppers in plots at Forsyth (1978) 6 weeks
post-application of bran treated with <u>Nosema</u> <u>locustae</u>.[a]

Treatment (No. spores/ha)	6 week $\frac{}{No./m^2}$
0	10.1
6.2 X 10^8	6.8
2.5 X 10^9	7.1
1.0 X 10^{10}	6.9
4.0 X 10^{10}	9.0

[a]Average based on 4 replications.

Table 21. Incidence of infection among grasshoppers in plots at Forsyth (1978) 6 weeks post-application of bran treated with <u>Nosema locustae</u>.[a]

Treatment (No. spores/ha)	All species		Melanoplus sanguinipes	
	No. examined	% infection	No. examined	% infection
0	247	0.0	63	0.0
6.2×10^8	313	1.0	67	3.0
2.5×10^9	340	3.2	78	9.0
1.0×10^{10}	454	5.9	123	16.3
4.0×10^{10}	315	6.7	86	17.4

[a]Average based on 4 replications.

Fig. 17.--Regressions based on percent infection among surviving
grasshoppers collected from plots at Forsyth (1978) 6 weeks
post-application of bran treated with Nosema locustae at 4
doses. (A) Melanoplus sanguinipes, (B) all species.

Response of <u>Nosema</u> <u>locustae</u> to ULV malathion sprays: Plots at Big

Timber, 1976

The density of all grasshoppers 4 and 6 weeks after application of

bran treated with <u>Nosema</u> <u>locustae</u>, and 40 days after bran application,

sprayed with ULV malathion, is presented in Table 22. Densities varied

between 5.2 to 8.9 grasshoppers per m^2 at the 4-week sample and

increased during the next two weeks on the check plots. Density

increased slightly during this time in plots receiving the pathogen

alone but declined in all plots treated with insecticide. Reduction in

density from the 4- to 6-week sample due to malathion was approximately

30 percent for the light doses (73-146 ml/ha), 60 percent for the

moderate dose (292 ml/ha) and 98 percent for the standard dose (585ml/

ha). Although <u>N</u>. <u>locustae</u> appeared to contribute to the decline in

grasshoppers during the two weeks, no effect of the pathogen on the

toxicity of malathion could be demonstrated on those plots treated

with both agents.

As shown in Table 23, grasshoppers of the subfamily Cyrtacanth-

acridinae, so-called spur-throated grasshoppers, were most abundant and

exhibited the highest incidence of infection. The Acridinae, slant-

faced grasshoppers, were next most abundant but had the lowest incidence

of infection and the Oedipodinae, banded-wing grasshoppers, were

least common and had intermediate incidence of infection.

Table 22. Density of grasshoppers in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with <u>Nosema locustae</u>. ULV malathion was applied 40 days after bran application.[a]

| Treatment | | 4 week | 6 week | 4 and 6 week avg. |
N. locustae (No. spores/ha)	ULV malathion (mls/ha)	No./m²	No./m²	No./m²
0	0	5.6	8.6	7.1
	73	5.2	4.7	5.0
	146	6.5	4.6	5.6
	292	6.5	3.5	5.0
	Avg. 170	6.1	4.3	5.2
	585	6.9	0.1	3.5
1.2×10^{10}	0	5.3	5.4	5.3
	73	8.9	5.3	7.1
	146	8.4	6.4	7.4
	292	6.8	1.6	4.2
	Avg. 170	8.0	4.4	6.2
	585	6.5	0.1	3.3

[a] Average based on 4 replications.

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Table 23. Relative frequency and incidence of infection of grass-
hoppers in plots at Big Timber (1976) 4 and 6 weeks post-
application of bran treated with Nosema locustae. ULV
malathion was applied 40 days after application of bran.[a]

	4 week		6 week	
Subfamily and species	% relative frequency	% infection	% relative frequency	% infection
Cyrtacanthacridinae	73.5	4.7	70.7	25.7
Melanoplus bivittatus	9.0	5.0	9.7	21.7
Melanoplus confusus	3.1	2.4	0.0	0.0
Melanoplus dawsoni	6.3	2.4	6.1	31.6
Melanoplus femurrubrum	0.0	0.0	0.2	0.0
Melanoplus infantilis	4.9	7.6	3.6	31.8
Melanoplus packardii	7.2	1.0	7.3	15.6
Melanoplus sanguinipes	42.1	5.5	43.6	27.0
Phoetaliotes nebrascensis	0.6	0.0	0.3	0.3
Acridinae	21.6	1.3	26.0	3.1
Aeropedellus clavatus	1.3	0.0	0.0	0.0
Ageneotettix deorum	6.9	0.0	10.7	3.0
Amphitornus coloradus	4.8	1.5	5.8	2.8
Aulocara elliotti	7.5	3.0	7.1	2.3
Hesperotettix viridis	1.0	0.0	2.4	6.7
Mermeria maculipennis	0.1	0.0	0.0	0.0
Psoloessa delicatula	0.1	0.0	0.0	0.0
Oedipodinae	4.8	0.0	3.2	5.0
Arphia pseudonietana	0.4	0.0	0.5	0.0
Cratypedes neglectus	0.1	0.0	0.2	0.0
Encoptolophus sordidus	0.4	0.0	0.6	0.0
Metator pardalinus	1.7	0.0	0.8	0.0
Spharagemon collare	0.1	0.0	0.2	100.0
Trachyrhachis kiowa	1.9	0.0	1.0	0.0
Trimerotropis campestris	0.1	0.0	0.0	0.0
Xanthippus corallipes	0.1	0.0	0.0	0.0

[a]Average based on 4 replications.

Examination of survivors collected from the plots at 4 and 6 weeks revealed that the incidence of infection increased as the season progressed (Table 24). Plots treated with N. locustae alone increased from 6.2 to 28.1 percent while plots treated with N. locustae and later sprayed with an average of 170 ml/ha malathion increased from 3.3 to 19.0 percent. Although the percent infection at 6 weeks was lower on those plots treated with insecticide, this reduced incidence of infection was also noted at 4 weeks and therefore was not attributable to differential selection of diseased grasshoppers by the insecticide treatment. The data presented in Table 25 for M. sanguinipes also shows that the incidence of infection in this species did not change as a result of insecticide treatment (both 0 ml/ha and avg. 170 ml/ha malathion plots exhibited nearly 27 percent infection at 6 weeks). Although changes in the relative frequency occurred for this species from the 4- to 6-week sample, treatment effects could not be demonstrated due to the variability between replications.

Integration of Nosema locustae with malathion: Plots at White Sulphur
        Springs, 1977

The densities of all grasshoppers 5, 30, and 48 days after application of bran treated with Nosema locustae, malathion, and N. locustae-malathion mixtures are presented in Table 26. Densities initially were low (between 2.4 to 2.6 grasshoppers per m^2 in the check plots) but then increased significantly in all treatments between 5 and 48 days

Table 24. Incidence of infection among grasshoppers in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with Nosema locustae. ULV malathion was applied 40 days after application of bran.[a]

| Treatment | | 4 week | | 6 week | | 4 and 6 week | |
N. locustae (No. spores/ha)	ULV malathion (mls/ha)	No. examined	% infection	No. examined	% infection	No. examined	% infection
0	0	384	0.3	82	1.2	466	0.4
	73	104	0.0	77	1.3	181	0.6
	146	173	0.6	40	0.0	213	0.5
	292	284	0.0	133	0.0	417	0.0
Avg.	170	561	0.2	250	0.4	811	0.2
	585	183	1.6	5	0.0	188	1.6
1.2×10^{10}	0	439	6.2	185	28.1	624	12.7
	73	387	4.4	209	18.7	596	9.4
	146	512	2.3	213	23.0	725	8.4
	292	609	3.4	198	15.2	807	6.3
Avg.	170	1508	3.3	620	19.0	2128	7.9
	585	347	6.6	7	0.0	354	6.5

[a]Average based on 4 replications.

