EFFECT OF THE FUNGAL PATHOGENS, *ERYNIA* SPP., ON THE ALFALFA WEEVIL, *HYPERA POSTICA*, AND THE PARASITE, *BATHYPLECTES CURCULIONIS* THOMSON

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AND THE PARASITE, BATHYPLECTES

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PREFACE

Since 1983, high mortality of the alfalfa weevil larvae, prepupae, and to a smaller proportion, pupae, due to infection by the fungal pathogens, Erynia spp., has been observed in Oklahoma. These studies were conducted to determine the extent of mortality of the alfalfa weevil due to infection by Erynia spp. on a seasonal basis and to determine the effect of fungal disease on parasitism by Bathyplectes curculionis.

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I dedicate this work to my family, for their support and understanding, and most of all, for their faith in me.

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CHAPTER I

INTRODUCTION

The eastern strain of the alfalfa weevil, *Hypera postica* (Gyllenhal), was first reported in Eastern Oklahoma in 1968 (Berberet et al. 1980). A year later, the western strain was reported in the northwest corner of the state (Berberet and Gibson, 1976). Since 1972, the weevil has been a serious pest of alfalfa across the state. Significant yield reduction in forage production of first and second alfalfa crops has been observed virtually every year (Berberet et al. 1980). An integrated control program for suppression of the weevil in Oklahoma has been developed (Berberet 1982).

Key elements of this program include cultural, chemical, and biological controls. Use of insecticides provides an effective and essential means of control for the weevil during peak densities of the host (Sholar et al. 1982). Studies have been conducted on a continuing basis to identify those insecticides that will provide the most cost effective weevil control. However, increasing costs have caused financially stressed producers to become receptive to alternative measures. To maintain weevil populations below economic threshold levels without sole reliance on

chemicals, alternative control procedures such as winter grazing of dormant alfalfa and growing tolerant varieties have been emphasized (Berberet et al. 1980). Additionally, the parasite, Bathyplectes curculionis Thomson has proved to be an effective biological control agent of the alfalfa weevil. With its natural dispersal into Oklahoma, the parasite has helped to reduce losses due to weevils (Berberet and Gibson, 1976). By comparison, a related species, B. anurus (Thomson) has not shown a tendency for rapid dispersal (Berberet et al. 1978). Thus, it may have limited potential as a biocontrol agent for H. postica.

A fungal pathogen of the alfalfa weevil has been reported in several geographic locations in the United States (Puttler et al. 1980, Barney et al. 1980, Nordin et al. 1983). This pathogen, classified as one of the Erynia spp. (Zygomycetes: Entomophthorales), was first reported in alfalfa weevil larvae in North America by Harcourt et al. (1974) during field investigation on the ecology of the alfalfa weevil in Eastcentral Ontario, Canada. In recent years, however, in over 30 counties in Oklahoma, more than one fungal pathogen has been observed to be the cause of high mortality of the larval, prepupal, and pupal stages of the alfalfa weevil.

Appearance of resting spores is a major criterion for classifying the pathogens. Samples of cadavers from this study were sent to Dr. R. A. Humber at the Boyce Thompson Institute in Ithaca, New York, for identification. The

prevalent spore type observed was dark and verrucose in appearance, similar to that seen in other studies. This pathogen is identified as Erynia punctata (Garbowski) unpublished comb. nov.. There were a few incidences of another pathogen with hyaline and smooth-walled spores, identified as Erynia phytonomi (Arthur) Humber, Ben-Ze'ev and Kenneth. In this study, these pathogens were collectively known as Erynia spp. unless specified.

Due to their recent appearance, few studies have been done in Oklahoma or elsewhere to show the effect of these fungi on the population levels of the alfalfa weevil. Further, few studies have been conducted to determine the effect of the pathogens on parasitism of the alfalfa weevil by B. curculionis. Although a high level of mortality of the alfalfa weevil has been observed, the potential of these fungal pathogens as control agents of the weevil remains to be seen in further investigations.

The objectives of this study were to:

1) Determine the seasonal mortality in the larval, prepupal, and pupal stages of the alfalfa weevil due to infection caused by Erynia spp.
2) Determine effects of fungal epizootics in the alfalfa weevil on the survival and total parasitism by Bathyplectes curculionis.

CHAPTER II

REVIEW OF LITERATURE

The Alfalfa Weevil

The alfalfa weevil, *Hypera postica* (Gyllenhal), was first introduced into the United States near Salt Lake City, Utah, in 1904 (Titus 1909). From there the population, which has become known as the western strain, spread rapidly into the other western states. In 1951, the weevil was found in the eastern United States near Baltimore, Maryland (Bissell 1952). The population has been identified as the eastern strain and has rapidly become the most destructive pest of alfalfa in the United States (App 1959).

The alfalfa weevil entered Oklahoma from both east and west. The eastern strain was first collected in 1968 from counties adjoining Arkansas and Missouri (Berberet et al. 1980). The western strain was not collected until 1969 although it was believed to have entered the Panhandle and extreme northwestern Oklahoma 2-3 years earlier (Berberet et al. 1980). The alfalfa weevil has been the primary insect threat to profitable alfalfa production in Oklahoma since 1972 (Berberet 1982).

The seasonal life history of the alfalfa weevil in Oklahoma involves one generation per year (Berberet et al.

1980). Egg deposition generally begins in November or December of each year as adult weevils return to alfalfa fields from summer aestivation sites in vegetation along fence rows, roadsides or other uncultivated areas. The eggs are deposited in stems of the fall growth of alfalfa and they overwinter successfully if weather is mild. Through March and April of each year, egg numbers decrease as hatching is accelerated due to warm weather and as the reproductive capacity of adults is exhausted.

Development time for the alfalfa weevil varies under different conditions with temperature acting as the chief influencing factor. Accumulated degree days toward development affect both ovipositional rates and time of hatching for eggs (Evans 1959). It was observed that in the warmer areas of the southern U.S., severe damage to early plant growth was attributed to larvae which hatched from eggs laid in the fall and the later spring damage was a result of larvae from spring laid eggs (Campbell et al. 1961). Sholar et al. (1982) have indicated that most damage is caused by the larval stage and includes defoliation or ragging of leaves around plant terminals.

Newly hatched larvae are tan or light yellow with black head capsules. The larvae feed within the plant terminals during the first two instars, then move to the mature leaves in the later instars (Manglitz and App 1957). The larvae occur over an extended period of time depending on temperature and more importantly, on egg-laying patterns

during the fall, winter, and spring. Larval feeding may therefore begin anytime from early spring to summer. Hsieh et al. (1974) determined that the mean degree days (F) required for completion of larval development is about 381 above a threshold of 48^{0}F for the eastern strain and about 372 above the threshold of 51^{0}F for the western strain.

Pupation begins from mid-March to mid-April and is usually about 2 weeks earlier in southern Oklahoma than in the northern counties (Berberet et al. 1980). The mature larvae typically crawl or drop to the ground where they spin a net-like cocoon of white, silken threads. Poos and Bissell (1953) observed that many larvae of the eastern strain had spun their cocoons and pupated on the host plant rather than at the soild surface as in the West. At a threshold temperature of 58^{0}F for both strains, the mean requirement for the development of the pupal stage is about 89 degree days (F) (Hsieh et al. 1974). In Oklahoma, the time interval for the pupal stage is about 10 days according to Berberet et al. (1980).

As the weevil adults emerge, they normally feed for a short period of time until the first harvest is taken, then move into vegetation along field margins to aestivate as temperature rises in harvested alfalfa fields.

Population levels of the weevil have been regularly recorded at two locations, one in northern Payne Co., Stillwater, and one in southern Grady Co., Chickasha, Oklahoma. High numbers of weevil eggs with peak larval

densities exceeding 5300/m^2 were recorded from 1972 to 1975 (Sholar et al. 1982). From 1976 to 1980, a decline in the weevil populations was seen. Two possible causes of this decline were parasitization of larvae by _B_. _curculionis_ (Thomson) and cold winter conditions which caused high weevil egg mortality.

Following 1980, however, a resurgence in weevil population has been observed (Doss and Berberet 1986). One possible reason for this resurgence has been warmer weather in winter which, with the exception of 1983, has allowed high survival of overwintering eggs. Another reason appears to be the decline in parasitism by _B_. _curculionis_.

A number of studies have been done involving yield and quality reductions by weevil larvae in alfalfa (Flessel and Niemczyk 1971, Hintz et al. 1976, Berberet et al. 1981). Yield reduction was chiefly due to extensive defoliation by larval feeding. Other factors contributing to losses included reduced growth and delayed maturity of infested alfalfa. Based on a regression analysis, research in Oklahoma has shown that for each increase in population of one weevil larva per stem, the adjusted first harvest yields of nonirrigated alfalfa were reduced by 188 kg/ha (Berberet et al. 1981). Residual effects in the second crop of alfalfa resulted in losses of approximately 157 kg/ha for each addition of 1 larva/stem (Berberet et al. 1981).

Feeding damage by the adult weevil was described by Titus in 1909. Hastings and Pepper (1953) reported that in

the southern U.S., when oviposition and adult feeding begin as the plant initiates spring growth, the adult can stunt the growth of the plant and cause reduced yields. Also, regrowth after the first harvest may be heavily damaged if newly emerged adult weevils remain in the fields when cool, damp weather conditions prevail (Sholar et al. 1982).

Several control measures have been used against the alfalfa weevil. When properly integrated, these control techniques would give an effective control strategy. Hence, studies have been directed toward development and evaluation of a management program for the alfalfa weevil in Oklahoma for the past 10 years (Berberet 1982).

Senst and Berberet (1980) studied the merits of winter grazing as a control for the weevil. They determined that grazing by livestock reduced overwintering egg populations by over 60% and resulted in much lower larval densities during the growing season. Though winter grazing may be beneficial in older stands, it is not practical on new plantings. Damage to plants may result from trampling since there is little plant cover established.

Four cultivars have been released which exhibit some tolerance to the alfalfa weevil. The first to be released was 'Team' (Barnes et al. 1970). It was followed by 'Arc' (Devine et al. 1975) and 'Liberty', and then by 'Cimarron'. The last three cultivars appear to possess a higher level of weevil tolerance than 'Team' and a wider range of multiple-pest resistant qualities (Berberet 1982). Characteristics

of tolerance exhibited by these varieties include rapid, vigorous growth in early spring and extensive lateral branching which helps to compensate for weevil damage in the growing terminals (Devine et al. 1975). Growing tolerant varieties has reduced the need for insecticide applications, thus reducing the costs of alfalfa production.

Long residual chlorinated hydrocarbon insecticides were once an important means of controlling the alfalfa weevil. However, these agents became less effective as a result of pesticide resistance in weevils (Dorsey 1966). Higher costs of production have further discouraged the use of insecticides. Presently, most alfalfa producers in Oklahoma still rely heavily on chemical insecticides to maintain both yield and quality of their forage. Efficacy of chemicals such as carbofuran and chlorpyrifos, have been sufficient to reduce weevil populations to levels less than the economic threshold of 1.5 larvae per stem (Doss and Berberet 1986). In an integrated program, chemical control will undoubtedly continue to be an essential means of control for the prevention of serious losses when larval populations exceed 1.5-2.0 larvae per stem (Berberet 1982).

The Parasites

Since the alfalfa weevil is believed to be of European origin (Chamberlain 1926), an important control measure adopted has been the importation of several species of parasites. Among these are two hymenopterous larval

parasites of the genus Bathyplectes spp. B. curculionis is an endoparasite that was released in Utah in 1911 (Chamberlain 1926). Through regular releases and natural dispersal, this parasite is now found throughout the entire range of its host (Dysart and Day 1976). A related hymenopterous species established in the United States for the control of the alfalfa weevil is B. anurus (Thomson). This parasite was initially released in New Jersey in 1960. It has subsequently become well established in 17 states to the north and east of Oklahoma (Dysart and Day 1976). In Oklahoma, B. anurus was established at two locations in 1972 through releases of adult parasites. Initially, it did not show great potential as an effective control agent due to slow rate of dispersal (Berberet et al. 1978).

B. curculionis completes one and a partial second generation per year (Armbrust et al. 1972). The 1st through 3rd instars of the weevil are equally preferred for oviposition by B. curculionis (Duodo and Davis 1974a). Death of the host larva occurs after it has spun its cocoon. The parasite then spins a cocoon within the cocoon of its host (Brunson and Coles 1968) and, in the case of diapausing forms, passes the summer and following winter in the prepupal stage before pupation and adult emergence in early spring. In laboratory studies of post-diapausing B. curculionis pupal stages, Caldwell et al. (1976) found a developmental threshold of 45^{0}F for the parasite. About 312 (F) degree days were required for pupal development.

In Oklahoma, _B_. _curculionis_ has become established in all alfalfa growing areas without aid of releases and has demonstrated increasing rates of parasitism. However, parasitism has decreased considerably since 1980. The hot, dry summer in 1980 caused extensive mortality of oversummering prepupae of the parasite (Doss and Berberet 1986) and might have begun this trend.

The effectiveness of _B_. _curculionis_ has been discussed in several studies. Duodo and Davis (1974b) observed that the total food consumption of larvae parasitized by _B_. _curculionis_ was lower than that of unparasitized larvae. A reduction of 1.14 mg/day in food consumption produces an immediate economic gain as determined by Armbrust et al. (1970). Further, a reduction in the population of the next weevil generation is brought about by mortality of the prepupae when the parasite emerges to spin its own cocoon (Duodo and Davis 1974a).

Effectiveness of this parasite is limited by the attack of hyperparasites on both larvae and pupae in cocoons (Puttler 1966). Further, eggs of _B_. _curculionis_ tend to be encapsulated by the larvae of the alfalfa weevil (Puttler 1967). Encapsulated eggs invariably die because of the interference with respiration and nutrition of the embryos by capsules (Berberet et al. 1976). Although successful biocontrol of the alfalfa weevil with this parasitic species has limitations, it will continue to play an important role in the control of the alfalfa weevil in many areas of the

United States.

The Fungal Pathogens

In recent years, the discovery of fungal pathogens in alfalfa weevil populations which cause high levels of mortality of the insect has prompted an increasing amount of interest in several areas of the U.S. (Puttler et al. 1980, Watson et al. 1981, Johnson et al. 1984). One of these pathogens was first reported in the weevil by Harcourt et al. (1974), after being discovered early in the summer of 1973 in Ontario, Canada. It was described by these authors as *Entomophthora phytonomi* Arthur. This fungus was first named by Arthur (1886), who found it infecting some larvae of the clover leaf weevil, *Hypera punctata* (Gyllenhal), in New York, in 1885.

Since Arthur (1886) never found resting spores in infected clover leaf weevils, Harcourt et al. (1974) thought that the resting spores they found in the alfalfa weevil belonged to the same fungus observed by Arthur (1886) because the conidia were similar to those described. In 1981, Harcourt et al. (1981) reported a second fungal species infecting *H. postica* larvae. Although the two species have similar conidial or asexual states, one of them possesses smooth-walled, hyaline resting spores whereas the other has thick-walled, verrucose resting spores, like those reported earlier.

The similarity of the conidial stage in these two

species has led to taxonomic difficulties. The species which has long been recognized as the natural control agent of the clover leaf weevils, was renamed Zoopthora phytonomi (Arthur) Batko by Ben-Ze'ev and Kenneth (1980). The discovery of smooth-walled resting spores in both species of weevil hosts which were similar to those produced in culture by Ben-Ze'ev and Kenneth (1980) supported the name change (Harcourt et al. 1981). The older generic name, Erynia Garbowski, was proposed by Humber and Ben-Ze'ev (1981) as the more correct name and regarded Zoopthora to be a more recent synonym. This pathogen, found in both the clover leaf and alfalfa weevil, is therefore named Erynia phytonomi (Arthur) Humber, Ben-Ze'ev and Kenneth. The species found only in the alfalfa weevil and described as Entomophthora phytonomi by Harcourt et al. (1974) has been designated Erynia punctata (Garbowski) (Humber unpublished).

Larval cadavers observed by Puttler et al. (1980) in Missouri usually appeared black and shrivelled and contained resting spores. Some cadavers were, however, tan-colored and covered with external mycelial growth which were conidiophores. These two types of cadavers were commonly observed as well in areas such as Kentucky, Canada, and Georgia where both Erynia spp. occurred (Nordin et al. 1983, Harcourt et al. 1977, Gardner 1982). Pupae were also affected by the fungi although the proportion affected was lower than that of the larval and prepupal stages (Harcourt et al. 1977). Although all larval instars were attacked,

Barney and Armbrust (1981) indicated that a slightly greater percentage of fourth than third instars were found to be diseased.

MacLeod et al. (1966) reported that wet weather was of great importance to the development of fungal epizootics. They stated that elucidation of the environmental requirements of entomogenous fungi would not only facilitate understanding of the mechanism of mycotic infection, but would also contribute to more effective use of these organisms in biological control. Availability of moisture is probably the most important enviromental factor affecting fungal epizootics of Erynia spp. in alfalfa weevil populations.

Wilding (1969) conducted an intensive study on the effect of humidity on conidial discharge of Entomophthora aphidis Hoffman and E. thaxteriana Hall and Bell. He found that both species discharged increasing numbers of conidia when relative humidity increased from 80 to 97.5% Based on this result, he hypothesized that other Entomophthora spp. require a similar saturated or nearly saturated atmosphere for conidial discharge. The same conclusion was expressed by Watson et al. (1981) with regard to Erynia spp., when they determined that high humidity was the primary factor initiating sporulation of the fungi in alfalfa weevil larvae. The condition of high humidity protects against rapid dessication and inactivation of the conidia (Newman and Carner 1976).

