SPRING EMERGENCE AND SEASONAL INFESTATION OF THE ALFALFA SEED CHALCID, *BRUCHOPHAGUS RODDI* GUSSAKOVSKII, IN OKLAHOMA.

By

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GUSSAKOVSKII, IN OKLAHOMA

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PREFACE

Several studies were conducted to gather information of the life cycle of the alfalfa seed chalcid (ASC) as it occurs in Oklahoma. The first study examined the spring emergence of the ASC using seed obtained from various locations in Oklahoma. The second study examined the adult field populations using field samples. The third study examined the seasonal infestation in alfalfa seeds at weekly intervals.

Early season alfalfa seed crops avoided peak populations densities of ASC and had low percentages of ASC damaged seed and low percentages of ASC that entered diapause. Late season seed crops had higher percentages of ASC damaged seed and percentages of ASC that entered diapause.

I would like to thank all of the people who assisted me with this project. Most of my gratitude is to my major advisor, Dr. Joseph O. Moffett, for his unique style of assistance, intelligent suggestions, and support. I would also like to thank the members of my committee, Dr. Richard Berberet and Dr. John Caddel, for their suggestions and straightening me out when I needed it. I would especially like to thank Dr. Ronald McNew for his assistance with the statistical analysis of the studies.

I would like to thank Johnson Seed Company in Enid, Hobart Seed Company in Hobart, Hal McCormick in Woodward, and Marcus Terry in Gate for supplying the seed used in the spring emergence study. I would like to thank Mr. John McCrosky, the O.S.U. Agronomy Department, Mr. Dale Stidham, and the South Central Research Station for the use of their alfalfa fields.

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CHAPTER I

INTRODUCTION

Statement of Problem

The alfalfa seed chalcid (ASC), *Bruchophagus roddi* Gussakovskii., is a cosmopolitan insect of economic importance wherever alfalfa (*Medicago sativa* L.) seed is produced. Adult ASC are 2-3 mm long and jet black in color. Immature ASC develop inside seeds, protected by the seed coat, and adults are active in fields at the same time as beneficial pollinators. As a result, there is no safe and effective chemical control for the ASC. Current ASC control methods in the Western states are cultural methods. There are currently no ASC control methods widely used in Oklahoma by alfalfa seed producers. Many producers are unaware of extent of seed loss due to ASC because damaged seed is lighter in weight and most of it is removed with other debris during threshing (Bacon et al. 1959). Estimates of ASC infested seed have ranged up to 1-91% in the United States (Urbahns 1920). In Oklahoma, Ahring et al. (1984) reported seed losses due to ASC ranging from 12-71%.

Alfalfa Seed Production in Oklahoma

Oklahoma is fifth among alfalfa seed producing states in terms of annual production. From 1929 to 1949 most alfalfa seed was produced in the plains states (Kansas, Oklahoma, Nebraska, and Minnesota) and Idaho. Oklahoma was the leading producer of seed in 1938, 1944, and 1949. Starting in 1950 and continuing to the present, California, Washington, and Idaho have dominated seed production (U.S.D.A. Agricultural Statistics 1929-1981). Oklahoma remains a leading alfalfa seed producer among the plains states. In 1978 to 1980, Oklahoma had an average production of 15 million kgs, worth about $5.4 million per year (Table I). Thus, alfalfa seed is an important crop in Oklahoma, and further understanding of the life system and control of the economically important ASC is needed.

TABLE I

OKLAHOMA ALFALFA SEED PRODUCTION
AND VALUE - 1978 TO 1980 *

Measurement	Units	1978	1979	1980	Average	Nat. Rank
Area Harvested	Hectares	27,000	24,000	27,000	26,000	4
Yield / Hct.	Kgs.	145	125	125	132	7
Production	1,000 Kgs.	17,582	12,400	15,157	15,046	5
Value	1,000 $$	6,380	4,669	5,225	5,425	7

* U.S.D.A. Agricultural Statistics 1981

Scope and Objectives

Before ASC control measures can be developed, the life system must be investigated. To aid the understanding of the ASC life system in Oklahoma, the following studies were established, each focusing on several factors.

The first study was designed to investigate spring emergence of ASC and its parasites from seed screenings obtained from four locations in Oklahoma during fall in 1982 and 1983. Objectives of the emergence study in 1982, were to determine spring emergence patterns of adult ASC and its parasites, and extent of parasitism present in overwintered seed. In 1983, the objectives included those of 1982 and were expanded to include species determination of ASC parasites present, and sex ratios and timing of male and female emergence of ASC and parasites. Also, the second year emergence of ASC and its parasites from the seed collected in 1982 was observed.

The second study involved field collections of ASC and its parasites with sweep net samples during the summer in 1983 and 1984. Objectives of the population study in 1983 were to determine seasonal occurrence of peak populations of adult ASC and parasites and estimate the number of ASC generations in Oklahoma. In 1984, the objectives included those of 1983 and were expanded to include species determination of ASC parasites present and sex ratios as well as seasonal population patterns for ASC and parasites.

The third study was designed to investigate ASC infestation levels in alfalfa seed from weekly samples throughout summer in 1983 and 1984. Objectives of this study were to determine the seasonal pattern of ASC infestations, and the seasonal occurrence of ASC in diapause.

Combining the information gained from these three studies, a time period was sought at which an alfalfa seed crop could be produced to avoid peak populations of adult ASC, and have the least amount of ASC damage. Also, it is desirable to allow the fewest ASC possible to reach the diapausing prepupal stage and survive the overwintering period. By using this information, alfalfa seed producers in Oklahoma may be able to modify their present seed growing practices to reduce ASC losses.

