

ALFALFA WEEVIL MODEL VALIDATION FOR THE
EASTERN AND WESTERN STRAINS
IN SOUTHCENTRAL KANSAS

by

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To my parents, Peter and Dorothy, for many years of
encouragement and love.

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INTRODUCTION

Kansas does not have an alfalfa weevil management program (or any guidelines) which considers alfalfa weevil densities, economics and yield loss to determine whether control is necessary. One way to establish such a program would be to develop or import a model which included these variables. Over the past eight years, several alfalfa weevil models have been developed throughout the United States, specifically in Illinois (Ruesink, 1974), Indiana (Miles et al., 1974), and New York (Shoemaker, 1977). To date, no model which describes alfalfa weevil population dynamics has been validated under Kansas field conditions. Field populations were monitored in southcentral Kansas to test the model made available by Ruesink.

It was hypothesized that the eastern and western alfalfa weevil strains converged in the central section of the state in the early 1970's (Dysart and Day, 1976; Sell et al., 1978). These strains are morphologically identical, but possess different physiological, behavioral and genetic traits. Ruesink's model contains the option of using either the eastern or western strain. The choice of strain affects spring densities, therefore, the predominant strain in the sampled field had to be determined. The model is intended to be used at various locations in Kansas, thus the geographic boundaries of the strains had to be defined. Prior to this study, the only reliable

strain determination technique consisted of crossing weevils with known mates, examining the viability of the offspring and comparing this data with known results. This technique is time consuming and labor intensive, so a quick, accurate strain determination method was needed.

The purpose of the research reported herein was to: (1) monitor alfalfa weevil populations in Kansas, (2) validate Ruesink's model for these field populations, (3) develop an alternate strain determination technique, and (4) use this technique to determine the geographic distribution of the strains in the state. The outcome of these investigations will determine if the model can be used to accurately predict populations and management practices anywhere in Kansas regardless of strain. The following literature review covers topics pertinent to this research; the alfalfa plant, the alfalfa weevil, the biological differences between strains, the use of electrophoretic keys and the alfalfa weevil model.

LITERATURE REVIEW

The Alfalfa Plant

Alfalfa is the world's most valuable forage crop and food source to livestock. The herbaceous perennial legume is cultivated for hay, pasture and silage on approximately 27.5 million acres (Kansas Crop and Livestock Rep. Ser., 1978) in the United States during 1978. In Kansas, about one million acres were harvested in 1978 yielding ca. 3 million tons at an estimated value of 130 million farm dollars (Kansas Crop and Livestock Rep. Ser., 1979).

Alfalfa has been called the "queen of forage crops" because of its high dry matter content, its protein yield potential, and its excellent animal intake. Alfalfa nutritive content exceeds all other crops grown for hay; it contains twice the amount found in grass (Hanson et al., 1962). The crop also contributes to soil improvement by supplying nitrogen, and enhancing soil structure resulting in reduced erosion (Armbrust and Gyrisco, 1974).

Alfalfa is attacked by a complex of pest species, including weevils, cutworms and leafhoppers. This review will focus solely on the alfalfa weevil. The amount of destruction depends upon the age of the plant when peak insect feeding occurs; mature plant stages can withstand a larger pest population than the younger developing stages. Wilson (1973) reported that a quality loss occurs before the first

sign of a yield loss, after which, it plateaus with an increasing pest population. Fick and Liu (1976) have shown that, in addition to the direct effects of weevil defoliation on quantity and quality, loss of leaves and injury to growing points may affect plant growth potential. Wilson (1973) determined that small weevil infestations result in an increase in harvestable yields which may be due to stimulation of plant growth by a low feeding density.

The alfalfa weevil has been managed primarily via insecticides and the proper timing of harvest (Wilson and Armbrust, 1970; Shoemaker, 1977; and Armbrust, 1978). Fields are usually sprayed in the spring before the first cutting when 20% tip damage is observed. This is followed by a stubble spray (Wilson and Armbrust, 1970). Niemczyk and Flessel (1969) developed a preventive spray program in which an initial application is made when the first instars are detected in the terminal buds with a second application 25-30 days later.

The number of harvests per season varies from two to eight depending upon geographical location. Three cuttings are typical for the north central region. The optimal time for the first harvest is between the bud and 10% bloom stage, when protein percentage and carotene content are high (Hanson and Davis, 1972). Because harvesting removes egg and larval stages from the field, manipulation of cutting time can control weevil populations, thus avoiding an insecticide application.

The Alfalfa Weevil

History and Background

The alfalfa weevil was first discovered in 1904 near Salt Lake

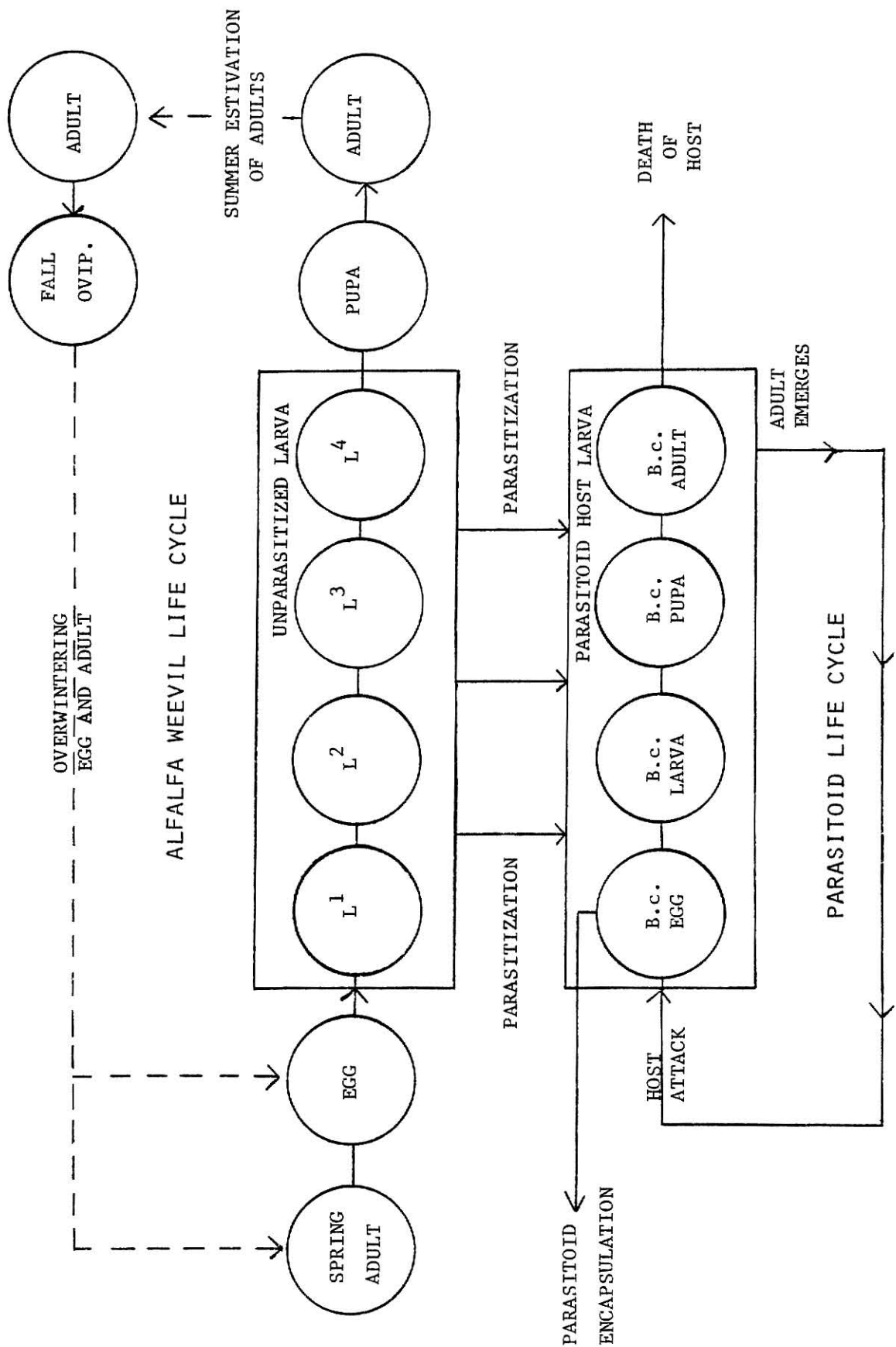
City, Utah (Titus, 1910). It spread approximately 10-20 miles/year, and remained confined mostly to the Great Basin for about the next 50 years (Davis, 1967). In 1932, it was reported in mid-California near the San Joaquin Valley representing a second introduction of the species (Essig and Michelbacher, 1933). In 1951, the alfalfa weevil was observed near Baltimore, Maryland, and unlike the weevil from the western portion of the country, the Maryland weevil rapidly dispersed northward, southward and westward at about 50 miles/year (Poos and Bissell, 1953; Davis, 1967). It is generally accepted that the separate introductions represent different populations, which may have originated from western Europe and the Middle East. The Utah and California introductions which stem from western Europe are referred to as the western strain, whereas the Maryland weevils which come from the Middle East are called the eastern strain (Koehler and Gyrisco, 1963; Blickenstaff, 1969; Davis, 1967; Pienkowski et al., 1969; Armbrust et al., 1970; Schroder and Steinhauer, 1976a, 1976b; Sell et al., 1978).

The first report of the western weevil invading Kansas came from Cheyenne and Hamilton counties (Peters, 1960). Since then, populations in southwestern Kansas remained light. The eastern strain was first observed in southeast Kansas, Cherokee county, in 1967 (Simpson, 1967). During 1974-1976 and 1981, populations in southeast Kansas were extremely heavy. The alfalfa weevil is currently distributed throughout North America in the 48 contiguous States and four Canadian providences (Ruesink, 1974).

Alfalfa Weevil Biology

The alfalfa weevil is a univoltine species with two overwintering

Figure 1. Life cycle of unparasitized and parasitized alfalfa weevil, and its parasitoid.



stages, egg and adult (Figure 1). Most of the eggs are laid during the spring in growing alfalfa stems. Newly emerged larvae move to the terminal leafbuds and commence feeding. Damage results in a skeletonized appearance and is primarily caused by the older larval instars. During the summer months, the adults migrate from the field, and aestivate in the adjacent hedgerows and woody areas. Upon return to the field in the fall, adult weevils mate, and some oviposition occurs before adults overwinter.

I. Egg Stage

The eggs are oval shaped being approximately .56 mm in length and .37 mm in diameter (Miller and Guppy, 1971). As development continues, the egg gradually changes color from yellow to dark brown as the head capsule becomes more evident within the structure (Litsinger, 1972).

Overwintering and early spring eggs are usually laid in dead stems, but as the season progresses the majority are deposited in green stems. Eggs have also been observed in alternate sites such as plant stubble, ground litter and grass (Niemczyk and Flessel, 1970). Litsinger (1972) noticed that 75%-90% of the eggs were located in the basal six inches of the alfalfa stem where temperature and humidity are slightly higher. Titus (1910) reported finding 1-28 eggs per oviposition puncture, whereas Litsinger (1972) observed that cluster size varied from 7-16 eggs.

Litsinger (1972) and Hsieh and Armbrust (1974) observed that the oviposition period commenced at 10-12°C, and terminated at 28°C in the field. Hintz (1972) noted that oviposition began in the fall when the daily maximum temperature did not exceed 79°F (26.1°C) for 12

consecutive days. Armbrust and Gyrisco (1974) reported that cold weather extended the duration of the egg laying period.

The weevil is more difficult to control in southern latitudes due to a longer oviposition period, a larger population of eggs, and an increased abundance of fall and winter laid eggs (Armbrust and Gyrisco, 1974). Researchers have noticed an increase in the incidence of fall oviposition in the southern latitudes of Illinois (Roberts et al., 1970; and Hsieh and Armbrust, 1974), Ohio (Niemczyk and Flessel, 1970) and Wisconsin (Litsinger, 1972). In Ohio, maximum viability was noted between October and December, while the greatest mortality occurred from February to mid-March (Niemczyk and Flessel, 1970). The survival percentage of overwintering eggs varies with winter severity, snow cover and alfalfa conditions (Armbrust et al., 1966).

Roberts et al. (1970) calculated the developmental threshold temperature to be 44.5°F (6.9°C) for the egg stage. In contrast, Litsinger and Apple (1973b) found the egg developmental threshold to be 50°F (10°C) for first and 50% hatch, which agrees with the findings of Melamed-Madjar (1962) who reported the initial temperature for development to be 11°C.

Using the 6.9°C (44.5°F) threshold, Roberts et al. (1970) estimated that embryonic development requires 174 degree-days (°C). Litsinger and Apple (1973b) reported that 176 and 206 thermal units were required for first and 50% hatch respectively at 10°C. In Israel, Melamed-Madjar (1962) found that development was complete in 23.4 days at 14.5°C, and decreased to 7.5 days at higher temperatures, 25°C. Guppy and Mukerji (1974) recorded occurrences of egg hatch between 8° to 35°C. They calculated egg hatchability at 10°C to be 75%; 100% was achieved

between 20^o-30^oC. They also reported that no larvae survived from eggs hatched at 8^oC, while larvae which hatched at 10^oC survived when reared at 20^oC.

Harcourt et al. (1974) proposed two methods for sampling the egg population. They devised a sampling plan to determine the number of quadrats/plot necessary to be sampled at various population densities to produce an acceptable estimate of the true population mean within a 10%, 15% or 20% confidence interval. This sampling procedure involves taking direct counts of the egg population, and has a time cost of 6.5 man-minutes per quadrat.

The second procedure relies on oviposition puncture counts which lessens sampling time, but is only effective until mid-June when growth and discoloration make detection difficult. The sampling scheme developed for oviposition puncture counts assumes 10.1 eggs/puncture for the eastern strain. This method is not acceptable for this investigation, because both strains are probably present in the area of experimentation. Latheef and Pass (1974) formulated a sampling policy to obtain population estimates of each age class, but it requires 80 man-hr/wk to examine enough samples to maintain a 10-15% confidence limit. Two techniques have been developed for making direct egg counts. The blender method (Pass and VanMeter, 1966) is labor-saving and yields higher counts than the stem dissection method.

II. Larval Stage

The first instar emerges from the shell via muscular contractions. It is approximately .8 mm long, pale yellow-green with minute black spots along the body, and a black head capsule. As larvae develop, they change to dark green with a prominent white dorsal stripe. Ultimate

larval size is about 1 cm (Titus, 1910; Armbrust and Gyrisco, 1974). Bartell and Roberts (1974) developed a head capsule caliper technique to determine larval instars which is quicker, but as accurate as using an ocular micrometer.

Upon eclosion, the first instar feeds within the stem for three to four days, then moves up the stem to conceal itself in terminal leafbuds. The third and fourth instars are most damaging. They skeletonize the exposed lower leaves.

Koehler and Gyrisco (1961) calculated the larval developmental threshold to be 9°C , which agrees with the threshold temperature, 9.5°C , established by Melamed-Madjar (1962). Guppy and Mukerji (1974) observed that it took 39, 77, 118 and 171 Centigrade degree-days for the first, second, third and fourth instars, respectively, to complete development (assuming a base of 10°C).

Hsieh et al. (1974) determined the mean time to complete development was 212 degree-days ($^{\circ}\text{C}$) in the eastern strain (base 8.9°C), and 207 degree-days ($^{\circ}\text{C}$) for the western strain (base 10.6°C). They also reported that the first and fourth eastern instars developed significantly slower than the second and third instars, whereas only the first instar developed significantly slower than the second and third instars in the western strain. Schroder and Steinhauer (1976a) found that larvae of the eastern strain had a shorter developmental period (18.9 days)¹ than the western strain, (20.3 days).

Mortality factors which affect the population dynamics of the larvae include lack of feeding, establishment, temperature, harvesting

¹The mean developmental period was calculated by averaging developmental times under four treatments: 18°C -8hr, 18°C -16hr, 24°C -8hr and 24°C -16hr.

and parasitoids, when abundant. In life table studies, Latheef et al. (1979) reported that larval mortality during establishment and feeding was a major factor affecting population dynamics. Temperature mortality is stage dependent. The first instar experienced the greatest mortality of all the larval stages when kept at a constant temperature ranging from 18.3°-30°C (Hsieh et al., 1974).

Harvest mortality can be severe. Litsinger (1972) found that the larval population can decrease up to 98% as a result of the first cutting. Time of cutting, weather conditions at cutting and length of time between cuttings all contribute to the mortality of the larval and pupal populations. Growers can take advantage of the situation by incorporating early harvesting into a pest management policy.

Larval mortality from the parasitoid, Bathyplectes curculionis, was slight in Kansas during 1979 and 1980 due to the parasitoid's trace density (Bell, 1979 and 1980). Thomas Burger (USDA-APHIS) also recorded low levels of B. curculionis in the midwest during this period. He speculates that these low trends are due to the females' inability to maintain their reproductive potentials (personal communication). Due to low parasitoid populations, B. curculionis was excluded from this investigation and will not be discussed further.

Guppy et al. (1975) suggested a sampling plan for estimating the mean alfalfa weevil larval population. They calculated the necessary number of ft² quadrats/plot to be sampled in order to achieve an accurate estimation of the mean within 10%, 15% or 20% confidence intervals. They calculated that sampling required about 7.5 man-minutes per 1 bouquet. Litsinger (1972) reported an 80% recovery of first larval instars from ft² quadrat samples placed in a Berlese

funnel.

III. Pupal Stage

The full grown larval spins a white "thread" cocoon which develops into a pupa in about two days. The pupal stage lasts from 6 to 14 days. Initially, it is a pale green color, then darkens as it matures. The adult emerges pale brown and soft. The elytra harden in about three days, and the color changes to dark brown (Titus, 1910).

