LIFE HISTORY OF THE ALFALFA WEEVIL, HYPERA POSTICA, AND TEMPORAL PREDICTIONS FOR LARVAL POPULATIONS EXCEEDING THE ECONOMIC THRESHOLD IN OKLAHOMA

By

JOSEPH ALAN STARK

Bachelor of Science
Arkansas Tech University
Russellville, Arkansas
1985

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1987

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Thesis approved:

Richard Berberet
Thesis Advisor

Gerrit W Cuperus

Jimmy F. Stritzke

David L Weeks

Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Alfalfa, Medicago sativa L., is one of Oklahoma's most important agricultural commodities. In 1988 ca. 1.2 million metric tons of forage were produced on over 165,000 ha. (Bellinghausen, 1988). Alfalfa stands in Oklahoma produce up to five crops per year with the highest yields being obtained at first harvest. At approximately the start of spring growth of the first crop of alfalfa, larvae of the alfalfa weevil, Hypera postica (Gyllenhal), begin to hatch and feed on plant terminals. The larvae routinely cause damage requiring treatment in order to avoid losses exceeding the cost of control. In some years, feeding may result in complete defoliation of the first crop, if the infestations are not controlled. Reduced yields may also be seen at later harvests due to loss of plant vigor (Berberet et al., 1981; Wilson et al. 1979).

The alfalfa weevil was first reported in Oklahoma in 1968 and had been reported in all counties of the state by 1971. Research was begun in 1971 to investigate the population ecology of the alfalfa weevil and develop an integrated management program. From this research, a great deal of information has been gained concerning the effectiveness of cultural practices (Berberet, 1982) and biological control agents (Berberet and Gibson, 1976) in reducing alfalfa weevil numbers. Studies have also been conducted that have described the relationship among larval population levels, alfalfa development stage, and forage losses (Berberet et al., 1981). Therefore past research has provided to the producer a set of management options with which to reduce damage caused by the alfalfa weevil and a method by which to determine when applications of insecticides will be profitable.

Little research has been conducted to explain variations in seasonal incidence and abundance of the alfalfa weevil in Oklahoma or in the southern United States. Timing of population events such as peak egg density, peak larval density, and the occurrence of economically damaging larval populations, may vary over a several month period. This high degree of variation requires that producers monitor larval populations over an 8-12 week period of time. Researchers in the northern United States and southern Canada have accurately predicted of population events based on day degree accumulations (Harcourt, 1981; Guppy and Mukerji, 1974; and Roberts et al., 1970). Efforts to describe the variation in the timing of population events with day degree models have not been successful in Oklahoma. A better understanding of the relationship between the weather and other factors that influence the population dynamics of the alfalfa weevil is needed to improve the prediction of key population events.

The past research on the alfalfa weevil in Oklahoma has resulted in an extensive database on weevil occurrence. It is the goal of this study to draw on this database to gain a better understanding of the population dynamics of the alfalfa weevil in Oklahoma and to apply this understanding to improve the prediction of key population events. The specific objectives of the study are:

I. To quantify the variation that occurs in the population dynamics of the alfalfa weevil in Oklahoma.

II. To identify sources of variation which must be measured in order to predict the timing of key events in the alfalfa weevil life cycle.

III. To test current assumptions concerning the temperature requirements for development of alfalfa weevil eggs using field-collected eggs.

IV. To assess the potential population density of the alfalfa weevil and the reproductive potential lost due to biotic and abiotic mortality factors.

V. To develop a method for predicting the timing of the first annual occurrence of alfalfa weevil larval populations exceeding economic thresholds.

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CHAPTER II

REVIEW OF LITERATURE

Alfalfa, Medicago sativa L., is the world's most valuable cultivated forage crop and an excellent forage for all classes of livestock. Approximately 12 million hectares of alfalfa are grown in the United States providing a higher protein production potential than corn, wheat, and soybeans (Armbrust, 1981).

Introduced into Oklahoma soon after 1900, alfalfa cultivation totaled about 101,000 ha by the 1920's. Production continued to increase and reached a peak of more than 240,000 ha in 1971 (Sholar et al., 1982). In 1988, about 1.2 million metric tons of forage were produced on over 165,000 ha. This represents a statewide average of 6.96 metric tons of forage per ha per year with county averages ranging from 3.53 to 17.47 metric tons per ha (Bellinghausen, 1988). In terms of total value, alfalfa ranks second among crops grown in Oklahoma with the forage and seed produced being valued at ca. $100 million per year (Stark et al., 1990).

Due to its high value, any pest that may reduce production of alfalfa causes a great deal of concern among producers. Of the species of insects infesting alfalfa, the alfalfa weevil, Hypera postica (Gyllenhal), causes the most damage in Oklahoma and in most alfalfa producing areas of North America. Damage in Oklahoma includes direct loss of forage at first harvest due to larval feeding, as well as reductions in yield in later cuttings due to loss of plant vigor (Berberet et al., 1981). In some cases, feeding by newly emerged adults can also slow the regrowth of alfalfa after the first cutting thus reducing the yield potential for the season (Bjork and Davis, 1984). While larval feeding may result in severe losses in Oklahoma, damage due to the feeding of adults weevils is not a serious problem if the larval populations are adequately controlled (Berberet et al., 1980).

The alfalfa weevil was first discovered in North America in Utah in 1904 near Salt Lake City (Titus, 1910). Until 1951, the alfalfa weevil remained a problem only in twelve western states. In 1951, this insect was first found in Maryland and had become a serious pest to first crop alfalfa in the Mid-Atlantic states by 1952 (Poos and Bissell, 1953). Both of these populations, referred to as the eastern and western strains, entered Oklahoma in the late 1960's. The eastern strain was first reported in 1968, and the western strain was collected for the first time in 1969. By 1971, the alfalfa weevil had been reported in all counties of the state with the two strains having merged in Northwest Oklahoma. First cutting yield losses ranging up to 8,600 kg/ha have occurred since 1972 (Berberet et al., 1980).

Adult alfalfa weevils return to alfalfa fields from summer estivation sites in the fall (Prokopy et al., 1967). Barney et al., (1978) found that this return begins as a gradual process, but that one or two peaks of migration usually occur. In studies on the Egyptian alfalfa weevil, Hypera brunneipennis (Boheman), Christensen et al. (1974) reported that the difference between the daily high and low temperature is the most important climatic factor that initiates this migration.

After returning to alfalfa fields, females deposit clusters of eggs in both dead and green alfalfa stems. Poinar and Gyrisco (1960) found that the weevils are most active at night with peak activity in the early evening between 8 p.m. and 10 p.m. in New York. When present, larger green stems are preferred since they provide a larger cavity for egg deposition (Norwood et al., 1967). Parks first reported in 1914 that there is a close relationship between temperature and ovipositional rate. Hsieh and Armbrust (1974) quantified this relationship and determined that the ovipositional threshold is 1.7 °C.

LeCato and Pienkowski (1970) demonstrated that female weevils respond quickly to temperatures above the ovipositional threshold and have been reported to be able to produce up to 1500 eggs/individual. Drea (1969) reported that after males were removed, individually caged females averaged 18.2 eggs/day over 60 days when held at ca. 24 °C. This average has been found to be as high as 48 eggs/day when females were maintained at similar temperatures, and males were made available at 2 week intervals (Coles and Day, 1977). These studies make it apparent that with optimal weather, a low population of adult weevils can produce enough eggs for a larval population exceeding the economic threshold of approximately 1.5 larvae per alfalfa stem (Mulder et al., 1988).

Temperatures below the threshold for ovipositional activity prevent egg deposition in many areas of the northern United States until late spring. In other areas, a significant number of eggs is deposited during warm winter periods, but due to periods of severe cold, few eggs deposited in the fall and winter remain viable and contribute to damaging spring larval populations (Townsend and Yendol, 1968). In Oklahoma, however, there may be extended periods in the fall and winter when oviposition occurs and egg numbers generally increase throughout the winter. There are also periods throughout the fall and the winter when embryogenesis proceeds. The developmental threshold for eggs is 9 °C (Guppy and Mukerji, 1974) which is frequently exceeded. In many years in Oklahoma, eggs laid in the fall and winter contribute to alfalfa damage and in some years economically damaging larval populations have occurred as early as February (Berberet et al., 1980).

After hatching, larvae of the alfalfa weevil go through four instars, each with a developmental threshold of about 9 °C (Litsinger and Apple, 1973). In northern regions, where most larvae result from spring-laid eggs, larval feeding usually begins after the alfalfa is 25 to 30 cm tall. In Oklahoma and other southern regions, larvae may be present as the alfalfa begins its spring growth (Armbrust, 1981). Numbers increase through the spring and peak prior to the first cutting of alfalfa taken in early to mid May. Yields at first cutting are decreased ca. 188 kg/ha for each addition of one larva/stem in the peak population. Even though the second crop of alfalfa is not generally infested, stress due to heavy feeding before the first harvest has an effect on second harvest yields. Yield reductions of 155 kg/ha are seen for each additional larva/stem feeding before first harvest (Berberet et al., 1981).

Fourth instars molt to the pupal stage after spinning a delicate cocoon either in ground litter or on the leaves of the plant (Harcourt and Guppy, 1975). The pupal stage occurs from late March into May in Oklahoma and new adults emerge about 10 days after pupae have formed. These adults stay in the field to feed for a period of time, but usually enter estivation sites by late May. There are reports of these adults laying eggs that produce a second generation of larvae before entering estivation (White et al., 1969 and Loan et al., 1983). This may occur in some years in Oklahoma when eggs begin hatching in January and February. There has not been evidence of infestations comprised of second generation larvae exceeding economic threshold levels. Estivating adults remain inactive until temperatures cool again in the fall and they then return to alfalfa fields to begin mating and oviposition (Prokopy et al., 1967).

Several parasites of the alfalfa weevil have been introduced into the United States. In Oklahoma, _Bathyplectes anurus_ (Thomson) is established in limited areas near release sites, while _Bathyplectes curculionis_ (Thomson) is found statewide and causes significant mortality in larvae during many years (Berberet and Gibson, 1976). Parasitism occurs in all four instars; however, the effectiveness of this parasitism is reduced by the ability weevil larvae to encapsulate _B. curculionis_ eggs. Encapsulation rates are low in first instars, but over 40 percent of third and fourth instars may exhibit this response to parasitism (Berberet, 1982).

Since 1983, infections of the fungus, _Zoophthora phytonomi_ (Arthur), have reduced numbers of weevil larvae, prepupae, and pupae in Oklahoma, with infection percentages as high as 100, 85, and 52, respectively. While this fungus has not consistently eliminated larval populations, epizootics have in some years reduced the need insecticide applications near the time of first harvest and may greatly reduce the number of weevils that reach the adult stage (Goh et al., 1989).

Although the temperature patterns during the winter in southern states allow egg and larval populations to begin building much earlier than in the north, periods of low temperatures also occur that slow and limit the growth of the weevil population. In general, the egg stage is more cold-hardy than the other immature life stages. Shade and Hintz (1983) concluded that, in general, mortality increases with the age of the embryo when development is interrupted by a cold period. They also found that mortality increases when the length of the interruption increases or the temperature decreases. These findings are in conflict with results given by Morrison and Pass (1974). They reported an increase in the tolerance to low temperatures and ability to survive longer durations of cold temperature as the eggs matured. Both sources did note one exception to this trend in that eggs that are near hatching are sensitive to cold temperatures.

Even though the duration of exposure to cold temperature and age of eggs at exposure affects the mortality rate, a lower limit has been defined in terms of the supercooling point. For 5 and 10 day old eggs the supercooling point is -21.9 °C and -23.6 °C, respectively. This lower limit is higher for larvae and increases with larval maturity before reaching its highest point in the pupal stage (Armbrust et al., 1969).

Many researchers have noted that, in order to predict the seasonal occurrence of population events in the alfalfa weevil life cycle, the relationship between temperature and insect development must be quantified. Few researchers, however, have applied these findings to the problem of prediction. Roberts et al. (1970) determined the developmental threshold and day degree requirements for completion of the egg stage in Illinois. This information was then used to predict the time at which eggs present in late January would hatch. Similarly, Guppy and Mukerji (1974) determined day degree requirements for development of all immature stages of the alfalfa weevil. They compared these day degree requirements to the accumulated day degrees between peak prevalence of life stages of the weevil for populations in the Bay of Quinte area and at Guelph in Ontario, Canada. They concluded that the timing of first occurrence or peak occurrence of eggs, larvae, or pupae can be estimated provided only that the daily temperature data are available and that the first occurrence or peak of an earlier stage is known.

Harcourt (1981) removed the requirement of having knowledge about earlier stages by relating his predictions to a fixed calendar date. He developed a model for predicting the time of peak occurrence of each life stage based on the number of day degrees accumulated after 1 April in southern Ontario. Using 4 years of data to validate the model, at no time, for any population event, did the observed and predicted dates differ by more than 2 days.

Clearly this model has great utility because predictions are based only on day degree values. However, there are some underlying assumptions that must be met for this model to be successful. Oviposition must begin on approximately the same date each year in order for it to be appropriate to begin the accumulation of day degrees on a specific date. Also, there must be a limited number of periods after day degree accumulation has begun in which weather occurs that causes mortality of eggs or larvae. Mortality of both eggs and larvae is dependent on the temperature level and the duration of the low temperature (Shade and Hintz, 1983; Armbrust et al., 1969). This mortality is not taken into account by the simple day degree based models.