CHAPTER II

GENERAL LITERATURE REVIEW

Classification

The alfalfa seed chalcid (ASC) is in the Order Hymenoptera, Superfamily Chalcidoidea, and Family Eurytomidae. A common misnomer is that this insect is a fly, with many alfalfa producers knowing the insect as the chalcis-fly. This misnomer occurs because early Farmer's Bulletins used this term (Sorenson 1930, 1934; Urbahns 1914, 1916, 1920).

Genus and species classifications of the ASC have changed several times since it was first described. Strong (1962a) gave the best synopsis of the name changes that have occurred. A summary of his findings is listed in the following paragraph.

The ASC was first described in Scandinavia as *Eurytoma gibba* by Boheman (1836). Howard (1880), working in North America, described the same species as *Eurytoma funebris*. Ashmead (1894) described the genus *Bruchophagus* and included *Eurytoma funebris* Howard under this classification. Girault (1916) renamed the insect as *Bruchophagus funebris* Howard. All subsequent work has used *Bruchophagus* as the genus for the ASC. Rodd et al. (1933) reported a chalcid species emerging from alfalfa and named it *Bruchophagus roddi* Gussakovskii, but this name was regarded as a synonym of *B. gibbus* (Boheman). Later, researchers reported differences in members of this species infesting different plants. Kolobova (1950) was first to suggest that *B. gibbus* might actually be a complex of species. Nikolskaya (1952) found morphological differences in the chalcids emerging from red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.). He split the classification into *B. roddi* for the chalcids from alfalfa and *B. gibbus* for the red clover chalcids. Fedoseeva (1954, 1956, 1958), Hansen (1955), and Neunzig and Gyrisco (1959) confirmed the validity of the division through morphological and host relationship studies. Fedoseeva (1956) also showed evidence of a third species, naming it *B. kolobovae* Fedoseeva. Strong (1962a) studied the female genitalia of the complex. He found evidence for three different species; *B. gibbus*, *B. kolobovae*, and *B. roddi*. He also did host specificity studies and found *B. gibbus* infested only red clover, *B. kolobovae* infested only trefoil (*Lotus* sp.), and *B. roddi* infested both alfalfa and burr clover (*M. hespida* Gaertner). The common names he assigned to the three species are the clover seed chalcid, trefoil seed chalcid, and alfalfa seed chalcid.

ASC Life Cycle and Description of Life Stages

The ASC is a small (2-3 mm) phytophagus wasp which infests seeds of certain *Medicago* species. Adults are mainly jet-black, with yellowish brown legs. Wings are clear with only one compound branched vein. Female and male adults are distinguished by differences in antennae. Antennae of males are moniliform and covered with whorls of long, light-colored hairs, on each of the 11 segments. Antennae of females also have hairs, but they are very fine and short and thus hard to observe. The 10 antennal segments of the female are much less pronounced than in the male (Sorenson 1930). Adult ASC are distinguishable from other wasps of the same general size by the jet-black color and a thorax covered with small thimble shaped projections (Butler and Hansen 1958).

Oviposition occurs while pods are still green and seeds are immature. Seeds are most suited for oviposition 8 to 10 days after they begin to form (Strong 1962b). The ovipositor is inserted through the pod and seed coat, and eggs are usually placed singly into the developing seed. Location of eggs in a green seeds can be determined by observing a brown scar of necrotic tissue caused by insertion of the ovipositor through the seed coat. The scar remains visable for about a week after the egg is inserted (Strong 1962b).

Eggs average 0.2 mm in length and are smooth sided and elliptical. One end is sharply pointed, while the other end terminates in a long slender tube (Sorenson 1930). The egg is milky-white when laid, but it gradually becomes translucent (Strong 1962b). More than one egg may be inserted into each seed, but only one ASC completes development (Tingley and Nielson 1974, 1975). Gravid females contain from 24 to 66 eggs with an average of 42 (Sorenson 1930). Developmental time for eggs ranges from 1-6 days (Strong 1962b, Sorenson 1930).

Each larva of the ASC feeds on the internal contents of a single seed, eventually hollowing it out. Only the seed coat is left intact. Sorenson (1930) found that only 1% of larvae (originating from eggs laid in older seeds) could not eat the endosperm faster than it developed. These larvae were eventually crushed within the seed. Developing larvae are completely white, except for dark mandibles. Sorenson (1934) observed no definite larval instars of the ASC and thought larvae molted irregularly until maturity was reached. Strong

(1962b) measured larval body and mandible lengths to distinguish larval instars. Batiste (1967a) found five larval instars in the trefoil seed chalcid and speculated that the ASC may also have five instars. Strong (1962b) reported the average length of time required for ASC to reach the last instar was 12 days at 24-27°C. He also found that early instars do not defecate, with the result being gray coloration in prepupae due to the fecal material contained in the body. Prepupae either defecate and molt to pupal stage, or enter diapause prior to pupation. If a prepupa enters diapause, defecation does not occur until diapause is terminated. Diapause can be entered at any time throughout the summer (Bacon et al. 1964), but Antonova and Bazvleva (1974) found most diapausing ASC came from the second seed crop of the year. Diapause is terminated by a period of cold temperature (Strong 1962b), or combination of cold and lengthening photoperiod (Nielson 1976). A period of intense cold may actually increase ASC vitality (Kralovic 1971).

Sorenson (1930) described various color changes that distinguish one substage of pupal development from another. Strong (1962b) distinguished one prepupal stage and four pupal substages as follows: prepupa - a short, white, thickened larva exhibiting no movement; pupa one - eyes and body white in color; pupa two - eyes pink to red in color with a yellowish body; pupa three - eyes brown in color with a black abdomen and a yellowish thorax; and pupa four - eyes brown in color with an entirely black body. A mature pupa has its legs, wings, and antennae folded closely to the body, which is encased in a thin transparent pupal skin. At 25°C, the average length of time for development from egg to adult emergence varied from 21 days (Butler et al.