Poss and Bissell (1953) and Harcourt and Guppy (1975) noted a difference in the location of cocoon placement between the eastern and western strains. All of the cocoons of the western strain were found on the ground, whereas two-thirds of the eastern strain cocoons were located in the foliage.

Studies by Melamed-Madjar (1962) and Litsinger (1972) yielded a threshold temperature for pupal development of 9.5°C . Hsieh et al. (1974) reported that the minimum temperature for pupal growth was 14.4°C for both the eastern and western strains. They found that the eastern strain required an average of 71 degree-days $(^{\circ}\text{C})^2$, whereas 72 degree-days $(^{\circ}\text{C})$ were needed for developmental completion in the western strain. Litsinger (1972) reported mean developmental periods of 27, 7, 3.5, and 2.5 days at 12° , 20° , 28° , and 36°C , respectively. Koehler and Gyrisco (1961) noted that mortality increased at low humidity and low temperatures, 12°C , as well as for moisture levels greater than 80% at 36°C .

Harcourt and Guppy (1975) developed two sampling plans to estimate the number of cocoons in the foliage and on ground litter.

²Calculated by averaging degree-days above 14.4°C for temperatures between 18.3° - 30.0°C .

The sampling procedure for foliage cocoons gives the number of ft^2 quadrats required to obtain an 80% probability of a true estimation of the population mean within a specified interval. Approximately 4.5 man-minutes is required for each quadrat sampled.

For litter examination, the sampling plan determined the number of $\frac{1}{4} \text{ ft}^2$ quadrats (= 1 unit) required to estimate the mean cocoon population within a certain precision. Sampling time is about 6 minutes per unit.

IV. Adult Stage

Upon emergence from a cocoon, the adult feeds for two to three weeks before entering aestivation (Litsinger and Apple, 1973a; Miller and Guppy, 1971). The adult is brown, and possesses a dark brown dorsal stripe along the elytra (Titus, 1910). The shape of the caudal abdominal sternite and the degree of projection of the abdominal tergite from a ventral view may be used to determine the sex of the adult (Pienkowski et al., 1969).

Hamlin et al. (1949) found estivating adults along the hedgerows and beneath ground litter. Manglitz (1958) detected the highest density of estivating weevils, ($1.18/\text{ft}^2$), two ft from the edge of the field in a woody environment. He recorded few adults, ($.73/\text{ft}^2$ and $.30/\text{ft}^2$), at distances of 62 ft and 126 ft, respectively, and none in the center of the field. Tysowsky and Dorsey (1970) discovered the majority of adults estivating .4-.8 inches beneath the soil surface. They could not detect any weevils in soil samples taken from the fence row or adjacent wooded surroundings.

Huggans and Blickenstaff (1964) suggested that summer diapause was induced by a photoperiod stimulus received during the larval stage.

DeWitt and Armbrust (1972) reported that larvae exposed to an uninterrupted 8 hr photoperiod did not diapause, while a 16 hr photoperiod induced diapause. Rosenthal and Koehler (1968) concluded that few larvae entered into diapause when subjected to a short photoperiod (9 hr). They reported that a long photoperiod (16 hr) treatment during the parental generation also resulted in less diapausing offspring, but they were not able to correlate the percentage of diapausing progeny with the tendency of the parental generation to estivate. Schroder and Steinhauer (1976a) cited Cothran (1967) for demonstrating that all larvae reared at 30°C exhibited diapause regardless of the short photoperiod effect. His findings agreed with Litsinger (1972) who observed that diapause was induced and prolonged at high temperatures (above 25°C).

Manglitz (1958) considered summer migration to be a period of true estivation rather than a period of sexual maturation, since no ovarian development occurred. Guerra and Bishop (1962) observed that weevils did not feed, fly or mate, and that development of the ovaries and egg tubes was slow during summer estivation. Considerable enlargement of the ovarioles and egg tubes, and distention of the oviduct transpired after diapause, succeeded by mating and oviposition. Diapausing weevils attained sexual maturity six months following emergence, while nondiapausing weevils required two and a half months. Litsinger (1972) characterized weevil diapause by low respiration, low body water content and high lipid concentration. In rediapausing females, the development of ovarioles did not regress, but hypertrophy of the fat bodies and oosorption occurred. Male testes underwent regression, and previously mated males showed no cyst development during estivation.

Melamed-Madjar (1962) determined the theoretical threshold of oviposition to be 11°C , and the average number of eggs/female to be 650 with a maximum of 1,560/female. Litsinger (1972) found that oviposition commenced at 10°C , and terminated at 28°C . LeCato and Pienkowski (1972a) observed that egg laying began 72 hours after copulation, and reached peak numbers three to four weeks later. Fecundity was severely reduced after 15 weeks in once mated females due to a decline in viable sperm.

Drea (1969) recorded an average of 1909 eggs per isolated female. Under 23°C , an average of 1,134 eggs per female was obtained (LeCato and Pienkowski, 1972c). Coles and Day (1977) reported the mean number of eggs laid per multiply mated female was 4,190 (50 eggs/female/day). One precocious female laid 6,438 eggs in 105 days at a rate of 61 eggs/day.

LeCato and Pienkowski (1972c) observed that maximum egg hatch occurred at 23°C , and was reduced at temperature extremes of 10° and 30°C . At 30°C , they observed a fragmentation of sperm tails in vitro, and abnormal oviposition behavior, (49% of the eggs were laid outside the stem). LeCato and Pienkowski (1972b) noticed that females exposed to low (-5°C) or high (35°C) temperatures exhibited a diminished oviposition period, a shortened life span, and reduced fecundity and fertility. They suggested that high temperatures, 35°C , interfere with oocyte maturation resulting in a few viable eggs. Females which were confined with males have shown reduced fecundity and longevity (LeCato and Pienkowski, 1972a). They hypothesized that males induced stress and increased mortality which intervened with oviposition.

After fall oviposition, adults seek shelter in the field, under litter and in weedy border areas up to a three mile distance from the

field (Litsinger, 1972). Tysowsky and Dorsey (1970) found 61.5% of the total adults hibernating at the base of the alfalfa plant about 2.5 cm below the soil surface. In New York, Helgesen and Cooley (1976) reported 25% survival in the overwintering adult population with the greatest mortality occurring mid-February to mid-March as a result of rapid temperature fluctuations. They were not able to detect any significant difference in survival between field and border overwintering sites.

Guppy and Harcourt (1977) devised a sampling plan to determine the average number of adults in the fields during the spring and summer. They calculated the number of quadrats needed to be sampled in order to accurately estimate the population mean within a specified confidence interval. The sampling policy required ca. six minutes to examine a quadrat using a D-Vac[®].

Alfalfa Weevil Strain Comparison

Because this investigation was conducted in southcentral Kansas where both the eastern and western strains were present (Dysart and Day, 1976; Sell et al., 1978), it was necessary to review strain differences, i.e., genetic, physiological and behavioral, to determine their affect upon the population dynamics of the species.

Blickenstaff (1965) demonstrated that a partial intersterility exists between the eastern and western strains. Eastern females crossed with western males produce infertile eggs, whereas western females mated with eastern males resulted in a female/male ratio of 5.6:1. Table 1 gives a complete summary of his results.

Table 1. F1 generation from various alfalfa weevil parental crosses.

CROSS	PROGENY
♀ ♂	
E x W	Infertile
W x E	Fertile (5.6 females:1 male)
E x EW	Infertile
W x EW	Embryos developed, but failed to hatch
EW x EW	1.9 females:1 male
EW -F1 hybrid from E X W parental cross	

Blickenstaff (1969) concluded that there was no significant mating preference between the sexes of the two strains. By contrast, Armbrust et al. (1970) found that eastern males mated more often and for a greater duration than the western males when restricted to a partner of the same strain. In free choice matings, eastern strain males mated more frequently with females of both strains than western males. In tests where the eastern and western males were competing for the same eastern female, eastern males tended to be more aggressive by 3:1. Eastern males outcompeted western males by 6:1 for western females. They hypothesized that the eastern male will contribute more to the gene pool, and the partial incompatibility from E (female) x W (male) will have little effect when the populations meet and mix.

Schroder and Steinhauer (1976b) demonstrated that matings between populations from Maryland (♀) x France (♂), Maryland (♀) x Germany (♂), and Maryland (♀) x Utah (♂) were partially intersterile, whereas the reciprocal crosses produced predominately females. The Utah population was shown to be completely compatible with the population from Germany and France, therefore it is believed that the western strain originated in western Europe, because of the incompatibility that exists between the Maryland, French and German populations.

Physiological strain differences in the hemocytes' response to the parasitoid egg have been reported. Host hemocytes surround the embryo inhibiting respiration and nutrient transfer, thus making the larvae immune to the parasitoid (van den Bosch, 1964; Puttler, 1967). Van den Bosch (1964) reported a distinct difference in the encapsulation capability between the alfalfa weevil and the Egyptian alfalfa weevil (Hypera brunneipennis) in California to Bathyplectes curculionis. The third and fourth instars of H. brunneipennis possess a high encapsulation ability, (55.5% and 78.9%), while that of H. postica is slight, (1.5% and 3.9%), thus making the western strain quite susceptible. Puttler (1967) obtained the opposite results from examining eastern strain parasitoid larvae in New Jersey and Delaware. This strain is more immune to the parasitoid. Strain response can vary with parasitoid species. Volker and Simpson (1975) noted that western alfalfa weevils displayed evasive behavior to Tetrastichus incertus and were not parasitized. Eastern weevils did not exhibit this behavior and were parasitized.

Researchers detected behavioral differences in cocoon placement (discussed previously), feeding reaction of new adults, and temperature-photoperiod responses between the two weevil strains. In laboratory experiments, Koehler and Gyrisco (1963) reported that California weevils consumed a total of 72.65 mg of alfalfa during 40.5 days, while New York weevils consumed a greater quantity, 82.88 mg, but fed for a shorter duration, 31 days. Schroder and Steinhauer (1976a) found that weevils from Utah, France and Germany all entered into diapause regardless of the temperature-photoperiod treatment, whereas Maryland weevils did not diapause under a 8 hour photophase.

Differences in strain developmental periods for the alfalfa weevil were reviewed in the discussion of the larval stage (above).

To date the only reliable technique for distinguishing these morphologically identical weevil strains is based on making crosses with an established strain colony. Pienkowski et al. (1969) took extensive morphometric measurements of the two strains, but in a personal communication (1978), he seriously doubted that this technique could be used to differentiate the strains.

Strain determination accomplished by the mating and rearing of offspring is labor intensive and time consuming, therefore, an efficient and accurate determination technique was sought. One approach involved SEM to compare larval morphological characteristics. This was done since no literature existed concerning the difference between the immature forms. Another strain determination method investigated the feasibility of gel electrophoresis based on preliminary findings of Blickenstaff (1965) and Sell et al. (1978). (See following section).

Electrophoretic Keys

Electrophoresis has been used to identify particular insects to the species or subspecies level when morphological characteristics are indistinguishable. Detectable protein differences in these organisms make identification possible. In an electric field, proteins migrate according to net charge and size, and thus can be differentiated by electrophoresis (Mauer, 1971). The protein patterns produced by electrophoresis can be selectively stained so that only bands of certain enzymes and their genetic variants are (e.g., esterase, malate dehydrogenase, etc.) are visible (Shaw and Prasad, 1970). These genetic

variants of a particular enzyme, called allozymes, are under the control of a single genetic locus and may migrate differently in an electric field. Conventionally, researchers have interpreted allozymes as representing alleles of a particular locus, and the electrophoretic pattern as the organism's genotype (Prakash et al., 1969; Ayala et al., 1972). This is not completely accurate since different polypeptides of an enzyme gene may have the same electrophoretic mobility and therefore go undetected. The term "electromorph" has been proposed to describe alleles which produce proteins with the same mobility, thus making the electrophoretic pattern a phenotypic description of an organism (King et al., 1975; Berlocher, 1980).

The use of biochemical characteristics for insect identification has several advantages and disadvantages. In some instances, electrophoretic techniques can be more objective than conventional taxonomic techniques. This occurs when the latter method uses only subjective traits like body form or color. In contrast, the former technique consistently relies on precise band mobilities. Another advantage of electrophoretic data is that the loci which are routinely used display little or no sex or age variation. Two apparent disadvantages of electrophoresis are its restriction to living organisms, and its requirement for some training and practice to accurately perform the technique (Avisé, 1974).

When classical taxonomic techniques can not distinguish morphologically identical species, researchers have resorted to electrophoresis. Ayala and Powell (1972) were able to identify six sibling species of Drosophila using starch gel electrophoresis. They determined that 15 to 32% of the examined loci could be used to identify

any two of these species. The use of several discrimination loci would make these identifications almost certain ($P > .9999$). In 1973, Ayala developed the first description of a taxon using electrophoretic patterns. Two pairs of subspecies of Drosophila willistoni group (D. willistoni willistoni and D. w. quechua, and D. equinoxialis equinoxialis and D. e. caribbensis) could be correctly determined 99.99⁺% of the time when five diagnostic loci were used. These loci represent approximately 20% of the examined loci. Saul et al. (1977) were able to distinguish two sibling species, Aedes triseriatus (the primary vector of LaCrosse encephalitis) and A. hendersoni (a nonvector mosquito) using the malate dehydrogenase locus. In 1979, Miles developed a biochemical key to identify six species of the Anopheles gambia complex from four enzyme loci. Berlocher (1980) was able to unambiguously determine eight species of the genus Rhagoletis while in the larval, pupal, or adult stage. This electrophoretic key could discriminate three economically important sibling species which have had a history of taxonomic controversy, R. pomerella (apple maggot), R. cingulata and R. cerasi. Prior to that, no key was available to distinguish these species as larvae or pupae using morphological traits.

The larval stage of the fall webworm, Hyphantria cunea, possess two races which are classified as either black-headed or orange-headed from its morphological traits, host plant range and behavioral characteristics. The adult forms are identical. McIntee and Wordin (1981) used starch gel electrophoresis to distinguish the adult stages from the B-esterase locus.

In a preliminary analysis of larval hemolymph, Blickenstaff (1965) detected differences between the two alfalfa weevil strains. Using gel

electrophoresis, eastern strain hemolymph separated into 19 protein fractions, whereas the western strain had 18. There also existed significant quantitative differences in three bands common to both strains.

Sell et al. (1978) reported significant strain differences in the gene frequencies of adult alfalfa weevils. Using starch gel electrophoresis, they observed four alleles at the esterase locus which were designated Est^a, Est^b, Est^c and Est^d.³ The eastern strain displayed the presence of Est^a and Est^b and the absence of Est^d, whereas the opposite occurred in the western population. Even though strain differences exist in regard to three esterase alleles, the frequency of the allele common to both strains, Est^c, is .894. This means 79.92% of the examined population (n=387) appear similar at this locus, therefore it can not be used for discrimination purposes. These results, however, support an investigation into finding other proteins to be used as diagnostic markers for strain separation.

Alfalfa Weevil Model

Ruesink (1974) has developed a model describing the population dynamics of the alfalfa weevil and its parasitoid, Bathyplectes curculionis. This model is coupled to an alfalfa crop model (Fick, 1975) which predicts plant consumption and yields. The user must supply the following data to run the model: (1) the densities of each weevil and parasitoid, (2) the strain of the weevil population, (3) biomass of the plant's stem, buds, leaves and total nonstructural carbohydrates, (4) the growing degree-days with a 5°C base received by the plant at the

³Est^a is the most cathodal allele, and Est^d the most anodal.

start of a simulation, (5) management practices (harvest or spray dates), (6) the latitude of the area of simulation, and (7) daily local minimum and maximum temperatures.

One day time steps update the state vector which depicts the density of each stage, and reduces it to account for mortality. The feeding potential is directly proportional to the developmental rate per instar. The actual amount of alfalfa consumed per instar is related to the ratio of available biomass to the calculated potential feeding rate. The model simulates harvest or spray practices when the actual dates of these practices are entered. The model also is designed to automatically invoke a spray procedure when the sum of third and fourth instar larvae (unparasitized and parasitized) exceeds 400 for a given day.

The output displays the population density for each life stage of the weevil and parasitoid, the parasitization percentage of large larvae, and the potential amount of consumed biomass. Trial simulations in Kentucky during 1972-1973 indicate a deviation in the egg and larval densities from the sampled field data (Ruesink, 1974). Ruesink believes that this discrepancy may be due to inadequate information about the termination of adult estivation and fall return to the field, mortality of ovipositing adults and oviposition rates in late spring and summer.

METHODS AND MATERIALS

The Sampling Program

Alfalfa weevil populations were monitored at Sandyland Experiment Field, St. John, Kansas, during the spring and fall of 1979 and 1980. In 1979, the sampled alfalfa variety, Kanza, was in its fourth year of cultivation. Sampling procedures followed the methods of Harcourt et al. (1974), Guppy et al. (1975) and Guppy and Harcourt (1977) for egg, larval and adult populations, respectively. The field, approximately 400 x 70 ft, was divided into 16 plots and a stratified random sampling procedure was implemented. The sample unit was a 1 ft² area of alfalfa foliage clipped at ground level.

Two subsamples/plot were collected twice a week and transported to the laboratory for analysis. One subsample was run through a low speed blender, then the processed plant material was rinsed and filtered. This was repeated two more times for each subsample. The eggs were then counted (Pass and Van Meter, 1966). The other subsample was placed in a Burlese funnel for two days to extract larvae. The temperature inside the funnel was recorded during this period, because it influences hatching and larval development. The resulting larvae were sized and tallied. Equipment was not available during the spring (1979) for adult collection.