In Oklahoma and other southern states, assumptions of typical day degree models do not apply. Oviposition usually begins soon after weevil adults return to fields from estivation sites in October. Oviposition continues and development occurs through the winter and spring, whenever temperatures exceed ovipositional or developmental thresholds. Depending on the timing of severe cold weather and subsequent mortality of eggs or larvae during winter, deposition of eggs that survive to yield larvae may begin as late as early March. During March and April, temperatures below the lethal limit for larvae occur relatively often. These factors prevent models such as that of Harcourt (1981) from being appropriate for Oklahoma.

Due to the amount of basic research that has been done on the developmental limits of the alfalfa weevil, it is probable that models can be developed to predict the timing of the important events in its life history in Oklahoma. It is apparent that these models will need to include parameters in addition to basic day degree accumulations from a fixed date in order perform reliably.

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CHAPTER III

SOURCES OF VARIATION THAT INFLUENCE THE POPULATION DYNAMICS OF THE ALFALFA WEEVIL, *HYPERA POSTICA* (GYLLENHAL), IN OKLAHOMA

Introduction

Alfalfa, *Medicago sativa* L., is one of Oklahoma's most important agricultural commodities. In 1988 ca. 1.2 million metric tons of forage were produced on over 165,000 ha. (Bellinghausen, 1988). At approximately the start of spring growth of the first crop of alfalfa, alfalfa weevil, *Hypera postica* Gyllenhal, larvae begin to hatch and feed on plant foliage. The larvae routinely cause damage requiring insecticidal treatment in order to avoid losses exceeding the cost of control. In some years, feeding may result in complete defoliation of the first crop if the infestations are not controlled. Reduced yields may also occur at later harvests due to loss of plant vigor (Berberet et al., 1981; Wilson et al., 1979).

Several researchers have noted that, in order to predict the seasonal occurrence of population events in the life cycle of the alfalfa weevil, the relationship between temperature and the development of the insect must be quantified. A few researchers have done this for specific geographic regions and have made use of that information for predictions. Roberts et al. (1970) determined the developmental threshold and day degree requirements for completion of the egg stage in Illinois. This information was then used to predict the time at which eggs present in late January would hatch. Similarly, Guppy and Mukerji (1974) determined day degree requirements for development of all immature stages of the alfalfa weevil. They compared these day degree requirements to the accumulated day degrees between peak prevalence of life stages of the weevil populations in the Bay of Quinte area and at Guelph in Ontario, Canada. They concluded that the timing of first occurrence or peak occurrence of eggs, larvae, or pupae can be estimated provided only that the daily temperature data are available and that the first peak or occurrence of an earlier stage is known.

Harcourt (1981) removed the requirement of having knowledge about earlier stages by relating his predictions to a fixed calendar date. He developed a model for predicting the time of peak occurrence of each life stage based on the number of day degrees accumulated after 1 April in southern Ontario. Using data taken over 4 years to validate the model, at no time, for any population event, did the observed and predicted dates differ by more than 2 days.

Clearly this model has great utility because predictions are based only on day degree values. However, there are some underlying assumptions that must be met for this model to be successful. Oviposition must begin on approximately the same date each year in order for it to be appropriate to begin the accumulation of day degrees on a specific date. Also, there must be a limited number of weather events after day degree accumulation has begun that cause mortality of eggs or larvae. Mortality of both eggs and larvae is dependent on the temperature level and the duration of the low temperature (Shade and Hintz, 1983; Armbrust et al., 1969). This mortality is not taken into account by the simple day degree based models.

In Oklahoma and other southern states, assumptions of the typical day degree models do not apply. Oviposition usually begins in October soon after weevil adults return to fields from estivation sites. Oviposition continues and development occurs through the winter and spring when the temperatures exceed the thresholds for oviposition (1.7 °C) or development (9 °C), respectively (Litsinger and Apple, 1973). Depending on the timing of lethal, low temperatures and subsequent mortality of eggs or larvae during winter, deposition of eggs that survive to yield larvae may occur as late as early March. During March and April, lethal temperatures for larvae occur relatively often. These weather events prevent single factor models such as that of Harcourt (1981) from being reliable in Oklahoma. It is apparent that models must include parameters in addition to basic day degree accumulations from a fixed date in order to perform consistently.

Many factors may influence the population dynamics of the weevil in Oklahoma. Extensive life history and temperature data were used to evaluate survival and development of different stages of the alfalfa weevil. The objective of this study is to document the variation that exists in these parameters. It is proposed that such variation mandates that additional parameters must be incorporated into predictive models for southern regions where larval populations often result from overwintered eggs.

Materials and Methods

Data Collected.

Data for this study were collected from sampling areas at Stillwater (North Central) and Chickasha (South Central), Oklahoma. Studies began with the 1971-72 year at Stillwater and 1975-76 at Chickasha and continued through 1987-88. In this context, a year refers to the period from onset of oviposition in fall until emergence of adults the following April or May. Alfalfa stands selected for sampling were from 2-5 years of age, had limited weed interference, and did not receive insecticide applications.

Sampling intervals for determining egg numbers were typically 2 weeks from October until February, then decreased to one week until cessation of sampling in May. Each sample consisted of plant material (dead and living alfalfa stems) removed from a 0.025 m^2 area. Twenty samples were selected at random on each date. The eggs were extracted from these samples using the blender technique of Pass and VanMeter (1966).

When numbers of newly hatched larvae in egg samples exceeded 5-10/0.1 m^2 of foliage, larval sampling was begun. This procedure involved collecting foliage from ten, 0.1 m^2 areas for estimation of larval numbers in plant terminals. Berlese funnels were used to extract larvae from the foliage prior to recording numbers of each instar.

For each year and location, the date when larval populations reached the economic threshold was estimated by noting when numbers of second, third, and fourth instars totaled more than 45/0.1 m^2 (Mulder et al., 1988). The number of day degrees that accumulated between 1 January and the date when larval populations peaked was calculated for for the 1980-81 through 1987-88 years using a sine wave approximation and the developmental threshold of 9 °C (Litsinger and Apple, 1973). These data were used to demonstrate the variation in the occurrence of larval numbers that necessitate chemical control both in terms of calendar days and day degrees.

Ovipositional Parameters.

Among the factors influencing the accumulation of eggs during the fall, winter, and spring in alfalfa stands is the number of adults present. Assessment of adult numbers is difficult, but an index of abundance was calculated based on egg numbers and temperatures to determine the extent of variation from year to year.

The adult abundance index (AAI) estimates the number of eggs laid per square meter per ovipositional day degree. As the AAI is calculated on a per day degree basis, it should provide an index to the number of female adults that are present and its magnitude is not dependent merely on the response of those weevils to temperature. The calculated values were compared with peak larval population levels to determine if the AAI is associated with the fluctuations in larval numbers over years.

In order to calculate the AAI, pairs of samples taken approximately 120 day degrees (threshold=9°C) apart were selected for each year and location. This represents the approximate incubation requirement for eggs (Morrison and Pass, 1974). Basic assumptions include that eggs present in the first of a selected pair of samples would have hatched before the second sample was taken and those present in the second sample were laid between the two sampling dates. First instars found in the second egg sample were likely those that had not yet left stems within which they hatched, and thus were included in the egg total as they probably resulted from eggs laid since the previous sample. The AAI was then calculated by dividing the number eggs and first instars present at the second sample date by the number of day degrees accumulated between the two dates. This division was done using day degrees based upon the ovipositional threshold of 1.7 °C (Hsieh and Armbrust, 1974).

For each year, AAI values were calculated for all pairs of sample dates that were ca. 120 day degrees apart. As the growing season progresses, the AAI decreases corresponding to depletion of the adults' reproductive capacity. Since the AAI decreases in the late spring and there is a difference in the frequency of samples taken during this period, the average AAI is not comparable for all years. For this reason, the maximum AAI for each year was used to compare the estimated adult abundance among years.

In addition to the estimated number of adults present, the egg numbers are related to temperature. Day degree accumulations based on the ovipositional threshold of 1.7° C were calculated using a sine wave approximation and totaled from 1 November until 1 April (Hsieh and Armbrust, 1974). These totals were used to compare the heat energy available for ovipositional activity.

In order to assess the variation in the heat energy available for development of eggs and larvae, day degrees values were also calculated based on a threshold of 9 °C

(Litsinger and Apple, 1973) and were totaled from 1 January until 1 April for each year and location.

Low Temperature Parameters.

Bass (1967) and Pitre (1969) found that eggs laid in fall and winter typically hatched by late winter and early spring. Since sub-freezing temperatures often occur during this time, a portion of the eggs and some of the larvae may be subjected to potentially lethal temperatures.

The effect of low temperatures on weevil populations is dependent upon the time of occurrence. For example, temperatures between -12 °C and -20 °C in January may have little effect on larval populations because those present at this time have a low probability of survival due to a lack of host material. By contrast, lethal temperatures in March would be likely to kill larvae that have become established in plant terminals. Also, a greater percentage of the larvae present in March are third and fourth instars and are more susceptible to temperature related mortality than other development stages (Morrison and Pass, 1974). The number of occurrences of temperature at or below -12 °C was totaled over 2 week intervals for each location. These frequencies were used to show the range of dates when low temperature occurs and the frequency with which it occurs.

Statistical Tests.

Mean values for population parameters were calculated for each location along with values for standard deviation and range. Paired t-tests were used to determine if the mean difference in the parameter values for the two sampling locations were significantly different from zero.

Results and Discussion

The timing of the first occurrence of larval populations exceeding the economic threshold of 45 larvae per 0.1 m^2 (Mulder et al., 1988) varied greatly in terms of calendar days. The date of the first occurrence of larval numbers exceeding 45 per 0.1 m^2 has ranged from February to April and has occurred almost as often in April as before the first of March (Figure 1). This variation in the timing of larval infestations requires that alfalfa fields be intensively monitored over a 2-3 month period in order to avoid economic losses.

Little consistency was seen in the day degree accumulations between 1 January and the date of peak larval populations over the different years and locations (Figure 2) with a mean of 215.5 and a standard deviation of 55.3. This inconsistency prevents simple day degree models from being effective in predicting events such as the time of peak larval populations in Oklahoma.

The adult abundance index (AAI) ranged from 0.1 to 2.1. during the study period (Figure 3). No consistent difference was seen in the adult abundance index (AAI) between Chickasha and Stillwater. AAI values for the two locations differed by up to 1.06. However, neither was consistently larger and the mean difference was not significant ($t=0.398$, $df=12$; $p>0.3$). AAI values were low for both locations in the late 1970's, before increasing in 1981-82. This pattern in the AAI is similar to that for peak larval populations which were relatively low from 1976-1981 (Figure 4). When the peak larval numbers were regressed against the AAI values over years, a strong positive correlation was evident (Figure 5, $r^2=0.692$). It should be noted that the regression equation:

$$\text{AAI} = 0.001092 \text{ X (Peak Larva)}$$

was calculated to demonstrate that an association does exist between the AAI and peak larval population. This association is described to lend creditability to the use of AAI as an index of the number of adult weevils that were present. It is not suggested that AAI be used as a means of predicting peak larval population levels. However, variations in adult abundance should have a direct effect on the potential size of the larval population and some measure of this factor should be incorporated into population dynamics models.

Figure 6 shows the number of day degrees, based on 1.7 °C, that accumulated between 1 November and 1 April during each year. Due to its more southern location, Chickasha had consistently higher day degree accumulations when compared to Stillwater, with means of 996.4 and 823.4 and standard deviations of 127.8 and 99.2, respectively. The mean difference between the two locations was significantly different from zero (t=12.19, df=12; p<0.001).

Variation was also seen when years were compared for the same location. A range of more than 450 and 350 day degrees/year occurred at Chickasha and Stillwater, respectively. Since a single female can lay in excess of 2 eggs per day degree (Coles and Day, 1977), these ranges may result in significant changes in the numbers of eggs deposited from year to year even when the number of adult females present does not change. For this reason, fluctuations in ovipositional day degree accumulation can effect the potential larval population.

At Chickasha the average for developmental day degrees between 1 January and 1 April was greater than at Stillwater, with means of 233.9 and 162.3 and standard deviations of 70.8 and 51.6, respectively (Figure 7). The mean difference was determined to be significant by a paired t-test (t=7.98, df=12; p<0.001). The difference in day degree accumulations over locations results in larval populations in excess of the economic threshold earlier in southern locations as noted by Berberet et al. (1980).

Fluctuations among the years at one location also have an effect on the timing of the alfalfa weevil population events such as peak larvae. Annual day degree accumulations between 1 January and 1 April at Chickasha ranged from 140 to 399, while at Stillwater, accumulations were between 104 and 299. Since the timing of larval population increase is dependent on the accumulation of developmental day degrees, accounting for this variation is vital to the success of development models and is the sole factor accounted for by models developed in more northern growing regions.

Frequency of occurrence of lethal, low temperatures may be critical, especially for prediction of larval numbers. Figure 8 shows the occurrences of temperatures below -12 °C in 2 week intervals. As expected, these occurrences were less frequent at Chickasha. They were most likely in January, followed by late December and early February. However, they also occurred in late February and March when later instars are more likely to be present. Since these are the stages which cause most yield losses and are most susceptible to cold temperatures, these later occurrences of lethal temperatures can delay the need for treatments to control larval populations. Simple day degree models do not account for these lethal temperatures and the delay in population increase that they may cause.

Conclusion

This study has shown that there are factors related to each life stage of the alfalfa weevil which vary significantly when compared over locations and years. Since day degree based models have been successful in northern growing regions, the developmental day degrees for eggs and larvae must be by far the most important of these variables. While developmental day degrees are also important in describing the population dynamics in southern regions, other factors related to the various life stages have a greater relative influence. The variation demonstrated in this study indicates that models of development for southern states need to include measures of adult abundance, ovipositional day degrees, and incidence of lethal, low temperatures as well as traditional developmental day degrees. Further research is needed to determine what variables should be measured to account for these sources of variation and how they can be incorporated into models to predict the occurrence of events in the alfalfa weevil life cycle.