1968), to 27 days (Strong 1962b).

Adult Behavior

The adult ASC chews a circular hole through the hardened seed coat (and pod, if present) and emerges through it. Males emerge first, and mating occurs soon after the females emerge (Sorenson 1930). Mating is not needed for oviposition, and the progeny of virgin females are always male (Urbahns 1920). Early sources of seed for ASC infestation are the volunteer plants which mav be growing in ditches and similar noncultivated areas. Usually cultivated alfalfa is managed so that a seed crop is not produced until several months later in the season. ASC may complete two generations on the noncultivated hosts before seed is available in cultivated alfalfa (Bacon et al. 1964). Adult populations increase from spring to late summer and high infestation levels may occur by late season (Antonova and Bazyleva 1974). As many as three or four overlapping generations may occur in warmer regions of the U.S. (Bacon et al. 1959). Two generations are reported from most of Russia (Antonova and Bazyleva 1974).

Urbahns (1914) observed that seed chalcids are strong fliers. Since they are small, ASC may be carried over long distances by wind. Carrillo and Dickason (1963) placed sticky traps at various angles to prevailing winds and found more chalcids in traps placed at right angles to the wind. They did not make any conclusions with this data due to low numbers of adults collected in the traps. Recapture studies using radioactively tagged ASC showed that in winds up to 8 km per hour, dispersal was in any direction, while in stronger winds it was predominantly downwind. This same study also showed the ASC leaving fields with few or no seeds available for oviposition by flying upward until a strong wind is encountered and then moving long distances downwind (Strong et al. 1963). Carrillo and Dickason (1963) found more chalcid activity in sunny areas. Johansen and Retan (1974) observed chalcids resting in any area that provided shade.

Hale and Fronk (1967) studied ASC reactions to light and found a great attractance to ultraviolet light frequencies of high intensity. Many of the very small parasitic Hymenoptera are attracted to black-light traps (Burbutis and Stewart 1979, Hollingsworth et al. 1970, and Stephen 1979), suggesting the use of black light traps as a possible sampling device of ASC populations. Strong (1962b) found that ASC females oviposit in darkness, suggesting activity at night when the black light traps would be in operation. Whether or not the ASC would be caught in the light traps depends on which attractance is stronger; ultraviolet light or alfalfa plants. Olfactory response of the ASC has been determined to be greatly involved in their attraction to seed pods of Medicago spp. and also in stimulation for oviposition (Brewer et al. 1982b, Kamm and Fronk 1964, Tingley and Nielson 1974). Buttery and Kamm (1980) and Paxton and Burkhardt (1970) reported on chemicals occurring in alfalfa that are attractive to ASC and may elicit an egg laying response. They reported positive attractance and ovipositional activity from 50% of female ASC when exposed to any of the following; citric acid, linoleic acid, oleic acid, or niacin.

Erdelyi and Manninger (1978) found the presence of a female pheromone in the ASC.

Damage by the ASC

The ASC is of economic importance wherever alfalfa is grown for seed. Many producers are unaware of the extent of losses due to the pest because the ASC infested seed is lighter than healthy seed and is removed with debris during the threshing process (Bacon et al. 1959). Loss estimates of 8-13% were reported by Sorenson (1934), 12-71% were reported by Ahring et al. (1984), and losses up to 91% were reported by Urbahns (1920).

Sorenson (1930) found ASC infested seeds are usually discolored, misshapen, and smaller than healthy seeds. Discoloration results in a mottled greenish-brown, brown, or dark brown appearance compared to a uniform shiny tan healthy seed. Misshaped seeds are shrunken and angular instead of the usual plump shape. Much damage may be caused by the breaking of the sterile environment of the seed by ovipositor insertion. This creates an opening for the attack of micro-organisms as well as producing mechanical injury. Evidence of chalcid damage includes presence of emergence holes in seeds or presence of larvae or pupae within seeds.

Frequently, more than one seed in a pod is attacked by the ASC. Erdelyi et al. (1979) found the maximum damage was inflicted to pods with four or fewer seeds.

Sampling for ASC Damage

Variation is found in methods in which seed samples are obtained and analyzed for infestation by different workers. Seed damaged by other insects, freezing, and disease may be confused with ASC damaged seed (Booth 1969, App and Manglitz 1972). Accurate determination of infestation is accomplished by microscopic examination of seeds (Strong 1960). He also suggested a sampling technique consisting of gluing seeds onto paper in thin columns to speed up microscopic determination and to decrease costs.

Several workers have developed other methods for identifying ASC damage. Watts et al. (1967) developed a uric acid (the main constituent of insect excrement) analysis wherein infested seeds undergo a noticeable color change when placed in an ammonia atmosphere. Booth (1969) developed a technique to calculate the percent of infested seeds based on the content of uric acid in a solution of crushed seeds. His technique involved incubating the crushed seed solution with the enzyme uricase, observing the optical density of the supernatant, and using this number in a regression equation to calculate the percent infestation.

Parasitism of the ASC

Parasites of the ASC include nine species of Hymenoptera (Table II). Butler and Hansen (1958) and Butler et al. (1968) published keys for the ASC parasites. All of parasites have similar life cycles. Females oviposit in seed containing ASC larvae or, on rare occasion, pupae. Parasitic larvae consume the entire immature ASC and either pupate or overwinter as diapausing pupae. Emergence is by chewing a hole in the seed coat in the same manner as the ASC.