Puttler et al. (1980) observed that the progressive increase in the incidence of fungal infection in a developing larval population indicated an apparently density-dependent relationship. However, as suggested in other studies (Los and Allen 1983, Brown and Nordin 1986, Nordin 1987), the occurrence of the fungi apparently increased as the population of the weevil was declining. Natural termination of Erynia spp. epizootics occurred when host densities fell below a critical threshold density of 1.7 larvae per stem (Nordin et al. 1983).

Even though the original source of Erynia spp. which infect alfalfa weevil populations is unexplained, they are a welcome addition to natural controls of the alfalfa weevil. However, few studies have been done to investigate the effects of Erynia spp. on the incidence of B. curculionis in alfalfa-growing areas in the United States. Deleterious effects to existing parasites could reduce somewhat the value of the fungal pathogens.

As a potential biological control agent of the alfalfa weevil, the fungal pathogens may be important in control of the weevil. More studies are therefore required to investigate all aspects of the interaction of these pathogens with the parasites and alfalfa weevil in order to determine their usefulness as part of an integrated program for the weevil.

CHAPTER III

SEASONAL MORTALITY OF THE ALFALFA WEEVIL LARVAE, PREPUPAE, AND PUPAE DUE TO INFECTION BY *ERYNIA* SPP.

Introduction

In Oklahoma, peak larval densities of *Hypera postica* (Gyllenhal) usually occur between March and early May, depending upon degree-day accumulations for development (Berberet et al. 1980). At the time of peak larval populations, over 60% of the larvae are typically third and fourth instars (Berberet et al. 1981). Onset of pupation occurs from late March to mid-April and is usually about 2 weeks earlier in Southern Oklahoma than in northern counties (Berberet et al. 1980).

The seasonal occurrence of *Erynia* spp. as observed in several localities has typically followed peak weevil larval densities (Puttler 1980, Nordin et al. 1983, Brandenburg 1985). As the decline of the weevil larval numbers occurs with onset of pupation, the percentage infection tends to increase. Los and Allen (1983) found that infection rates rose from 49 to 89% in declining weevil populations in Virginia, whereas Barney and Armbrust (1981) saw an increase from 6% in April to 22% in May in Illinois.

All instars may be infected, but Harcourt et al. (1984)

observed higher mortality in the later instars. Larvae infected as first and second instars generally formed tan-colored cadavers from which conidiophores were produced whereas infected third and fourth instars formed either tan or black cadavers with the latter containing resting spores (Harcourt et al. 1977, Watson et al. 1981, Nordin et al. 1983). Those weevils that died as prepupae or pupae invariably contained resting spores. A relatively small proportion of the pupal stage succumbs to the pathogens (Harcourt et al. 1977).

The conditions necessary for initiation of fungal infection were found to be high humidity resulting from moisture due to precipitation or irrigation (MacLeod et al. 1966, Barney and Armbrust 1981, Millstein et al. 1983). In addition, Brown and Nordin (1986) suggested that the extent to which infections spread through the weevil populations depended on host densities and the amount of inoculum present in the enviroment. Subsequently, natural termination of disease occurred when larval numbers fell below a threshold density of 1.5-1.7 per stem (Nordin et al. 1983). Although temperature might be related to the increase in infection, Millstein et al. (1982) observed that the pathogens were relatively impervious to direct influences of temperatures during an epizootic.

This study was conducted to determine the extent of mortality of the larvae, prepupae, and pupae of the alfalfa weevil due to infection by <u>Erynia</u> spp. on a seasonal basis

in Oklahoma.

Materials and Methods

My research was conducted at the Agronomy Research Station at Stillwater (Payne Co.) during 1986 and 1987. Prior to this study, data were collected from 1983 to 1985 and these were included to give a better long-term analysis of effects of _Erynia_ spp. on the weevil population. The site (ca 2 ha.) was maintained without insecticides from 1983 to 1985. During 1986-1987, one half of the area was sprayed with chlorpyrifos insecticide (0.55 kg ai/ha) to prevent complete defoliation of the alfalfa by weevils, such as occurred on the untreated portion. Sampling could then be extended until development of all weevils was completed without high mortality levels due to starvation.

Estimation of weevil larval population densities was conducted from March to May of each year. As larvae reached the third and fourth instars, samples were collected for rearing to determine fungal infection. In addition, numbers of cocoons were estimated as pupation began and samples of prepupae and pupae were held in the laboratory to determine percentages of fungal infection.

To estimate weevil larval population densities, samples of alfalfa foliage from 10, $0.1m^2$ areas were collected weekly in 1983-1985. During 1986-1987, sampling was done every 3-4 days to give more complete data on fluctuations in populations. Larvae were extracted from these samples by

use of Berlese funnels and stored in 50% alcohol for instar identification and count. Numbers of cocoons which contained healthy or diseased weevils were estimated from the $0.1m^2$ areas sampled for larvae after the foliage was removed.

Following the first occurrence of fungal infections, collections of 50 larvae for rearing were made at 2-4 day intervals during 1983 to 1985. To increase the sampling precision, 100 larvae were collected on each date in 1986 and 1987. Larvae were placed individually in sterile vials and then reared on greenhouse-grown alfalfa trifoliolates to limit occurrence of infections to those larvae which had begun in the field.

Cocoons (100/date) collected for rearing were taken from the soil surface about crowns in the areas where samples of foliage for larval density estimates had been removed. These were then placed individually in vials after the life stage in each (prepupa, pupa, or adult) was recorded. Empty cocoons were not included in collections. All stages were checked at 1-2 day intervals for adult emergence or death due to fungal infection.

Weevils which died as a result of fungal infection had characteristic tan (conidiospore formation) or black (resting spore formation) coloration. Fungal infection was verified by preparing wet mounts of dead larvae, prepupae, and pupae for microscopic observation. Infection was indicated by the presence of conidiophores and mycelia or

resting spores, and the percentage of weevils of each life stage which had been infected was recorded. The types of resting spores present were also recorded to detect incidence of the two Erynia spp.

Graphs were constructed for each year to illustrate the seasonal trends of weevil populations based on the larval population estimates and the incidence of fungal infection based on larval rearing. The first and second instars, which in my observations were seldom infected by fungi, were grouped together as small larvae. The third and fourth instar, which frequently were infected by fungi were combined as large larvae. A prediction curve was drawn for the incidence of large larvae assuming there had been no mortality due to fungi or other causes. Positions of points along this prediction line were determined on the vertical axis corresponding to densities of small larvae and on the horizontal axis according to dates after which 200 degrees days (dd) had accumulated from the respective sampling dates for small larvae. The 200 dd value is approximately equivalent to the developmental time required for two instar periods (or growth from small to large larvae). Calculation of dd for alfalfa weevil development used in positioning points along the prediction line was done according to tables published by Wedburg et al. (1977). The accumulated dd on a monthly basis for February through May from 1983 to 1987 and the long-term (1973-1982) average degree-days for these months were calculated.

Tables were constructed to show the percentage of fungal-infected prepupae and pupae from rearing. Assistance in identification of fungal pathogens based on characteristics of spores was provided by Dr. R. A. Humber, Boyce Thompson Institute, Cornell University, New York.

Results

In the laboratory, tan-colored cadavers were found attached by rhizoids to leaves or sides of plastic vials in which weevil larvae were reared. In some instances, conidia had been discharged in a white halo around the cadavers. A few third and fourth instars as well as all infected prepupae and pupae were blackened and shrivelled, with no evidence of rhizoid formation. Recently killed black cadavers were soft and filled with a brownish-black fluid. After a period of several days, they became dry and tended to crumble. *Erynia punctata* (Garbowski) was the predominant pathogen found in these blackened cadavers. Few cadavers containing resting spores of *E. phytonomi* were observed. Two other types of resting spores, one with thin walls and the other with reticulations on the surface, were also observed. The identities of these resting spores are not known.

In 1983, intermittent cold weather conditions were observed in March and April with minimum temperatures averaging ca. $-1^{0}C$ from 17-24 March. The degree-day accumulation was far less than average for the month (Table

I). Frequent rainfall was recorded during the period that weevil larvae were present and conditions were favorable for fungal development.

Larval numbers increased throughout March, but a sharp decline for large larvae occurred following a hard freeze on April 5. Large larvae on the exposed parts of the plants were apparently more affected by the cold weather than the smaller instars which occur in the terminals. The peak density for small larvae was $258/0.1m^2$ in early April whereas large larval numbers were $190/0.1m^2$ on 7 May (Fig. 1). A week later, fungal infection was estimated at 85% and incidence of disease remained high until virtually all larvae were killed or had pupated. On 22 May, weevil larvae numbered less than $seven/0.1m^2$. The predicted curve indicated a longer period of occurrence for large larvae had disease not been a factor.

A warmer spring with about 123 dd in February (Table I) of 1984 allowed rapid hatching of weevil eggs which resulted in the presence of high numbers of small larvae early in March (Fig. 2). Large larvae also increased rapidly in numbers and approximated the population of small larvae ($500/0.1m^2$) by mid-April. Prior to this peak, fungal infection was detected at low levels as early as 29 March. The increase in disease spanned a period of 3 weeks and an estimated 91% infection was observed on 9 May. A distinct difference between the observed and predicted curves for large larval incidence resulted due to extensive mortality

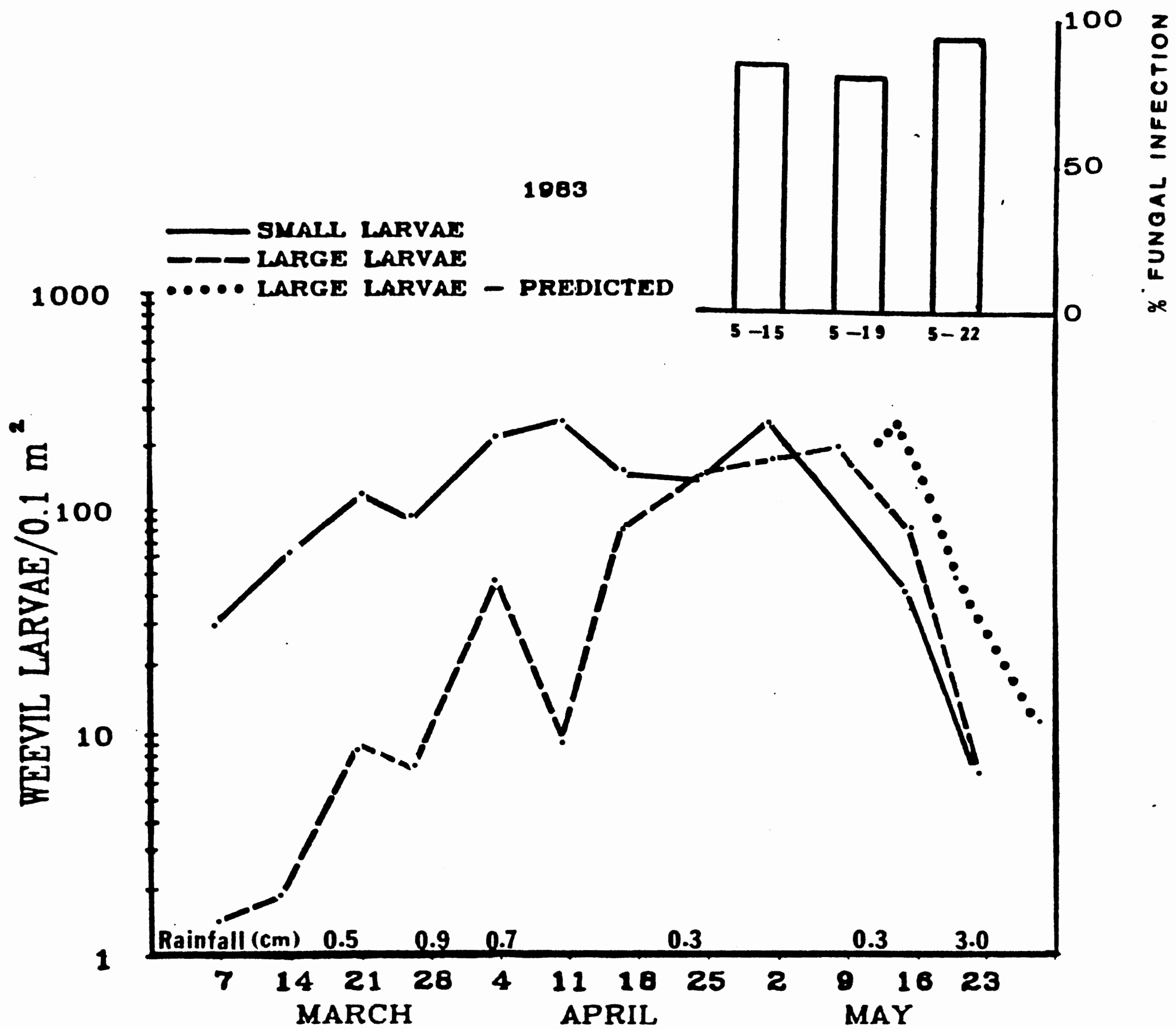
TABLE I

MONTHLY DEGREE-DAY ACCUMULATIONS (^{0}F) FOR FEBRUARY TO MAY DURING 1983-1987 WITH LONG-TERM AVERAGES (1973-1982) CALCULATED FOR THESE MONTHS, STILLWATER, PAYNE CO., OKLAHOMA.

YEAR	FEBRUARY	MARCH	APRIL	MAY[a]
1983	44	130	214	239
1984	123	116	272	269
1985	41	221	451	298
1986	172	282	416	332
1987	85	186	440	373
1973-1982 (Averages)	67	193	357	301

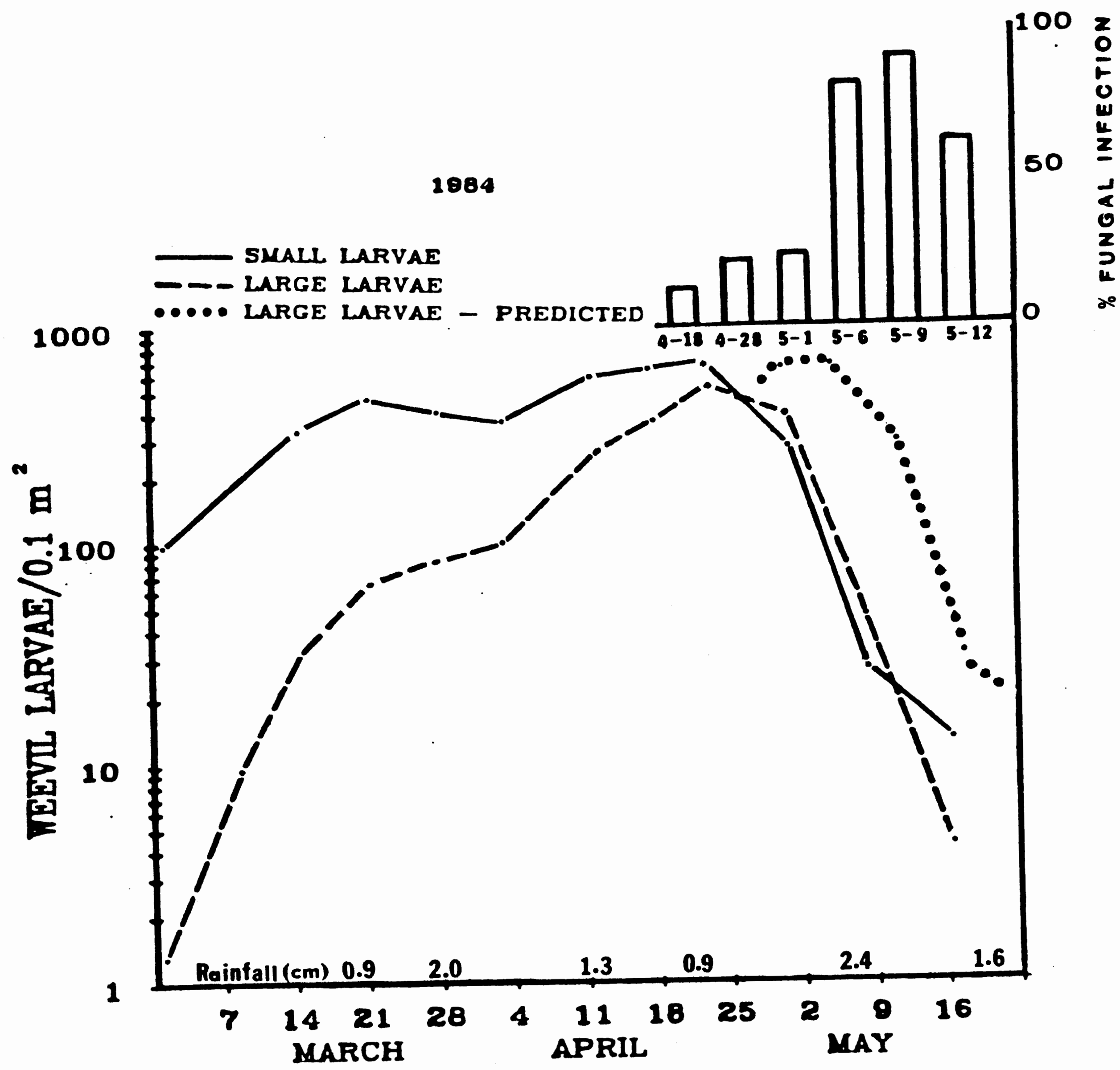
a Degree-days accumulated to 15 May

Figure 1. Population Densities of Alfalfa Weevil and % Fungal Infection of *Erynia* spp. in Larval Rearing at Stillwater, 1983. (Predicted line indicates theoretical population density for large larvae without fungal-induced mortality)



1983
SMALL LARVAE
LARGE LARVAE
LARGE LARVAE – PREDICTED
WEEVIL LARVAE/0.1 m²
1000
100
10
1
% FUNGAL INFECTION
100
50
0
5 –15
5 –19
5 – 22
Rainfall (cm) 0.5 0.9 0.7 0.3 0.3 3.0
7 14 21 28 4 11 18 25 2 9 16 23
MARCH
APRIL
MAY

Figure 2. Population Densities of Alfalfa Weevil and % Fungal Infection of *Erynia* spp. in Larval Rearing at Stillwater, 1984. (Predicted line indicates theoretical population density for large larvae without fungal-induced mortality)


1984
SMALL LARVAE
LARGE LARVAE
LARGE LARVAE – PREDICTED
WEEVIL LARVAE/0.1 m²
1000
100
10
1
% FUNGAL INFECTION
100
50
0
4-18
4-28
5-1
5-6
5-9
5-12
Rainfall (cm)
0.9
2.0
1.3
0.9
2.4
1.6
7
14
21
28
4
11
18
25
2
9
16
MARCH
APRIL
MAY

caused by the pathogens.