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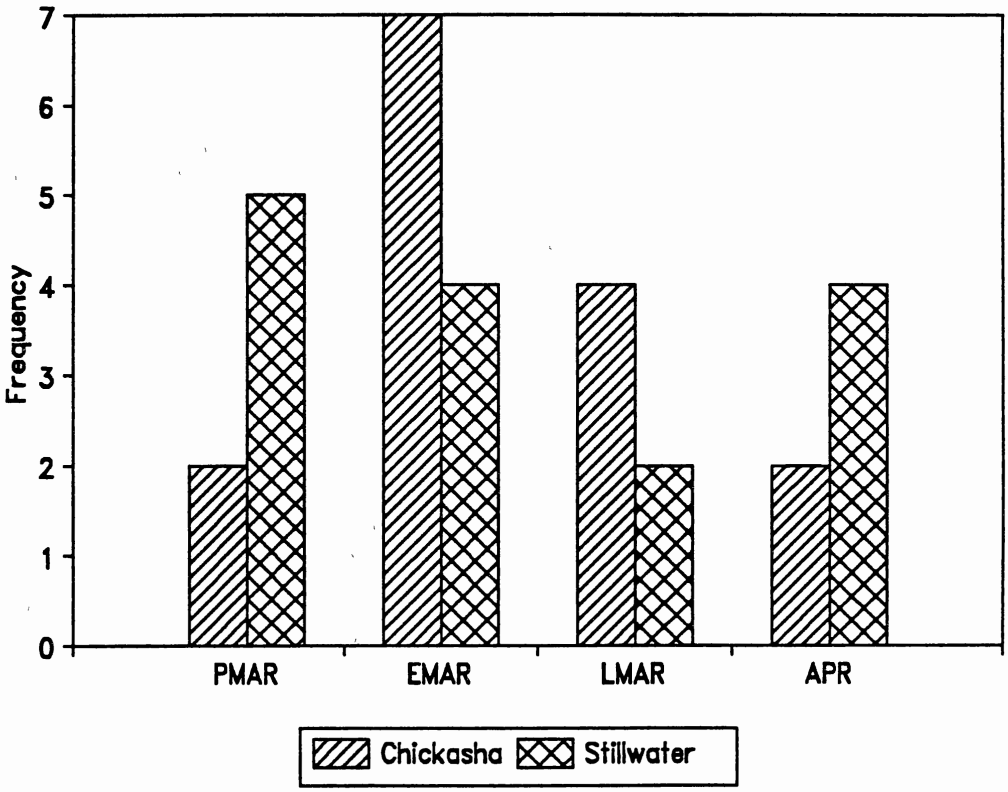

Figure 1. Times when alfalfa weevil larval populations first exceeded the economic threshold at Chickasha and Stillwater, Oklahoma, 1972-88.

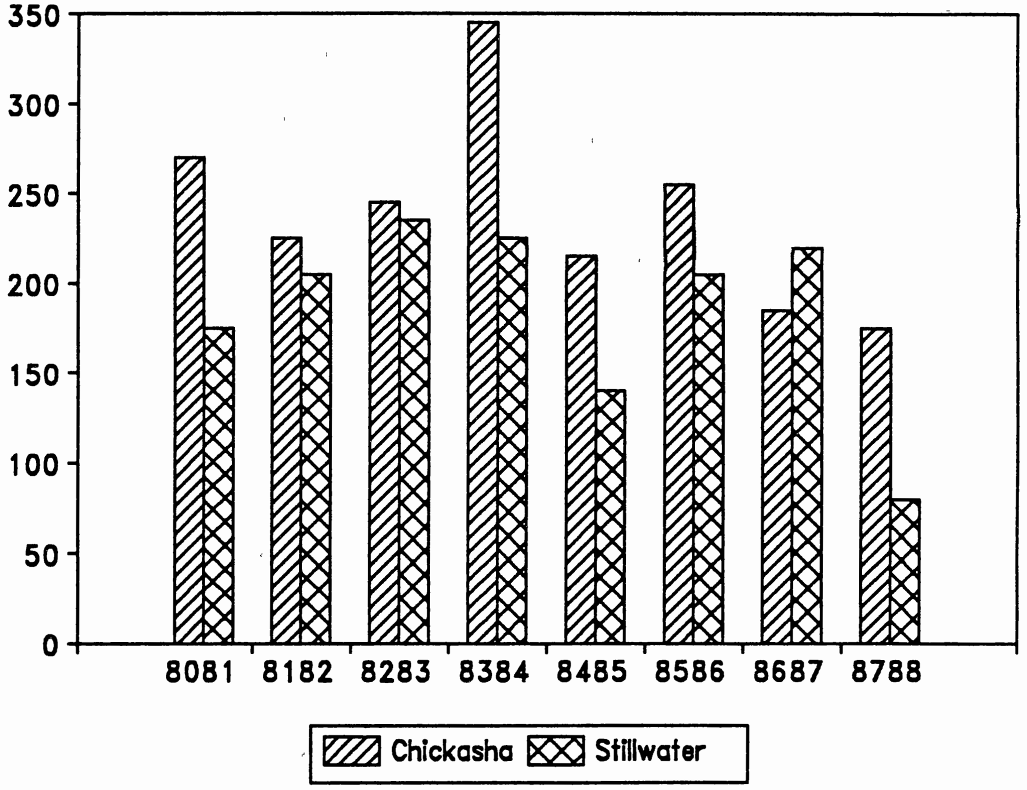

Figure 2. Accumulated day degrees between 1 January and the time of peak larval populations at Chickasha and Stillwater, Oklahoma, 1981-88.

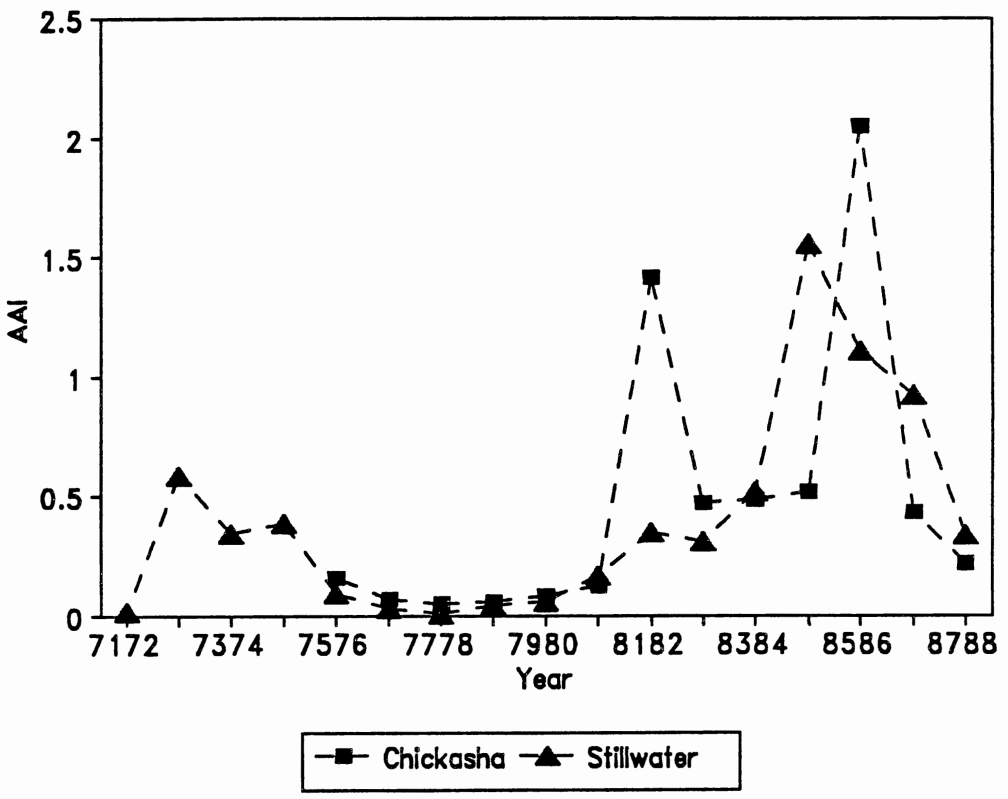

Figure 3. Maximum adult abundance index (AAI) at sampling locations at Chickasha and Stillwater, Oklahoma, 1972-88.

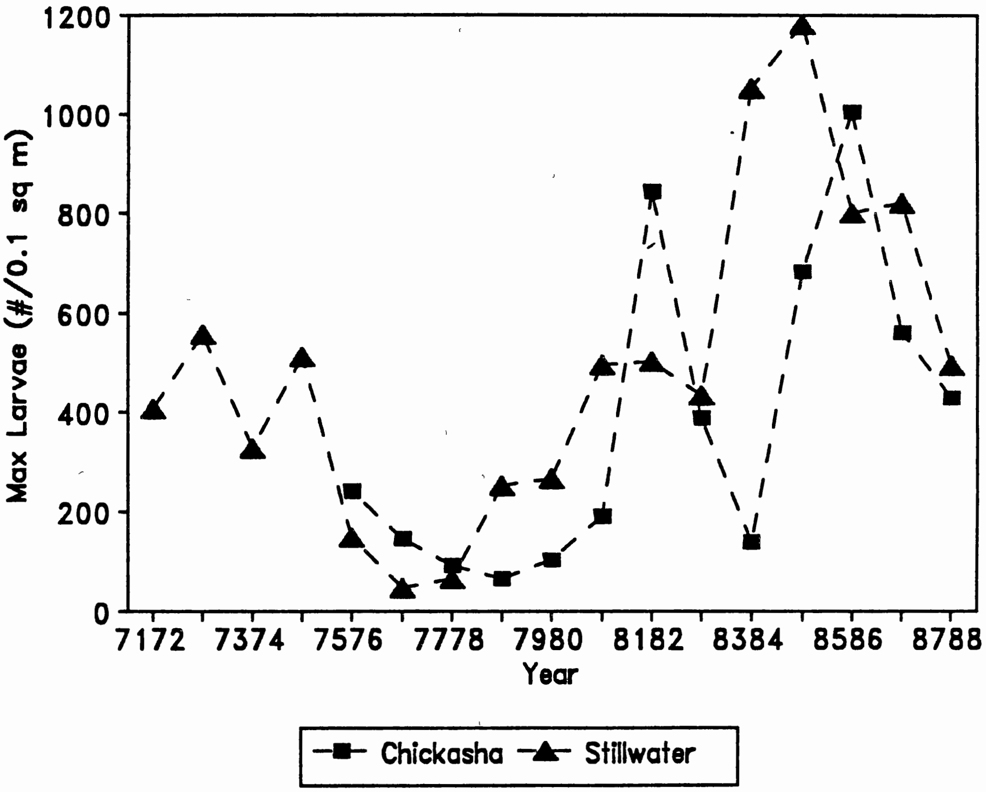

Figure 4. Peak larval population densities at Chickasha and Stillwater, Oklahoma, 1972-88.

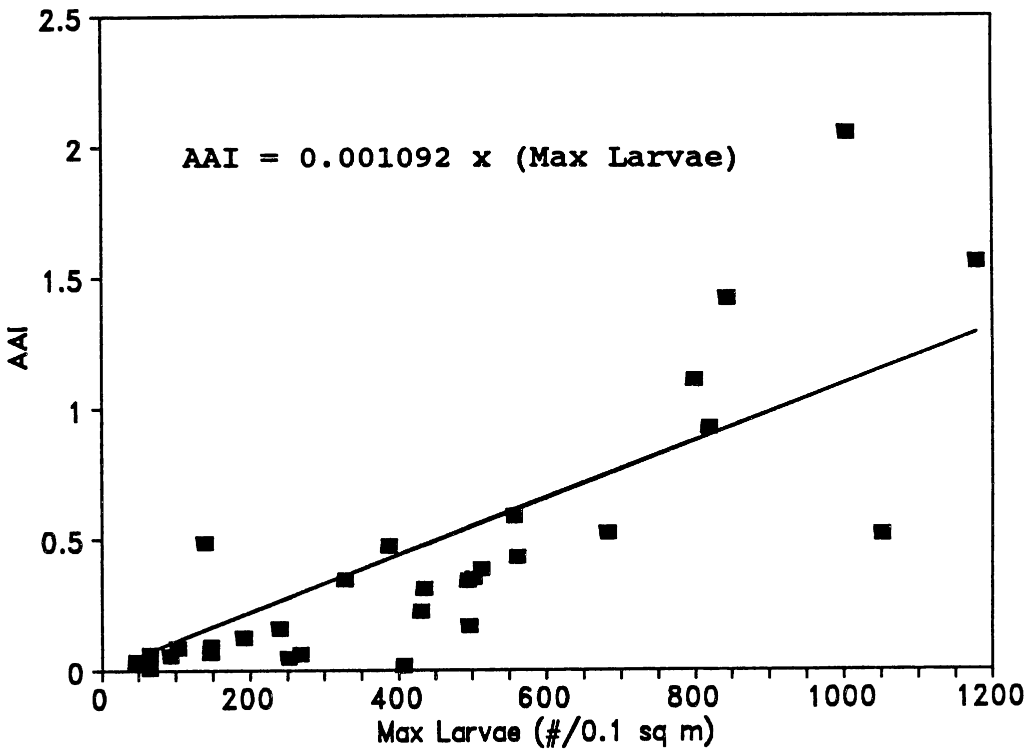

Figure 5. Association between maximum adult abundance index (AAI) and peak larval population levels at Chickasha and Stillwater, Oklahoma, 1972-88.