Table 25. Relative frequency and incidence of infection among <u>Melanoplus sanguinipes</u> in plots at Big Timber (1976) 4 and 6 weeks post-application of bran treated with <u>Nosema locustae</u>. ULV malathion was applied 40 days after application of bran.[a]

N. locustae (No. spores/ha)	ULV malathion (mls/ha)	4 week		6 week		4 and 6 week	
		% relative frequency	% infection	% relative frequency	% infection	% relative frequency	% infection
0	0	34.6	0.0	24.4	0.0	32.8	0.0
	73	18.3	0.0	31.2	0.0	23.8	0.0
	146	45.7	0.0	42.5	0.0	45.1	0.0
	292	52.5	0.0	38.8	0.0	46.5	0.0
	Avg. 170	44.0	0.0	34.4	0.0	41.1	0.0
1.2×10^{10}	0	46.9	8.7	40.0	27.0	44.9	13.6
	73	43.9	7.1	57.9	21.5	48.8	13.1
	146	35.9	4.3	45.1	27.1	38.6	12.1
	292	34.5	5.2	26.3	40.4	32.5	12.2
	Avg. 170	37.4	5.5	43.4	27.1	39.1	12.5

[a]Average based on 4 replications.

Table 26. Density of grasshoppers in plots at White Sulphur Springs (1977) 5, 30, and 48 days post-application of bran treated with Nosema locustae, malathion and N. locustae-malathion mixtures.[a]

| Treatment | | Days post-application | | | Avg. post-application No./m² | |
N. locustae (No. spores/ha)	% malathion (wt. tech/ wt. bran)	5 No./m²	30 No./m²	48 No./m²	malathion only	malathion+ N. locustae
0	0.00	2.6	3.1	3.7	3.1	
7.4 X 10⁹	0.00	2.4	2.6	3.7		2.9
0	0.03	2.2	2.4	3.6	2.7	
7.4 X 10⁹	0.03	2.3	3.2	3.8		3.1
0	0.09	1.4	2.1	2.6	2.1	
7.4 X 10⁹	0.09	1.9	2.1	2.7		2.2
0	0.37	1.2	1.1	2.4	1.6	
7.4 X 10⁹	0.37	1.1	1.1	2.7		1.7

[a]Average based on 4 replications.

post-treatment ($p \leq .05$). At 5 days post-application, the lowest

densities were recorded in those plots treated with either 0.37 percent

malathion or 7.4×10^9 spores/ha + 0.37 percent malathion. Analysis

showed that the densities of grasshoppers were significantly lower in

these plots than in either the check or in plots treated with $\underline{N}$.

$\underline{locustae}$ ($p \leq .05$). The calculated control was 51 percent for the 0.37

malathion formulation and 56 percent for the 7.4×10^9 spores/ha + 0.37

percent malathion formulation. The same relationship was observed 48

days post-application although higher densities were recorded at this

time. Only between 27 to 35 percent control could be demonstrated in

those plots which had received the highest dose of malathion.

Bran treated with 0.03 or 0.09 percent malathion also caused

reduction in densities 5 days after application, but the amount of

control was not as great. For example, treatments of 0.03 to 0.09

percent malathion resulted in 10 to 44 percent control with signifi-

cant differences evident only between densities in plots treated with

0.09 percent malathion and those in check plots ($p \leq .05$). At 48 days

post-application, control dropped to 2 to 30 percent in the plots

treated with the lower doses of malathion.

As shown in Table 27, grasshoppers of the subfamily Cyrtacanth-

acridinae were most abundant and exhibited the highest incidence of

infection by $\underline{N}$. $\underline{locustae}$. The Acridinae were next most abundant but

had the lowest incidence of infection and the Oedipodinae were least

Table 27. Relative frequency and incidence of infection among grass-
hoppers in plots at White Sulphur Springs (1977) at 30 and
48 days post-application of bran treated with Nosema
locustae or N. locustae-malathion mixtures.[a]

Subfamily and species	30 days		48 days	
	% relative frequency	% infection	% relative frequency	% infection
Cyrtacanthacridinae	79.2	8.0	75.7	20.8
Melanoplus dawsoni	2.4	6.9	2.8	15.6
Melanoplus gladstoni	36.5	2.2	44.4	14.3
Melanoplus infantilis	36.1	14.0	25.0	33.4
Melanoplus packardii	1.5	5.3	1.6	11.1
Melanoplus sanguinipes	2.7	9.1	2.0	21.7
Acridinae	17.5	0.5	20.1	1.3
Aeropedellus clavatus	0.8	0.0	0.0	0.0
Ageneotettix deorum	3.1	0.0	2.8	3.1
Amphitornus coloradus	1.1	7.7	1.5	0.0
Aulocara elliotti	0.2	0.0	0.1	0.0
Bruneria brunnea	12.3	0.0	15.8	1.1
Oedipodinae	3.3	0.0	4.2	8.3
Acrolophitus hirtipes	0.2	0.0	0.2	0.0
Arphia psuedonietana	0.2	0.0	1.5	5.9
Cratypedes neglectus	0.3	0.0	0.0	0.0
Metator pardalinus	0.8	0.0	0.3	25.0
Spharagemon equale	0.1	0.0	0.0	0.0
Trachyrhachis kiowa	0.2	0.0	0.0	0.0
Trimerotropis campestris	1.5	0.0	1.9	9.1
Xanthippus corallipes	0.0	-0.0	0.3	0.0

[a]Average based on 4 replications.

common and had intermediate incidence of infection. The change in the
relative frequency of the Cyrtacanthacridinae from 79.2 to 75.7 per-
cent during the two weeks may have been caused by N. locustae. In
particular, two species of this subfamily, Melanoplus infantilis and
Melanoplus sanguinipes, decreased in relative frequency and also
exhibited the highest incidence of infection. Due to its abundance and
high incidence of infection, M. infantilis was used as an indicator of
differences between treatments.

Significant reductions in the density of all grasshoppers in plots
treated with only N. locustae as compared to the check plots could not
be demonstrated for any of the post-application sampling periods ($p \geq .05$).
The calculated percent control was 7 percent at 5 days after treatment,
18 percent at 30 days after treatment, and 4 percent at 48 days after
treatment. Examination of the species composition data, however,
revealed that changes in the relative frequency of M. infantilis
occurred in those plots treated with only N. locustae. At 48 days
post-application, 20.7 percent of the grasshoppers in plots treated
with the pathogen were M. infantilis, while this species comprised
33.6 percent of the population in the check plots (Table 28).