In 1985, cool conditions with lower than normal degree-day accumulation for February (Table I) resulted in later hatching of eggs than had occurred in 1984. This was followed by warm weather during the second week of March which caused a rapid increase in numbers of small larvae (Fig. 3). Large larvae were not detected until 23 March. Fungal infections were detected at high levels as larval numbers declined in April and 100% mortality was estimated on 1 May. The expected decline in numbers of large larvae due to pupation was hastened considerably by the fungal pathogens as indicated by comparison of the lines for predicted and actual population densities (Fig. 3).

Unusually warm temperatures with more than 280 dd accumulated were recorded for both March and April of 1986 (Table I). Rapid hatching of weevil eggs resulted in a density of 635 small larvae/0.1m^2 as early as 22 March (Fig. 4). Large larvae failed to attain a similar population density due to occurrence of fungal disease. A sharp decline in numbers of large larvae came after a peak of about 200/0.1m^2 when over 90% of larvae reared were infected. In the area treated with chlorpyrifos (not shown on graph), an infection percentage of more than 80% was observed from 17 to 23 April and weevil populations did not exceed 100/0.1m^2. Weather conditions in 1986 were optimal for an earlier and more prolonged fungal epizootic than in previous years.

Figure 3. Population Densities of Alfalfa Weevil and % Fungal Infection of _Erynia_ spp. in Larval Rearing at Stillwater, 1985. (Predicted line indicates theoretical population density for large larvae without fungal-induced mortality)

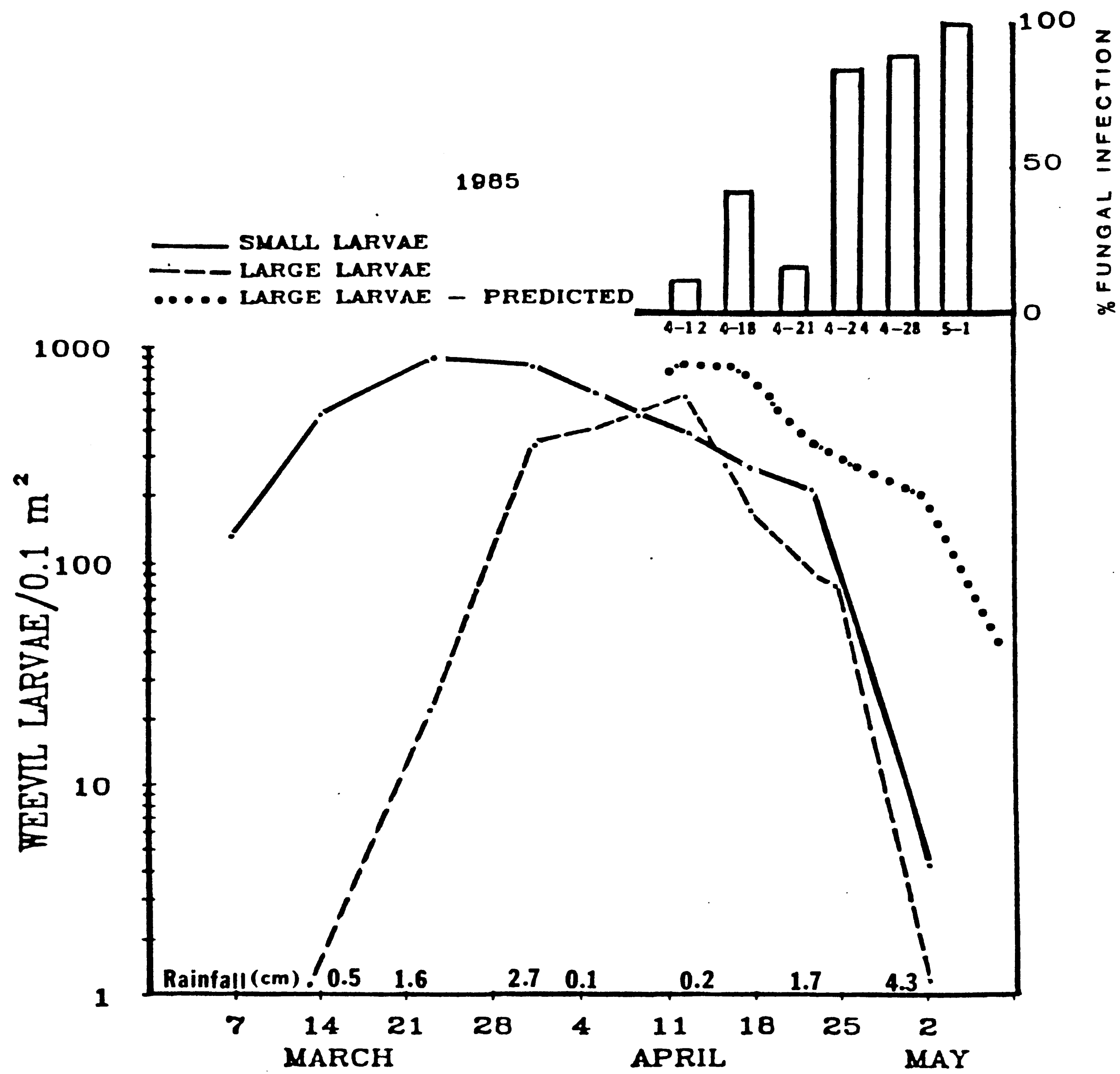
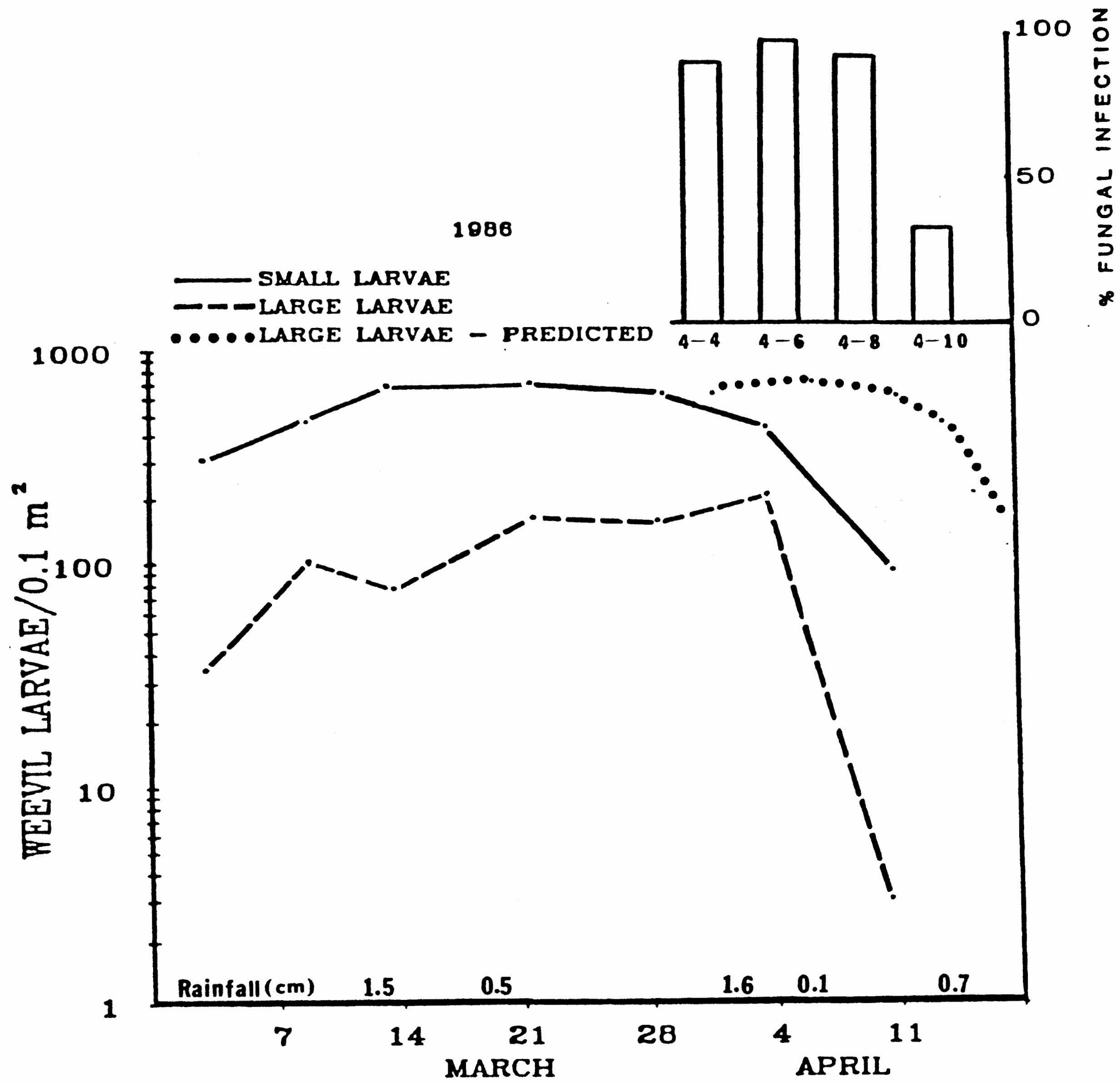

1985
SMALL LARVAE
LARGE LARVAE
LARGE LARVAE – PREDICTED
100
50
0
% FUNGAL INFECTION
4-12
4-18
4-21
4-24
4-28
5-1
1000
100
10
1
WEEVIL LARVAE/0.1 m²
Rainfall (cm)
0.5
1.6
2.7
0.1
0.2
1.7
4.3
7
14
21
28
4
11
18
25
2
MARCH
APRIL
MAY

Figure 4. Population Densities of Alfalfa Weevil and % Fungal Infection of *Erynia* spp. in Larvae Collected for Rearing from the Untreated Area at Stillwater, 1986. (Predicted line indicates theoretical population density for large larvae without fungal-induced mortality).



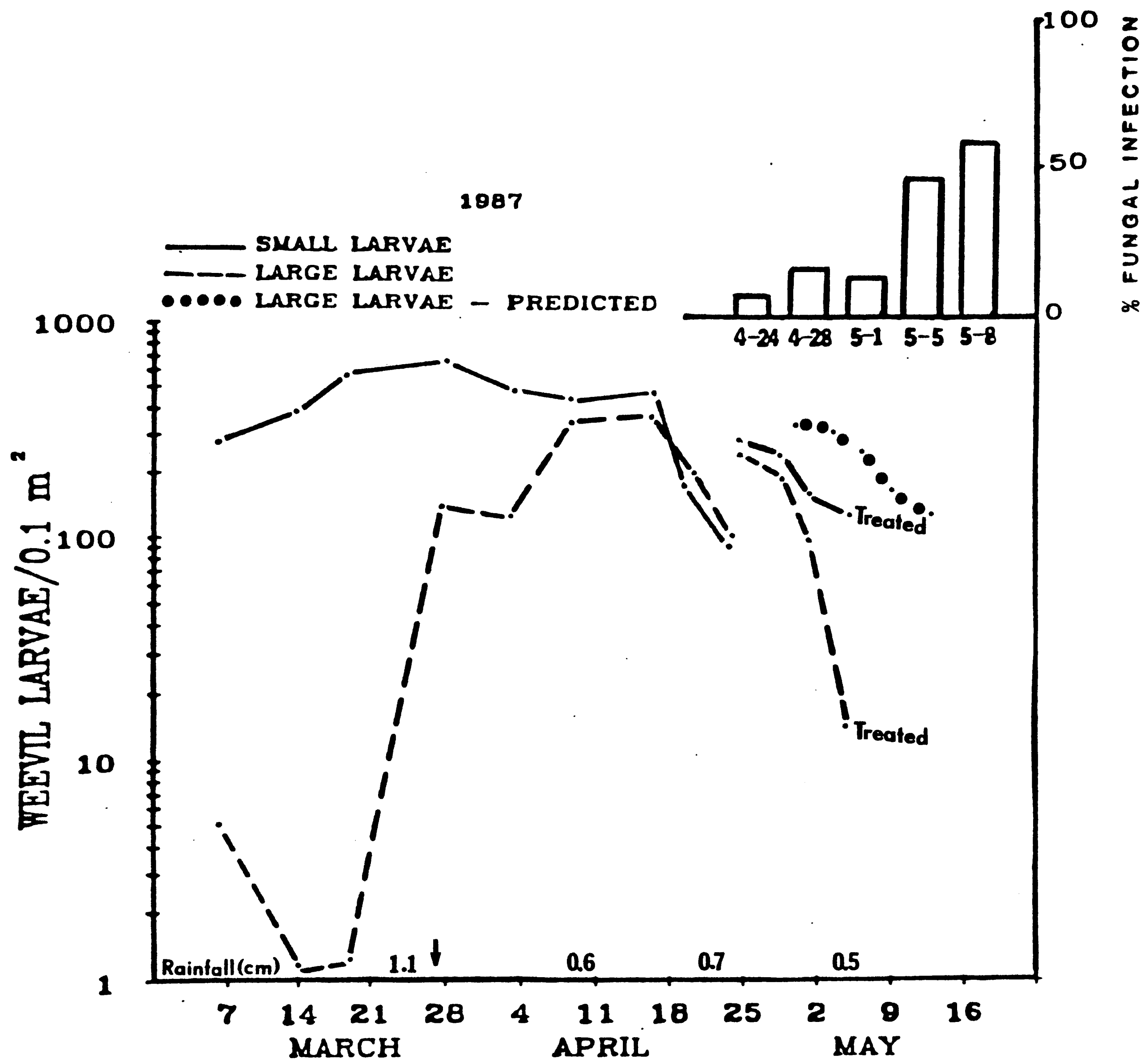
1986
SMALL LARVAE
LARGE LARVAE
LARGE LARVAE – PREDICTED
% FUNGAL INFECTION
100
50
0
4-4
4-6
4-8
4-10
WEEVIL LARVAE/0.1 m²
1000
100
10
1
Rainfall (cm)
1.5
0.5
1.6
0.1
0.7
7
14
21
28
4
11
MARCH
APRIL

In 1987, a period of freezing weather with minimum temperatures near -7^{0}C occurred from 27 March to 3 April. Heavy damage to the alfalfa plants and mortality of large larvae resulted (Fig. 5). Despite the fact that degree-day accumulations were higher than average for March and April (Table I), numbers of large larvae did not reach levels that might have been expected. With the return of warmer conditions, large larvae increased to a peak of 379.5/0.1m^2 by 18 April. Infection by Erynia was estimated at 2% on 16 April. When sampling was continued in the treated area (Fig. 5-treated), fungal infections reached 60% on 8 May and large larvae numbered less than 10/0.1m^2. Extremely dry conditions during March, April, and May, and low humidities apparently prevented the occurrence of extensive fungal epizootics in 1987.

Rearing of Prepupae and Pupae

Blackened cadavers were enclosed in cocoons that were usually intact. In cases where rainfall had occurred after death, cadavers were decomposed with traces of brownish-black fluid remaining in cocoons. Over the years 1983-1987, the percentage of infection in prepupae frequently exceeded 50% whereas infection of pupae rarely exceeded 20% (Tables II-VI). In all years except 1987, cocoon numbers were much lower than the larval population densities due to mortality of the large larvae before cocoons were spun. There were higher numbers of infected pupae later in each season

Figure 5. Population Densities of Alfalfa Weevil and % Fungal Infection of *Erynia* spp. in Larvae Collected for Rearing from the Area Treated with Chlorpyrifos (0.55 kg ai/ha) at Stillwater, 1987. (Arrow indicates date of Chlorpyrifos application; Predicted line indicates theoretical population density for large larvae without fungal-induced mortality).