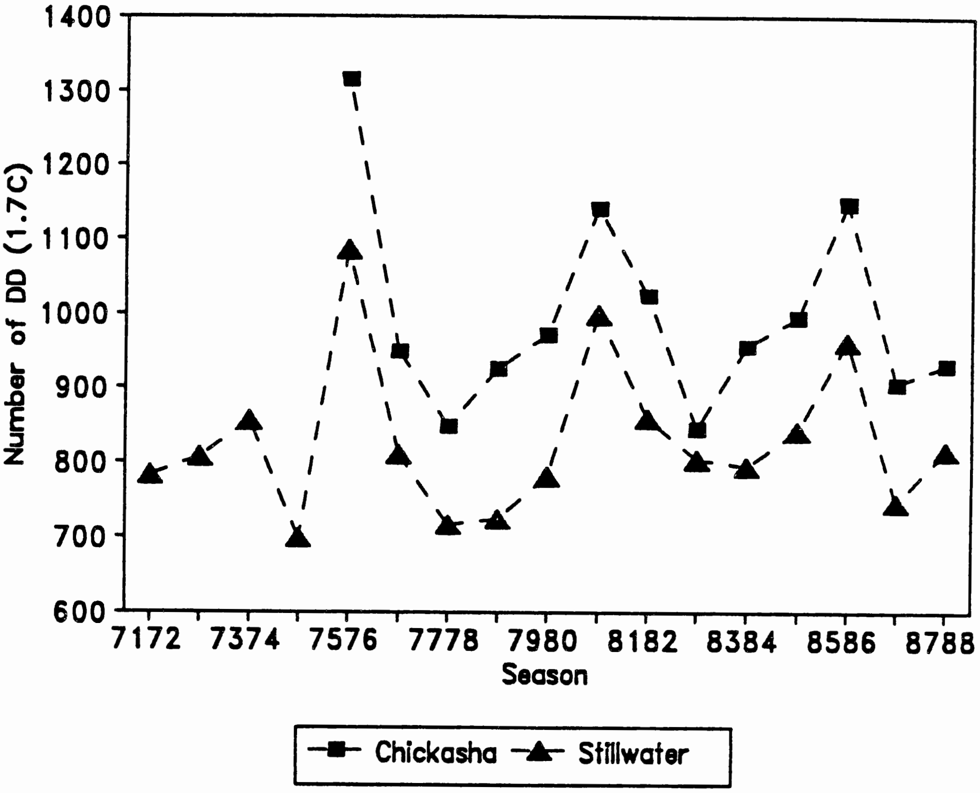

Figure 6. Accumulated day degrees between 1 November and 1 April using an ovipositional threshold of 1.7 °C at Chickasha and Stillwater, Oklahoma, 1972-88.

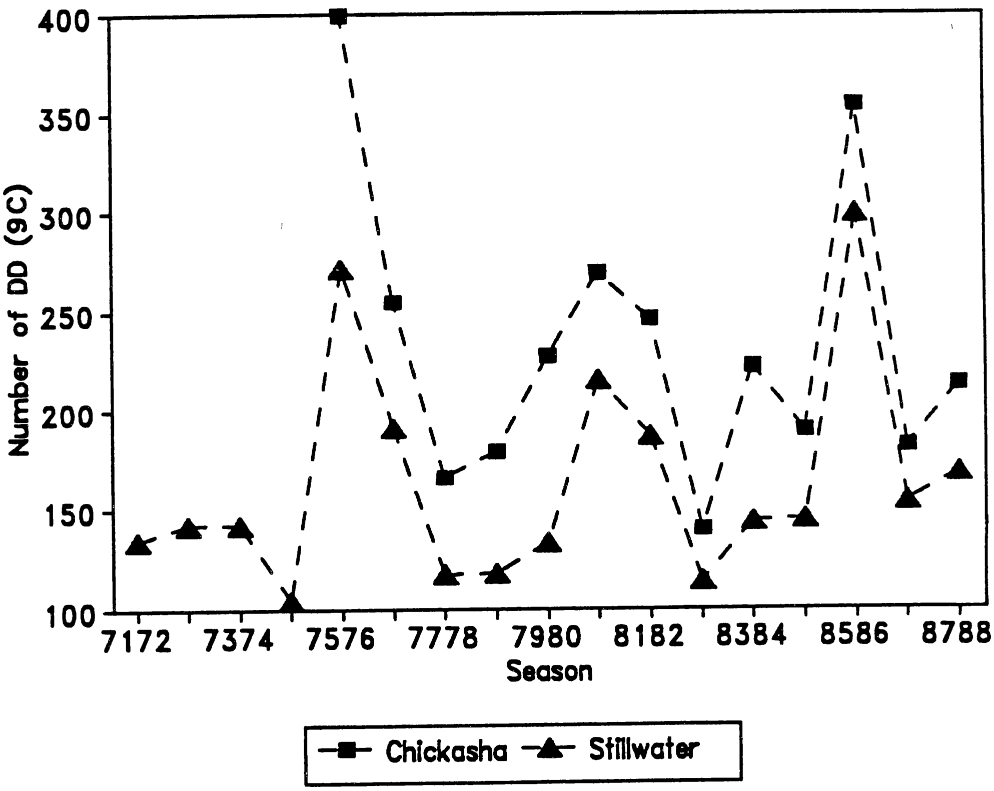

Figure 7. Accumulated day degrees between 1 January and 1 April using a developmental threshold of 9 °C at Chickasha and Stillwater, Oklahoma, 1972-88.

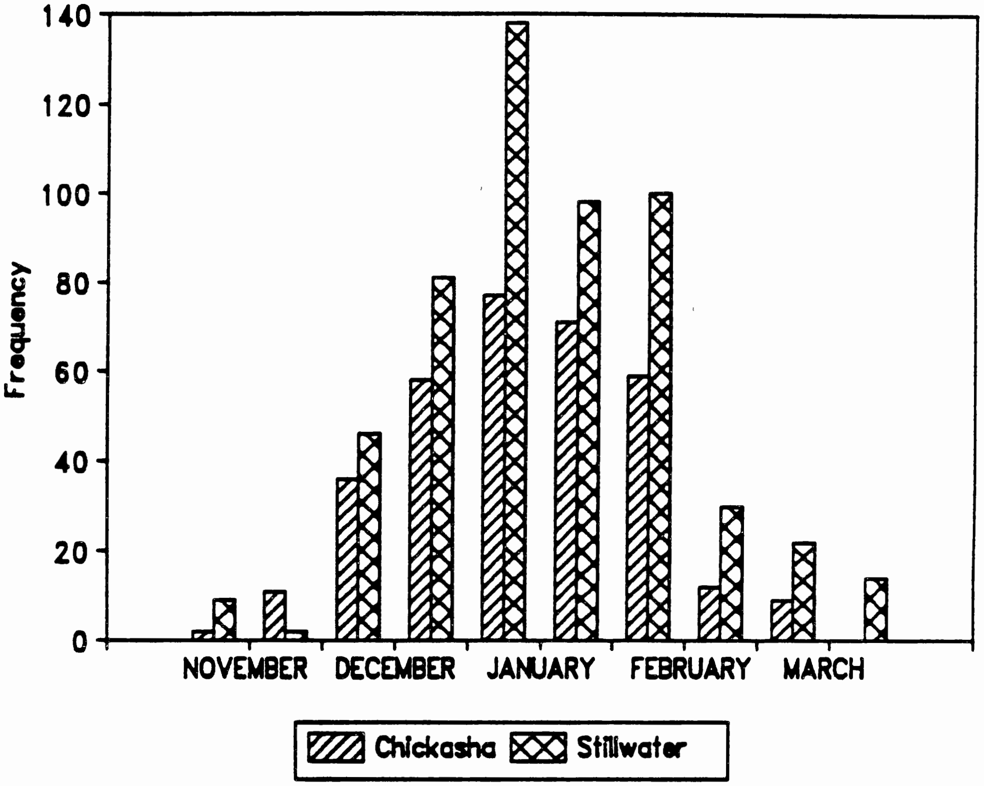

Figure 8. Frequency of days with minimum temperature at or below the lethal limit (-12 °C) for eggs of the alfalfa weevil, Chickasha and Stillwater, Oklahoma, 1972-88.

CHAPTER IV

FIELD VALIDATION OF DEVELOPMENTAL REQUIREMENTS OF ALFALFA WEEVIL, *HYPERA POSTICA* (GYLLENHAL), EGGS

Introduction

The relationship between temperature and alfalfa weevil population dynamics was first analyzed when Parks (1914) found a strong association between temperature and ovipositional rate. As the alfalfa weevil became a more important pest of alfalfa in the United States, many researchers sought to quantify the relationship between temperature and its developmental rate. These studies are essential for preparation of predictive models relating to the timing of events in the alfalfa weevil life history.

Researchers in northern states have determined the amount of heat energy that is required for eclosion in terms of day degrees above an established threshold. Studies of this type are usually conducted using eggs from a laboratory colony and record required developmental times at a series of constant temperatures. There has been some discrepancy in the results reported. Roberts et al. (1970) reported that 174 day degrees were necessary with a developmental threshold of 6.9°C. Litsinger and Apple (1973) found that

119 day degrees were required with a threshold of 9°C and a 111 day degree requirement was reported by Guppy and Mukerji (1974) using a developmental threshold of 10°C. Finally Morrison and Pass (1974) reported a requirement of 120 day degrees based on a threshold of 9°C.

In southern states, constant temperature studies have not been conducted. It has been assumed that the day degree requirements established elsewhere are applicable to southern regions. Of particular concern in the south is the fact that eggs are laid and development occurs from October until the following April. Since the reported results involved lab colonies, it is not known if significant variation exists in the day degree requirements for eggs laid during different months of the year from October to April. The purpose of this study is to determine if requirements reported in previous studies are applicable for predictions in Oklahoma using field collected eggs. Another objective is to assess whether these requirements vary significantly during the year.

Materials and Methods

Data were collected from sampling areas at Stillwater (North Central) and Chickasha (South Central), Oklahoma from 1988 to 1990. Alfalfa stands selected for sampling were from 2-5 years of age, had limited weed interference, and did not receive insecticide applications.

Sampling intervals for alfalfa weevil eggs were typically 2 weeks through the winter and decreased to 1 week during March and April. Sampling was begun in December of 1988 and in November of 1989. Each sample consisted of all dead and living alfalfa stems clipped from a 0.025 m^2 area. Twenty samples were selected at random from the field on each date. The eggs were extracted from these samples using the blender technique of Pass and VanMeter (1966).

Eggs were sorted by color into three classes. Yellow eggs are those most recently oviposited and have completed virtually no embryonic development. Brown eggs are those with more than 26 day degrees accumulated for development (Morrison and Pass, 1974). The brown coloration, resulting from cellular structure of the embryo, persists throughout most of embryogenesis. When the time for hatching nears, the head capsule of the developing larva can be seen through the chorion. This third stage of egg development is referred to as the blackhead stage.

Yellow eggs taken in field sampling were utilized to determine the day degree requirement for completion of the brown stage of development. They were available from January through March in both years and were also collected in December, 1989, and April, 1990, at Stillwater. Low numbers of eggs at Chickasha during the winter of 1989-90 years resulted in the collection of few yellow eggs. For this reason, no samples from Chickasha in 1989-90 were used for this study.

All of the yellow eggs found on each sample date were transferred to a petri dish lined with moist filter paper. Up to one hundred of these eggs were transferred, at a rate of twenty per dish, to petri dishes lined with gridded filter paper. A small amount of 5% cupric sulfate solution was added to the dishes to inhibit the growth of mold. If more than 100 eggs were collected, 100 were selected at random from the available eggs with any egg that appeared damaged or inviable being discarded.

The petri dishes containing the eggs were then placed in a darkened growth chamber at 22±2°C. At ca. 12 hour intervals, the eggs were checked for changes in color and the filter paper was moistened to prevent desiccation. The time required for each egg to reach the brown or blackhead stage was recorded and those at the blackhead stage were discarded. The number of day degrees that occurred between the start of brown and blackhead stages was calculated for each. A developmental threshold of 9°C was used for the calculations resulting in the accumulation of ca. 0.5 day degree/hour at the temperature of 22 °C. By the choice of incubation temperature and limiting the time between observations, the possibility of over-estimation of the day degree requirements was reduced.

From these data, mean brown stage developmental requirements were calculated for all eggs sampled and for each location-year-month combination. The overall mean was compared to published values using a t-test to determine if the values found in this study are significantly different from the accepted values. Based on data reported by Morrison and Pass (1974), 67% of the day degree requirements for egg development accrue during the brown stage. Day degree requirements for total egg development reported in the literature were reduced by 33% in order to compare them with the results of this study. Data from literature sources were also adjusted such that all values used for comparison are based on a developmental threshold of 9 °C.

The day degree requirements for the groups of eggs were also compared to determine if significant differences occurred. Eggs were classified by location-year combinations and by months. Location and year groups were not examined separately because of the lack of data for 1989-90 at Chickasha. A general linear models procedure was conducted to detect significant differences in development times among the groups of eggs (SAS, 1988).

Results and Discussion

When the brown stage day degree requirements for all eggs collected were averaged, the grand mean was 78.0 with a standard deviation of 0.57 based on a total 286 eggs. Due to the large number of observations used to calculate the mean, small differences can be declared significant when comparing this mean to published values. Even with this high level of power, the value found in this study is not significantly different at the 0.01 level from the values reported by Litsinger and Apple (1973). It is significantly different from the 80 DD value of Morrison and Pass (1974), 74 DD value of Guppy and Mukerji (1974), and the 98 DD value of Roberts et al. (1970) (Table 1). However, with the exception of the results of Roberts et al. (1970), the greatest deviation from any of the published means was four day degrees. Since the results of Morrison and Pass (1974) and Guppy and Mukerji (1974) differ by six day degrees, the statistically significant difference between these published and observed means is a product of the power of the statistical test and has no practical significance. The mean day degree requirement reported by Roberts et al. (1970) was 20 day degrees higher than the observed mean. However, those results were reported in terms of days required to complete development and the incubation temperatures used were higher than for the other studies. Both of these factors increase the number of day degrees accumulated between times that the eggs are checked for developmental changes, which increases the probability of overestimating the day degree requirements.