The above procedure including adult sampling was repeated during

the three remaining sampling periods. A D-Vac[®] was used to gather adults from the plant crowns and the soil surface of the larval subsample. These adults were added to the number found in the foliage above this area. The selected sampling schemes assured an 80% probability of being within 10% of the population mean at moderate infestations.

Plant height of each subplot was measured each time the field was sampled. Weather instruments recorded daily maximum and minimum temperatures at the field site for both years.

Model Validation under Kansas Field Conditions

Ruesink's model (1974) was validated using alfalfa weevil populations monitored at St. John, Kansas, in 1979 and 1980 (see Sampling Program). A series of runs were performed, each initiated by supplying information about field conditions on a selected starting date (see input requirements, p.23).

The model was designed to be run using either the eastern or the western strain. The sampling site represents an area where the strains met in the early 1970's (Dysart and Day, 1976; Sell et al., 1978). It was not known to what extent the population was comprised of each strain when samples were collected in 1979 and 1980. This information was obtained from an electrophoretic strain determination technique (see following section). The populations at St. John subsequently were determined to be 88.0% and 85% of the eastern strain during spring 1980 and 1981, respectively (see Results). Therefore the model was run using solely the eastern strain.

Because of time and resource constraints, data on adult densities

and plant biomass were not collected. Other researchers have also encountered difficulties in sampling the adult stage (Ruesink, 1974; Armbrust, 1978). Estimates were established by finding densities for which predicted egg populations existed within a 95% confidence limit of the observed population. These estimates were used to start the model. To preserve the integrity of the statistical tests used for validation, progeny which resulted from these adults were excluded from the analysis.

Plant densities were obtained by running the plant components of Ruesink's model with recommended initial plant conditions for March 1 (Fick, 1975). The model produced biomass values which were used as input for each model run.

Model options were selected to mimic field conditions during a run. Actual harvest dates were entered into the model to simulate the field's cutting schedule. The field was not sprayed during the sampling period, therefore the spray option was not utilized. The model's parasitoid option was not employed since B. curculionis was reported as trace in Kansas during this period (Bell, 1979 and 1980).

The most critical decision making period for weevil control occurs in spring before the first cutting, so this became the focus of validation. Separate runs were made starting on each of the dates when samples were collected. Each run used the values measured on that date as initial conditions. Each model run terminated two days after the sampling date it was intended to predict. The two additional days were included to simulate extraction using a Burlese funnel. Temperatures monitored inside the funnel during 48 hours were entered into the model. No runs were made past the first cutting. This procedure of multiple

runs assured that validation was independent of initial conditions.

Model predictions were compared to observed fall densities to determine if a late harvest for removal of potential overwintering eggs was necessary. Runs started with previous spring field densities and terminated on the last sampling date in November. Fall populations which were affected by the estimated spring adult population were not included. As before, multiple runs beginning on different sampling dates were employed.

Another way to determine if a late harvest is needed for egg removal is to start a model run in the fall and terminate it the following year after the spring growing season. To do this, the density of fall adults in each physiological state must be determined. During the fall, adults terminate diapause, return to the field to mate, oviposit, and then overwinter. Difficulty, however, in sampling and estimating the proportion of field reproducing adults precluded making these model runs.

Welch et al. (1981) proposed two types of validation techniques related to model use. One method is applicable for research models, whereas the other is suitable for management models. Research models attempt to describe a selected system with high accuracy. They can be used to determine the importance of biological and environmental variables, and to identify strengths and weaknesses in existing knowledge. Welch maintains that these models should be validated using rigorous statistical procedures. By contrast, management models contain decision making features designed to maximize producer's profits with minimal risk. Cost/benefit ratios and user risk criteria (when applicable) should be used to validate management models.

Two statistical tests were used to evaluate the model from a research perspective. An easier, but less powerful, technique is based on a nonparametric test of regression slope developed by Theil (cited in Hollander and Wolfe, 1973). If the model perfectly mimicked the actual system, a graph of model output vs. observed results would display a straight line with a slope of 1 ($\theta = 45^\circ$). The null hypothesis is that all observations fall on this line. The model is rejected when a significant number of points deviate from this slope. The test was performed on both seasons of data (spring and fall) for 1979 and 1980.

A more powerful parametric test devised by Welch et al. (1981) was used to validate model use for research objectives. Confidence limits are calculated from initial conditions and observed results. The model is rejected if the confidence limit around the difference between the observed and predicted results does not include zero. The test's advantage is that it considers the effect of initial sampling error on predicted results. The test's disadvantages are its complexity and tediousness, therefore it was performed only on spring data for both years.

The Kruskal-Wallis test for ties was performed to determine if differences existed among the instar stages in regards to the results from the above parametric test. This nonparametric test was used instead of an unbalanced ANOVA because the data was not normally distributed.

Examination of the model in a management context involved comparing the model's spray predictions to recommendations from New York (Helgesen and Muka, 1978), Pennsylvania (Gesell, 1978) and Illinois (Wedberg et al., 1977) alfalfa pest management programs. Total larval population

(larvae per 30 stems), plant height and degree days (for Illinois only) were variables used by these programs to determine management recommendations from charts. These recommendations were compared to predicted model advisories. Model accuracy was judged according to the frequency with which model management predictions agree with the type and timing of advisories from the above pest management programs.

Alternate Methods of Alfalfa Weevil Strain Determination

Two techniques, scanning electron microscopy and disc electrophoresis, were investigated as potential strain determination procedures.

Scanning Electron Microscope (SEM)

SEM was used to observe external morphological characteristics of both strains. The examined larvae were from the same source as those of Data Set 1 in the electrophoresis study (see below).

Two replications of both strains of four instars were sonically cleaned in a distilled water and detergent mixture, then transferred to a buffered formalin solution for preservation. One-half week prior to a SEM run, the samples were dehydrated using the selected alcohol series according to Humason (1972). A critical point drying apparatus (DCP-1 Penton Vacuum, Inc.) was used to remove remaining alcohol from the samples which were subsequently stored in a dessicator. The larvae were coated with a gold and palladium alloy (60%-40%) about an hour before viewing (Hayes and Pawley, 1975).

The dorsal side, lateral side, ventral side, head, mouthparts and posterior view were examined at 80X magnification. This pilot

investigation revealed no obvious morphological distinctions. Difficult specimen preparation caused many samples to collapse and become wrinkled making characteristics obscure. For these reasons, this strain determination technique was abandoned, and will not be discussed further.

Electrophoresis

Several steps were necessary to develop a strain determination technique based on gel electrophoresis: (1) perform gel electrophoresis using hemolymph from eastern and western larvae, (2) quantify observed protein patterns (Data Set 1), (3) develop several methods to differentiate the strains based on these patterns, (4) evaluate these classification methods using an independent set of larvae of known strains (Data Set 2), (5) develop mathematical discriminant model(s) to classify unknown larvae based on the most successful method(s) selected above, and (6) program the discriminant model(s) into a calculator.

Eastern strain larvae used in Data Set 1 were the F1 generation from larvae collected in Wayne Co., Ohio, on April 22, 1980, by John Flessel (Ohio Agr. Res. and Devel. Center). Eastern strain larvae of Data Set 2 were the F2 generation reared from larvae gathered in Cass Co., Michigan, during July, 1980, by Milton Holmes (USDA-APHIS). The western larvae which were used to establish Data Set 1 represent the F2 generation of weevils collected near Logan, Utah, on July 3, 1980. Western data set 2 consisted of larvae gathered from two locations, Lehi and Logan, Utah, on May 15 and May 18, 1981. All western larvae were collected by Donald Davis (Utah St. Univ.).

Gel electrophoresis was performed on hemolymph obtained from fourth

instar larvae which were starved 24 hours prior to extraction. Larvae were placed in a warm water bath, 50°C, for one minute to stun their movement, then individually transferred to a porcelain spot depression plate containing 10 μ l of tris-glycine buffer. Approximately 2.0 mg of phenyl thiocarbamide and 2.0 mg of phenylmethanesulfonyl fluoride were added to prevent tyrosinase (melanization) and proteinase (clotting) activity, respectively. The ventral side of each larva was gently scraped with a sharp handmade pipette until the surface was punctured and hemolymph was released. The hemolymph was allowed to mix with the buffer and added chemicals, then stored at 25°C until needed.

Gel electrophoresis using a 7% acrylamide separating gel (9.5 pH) was carried out in 75 mm X 5.0 mm (i.d) tubes at room temperature using a tris-glycine electrode buffer (8.5 pH). A constant current of 2 milliamps per gel was applied until the bromophenol blue tracking dye migrated to the base of the tubes, ca. 2 hours (Davis, 1964). The gels were removed from their tubes, and placed in a 12% trichloroacetic solution for one-half hour to fix the protein sample. After protein fixation, the gels were rinsed several times with distilled water, then placed in a Coomassie blue stain for one hour. The gels were stored in 7% acetic acid at 4°C (Canalco, 1969).

The gels' protein patterns were quantified by recording three variables for each band present in the gel. These variables include the distance a band had migrated from the origin (the cathode), the band's width and a rating of band intensity (3-strong, 1-faint). The absolute distance a band migrates may vary depending on exact duration of a run, current, and several other factors. Therefore, a band's distance from the origin is best expressed as a relative mobility (R_f) which is the

distance the band migrates divided by the distance the bromophenol blue tracking dye migrates.

An unbalanced analysis of variance (ANOVA) using the three variables mentioned above was performed on seven bands common to each gel of Data Set 1 (N=35). This test was to determine quickly which of these bands were significantly different between strains. The underlying assumptions of an ANOVA (normality and homoscedasticity of variances) were also tested.

Two bands (band 6 and 7) showed significant differences (see Results). These bands were used to develop a set of possible methods from which the gels could be classified. The following variables were examined as possibilities:

- (1) the Rf of band 7, Method A
- (2) the Rf of band 6 and band 7, Method B
- (3) the relative distance between band 6 and band 7 (the distance between bands divided by the distance of the tracking dye), Method C
- (4) the ratio of band 6 to band 7 (band 6 divided by band 7), Method D,
- (5) 2 and 3 (above), Method E
- (6) 2 and 4, Method F
- (7) 3 and 4, Method G
- (8) 2, 3 and 4, Method H.

The eight different techniques were each separately tested using an independent data set (Data Set 2, N=124). Methods which did not correctly classify at least 95% of the gels were eliminated from consideration.

Discriminant models were developed based on successful classification methods. Generalized squared distance functions serve as model equations (SAS User's Guide, 1979, p.183). The models use the variable means of each strain and calculated inverse covariance

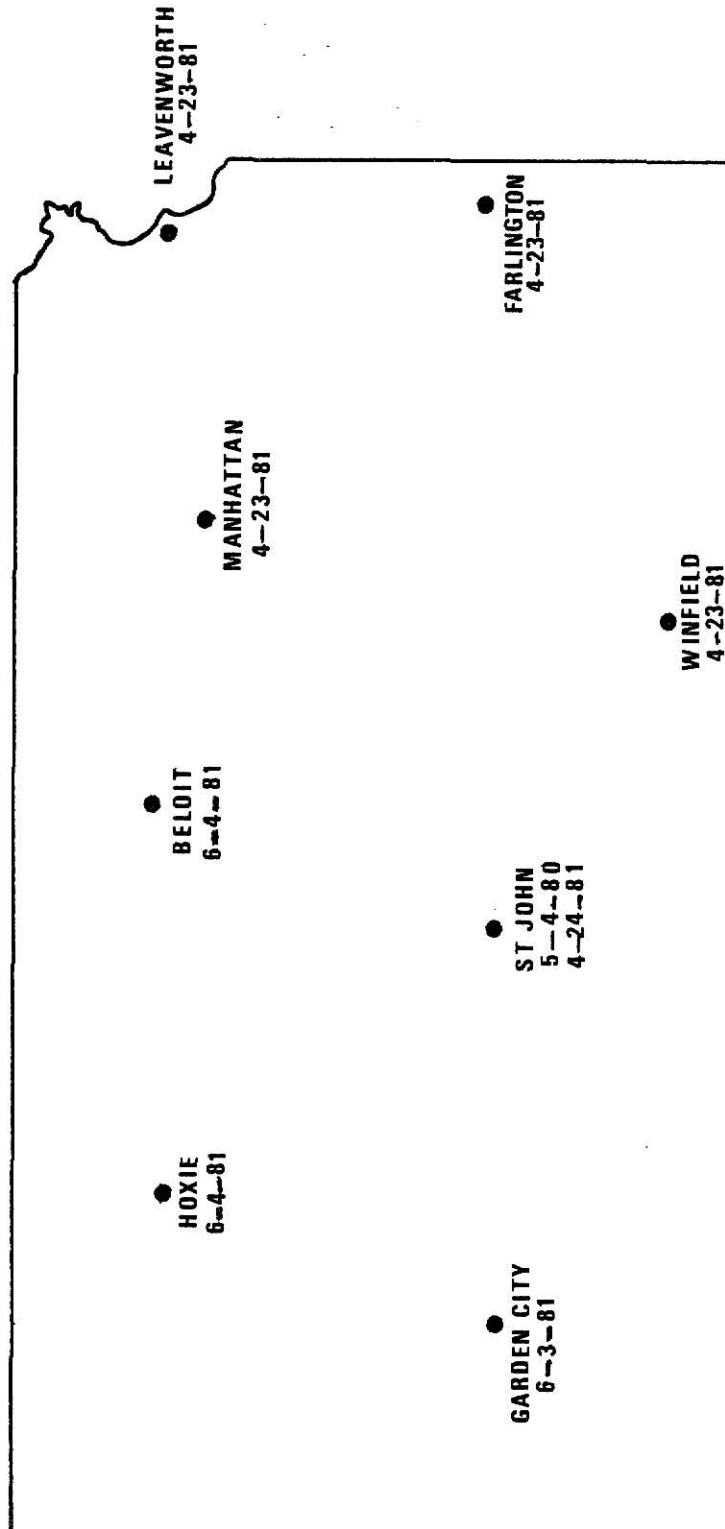
matrices. Programs were then written for a Texas Instruments SR-59TM calculator which would classify unknown weevils using the distances band 6, band 7 and the tracking dye had migrated. These programs are described in Appendix I-IV.

Geographical Distribution of the Strains in Kansas

Eight sites distributed in the northern and southern portions of eastern, central and western Kansas were visited to collect alfalfa weevil larvae (Figure 2). Larvae in eastern and central Kansas were gathered from five sites during April 23-24, 1981. Larvae were collected from three locations in western and central Kansas on June 3-4, 1981. More sites were selected from the eastern two-thirds of Kansas due to the higher concentration of alfalfa plantings. The month lapse in collecting reflects the timing of peak larval populations across the state. Larvae were also gathered the previous year (May 4, 1980) at St. John in the field where alfalfa weevil populations were being monitored for model validation.

The collected larvae were transported to the laboratory for analysis. At least 30 larvae per site were used to ensure an adequate sample size. The strain of each individual was determined using gel electrophoresis and calculator programs discussed above.

Figure 2. Alfalfa weevil collecting sites (and date of collection) for the geographic strain distribution study.



RESULTS

The Sampling Program

1979

Spring egg densities remained low until early May when they began to increase. Overall, however, the season's infestation of eggs was light (Figure 3).

Figures 4 and 5 represent the mean densities of small (first and second instars) and large (third and fourth instars) alfalfa weevil larvae, respectively. The field infestation of larvae was low, and Kansas growers were not advised to spray during the spring. All four stages displayed bimodal peaks on May 1 and May 12. The first peak occurred at about the same density for each instar, whereas the height of the second peak increased in older larvae. The temperature recorded inside the Burlese funnel was consistently between 37-40°C.

The field was cut four times (May 17, June 29, July 20, and Sept. 4) between spring and fall sampling. The egg population increased after the last harvest, but remained at low levels during the fall, Figure 6. The confidence interval about the mean shows a consistent statistical difference from zero for eggs after Oct. 16. The mean densities of the remaining life stages were not significantly different from zero (Table 2).

Figure 3. Alfalfa weevil egg densities at St. John,
Kansas during spring 1979.