1987
SMALL LARVAE
LARGE LARVAE
LARGE LARVAE – PREDICTED
100
50
0
% FUNGAL INFECTION
4-24 4-28 5-1 5-5 5-8
1000
100
10
1
WEEVIL LARVAE/0.1 m²
Treated
Treated
Rainfall(cm)
1.1
0.6
0.7
0.5
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY

TABLE II

ESTIMATED DENSITY OF ALFALFA WEEVIL COCOONS AND PERCENT PREPUPAE COLLECTED FOR REARING[a] WITH PERCENT FUNGAL INFECTIONS IN PREPUPAE AND PUPAE, STILLWATER, 1983.

	WEEVIL COCOONS		% W/RESTING SPORES	
DATE	#/0.1M^2	% W/PREPUPAE	PREPUPAE	PUPAE
MAY 07	20	49	0.0	0.0
MAY 15	60	44	17.0	1.0
MAY 19	-[b]	44	36.0	5.0
MAY 22	-	43	32.0	3.0

a Based on a sample size of 100 cocoons
b Density of cocoons not estimated

TABLE III

ESTIMATED DENSITY OF ALFALFA WEEVIL COCOONS AND PERCENT PREPUPAE COLLECTED FOR REARING[a] WITH PERCENT FUNGAL INFECTIONS IN PREPUPAE AND PUPAE, STILLWATER, 1984.

	WEEVIL COCOONS		% W/RESTING SPORES	
DATE	#/0.1M^2	% W/PREPUPAE	PREPUPAE	PUPAE
APR 12	3	39	5.1	0.0
APR 18	5	35	0.0	0.0
APR 24	10	61	1.6	7.7
APR 28	-[b]	44	1.8	9.1
MAY 01	100	47	10.6	3.8
MAY 05	-	17	5.9	19.3
MAY 09	250	28	60.7	13.9
MAY 12	-	38	68.4	20.9

a Based on sample size of 100 cocoons
b Density of cocoons not estimated

TABLE IV

ESTIMATED DENSITY OF ALFALFA WEEVIL COCOONS AND PERCENT PREPUPAE COLLECTED FOR REARING[a] WITH PERCENT FUNGAL INFECTIONS IN PREPUPAE AND PUPAE, STILLWATER, 1985.

	WEEVIL COCOONS		% W/RESTING SPORES	
DATE	#/0.1M^2	% W/PREPUPAE	PREPUPAE	PUPAE
APR 12	20	98	9.2	0.0
APR 18	80	35	48.6	27.0
APR 21	-[b]	24	86.4	11.0
APR 24	200	32	84.4	14.5
APR 27	-	21	100.0	23.0
MAY 01	-	28	91.7	52.2

a Based on a sample size of 100 cocoons
b Density of cocoons not estimated

TABLE V

ESTIMATED DENSITY OF ALFALFA WEEVIL COCOONS AND PERCENT PREPUPAE COLLECTED FOR REARING[a] WITH PERCENT FUNGAL INFECTIONS IN PREPUPAE AND PUPAE, STILLWATER, 1986.

	WEEVIL COCOONS		% W/RESTING SPORES	
DATE	#/0.1M^2	% W/PREPUPAE	PREPUPAE	PUPAE
UNTREATED AREA				
MAR 22	5	100	0.0	0.0
MAR 29	45	78	0.0	0.0
APR 04	100	28	7.1	12.5
APR 06	-[b]	71	62.0	23.1
APR.08	-	37	86.5	12.3
APR 10	-	15	26.7	14.7
APR 12	-	34	85.3	10.0
APR 15	-	33	60.6	10.4
TREATED AREA[c]				
APR 12	23	37	16.2	0.0
APR 15	-	25	56.0	5.6
APR 17	28	21	66.7	0.0
APR 19	-	16	68.8	1.2
APR 21	-	18	83.3	5.0
APR 23	5	12	50.0	5.8

a Based on a sample size of 100 cocoons
b Density of cocoons not estimated
c Area treated with chlorpyrifos (0.55 kg ai/ha)

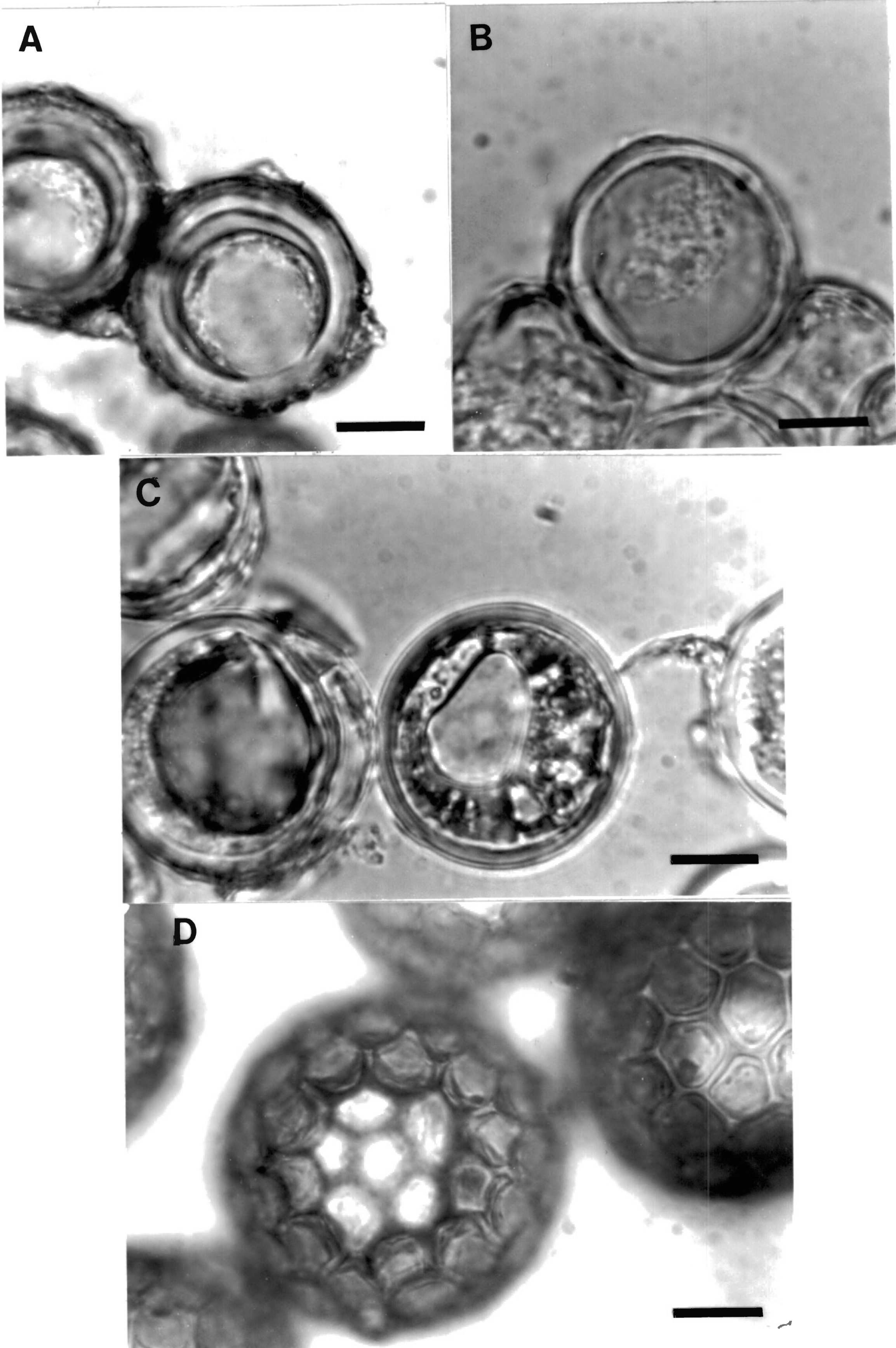
TABLE VI

ESTIMATED DENSITY OF ALFALFA WEEVIL COCOONS AND PERCENT PREPUPAE COLLECTED FOR REARING[a] WITH PERCENT FUNGAL INFECTIONS IN PREPUPAE AND PUPAE, STILLWATER, 1987.

	WEEVIL COCOONS		% W/RESTING SPORES	
DATE	#/0.1M^2	% W/PREPUPAE	PREPUPAE	PUPAE
UNTREATED AREA				
APR 03	-[b]	100	0.0	0.0
APR 10	4	100	0.0	0.0
APR 16	23	83	0.0	0.0
APR 20	300	31	0.0	0.0
APR 24	450	21	9.5	4.0
TREATED AREA[c]				
APR 20	11	43	0.0	0.0
APR 24	23	54	1.9	0.0
APR 28	27	41	0.0	1.8
MAY 01	-	43	0.0	0.0
MAY 05	70	16	6.3	1.3
MAY 08	-	19	36.8	5.3

a Based on a sample size of 100 cocoons
b Density of cocoons not estimated
c Area treated with chlorpyrifos (0.55 kg ai/ha)

Figure 6. Types of Resting Spores Observed in Fungal-Infected Alfalfa Weevil - (**A**) *Erynia punctata*, (**B**) Thin-walled spore (Unknown), (**C**) *Erynia phytonomi*, and, (**D**) Reticulated spore (unknown). Bar= 10 μ


A
B
C
D

because most healthy pupae had already molted to adults and predominantly cocoons with dead pupae remained.

Four types of resting spores with different morphological forms were observed (Fig. 6). The prevalent type, being dark brown and verrucose in appearance, was identified by Dr. R. A. Humber as E. punctata. The diameter of these spores ranged from 27.5-40 μ. The smooth-walled and hyaline spores identified as E. phytonomi, were seen in few cadavers and ranged in diameter of 25-40 μ. The third type was thin-walled and yellowish-brown in color. These spores were variable in diameter ranging from 25-42.5 μ and were common in light-brown cadavers that crumbled easily. The fourth type of resting spore was uncommon and much larger (diameter > 70 μ). This resting spore appeared to subdivide within a membranous cover that came off easily when the spore was pressed lightly. The identities of these last two pathogens are unknown.

Discussion

Berberet et al. (1980) observed that alfalfa weevil egg numbers tended to decrease through March and April of each year as hatching accelerated due to warm temperatures and as reproductive capacity of adults was exhausted. In my study, the densities of small larvae tended to be high in March and early April and then declined gradually as hatching was completed. In all years except 1983, the decline in larval populations during April was due in part to starvation as

alfalfa was completely defoliated as a result of weevil feeding. Brandenburg (1985) suggested that pupation was the most likely reason for declining weevil populations even when a high incidence of disease occurred. However, I believe that reductions in large larvae resulted from a combination of pupation, starvation, and fungal infection, depending on the time of occurrence of epizootics.

Except for 1984 and 1986, the pattern of disease incidence that I observed was similar to that reported by Los and Allen (1983) with low percentages of infection occurring at peak host densities followed by an increase in percent mortality as weevil larvae declined in numbers. The decline in disease incidence later in the season of 1984 and 1986 probably signaled the termination of fungal epizootics as less than 5 larvae/0.1m^2 were available. This supported the result of Nordin et al. (1983) who suggested a threshold of 1.5-1.7 larvae/stem for the occurrence of epizootics by Erynia spp.

In addition, the pathogens usually infected larvae too late to prevent serious weevil damage to alfalfa. Economic losses were incurred prior to mortality of the larvae from disease. This observation concurs with that made by Brandenburg (1985) and Johnson et al. (1984). In my study, disease also occurred at the time when oviposition by weevils was almost completed. This was the main reason for a relatively low number of larvae in the area treated with chlorpyrifos during 1986 and 1987. Fungal disease then

further helped to reduce weevil numbers.

Results from the study made by Watson et al. (1981) indicated that when infected as first or second instars, conidial production from cadavers generally resulted. In my observation, the tan-colored cadavers were usually in the third and fourth instars. Although a few infected third and fourth instars also produced resting spores, this fungal stage was predominant in all infected prepupae and pupae. It is apparent that the physiological age of the weevil larvae is an important determinant for the spore form found in cadavers as hypothesized by Newman and Carner (1975). Certain physiological cues related to the maturation of the host as it nears pupation may be the means for triggering resting spore production. These spores are an essential survival stage, providing the pathogens the capability for prolonged dormancy between weevil infestations until favorable conditions such as moisture and susceptible hosts are again available (Nordin 1987). Germination of these resting spores then provides the inoculum for the initiation of infections each year (Brown and Nordin 1986).

The observation that a higher proportion of prepupae than pupae was infected was also made by Harcourt et al. (1977). It is probable that those which died as prepupae were infected as fourth instars prior to spinning cocoons. Watson et al. (1981) observed that infected larvae died within 7 days, whereas an incubation period of about 5 days was suggested by Nordin et al. (1983). There is therefore a

high likelihood that infected larvae would succumb to the disease before developing into the prepupal stage. As few pupae were usually found to have been killed by the disease, infection in the pupal stage was probably much less common than during the fourth instar.

High host densities and sufficient moisture from rainfall were available to promote epizootics in all years except for 1987. The prolonged disease incidence in 1984 and 1985 resulted from this combination of optimal conditions. The importance of rainfall was evident in results by Johnson et al. (1984) who observed that heavy rains initiated an epizootic, and those of Barney and Armbrust (1981) who stated that rainfall was the overriding factor in seasonal larval infection rates. Although the influence of temperature is still not fully established, unusually warm temperatures might have aided the infection process of the fungi in 1986.

A relatively high proportion of the weevil population appeared to have died from fungal infection from 1983-86. However, estimated mortality on a generational basis indicates that this may not have been the case. As an example, in 1984, large larvae numbered at $373/0.1m^2$ on 1 May (Fig. 2). By 9 May, 250 cocoons/$0.1m^2$ (Table II) were estimated to be present which suggested that decline in large larvae in the first week of May was due mainly to pupation and that most larvae escaped the extensive fungal disease epizootic observed from 6-12 May. Since a high

percentage of weevils in cocoons were pupae and pupal mortality was less than 20% during the epizootic, at least 200 weevils/0.1m^2 might have survived to the adult stage. Despite the fact that fungal mortality in larvae once exceeded 90% (9 May), the proportion of the larvae for the weevil generation that actually died from fungal disease was therefore much lower.

To use *Erynia* spp. effectively in the control of the alfalfa weevil, various factors such as time of initiation and termination of disease, the requirements for formation of resting spores, and the most optimal conditions for infection to occur, should be further determined. The essential requisite of a biological control program is to synchronize infection by these fungi with the increase of weevil larvae prior to occurrence of economic damage to the alfalfa.

CHAPTER IV

EFFECT OF FUNGAL EPIZOOTICS IN THE ALFALFA WEEVIL POPULATION ON SURVIVAL AND TOTAL PARASITISM OF BATHYPLECTES CURCULIONIS

Introduction

In Oklahoma, average parasitism of Hypera postica Gyllenhal by Bathyplectes curculionis Thomson at peak weevil density ranged from 2.9% in 1973 to 50% in 1977 (Berberet et al. 1980). A decline in rates of parasitism has been observed over the past 7 years which seems to have begun with extensive mortality of oversummering parasites due to the hot, dry weather conditions in 1980 (Doss and Berberet 1986). With the appearance of the fungal pathogens, Erynia spp., in Oklahoma since 1983, numbers of parasites may decline further due to competition of the two types of natural enemies.

The emergence of first generation B. curculionis adults in March typically coincides with increasing weevil larval densities prior to mid-April (Berberet et al. 1978). Nondiapausing parasites begin a second generation with emergence of adults in April and May (Berberet et al. 1978). The appearance of these adults coincides with peak incidence of fungal disease in declining weevil populations which results in direct competition for hosts by both natural

enemies of the weevil. Since few studies have been done to show the results of such competition between fungal agents and parasites, this study was designed to determine the effect of fungal epizootics in weevil populations on effective parasitism and survival of B. curculionis.

Methods and Materials

This study was conducted in conjunction with the work described in Chapter III during 1986 and 1987. Data collected in 1983-1985 at Stillwater were analyzed together with my studies from 1986 to 1987. In addition, data for 5 years (1983-1987) collected from the South Central Research Station in Chickasha, (Grady Co.) were included. Sampling was conducted in unsprayed alfalfa except when chlorpyrifos (0.55 kg/ha) insecticide was used in 1986 and 1987 at Stillwater to prevent complete loss of the foliage to weevil larvae. Sampling was continued in the treated area after most larvae in the untreated area died of starvation from lack of available alfalfa plants.

Larvae were individually collected in sterile plastic vials for rearing. The numbers of larvae collected and the procedures used in sampling and rearing were the same as described in Chapter III. Percentages of larvae parasitized and/or infected were recorded for each date.

At 2-3 day intervals, 300-500 larvae were collected using a sweep-net from which 100 larvae were subsampled for dissection. During 1986-1987, this subsample was increased

to 200 after first occurrence of fungal disease. After collection, live larvae were stored at 5^{0}C until they were dissected. Larvae were placed in distilled water and dissected under a stereomicroscope. Parasitized larvae contained eggs or larvae of B. curculionis whereas fungal infection was indicated by presence of hyphae. The numbers of larvae that contained hyphae and/or parasites were then recorded and percentages were calculated to reflect occurrence of each mortality agent.

Graphs were drawn for each location by year to compare the percent occurrence of fungal infection and parasitism from the rearing of larvae and dissection process. The trends of parasitism during years prior to and after the appearance of Erynia spp. in Stillwater were also illustrated on a series of graphs using data from the dissections of larvae collected from 1978 to 1987.