Mean day degree requirements by month ranged from 66 for March at Stillwater, 1989 to 83 for March at Chickasha, 1989 (Table 2). Also listed in Table 2 are the F values for comparing the monthly means within each location/year combination. The monthly day degree requirements were not significantly different in 1988-89, with p values of 0.116, and 0.675 for Stillwater and Chickasha, respectively. In 1989-90 at Stillwater, there was a significant difference among the months (p=0.0082); however, the difference between highest and lowest monthly day degree requirements is only 7 day degrees.

A single analysis of variance table for all three location/year combinations is shown in Table 3. As noted before, months did not have a consistent effect. For this reason, the interaction between locations and month was present with the F statistic having a p-value of 0.100. Since this interaction is not highly significant, some information can be gained by examining the simple effects for locations and months. As expected from the results of the one-way analysis of variance, the simple effect for month was significant, having a p-value of 0.019. The simple effect for location/year combinations was not significant having a p-value of 0.72 for the F statistic, indicating that when averaged over the years, there was little difference in the day degree requirements for the combinations. This is further demonstrated by the yearly means shown in Table 2, with the three means having a range of 2 day degrees.

Conclusions

Even though significant variation was present among the day degree requirements for the development of brown eggs collected in different months, there was no set pattern for the variation. For this reason, the results of this study do not cause concern that the developmental day degree requirements of alfalfa weevil eggs varies with the time of oviposition. Also, the yearly means of these field-collected eggs were consistent and the overall mean day degree requirement agreed with most published reports for eggs from lab colonies. This study provides little evidence that the published day degree requirements are not applicable for Oklahoma weevil populations.

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Table 1. Deviation of published day degree requirements from the observed mean from Chickasha and Stillwater, Oklahoma.

Literature Source	Brown Stage DD 1/	Deviation 2/	t-value 3/	p
Guppy & Mukerji	74	-4	7.01	0.0000
Litsinger & Apple	79	1	1.75	0.0400
Morrison & Pass	80	2	3.50	0.0001
Roberts et al.	98	20	35.03	0.0000

1/ 67% of reported day degree requirement for egg hatch, corrected if necessary, to a developmental threshold of 9°C.
2/ (Brown DD - 78)
3/ (Brown DD -78)/0.57

Table 2. Mean and standard deviations of each location/year and month combination from Chickasha and Stillwater, Oklahoma.

	Stillwater		Chickasha
Month	1988-89	1989-90	1988-89
November	- -	81 (12.5)	- -
December	80 (12.2)	82 (12.0)	77 (3.8)
January	77 (7.42)	75 (6.5)	79 (8.2)
February	79 (9.20)	75 (11.6)	78 (10.9)
March	66 (5.02)	77 (5.6)	85 (12.9)
April	- -	74 (9.4)	- -
F-Value (Month)	2.03	3.26	0.51
p-Value (Month)	0.116	0.0082	0.675
Yearly Mean	78 (9.0)	78 (10.3)	79 (9.1)
Grand Mean		78 (9.7)	

Table 3. Analysis of variance for location/year and month combinations.

Source	df	Mean Square	F	p
Location/Year	2	97.6	0.33	0.720
Month	5	820.4	2.77	0.019
LY*Month	6	533.6	1.80	0.100
Within LY*Month (Error)	272	296.6		

CHAPTER V

ASSESSMENT OF THE POPULATION POTENTIAL OF ALFALFA WEEVIL, *HYPERA POSTICA* (GYLLENHAL), IN OKLAHOMA

Introduction

The alfalfa weevil, *Hypera postica* (Gyllenhal), was first documented in Oklahoma in 1969 and by 1972 had been reported in all counties of the state. Since 1972, larval populations have caused severe damage to the first crop of alfalfa and significant subsequent losses at second cutting due to reduced plant vigor (Berberet et al., 1981).

Oviposition by adult weevils begins in the fall and continues through the following spring with the rate of oviposition being related to the temperature. LeCato and Pienkowski (1970) demonstrated that female alfalfa weevils respond quickly when the temperatures rise above the ovipositional threshold of 1.7°C.

In northern regions, researchers have reported that eggs laid in the fall and winter do not contribute significantly to the larval populations in spring (Townsend and Yendol, 1968; Blickenstaff, et al., 1972). Temperatures in these areas frequently fall below the lower lethal limit and cause mortality of many of the eggs that are present. In southern regions, this does not typically occur. Bass (1967) reported ovipositional activity reached its peak during January in Alabama and Pitre (1969) found that eggs were most abundant in January and February. In both cases, larvae resulting from these eggs were present in late winter and early spring. In Oklahoma, fall and winter oviposition has resulted in economically damaging populations of larvae (1.5 larvae per alfalfa stem) as early as late January, with peak densities having exceeded 1300 eggs/0.1 m^2 (Mulder et al., 1988).

While conditions exist that permit deposition of large numbers of eggs, there are also several factors which may limit the survival of these eggs and the larvae that hatch from them. Low temperatures often occur that render a portion of the eggs inviable. Also, larvae hatching from eggs before alfalfa growth has begun in spring may have difficulty in locating a living alfalfa terminal. Newly hatched larvae are quite susceptible to low temperatures and either desiccation or drowning, depending on weather conditions, unless they can rapidly locate a suitable feeding site. This is especially a problem for larvae hatching from eggs laid in dead stems.

In general, the larval stage is more susceptible to low temperatures than the egg stage with cold-hardiness decreasing with each successive instar (Armbrust et al., 1969). In years when sub-freezing temperatures occur in

April, there may be extensive mortality of third and fourth instars.

Since 1983, infections of the fungus, Zoophthora phytonomi (Arthur), have reduced numbers of weevil larvae, prepupae, and pupae in Oklahoma, with infection percentages of field-collected larvae as high as 100, 85, and 52, respectively. While this fungus has not consistently eliminated larval populations, epizootics have in some years reduced the need for insecticide applications near the time of first harvest and may greatly reduce the number of weevils that reach the adult stage (Goh et al., 1989).

When larval populations are not controlled with insecticides, survival is often reduced by starvation as numbers in excess of 500/0.1 m^2 often completely strip plants of foliage. While starvation of weevils does not reduce the damage to alfalfa plants, it does diminish the numbers of weevils reaching the adult stage.

Based on life table studies in Ontario, Canada, Harcourt et al. (1977) reported that several mortality factors combined to prevent more than 90% of the potential alfalfa weevil population from reaching the adult stage. The extent that weevil numbers are reduced by various mortality factors has not been quantified in Oklahoma. The purpose of this paper is to estimate the population potential of the alfalfa weevil in the absence of these limiting factors, to assess the degree to which they limit population size, and to examine their relative importance.

Materials and Methods

Data for this study were collected from sampling areas at Stillwater (North Central) and Chickasha (South Central), Oklahoma. Studies began with the 1980-81 year and ended with the 1989-90 year in both locations. In this context, a year refers to the period from onset of oviposition in fall of one year until emergence of adults the following April or May. Alfalfa stands selected for sampling were from 2-5 years of age, had limited weed interference, and did not receive insecticide applications.

Sampling intervals for determining egg numbers were typically 2 weeks from October until February, then reduced to one week until cessation of sampling in May. Each sample consisted of plant material (dead and living alfalfa stems) removed from a 0.025 m^2 area. Twenty samples were selected at random on each date. The eggs were extracted from these samples using the blender technique of Pass and VanMeter (1966). For sampling dates after 31 January, a subset of ca. 100 eggs was reared in an incubator to determine the percent of the eggs which were viable.

When numbers of newly hatched larvae recovered in egg samples exceeded 5-10/0.1 m^2 of foliage, larval sampling was begun. This procedure involved collecting foliage from ten, 0.1 m^2 areas for estimation of larval numbers. Berlese funnels were used to extract larvae from the foliage prior to recording numbers of each instar.

In addition to the egg and larval data for each year, daily temperature data were gathered from a reporting station near each sampling location. Developmental day degree accumulations were calculated for each day from fall to spring using a sine wave approximation. Day degree calculations were based on a developmental threshold of 9°C (Litsinger and Apple, 1973).

In order to estimate the total number of eggs that were deposited per unit area (0.1 m^2) each year, counts for several sampling dates occurring at specific day degree intervals before and after the date of peak egg density were summed. Dates were selected having an approximately 105 day degree accumulation between them. As 105 day degrees are required for hatching (Litsinger and Apple, 1973), the eggs present on one date would be expected to have hatched before the next date and those present on the second date would have been laid in the interval between the two dates. For each of the selected sampling dates, the number of viable eggs was estimated by multiplying the total number of eggs times the percent viability determined for that date. Viable egg totals were also summed over the selected sampling dates as an estimate of the number of eggs per m^2 that survived to hatching.

First instars found in egg samples were those that had not yet left stems and they were included in both the total and viable egg counts. Beginning the selection process with the sample having the peak egg numbers insured that the estimated total eggs for each year included all present at the peak as well as those that hatched earlier or were laid later.

The same procedure was used to estimate the total number of larvae per 0.1 m^2 in three groups; first and second instars, third instar, and fourth instar that were present during the spring. Sample dates were selected before and after each instar peak at 71, 39, and 50 day degree intervals, respectively (Litsinger and Apple, 1973). Since the larval numbers of each instar peaked on different sample dates and the day degree requirements differ for the instars, this calculation was done separately for each group.

The first and second instars were combined in order to better match the day degree requirements to the accumulation of day degrees between the selected samples. If the number of day degrees that accumulated between sample dates is consistently larger than the number of day degrees between selected samples, the number of weevils that occurred in that life stage will be underestimated. However, numbers of third and fourth instars were not combined as the interval between samples would have been quite wide and it would not have been possible to estimate mortality in third instars.

During some years, the larval samples from the earliest selected sample date for a given instar contained individuals from later instars. These later instars are larvae which had molted prior to the first selected sample date and thus were not counted when they were earlier instars. Specifically, the number of third and fourth instars present in the earliest selected sample date for first and second instars was included in the first and second instar total. On the earliest date selected for third instars, the number of fourth instars present was also included in the third instar total.

Preliminary comparisons of data for egg and larval counts indicated that the numbers of eggs being recovered from samples was consistently lower than what the actual numbers should have been based on the numbers of larvae recovered. Total numbers of first and second instars frequently exceeded numbers of eggs estimated to have been present. For an accurate adjustment to reflect the number of eggs laid during the year, it was assumed that the estimated number of first and second instars recovered and the estimated number of viable eggs should be equal. This assumption results in a conservative estimate of total egg numbers because it does not include larvae that die before becoming established in plant terminals.

When the number of first and second instars was initially estimated to be greater than the number of viable eggs, the percent difference was assumed to be equal to the percentage by which egg numbers were underestimated in the sampling process. The estimated egg total (viable + inviable) was increased by this same percentage. In four instances where the initial estimate (from sampling) for total eggs was greater than the number of first and second instars, the estimate was left unchanged.

If no mortality occurred, the four population densities determined for weevils in each generation (total eggs, first and second instars, third instars, and fourth instars) would have the same value. However, mortality obviously occurs and the following are procedures for estimating losses for each stage.

Egg inviability was estimated by comparing the adjusted total eggs to the number of first and second instars. The adjustment made for the underestimation of eggs assumed the number of viable eggs to be equal to the number of established (sampled) first and second instars. Therefore, the difference between the viable egg total and the first and second instars total is always zero, preventing mortality of larvae prior to establishment from being estimated in this study. Harcourt et al. (1977) reported a 25.2% mortality of weevils due to failure to establish. Also, Bartell and Pass (1978) found that 32% of first instars in lab colonies failed to establish in plant terminals. The differences between the subsequent instar totals are estimates of the mortality of individuals in the earlier stages. In two years of the study period, the estimated number of individuals in a later life stage exceeded the number in an earlier life stage. In these cases, it was assumed that the earlier life stage was underestimated and the total of the earlier life stage was set equal to the total of the later life stage. In all years the difference between life stage totals was divided by the adjusted total number of eggs that were laid, thus estimating the proportion of the population potential that was lost between life stages. This value is referred to as the mortality proportion for the earlier life stage. Finally, these proportions were totaled for each year and location to estimate the proportion of the population that died prior to reaching the fourth instar.

Data on percent infection by Z. phytonomi (Goh et al., 1989) were examined along with weather records to identify possible sources for the estimated mortality of eggs and larvae.

Results and Discussion

Numbers of viable eggs estimated for years ranged from 153.1 to 1565.3 /0.1 m^2 with means of 602.32 at Chickasha and 610.48 at Stillwater (Tables 1 and 2). In 16 of the 20 year/location combinations, the number of first and second instars was greater than the viable egg total. These differences indicate that the number of eggs laid was underestimated by an average of 28 and 35 percent for Chickasha and Stillwater, respectively. The total numbers of eggs (viable and inviable) were adjusted to reflect this underestimation and the adjusted totals are given (Tables 1 and 2). The adjusted estimates show an average of more than 1300 eggs/0.1 m^2 were deposited per year at Stillwater, while at Chickasha the average was 1127.05 eggs/0.1 m^2. The average number of viable eggs was about the same for the two locations; however, Stillwater had greater numbers of first and second instars. This difference resulted in smaller adjustments in the total egg estimates for Chickasha. Even though these adjustments result in large increases in the estimated egg totals, the true number of eggs deposited during the year was in all likelihood higher. No adjustment was made for establishment losses of first instars. Since these losses have been reported to be 25-35% (Harcourt et al., 1977; Bartell and Pass, 1978), the true mean egg total may be in excess of 1500 eggs/0.1 m^2.