The contribution of N. locustae in reducing densities of grass-
hoppers in plots treated with mixtures of the pathogen and insecticide
was difficult to assess. A greater reduction in the relative frequency
of M. infantilis during the 30-day to the 48-day sampling periods in

Table 28. Relative frequency and incidence of infection among <u>Melanoplus infantilis</u> in plots at White Sulphur Springs (1977) 30 and 48 days post-application of bran treated with <u>Nosema locustae</u>, malathion and <u>N. locustae</u>-malathion mixtures.[a]

| Treatment | | 30 days | | 48 days | | 30 and 48 days % relative frequency | | |
N. locustae (No. spores/ ha)	% malathion (wt. tech/ wt. bran)	% relative frequency	% infection	% relative frequency	% infection	malathion only	malathion+ N. locustae	% infection
0	0.00	46.6	0.0	33.6	0.0	40.2		0.0
7.4 X 10^9	0.00	37.1	18.8	20.7	48.3		29.8	28.0
0	0.03	43.2	0.0	34.4	1.1	38.8		
7.4 X 10^9	0.03	34.3	14.4	26.7	32.1		30.8	21.4
0	0.09	33.1	0.0	17.7	0.0	26.0		
7.4 X 10^9	0.09	38.6	17.5	27.7	37.9		33.5	25.4
0	0.37	30.1	0.0	32.6	0.0	31.4		
7.4 X 10^9	0.37	34.4	3.2	25.1	21.2		29.2	11.8

[a]Average based on 4 replications.

those plots treated with the mixed formulations as compared to those plots receiving only malathion suggests, however, that N. locustae continued to cause mortality among the grasshoppers.

Examination of survivors collected 30 and 48 days after application revealed that the incidence of infection for all species of grasshoppers (Table 29) and for M. infantilis (Table 28) increased significantly as the season progressed ($p \leq .05$). The low incidence of infection observed among grasshoppers in the plots treated with only malathion and in the check plots indicated that virtually no grasshopper migration occurred during the test. The highest rates of infection were noted among grasshoppers from those plots treated with only N. locustae. Within 48 days of treatment, 20.0 percent of all grasshoppers and 48.3 percent of the M. infantilis showed infection. The incidence of infection was lower among the grasshoppers from plots treated with a mixture of 7.4 X 10^9 spores/ha + 0.37 percent malathion. For example, only 10.3 percent of all grasshoppers and 21.2 percent of the M. infantilis were infected. These data, presented graphically in Fig. 18, show that mixtures of N. locustae with 0.37 percent malathion resulted in significantly reduced rates of infection during the season as compared to those formulations of the pathogen alone ($p \leq .05$). The mixed formulations of N. locustae with 0.03 and 0.09 percent malathion were also found to have reduced rates of infection although significant differences were not evident between these treatments and the

Table 29. Incidence of infection among grasshoppers in plots at White Sulphur Springs (1977) 30 and 48 days post-application of bran treated with Nosema locustae, malathion and N. locustae-malathion mixtures.[a]

| Treatment | | 30 days | | 48 days | | 30 and 48 days | |
N. locustae (No. spores/ha)	% malathion (wt. tech/ wt. bran)	No. examined	% infection	No. examined	% infection	No. examined	% infection
0	0.00	238	0.0	232	0.9	470	0.4
7.4×10^9	0.00	345	8.1	280	20.0	625	13.4
0	0.03	264	0.0	256	2.0	520	1.0
7.4×10^9	0.03	344	6.7	292	19.9	636	12.7
0	0.09	284	0.0	243	0.0	527	0.0
7.4×10^9	0.09	267	9.0	238	17.2	505	12.9
0	0.37	209	0.0	215	0.9	424	0.5
7.4×10^9	0.37	270	2.6	339	10.3	609	6.9

[a]Average based on 4 replications.

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Fig. 18.--Incidence of infection of <u>Nosema locustae</u> among grasshoppers in plots at White Sulphur Springs (1977) 30 and 48 days post-application of bran treated with 7.4 X 10^9 spores/ha and 7.4 X 10^9 spores/ha + 0.37% malathion. (A) all species, (M) <u>Melanopous infantilis</u>.

treatment of N. locustae alone (p≥.05).

Integration of Nosema locustae with malathion: Plots at Forsyth, 1978

The densities of all grasshoppers before treatment and 3 days, 4 weeks and 6 weeks after application of bran treated with Nosema locustae, malathion, and N. locustae-malathion mixtures are presented in Table 30. As evident, the densities varied from 5.9 to 13.4 grasshoppers per m^2 before treatment. A slight increase in the density was noted for the 3-day post-application sample in the check and 4.9×10^9 spores/ha plots although these increases were not significant (p≥.05). At 3 days post-application, significantly lower densities occurred in plots treated with 4.9×10^9 spores/ha + 0.39 percent malathion as compared to densities in plots treated without malathion or with 0.06 percent malathion (p≤.05). The calculated percent control was 28 percent for the highest dose of malathion. Treatments of 0.13 percent malathion resulted in significantly lower densities than in check plots, but differences were not evident between any malathion treatments (p≥.05). The percent control for plots receiving treatments of 0.13 percent malathion resulted in 16 to 24 percent control. No controlling activity was evident at 4 and 6 weeks post-application.

As shown in Table 31, the species complex of grasshoppers was considerably different in these tests from that of the previous year. Grasshoppers of the subfamily Cyrtacanthacridinae were most abundant

Table 30. Density of grasshoppers in plots at Forsyth (1978) prior to application and at 3 days, 4 weeks and 6 weeks post-application of bran treated with <u>Nosema locustae</u>, malathion and <u>N. locustae</u>-malathion mixtures.[a]

| Treatment | | Pre-application | Post-application | | | Avg. post-application |
N. locustae (No. spores/ha)	% malathion (wt. tech/ wt. bran)	No./m²	3 days No./m²	4 week No./m²	6 week No./m²	No./m²
0	0.00	5.9	6.4	6.1	3.4	5.3
4.9 X 10⁹	0.00	7.7	8.1	4.6	2.6	5.1
4.9 X 10⁹	0.06	10.2	9.9	7.6	5.0	7.5
4.9 X 10⁹	0.13	13.4	11.2	8.3	3.9	7.8
4.9 X 10⁹	0.39	11.0	7.9	6.7	4.9	6.5
0	0.13	7.1	5.4	6.4	3.0	4.9

[a]Average based on 4 replications.

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Table 31. Relative frequency and incidence of infection of grass-
hoppers in plots at Forsyth (1978) 4 and 6 weeks post-
application of bran treated with Nosema locustae or N.
locustae-malathion mixtures.

Subfamily and species	4 week		6 week	
	% relative frequency	% infection	% relative frequency	% infection
Cyrtacanthacridinae	45.7	2.5	49.6	6.2
Melanoplus angustipennis	0.9	22.2	0.1	0.0
Melanoplus bowditchi	0.1	0.0	0.0	0.0
Melanoplus confusus	0.3	0.0	0.0	0.0
Melanoplus femurrubrum	3.7	0.0	4.1	0.0
Melanoplus gladstoni	1.9	0.0	1.9	7.1
Melanoplus infantilis	0.1	0.0	0.1	100.0
Melanoplus keeleri	1.0	0.0	0.8	0.0
Melanoplus packardii	2.1	0.0	1.9	0.0
Melanoplus sanguinipes	26.1	2.7	26.2	7.3
Phoetaliotes nebrascensis	9.5	2.1	14.4	1.9
Acridinae	40.3	0.4	29.8	1.5
Ageneotettix deorum	27.4	0.0	19.2	2.1
Amphitornus coloradus	0.5	0.0	0.4	0.0
Aulocara elliotti	0.2	0.0	0.3	0.0
Dactylotum pictum	0.4	0.0	0.4	0.0
Drepanopterna femoratum	2.4	0.0	1.4	0.0
Eritettix simplex	0.4	0.0	0.3	0.0
Hesperotettix viridis	0.2	0.0	0.5	0.0
Hypochlora alba	0.7	0.0	0.5	0.0
Mermiria maculipennis	0.6	0.0	1.1	0.0
Opeia obscura	6.2	0.0	3.8	0.0
Orphulella compta	0.9	0.0	1.4	0.0
Phlibostroma quadrimaculatum	0.4	0.0	0.5	0.0
Oedipodinae	14.0	0.0	20.5	0.7
Arphia pseudonietana	1.6	0.0	3.3	0.0
Encoptolophus sordidus	0.0	0.0	0.5	0.0
Hadrotettix trifaciatus	1.5	0.0	2.2	0.0
Hippiscus rugosus	0.0	0.0	0.1	0.0
Mestobregma plattei	0.1	0.0	1.1	0.0
Metator pardalinus	0.8	0.0	1.0	0.0
Spharagemon collare	0.7	0.0	1.0	14.3
Spharagemon equale	6.9	0.0	9.9	0.0
Trachyrhachis kiowa	2.0	0.0	1.4	0.0
Xanthippus corallipes	0.3	0.0	0.1	0.0