Reports of total ASC parasitism (all species combined) varied among studies, as follows: 7-47% (Sorenson 1930), 6-21% (Sorenson 1934), 3-63% (Butler and Hansen 1958), and under 2% (Brewer and Horber

1982). Predominate parasitic species in an area may change from year to year (Brewer and Horber 1982). Sorenson (1930) and Bacon et al. (1959) reported that parasites were of minor importance in regulating ASC populations, while Wildermuth (1931) reported that parasites often reduced the number of ASC which resulted in less damage to the seeds. Saunders and Hsiao (1970) created a successful means of rearing the parasite *Amblymerus bruchophagi* (Gahan) under laboratory conditions.

TABLE II

BIBLIOGRAPHY OF CHALCIDOIDEA PARASITES OF THE ASC

Family	Scientific Name (Plus Synonyms)	Citing Literature
Pteromalidae	*Mesopolobus (=Amblymerus, =Eutelus) bruchophagi* (Gahan)	a, b, c, d, e, f, g
	Trimeromicrus maculatus Gahan	a, b, f, g
	Habrocytus medicaginis Gahan	a, b, c, d, e, f, g
Torymidae	*Liodontomerus insuetus* Gahan	a, b, g
	L. perplexus Gahan	a, b, c, d, e, f, g
Eulophidae	*Tetrastichus bruchophagi* Gahan	a, b, d, e, f, g
	T. venustus Gahan	a, b, g
Eupelmidae	*Eupelmella (=Macroneura) vesicularis* (Retzius)	a, b, e, f, g
	Eupelmus allynii (French)	a, b, g

a, Butler and Hansen (1958); b, Urbahns (1920); c, Sorenson (1930); d, Sorenson (1934); e, Neunzig and Gyrisco (1959); f, Peck (1963); g, Krombien et al (1979)

ASC Control Methods

Traditional ASC control has consisted of cultural methods. Cultural control methods fall into two major types: sanitary and crop timing. Sanitary methods include: cultivation to bury infested seed, eliminating harvest residue (mainly by burning), keeping field margins free of volunteer alfalfa, and destroying screenings after seeds have been cleaned. Methods of timing seed production include: using co-operation between producers to adopt a uniform plan for timing of seed production and harvest over large areas of land, management of seed crop so that seeds are set early and over a minimum period of time, and producing the crop early in summer to avoid the major populations of the ASC in the field (Bacon et al. 1964, Harpaz 1978, Homan and Water 1974, Johansen and Retan 1974, Sorenson 1930, Wildermuth 1931). Combinations of several of the above listed cultural methods are commonly used. Cultural control methods do not provide 100% control of the ASC, but have reduced losses from 70% to 4% (Harpaz 1978).

Many insecticides are effective for control of ASC, but they are also destructive to the alfalfa pollinators (Antonova and Bazyleva 1974, Bacon et al. 1964, Mateias et al. 1979, Naidenov 1977, Sapanaru and Sandru 1973, Shelikhov 1978, Stokovskaya et al. 1977). Benedek et al. (1972) studied the diel activity of ASC adults and found them in the upper foliage at night, where they could be accessible to insecticides when the pollinators are not present.

Procedures of screening for ASC resistant plants were formulated (Nielson 1967, Nielson and Schonhorst 1965). Howe and Manglitz (1961) reported differences in the amount of ASC infestation on different alfalfa cultivars. Strong (1962c) tested 40 alfalfa cultivars for ASC resistance and found the lowest infestation levels on non-dormant, non-variegated types such as 'Lahontan' and 'Hairy Peruvian'. The most susceptible cultivars were the dormant, variegated cultivars originating from northwest Europe. He found no so called "light" (less than 10%) seed infestations among selections, but thought his findings warranted further investigation. Rowley and Haws (1964) found resistant strains originating from Afghanistan while the most susceptible strains were from Europe and 'more northern countries'. Nielson and Schonhorst (1967) tested thousands of alfalfas from around the world and found significant differences in ASC susceptibility. The most resistant cultivars were Lahontan, Hairy Peruvian, 'Zia', 'Ranger', and 'A-224'.

Tingley and Nielson (1975) determined that resistance in alfalfa to the ASC is through non-preference and that antibiosis is not a factor. Recent work has concentrated on natural features of various alfalfa clones which inhibit ASC oviposition. Small and Brooks (1982) found that tightness of the pod's coil could protect seeds from oviposition. Brewer et al. (1983a) found a negative correlation of ASC infestation with the density and length of erect glandular hairs of the pods.

CHAPTER III

SPRING EMERGENCE AND INTERACTION OF THE ASC AND ITS PARASITES IN OKLAHOMA

Introduction

The alfalfa seed chalcid (ASC), *Bruchophagus roddi* Gussakovskii, overwinters as diapausing prepupae in seeds of alfalfa, *Medicago sativa* L. (Urbahns 1920). Termination of diapause in the ASC was investigated by Nielson (1976) who reported that termination was caused by factors of chilling as well as lengthening photoperiod, with some ASC staying in diapause for up to two years. Sorenson (1930) reported that male ASC emerged faster and were more numerous than female ASC. Spring emergence of ASC parasites in relation to ASC emergence has not been investigated for every parasite species, but Saunders and Hsiao (1970) reported that peak emergence of *Amblymerus bruchophagi* (Gahan) occurred before the ASC peak emergence while peak emergence of *Liodontomerus perplexus* Gahan occurred after the ASC.