Totals for first and second instars ranged from 122 to 2227.5 /0.1 m^2/year at Chickasha with a mean of 792.57. The totals were higher at Stillwater, ranging from 414.2 to 1879.8 /0.1 m^2/year with a mean of 926.85. Estimated totals for the third instar were much lower than those for the first and second instars with means of 343.29 and 452.73 /0.1 m^2/year at Chickasha and Stillwater, respectively (Tables 1 and 2). The fourth instar totals decreased further with means of 144.74 and 215.97 /0.1 m^2/year, respectively.

To compare the extent of mortality among locations, years, and life stages, the decline in numbers between consecutive life stages was converted to a proportion of the total number of eggs that were present in that year (Table 3). Therefore, these proportions represent the relative amount of the population that was lost between those two stages. The mortality estimated by these proportions is attributed in each instance to the earlier life stage; however, the decrease in numbers may have been partially due to individuals that had reached the later life stage, but died prior to being sampled (Table 3).

Despite the larger numbers of eggs and larvae at Stillwater, the average mortality proportions were similar for the two locations with the greatest difference in means being 0.021 for eggs (Table 3). Averaged over the two locations, there was 31.8% mortality of eggs. Losses in this stage can be attributed to lethal, low temperatures, to desiccation of eggs, and the inability of some newly hatched larvae to locate suitable feeding sites in plant terminals. While all of these factors result in mortality that should be attributed to the egg stage, the data presented here measure only the mortality due to egg inviability.

The data for the 1983-84 year exemplify the effects of lethal, low temperatures on eggs, as well as, the moderating influence of snow cover. Approximately 72% of the eggs were rendered inviable at Chickasha due to 4 days with maximum temperatures below -7 °C in December, while at Stillwater, where ambient temperatures were below -7 °C for 11 days, there was only a 36% loss of eggs. The only apparent difference was the presence of 12-15 cm snow at Stillwater during the time when lethal temperatures occurred. The snow appears to have acted as an insulating layer that helped to prevent mortality of the eggs within alfalfa stems.

Mortality of first and second instars that could be directly attributed to low temperatures appeared to be infrequent. They are relatively cold-hardy and have supercooling points below -16 °C (Armbrust et al., 1969). One possible example occurred during 1985-86 when egg hatch began early and numbers of first and second instars were high. A 2 day period with minimum temperatures below -12 °C occurred on February 11 and 12, contributing to mortality proportions of 0.64 and 0.42 for Chickasha and Stillwater, respectively. The difference in the mortality between the two locations may again be attributed to 25 cm snow cover present at Stillwater.

In spite of the lack of clear examples, there is indirect evidence of mortality in first and second instars due to low temperatures. Table 5 shows that about 35% of the weevil population is lost in these life stages. These larvae are present during a period when the activity of other possible mortality factors has been reported to be low. While Bathyplectes curculionis (Thomson) may parasitize first and second instars, it does not kill hosts until they have reached the prepupal stage (Berberet and Gibson, 1976). The earliest reported infections by Z. phytonomi occurred ca. 20 March and no epizootics have occurred prior to April (Goh et al., 1989). Also, since the forage consumption by first and second instars is relatively low, starvation of these larvae is unlikely unless third and fourth instars have previously destroyed plant terminals. There are usually relatively few predaceous insects observed during times that early instars predominate. Therefore, the only remaining mortality factor that has been identified for first and second instars is temperature.

Mortality of third instars due to temperature was not verified in this study. Armbrust et al. (1969) reported that third and fourth instars were more susceptible than earlier instars to low temperature with supercooling points of -9.8°C and -8.2°C, respectively. However, occurrences of temperatures below -8°C are rare during late March and April when larger instars are prevalent.

Mortality of third instars could be attributed to infection by <u>Z. phytonomi</u>, predators and starvation. The mortality estimate for third instars ranged from 0.0 to 0.41 at Chickasha and from 0.05 to 0.47 at Stillwater with means of 0.169 and 0.160, respectively. While it is difficult to separate the effects of these factors, there seems to be a relationship between the number of first and second instars and the third instar mortality proportion. In years when the first and second instars totaled more than 500 /0.1 m^2, the third instar mortality proportion averaged 0.213 at Chickasha and 0.203 at Stillwater. However, the third instar mortality proportion averaged 0.095 at both locations in years when the first and second instars totaled less than 500 /0.1 m^2. This difference is also evident in the data for 1983-84. The first and second instars totaled 1043.3 /0.1 m^2 at Stillwater (Table 2) while at Chickasha there were just 262.2 /0.1 m^2 (Table 1). The higher numbers of larvae had completely defoliated plants at Stillwater resulting in the starvation of third instars. The mortality proportion was 0.47 at Stillwater compared to 0.20 at Chickasha where larval numbers were not high enough to completely defoliate the alfalfa and cause starvation.

The proportions of the population potential of the weevil lost during the egg and larval stages are consistent between the two locations (Table 4). The average mortality proportion for the egg stage was 0.328 at Chickasha and 0.307 at Stillwater. The egg mortality proportions are much higher than the 2.1% reported by Harcourt et al. (1977). However, they reported 25.2% mortality due to establishment loss, which was not estimated in this study. At Chickasha the average first through third instar mortality proportion was 0.512, while at Stillwater it was slightly higher at 0.517. Harcourt et al. (1977) report similar results with 62% of the population being lost in first through fourth instars.

However, the sources of mortality differed in the two studies. Harcourt attributed virtually all larval mortality to Z. phytonomi and starvation was not a factor. Data from both studies indicate that larval mortality factors are more important in limiting the success of the alfalfa weevil. However in Oklahoma, since much of the mortality in the larval stage is due to starvation, the mortality in the egg stage may be of more importance in terms of preserving alfalfa production.

The number of larvae reaching the fourth instar provides a measure of the carrying capacity of alfalfa stands in Oklahoma. While estimates of the potential larval population ranged from 311.0 to 2908.9 (adjusted egg totals in Tables 1 and 2), the total fourth instar numbers exceeded 400 /0.1 m^2 in only 2 years and in most years were under 300 /0.1 m^2 (Table 5). It seems that even if very large numbers of larvae survive to the third instar, the alfalfa stand can not support more than 500 larvae/0.1 m^2.

The average proportion of the eggs laid that survived to the fourth instar was 0.160 and 0.176, for Chickasha and Stillwater, respectively (Table 5). Even though these data indicate that over 80% of the potential population of the weevil was lost prior to the fourth instar, the proportion surviving to adulthood is even lower. Data were not available in this study to assess the mortality in the fourth instar, prepupae, and pupal stages. Since mortality due to parasitism and fungal infections is more prevalent after most larvae have reached the third and fourth instars, the effect of these factors has not been fully quantified in this study.

Conclusions

The reproductive potential of the alfalfa weevil is very high and the winter and spring climate in Oklahoma allows much of this potential to be realized with large numbers of eggs being deposited. However, more than 30 percent of the eggs are rendered inviable. Subsequently, 50 percent of the eggs deposited result in established larvae that are lost before they reach the fourth instar. Finally, unmeasured mortality factors effecting the fourth instars, prepupae, and pupae probably further reduce the actual adult population potential of the weevil to less than 10% of total eggs actually laid.

While the average mortality was fairly consistent over the years, different mortality factors came into play in different years. The mixture of mortality factors seem to act as a system to limit the survival of weevils to the adult stage. Unfortunately, the final limiting factor before the fourth instar is reached is starvation of the larvae due to lack of host material and this limiting factor must be replaced with some combination of cultural and chemical controls to allow profitable alfalfa production.

Further research is needed to directly link the mortality demonstrated in this study with specific mortality factors, especially in the larval stage. Also additional data are needed in order to assess the mortality of fourth instars, prepupae, and pupae.

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Table 1. Estimated total numbers of individuals by life stage for Chickasha, Oklahoma, 1981-90.

	Actual Egg Counts		Proportion	Adjusted	1st & 2nd	Third	Fourth
Year	Total	Viable	Missed	Total Eggs	Instars	Instars	Instars
8081	186.0	153.1	0.40	311.0	256.0	174.7	174.7
8182	1837.8	1243.8	0.13	2121.8	1436.0	749.2	474.6
8283	694.4	553.4	0.20	872.5	695.3	246.2	192.8
8384	933.4	421.9	0.00	933.4	262.2	262.2	72.4
8485	767.8	579.8	0.39	1258.8	950.6	696.2	174.9
8586	2015.0	1543.0	0.31	2908.9	2227.5	371.3	46.0
8687	939.8	795.7	0.17	1126.4	953.7	310.5	131.3
8788	272.8	234.2	0.67	821.3	705.1	492.5	158.9
8889	591.2	312.0	0.02	601.2	317.3	103.3	22.6
8990	315.2	186.3	0.00	315.2	122.0	26.8	9.7
MEAN	855.3	602.3	0.28	1127.0	792.5	343.2	145.8
STD	592.09	443.16	0.193	773.25	615.29	227.07	127.06

Table 2. Estimated total numbers of individuals by life stage for Stillwater, Oklahoma, 1981-90.

Year	Actual Egg Counts		Proportion Missed	Adjusted Total Eggs	1st & 2nd Instars	Third Instars	Fourth Instars
	Total	Viable					
8081	256.2	174.4	0.70	861.9	586.7	435.3	310.3
8182	515.0	341.2	0.58	1233.9	817.5	304.3	243.7
8283	511.8	399.5	0.33	764.9	597.1	321.4	304.5
8384	1005.8	645.5	0.38	1625.6	1043.3	993.5	234.4
8485	2164.8	1565.3	0.17	2599.8	1879.8	848.4	344.2
8586	1263.2	850.5	0.36	1959.2	1319.1	504.4	99.2
8687	1091.4	930.3	0.36	1695.0	1444.8	522.1	416.5
8788	430.4	339.0	0.59	1057.7	833.1	391.2	139.4
8889	649.4	420.2	0.00	649.4	414.2	139.2	30.1
8990	674.0	438.9	0.00	674.0	332.9	67.5	37.4
MEAN	856.2	610.5	0.35	1312.1	926.9	452.7	216.0
STD	529.91	389.79	0.227	611.63	469.53	273.23	126.66

Table 3. Estimated mortality proportion for eggs, first and second instars, and third instars for Chickasha and Stillwater, Oklahoma, 1981-90.

Year	Egg		First and Second Instars		Third Instar	
	Chick.	Still.	Chick.	Still.	Chick.	Still.
8081	0.18	0.32	0.26	0.18	0.00	0.15
8182	0.32	0.34	0.32	0.42	0.13	0.05
8283	0.20	0.22	0.51	0.36	0.06	0.02
8384	0.72	0.36	0.00	0.03	0.20	0.47
8485	0.24	0.28	0.20	0.40	0.41	0.19
8586	0.23	0.33	0.64	0.42	0.11	0.21
8687	0.15	0.15	0.57	0.54	0.16	0.06
8788	0.14	0.21	0.26	0.42	0.41	0.24
8889	0.47	0.36	0.36	0.42	0.13	0.17
8990	0.61	0.51	0.30	0.39	0.05	0.04
Mean	0.328	0.307	0.343	0.357	0.167	0.160
Std	0.194	0.095	0.179	0.139	0.133	0.126

Table 4. Estimated mortality proportion for eggs and first through third instars and overall mortality proportion for Chickasha and Stillwater, Oklahoma, 1981-90.

Year	Egg		First - Third Instars		Overall	
	Chick.	Still.	Chick.	Still.	Chick.	Still.
8081	0.18	0.32	0.26	0.32	0.44	0.64
8182	0.32	0.34	0.45	0.47	0.78	0.80
8283	0.20	0.22	0.58	0.38	0.78	0.60
8384	0.72	0.36	0.20	0.50	0.92	0.86
8485	0.24	0.28	0.62	0.59	0.86	0.87
8586	0.23	0.33	0.75	0.62	0.98	0.95
8687	0.15	0.15	0.73	0.61	0.88	0.75
8788	0.14	0.21	0.67	0.66	0.81	0.87
8889	0.47	0.36	0.51	0.59	0.98	0.95
8990	0.61	0.51	0.36	0.44	0.97	0.94
Mean	0.328	0.307	0.512	0.517	0.840	0.824
Std	0.194	0.095	0.181	0.107	0.154	0.118

Table 5. Estimated number of fourth instars and proportion surviving to fourth instar for Chickasha and Stillwater, Oklahoma, 1981-90.

	Fourth Instar		Survival	
Year	Chickasha	Stillwater	Chickasha	Stillwater
8081	174.7	310.3	0.56	0.36
8182	474.6	243.7	0.22	0.20
8283	192.8	304.5	0.22	0.40
8384	72.4	234.4	0.08	0.14
8485	174.9	344.2	0.14	0.13
8586	46.0	99.2	0.02	0.05
8687	131.3	416.5	0.12	0.25
8788	158.9	139.4	0.19	0.13
8889	12.1	30.1	0.02	0.05
8990	9.7	37.4	0.03	0.06
Mean	144.74	215.97	0.160	0.176
Std	128.108	126.662	0.154	0.118

CHAPTER VI

A MULTIVARIATE METHOD FOR PREDICTING THE TIMING OF ALFALFA WEEVIL, HYPERA POSTICA (GYLLENHAL), LARVAL POPULATIONS EXCEEDING THE ECONOMIC THRESHOLD IN OKLAHOMA

Introduction

The alfalfa weevil, *Hypera postica* (Gyllenhal), is the most severe pest of alfalfa in Oklahoma and in most alfalfa producing areas of North America. Damage includes direct loss of forage in the first harvest due to larval feeding as well as reductions in yield in later cuttings due to loss of plant vigor (Berberet et al., 1981; Wilson et al., 1979). Due to its high loss potential, much research has been conducted with the goal of understanding the population dynamics of the weevil and predicting the occurrence of damaging populations.