and comprised nearly half of the entire population. The Acridinae were
next most abundant and the Oedipodinae were the least common. As in
the previous test, grasshoppers of the subfamily Cyrtacanthacridinae
showed the highest incidence of infection by $\underline{N}$. locustae although the
variety and abundance of species was altogether different. This fact
together with the differences in developmental stage of the grass-
hoppers at the time of applications between the two tests (the popula-
tion was predominently third-instar in 1977 but fifth-instar in 1978)
combined to produce a lower incidence of infection in 1978. The most
abundant species of the Cyrtacanthacridinae was Melanoplus sanguinipes
and since it was infected with $\underline{N}$. locustae at both the 4- and 6-week
samples, this species was used as an indicator of differences between
treatments. The relative frequency of the Cyrtacanthacridinae
increased from 45.7 to 49.6 percent during the two weeks. Changes in
the relative frequency of the Acridinae from 40.3 to 29.8 percent
during the same period were primarily the result of changes in one
species, Ageneotettix deorum. This species decreased in abundance
from 27.4 to 19.2 percent and since grasshoppers were infected with $\underline{N}$.
locustae, the pathogen may have contributed to this decline.

The first evidence of control by $\underline{N}$. locustae was at 4 weeks post-
application. At that time, reductions of 40 percent were observed in
plots treated with $\underline{N}$. locustae alone whereas the percentage reduction
in plots treated with $\underline{N}$. locustae-insecticide formulations varied from

26 to 39 percent. By 6 weeks post-application the numbers of grass-
hoppers in the check plots had dropped by 42 percent and although the
plots treated with $\underline{N}$. $\underline{locustae}$ either alone or in combination with
malathion averaged 61 percent control, differences were not significant
($p \geq .05$).

Examination of survivors collected 4 and 6 weeks after applica-
tion revealed that the incidence of infection for all species (Table
32) and for $\underline{M}$. $\underline{sanguinipes}$ (Table 33) increased significantly as the
season progressed ($p \leq .05$). Infection by $\underline{N}$. $\underline{locustae}$ was not observed
in grasshoppers in samples collected from the check plots, indicating
that little migration of grasshoppers took place during the test. The
highest infection rates were noted for those plots treated with $\underline{N}$.
$\underline{locustae}$ alone as in the previous test, however, only 14.3 percent of
the $\underline{M}$. $\underline{sanguinipes}$ in these plots showed signs of infection at 6 weeks
post-application. The lowest incidence of infection was observed
among grasshoppers from plots treated with a mixture of 4.9×10^{10}
spores/ha + 0.39 percent malathion. For example, only 1.7 percent of
all grasshoppers and 6.0 percent of the $\underline{M}$. $\underline{sanguinipes}$ were infected
at 6 weeks post-application. Fig. 19 shows the effect of mixed
formulations of $\underline{N}$. $\underline{locustae}$ with 0.39 percent malathion on the
incidence of infection 5 and 6 weeks after treatment. Analysis of
these data revealed that this formulation resulted in significantly
lower infection rates than $\underline{N}$. $\underline{locustae}$ alone ($p \leq .05$). The mixed

Table 32. Incidence of infection among grasshoppers in plots at Forsyth (1978) 4 and 6 weeks post-application of bran treated with Nosema locustae, malathion and N. locustae-malathion mixtures.[a]

| Treatment | | 4 week | | 6 week | | 4 and 6 week | |
N. locustae (No. spores/ha)	% malathion (wt. tech/ wt. bran)	No. examined	% infection	No. examined	% infection	No. examined	% infection
0	0.00	150	0.0	98	0.0	248	0.0
4.9×10^9	0.00	240	0.8	158	3.8	398	2.0
4.9×10^9	0.06	208	1.9	220	4.1	428	3.0
4.9×10^9	0.13	284	1.8	176	2.3	460	2.0
4.9×10^9	0.39	262	0.4	176	1.7	438	0.9
0	0.13	191	0.0	117	0.0	308	0.0

[a]Average based on 4 replications.

Table 33. Incidence of infection among Melanoplus sanguinipes in plots at Forsyth (1978) 4 and 6 weeks post-application of bran treated with Nosema locustae, malathion and N. locustae-malathion mixtures.[a]

N. locustae (No. spores/ha)	Treatment % malathion (wt. tech/ wt. bran)	4 week No. examined	4 week % infection	6 week No. examined	6 week % infection	4 and 6 week No. examined	4 and 6 week % infection
0	0.00	28	0.0	24	0.0	52	0.0
4.9×10^9	0.00	46	4.3	21	14.3	67	7.5
4.9×10^9	0.06	49	2.0	71	7.0	120	5.0
4.9×10^9	0.13	103	2.9	49	6.1	152	3.9
4.9×10^9	0.39	61	1.6	50	6.0	111	3.6
0	0.13	42	0.0	34	0.0	76	0.0

[a]Average based on 4 replications.

Fig. 19.--Incidence of infection of <u>Nosema</u> <u>locustae</u> among grasshoppers in plots at Forsyth (1978) 4 and 6 weeks post-application of bran treated with 4.9 X 10^9 spores/ha and 4.9 X 10^9 spores/ha + 0.39% malathion. (A) all species, (M) <u>Melanoplus</u> <u>sanguinipes</u>.

formulations of $\underline{N}$. $\underline{locustae}$ with 0.09 and 0.13 percent malathion were also found to produce reduced rates of infection in $\underline{M}$. $\underline{sanguinipes}$ although no significant differences could be demonstrated ($p \geq .05$).

DISCUSSION

Although grasshoppers have long been economically important on western rangeland, public interest was not focused on their control until the late forties (Wakeland 1951). Poison baits were most commonly recommended although sprays later replaced their use and considerable effort was spent in the improvement of their effectiveness. The goal of much research during this period was to perfect methods that would result in the greatest numbers of grasshoppers being killed as is evidenced in the following statement by Parker (1952): "Almost complete destruction of grasshoppers by spraying half a gallon of kerosene containing 2 ounces of poison over an acre seems almost incredible, but that is what aldrin does." The availability of many new synthetic organic insecticides led to numerous spectacular pest control successes but at the same time may have increased our dependence on repeated applications of insecticides. Such dependence, in the case of certain other insect pests, has resulted in insect resistance, emergence of secondary pests and the resurgence of pest populations (Glass 1975). Thus, the ecologically-based philosophy of Integrated Pest Management emerged in which the associated environment and the population dynamics of the pest species are considered together and all techniques and methods are utilized to maintain pest populations below levels causing economic injury. In retrospect, it is obvious that the approach used during the past 30 years to control

grasshoppers was deficient from the start since it ignored the fact that grasshoppers are part of a life system. The present studies showed the potential of using Nosema locustae either by itself or in combination with insecticides or virus in grasshopper management programs.