Using data obtained from dissections of weevil larvae at Stillwater, Chi-square (χ^2) tests were performed to determine if there was a statistically significant relationship between occurrence of fungal infections and parasitism for the years 1983-1987. The χ^2 value was calculated using this formula (Little and Hills 1978):

$$\chi^2 = \text{SUM OF } \frac{(\text{OBSERVED FREQUENCY} - \text{EXPECTED FREQUENCY})^2}{\text{EXPECTED FREQUENCY}}$$

Significant values of the χ^2 tests suggest rejection of the hypothesis of independent occurrence of pathogens and the parasite. A table was constructed to show the observed and expected frequencies of the two variables with the

respective χ^2 values. Frequency values in the table refer to the observed numbers of larvae that were parasitized or infected, as well as those containing both agents, or having neither parasites and infection.

Results

Different results were observed for the rearing and dissections of weevil larvae. The rearing process gave a good estimation of fungal infection whereas larval dissection was a better method for the estimation of parasitism. When infected, larvae in rearing inevitably died of disease even though they might have been parasitized. As a result, a low percentage of parasitism was detected during the occurrence of fungal disease (Figs. 6-15). An inverse relationship apparently existed between levels of fungal infection and parasitism in larval rearing. Parasitism tended to increase only when disease incidence was at relatively low levels.

In dissections, parasites could be detected even in infected larvae. More parasites were actually present than indicated in the rearing process. The eggs and/or larvae of B. curculionis were usually easy to discern even when fungal hyphae were present. In contrast, fungal disease tended to be underestimated because the presence of hyphae could not be detected in some parasitized larvae that might be in the early stages of infection.

At Stillwater, in all years except 1987, fungal

Figure 7. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Stillwater OK., during 1983.

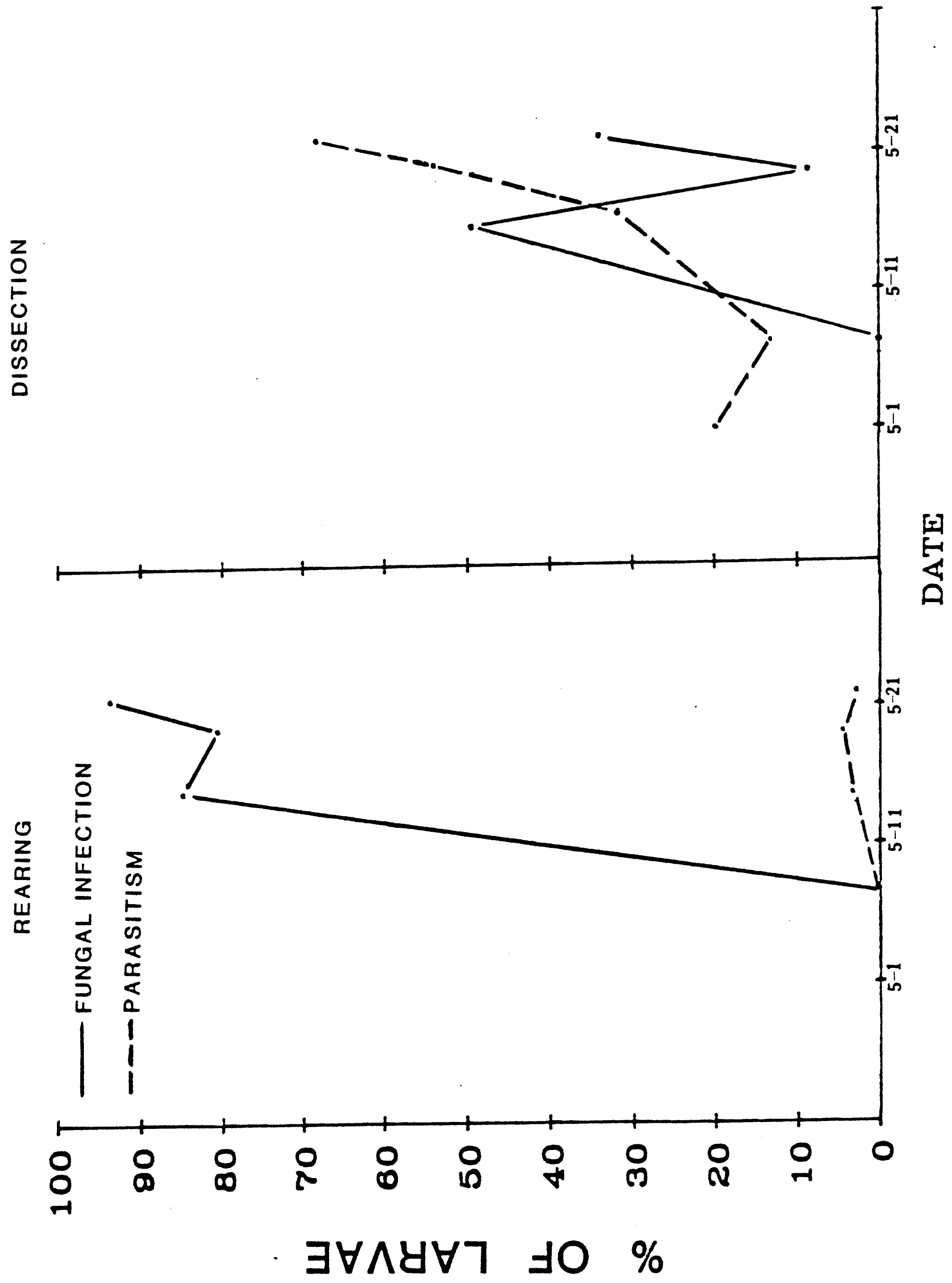

REARING
DISSECTION
FUNGAL INFECTION
PARASITISM
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
5-1
5-11
5-21
5-1
5-11
5-21
DATE

Figure 8. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Stillwater OK., during 1984.

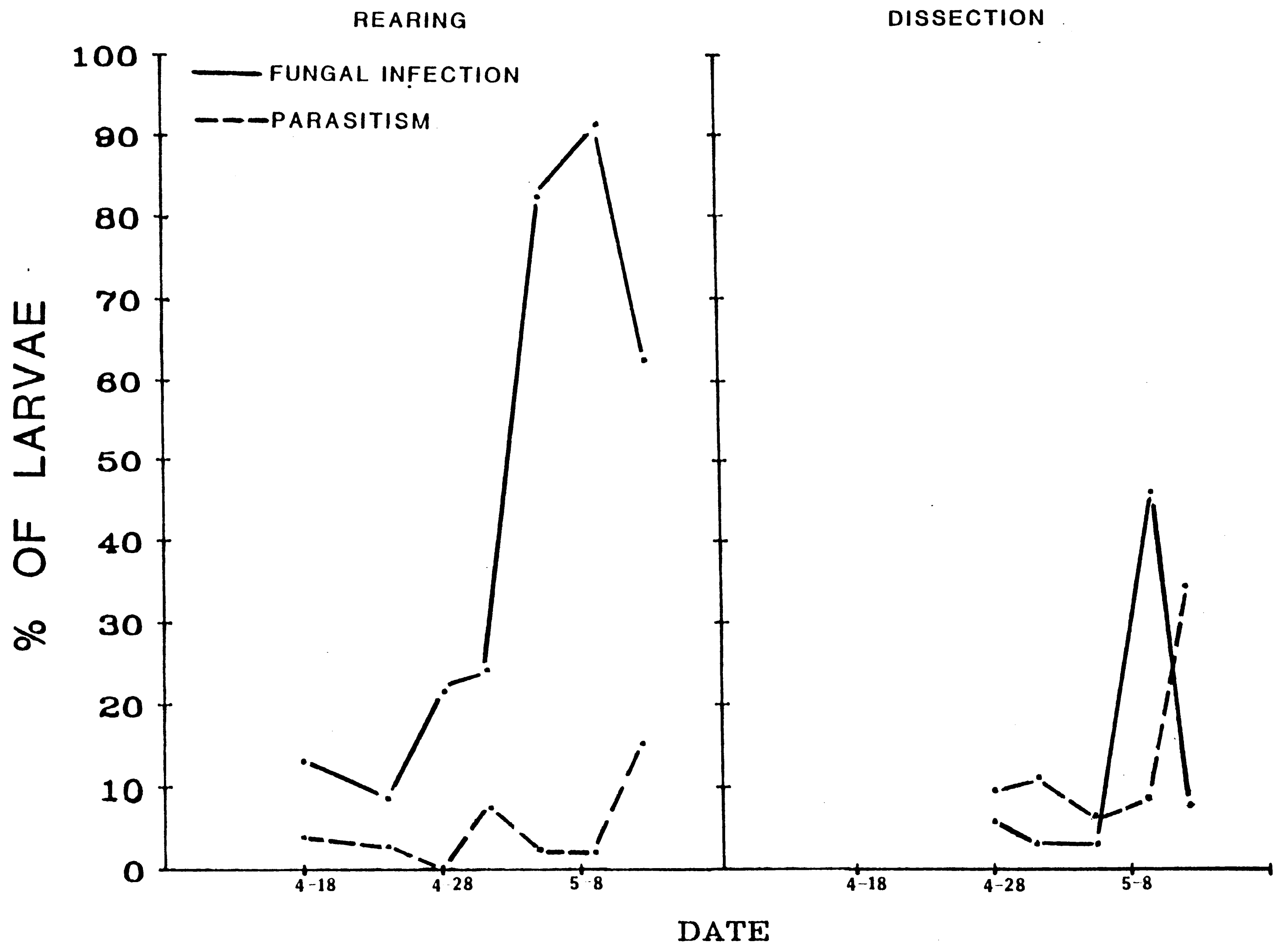
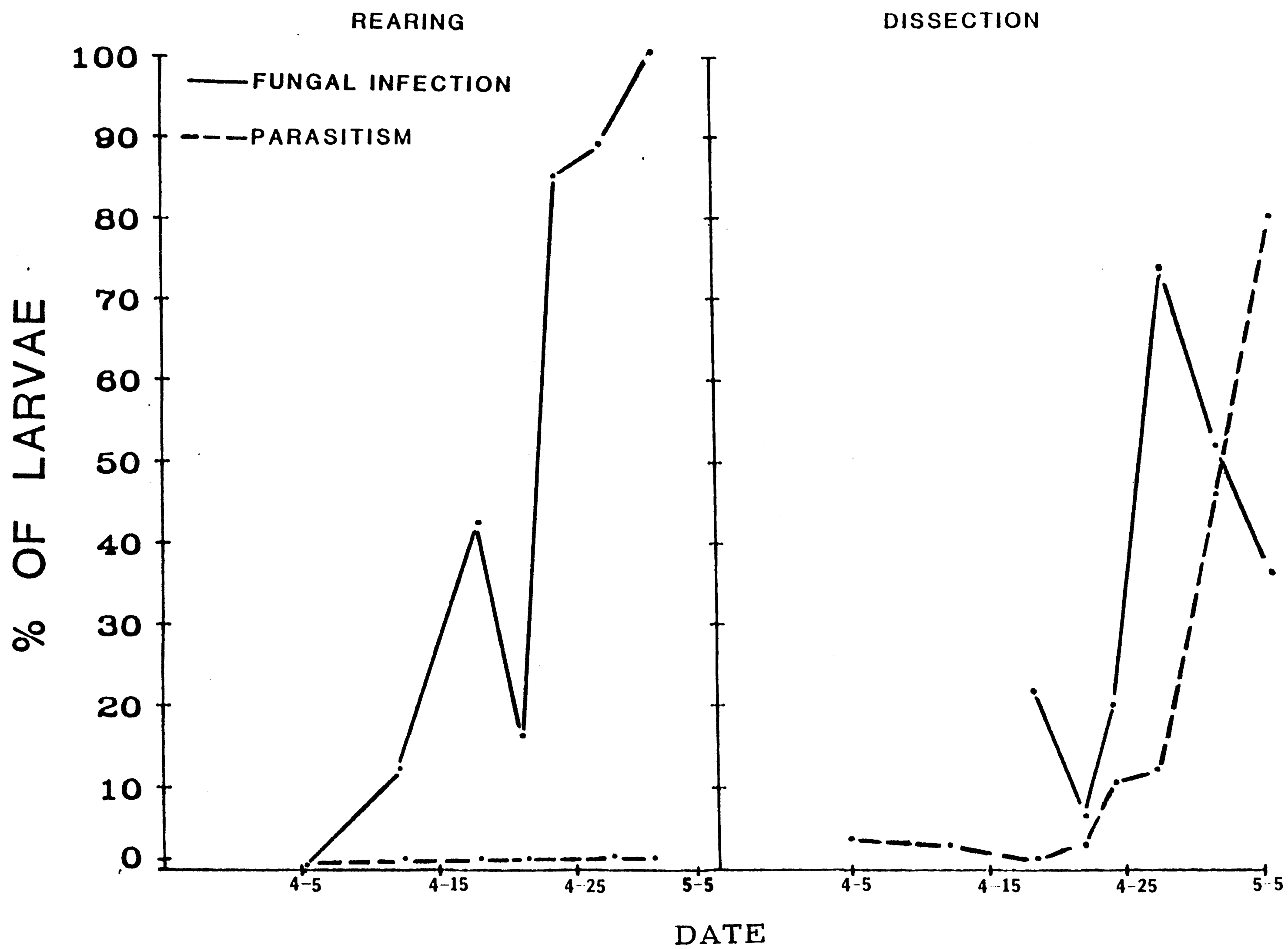
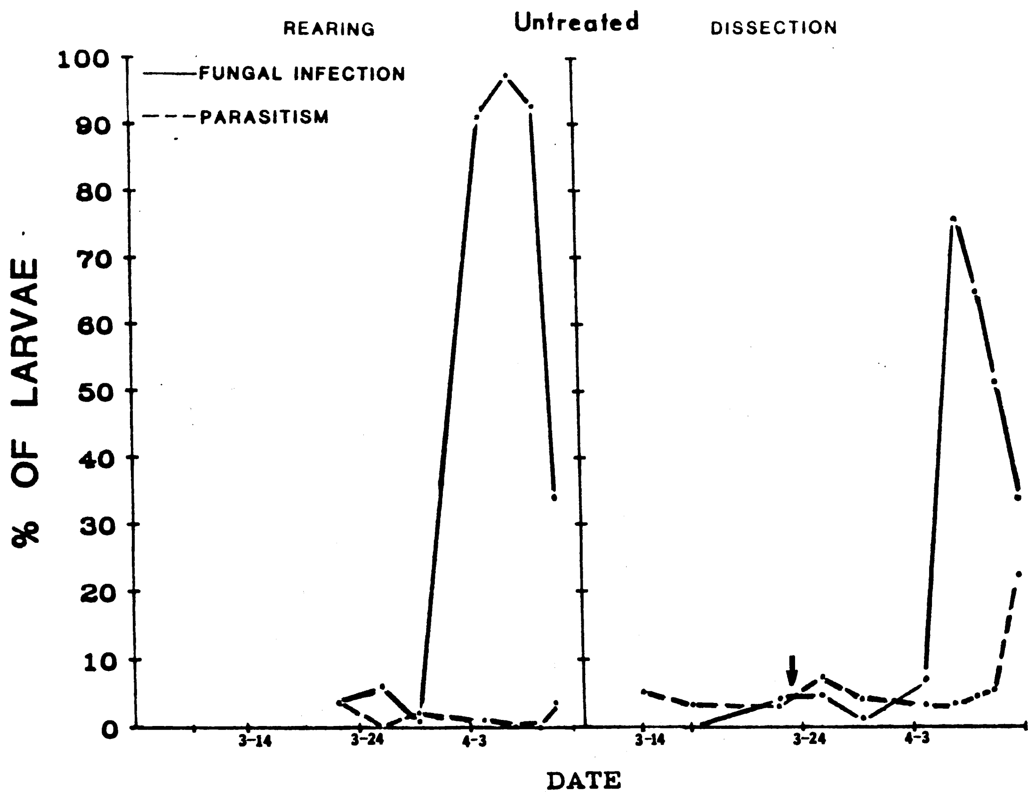

REARING
DISSECTION
FUNGAL INFECTION
PARASITISM
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
4-18
4 28
5 8
4-18
4-28
5-8
DATE

Figure 9. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Stillwater OK., during 1985.



REARING
DISSECTION
FUNGAL INFECTION
PARASITISM
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
4-5
4-15
4-25
5-5
DATE

Figure 10. Percentages of Infection by _Erynia_ spp. and Parasitism by _Bathyplectes curculionis_ in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted for the Untreated and Chlorpyrifos-Treated Areas at Stillwater OK., during 1986. (Arrow indicates date of chlorpyrifos application).