Temperature based models have been developed which predict the occurrence of peaks of the life stages of the weevil. Harcourt (1981) developed a model based on the number of day degrees accumulated after 1 April. Using four years of data to validate the model, at no time, for any population event, did the observed and predicted dates differ by more than 2 days. However, the timing of population events is fairly consistent in northern growing regions. Harcourt also reported that peak larval populations occurred over the same 7 day span during these 4 years.

In southern regions, the timing of population events occurs over a much wider range. The time at which larval populations first exceed the economic threshold at Chickasha and Stillwater, Oklahoma, has occurred almost as often in April as it has before 1 March. Depending on the timing of temperatures below the lower lethal limit, oviposition that results in the establishment of larvae may begin as early as November or as late as early March. This wide range was attributed to the high degree of variation in temperature conditions through the winter months. Also, the numbers of overwintering adult weevils varies from year to year as does the potential population size for eggs and larvae. After larval populations begin to increase in February and March, lethal temperatures are not uncommon (Chapter III).

These factors prevent models such as that of Harcourt (1981) from being successfully implemented in Oklahoma. This is illustrated by a lack of consistency in the number of day degrees accumulated between 1 January and the peak of the larval populations in Stillwater and Chickasha, Oklahoma. It is apparent that models will need to include parameters in addition to basic day degree accumulations from a fixed date in order perform consistently (Chapter III).

Discrimination analysis is a statistical technique that derives rules for classifying individuals into two or more groups. Each rule is based on an algebraic combination of variables measured on individuals known to belong to those groups. If a rule adequately separates the groups, it can be used to classify new observations based on the variables used to derive it (Johnson and Wichern, 1988). Fisher (1938) developed this method using the separation of two iris species, Iris setosa (Pall. ex Link) and Iris versicolor L., based on measurements of flower characteristics, as an example. It has been used in morphometric studies to separate individuals of different groups of organisms that can not be easily distinguished visually (Pimentel, 1979). Inayatullah et al. (1987) used this method to separate greenbug, Schizaphis graminum (Rondani), biotypes based on measurements of body parts. Pearson and Meyer (1990) used discrimination analysis to identify sites infested with blueberry maggots, Rhagoletis mendax (Curran), based on physical site characteristics such as bush height, shade percentage, and soil organic matter and sand percentages.

The purpose of this study is to develop a method to predict the time of the first annual occurrence of alfalfa weevil larval populations exceeding the economic threshold. Multivariate discrimination analysis was used to make predictions based on variables which could be measured on or before 15 February. As larval populations rarely reach economic threshold levels prior to 15 February, these predictions can assist producers in planning sampling and control strategies.

Materials and Methods

Data for this study were collected from sampling areas at Stillwater (North Central) and Chickasha (South Central), Oklahoma. Studies began with the 1971-72 year at Stillwater and 1975-76 at Chickasha and continued through 1989-90. In this context, a year refers to the period from onset of oviposition in fall of one year until emergence of adults the following April or May. Alfalfa stands selected for sampling were from 2-5 years of age, had limited weed interference, and did not receive insecticide applications.

Sampling intervals for eggs were typically 2 weeks from October until February and reduced to one week until cessation of sampling in May. Each sample consisted of plant material (dead and living alfalfa stems) removed from a 0.025 m^2 area. Twenty samples were selected at random for each location and date. The eggs were extracted from these samples using the blender technique of Pass and VanMeter (1966). For sampling dates after 31 January, a subset of ca. 100 eggs was placed in an incubator in order to determine the percentage of the eggs which were viable.

When numbers of newly hatched larvae in egg samples exceeded 5-10/0.1 m^2, larval sampling was begun. This procedure involved collecting foliage from 10, 0.1 m^2 areas for estimation of larval numbers in plant terminals. Berlese funnels were used to extract larvae from the foliage prior to recording numbers of each instar.

Each year/location combination of the data set was classified into one of four classes depending on when the larval populations first exceeded the economic threshold. This designation was made when the 2nd, 3rd and 4th instars combined totaled more than 45 larvae per 0.1 m^2 (Mulder et al., 1988). The four classes were Pre-March (PMAR), Early-March (EMAR, 1 March -15 March), Late-March (LMAR, 16 March - 31 March), and April (APR).

In addition to the egg and larval data for each year, daily temperature data were gathered from a reporting station near each sampling location. These data were used in the calculation of temperature related quantities as detailed below.

Several candidate variables were evaluated to determine effectiveness in separating the four classes. Candidate variables were selected to account for variation in the abundance of adults, the amount of oviposition, the amount of heat energy available for oviposition and development, and the occurrence of temperatures below the lower lethal limit for survival. A set of six variables was selected which was related to these sources of variation. The utility of this set for separating the four classes of years was assessed using multivariate analysis of variance and discrimination analysis as detailed below.

Selected Discrimination Variables

In order to estimate the number of weevil adults ovipositing during a given time frame, the adult activity index was calculated for each year as described in Chapter III. The adult abundance index (AAI) measures the number of eggs laid per square meter per ovipositional day degree. As the AAI is calculated on a per day degree basis, it is intended to provide an index of the number of active adults that were present and is not dependent solely on the response of those weevils to temperature.

The estimated number of eggs present on 1 January and the number of viable eggs on 1 February was recorded for each year. Total egg numbers were used on 1 January because viability data were not always taken in early January.

Temperature-related variables were also measured to monitor the ovipositional activity and egg development. To account for the amount of heat energy available for oviposition and egg development, day degree accumulations from 1 January until 15 February were calculated using a sine wave approximation. Ovipositional day degrees (DD1) were based on a threshold of 1.7°C (Hsieh and Armbrust, 1974) and developmental day degrees (DD9) were based on a 9 °C threshold (Litsinger and Apple, 1973). To estimate effects of lethal, low temperatures on weevil populations, the number of days in which the minimum temperature was at or below -12°C (SUB12) was also totaled between 1 January and 15 February.

Statistical Analysis

Univariate and multivariate analysis of variance procedures were conducted to confirm that the four classes differed in regard to the six variables measured: AAI, 1 January egg total, 1 February viable egg total, DD1, DD9, and SUB12. Four different test statistics were calculated as a result of the multivariate analysis of variance procedure (SAS, 1988). Significant differences among the classes, at least in the multivariate tests, are necessary for there to be a reasonable chance for the discrimination analysis to be successful.

Due to the low number of observations in the classes, it was not possible to estimate the error rates of the discrimination analysis. For this reason, the discrimination procedure was based on simulated data and tested using the observed data, as detailed below. The sample means and standard deviations of each variable were calculated for each of the four classes along with the sample covariance matrix for the six variables. Based on these calculations, 10 sets of observations (six variables each) were generated for each of the four classes. In each class, the variables were generated so that they represent a six-dimensional normal distribution having the same mean vector and covariance structure as was estimated for that class. The interactive matrix language program (SAS, 1985) used for generation on multi-dimensional normal variates was developed based on a procedure outlined by Weeks and Spradling (personal communication, Oklahoma State University, 1991). A listing of the program is given in the appendix.

The covariance matrices of the four classes were compared to see if they were equal using a chi-square test for homogeneity of covariance (Morrison, 1976). Based on the results of this test, the appropriate discrimination procedure was selected.

Discrimination analysis was conducted based on the 40, six variable observations (SAS, 1988). This technique derived a rule which can be used to classify years into one of the four classes. Because the day degree values and egg samples may contain most of the information contained in the adult abundance index, a second analysis was run using only five variables. Since the calculation of the AAI requires additional egg sampling, it would be desirable for the discrimination rule to be based on only the five other variables.

In both cases, the discrimination rule was evaluated by classifying the original 34 observations and comparing the model's predictions to field observations. In cases of misclassification, the values of the discrimination variables and weather data were examined for possible explanations.

The performance of the discrimination rule was also evaluated by examining the posterior probabilities. For each observation, the set of four posterior probabilities was calculated. These probabilities estimate the likelihood of a given observation belonging to each of the four classes (SAS, 1988). The observation is placed in the class corresponding to the highest posterior probability. If all observations were classified correctly with a posterior probability of 1, the discrimination function has performed well.

The generalized squared distances between all pairs of classes were calculated to measure the relationships among the classes. This method is used to determine the similarities between the four time classes with reference to the variables included in the analysis. By knowing the relative similarities of the classes, it is possible to evaluate which are most likely to be misclassified and into which class misclassified observations are most likely to fall.

Results and Discussion

The EMAR class was the most prevalent at both Chickasha and Stillwater with a total of 14 observations (Table 1). Approximately the same number of year/location combinations fell into each of the other three classes.

Means and standard deviations for the six discrimination variables are given for each class in Table

2. The adult abundance index (AAI) was generally lower for the years in which the larval numbers increased later. The values ranged from 0.086 among the years in the LMAR class to 0.625 eggs per day degree for the years in the PMAR class. The average index for APR years was slightly higher than the LMAR years. This trend supports the effectiveness of the AAI for estimating the relative abundance of ovipositing weevils. When the index is lower, it is expected that more time would be required for damaging larval populations to develop.

Egg numbers both at 1 January and at 1 February followed the same pattern. They were highest during years that had damaging larval populations during early March. As was expected, they were lowest in the APR class indicating that during those years, oviposition began slowly. However, it was unexpected that the egg numbers for the PMAR class would be lower than for the EMAR class.

Day degree values for both oviposition and for development showed that a larger accumulation of day degrees during January and early February is associated with earlier development of larval populations. However, the highest mean for both day degree values occurred in the EMAR class instead of the PMAR class.

The values of SUB12 did not match the pattern that was expected. While the highest mean did occur for the APR class, the next highest occurred in the PMAR class. The lowest value of 3.7 days was the mean for the LMAR class.

The observed SUB12 values deviated from the expected pattern more than the other variables. This deviation may demonstrate the interaction of low temperatures with the other variables. While there was relatively frequent occurrences of lethal, low temperatures for observations in the PMAR class, the egg numbers were sufficiently high in each instance that large numbers of viable eggs remained despite temperature related mortality. Conversely, the low temperatures probably compounded the effects of low egg and day degree values in years classified APR.

For five of the six variables, the relative values among classes were generally consistent with the time of development. However, in each case there was one class whose mean deviated from the expected pattern. These deviations from expected patterns of means point out the difficulty in using a single variable to predict the timing of the occurrence of weevil populations exceeding the economic threshold.

Table 3 shows the results of one way analysis of variance procedures conducted to determine if the classes differ significantly in relation to the six discrimination variables. For each variable, the analysis was weighted by the reciprocal of the within class standard deviation for that variable. Weighted analysis of variance was used due to the unequal variances of the variables across classes. AAI and egg numbers from both estimation dates were significantly different among the four classes ($P<0.001$).

The three temperature variables failed to show significant class differences with observed significance levels ranging from 0.1257 to 0.1692.

While the one-way analysis of variance does not indicate significant differences between the classes for all six variables, the multivariate analysis of variance demonstrates that the classes do differ in respect to the six selected variables, when their covariance structure is considered (Table 4). For the combination of variables, all four test statistics indicate that the four classes differ significantly.

The test for homogeneity of the within class covariance matrices resulted in a chi-square value of 372.7 with 63 degrees of freedom. This test statistic value indicates that the covariance matrices are significantly different ($P<0.0001$) and that a quadratic discrimination function is appropriate for these data.

A quadratic discrimination function was derived based on the 40, six variable, simulated observations. The performance of this function was evaluated by using it to classify the original 34 observations. Table 5 lists the year/location combinations that were not correctly classified with a posterior probability of 1.000. Two PMAR years and one LMAR were classified correctly (the class with the highest posterior probability was the correct class), but with posterior probability less than 1.000.

Two observations were misclassified resulting in an estimated error rate of 5.9%. The 1987-88 year at Chickasha was classified as an EMAR, when the larval numbers actually exceeded the economic threshold before 1 March. During this year, the egg numbers were relatively low and declined from 68 to 50 eggs/0.1 m^2 between 1 January and 1 February. The model apparently responded to these egg numbers and classified this year as an EMAR when in reality there was sufficient adult activity and day degree accumulation for the larval populations to develop to damaging levels before 1 March.

The 1989-90 year at Chickasha was also classified as an EMAR, but it was actually an APR year. The discrimination variables for this year indicated that it would have early larval abundance. The day degree values were the highest of all the APR years, SUB12 was zero, the AAI was second highest for the class, and the 1 January egg total was the highest for the class at 142 eggs/0.1 m^2. There were no temperatures below the lethal limit after the 15 February classification date that might have delayed the development of the larval populations. However, very few of the eggs present on 1 January were viable due to temperatures below -20 °C in late December of 1989. This event was missed since SUB12 is totaled after 1 January. Only the February egg number (28 eggs/0.1 m^2) reflected this event and was near the APR class mean. The model did not respond to the reduced egg number and classified this year as an EMAR.

When the AAI was deleted from the discrimination variables, the discrimination function derived was slightly less accurate. Table 6 shows the years that were not correctly classified with a posterior probability of 1.000. The 1979-80 years at Chickasha and Stillwater and the 1987-88 year at Chickasha had posterior probabilities of less than 1.000, but were correctly classified.