The objectives of the 1982 study were to determine the spring emergence patterns of ASC and its parasites from alfalfa screenings obtained the previous fall, and estimate the extent of parasitism found in overwintered seed. In 1983, the objectives of this study included those of 1982, and were expanded to include species determination of ASC parasites present, as well as sex ratios and timing of male and female emergence of ASC and parasites. Also, the second year emergence of ASC and its parasites from the seed collected in 1982 was observed.

Materials and Methods

Alfalfa screenings from the fall harvest were used as a concentrated source of infested seeds because screenings frequently contain large numbers of diapausing larvae of both ASC and ASC parasites (Urbahns 1914). Also, screenings may contain 40 to 50 infested seeds per 1000, while as few as one to two seeds per 1000 in cleaned seed may be infested (Antonova and Bazyleva 1974). Screenings were obtained from four areas in Oklahoma after fall harvest in 1982 and 1983. The areas were: Hobart (Southwest), Enid (Northcentral), Woodward (Northwest), and Gate (far Northwest). Screenings were cleaned in the laboratory with a Dakota seed blower and by hand to remove chaff, dirt, and foreign seeds.

A one liter sample of cleaned seed was used from each source in each year. Each sample was subdivided into 10 equal parts of 100 ml (ca. 50,000 seeds). Each subsample was spread evenly in a one liter carton to a depth of not more than 1.5 cm so that all adults emerging could free themselves. Cartons were painted black (to reduce light passage through the cardboard), and an easily removable glass vial (four dram size) was inserted into an opening cut in the side of each carton (Figure 1 and Figure 2). Brewer (1982) reported that emerging adult ASC are positively phototropic, and the vial serves as both an attractive light source and a collecting trap.

Cartons were placed in an outdoor cage located on the Agricultural Research Station in Stillwater, Oklahoma. The cage was constructed of four 10x10 cm posts, a tilted plywood roof (to shed rain and snow),

Figure 1. Outside View of a Rearing Carton

Figure 2. Inside View of a Rearing Carton

and chicken wire sides. Bacon et al. (1964) found significantly higher emergence of ASC from seeds placed in rearing cartons than directly on soil and attributed soil moisture as the cause. To keep rearing cartons as dry as possible they were elevated ca. 10 cm from the soil surface and covered with plywood.

Cartons containing alfalfa seed from fall harvest in 1982 were placed in the cage in early November, 1982. Adult ASC and parasite emergence was observed from these seeds during the spring in 1983, and again in 1984. Additional cartons containing alfalfa seed from fall harvest of 1983 were placed in the cage in early November of that year. In 1983 the cartons were placed directly on top of those from the previous year (Figure 3). Arrangement of cartons consisted of four rows of 10 cartons. A single row of cartons was from a single source. Placement of the different sources was from east to west with Gate to the far east followed by Enid, Woodward, and Hobart on the far west.

Starting on April 1 each year, cartons were checked daily until emergence started (Figure 4). Samples were collected weekly during 1983, and three times a week (Monday, Wednesday, and Friday) during 1984. Samples of adults were obtained by removing (and quickly capping) any occupied vials and replacing them with empty ones. Occupied vials were brought back to the laboratory and placed in a freezer overnight to kill the insects. Identifications were made using a stereomicroscope. Samples were sorted as to ASC or ASC parasite in 1983. Data recorded were a composite total of all vials within a location. Samples were sorted as to sex of ASC and sex of each species of ASC parasite in 1984. Data were recorded for each individual vial. Sampling continued until emergence ceased. There was no emergence

Figure 3. Rearing Cartons Inside Cage

Figure 4. Checking for Adults

of adults during fall and winter. Parasitic species identifications were made by comparison with series identified by E. E. Grissell (Systemic Entomology Laboratory, USDA) and located in the K. C. Emerson Entomological Museum at Oklahoma State University

Numbers of adults emerging weekly were graphed on a log scale in order to illustrate periods of greatest emergence from all four sources. Percentage of emergence for each week was calculated by dividing the numbers of adults that emerged from a source during a given week by the total number for that season.

In 1984, counts of ASC and parasites emerging from each carton were recorded seperately. Thus, the different seed sources served as treatments and cartons were replications. Means for location were calculated weekly by totaling all observations made during each week (three dates x 10 vials = 30) and dividing by 10. Standard error for each mean was calculated. A 95% confidence interval was calculated for each mean by ± 2 x standard error.

Results

In 1983, the first adult ASC emerged on May 2 and emergence continued until the week of August 5 from seeds obtained in Woodward and Gate. Emergence from Enid and Hobart occurred from May 2 to the week of July 12, and the week of May 18 to the week of July 20, respectively. Peak emergence occurred between June 2 and July 4 (Figure 5). A total of 17,666 ASC emerged from seeds collected at Woodward, of this 12,262 (69.3%) emerged between June 10 and 26 (Table III). From seeds collected at Gate a total of 4,495 ASC emerged with 2,768 (61.5%) emerging from June 10 to 26. From seeds obtained at Enid a total of

500 ASC emerged with 362 (72.4%) emerging from June 10 to 26. Although relatively few ASC emerged from seed obtained at Hobart, the seasonal pattern was similar to other locations.

In 1983, ASC parasites emerged from May 2 through August 5 from seed obtained at Woodward, Gate, and Enid and from June 26 through August 5 at Hobart. Peak emergence of ASC parasites was from June 10 to July 12 (Figure 6). From seeds collected at Woodward a total of 714 parasites emerged, with 493 (71.6%) emerging between June 18 and July 4 (Table IV). From seeds collected at Gate a total of 973 parasites emerged, with 728 (74.8%) emerging between June 18 and July 4. From seeds collected at Enid a total of 2150 parasites emerged, with 1627 (75.6%) emerging between June 18 and July 4. From seeds collected at Hobart a total of 43 parasites emerged, with 33 (76.7%) emerging between June 26 and July 12. Peak emergence of ASC parasites occurred one week later than the peak emergence of ASC.