REARING
Untreated
DISSECTION
100
90
80
70
60
50
40
30
20
10
0
—FUNGAL INFECTION
---PARASITISM
% OF LARVAE
3-14
3-24
4-3
3-14
3-24
4-3
DATE

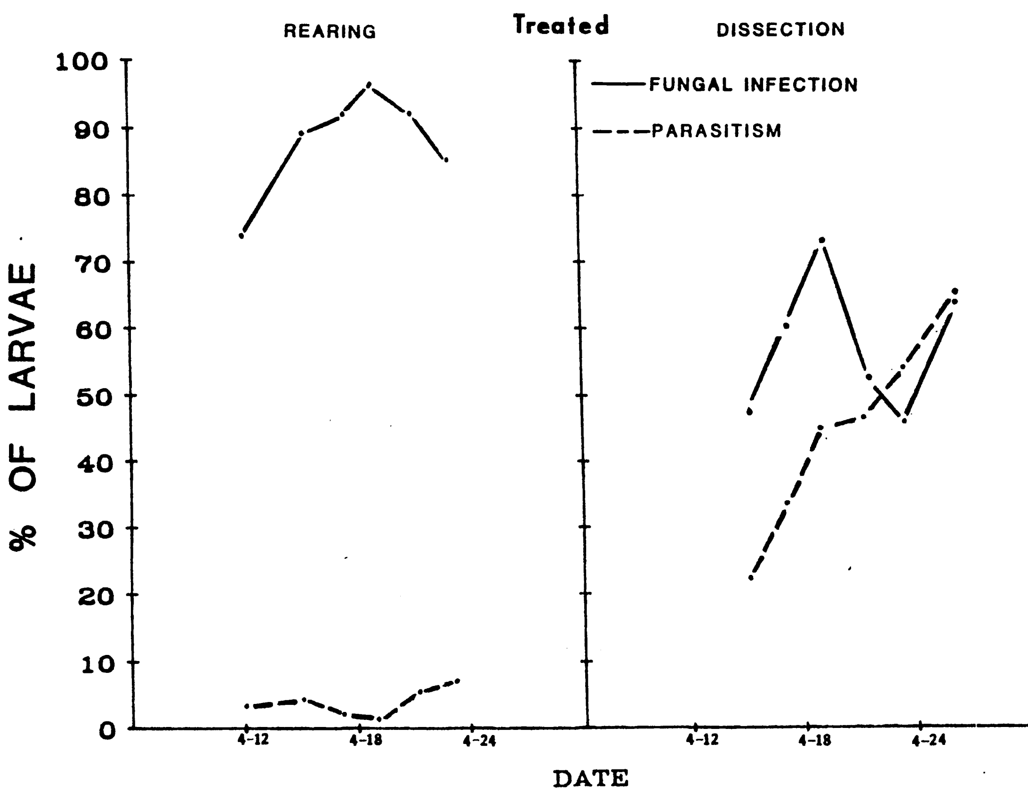
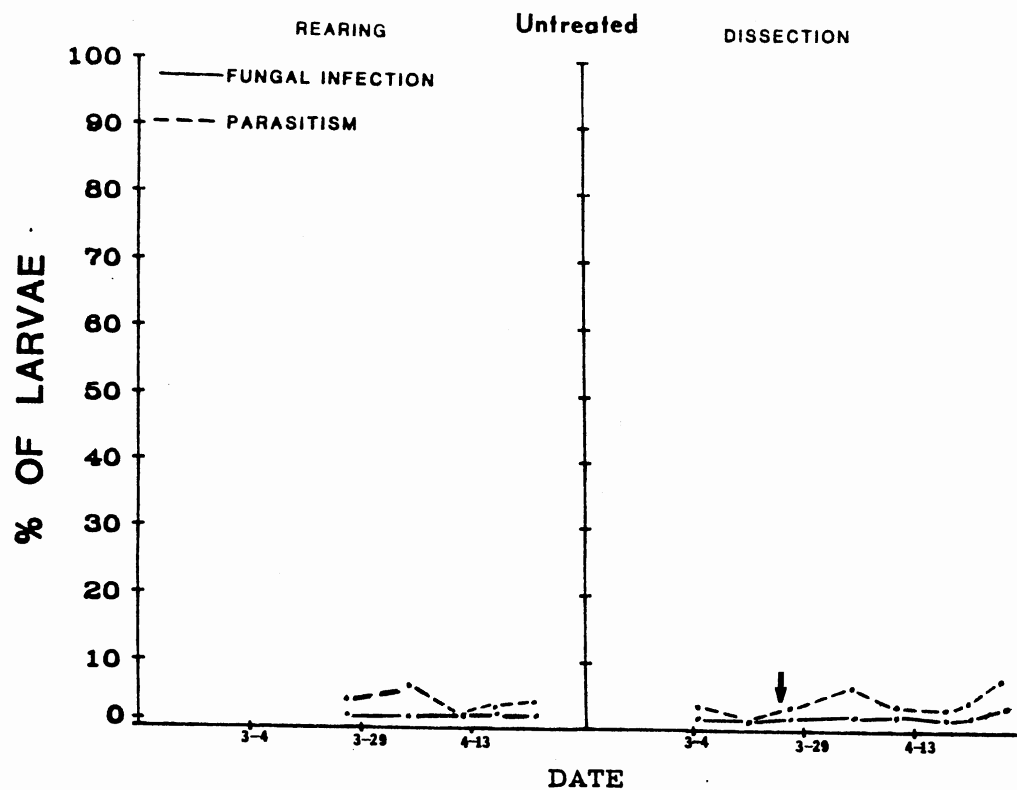

REARING
Treated
DISSECTION
100
90
80
70
60
50
40
30
20
10
0
—FUNGAL INFECTION
---PARASITISM
% OF LARVAE
4-12
4-18
4-24
4-12
4-18
4-24
DATE

Figure 11. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted for the Untreated and Chlorpyrifos-Treated Areas at Stillwater OK., during 1987. (Arrow indicates date of chlorpyrifos application).


REARING
Untreated
DISSECTION
FUNGAL INFECTION
PARASITISM
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
3-4
3-29
4-13
3-4
3-29
4-13
DATE

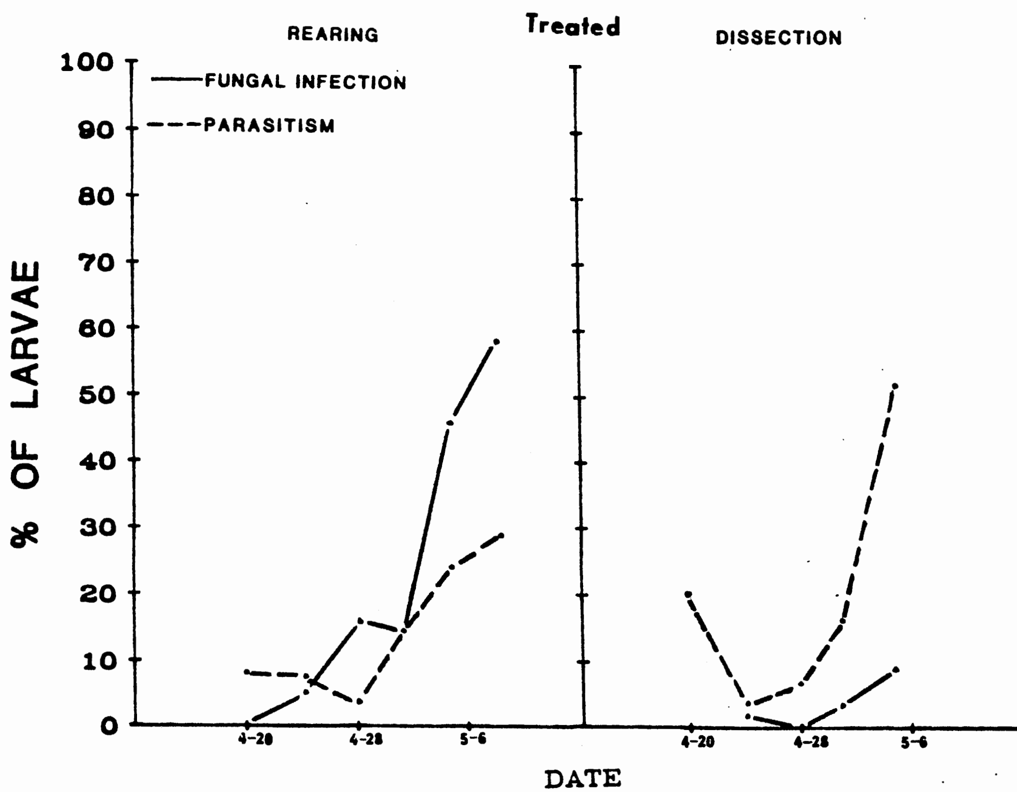
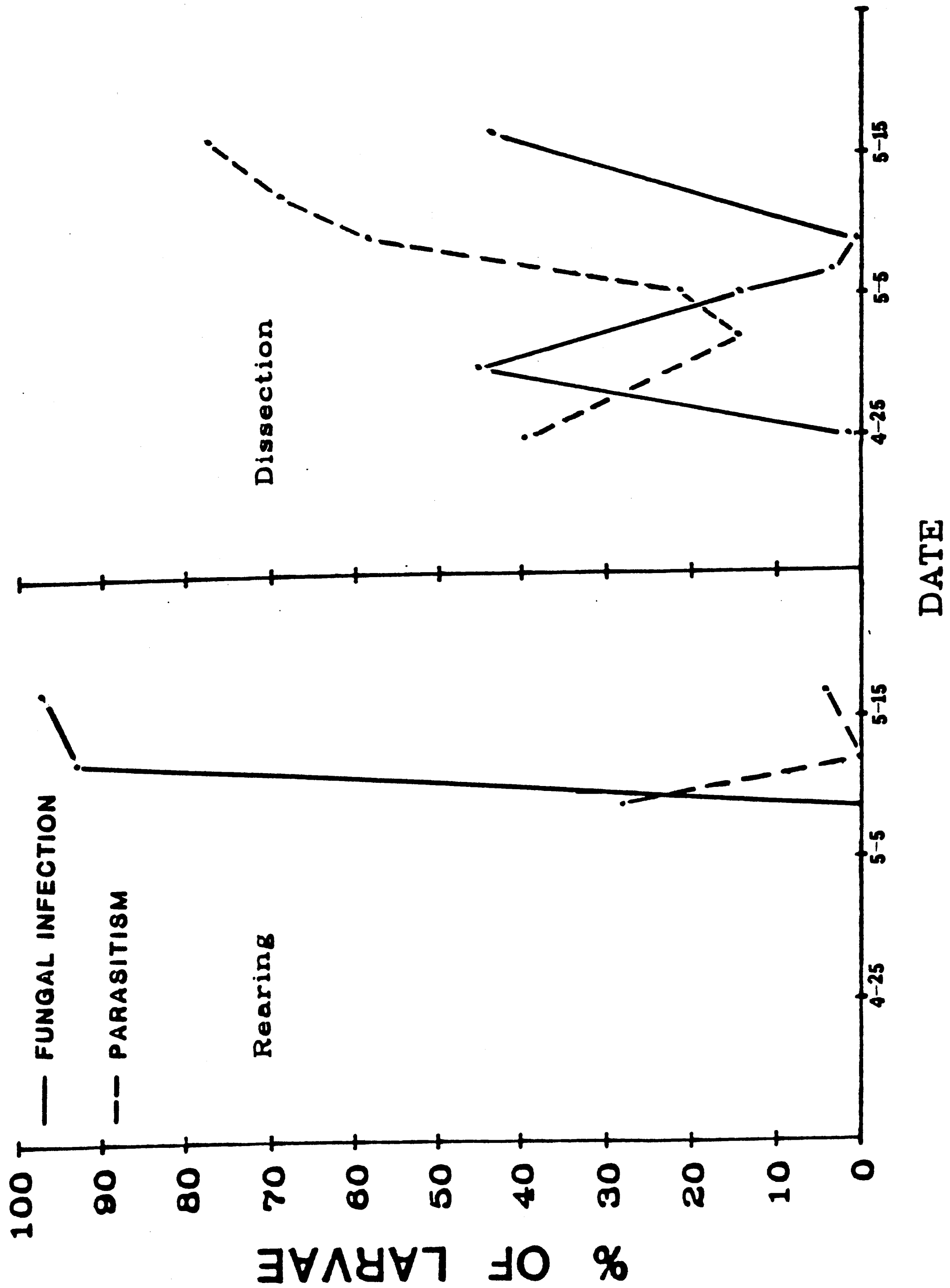
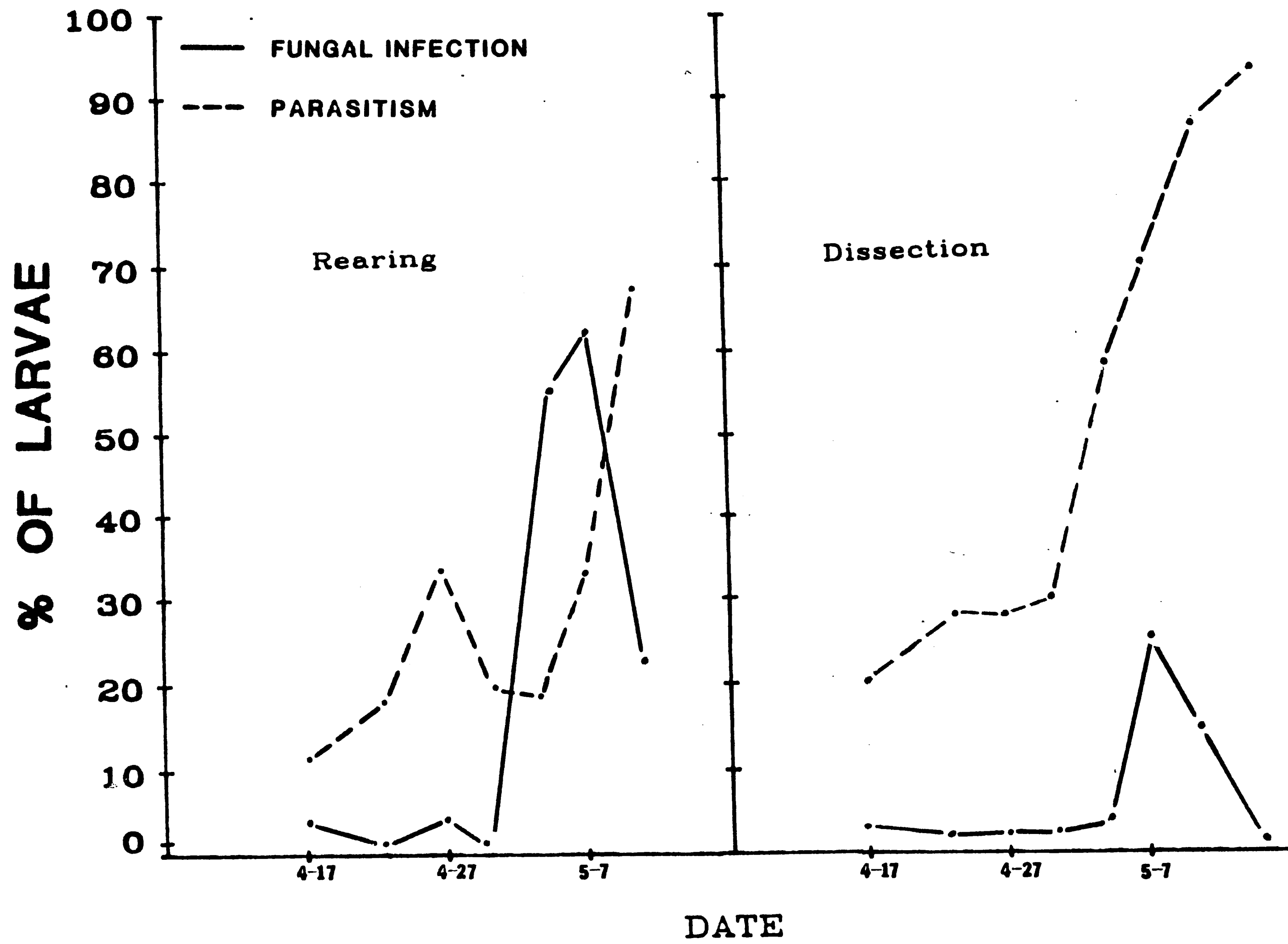

REARING
Treated
DISSECTION
FUNGAL INFECTION
PARASITISM
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
4-20
4-28
5-6
4-20
4-28
5-6
DATE

Figure 12. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Chickasha, OK., during 1983.