The 1975-76 and 1985-86 years at Stillwater were misclassified as EMAR when both actually belong to the PMAR class. In 1975-76 year, conditions were much like those at Chickasha in 1987-88 (misclassified by the six variable model). The model again apparently responded to the low egg numbers and predicted that the larval populations would develop more slowly. The 1985-86 year at both Chickasha and Stillwater was exceptional. At both locations, the larval populations had exceeded economic thresholds before the February classification date. The model correctly classified the Chickasha observation, but placed the Stillwater observation into the EMAR class.

Also, the Stillwater 1971-72 year was classified as a APR year, but was actually a LMAR year. SUB12 was ten, with these low temperatures occurring in January and contributing to the reduction in egg numbers from 30 to 15 eggs/0.1 m^2. The model apparently responded to the low egg values and placed this year in the APR class. Based on these three misclassified observations, the estimated error rate for the five variable discrimination function is 8.8%.

The generalized squared distances between the six variable class means are given in Table 7. These values give an indication of where observations are likely to be misclassified. Each column of the table shows the distance from the other classes to the class indicated by that column. Within a column, the distances shown indicate the relative likelihood of an observation from another class being misclassified into the class indicated by that column. For example, in the PMAR column, LMAR has a value of 142, while the distance from EMAR to PMAR is 2530. This indicates that observations belonging to the EMAR class are less likely than observations from the LMAR class to be misclassified into the PMAR class. In each of the columns, the distance corresponding to LMAR is the smallest, which may indicate that observations from the LMAR class are the most likely to be misclassified. However, this observation is not supported by the observed misclassifications shown in Table 5.

Comparing across columns, the means in Table 7 show the average distance to each of the columns from the other three columns. Since EMAR has by far the lowest mean distance (67.3), when an observation is misclassified, the discrimination function is most likely to place it into the EMAR class. These mean values also indicate that observations are not likely to be misclassified into the LMAR class.

Table 8 shows the generalized squared distances for the five variable discrimination analysis. Since these values are distances between five dimensional means as opposed to six dimensions, they are in general much smaller than the values in Table 7. This should not necessarily be interpreted as an indication that misclassifications are more likely with the five variable model than with the six variable model.

As with the six variable model, the distances indicate that observations, if misclassified, are most likely to be placed into the EMAR class. This is supported by the two PMAR observations that were misclassified into the Early March class as shown in Table 6. The other observed misclassification in Table 6 is also consistent with the distance values. While the mean distances indicate that observations are not likely to be misclassified into the APR class, the distance from the LMAR class to the APR class is relatively short. This corresponds to the misclassification for the 1971-72 year from Stillwater shown in Table 6.

Conclusions

The estimated error rates of 5.9% and 8.8% of the five and six variable discrimination functions indicate that they performed well. The observations made based on the generalized squared distances indicated that each class was most similar to its neighboring classes and that any outlying observations are most likely to be classified as EMAR. Such a misclassification is not alarming if the year/location actually falls in the LMAR or APR class. It does present a problem when the year misclassified is actually a PMAR. Since three of the five misclassifications were of this type, it is important that the use of this model be coupled with some monitoring for early season development of larval populations.

The five variable model performed almost as well as the six variable model. Since the calculation of the AAI requires several egg samples, the five variable model, in not utilizing this parameter, was selected as superior.

In either case it should be noted that these discrimination functions were derived for only two locations where adequate historic population data were available. Two extensions of these predictions may be possible. The predictions for these two locations might be used to forecast the development of larval populations in other geographic areas. Also, the discrimination function might be used to make predictions based on data collected at other sites. Further research will be necessary to assess the limits and validity of these extensions of the discrimination functions presented in this study.

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Table 1. Number of occurrences of each class for year/location combination.

Class	Location		Total
	Chickasha	Stillwater	
Pre-March (PMAR)	2	5	7
Early March (EMAR)	8	6	14
Late March (LMAR)	4	2	6
April (APR)	3	4	7

Table 2. Mean (standard deviation) values for the six discrimination variables for year/location classes.

	CLASS			
Variable	PMAR	EMAR	LMAR	APR
AAI	0.625(0.7037)	0.539(0.3889)	0.086(0.0496)	0.100(0.1172)
Eggs- 1 Jan.	271.4(339.44)	358.4(267.08)	67.2(105.90)	31.58(49.21)
Eggs- 1 Feb.	169.4(159.52)	285.3(297.03)	88.0(98.00)	19.8(13.52)
DD1	303.6(109.53)	323.1(120.16)	258.7(34.63)	192.3(181.96)
DD9	95.9(52.60)	100.8(63.14)	60.7(14.53)	52.6(72.08)
SUB12	6.0(4.00)	4.4(2.92)	3.7(4.32)	9.3(6.60)

Table 3. F-values and significance levels for the comparison of classes using each of the six discrimination variables separately.

Variable	F	Pr > F
AAI	7.87	0.0005
Eggs-1 Jan.	7.46	0.0007
Eggs-1 Feb.	7.02	0.0010
Day Degrees 1	1.80	0.1692
Day Degrees 9	2.06	0.1260
SUB12	2.07	0.1257

Table 4. Multivariate test statistics for testing the hypothesis that class had no effect based on all discrimination variables.

Statistic	Value	F	Numerator DF	Denominator DF	Pr > F
Wilks' Lambda	0.33864551	1.8449	18	71.2	0.0360
Pillai's Trace	0.87282721	1.8465	18	81	0.0331
Hotelling-Lawley Trace	1.38100011	1.8158	18	71	0.0399
Roy's Greatest Root	0.83334983	3.7501	6	27	0.0076

Table 5. Year/location combinations with a posterior probability less than 1.000 using six discrimination variables.

Location	Year	Actual Class	Classified Into Class	PMAR	EMAR	LMAR	APR
Stillwater	7172	LMAR	LMAR	0.0	0.0	0.974	0.026
Stillwater	7576	PMAR	PMAR	0.590	0.410	0.0	0.0
Stillwater	8081	PMAR	PMAR	0.998	0.002	0.0	0.0
Chickasha	8788	PMAR	EMAR*	0.134	0.867	0.0	0.0
Chickasha	8990	APR	EMAR*	0.0	0.593	0.0	0.407

*Misclassified observation.

Table 6. Year/location combinations with posterior probability less than 1.000 using five discrimination variables.

Location	Year	Actual Class	Classified Into Class	PMAR	EMAR	LMAR	APR
Stillwater	7172	LMAR	APR *	0.0	0.0	0.042	0.958
Stillwater	7576	PMAR	EMAR*	0.014	0.986	0.0	0.0
Chickasha	7980	LMAR	LMAR	0.0	0.041	0.927	0.033
Stillwater	7980	LMAR	LMAR	0.0	0.012	0.988	0.001
Stillwater	8586	PMAR	EMAR*	0.066	0.934	0.0	0.0
Chickasha	8788	PMAR	PMAR	0.995	0.0	0.005	0.0

*Misclassified observation.

Table 7. Generalized squared distances between classes based on the six variable discrimination analysis.

From Class	To Class Pre-March	 Early March	 Late March	 April
Pre-March	-	36.2	72,992,069	13,016
Early March	2,530	-	4,238,684	12,158
Late March	142	33.7	-	1,343
April	1,791	132.0	3,716,649	-
Mean	1,488	67.3	26,982,467	8,839

Table 8. Generalized squared distances between classes based on the five variable discrimination analysis.

From Class	To Class Pre-March	Early March	Late March	April
Pre-March	-	40.7	954	29,108
Early March	177	-	510	3,311
Late March	165	43.4	-	410
April	603	354.0	303	-
Mean	315	146.0	589	10,943

APPENDIX

SAS/IML Program for Simulation Data

```
data fast.class4;
length class $ 4;
set fast.since;
if location="CHICKASH" and season=8283 then class="EMAR";
if location="CHICKASH" and season=8384 then class="EMAR";
if location="STILLWAT" and season=7172 then class="LMAR";
if location="STILLWAT" and season=7374 then class="EMAR";
if location="STILLWAT" and season=7475 then class="LMAR";
if class=' ' then delete;
;
proc sort data=fast.class4;
by class;
;
proc anova noprint outstat=out data=fast.class4;
     by class;
     model jleggs vfleggs dd35c dd48c subtens=;
     manova;
;
data out;set out;
    stat='VAR';
    jleggs=jleggs/df;
    vfleggs=vfleggs/df;
    dd35c=dd35c/df;
    dd48c=dd48c/df;
    subtens=subtens/df;
    if _source_='ERROR';
    keep class stat jleggs vfleggs dd35c dd48c subtens;
;
proc print data=out;
;
proc means noprint mean data=fast.class4;
by class;
var jleggs vfleggs dd35c dd48c subtens;
output out=mout mean=jleggs vfleggs dd35c dd48c subtens;
;
data mout;
set mout;
stat='MEAN';
;
proc print data=mout;
;
data in;
set mout out;
ran1=rannor(1);
ran2=rannor(2);
ran3=rannor(3);
ran4=rannor(4);
ran5=rannor(5);
ran6=rannor(6);
ran7=rannor(7);
ran8=rannor(8);
ran9=rannor(9);
ran10=rannor(10);
rename jleggs=v1 vfleggs=v2 dd35c=v3 dd48c=v4 subtens=v5;
;
```

```
proc iml;
use in;
read into mup all where(class='APR' & stat='MEAN')
     var {v1 v2 v3 v4 v5};
mu=mup`;
read into var all where(class='APR' & stat^='MEAN')
     var {v1 v2 v3 v4 v5};
read into ran all where(class='APR' & stat^='MEAN')
     var {ran1 ran2 ran3 ran4 ran5 ran6 ran7 ran8 ran9 ran10};
call eigen(val,vec,var);
p=vec;
d=diag(val);
dh=d##0.5;
mb=repeat(mu,1,10);
agp=mb+p*dh*ran;
ag=agp`;
ac=repeat("APR",10,1);
;
read into mup all where(class='EMAR' & stat='MEAN')
     var {v1 v2 v3 v4 v5};
mu=mup`;
read into var all where(class='EMAR' & stat^='MEAN')
     var {v1 v2 v3 v4 v5};
read into ran all where(class='EMAR' & stat^='MEAN')
     var {ran1 ran2 ran3 ran4 ran5 ran6 ran7 ran8 ran9 ran10};
call eigen(val,vec,var);
p=vec;
d=diag(val);
dh=d##0.5;
mb=repeat(mu,1,10);
Egp=mb+p*dh*ran;
Eg=Egp`;
ec=repeat("EMAR",10,1);
;
read into mup all where(class='LMAR' & stat='MEAN')
     var {v1 v2 v3 v4 v5};
mu=mup`;
read into var all where(class='LMAR' & stat^='MEAN')
     var {v1 v2 v3 v4 v5};
read into ran all where(class='LMAR' & stat^='MEAN')
     var {ran1 ran2 ran3 ran4 ran5 ran6 ran7 ran8 ran9 ran10};
call eigen(val,vec,var);
p=vec;
d=diag(val);
dh=d##0.5;
mb=repeat(mu,1,10);
Lgp=mb+p*dh*ran;
Lg=Lgp`;
lc=repeat("LMAR",10,1);
;
```

```
read into mup all where(class='PMAR' & stat='MEAN')
     var {v1 v2 v3 v4 v5};
mu=mup`;
read into var all where(class='PMAR' & stat^='MEAN')
     var {v1 v2 v3 v4 v5};
read into ran all where(class='PMAR' & stat^='MEAN')
     var {ran1 ran2 ran3 ran4 ran5 ran6 ran7 ran8 ran9 ran10};
call eigen(val,vec,var);
p=vec;
d=diag(val);
dh=d##0.5;
mb=repeat(mu,1,10);
Pgp=mb+p*dh*ran;
Pg=Pgp`;
Pc=repeat("PMAR",10,1);
;
C=AC//EC//LC//PC;
G=AG//EG//LG//PG;
create test from g;
append from g;
create ctest from c;
append from c;
quit;
;
data ctest;
set ctest;
class=col1;
keep class;
;
data test;
set test;
jleggs=col1;
vfleggs=col2;
dd35c=col3;
dd48c=col4;
subtens=col5;
keep jleggs vfleggs dd35c dd48c subtens;
;
data fast.weeks_c4;
length class $ 4;
merge ctest test;
;
proc discrim pool=test testlist testdata=fast.class4 data=fast.weeks_c4;

class class;
priors APR=.21 EMAR=.40 LMAR=.18 PMAR=.21;
var jleggs vfleggs dd35c dd48c subtens;
;
run;
```

2

VITA

Joseph Alan Stark

Candidate for the Degree of

Doctor of Philosophy

Thesis: LIFE HISTORY OF THE ALFALFA WEEVIL, *HYPERA POSTICA*, AND TEMPORAL PREDICTIONS FOR LARVAL POPULATIONS EXCEEDING THE ECONOMIC THRESHOLD IN OKLAHOMA

Major Field: Entomology

Biographical:

Personal Data: Born in Heber Springs, Arkansas, February 1, 1963, the son of Major and Mary Stark. Married on June 9, 1991, to Felicia Rae Nichols.

Education: Graduated from Russellville High School, Russellville, Arkansas, in May, 1981; received Bachelor of Science Degree in Mathematics from Arkansas Tech University in December, 1984; received Master of Science Degree in Statistics from Oklahoma State University in July, 1987; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1991.

Professional Experience: Teaching Assistant, Department of Statistics, Oklahoma State University, January, 1985, to June, 1985; Research Assistant, Department of Entomology, Oklahoma State University, June, 1985, to April, 1987; Acting Data Services Coordinator, Department of Entomology, Oklahoma State University, April, 1987, to April, 1988; Data Services Coordinator, Department of Entomology, Oklahoma State University, April, 1988, to August, 1991.