Total number of adults (ASC and parasites combined) that emerged from seeds collected at each location in 1982 were: 18,380 from Woodward, 5,468 from Gate, 2,650 from Enid, and 82 from Hobart. Percentages of the above listed total numbers that were composed of ASC parasites were: 81.1% from Enid, 52.4% from Hobart, 17.8% from Gate, and 3.8% from Woodward.

In 1984, the first adult ASC emerged on April 16 from all locations and emergence continued until the week of July 13 for Woodward, July 20 for Hobart and Enid, and August 3 for Gate. The general pattern of ASC emergence apparently shows two peaks for both males and females: the major peak of emergence occurred in mid-June, which was prededed by a small peak in mid-May (Figure 7 and 8). However, the

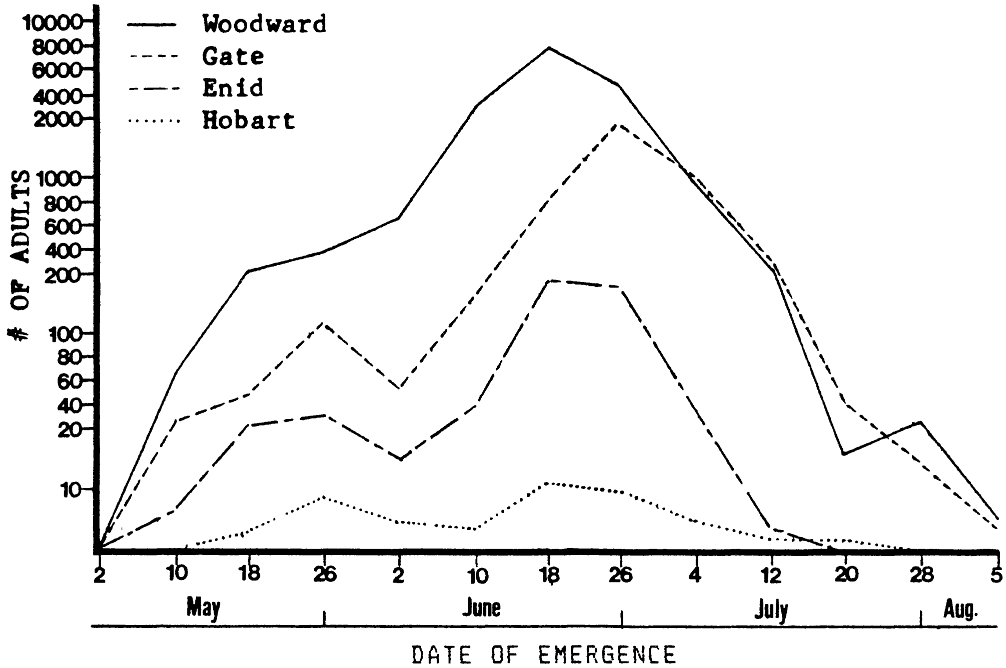

Figure 5. Emergence of ASC from Four Seed Sources in 1983

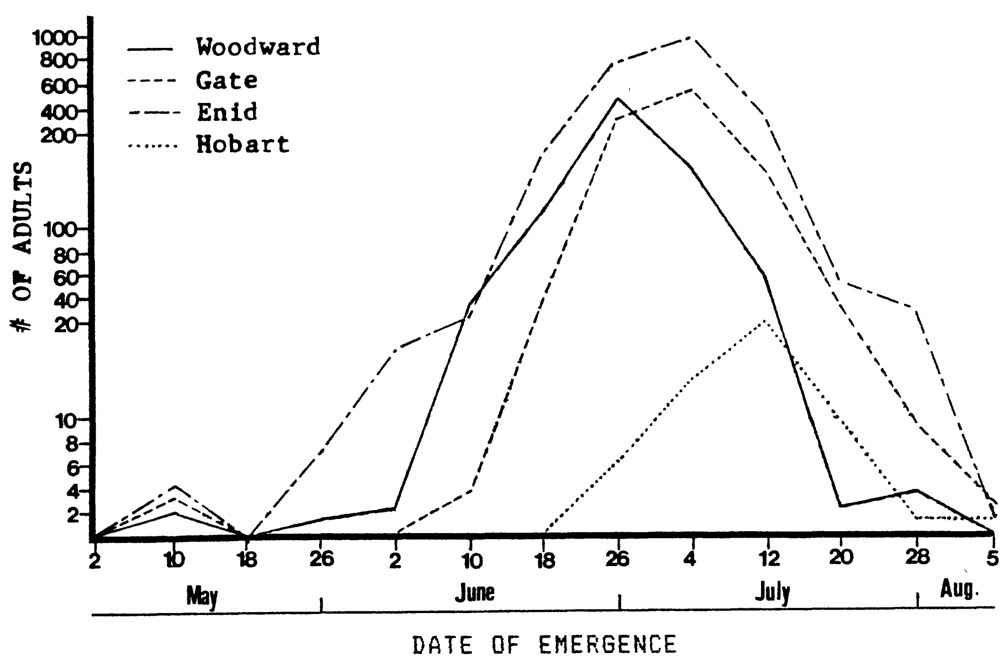

Figure 6. Emergence of ASC Parasites from Four Seed Sources in 1983

TABLE III

PERCENTAGE OF ASC EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1983

Date	Location			
	Hobart	Enid	Gate	Woodward
May 10	0	1.2	0.4	0.4
18	5.1	4.0	1.1	1.1
26	20.5	4.6	2.4	1.8
June 2	7.7	2.8	1.1	3.5
10	5.1	7.0	3.7	17.0
18	25.6	37.2	17.5	41.9
26	23.1	35.2	44.0	27.5
July 4	7.7	7.6	22.0	5.5
12	2.6	0.4	6.6	1.1
20	2.6	0	0.8	>0.1
28	0	0	0.3	0.1
Aug. 5	0	0	>0.1	>0.1