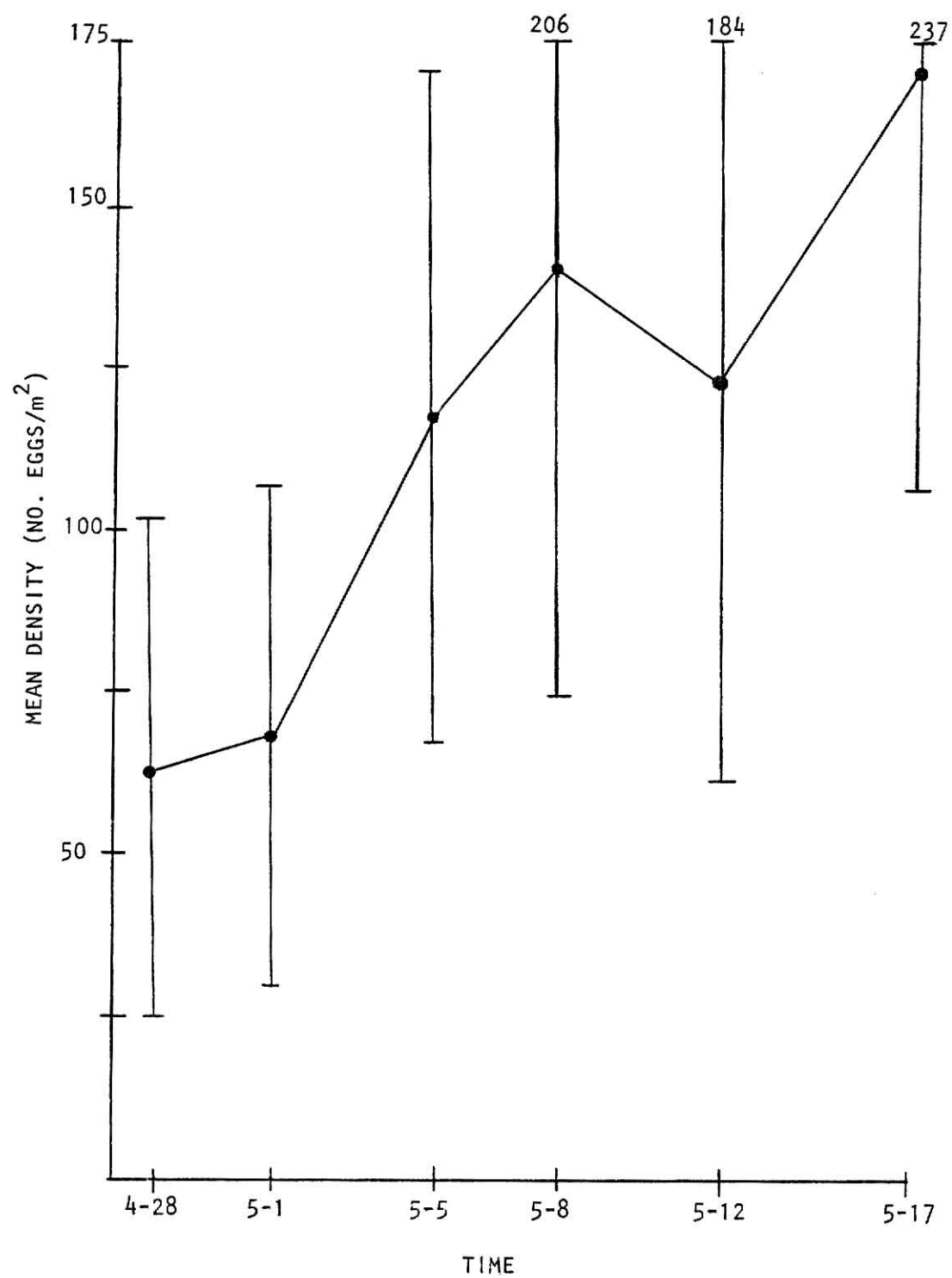


Figure 4. Mean density of alfalfa weevil first and second instars at St. John, Kansas during spring 1979.

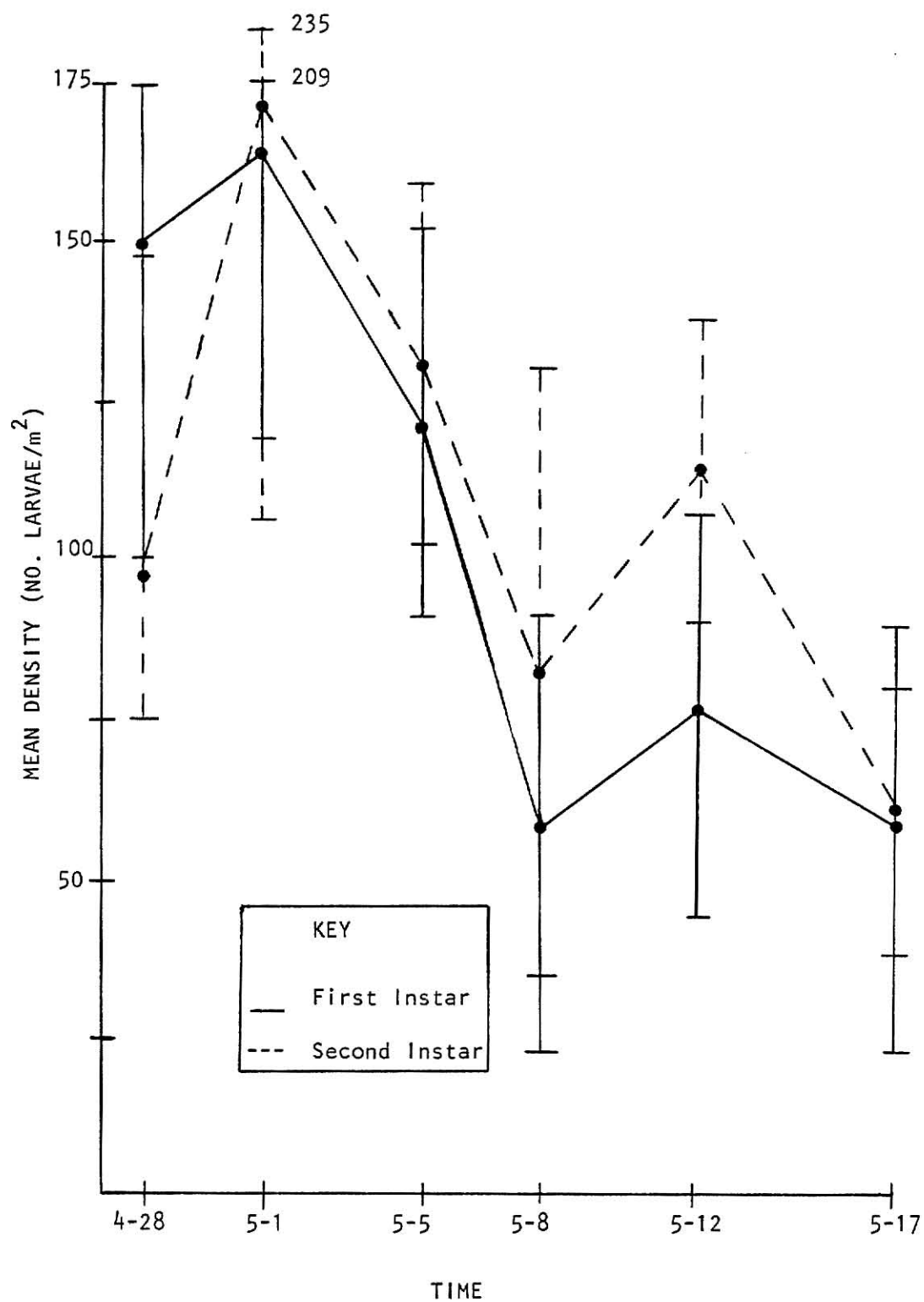


Figure 5. Mean density of alfalfa weevil third and fourth instars at St. John, Kansas during spring 1979.

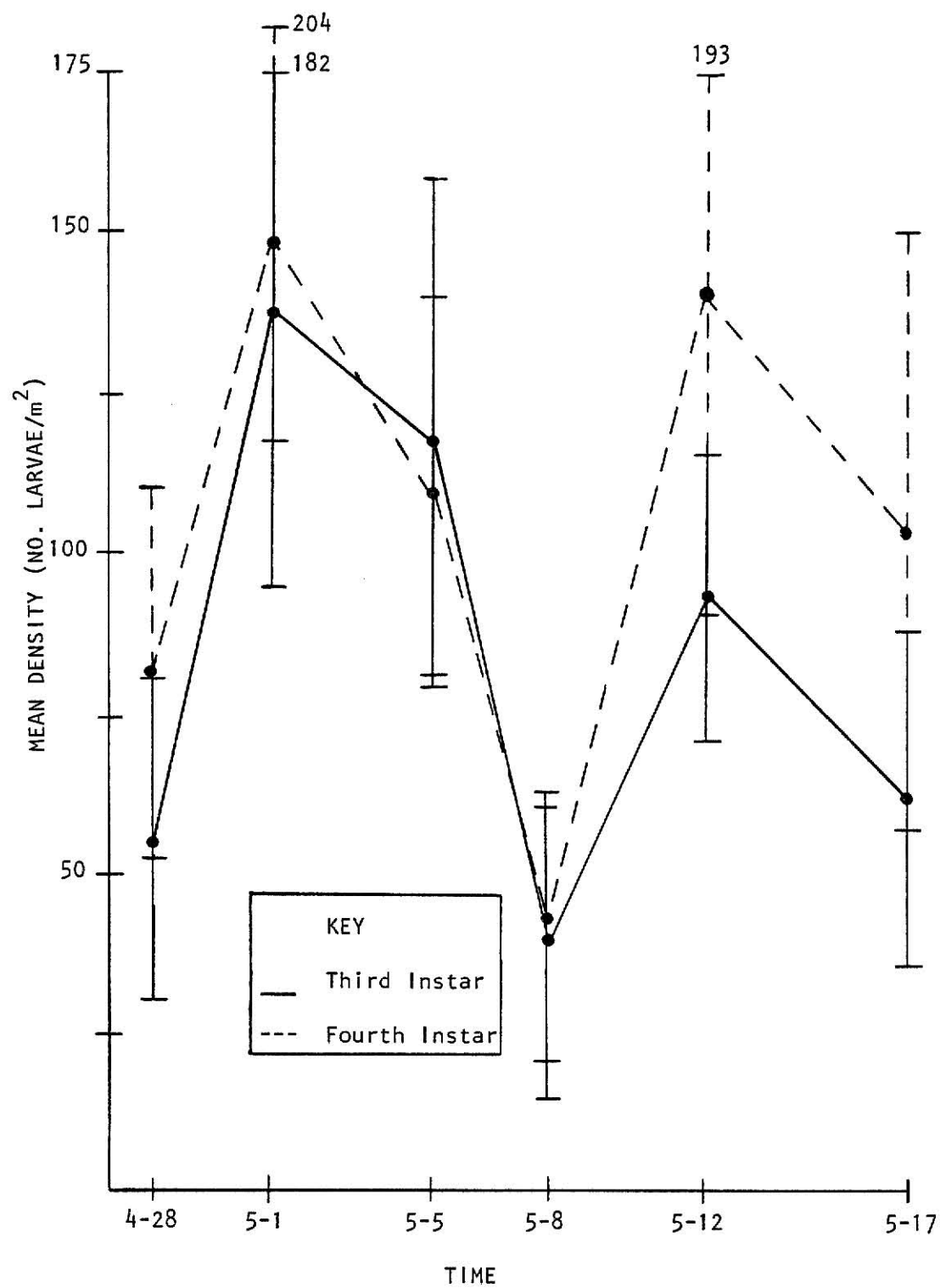


Figure 6. Alfalfa weevil egg densities at St. John,
Kansas during fall 1979.

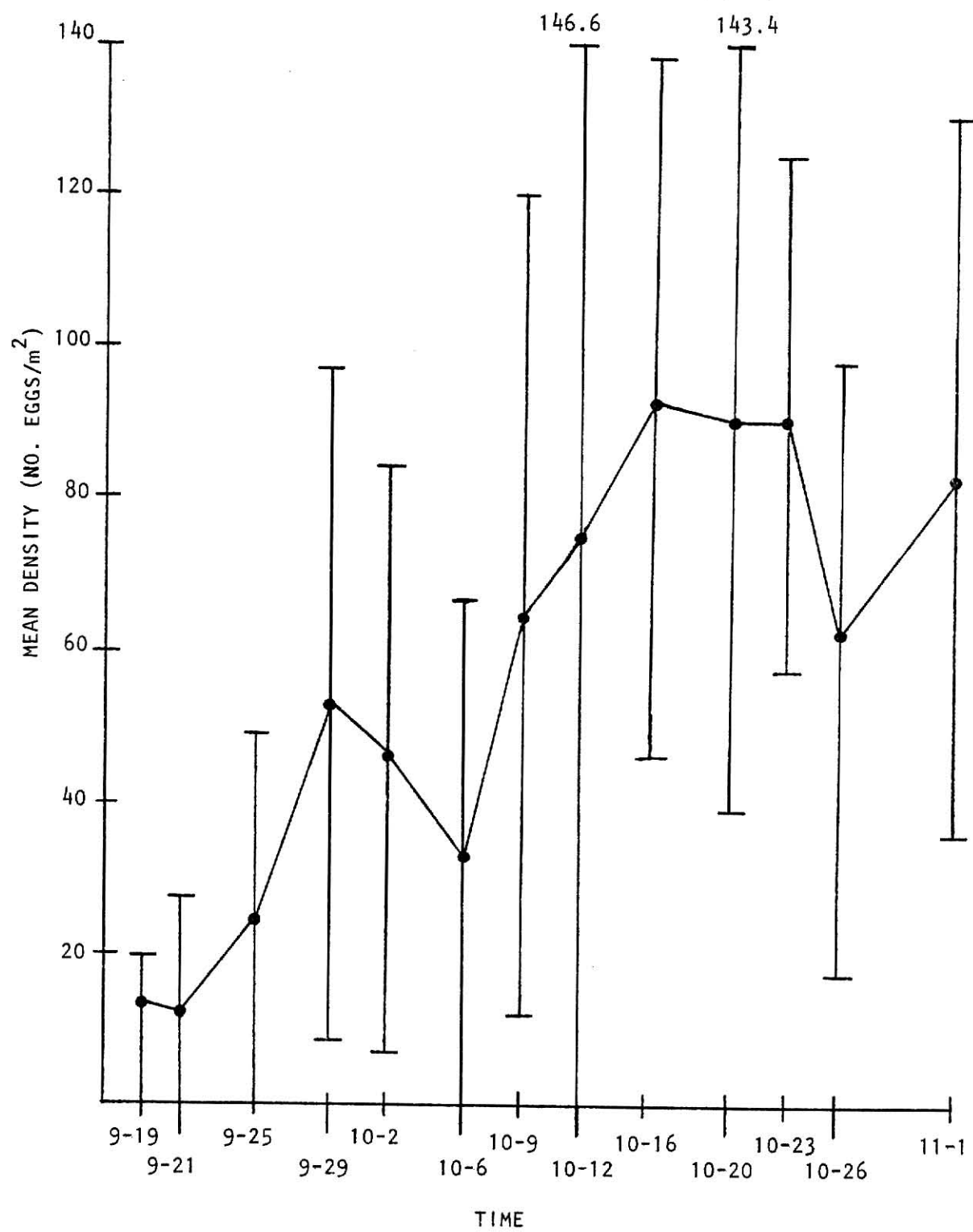


Table 2. Mean density^a of larvae and adults during fall 1979 at St. John, Kansas.

DATE	LARVAL INSTARS				ADULTS
	1	2	3	4	
9-19-79	0	0	0	0	0
9-21-79	0	0	0	0	0
9-25-79	0	0	0	0	0
9-29-79	1.35±.925	0	0	0	0
10-2-79	0	.677±.667	0	0	.667±.667
10-6-79	1.35±1.35	1.35±.925	0	0	.667±.677
10-6-79	0	1.35±.925	0	0	0
10-12-79	6.77±3.26	0	0	0	0
10-16-79	8.63±4.22	1.99±1.09	.677±.667	0	0
10-20-79	2.66±1.56	1.99±1.09	0	0	0
10-23-79	.677±.667	.677±.667	0	0	.677±.667
10-26-79	.677±.667	1.35±.925	0	0	0
11-1-79	0	0	0	0	0

^aMean density (individuals/m²) ± S.E.

1980

The spring egg population (Figure 7) was higher than 1979's on every sampling date except one, May 8. Mean densities during early April 1980 were similar to 1979 densities recorded in late April of the previous year. This suggests that the 1980 population was either earlier or higher. Egg densities sampled late in March 1980 coincided with previous fall densities.

The 1980 spring densities of small and large larvae (Figures 8 and 9) were significantly higher than in 1979. Some spraying occurred in Kansas for larval control in 1980. The first three instars had differing densities in the two years but peaked at very similar times (May 1, 1979, and May 4, 1980). The fourth instar did not differ between years. Its 1980 peak was within eight days of the 1979 peak data. Overall, densities of younger larvae exceeded those of older larvae. No adults were collected during this season. Temperatures inside the Burlese funnel were approximately 40°C. For a given date, mean plant height in 1979 was greater than 1980 by 7-10 cm (Figure 10).

Fall egg densities significantly differed from zero after Oct. 28 (Figure 11). Total egg densities in 1979 were greater than 1980 during the duration of the 1979 sampling period which ended Nov. 1. Larvae and adult populations remained at trace levels during fall (Table 3).

Model Validation Under Kansas Field Conditions

Estimated adult densities and plant values used to start a model run are listed in Table 4. Both 1979 and 1980 adult densities represented low levels which agree with sweep net counts obtained from the field. Values for buds and total nonstructural carbohydrates (TNC)

Figure 7. Alfalfa weevil egg densities at St. John,
Kansas during spring 1980.