Three criteria of microbial agents are listed by Steinhaus (1954) as important for their success in causing natural epizootics. These are the ability to (1) invade and infect the host, (2) survive in the environment, and (3) spread from one insect to another. McLaughlin (1973) suggested that the characteristics possessed by protozoa, for example, their longer period of time required to kill a host after infection, would allow for spread by the host and result in a continuous source of inoculum. Henry (1978) also considered these attributes important in selecting an agent for applied use and, in fact, based the development of the applied use of N. locustae on these principles. He reported that N. locustae is one of the least virulent pathogens of grasshoppers, including fungi, viruses and other pathogens, and that this characteristic makes this pathogen especially useful for long-term control of grasshoppers (Henry, 1970). The symptoms shown by grasshoppers heavily infected by N. locustae in these studies were similar to those reported by Canning (1953) and Henry (1972). The pathogen was found to primarily infect the fat body and in so doing debilitated the host. In the present studies, the development of N.

locustae was found to be relatively slow, requiring approximately 16 days (at 30° C) before mature spores were observed. At 24 days post-inoculation little or no fat remained and grasshoppers became lethargic, fed less and were pale in color due to the presence of spores. Although not measured in these studies, the sublethal effects of infection by N. locustae also occur. Henry (1969, 1971) noted that female grasshoppers in the field, even lightly infected with the pathogen, rarely contain eggs. Other workers have reported that both the reproductive and diapause potentials of insects are lowered as a result of microsporidosis (Gaugler and Brooks 1975). Increased cannibalism has also been observed among grasshoppers which are diseased and group-reared. Henry (1969) attributed this behavioral change to the weakened condition of the victim, especially during moulting, since deformities, such as twisted wings and legs, are common among infected grasshoppers.

Although the host range for N. locustae is considered to include 58 species of Orthoptera (Henry 1969), the pathogen exhibits differences in virulence depending on the host (Henry 1972). Some authors have even suggested that races of N. locustae may exist (Canning 1962 a,b), although conclusive evidence is lacking. Laboratory assays in the present studies showed the virulence of N. locustae to differ not only between species (Melanoplus sanguinipes, Melanoplus bivittatus, and Melanoplus differentialis) but also between strains of the same

species (non-diapause and diapause strains of M. sanguinipes). In laboratory studies the non-diapause strain of M. sanguinipes was found to exhibit a higher rate of mortality and a lower rate of infection than field collected diapausing grasshoppers of the same species. Similar LD_{50} values for the pathogen in field collected M. sanguinipes as those reported here (5.3 X 10^5 spores/grasshopper at 24 days) have also been noted by Henry (5.5 X 10^5 spores/grasshopper at 20 days) (unpublished data). Differences in the pathogenicity of N. locustae in this strain may be the result of selection. Pickford and Randell (1969) stated that over the 12-year period during which the strain was developed, the population was reduced to very small numbers. Although their criteria of fecundity and hatchability were met, other biological factors, for example, susceptibility to disease, were ignored. When other criteria are used, however, such as in the selection of a non-diapause strain of the gypsy moth [Lymantria dispar (L.)] for nuclear polyhedrosis virus production, it is possible for selection to be accomplished without altering the insect's susceptibility to disease (Hoy 1978). The colony of non-diapausing M. sanguinipes used in the present study, then, could conceivably be re-selected to include those grasshoppers which respond similarly to field collected M. sanguinipes in their susceptibility to infections by N. locustae.

Differences in infectivity were likewise noted between species and between subfamilies of grasshoppers in field plots treated with

the pathogen. Examination of the species composition in the present studies revealed the incidence of infection to be higher for species of the subfamily Cyrtacanthacridinae than for species of either the Oedipodinae or Acridinae. It was also noted that the incidence of infection varied between species within the Cyrtacanthacridinae. Similar differences in infectivity were reported by Henry (1972) under natural epizootic conditions of the disease. Although the reasons for these differences are not clear, cannibilism, feeding habits, and seasonal development may be factors which influence infection between species within a population of grasshoppers (Henry, 1972).

The relationships between dosage of <u>N</u>. <u>locustae</u> and both mortality and incidence of infection were demonstrated in the present studies both in laboratory assays and in field tests. Increased doses of the pathogen resulted in increased mortality and infection to the grasshoppers. Henry and Oma (1974 a) found a similar relationship in bio-assays comparing <u>N</u>. <u>locustae</u> with two other species of <u>Nosema</u> in <u>M</u>. <u>bivittatus</u>. Field studies in which <u>N</u>. <u>locustae</u> was applied at different doses further demonstrated a dosage response to reductions in density and incidence of infection (Henry 1971; Henry et al. 1973). These workers reported that the concentration of spores was considered of greater importance than either the timing of applications or levels of wheat bran carrier in applications of <u>N</u>. <u>locustae</u> to rangeland.

In the present studies, a logarithmic relationship between the dosage of N. locustae used for initiating infection and the mortality and incidence of infection was demonstrated both from laboratory and field tests.

The development of the infection, however, is also dependent upon factors acting during the period of time after inoculation. Surtees (1971) has described this relationship within a natural population by the equation:

$$y = 1 - e^{-rt}$$

where y is the proportion of the population infected and r is the rate of effective infecting contact per individual in time t. In an unpublished report, Henry and McCleave (1963) reported that in laboratory assays, higher densities of grasshoppers also produced a greater incidence of infection by N. locustae. In the present laboratory studies, in order to minimize the effects of density and standardize the assay, a constant density of 5 grasshoppers per rearing tube was established and maintained for only the first 24 days after treatment. For field studies, sampling for changes in density and incidence of infection was restricted to specific periods after application; this enhanced the validity of comparisons between tests.

The application of from 6.2 X 10^8 to 4.0 X 10^{10} spores/ha showed the same relationship between the initial dosage of N. locustae and the incidence of infection in both 1976 and 1978. Due to the advanced

stage of development of the grasshoppers at the time applications were made at Forsyth in 1978 (50% of the M. sanguinipes in the population were fifth-instar), however, the effects of the pathogen were not as pronounced. This effect of proper timing of applications on the basis of grasshopper development has been reported by other workers (Henry et al. 1973). Reductions in density were found to be 30 percent or less in these tests and this compared favorably with the results of Henry. He reported reductions for all grasshoppers of between 13.1 to 27.5 percent for the 4- to 6-week period following application of N. locustae (Henry 1971). In a later study by Henry et al. (1973), however, he reported that N. locustae caused considerably greater reductions in grasshoppers (50-60%). The species complex in the study plots at Big Timber and Forsyth was considerably different from that reported by these workers, however, and may account for the differences observed. Treatments of 4.0 X 10^{10} spores/ha produced infections 6 weeks later in 21 percent of all grasshoppers at Big Timber but only one-third this amount at Forsyth. The incidence of infection in either year was less than that reported by other workers who found that from 35 to 60 percent of all grasshoppers treated with similar doses were infected by 6 weeks post-application (Henry et al. 1973). Differences in the formulation and application of bran as well as differences in sampling techniques could have been responsible for the reduced rates of infection observed in the present studies. The

continuous flow augering system used for formulation and the modified
seed spreader used for application in the present study may not
have provided as complete coverage of the pathogen as did the equip-
ment used by Henry. He used a batch mixer and Buffalo Turbine in his
attempts to obtain complete coverage. Despite these difficulties,
however, these tests were useful in determining an efficient level of
inoculum to use for the remainder of the field studies.