TABLE IV

PERCENTAGE OF ASC PARASITES EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1983

Date	Location			
	Hobart	Enid	Gate	Woodward
May 10	0	0.2	0.3	0.6
18	0	0	0	0
26	0	0.3	0	0.1
June 2	0	0.8	0	0.3
10	0	1.0	0.3	3.8
18	0	7.5	3.5	15.6
26	14.0	35.6	23.9	48.5
July 4	32.6	40.0	50.9	23.1
12	44.2	11.4	16.5	7.3
20	4.6	2.1	3.4	0.3
28	2.3	1.0	0.9	0.4
Aug. 5	2.3	>0.1	0.3	0

peak in mid-May was composed of significant higher numbers of emerging ASC only from seeds collected at Enid and Woodward (Table V). A large percentage of all ASC emergence from each location occurred during the month of June, particularly in mid-June during the peak (Table VI). From seeds collected at Woodward a total of 5252 ASC emerged, with 4206 (80.1%) emerging between June 1 and June 22. From seeds collected at Gate a total of 3296 ASC emerged, with 2430 (73.7%) emerging between June 1 and June 22. From seeds collected at Enid a total of 2579 ASC emerged, with 1640 (63.5%) emerging between June 1 and June 22. Only 96 ASC emerged from seeds collected at Hobart and the emergence was spread out with no peak emergence. Male to female sex ratios for the ASC that emerged in 1984, were 1:1.75 for Woodward, 1:1.4 for Gate, 1:1.1 for Enid, and 1:1 for Hobart. Male ASC (Figure 7) emerged in greater numbers than females (Figure 8) at all locations through June 8, after which females became more numerous. Males started to emerge from all locations during the week of April 20, while females from Hobart and Gate started to emerge during the week of April 27 and May 4, respectively. No males emerged after July 20, and only one female ASC from Gate emerged after July 20.

A total of five species of parasites emerged in 1984; L. perplexus, Liodontomerus insuetus Gahan, E. allynii, T. bruchophagi, and T. maculatus. A picture key to the ASC and its parasites found in Oklahoma is located in the Appendix. The two major species of parasites were L. perplexus (90.4% of the parasites) and T. bruchophagi (9.2%). Other parasitic species occurred very rarely.

Liodontomerus perplexus emerged from April 13 through August 3, but there was little emergence before June 8. Peak emergence occurred