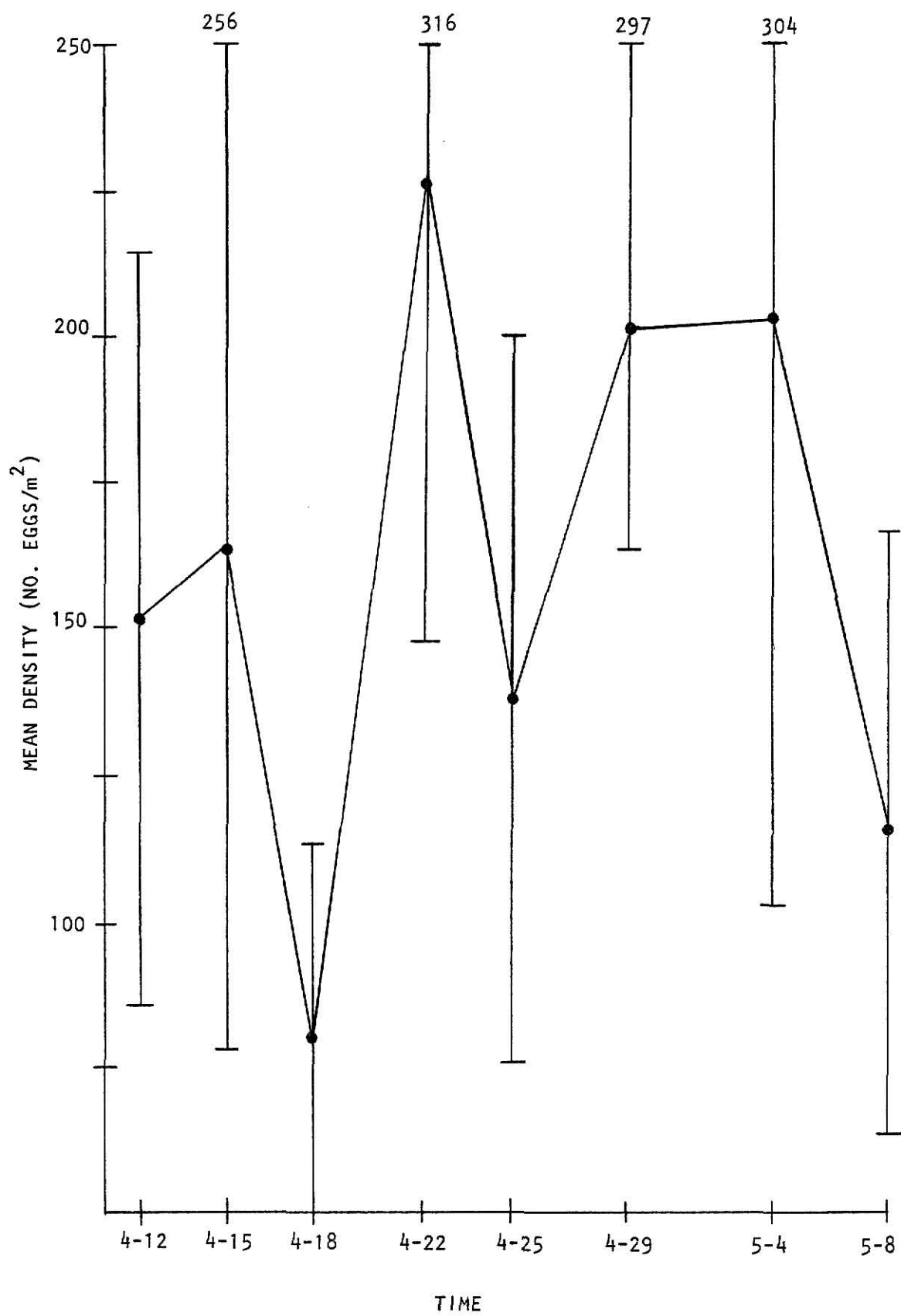


Figure 8. Mean density of alfalfa weevil first and second instars at St. John, Kansas during spring 1980.

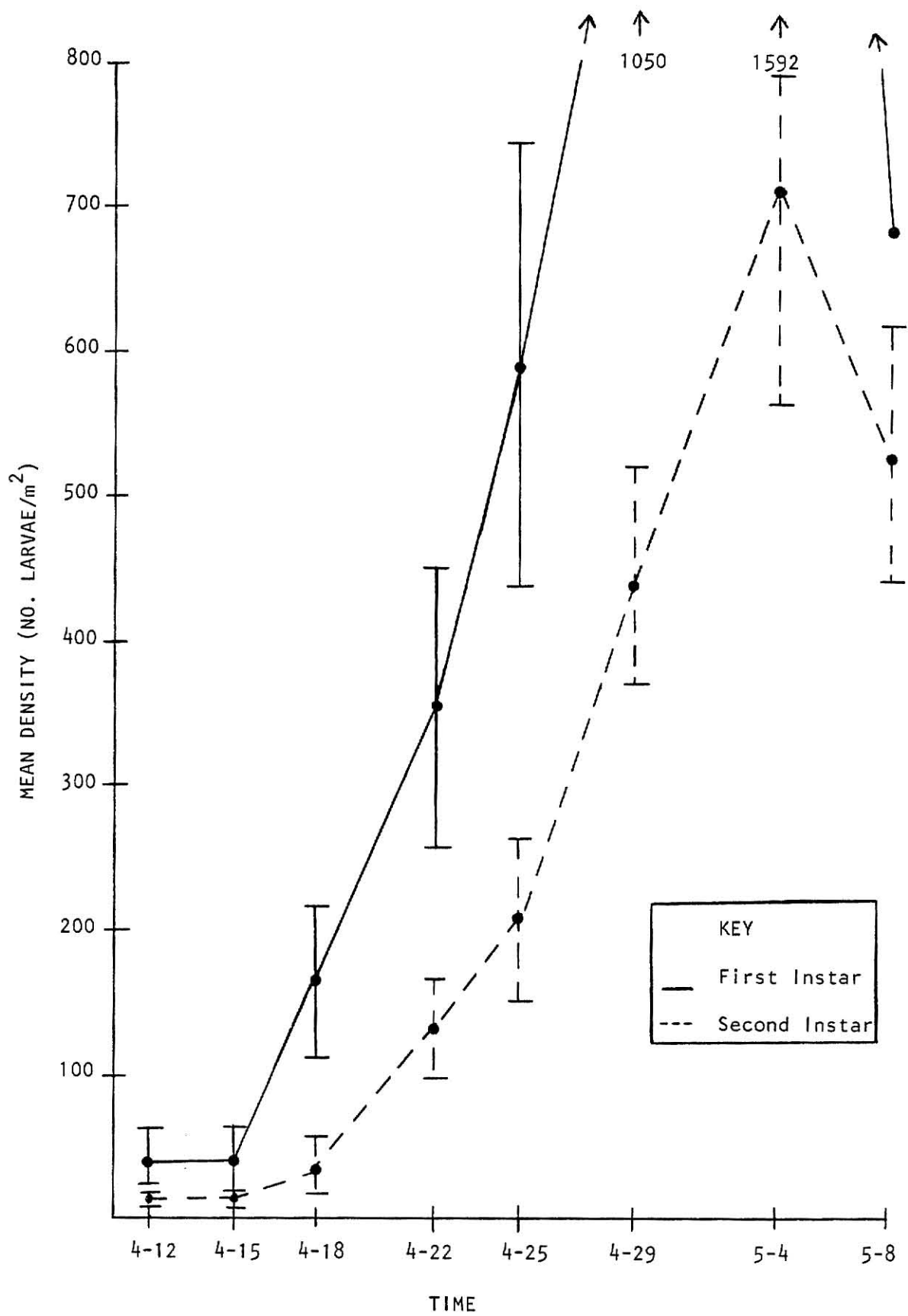


Figure 9. Mean density of alfalfa weevil third and fourth instars at St. John, Kansas during spring 1980.

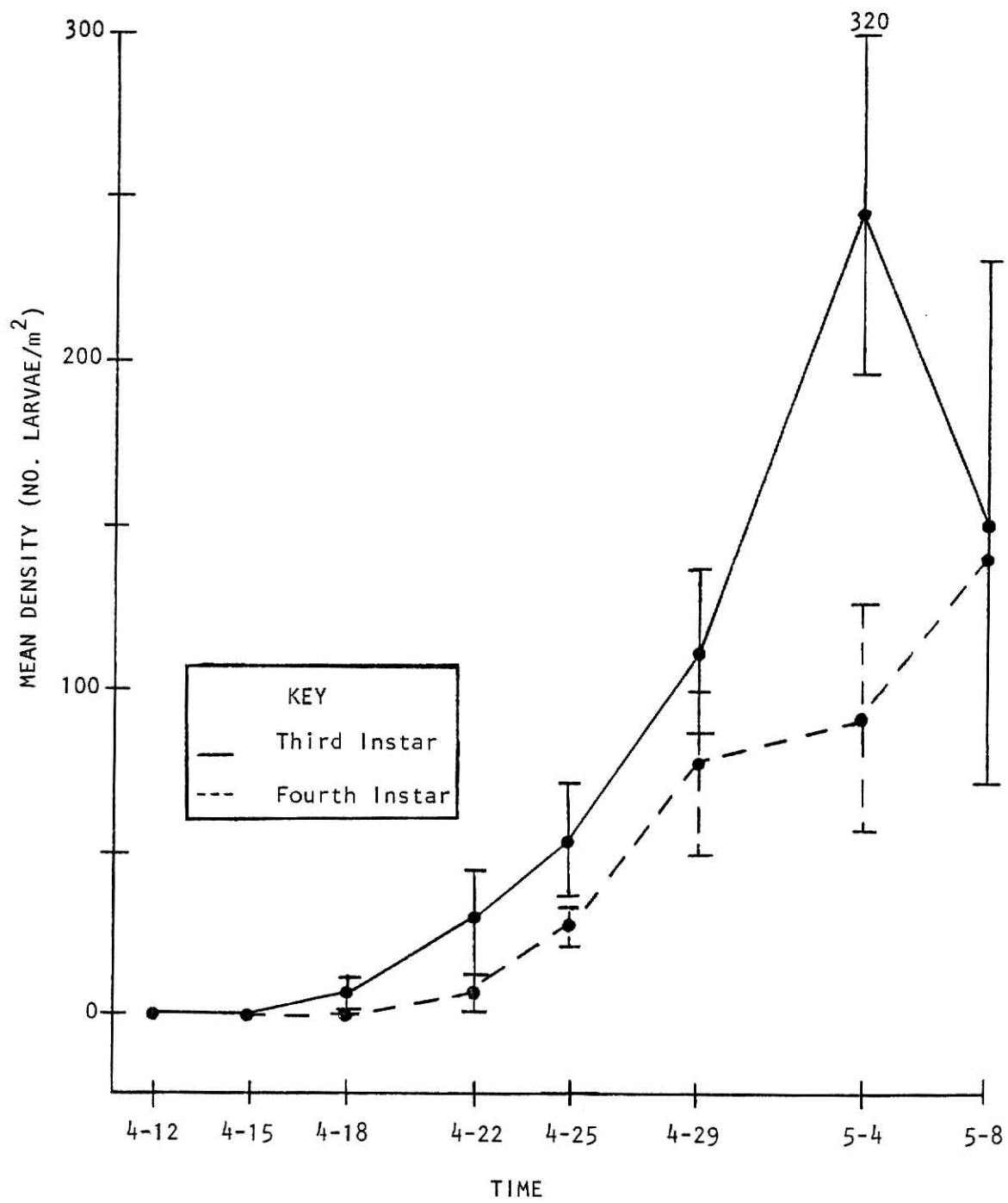


Figure 10. Mean alfalfa height at St. John, Kansas during
spring 1979 and 1980.

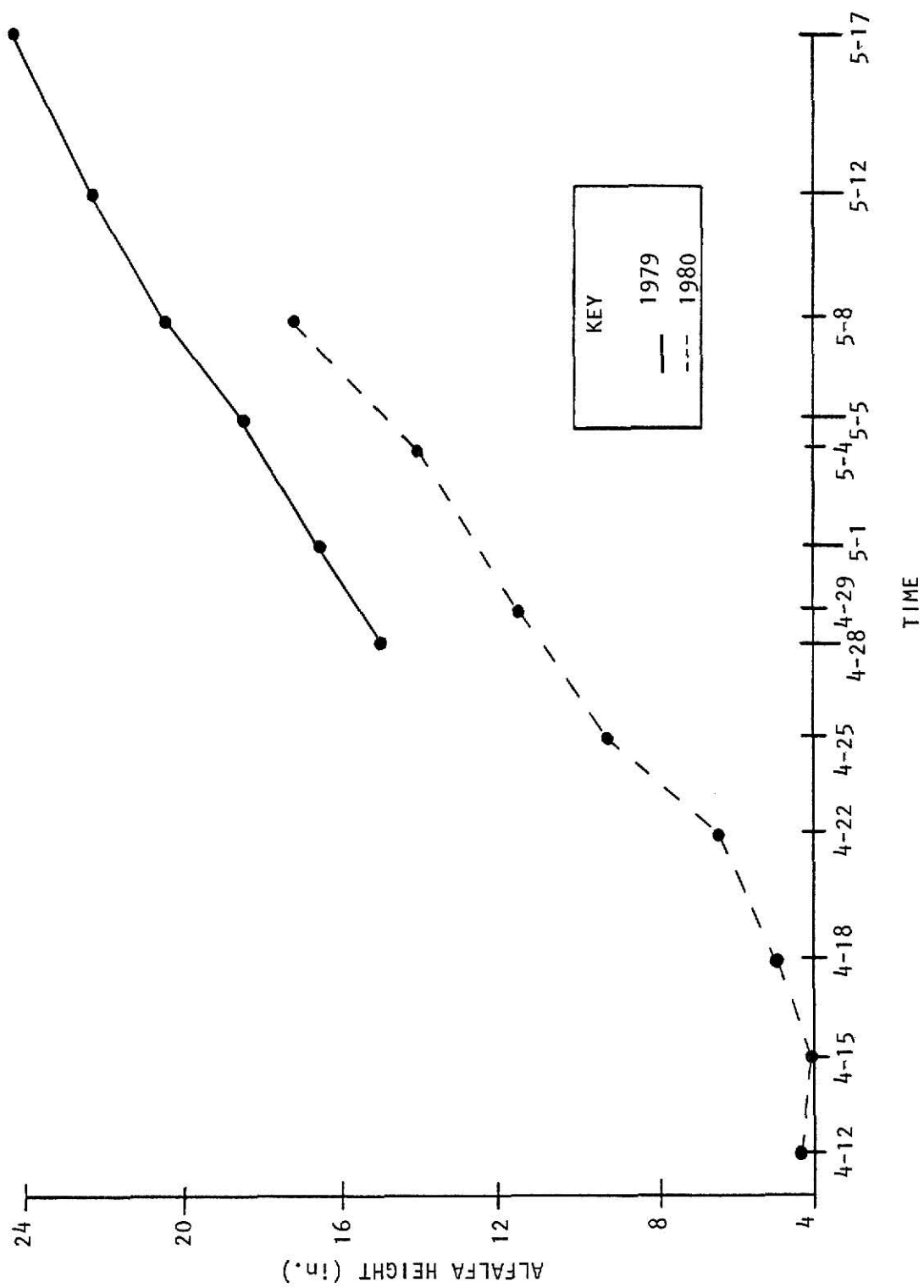


Figure 11. Alfalfa weevil egg densities at St. John,
Kansas during fall 1980.

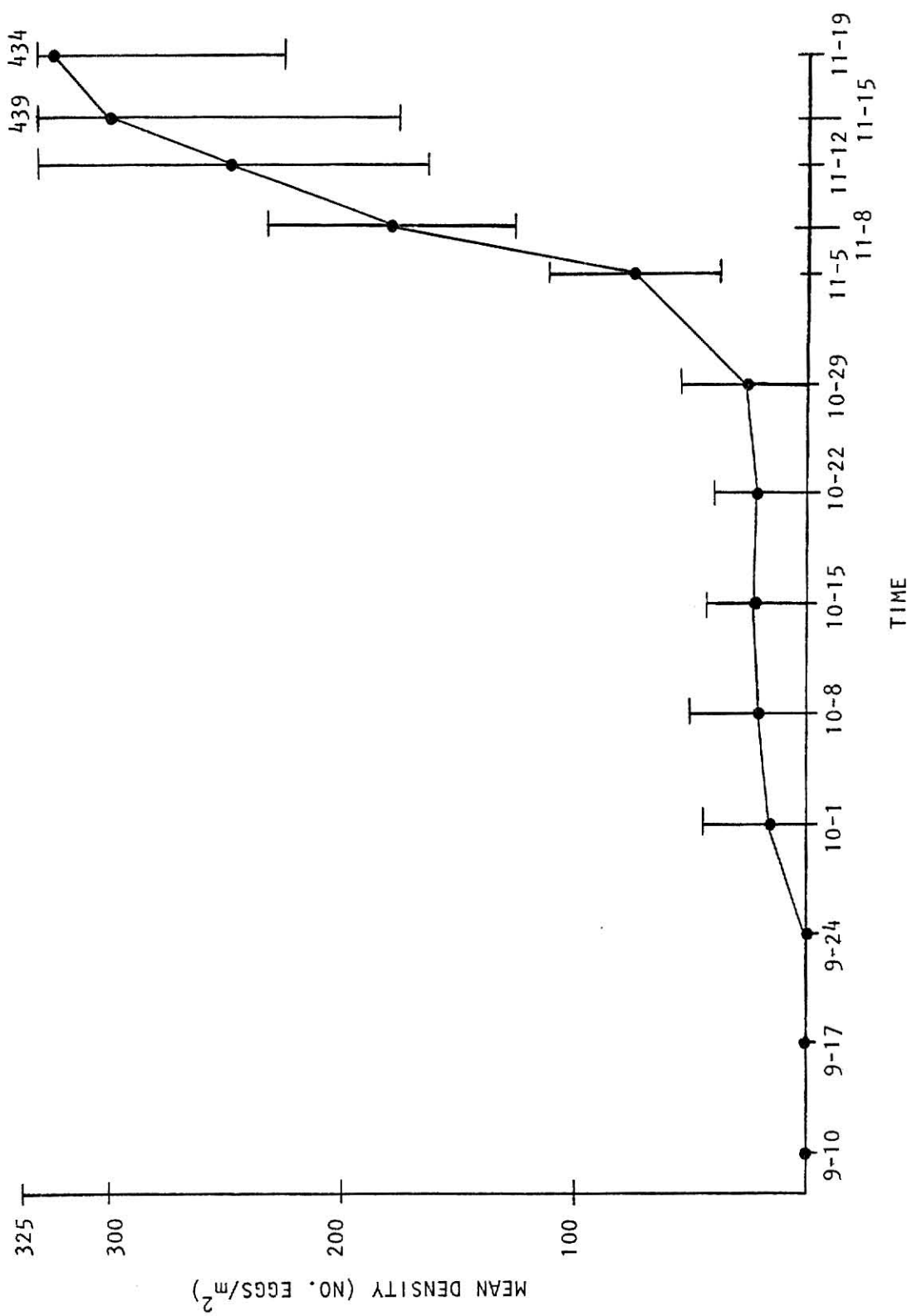


Table 3. Mean density^a of larvae and adults during fall 1980 at St. John, Kansas.