Although the use of N. locustae in control programs could maintain
populations of grasshoppers at non-economic levels for prolonged
periods, occassional outbreaks would probably continue to occur (Henry
1970). Such outbreaks would require either more virulent microbial
agents (i.e., viruses) or chemical treatment to reduce populations to
levels below economic thresholds. Temporary reduction in numbers could
be accomplished either early in the season (during the nymphal period)
before forage losses occurred, or later in the season (before ovi-
position) to prevent outbreaks from occurring the following year. It
is conceivable that both approaches could be valuable in a scheme for
managing grasshopper populations. For example, the individual rancher
experiencing a localized outbreak of grasshoppers could reduce forage
losses by applying a N. locustae-virus or N. locustae-insecticide
formulation early in the season. Federal control programs, on the other
hand, attempting to maintain non-economic populations over a larger
area could utilize both early season applications (virus or insecticide

with N. locustae) and later season applications (ULV insecticide) to

achieve their objective.

The role of stress in the diseases of man has been recognized for

a long time (Selye 1950, 1952, 1955). Although stress has not been

studied as widely in invertebrates, Steinhause (1958) recognized that

it does play a role in the occurrence of disease in insects. The term

"stressor" in insect pathology is defined as any stimulus that tends

to disrupt the homeostasis of an insect or any adverse force or con-

dition or inconvenience to which the insect is subject (Steinhaus 1958,

1960). Stress-induced viral infections have been reported for several

insects (Jaques 1962; Bucher & Harris 1968; Biever & Wilkinson 1978).

Since an insect infected by a pathogen would be under stress, it would

seem reasonable to assume that the addition of another stressor (i.e.,

and insecticide), would increase the overall effect. Girardeau and

Mitchell (1968) reported such an influence of a nuclear polyhedrosis

infection in the cabbage looper [Trichoplusia ni (Hübner)] on its

susceptibility to chemical insecticides, and Bell and McLaughlin (1970)

reported that a protozoan infection increased the toxicity of four

different insecticides in the boll weevil (Anthonomus grandis Boheman).

In the present studies, grasshoppers stressed by infection with N.

locustae appeared to show a similar response when fed oral doses of

malathion. Non-diapause M. sanguinipes infected with N. locustae for

15 days may have been more susceptible to the insecticide than those in

the healthy state. The effect of N. locustae, however, on the toxicity of malathion in M. differentialis, even 39 days post-inoculation, could not be demonstrated. It is likely that differences in the "stressed state" occur between species, however, since specific host-pathogen relationships have evolved over time. Further research of the stress phenomenon resulting from infection by N. locustae, however, will have to be accomplished before it is fully understood.

Field tests were also unable to demonstrate the effects of N. locustae on malathion toxicity. Although 27 percent of the grasshopper population was infected with N. locustae at the time the mid-season ULV malathion treatment was applied, the mortality rate among grasshoppers in plots pretreated with N. locustae was not different from that in plots without N. locustae. The reason for lack of a higher mortality rate among grasshoppers in those plots pretreated with the pathogen could have resulted from variation between treatments or because of the involvement of an entire species complex. In effect, those species which became stressed as a result of infection might have been diluted by those species which although infected did not exhibit a stressed state. Another possibility was that the mode of entry of malathion into the grasshopper was different in laboratory and field tests. Malathion was administered orally in the lab and topically in the field. At any rate, these tests show that mid-season applications of ULV malathion may not prove to be as disruptive to the diseased portion of the population

as was first hypothesized. If this were the case, both N. locustae
and ULV malathion could be used for treating the same area in the same
season without adverse effects to one another. For example, grass-
hopper damage could be assessed 4 to 6 weeks after N. locustae had been
applied to an area and those regions within the area still heavily
infested could be sprayed with insecticide to reduce numbers below
those levels causing economic injury. If the assessment were made prior
to ovipostion, grasshopper populations would also be prevented the next
year. A more economical approach, however, would be to combine both
materials on bran and to apply them simultaneously early in the season
before damage to forage had occurred.

Although Cowan (1958) estimated that grasshoppers annually con-
sume from 6 to 12 percent of the available forage in the Western States,
accurate measurements of forage losses and economic injuries have been
difficult to obtain (Hewitt 1977). Despite these difficulties,
estimates have been used successfully to predict the amount of forage
consumed by grasshoppers during the season (Hewitt et al. 1976) and to
assess the economics of applying insecticides (Onsager 1978). Such
studies have indicated that early season applications made before the
grasshopper population consists mainly of adults are more efficient.

The concept of integrated control with insecticides and pathogens
is not new and reports have appeared in the literature over the past 30
years. Telenga (1952) reported that the combined use of bacteria,

fungi, and insecticides resulted in greater mortality among some
lepidopterous larvae than when the agents were used separately. Studies
prior to 1968 on the combined effects of mixtures of pathogens and
insecticides were reviewed by Benz (1971). Since this review several
workers have investigated the area of integrated control with varying
results. McLaughlin (1973) expressed the view that, "the role of
protozoans in an integrative program should be studied", but only
recently have any reports appeared that show the potential of combining
microsporidians with other agents (Wilson 1978; Fuxa 1979). In the
present studies, two chemical insecticides were tested in combination
with N. locustae and none of the mixtures were found to be antagonistic.
The pathogen appeared to act normally in the grasshopper and its effects
were additive to the action of the insecticide. This result was partic-
ularly encouraging since some other pathogens, notably bacteria and
viruses, have been reported to act antagonistically with chemicals
(Morris 1972; Komolpith 1975; Hamilton & Attia 1977; Luttrell et al.
1979). It is possible that due to their unique mode of action in the
insect, microsporida may prove to be a better candidate than other
microbials for integration with other agents (Fuxa 1979). When large
numbers of spores are ingested, the polar filaments can damage the mid-
gut epithelium sufficiently to facilitate invasion of bacteria into the
hemocoel from the gut lumen and this may aid insecticide entry.

In the present laboratory assays in which combinations of
insecticide and $\underline{N}$. $\underline{locustae}$ were fed to grasshoppers, the toxicity of
malathion was found to differ in the two species tested. The LD_{50} for
$\underline{M}$. $\underline{sanguinipes}$ was 0.72 µg per third-instar while the LD_{50} for $\underline{M}$.
$\underline{bivittatus}$ was 1.42 µg per third-instar. $\underline{M}$. $\underline{bivittatus}$ is a larger
species and therefore requires greater amounts of insecticide to effect
the same degree of mortality. For both species, however, malathion
acted rapidly in causing mortality and was as effective alone as it was
in combination with $\underline{N}$. $\underline{locustae}$. Carbaryl produced similar results in
$\underline{M}$. $\underline{sanguinipes}$ as malathion, with 0.47 µg per third-instar causing 50
percent mortality. These results are consistent with the work of others
(Mazuranich, personal communication). Since both insecticides were
capable of producing significant mortality in $\underline{M}$. $\underline{sanguinipes}$ within 24
hours, either alone or in combination with $\underline{N}$. $\underline{locustae}$, they were con-
sidered likely candidates for field testing. Malathion is the most
widely used material for grasshopper control at the present time and
therefore it was selected for field evaluation in the present studies.
Carbaryl, however, is also registered for use against grasshoppers and
it may prove to be a more effective insecticide due to its greater
persistence (Onsager 1978).