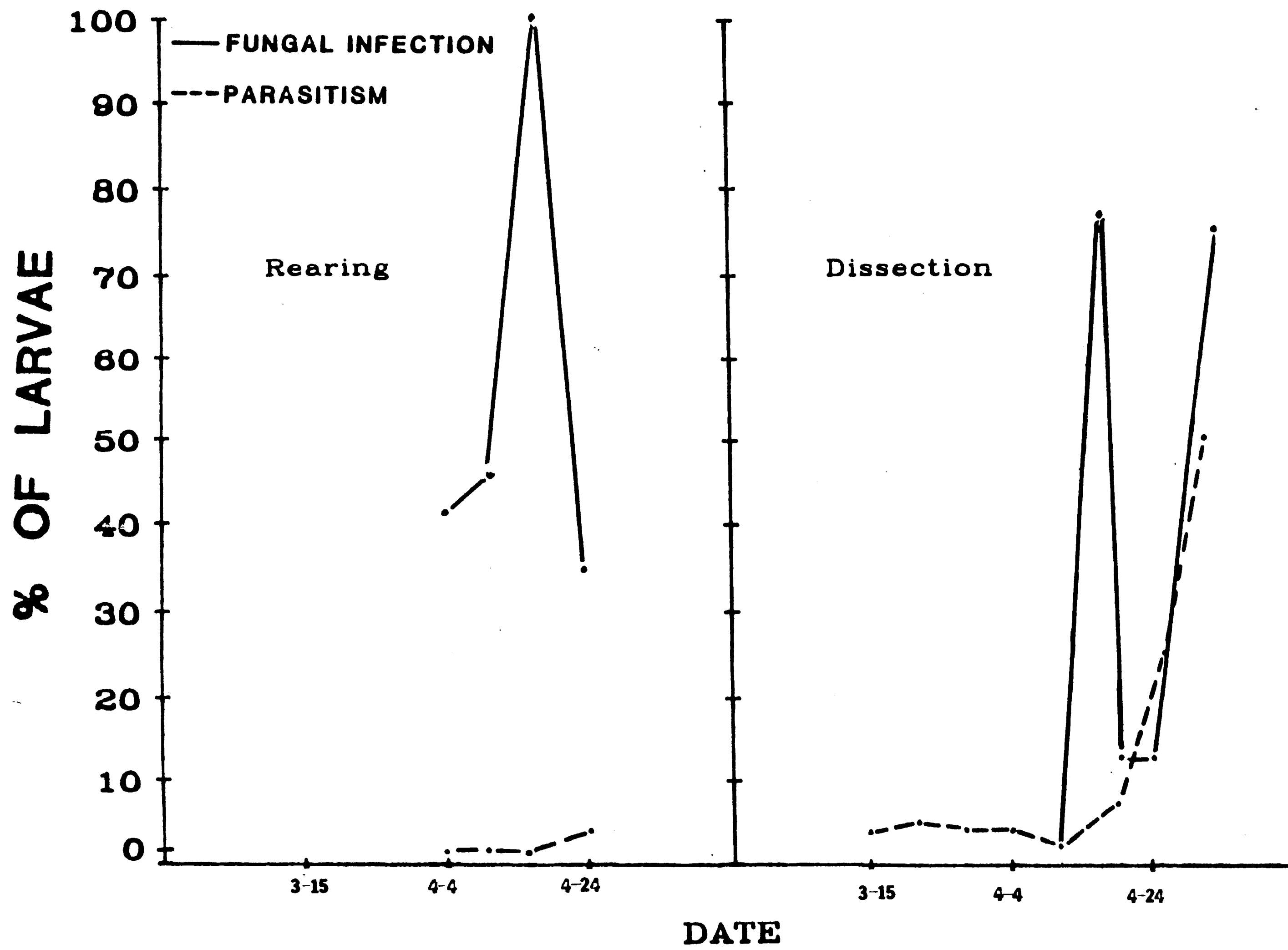
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
FUNGAL INFECTION
PARASITISM
Rearing
Dissection
4-25
5-5
5-15
4-25
5-5
5-15
DATE

Figure 13. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Chickasha, OK., during 1984.



FUNGAL INFECTION
PARASITISM
Rearing
Dissection
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
4-17
4-27
5-7
4-17
4-27
5-7
DATE

Figure 14. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Chickasha, OK., during 1985.



100
90
80
70
60
50
40
30
20
10
0
FUNGAL INFECTION
PARASITISM
Rearing
Dissection
% OF LARVAE
3-15
4-4
4-24
3-15
4-4
4-24
DATE

Figure 15. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Chickasha, OK., during 1986.

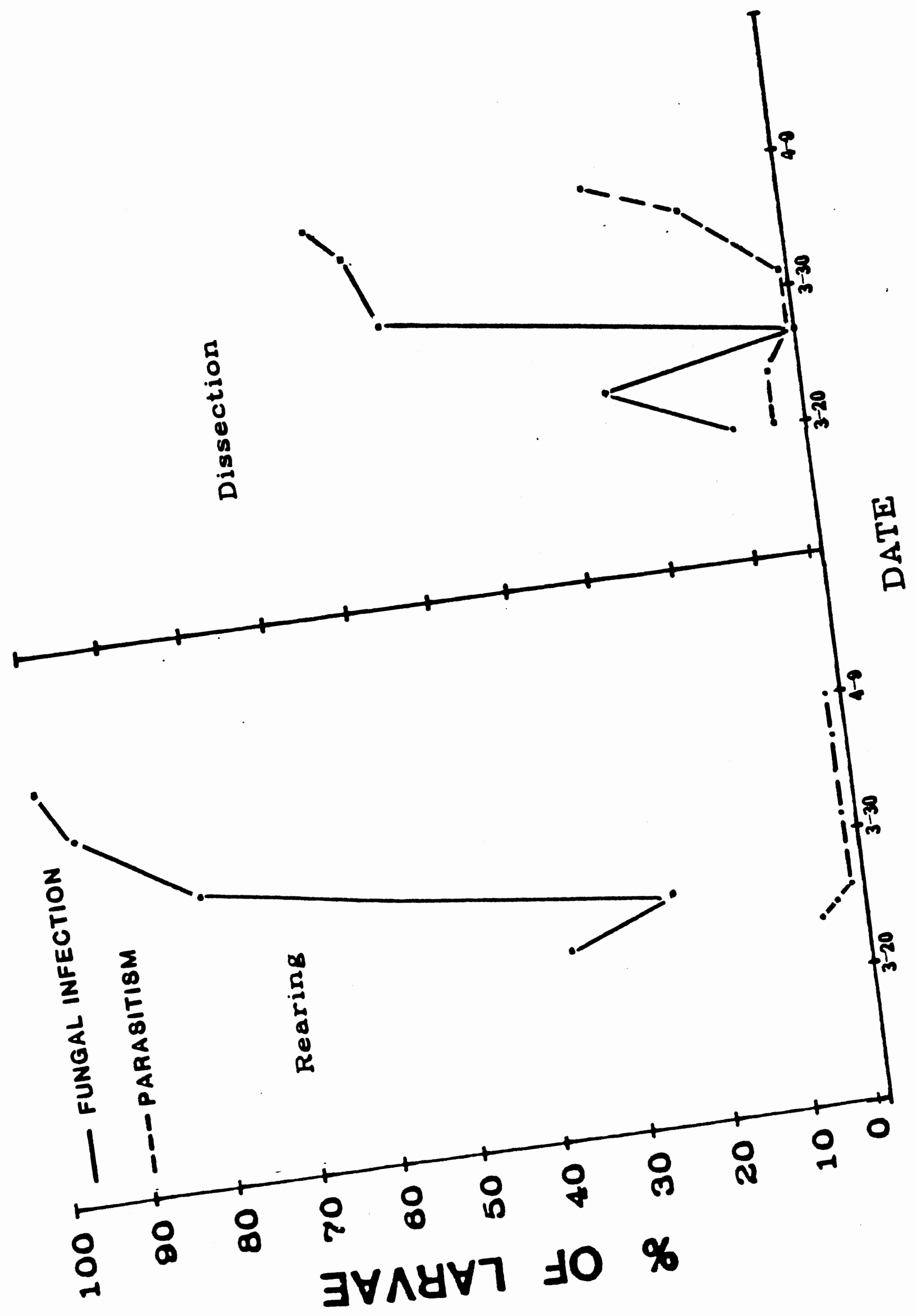
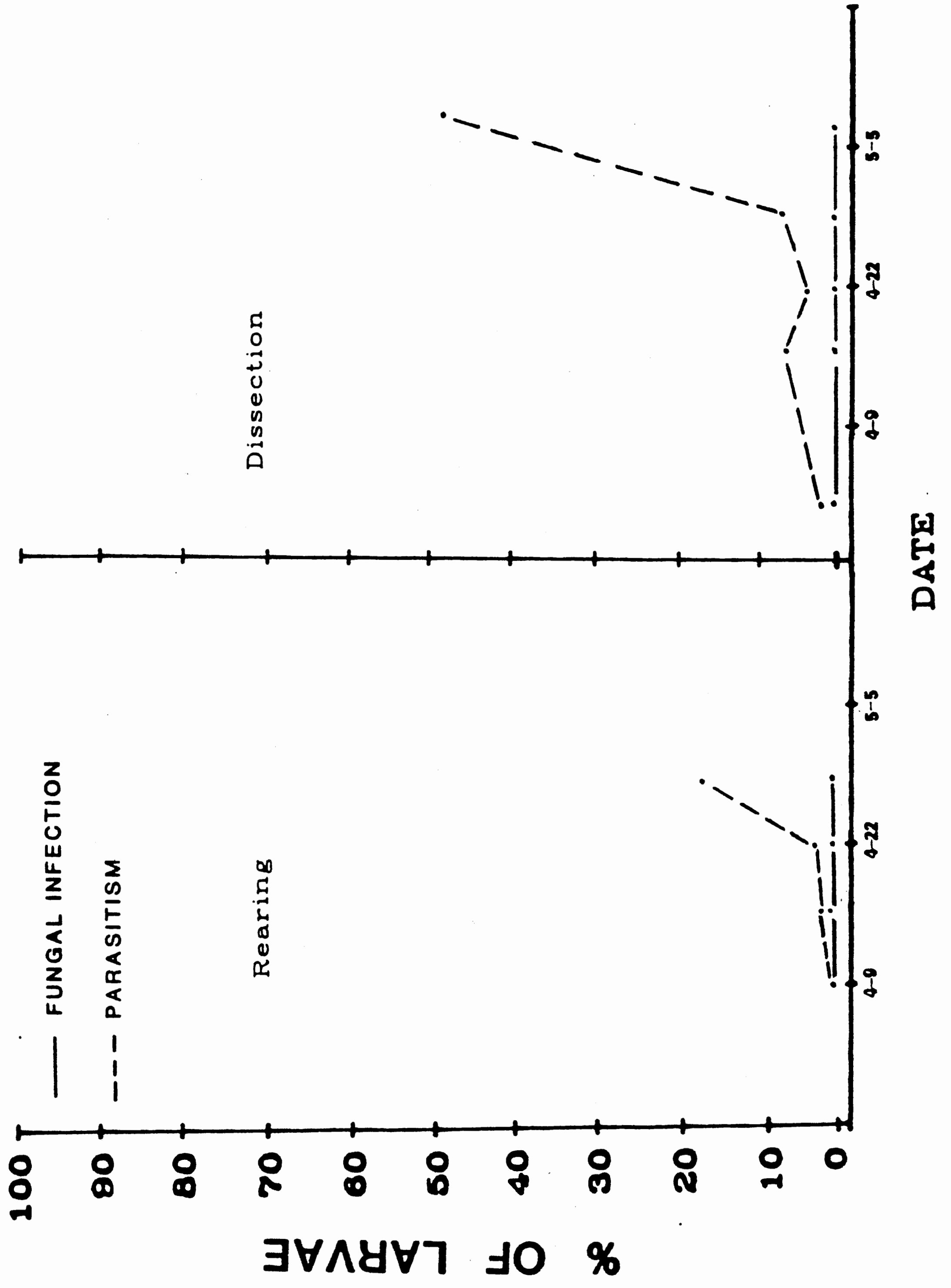

— FUNGAL INFECTION
---- PARASITISM
Rearing
Dissection
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
3-20
3-30
4-9
3-20
3-30
4-9
DATE

Figure 16. Percentages of Infection by *Erynia* spp. and Parasitism by *Bathyplectes curculionis* in Alfalfa Weevil Larvae from the Rearing and Dissection Experiments Conducted at Chickasha, OK., during 1987.


FUNGAL INFECTION
PARASITISM
Rearing
Dissection
% OF LARVAE
100
90
80
70
60
50
40
30
20
10
0
4-9
4-22
5-5
DATE

infection during rearing increased from a low percentage to a peak of more than 90%. No fungal infection was detected in the untreated area in 1987 because of extremely dry conditions (Fig. 10). In 1985, no larvae that were reared yielded parasites because of a high incidence of disease throughout the season in which mortality reached 100% on 1 May (Fig. 8). However, as high as 80% parasitism could be detected in the dissections process this year.

At Chickasha, except 1984 and 1987, mortality observed in rearing also peaked above 90% during fungal epizootics (Figs. 11-15). No disease was detected the entire season in this location in 1987 (Fig. 15). Compared to other years, a higher percentage of parasitism was observed in 1984 in larvae that were reared since fungal infection was never higher than 65% (Fig. 12). Dry weather conditions during this period apparently limited germination of spores.

In rearing, the percentage of infection in larvae tended to increase rapidly within four to five days. This indicated the likelihood that weevil larvae, detected with only parasites during dissections, would inevitably die during the time that a high incidence of disease occurred. Then, as sampling drew to a close for the season, fungal infection and in particular, the percentage of parasitism reached a peak.

The number of parasitized larvae was synchronized with weevil populations in 1978 and 1979 during the latter part of the season (Fig. 17, A-B). Since 1981, there were higher

Figure 17. Estimated Total Populations of the Alfalfa Weevil Larvae/$0.1m^2$ (o---o) and the Larvae Parasitized by _Bathyplectes_ _curculionis_/$0.1m^2$ (●---●) Observed from Dissections at Stillwater, OK., during 1978-1981 (A-D).

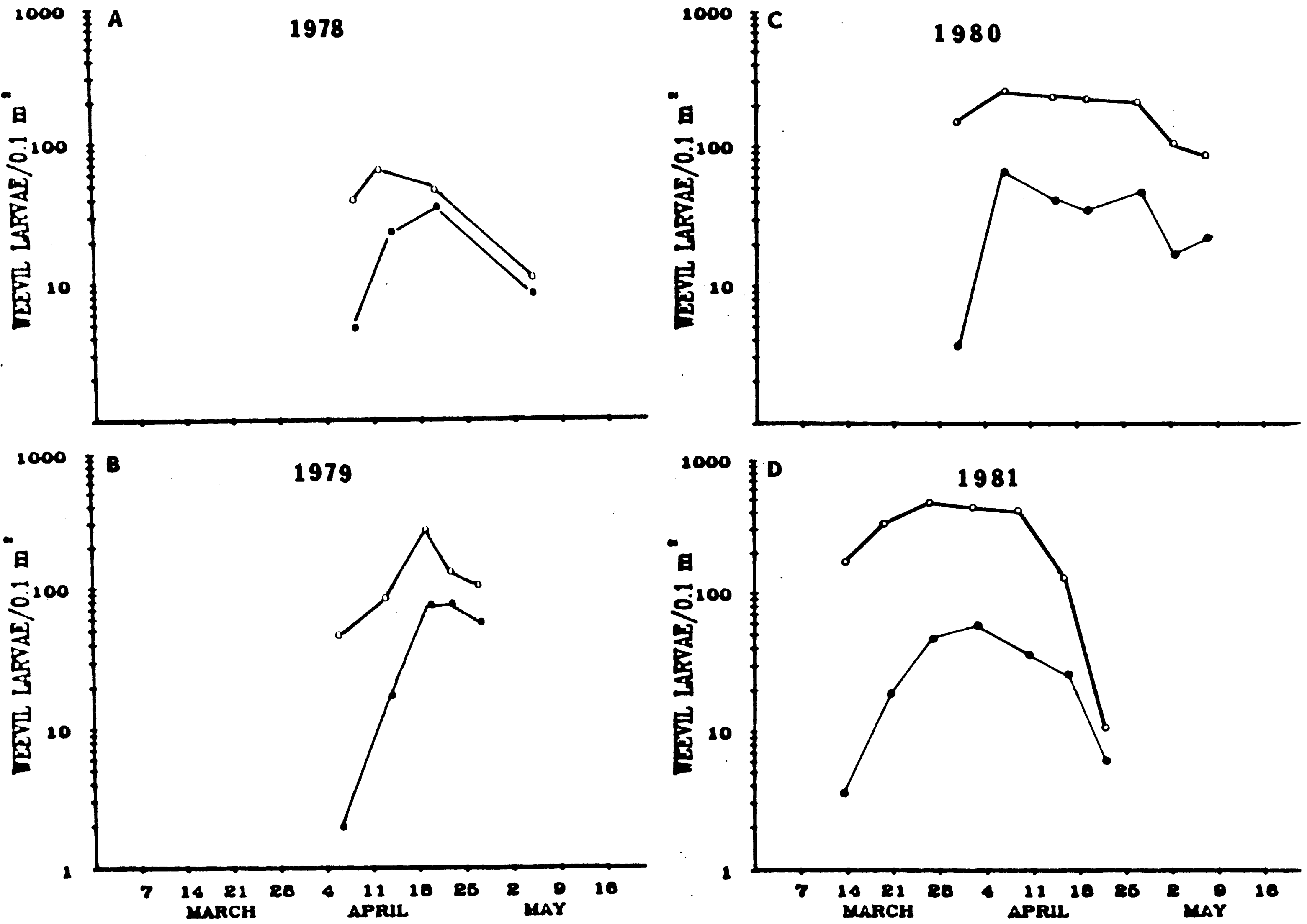
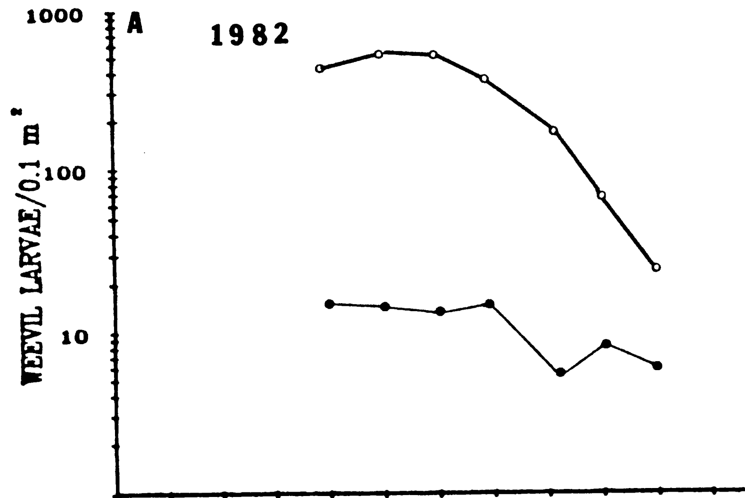
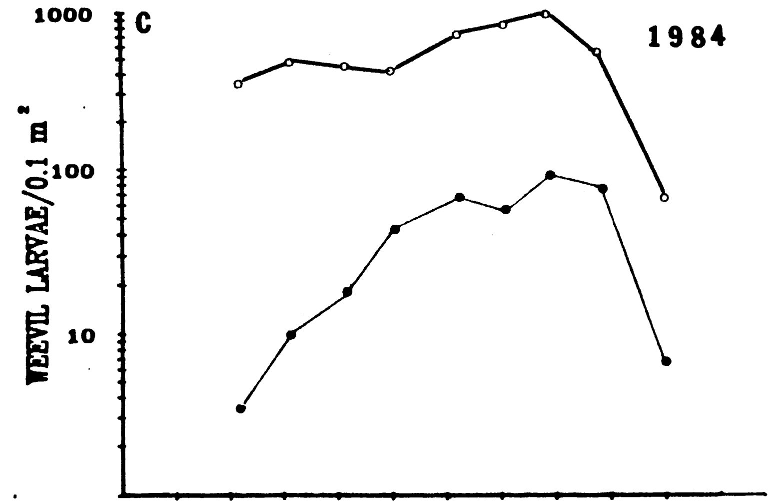
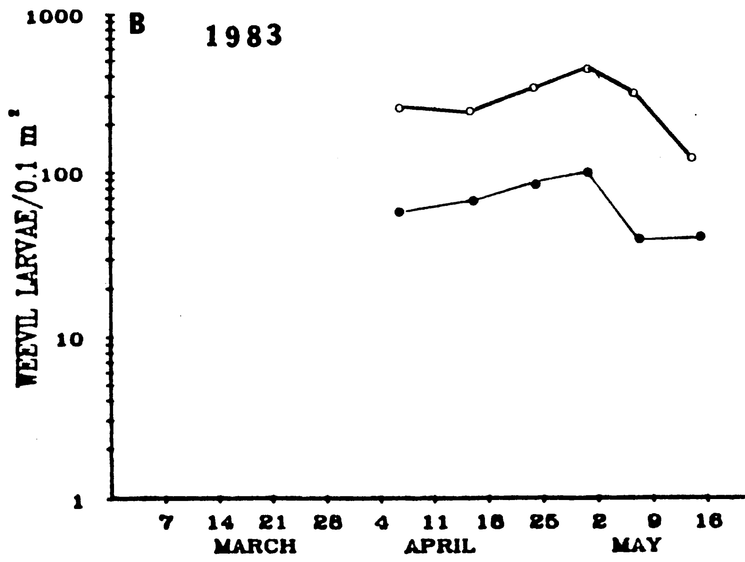