DATE	LARVAL INSTARS				ADULTS
	1	2	3	4	
9-10-80	8.63±4.45	3.98±1.95	0	0	0
9-17-80	3.32±2.15	0	0	0	0
9-24-80	0	0	0	0	0
10-1-80	.677±.667	0	.677±.667	0	0
10-8-80	1.35±.925	0	0	0	0
10-15-80	3.98±4.33	0	0	0	0
10-22-80	2.66±1.85	0	0	0	.677±.667
10-29-80	.677±.667	0	0	0	---
11-5-80	3.98±2.40	0	0	0	3.98±1.68
11-8-80	3.32±1.91	0	0	0	---
11-12-80	5.98±2.97	0	0	0	2.66±1.21
11-15-80	5.98±3.33	1.35±.925	0	0	0
11-19-80	0	0	0	0	.677±.667

^aMean density (individuals/m²) ± S.E.

Table 4. Adult alfalfa weevil densities and plant value estimates for each starting date in spring 1979 and 1980 at St. John, Kansas.

Date	Adults ₂ (no./m ²)	Stem ₂ (gm/m ²)	Buds ₂ (gm/m ²)	Leaf ₂ (gm/m ²)	TNC ^a (gm/m ²)	GDDB5 ^b
4-28-79	1.62	124.00	11.00	166.00	92.30	324.00
5- 1-79	1.52	164.00	11.00	173.00	95.80	352.00
5- 5-79	1.46	201.00	12.00	177.00	99.70	382.00
5- 8-79	1.30	248.00	12.00	186.00	102.00	429.00
5-12-79	1.30	281.00	12.00	194.00	103.00	444.00
4-12-80	3.50	0.00	11.00	0.00	87.00	49.73
4-15-80	3.00	0.81	11.00	6.50	86.40	60.80
4-18-80	3.00	2.70	11.00	11.00	79.80	81.70
4-22-80	2.75	13.00	11.00	23.00	86.20	133.00
4-25-80	2.50	24.00	11.00	40.00	90.40	167.00
4-29-80	2.25	46.00	12.00	83.00	95.20	190.00
5- 4-80	2.00	94.00	12.00	157.00	96.20	245.00

^aTNC--Total nonstructural carbohydrates.

^bGDDB5--Growing degree days base 5°C.

were similar for both years. Stem biomass in 1979 was twice the amount for comparable dates in 1980, whereas leaf biomass was slightly higher in 1979. A greater accumulation of growing degree days (base 5°C) by each sampling date in 1979 could explain the difference in stem growth. Field measurements of plant height confirm the difference in stem biomass (Figure 10).

The following discussion concentrates on the outcome of model validation to densities sampled in Kansas during 1979 and 1980. The results of the various statistical tests are stated in the following order for each year: (1) spring model validation includes results from, Theil's nonparametric test, a parametric test, and Kruskal-Wallis' test, (2) spring validation for management usage reports a comparison of model recommendation to three pest management programs, and (3) fall validation states results using Theil's nonparametric test.

Spring 1979

Theil's nonparametric test indicated no significant differences at 95% probability between observed and predicted densities for each larval instar in spring 1979. These results were calculated from model runs starting on 4-28 and 5-1. Runs starting on 5-5, 5-8, or 5-12 were not included because probability tables for this test statistic require at least four sampling dates (degrees of freedom) to reject with 95% probability.

Unlike the previous test, validation of spring populations using the parametric test (Welch et al., 1981) demonstrated that 48% of the larval populations differed significantly from observed values (Table 5). This value (29 runs in 60) was calculated by including runs from

Table 5. Model validation to spring 1979 densities using the parametric test developed by Welch et al. (1981).

Model Run Starting Date	Number Model Rejects per Instar				Percent Rejects for ¹ Each Starting Date
	1	2	3	4	
4-28-79	3/5	5/5	3/5	1/5	12/20 = 60.0%
5- 1-79	2/4	2/4	2/4	4/4	10/16 = 62.5%
5- 5-79	0/3	2/3	1/3	2/3	5/12 = 41.7%
5- 8-79	0/2	1/2	0/2	1/2	2/8 = 25.0%
5-12-79	0/1	0/1	0/1	0/1	0/4 = 0.0%
OVERALL PERFORMANCE	5/12	10/15	6/15	8/15	29/60 = 48.3%

¹Weighing runs from each starting date equal, overall performance is:
 $((.60 + .625 + .417 + .25 + .0) / 5) \times 100 = 37.8\%$ rejects

five starting dates. Runs started early in the season generated predictions for more sampling dates than did runs started closer to harvest. Consequently, early starting runs had a greater apparent effect on the model's overall performance. Equalizing the number of runs from each starting date, the model accurately predicted 62% of the observations. There was no statistical difference ($P < .91$) in the frequency of model rejection by instar stage from the Kruskal-Wallis test. Model runs with later starting dates correctly predicted larval densities more often than runs started at earlier dates. Because of its lower power, the nonparametric test did not reveal this trend. Examining the rejected runs (classified by instar) showed that first and second instar predictions were underestimated. Third instar predictions were rejected equally for over- and underestimating observed populations, whereas model estimates of fourth instars were more frequently rejected for overpredicting densities in this stage (Table 6).

Model management predictions for spring 1979 matched recommendations from three alfalfa pest management programs. All four advisories predicted no damage, thus spraying was not necessary.

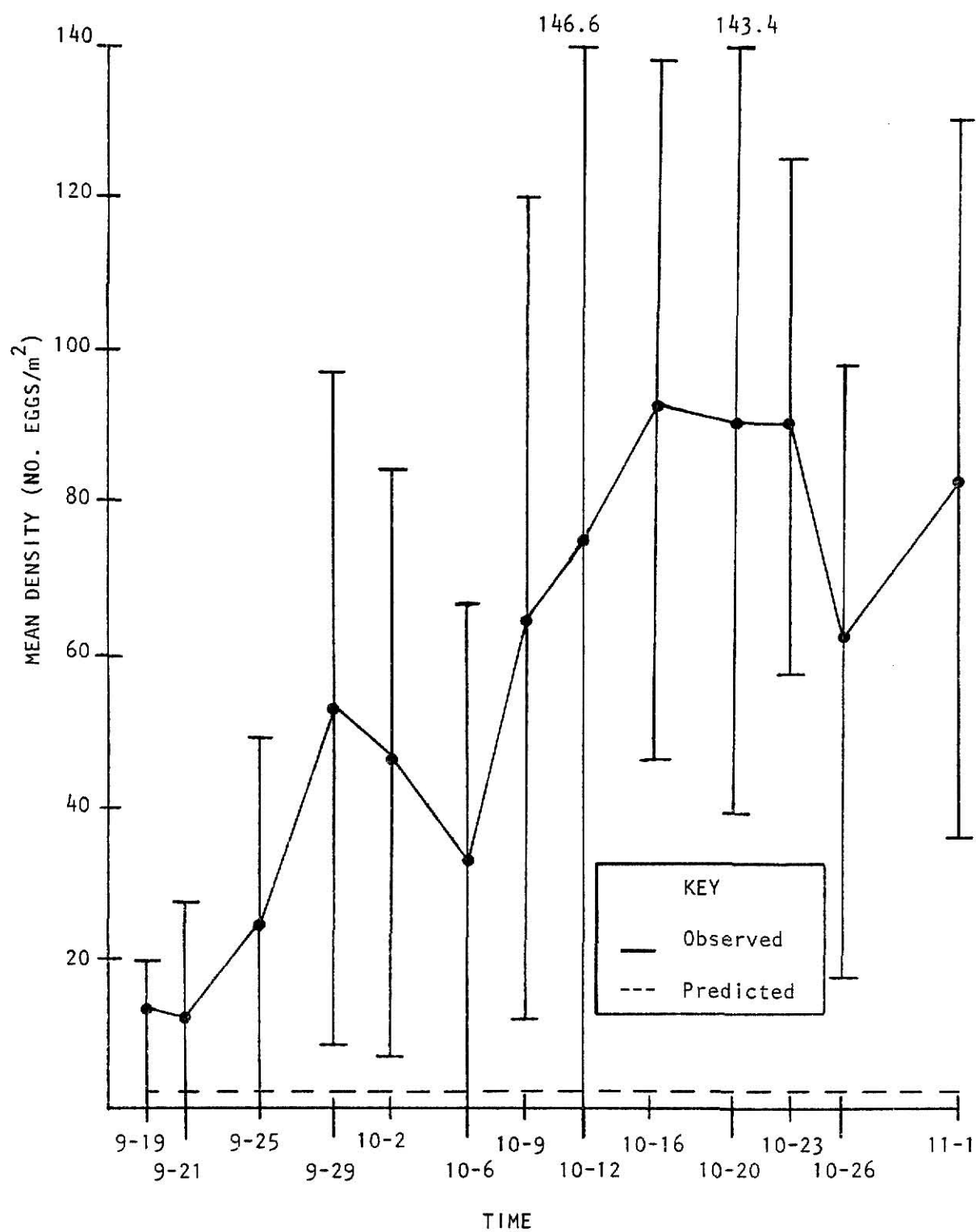
Fall 1979

The model predicted zero densities for fall egg and larval populations (Figure 12). These results were consistent for six model runs. Theil's test rejected the model for inaccurate egg predictions with 95% probability. All larval predictions coincided with sampled densities, therefore the model was not rejected for this stage.

Table 6. Classification of model rejects by instar for spring 1979.

INSTAR STAGE	TYPE OF REJECT		TOTAL PER INSTAR
	UNDERPREDICTION	OVERPREDICTION	
1	5	0	5
2	10	0	10
3	3	3	6
4	1	7	8
TOTAL PER REJECTION TYPE	19	10	29

Figure 12. Observed and model predicted fall alfalfa
weevil egg populations during 1979.



Spring 1980

Theil's test indicated that the model could not accurately predict 47% of the larval observations with 95% probability (Table 7). Model runs starting on 4-12, 4-15, 4-18, 4-22, and 4-25 were used in these calculations. First instar predictions for the 4-15, 4-18, and 4-25 runs could not be tested because after deleting data influenced by spring adults (which were estimated), too few degrees of freedom remained. Model runs using earlier starting dates were rejected more than runs of a later date. This trend appears to be related to the statistical procedure, because this tendency did not exist with the parametric test applied to the same data set.

Again, the parametric test rejected the model more frequently than Theil's test in validating spring densities. Approximately 65.6% of the model runs were rejected with 95% probability with the parametric test (Table 8). This figure was calculated from a total of 96 runs using seven starting dates. Equalizing the weight of runs from each starting date, the model was rejected 73.4%. The model appeared to be rejected more for later starting dates, but as stated above, this trend did not correspond to the results from Theil's test. Differences in rejection by instar were close to significant ($P < .061$). The first instar was rejected more frequently than the second instar, and so forth. Of the rejected predictions, the model underpredicted the first and second instar stages 35 out of 36 estimates (Table 9). There was no trend for under- or overpredicting the third or fourth instars.

The model accurately predicted the need to spray immediately on three of four sampling dates where model advisories were compared to New York, Pennsylvania, and Illinois recommendations. The cost related to

Table 7. Theil's nonparametric test for slope regression applied to spring 1980 alfalfa weevil populations at St. John, Kansas.

MODEL RUN STARTING DATE	MODEL ACCURACY ¹ PER INSTAR				NUMBER REJECTED PER MODEL RUN
	1	2	3	4	
4-12-80	Not Rejected	Rejected	Rejected	Rejected	3
4-15-80	NA	Rejected	Rejected	Rejected	3
4-18-80	NA	Rejected	Not Rejected	Not Rejected	1
4-22-80	Not Rejected	Not Rejected	Rejected	Not Rejected	1
4-25-80	NA	Not Rejected	Not Rejected	Not Rejected	0
Number Rejected per Instar	0	3	3	2	8/17 = 47%

NA - not applicable

¹ 95% Confidence Level.

Table 8. Model validation to spring 1980 densities using the parametric test developed by Welch et al. (1981).

Model Run Starting Date	Number Model Rejects per Instar				Percent Rejects for ¹ Each Starting Date
	1	2	3	4	
4-12-80	3/4	3/5	2/6	2/7	10/22 = 45.5%
4-15-80	3/3	3/4	2/5	2/6	10/18 = 55.6%
4-18-80	3/3	3/4	3/5	2/4	11/16 = 68.8%
4-22-80	4/4	3/4	2/4	1/4	10/16 = 62.5%
4-25-80	3/3	2/3	3/3	2/3	10/12 = 83.3%
4-29-80	2/2	2/2	2/2	2/2	8/8 = 100%
5- 4-80	1/1	1/1	1/1	1/1	4/4 = 100%
OVERALL PERFORMANCE	19/20	17/23	15/26	12/28	63/96 = 65.6%

¹Weighing runs from each starting date equal, overall performance is:
 $((.455 + .556 + .668 + .625 + .833 + 1.00 + 1.00) / 7) \times 100 = 73.4\%$ rejects.

Table 9. Classification of model rejects by instar for spring 1980.

INSTAR STAGE	TYPE OF REJECT		TOTAL PER INSTAR
	UNDERPREDICTION	OVERPREDICTION	
1	19	0	19
2	16	1	17
3	6	9	15
4	5	7	12
TOTAL PER REJECTION TYPE	46	17	63

an incorrect model recommendation could not be determined because field experiments were not designed to evaluate alfalfa yields from sprayed and unsprayed plots.

Fall 1980

Nine model runs predicted zero fall egg populations until 10-29. Validation could not be accomplished with populations after this date because predicted egg populations were influenced by the estimated adult density selected the previous spring. The model was not rejected for egg and larval predictions during early fall using Theil's test. These results are identical to the previous year for the larval stage.

Alternate Methods of Alfalfa Weevil Strain Determination

Electrophoresis

Average relative mobility (Rf), width and intensity for each of the seven bands common to each gel of Data Set 1 are displayed in Figure 13 and listed in Table 10. Two comparisons were significant, intensity of band 6 ($P < .05$) and Rf of band 7 ($P < .01$). Strain classification based on band 6's intensity was inaccurate. The reason is that the mean difference between strain for this variable was .48 which made detection subjective when using an integer rating scale (1 = faint, 3 = strong). Strain categorization using band 7's Rf produced better results. Identification was 83% correct when this band was used as the sole discriminating variable. Examination of this variable indicated that 17% error was caused by similar Rf for six gels of both strains. A higher degree of accuracy was desired, thus an additional variable was sought.

Figure 13. Typical polyacrylamide gels for eastern and western alfalfa weevil strains.

EASTERN

WESTERN

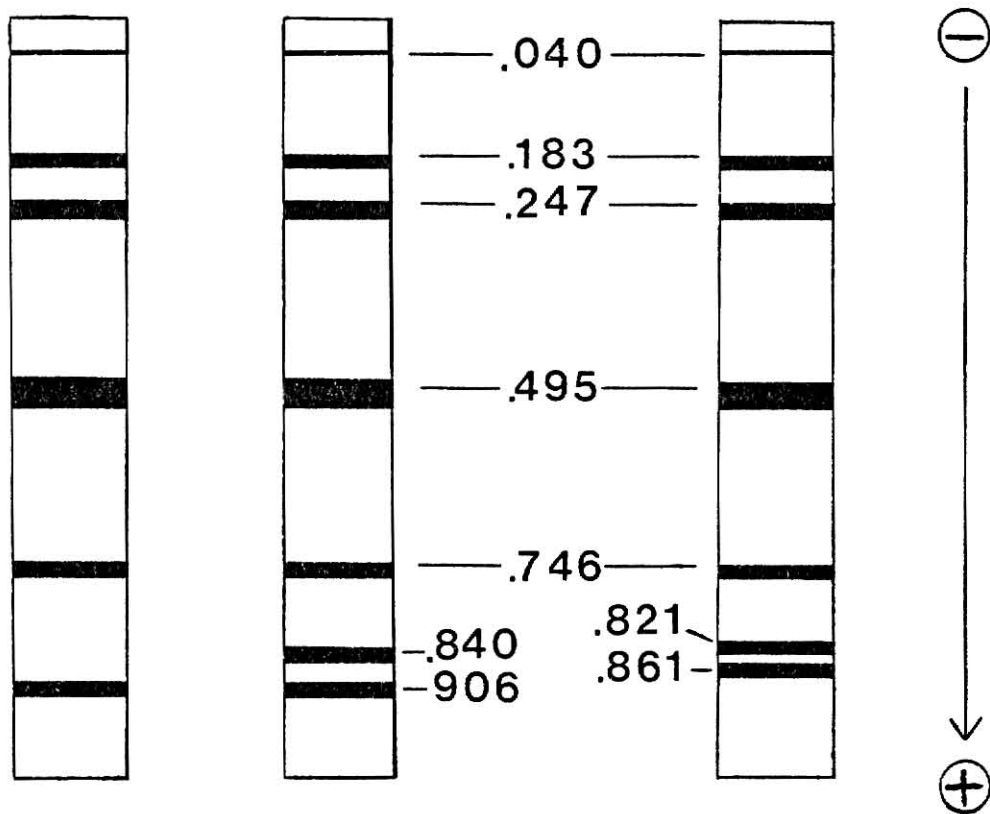


Table 10. Relative mobility (Rf), width and intensity for seven electrophoretic bands of the alfalfa weevil.