Infection by $\underline{N}$. $\underline{locustae}$ in both species of grasshoppers assayed in
the laboratory was not found to be adversely affected by either of the
insecticides. Development of the pathogen appeared to continue

normally even after spores used for inoculation were soaked for 48

hours in solutions containing malathion or carbaryl. Differences in

the virulence of N. locustae in M. sanguinipes and M. bivittatus,

however, were observed although this was attributed to its pathogenicity

in relation to the host. The LD_{50} for M. bivittatus was 20 times

greater than that for M. sanguinipes.

Since a generalized concept has not as yet emerged concerning the

compatibility between insecticides and bacteria or insecticides and

viruses, it is necessary that each specific combination of chemical and

pathogen be considered separately. Microsporidians may also react

differently from the pathogens thus far reported and their mode of

action needs to be investigated further. In addition, a range of

inoculum levels must be investigated in order to detect any synergistic

or antagonistic effects which might otherwise be masked by the action

of the pathogen (Fuxa 1979).

In the laboratory assays in which combinations of GIBV and N.

locustae were fed to M. sanguinipes, the pathogens apparently developed

independently of one another. Multiple infections in insects have been

reported for microsporida-virus mixtures (Fuxa 1979), bacteria-virus

mixtures (McVay et al. 1977), and virus-virus mixtures (Tanada 1959;

Tanada & Chang 1964; Whitlock 1977). Antagonism, additivity, and

synergism have all been noted by these workers thereby precluding any

generalized conclusions. In the present studies, the results of multi-

ple infections by a virus and protozoan were shown to be additive
although both pathogens infect the fat body. GIBV caused significant
mortality within 7 days after inoculation while N. locustae acted more
slowly. Histological studies would have to be done to confirm the
particular mode of action of N. locustae-GIBV mixtures and illucidate
the interactions between pathogen and host. Although field tests of N.
locustae-GIBV mixtures were not attempted in the present studies, such
a formulation appears promising for field applications as well. The
virus is highly infectious and causes rapid mortality in several species
of grasshoppers (Henry and Jutila 1966). Grasshopper populations could
be reduced in numbers early in the season with GIBV and maintained at
low levels for several years by the effects of N. locustae.

Field tests of N. locustae-malathion mixtures on bran showed that
integration of these agents would be a feasible alternative to total
reliance on chemical insecticides to control grasshoppers on rangelands.
Reductions in density of approximately 50 percent occurred within 5 days
after malathion was applied as a 0.37 percent (wt tech/wt bran) bran
bait either alone or in combination with N. locustae when grasshoppers
were in the third-instar of development. When similar formulations
were applied to plots when grasshoppers were predominently in the fifth-
instar of development, a reduction in density of approximately 25
percent was observed. It is important to note that in both of these
tests, only a very small quantity of malathion was required to achieve a

measurable degree of mortality. The standard ULV malathion treatments

of 8 fluid oz/A (585 ml/ha) by comparison require about 100 times the

amount of technical material. Skoog et al. (1961) reported that 8 oz

of malathion per acre caused mortality of between 77 and 92 percent

when applied as a ULV spray. By administering malathion orally rather

than topically, a more efficient use of the chemical could be obtained.

In addition, concentration of malathion on bran would prevent indiscrim-

inant dispersal of the insecticide in the environment and thereby

preserve existing natural enemies.

It has been estimated that the reduction in forage due to a high

population of Aulocara elliotti can reach 35 percent during the four

weeks following the appearance of third-instar nymphs (Hewitt et al.

1976). In assessing the economics of forage losses due to grasshoppers,

Onsager (1978) has shown that density reductions later in the season

were the least economical even if higher mortalities were obtained. In

the present study, low doses of malathion were purposely chosen to

cause a reduction in density of 50 percent or less to permit the

survival and multiplication of N. locustae in the residual grasshopper

population. Higher doses of the insecticide could be used if a greater

degree of control were desired. Contrary to prior philosophy, elimina-

tion of most of the grasshoppers, however, would not be necessary so

long as treatments were applied early in the season. By reducing

densities with small doses of malthion (or carbaryl) before maximum

forage loss occurred, economic losses could be prevented in areas of grasshopper outbreaks.

Changes in the species complex demonstrated the effects of N. locustae on grasshopper populations in the present studies. Although the pathogen did not act as rapidly as malathion in causing mortality, its debilitating effects became evident during the weeks following treatment. The tests at White Sulphur Springs in 1977 showed a reduction in the abundance of the Cyrtacanthacridinae during the season. Since this subfamily also exhibited a higher incidence of infection than either the Acridinae or Oedipodinae, these changes in the relative frequency were attributed to the effects of N. locustae. Changes in the relative frequency of Melanoplus infantilis in plots treated with either N. locustae alone or in combination with malathion showed that the pathogen continued to cause mortality in this species. Examination of those surviving showed that nearly 50 percent of the M. infantilis were infected with N. locustae 48 days after the pathogen had been applied. Of the other three species infected in the subfamily Cyrtacanthacridinae, Melanoplus dawsoni and Melanoplus gladstoni increased in abundance while Melanoplus packardii remained nearly the same. M. dawsoni and M. gladstoni were both observed to hatch later than either M. infantilis or M. sanguinipes. M. packardii, although similar in hatching time, was more tolerant to infection by N. locustae (exhibited less mortality). Had the effects of N. locustae on mortality

of these species not been diluted by these factors, the change in the
relative frequency of the Cyrtacanthacridinae might have even been
greater. In the tests at Forsyth in 1978, although the species complex
differed, it also reflected changes during the season which may have
been attributable to infection by N. locustae. The Acridinae showed
the largest change in abundance and this change was in large part due
to a single species, Ageneotettix deorum. Although the Cyrtacanth-
acridinae were infected with N. locustae, their relative frequency
increased during the 4- to 6-week sampling periods and therefore con-
trol by the pathogen could not be demonstrated for this subfamily. A
higher incidence of infection in these species may be necessary to
effect measurable control.

Grasshopper populations treated with formulations of N. locustae
and malathion displayed lower incidences of infection than when the
pathogen was applied by itself. Integrated field tests of microbial-
insecticide formulations (Jaques 1973; Hussain & Askari 1976; Morris
1977 a, c) have not reported this effect on the pathogen. These
workers, however, have only used percent control to measure the efficacy
of treatments and have not observed infection rates in the survivors as
was done in the present study. Reducing the density of grasshoppers
with an insecticide also reduces the number of potentially infectable
units in a population. Only 25-50 percent of the population was killed
by malathion in the present studies, but this was apparently sufficient

to slow the spread of infection by N. locustae. Surtees (1971) has

considered several factors limiting natural epizootics and has shown

that changes in the host as well as the pathogen can modify the progress

of infection within a population. It is also possible that the higher

doses of malathion required in the field tests may have acted directly

on the pathogen to reduce its infectivity. Concentrations of malathion

in field formulations were approximately 100 times greater than the

mixtures assayed in the laboratory.

The disruption to an induced epizootic by simultaneously applying·

N. locustae with malathion, however, may only be temporary and not

reduce the overall benefits of this management approach. The long-term

effects of virus-fenitrothion combinations have been reported to

suppress spruce budworm (Choristoneura fumiferana) populations for

three years (Morris 1977 a). In studies of the epizootiology of

infections by N. locustae, Henry (1972) suggested that once the disease

is established in a population it might persist indefinitely. The

debilitating effects on the host of microsporidosis might be sufficient

to suppress populations below economic thresholds for several years.