A
1978
C
1980
B
1979
D
1981
WEEVIL LARVAE/0.1 m²
1000
100
10
1
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY

Figure 18. Estimated Total Populations of the Alfalfa Weevil Larvae/0.1m^2 (o---o) and the Larvae Parasitized by *Bathyplectes curculionis*/0.1m^2 (●---●) Observed from Dissections at Stillwater, OK., during 1982-1985 (A-D).


A
1982
1000
100
10
WEEVIL LARVAE/0.1 m²


C
1984
1000
100
10
WEEVIL LARVAE/0.1 m²


B
1983
1000
100
10
1
WEEVIL LARVAE/0.1 m²
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY

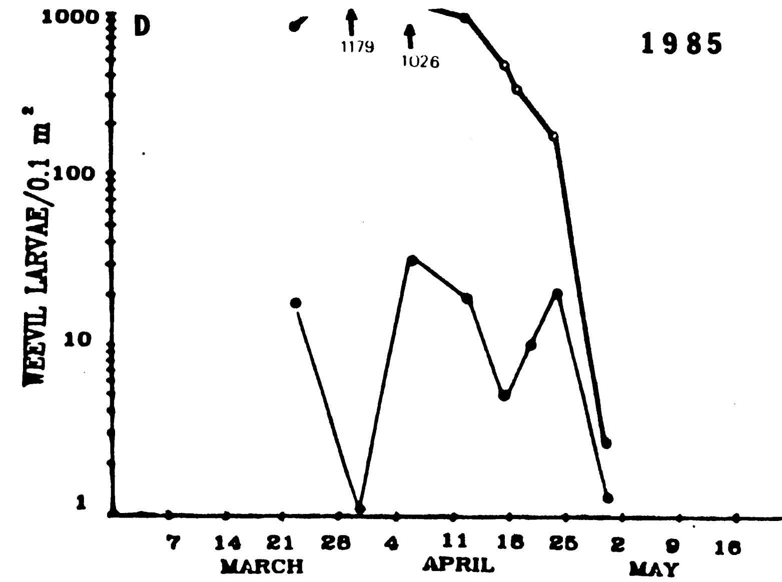
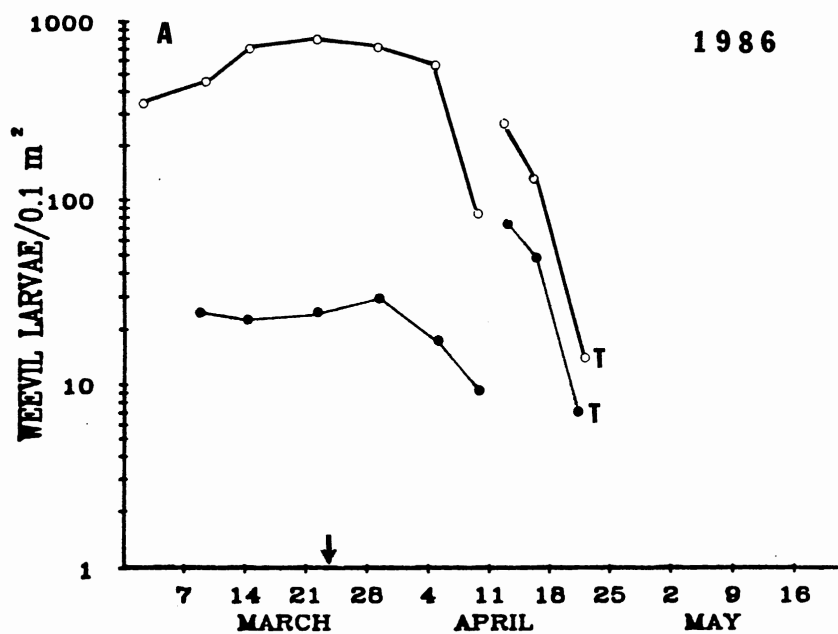
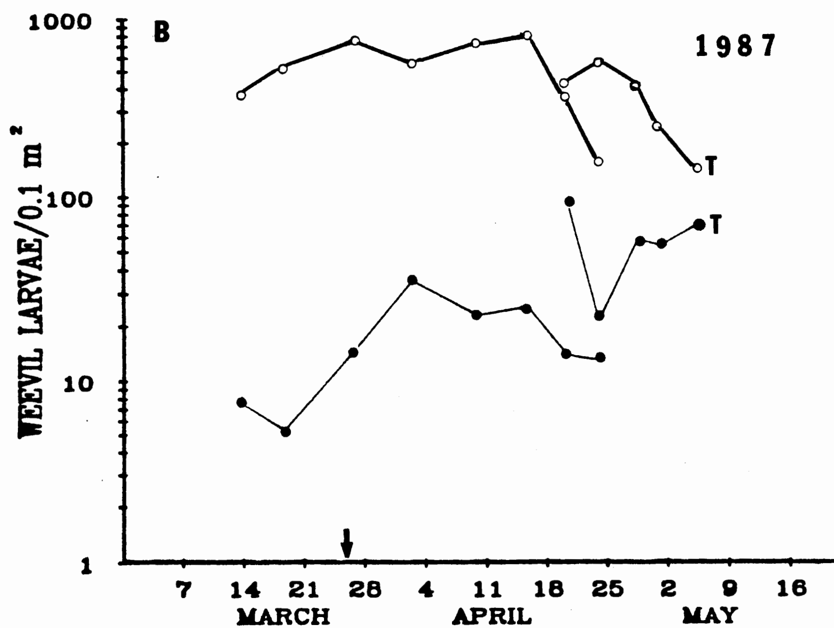

D
1985
1179
1026
1000
100
10
1
WEEVIL LARVAE/0.1 m²
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY

Figure 19. Estimated Total Populations of the Alfalfa Weevil Larvae/0.1m^2 (o---o) and the Larvae Parasitized by _Bathyplectes_ _curculionis_/0.1m^2 (●---●) Observed from Dissections of Larvae Collected from both Untreated and Chlorpyrifos-Treated (T) Areas at Stillwater, OK., during 1986-1987 (A-B). (Arrow indicates date of chlorpyrifos application).



A
1986
WEEVIL LARVAE/0.1 m²
1000
100
10
1
T
T
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY



B
1987
WEEVIL LARVAE/0.1 m²
1000
100
10
1
T
T
7 14 21 28 4 11 18 25 2 9 16
MARCH
APRIL
MAY

weevil densities such as seen in 1985 when a peak of 1,179/0.1m^2 was estimated (Figs. 18-19). A big difference between the number of weevil larvae/0.1ml^2 and the number of parasitized larvae/0.1m^2 resulted. Peak number of parasitized weevil larvae, however, was estimated at 30-90/0.1m^2 over the 10 years that data were collected. Therefore, even with the occurrence of Erynia spp., there did not appear to be a definite decline in the total number of parasitized larvae since 1983.

Discussion

Rearing of individual larvae gave an excellent indication of the trend of fungal disease during the sampling season each year. The percentage of infected larvae was observed to increase from a low to a high level during each year. Rearing was, however, a poor method for the estimation of parasitism. Fungal disease typically killed weevil larvae before development of immature parasites could be completed. As a result, no parasites were recovered from samples taken for rearing at Stillwater in 1985 because of high percentages of fungal infection throughout the season (Fig. 8). A high estimation of parasitism was detected in the rearing experiment only when there was a low incidence of disease. As in 1987, due to the dry spring, there was a reduced incidence of the fungal disease in the area treated with chlorpyrifos at Stillwater and rearing yielded up to 27% parasitism. In the untreated

area and at Chickasha, no infected larvae were seen. In this respect, the rearing process does not give a good estimation of parasitism.

The dissections gave better estimates of the effects of the pathogens in destroying weevils parasitized by B. curculionis because parasites could be detected in diseased larvae. However, dissections, tended to give lower estimates of disease incidence. Infected weevil larvae in which growth of hyphae was very limited were often not detected as diseased. The presence of parasites in diseased weevil larvae nonetheless showed that B. curculionis was generally limited by disease. Apparently, those weevil larvae collected for dissections would have been infected and died in the field within a few days during an epizootic. This was evident in the rapid increase in the percentage of infected larvae that were reared.

Although Berberet et al. (1978) observed that the 1st-generation B. curculionis was normally synchronized with increasing weevil populations, I detected low percentages of parasitism during the early part of the season in both locations for most years (except Chickasha-1985). The high percentages of mortality of weevil larvae from fungal infection prior to emergence of parasites in the previous year could have caused these low levels. This concurred with results made by Ullyett and Schonken (1940) who noted that few parasites survived to control severe outbreaks of the diamondback moth the following year after epizootics of

Entomophthora sphaerosperma Fres. occurred.

Incidence of both natural enemies of weevil larvae observed in dissections tended to increase greatly after the highest population density for the larvae had been reached in April or early May. The higher pecentages of parasitism resulted from the presence of second-generation B. curculionis which coincided with conditions favorable for fungal infections at this time in the season. According to Loan (1981), since Microctonus colesi Drea must compete directly with two species of fungi for the same weevil hosts, peak attack of the parasites might be delayed until epizootics of the disease subside. As in 1984 at Chickasha (Fig. 12), parasitism by B. curculionis increased to a high percentage after the termination of the fungal epizootic in a manner similar to that reported for M. colesi.

As reported by Doss and Berberet (1986), the parasites may never have fully recovered from the extensive mortality caused by the hot, dry summer weather in 1980. This might have resulted in part in the higher weevil densities, which in turn caused the big difference between the total number of larvae/0.1m^2 and the number of parasitized larvae/0.1m^2. However, the estimated peak number of parasitized larvae, remarkably, remained the same (30-90/0.1m^2) throughout the years 1978-1987. This suggested that total parasitism was not related to host density which supported the observation that B. curculionis tended to act in a density-independent manner and contributed little to weevil population trend

(Harcourt et al. 1977). Although fungal disease was observed to cause high mortalities of weevil larvae since 1983 and could have reduced parasitism to an extent, the overall effect of the pathogens on B. curculionis was not as highly limiting as the data implied.

To use both biological agents effectively in the control of the alfalfa weevil, the occurrence of fungal epizootics should be manipulated so that the parasite will appear only as disease is subsiding. In doing so, competition for the host could be reduced without any adverse effect of Erynia spp. on the development of the parasites.

Chi-Square Tests

On 22 May (1983) and 5 May (1985), a higher observed than expected number of larvae with both parasitism and fungal infection were observed (Table 7). The significant χ^2 values therefore reject the hypothesis of no relation between fungal infection and parasitism on these dates. Significant χ^2 values were, however, also observed when the expected frequency of larvae with both mortality agents was much higher than the observed value. Since no evidence was available or obvious enough to suggest the involvement of other factors that might have caused the higher expected values, the null hypothesis was also rejected.

The trend of significant χ^2 such as in 1983, and among the other years, was highly inconsistent and it is therefore

TABLE VII

DEGREE OF ASSOCIATION BETWEEN PERCENT PARASITISM AND FUNGAL INFECTION IN ALFALFA WEEVIL LARVAE AS DETERMINED BY χ^2 TESTS, FOR UNTREATED AND TREATED (T) AREAS, STILLWATER, 1983-1987

		FREQUENCY VALUE				
YEAR	DATE	# PAR	# FUNG	# BOTH	# NONE	χ^2
1983	5-15	22 (16)	40 (34)	10 (16)	28 (34)	6.618*
	5-19	51 (49)	06 (4)	03 (5)	40 (42)	1.701
	5-22	41 (46)	06 (11)	28 (24)	25 (21)	4.294*
1984	4-28	09 (9.4)	05 (5.4)	01 (.6)	85 (84.6)	0.315
	5-01	11 (10.7)	03 (2.7)	00 (.3)	86 (86.3)	0.382
	5-05	07 (6.8)	03 (2.8)	00 (.2)	90 (90.2)	0.233
	5-09	08 (5)	46 (43)	01 (4)	45 (48)	5.114*
	5-12	34 (31)	08 (5)	00 (3)	58 (61)	4.480*
1985	4-18	01 (.8)	21 (20.8)	00 (.2)	78 (78.2)	0.269
	4-21	03 (2.8)	07 (6.8)	00 (.2)	90 (90.2)	0.233
	4-24	11 (9)	20 (18)	00 (2)	69 (71)	3.090*
	4-27	02 (3)	63 (64)	10 (9)	25 (24)	0.739
	5-01	25 (22)	31 (28)	21 (24)	23 (26)	1.375
	5-05	40 (36)	05 (9)	40 (44)	15 (11)	4.040*
1986	3-22	06 (5.8)	08 (7.8)	00 (.2)	186(186.2)	0.258
	3-26	08 (17)	10 (9.1)	00 (.9)	172 (173)	1.041
	3-29	08 (7.9)	03 (2.9)	00 (.1)	189(189.1)	0.127
	4-04	06 (5.5)	17 (16.5)	00 (.5)	177(177.5)	0.575
	4-06	02 (1.5)	147 (146.5)	04 (4.5)	47 (47.5)	0.261
	4-08	03 (2.8)	124 (123.8)	05 (5.2)	68 (68.2)	0.015
	4-10	05 (5.4)	109 (109.4)	08 (7.6)	78 (77.6)	0.053
	T 4-12	35 (30)	58 (53)	10 (15)	97 (102)	3.589*
	T 4-15	17 (22)	71 (76)	26 (21)	86 (81)	3.140*
	T 4-17	36 (29)	81 (74)	29 (36)	54 (61)	4.196*
	T 4-19	29 (24)	87 (82)	60 (65)	24 (29)	3.048*
	T 4-21	47 (46.5)	54 (53.5)	48 (48.4)	51 (51.4)	0.016
	T 4-23	51 (47)	42 (46)	56 (52)	51 (55)	1.025
	T 4-26	21 (22)	22 (23)	44 (43)	13 (12)	0.237
1987	T 4-24	08 (7.8)	04 (3.8)	00 (.2)	188 (188.2)	0.170
	T 5-01	33 (33)	05 (05)	01 (01)	161 (161)	0.000
	T 5-05	98 (94)	13 (9)	06 (10)	83 (87)	3.508*

* = Significant χ^2
# = Numbers of larvae dissected
T = Area treated with chlorpyrifos (0.55 kg ai/ha)

inconclusive to state that both parasitism and infection are related. Further studies are necessary to determine if this relationship exists.

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