STRAIN	BAND	b n	BAND VARIABLES ^a		
			Rf	WIDTH	INTENSITY
E	1	12	.044±.0003	.081±.0007	2.08±.182
W	1	16	.037±.0005	.083±.0007	2.56±.099
E	2	16	.182±.0002	.095±.0013	2.44±.132
W	2	19	.184±.0001	.111±.0020	2.45±.058
E	3	16	.250±.0002	.103±.0003	2.00±.167
W	3	19	.244±.0001	.094±.0002	2.60±.056
E	4	15	.502±.0003	.197±.0026	2.53±.106
W	4	19	.489±.0002	.239±.0169	2.79±.066
E	5	16	.751±.0002	.128±.0002	.87±.096
W	5	19	.741±.0005	.168±.0013	.63±.107
E	6	5	.840±.0003	.160±.0030	1.57±.128*
W	6	19	.821±.0001	.160±.0030	2.05±.054*
E	7	16	.906±.0003**	.150±.0030	1.30±.450
W	7	19	.861±.0001**	.180±.0040	1.42±.257

^aMean ± S.E

^bEastern n=16, Western n=19; n less than these values indicates a missing band.

*Significant at =.05.

**Significant at =.01.

Instead of selecting the comparison with probability closest to .05, qualitative characteristics were examined. The 11 gels which did not have band 6 were eastern strain, Table 10. Regarding Data Set 1, 68% of the easterns could be correctly determined by the absence of this band. Seven gels were missing band 1, but this could not be correlated to strain.

The accuracy of eight methods which use band 6 and 7 to discriminate both data sets was determined, Table 11. Two methods proved accurate 95% of the time. The first, Method C (see Methods section), used the Rf between band 6 and 7. The other, Method F, employed three variables, the Rf of band 6 and band 7 and the Rf of Band 6 divided by that of band 7. Two Texas Instrument SR-59TM calculator programs were written to classify unknown alfalfa weevil larvae based on Methods C and F, Appendix I-IV.

Geographical Distribution of the Strains in Kansas

Figure 14 displays the proportion of strains at eight sites in Kansas which was determined by averaging the results of the two calculator programs. In 1981, the eastern strain predominated. The St. John site indicated that the same trend may have existed in 1980. The northeast site (Leavenworth) was 100% eastern, whereas southwest Kansas (Garden City) had the lowest percentage of eastern weevils (71%). Except for these two locations, no definite pattern was present for the remaining sites with respect to the percentage of each strain.

Table 11. Evaluation of the eight methods used to discriminate Data Sets 1 and 2.

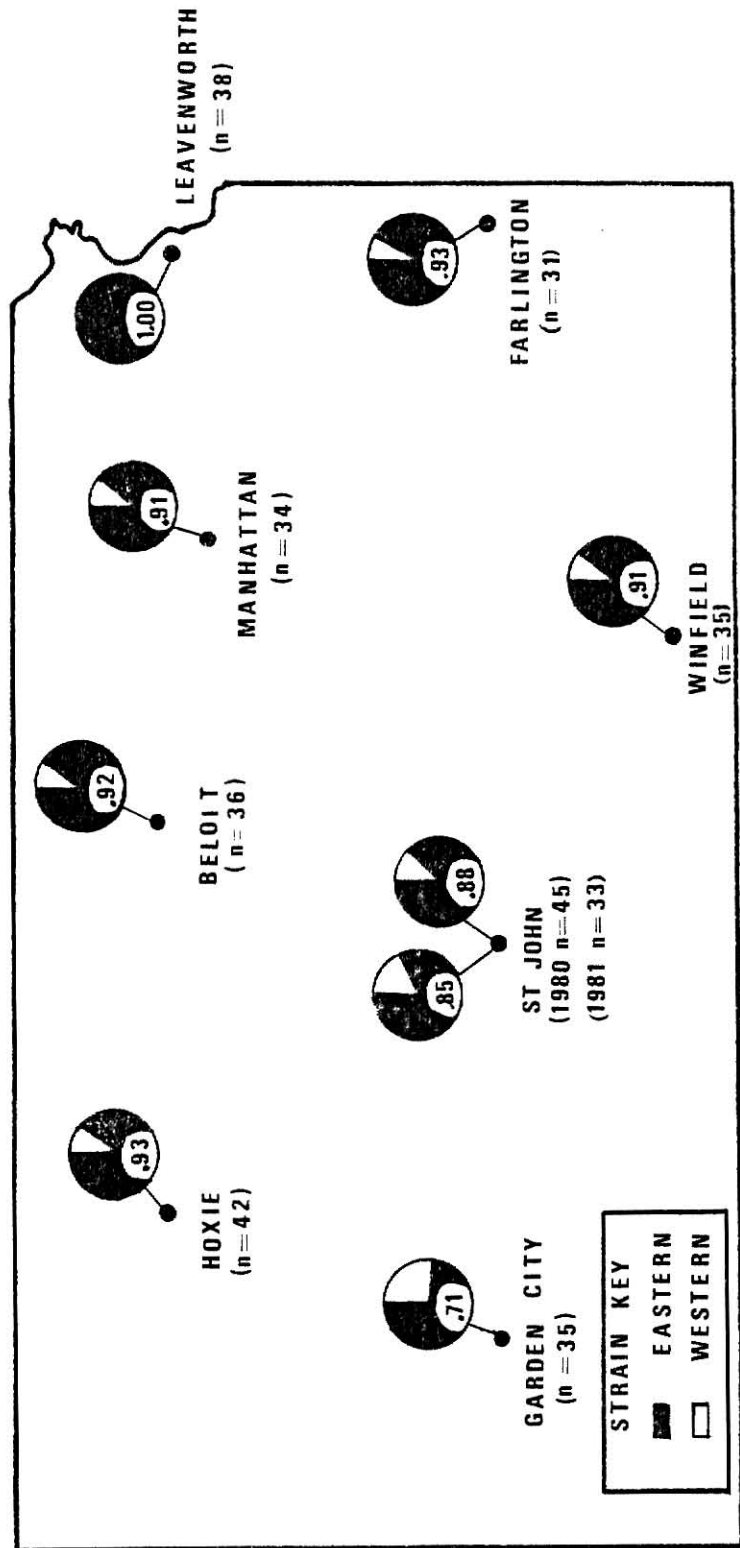
METHOD	NUMBER MISCLASSIFICATIONS		PERCENT ACCURACY Total Correct 159
	Data Set 1 (N=35)	Data Set 2 (N=124)	
A(1)	6	26	79.9%
B(2)	4	8	92.5%
C(3)	3	5	95.0%*
D(4)	2	13	90.6%
E(2,3)	4	16	87.4%
F(2,4)	2	5	95.6%*
G(3,4)	4	14	88.7%
H(2,3,4)	3	6	94.3%

* Acceptable at 95% accuracy.

Where method:

- 1 -- Rf band 7.
- 2 -- Rf band 6 and band 7.
- 3 -- Rf between band 6 and band 7.
- 4 -- band 6 divided band 7.

Figure 14. Percentage of each alfalfa weevil strain at each collecting site during spring 1981.



DISCUSSION

Model Validation under Kansas Field Conditions

Model performance was evaluated in terms of three objectives to 1979 and 1980 field densities. These are the model's ability to: (1) predict accurately spring densities, (2) recommend proper spring management practices, and (3) estimate precisely fall populations.

To accomplish objective 1, it is necessary to examine, in detail, the cases where the model was statistically rejected. Not all rejected runs are caused by model errors. Those which are not must be identified and eliminated from consideration. Further, rejections resulting from model inaccuracies serve to delineate areas where further refinement may be needed to relate to Kansas conditions, and/or to increase our general knowledge about this biological system.

Model predictions for the spring were rejected in 48% and 66% (1979 and 1980) of the runs using the parametric test (Welch et al., 1981). Because the parametric test is statistically more powerful than Theil's test in detecting differences, only the results from this test will be discussed henceforth for spring validation.

First and second instar predictions were underestimated for 56/57 of the runs during 1979 and 1980. Comparing these densities to spring egg populations, more total small larvae were sampled than could be accounted for by total eggs. This occurred in both years. (Figures

2,3,6 and 7). Two reasons could have caused model underprediction. Samples of first and second instar populations could have been contaminated with the cloverleaf weevil, or the egg populations may have been sampled inaccurately and thus underestimated.

Cloverleaf weevil contamination would be more likely with third and fourth instars instead of earlier instars, because of the cloverleaf weevil's larger size. This misidentification probably occurred in 1979 (discussed later).

Egg density underestimation could have occurred for several reasons. Adults oviposit some eggs in alfalfa crowns and litter making recovery extremely difficult (Bell, personal communication). This is likely to occur in early in April when plants are small and stem biomass is limited. Another source of underestimation is the duration and temperature during the time interval after a sample is collected but before it is analyzed. During this period, eggs continue to hatch into larvae. The number of eggs per sample will decline, whereas the number of larvae per sample will increase. A third reason for egg underestimation is related to the blender procedure used to extract eggs from plant stems. Although a rheostat was used to regulate blender speed, it is inevitable that some eggs were destroyed. The majority of cases when early instar predictions were rejected occurred in runs where egg density inputs were suspect. Because these samples do not contribute to an understanding of model accuracy, they will not be considered further.

The model overpredicted densities of the third and fourth instars for 24 of 30 among the rejected predictions for 1979 and 1980 (Table 12). In 1979, the third and fourth instar populations may have been

Table 12. Summary of model runs for 1979 and 1980.

SUMMARY OF RUNS	<u>RUESINK'S MODEL</u>	
	1979	1980
Accepted Runs	13	24
Rejected		
Overpredicted	9	15
Underpredicted	5	1
Total Number Runs	27	40
Percent Model Error	51.9%	40.0%

sampled inaccurately because of contamination by cloverleaf weevils. Dr. K. O. Bell, state survey entomologist, reported a higher cloverleaf weevil population than normal during this spring sampling period (personal communication). Despite field overestimation of the third and fourth instars, the model predicted even higher densities. While true alfalfa weevil densities are not known, the high predictions reveal true model errors.

Model variables which affect larval predictions are developmental rate and mortality. A density would be overpredicted if model developmental time was longer than actual development time. If this was the case, however, then predicted peak densities would occur later than observed peak densities. This trend was not noticed for the field data.

Overprediction of the third and fourth instars could also result if model survivorship rates were set too high. Model survivorship values were 1.0 for the egg and for all larval stages, which is clearly unrealistic. Harcourt and Guppy (1977) reported 26% mortality during larval establishment and feeding. To test the effect of survivorship, these values were varied from 1.0 to .75 for the third and fourth instars. Table 13 demonstrates that the number of high predictions decreased whereas the number of low predictions increased as survivorship was reduced. The total number of rejections fell drastically. The decline in total number of rejections from a decrease in survivorship can be explained biologically. Early instars (first and second) conceal themselves in the buds of the alfalfa plant where they exist in a protected environment. Older larvae (third and fourth instars) move from these sites to feed on leaves where they are exposed to abiotic and biotic factors (e. g., predators, rain, wind,

Table 13. Outcome of varying survivorship for both third and fourth instars based on 1980 data.

SURVIVORSHIP	1.0	1980 DATA	.75
		.85	
Accepted Runs	24	29	33
Rejected			
Overpredicted	15	10	3
Underpredicted	1	1	4
Total Number Runs	40	40	40
Percent Model Error	40.0%	27.5%	17.5%

dessication, etc.) which can kill larvae.

The remaining 6 of 30 rejected third and fourth instar predictions for 1979 and 1980 were underpredicted. No biological explanation is possible for these rejections at the present time.

Ruesink's model predicted correctly 24 of 40 runs (60% accuracy) (Table 13). Decreasing larval survivorship caused the model to estimate precisely 33 of 40 runs. This adjustment increased model accuracy to 82.5%. This means that the model can successfully describe spring alfalfa weevil populations with 95% accuracy in 82.5% of the cases. This figure, however, does not include validation of the model's management algorithm.

Management validation examined the model under two different field situations in 1979 and 1980. For the two years of testing, the model made accurate management decisions in 7 of the 8 cases. In 1979, weevil densities were low and the model recommended accurately the no spray option on four dates. In 1980, populations were high and the model selected correctly the spray immediately option on 3 of 4 occasions. Overall, model advisories were accurate when weevil populations were low or high.

The model's management algorithm remains to be tested against intermediate densities. In such a situation it would be difficult for the model to produce a correct recommendation due to the simplicity of the spray algorithm. The model recommends spraying when the sum of the third and fourth instar densities (parasitized plus unparasitized) exceeds $400 \text{ individuals/m}^2$. Because the spray decision is based only on weevil densities, it is limited and in need of improvement.

The economic impact of a pest depends on pest densities, stage of

plant development, damage-yield relationship, crop market value and management costs. A spray decision determined from the factors listed above would be a sound advancement over the model's current spray algorithm. The alfalfa weevil model was modified to incorporate a management decision based on cost/benefit criteria. The model was adjusted to accept additional variables (market values, management costs and predicted yields) as input so that current figures could be used and updated when necessary. Ruesink's model calculates the amount of food consumed by weevils (gm/m^2) and the resulting yield loss. Using the terminology of Stone and Pedigo (1972), a gain threshold was calculated. This is the number of tons that yield must be increased (from control) to compensate for the total cost of management. The equation is:

$$\text{Gain Threshold} = \frac{\text{Management costs (\$/acre)}}{\text{Market Value (\$/ton)}} \quad (1)$$

The model would need to be run to predict yield loss from the sampled field densities. If the loss in yield is greater than the gain threshold, then intervention is necessary to avoid significant crop loss. If yield loss is less, then plant damage is economically tolerable.

Currently Kansas does not have an alfalfa weevil management program (or any guidelines) which considers pest densities, economics and yield loss in deciding whether control is needed. If alfalfa weevil management criteria did exist for Kansas, model use would be advantageous over pest recommendation charts despite model weakness at intermediate pest densities. The model has the capability of projecting population levels early in the season during critical decision making

periods, thus giving growers advanced warnings. Pest recommendation charts either advise immediate control or resampling in 50 dd ($^{\circ}$ C) (3-5 days) which may not give growers enough time to schedule a spraying operation. The model also accounts for weevil control by B. curculionis. During 1979 and 1980 B. curculionis populations were trace and only slightly influenced the weevil population. The parasitoid population could increase and significantly affect weevil densities. Grower charts do not consider control by parasites. The use of model recommendations instead of charts has disadvantages in terms of distributing information to growers. Some type of delivery system must exist to disseminate these model advisories.

The results of fall validation studies conflict for the egg stage but agree for larval populations. The model was rejected (Theil's test) in 1979 but not in 1980 for fall egg predictions. Despite an underestimation of the fall egg populations (discussed earlier), the model predicted a zero population which was rejected in 1979. The cause of poor predictions for Kansas field conditions is related to the diapause section of the model. This reflects a basic lack in understanding the mechanisms of diapause termination. Ruesink (1974) acknowledges a weakness in this section of the model.

The set of diapause termination factors for this species may vary throughout the country and between years at the same location. Weevils located in southern latitudes oviposit more fall eggs which survive through mild winters, thus making spring populations more difficult to control (Armbrust and Gyrisco, 1974; Roberts et al., 1979; Hsieh and Armbrust, 1974; Litsinger, 1972; and Niemczyk and Flessel, 1970). A latitudinal gradient for fall oviposition is not incorporated in the

model. The model terminated diapause (measured by adult return to the field) before Oct. 27 by a chilling mechanism (degree days ($^{\circ}\text{F}$) below 42°F). For 1979 and 1980, this chill requirement was not fulfilled, therefore oviposition in the model could only commence after Oct. 27. This is the reason the model predicted zero egg population during this interval. For 1979, field oviposition began in September, therefore these predictions were rejected. In 1980, this did not occur. The field population differed significantly from zero around Nov. 1 (Figure 10), which agrees with model estimations.

I can only speculate on the reason that fall oviposition began about 20 days earlier in 1979. In 1980, average maximum and minimum temperatures (May-October) were higher than in 1979. In 1980, maximum temperatures were about 100°F for about 6 weeks. A hot, dry environment places more stress on weevils in the field, thus causing them to remain longer in protective habitats along hedgerows and woody areas before returning to the field in fall. Further research is necessary to determine the mechanisms terminating diapause before adults return to the field and oviposition can be accurately predicted.

Alternate Methods of Alfalfa Weevil Strain Determination

Electrophoresis has frequently been used to classify organisms to species, but few researchers have used this technique to identify subspecies, races, or strains (Ayala, 1973; McIntee and Wordin, 1981). This is the first report of using electrophoresis to discriminate eastern and western alfalfa weevil strains. The procedure developed here can classify weevil larvae to strain with 95% correctness. This accuracy is certainly acceptable for field surveys when it is necessary

to determine if the population is of pure or mixed strain, or to ascertain which strain predominates.

This technique has several advantages and disadvantages. The electrophoretic discriminant procedure involves less work and is quicker than the correct strain classification method of cross-mating weevils and examining the progeny for percent egg hatch. Colony rearing to provide controls for each electrophoretic run is minimal, because once the larvae reach third or fourth instar, hemolymph can be collected and stored. Depending on the available electrophoretic equipment, 20-40 weevil samples can be processed per day. Up to 20 samples can be run on a slab gel (2 slabs per run), which results in less experimental variation. This is a further refinement over tube gels (present technique). By contrast, cross-mating requires a large weevil colony to provide a mate of known strain for each undetermined weevil. It takes months to obtain strain results from field collected larvae. The reason is that virgin adults must be used which entails rearing field larvae to adults and isolating them before mating can begin. This requires approximately one month for nondiapausing larvae, or 3-4 months for diapausing larvae.