The sublethal effects of microsporidan infections on the reproductive

potential in insects have been well documented (Veber and Jasic 1961;

Tanabe and Tamashiro 1967; Smirnoff and Chu 1968; Gaugler and Brooks

1975). The improvement of bait formulations might also increase the

effectiveness of N. locustae since certain species of grasshoppers do

not eat bran (Parker 1952). Innovative approaches involving behavioral

modifiers, such as pheromones and feeding stimulants have already been

attempted for protozoan pathogens (McLaughlin et al. 1969; Shapas et al.

1977). More economical application equipment will also have to be

developed for dissemination of N. locustae (Mussgnug & Henry 1979).

The development of resistance in insects to microbial agents has

not yet been fully investigated but this has not been reported to occur

in natural populations. Laboratory selection of resistant strains has,

however, been reported for a number of different genera (Surtees 1971).

Further studies will have to be conducted in order to assess the long-

term effects of integrated insecticide-pathogen or pathogen-pathogen

bait formulations on grasshopper populations. In any case, an inte-

grated approach such as the one proposed in these studies could provide

another management tool for effectively suppressing densities of grass-

hoppers on rangeland.

SUMMARY

These studies showed the effects of Nosema locustae and formula-
tions of N. locustae with insecticides or virus on grasshoppers.
Utilization of N. locustae either exclusively or in combination with
chemicals or other pathogens appeared to be a promising approach for
the management of grasshopper populations on rangeland. An integrated
pest management scheme utilizing insecticides and pathogens could pro-
vide both short- and long-term control of grasshoppers without harmful
effects to the environment.

N. locustae primarily infects the fat body and causes grasshoppers
to become lethargic, feed less and eventually die. Development of the
pathogen was relatively slow, requiring approximately 16 days for spore
formation at 30° C. The virulence of N. locustae varied between
species as well as strains of the same species. For the non-diapause
strain of Melanoplus sanguinipes, 0.9×10^5 spores per third-instar,
for the diapause strain of M. sanguinipes, 5.3×10^5 spores per third-
instar, and for Melanoplus bivittatus, 3.2×10^6 spores per third-
instar were required to produce a mortality of 50 percent at 24 days
post-inoculation. Differences in the pathogenicity of N. locustae were
also noted between species and subfamilies of grasshoppers in field
tests. The pathogen exerted a greater influence on grasshoppers of the
Cyrtacanthacridinae than either the Acridinae or Oedipodinae.

A logarithmic relationship between the dosage of N. locustae used
for initiating infection and the mortality and incidence of infection

was demonstrated both from laboratory and field tests. Increased

doses of the pathogen resulted in increased mortality and infection to

the grasshoppers. Between 2.5 and 7.4 billion spores per hectare on

1.68 to 3.36 kg bran was considered to be an efficient level of

inoculum to apply for significant control of grasshoppers on rangeland.

Applications of the pathogen when grasshoppers were in the third-instar

of development resulted in better control than when the pathogen was

applied later in the season. After grasshoppers had become infected

with N. locustae, the infection continued to spread throughout the

population and the incidence of infection continued to increase during

the remainder of the season. Reductions in density of 30 percent with

30 percent of the survivors showing infection were noted 6 weeks post-

application. Modification of the augering system for formulation and

the seed spreader for application to obtain complete coverage could

improve these results.

Infection by N. locustae may increase the toxicity of malathion

fed orally to some species of grasshoppers. The non-diapause strain of

M. sanguinipes stressed with the disease for 15 days may have been more

susceptible to the insecticide than those grasshoppers in the healthy

state. The effects of the disease on the toxicity of malathion in

Melanoplus differentialis could not be demonstrated. There was no

evidence from field tests of a stress phenomenon induced by N. locustae.

Although 27 percent of the grasshopper population was infected with N.

locustae at the time mid-season ULV malathion treatments were applied,
a differential selection of diseased grasshoppers over those in the
healthy state could not be demonstrated. It appeared that mid-season
spraying with ULV malathion (avg 170 ml/ha) to control outbreaks of
grasshoppers (and prevent oviposition) might not be disruptive to
epizootics of N. locustae induced earlier in the season.

Combinations of N. locustae with the insecticides malathion or
carbaryl, fed orally to third-instar grasshoppers were found to be non-
antagonistic. The pathogen appeared to develop normally and its
effects were additive to the action of the insecticide. The toxicity
of malathion was found to differ between the non-diapause strain of M.
sanguinipes (LD_{50} = 0.72 µg per third-instar) and M. bivittatus (LD_{50} =
1.42 µg per third-instar). For both species, however, malathion acted
rapidly and caused mortality within 24 hours after ingestion. Carbaryl
produced similar results in the non-diapause strain of M. sanguinipes
(LD_{50} = 0.47 µg per third-instar). Mortality from mixtures of the path-
ogen and insecticide by 24 days post-inoculation was attributed to N.
locustae. Infection by N. locustae was not found to be adversly
affected by either malathion or carbaryl in the laboratory. Soaking
spores in solutions of the insecticides for up to 48 hours did not
significantly reduce the viability of the pathogen. Simultaneous treat-
ment of the pathogen with the insecticide did not reduce infection
rates in the survivors.

Combinations of N. locustae with GIBV fed orally to third-instar

grasshoppers were also found to be non-antagonistic. Both pathogens

infected the fat body but appeared to develop normally and their

effects were additive. Mortality due to infection by the virus was

more rapid than N. locustae, but slower than malathion or carbaryl.

For the non-diapause strain of M. sanguinipes, the LD_{50} of GIBV at 7

days post-inoculation was determined to be 1.17 X 10^6 inclusions per

third-instar. Infection by N. locustae was not reduced by simultaneous

treatment with GIBV and many grasshoppers exhibited duel infections.

Field tests of N. locustae-malathion formulations on bran showed

that integration of these agents rapidly reduced densities of grass-

hoppers and initiated infection in the residual population. Reductions

in density of 50 percent occurred within 5 days after malathion was

applied as a 0.37 percent (wt tech/wt bran) bran bait either alone or

in combination with N. locustae when grasshoppers were in the third-

instar of development. Similar formulations applied when grasshoppers

were predominently in the fifth-instar of development reduced densities

by approximately 25 percent within 3 days. The effects of N. locustae

on the population became evident during the weeks following treatment.

Nearly half of the Melanoplus infantilis examined 48 days post-applica-

tion were infected with N. locustae while later season applications

produced infection rates in M. sanguinipes of 15 percent at 6 weeks

post-application. Mortality caused by the pathogen resulted in changes

in the species composition. Grasshoppers of the subfamilies
Cyrtacanthacridinae and Acridinae were reduced in abundance as a result
of the disease.

Grasshopper populations treated with formulations of $\underline{N}$. $\underline{locustae}$-
malathion displayed lower infection rates than when the pathogen was
applied by itself. Although the incidence of infection increased in
the population, it never fully recovered during the season. Reducing
the population with an insecticide apparently slowed the spread of
infection by $\underline{N}$. $\underline{locustae}$. The disruption to an induced epizootic by
simultaneously applying malathion with $\underline{N}$. $\underline{locustae}$, however, does not
reduce the overall benefits of the approach since the effect is only
temporary and the disease continues to spread within the population.
Improvement of methods used to formulate and apply these baits could
make such an approach an effective and efficient way of managing grass-
hopper populations on rangeland.

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