One disadvantage of the alfalfa weevil strain determination technique is that one must learn electrophoretic methods and techniques to obtain accurate and reproducible results. Another disadvantage is that a Texas Instruments Programmable SR-59TM calculator must be used to run the two calculator discriminate programs (Appendix I-IV). Additionally, electrophoresis has the limitation that the specimen is killed as a result of collecting a hemolymph sample.

The possibility exists that the discriminating protein bands were

produced by artifacts other than strain differences (e.g., antibodies). For this to occur, the following conditions must be met by these artifacts: (1) they had to exist in field weevil populations, since all samples were processed using the same laboratory technique, (2) they had to be selectively present in one strain of alfalfa weevils collected from Ohio, Michigan and Utah during 1980 and 1981, (3) the pattern of artifact presence and absence in Kansas weevils had to exhibit frequencies which mirror results obtained from two independent methods (cross-mating and parasitism rates) at Nebraska (Klostermeyer and Manglitz, 1979) and Oklahoma (Berberet and Gibson, 1976). Since it is very unlikely that all of these conditions exist, the possibility of artifacts has been ruled out.

Further refinement and research on the technique discussed here is possible. These results are based on weevils collected from Ohio, Michigan, and Utah. Variation in electrophoretic patterns of weevils collected from different geographical locations has not been examined. Additional research should involve collecting and analyzing weevils from field populations of pure strains across the country. The discriminant calculator programs may have to be modified to be applicable for a broader region. Biochemically identifying the protein(s) currently being used to discriminate strains may possibly elucidate the physiological and/or behavioral differences between the strains.

The classification procedure developed here was not designed to determine if field weevils are of pure or hybrid strain. Hybrid weevils have been produced in the laboratory, but it is unknown whether this occurs under field conditions (Blickenstaff, 1965, 1969; Armbrust et al., 1970; White et al., 1972). To determine if a particular

individual was hybrid, gels would have to be selectively stained to observe genetic variants of some particular enzyme (Shaw and Prasad, 1970). Enzymes of hybrids, which display bands of intermediate relative mobility compared to bands from homozygous individuals, could be used to detect hybridization.

Geographical Distribution of the Strains in Kansas

Klostermeyer and Manglitz (1979) determined that the eastern strain had migrated to 100° longitude and the western strain had moved east to 97° longitude as of 1977. They found that the populations were mixed between these two boundaries for about 150 miles in central Nebraska. This study confirms the hypothesis that the eastern and western populations have mixed in Kansas. By 1981, the overlap has entirely covered Kansas (except Leavenworth).

This investigation revealed that the eastern strain predominates in Kansas. The history of the alfalfa weevil in Kansas indicates a difference in the economic impact of the strains. The eastern strain has been present in Kansas for at least 14 years (Simpson, 1967). During this period, populations have increased to seriously damaging densities on two occasions in southeast Kansas where the eastern strain has been present. Pesticide applications were required during 1974-1976 and again in 1980-1981. The western strain has been present in southwest Kansas for at least 21 years (Peters, 1960). This section of the state has not experienced weevil outbreaks until 1980 (this was probably due to the migration of easterns to this region). It appears that under Kansas field conditions, western populations have not been able to increase in numbers to the extent of eastern weevils.

The abundance of easterns in Kansas may also be affected by migration rate. Researchers (Davis, 1967; Klostermeyer and Manglitz, 1979) have calculated that easterns migrate about 50 miles/year, whereas westerns move at 10-20 miles/year. The predominance of easterns in Kansas can be due to their ability to migrate faster and become more established than westerns.

Similar strain dynamics have occurred in Oklahoma. Berberet and Gibson (1976) maintain that the western strain has remained at low densities in the Oklahoma panhandle until 1970. They believe that the weevils merged in 1971 because alfalfa weevils were detected in all counties. Using actual and effective parasitism and encapsulation rates, they concluded that the eastern strain occupied and predominated in the northeast and northwest sections of the state by 1974-1975.

Laboratory studies indicate that other factors may have contributed to the numbers of easterns. Armbrust et al. (1970) were able to show that eastern males were more aggressive and mated more frequently than western males in the laboratory. They predicted that the eastern strain would add more genes to the population than the western strain when the populations met. Although the eastern strain has prevailed in Kansas, the results of Armbrust's studies are inconclusive. Blickenstaff (1969) was not able to detect any mating preferences or differences in competitiveness between the strains.

The presence of eastern weevils in western Kansas suggests that this strain may have migrated into eastern Colorado, since it disperses about 50 miles/year. The populations in eastern Colorado have fluctuated from being light to heavy and, at times, have required chemical control. Despite the yearly fluctuations, the populations have

appeared to be slowly building up over the last seven years (Wm. Hantsbarger⁴, personal communication). This trend will probably continue to cause further problems for Colorado alfalfa growers. Western Missouri probably has trace presence of the western strain, but in numbers insufficient to alter population dynamics.

The outcome of the various studies reported herein enables a contribution to be made to alfalfa growers in Kansas. The overall objective of these investigations is to have a precise alfalfa weevil model which can be used at several locations in Kansas. Presently, a model is available which can accurately recommend management practices when spring populations are high or low. The model has been adjusted to handle intermediate densities. To run the model, the predominant strain at a location must be known. The distribution and abundance of each strain throughout Kansas has been determined. Therefore, the essential components to run the model under Kansas field conditions have been provided. Kansas alfalfa growers will judge how well this objective has been accomplished.

⁴1982, Dept. of Zoology and Entomology. CSU, Fort Collins, CO.

CONCLUSIONS

Ruesink's alfalfa weevil model was validated by comparison with 1979 and 1980 Kansas field populations. For spring validation, rejected model runs were either underpredicted for early instars or overpredicted for older instars. Early instar underpredictions were attributed to an inadequate egg sampling procedure. Overpredictions were the result of unrealistic model survivorship rates. After adjusting survivorship, the model functioned adequately, since only 17.5% of all spring runs were rejected due to model error.

The model performed well in predicting management recommendations when field populations were low or high. The model is an advancement over management recommendation charts, because it can forecast populations levels early in the season during critical decision making periods, and it includes control by B. curculionis. A disadvantage of model use is a need for a delivery system. The opportunity did not exist to validate advisories under intermediate weevil densities. To produce accurate recommendations for such populations, the simplified model spray algorithm was adjusted.

For fall validation, the model poorly predicted return of adults to the field. This reflects a lack of adequate knowledge and the necessity for additional research on the mechanisms of diapause termination.

A strain determination technique was successfully developed to differentiate eastern and western alfalfa weevils. The electrophoretic classification procedure can be used to determine if weevil populations are pure or mixed and which strain predominates. The technique can be easily modified to ascertain whether field hybridization occurs.

The electrophoretic technique was used to determine the geographical distribution of strains in Kansas. This study revealed that both strains are present, but that the eastern strain predominates across the state. Eastern abundance is probably the result of a faster migration rate and more successful establishment under Kansas field conditions. Because of eastern predominance, the model can be run under Kansas field conditions using solely the eastern strain as input. It appears that the eastern strain has migrated into eastern Colorado where it will continue to cause problems to alfalfa growers. The western strain is probably in western Missouri, but in densities too low to effect yield loss.

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A P P E N D I X

APPENDIX I

Alfalfa Weevil Discriminant Programs using a Texas Instrument SR-59TM Calculator

Procedure to Run the Programs

Enter Program

Select either the one or three variable discriminant program listing displayed in Appendix IV. Store the required constants in the assigned memory registers. Refer to the SR-59TM owner's manual⁵ for instructions on keying in a program and storing data.

Record Program using a SR-59TM Magnetic Card

- A. One variable program
 - 1. Enter the program and store it in Bank 1 of a magnetic card.
 - 2. Record the data memory in Bank 4.
 - 3. See the owner's manual for detailed instructions.
- B. Three variable program
 - 1. Enter the program and store the first section in Bank 1 and the remaining portion in Bank 2 on another card.
 - 2. Record the data memory in Bank 4.

Read Program from a SR-59TM Magnetic Card

Read the selected program from the appropriate banks. See owner's manual for additional instructions.

Enter Variables

- 1. Enter the distance band 6 migrated, then press "A".
- 2. Enter the distance band 7 migrated, then press "B".
- 3. Enter the distance the tracking dye migrated, then press "C".
- 4. Read display.

⁵ Personal Programming. A complete owner's manual for a TI programmable 58/59.

Interpret Output

1. "0" display designates the EASTERN strain.
2. "1" display represents the WESTERN strain.
3. Reset program to beginning, press "RST".

Appendix II

Sample Runs

5.1 press A
5.45 press B
6.1 press c
0 display EASTERN

5.15 press A
5.45 press B
6.2 press C
1 display WESTERN

Appendix III

Program Listings

One Variable Program

<u>Listing</u>	<u>Comments</u>
2ND LBL A STO 4 R/S	Define label key "A" to represent variable, band 6 store value in register 4;
2ND LBL B STO 5	Define "B" to represent variable, band 7; store value in register 5;
2ND LBL C STO 6	Define "C" to represent the tracking dye variable; store value in register 6;
(RCL5-RCL4)/RCL6= STO 7	Calculate the relative distance between band 6 and band 7, X; store value in register 7;
(RCL7-RCL1)X ² *RCL3= STO 20	$D_E^2(X) = (X - \bar{X}_E)^2 * COV^{-1}$; store in register 20;
(RCL7-RCL2) ² *RCL3= STO 21	$D_W^2(X) = (X - \bar{X}_W)^2 * COV^{-1}$; store in register 21;
RCL20 X↔T RCL 21	Store register 20 value in the T-register; Call register 21 to the X register;
X> T D GTO E	If X is greater than the value in T then go to D, else go to E;
2ND LBL D RCL 10 R/S	Define label key to represent EASTERN strain; recall "0" to the display; end program.
2ND LBL E RCL 11 R/S	Define label key to represent WESTERN strain; recall "1" to the display; end program.

Three Variable Program

<u>Listing</u>	<u>Comments</u>
2ND LBL A STO 20 R/S	Define "A" to represent variable band 6; store value in register 20;
2ND LBL B STO 21 R/S	Define "B" to represent variable band 7; store value in register 21;
2ND LBL C STO 22	Define "C" to represent the tracking dye variable; store value in register 22;
RCL 25 / RCL 22 = STO 25 RCL 21 / RCL 22 = STO 26 RCL 20 / RCL 21 = STO 27	Rf band 6, X_1 --store value in 25 Rf band 7, X_2 --store value in 26 band 6 / band 7 = X_3 --store value in 27
RCL 25 - RCL 1 = STO 31 RCL 26 - RCL 3 = STO 32 RCL 27 - RCL 5 = STO 33	$(X_1 - \bar{X}_1)$ --store value in 31 $(X_2 - \bar{X}_2)$ --store value in 32 $(X_3 - \bar{X}_3)$ --store value in 33
RCL 25 - RCL 2 = STO 34 RCL 26 - RCL 4 = STO 35 RCL 27 - RCL 6 = STO 36	$(X_1 - \bar{X}_1^W)$ --store value in 34 $(X_2 - \bar{X}_2^W)$ --store value in 35 $(X_3 - \bar{X}_3^W)$ --store value in 36
(1) EASTERN (RCL 31 * RCL 11) + (RCL 32 * RCL 14) + (RCL 33 * RCL 17) = STO 41 (RCL 31 * RCL 12) + (RCL 32 * RCL 15) + (RCL 33 * RCL 18) = STO 42 (RCL 31 * RCL 13) + (RCL 32 * RCL 16) + (RCL 33 * RCL 19) = STO 43	
(2) EASTERN (RCL 41 * RCL 31) + (RCL 42 * RCL 32) + (RCL 43 * RCL 33) = STO 50	

Comments: $D_E^2(X) = [X_j - \bar{X}_{jE}]^T \text{COV}^{-1} [X_j - \bar{X}_{jE}] \quad j=1,2,3$

$$(1) [(X_1 - \bar{X}_{1E}) \quad (X_2 - \bar{X}_{2E}) \quad (X_3 - \bar{X}_{3E})] \begin{bmatrix} X_1^2 & X_2 X_1 & X_3 X_1 \\ X_1 X_2 & X_2^2 & X_3 X_2 \\ X_1 X_3 & X_2 X_3 & X_3^2 \end{bmatrix}^{-1}$$

$$(2) \text{ product of (1) } * \begin{bmatrix} X_1 - \bar{X}_{1E} \\ X_2 - \bar{X}_{2E} \\ X_3 - \bar{X}_{3E} \end{bmatrix} = D_E^2(X) = \text{STO } 50$$

(1) WESTERN

(RCL 34 * RCL 11) + (RCL 35 * RCL 14) + (RCL 36 * RCL 17) = STO 44

(RCL 34 * RCL 12) + (RCL 35 * RCL 15) + (RCL 36 * RCL 18) = STO 45

(RCL 34 * RCL 13) + (RCL 35 * RCL 16) + (RCL 36 * RCL 19) = STO 46

(2) WESTERN

(RCL 44 * RCL 34) + (RCL 45 * RCL 35) + (RCL 46 * RCL 36) STO 51

Comments: $D_W^2(X) = [X_j - \bar{X}_{jW}]^T \text{COV}^{-1} [X_j - \bar{X}_{jW}] \quad j=1,2,3$

$D_W^2(X) = \text{STO } 51$

<u>Listing</u>	<u>Comments</u>
RCL 50 X $\leftrightarrow$ T RCL 51	Store register 50 value in the T-register; call register 51 to the X register;
2ND X $\gg$ T 2ND A' GTO 2ND B'	If X is greater than the value in T then go to A', else go to B';
2ND LBL 2ND A' RCL 7 R/S	Define label key to represent EASTERN strain; recall "0" to the display; end program.
2ND LBL 2ND B' RCL 8 R/S	Define label key to represent WESTERN strain; recall "1" to the display; end program.

Appendix IV

Register Allocation

One Variable Program

<u>Permanent Memory</u>		
<u>Constants</u>	<u>Register</u>	<u>Comments</u>
.060	1	X_E for the relative distance between band 6 ^E and band 7
.041	2	X_W for the relative distance between band 6 ^W and band 7
4412.089	3	COV^{-1}
0	10	depicts EASTERN strain
1	11	depicts WESTERN strain

<u>Temporary Memory</u>	
<u>Register</u>	<u>Comments</u>
4	band 6 variable, label A
5	band 7 variable, label B
6	tracking dye variable, label C
7	relative distance between band 6 and band 7, X
20	$D_E^2(X)$
21	$D_W^2(X)$

Used Memory Registers: 8-9, 12-19, 22-59.

Three Variable Program

Permanent Memory

<u>Constants</u>	<u>Register</u>	<u>Comments</u>
.840	1	X_{1E} for band 6
.821	2	X_{1W} for band 6
.906	3	X_{2E} for band 7
.861	4	X_{2W} for band 7
.928	5	X_{3E} band 6 / band 7 ratio
.954	6	X_{3W} band 6 / band 7 ratio
0	7	depicts EASTERN strain
1	8	depicts WESTERN strain
958764	11	
-916461	12	$\text{COV} \begin{bmatrix} X_1^2 & X_1X_2 & X_1X_3 \\ (11) & (12) & (13) \\ X_2X_1 & X_2^2 & X_2X_3 \\ (14) & (15) & (16) \\ X_3X_1 & X_3X_2 & X_3^2 \\ (17) & (18) & (19) \end{bmatrix}^{-1}$ where X_1^2 is element X_{11}, X_2X_1 is element X_{21}, etc.
-883583	13	
-916461	14	
877712	15	
846613	16	
-883583	17	
846613	18	
822521	19	

Temporary Memory

Register	Comments
20	band 6 variable, label A
21	band 7 variable, label B
22	tracking dye variable, label C
25	Rf band 6, X_1
26	Rf band 7, X_2
27	band 6 / band 7 ratio, X_3
31	$(X_1 - \bar{X}_{1E})$
32	$(X_2 - \bar{X}_{2E})$
33	$(X_3 - \bar{X}_{3E})$
34	$(X_1 - \bar{X}_{1W})$
35	$(X_2 - \bar{X}_{2W})$
36	$(X_3 - \bar{X}_{3W})$
41	$[X_j - \bar{X}_{jE}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j1}]$
42	$[X_j - \bar{X}_{jE}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j2}]$
43	$[X_j - \bar{X}_{jE}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j3}]$
44	$[X_j - \bar{X}_{jW}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j1}]$
45	$[X_j - \bar{X}_{jW}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j2}]$
46	$[X_j - \bar{X}_{jW}]^T \text{COV}^{-1} [\sum_{j=1}^3 X_{j3}]$
50	$D_E^2(X)$
51	$D_W^2(X)$

Used Memory Registers: 9-10, 23-24, 28-30, 37-40, 47-49, 52-59.

ALFALFA WEEVIL MODEL VALIDATION FOR THE
EASTERN AND WESTERN STRAINS
IN SOUTHCENTRAL KANSAS

by

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ALFALFA WEEVIL MODEL VALIDATION FOR THE
EASTERN AND WESTERN STRAINS
IN SOUTHCENTRAL KANSAS

An alfalfa weevil model was validated under Kansas field conditions in 1979 and 1980. Model accuracy was determined using two criteria, statistical procedures and frequency of correct management recommendations. For both years, spring model runs were rejected for either underpredicting early instars or overestimating older instar densities. Reasons include problems with egg sampling, and model overestimates of survival. Adjusting survivorship, the model was 82.5% accurate. In the fall, the model did not accurately represent diapause termination.

Model recommendations were compared to advisories of three alfalfa weevil management programs. The model predicted correctly 7 out of 8 recommendations for both years.

The model can be used for either strain, therefore the distribution of these strains in Kansas was determined. The strains have mixed throughout most of Kansas with easterns predominating. To facilitate this study, an electrophoretic strain determination technique was developed which separated larval hemolymph proteins in a 7% polyacrylamide gel.