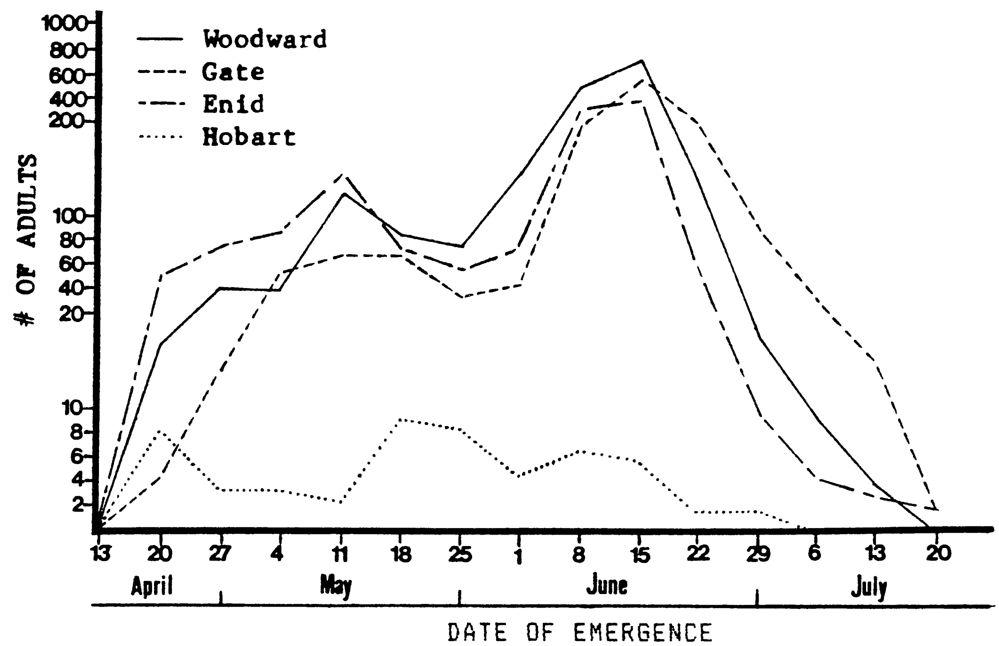

Figure 7. Numbers of Male ASC that Emerged Weekly from Four Seed Sources in 1984

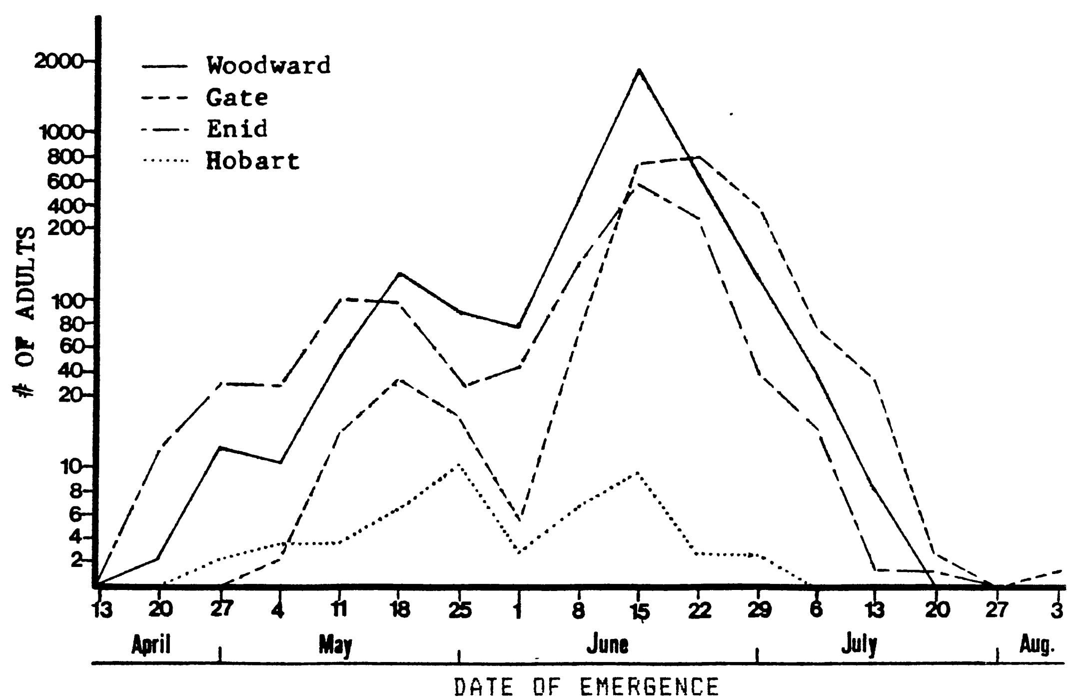

Figure 8. Number of Female ASC that Emerged Weekly from Four Seed Sources in 1984

TABLE V

MEAN NUMBERS OF ASC EMERGING WEEKLY
FROM FOUR SEED SOURCES IN 1984

	Location			
	Hobart	Enid	Gate	Woodward
Date	Mean 95%CI	Mean 95%CI	Mean 95%CI	Mean 95%CI
Apr. 20	0.8 ± 0.2	6.1 ± 2.6	0.4 ± 0.4	1.9 ± 1.8
27	0.5 ± 0.3	10.4 ± 5.9	1.3 ± 0.8	5.0 ± 3.7
May 4	0.6 ± 0.4	10.8 ± 3.5	3.7 ± 2.5	4.7 ± 2.5
		*		*
11	0.5 ± 0.4	24.0 ± 3.8	7.8 ± 2.3	16.8 ± 3.1
18	1.5 ± 0.8	16.4 ± 3.9	9.4 ± 2.1	20.7 ± 3.6
		*	*	
25	1.8 ± 1.1	8.3 ± 1.4	4.6 ± 1.6	16.0 ± 4.0
June 1	0.6 ± 0.4	11.0 ± 1.6	4.5 ± 2.6	21.2 ±10.4
		*	*	*
8	1.2 ± 0.6	43.4 ± 5.8	26.7 ± 9.1	91.9 ±28.7
		*	*	*
15	1.4 ± 0.6	92.1 ±16.6	121.0 ±28.8	248.9 ±73.9
	*	*		*
22	0.3 ± 0.3	28.5 ± 6.2	95.3 ±14.7	79.8 ±32.3
		*	*	*
29	0.3 ± 0.3	4.6 ± 3.8	39.3 ±11.2	12.7 ± 3.0
			*	*
July 6	0 ± 0	1.9 ± 0.9	10.5 ± 3.1	4.7 ± 3.1
		*	*	*
13	0 ± 0	0.3 ± 0.3	4.8 ± 2.0	0.8 ± 0.6
			*	
20	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0 ± 0
27	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Aug. 3	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0

* Values above and below symbol are significantly different at $P \leq 0.05$

TABLE VI

PERCENTAGE OF ASC EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1984

Date	Location			
	Hobart	Enid	Gate	Woodward
Apr. 20	8.3	2.4	0.1	0.4
27	5.2	4.0	0.4	1.0
May 4	6.3	4.2	1.1	0.9
11	5.2	9.3	2.4	3.2
18	15.6	6.4	2.8	3.9
25	18.8	3.2	1.4	3.0
June 1	6.3	4.3	1.4	4.0
8	12.5	16.8	8.1	17.5
15	14.6	35.7	36.7	47.4
22	3.1	11.0	28.9	15.2
29	3.1	1.8	11.9	2.4
July 6	0	0.7	3.2	0.9
13	0	0.1	1.5	0.2
20	1.0	>0.1	0.1	0
27	0	0	0	0
Aug. 3	0	0	>0.1	0

between June 8 and July 6 for males (Figure 9) and June 15 to July 13 for females (Figure 10). The period of greatest emergence was from June 8 to July 6 for Woodward, June 15 to July 13 for Gate, and June 8 to July 22 for Enid (Table VII). Approximately 90% of the L. perplexus emerged between June 8 and July 6 (Table VIII). Sex ratio of males to females was 1:1.6 for Woodward, 1:1.3 for Gate, 1.2:1 for Enid, and 1:2.4 for Hobart. Male L. perplexus occurred in greater numbers up to June 22 and the females were more numerous after June 22. Peak emergence of L. perplexus was two weeks later than the ASC peak emergence.

Tetrastichus bruchophagi emerged from April 13 through July 20, but there was little emergence before June 1. Peak emergence of male

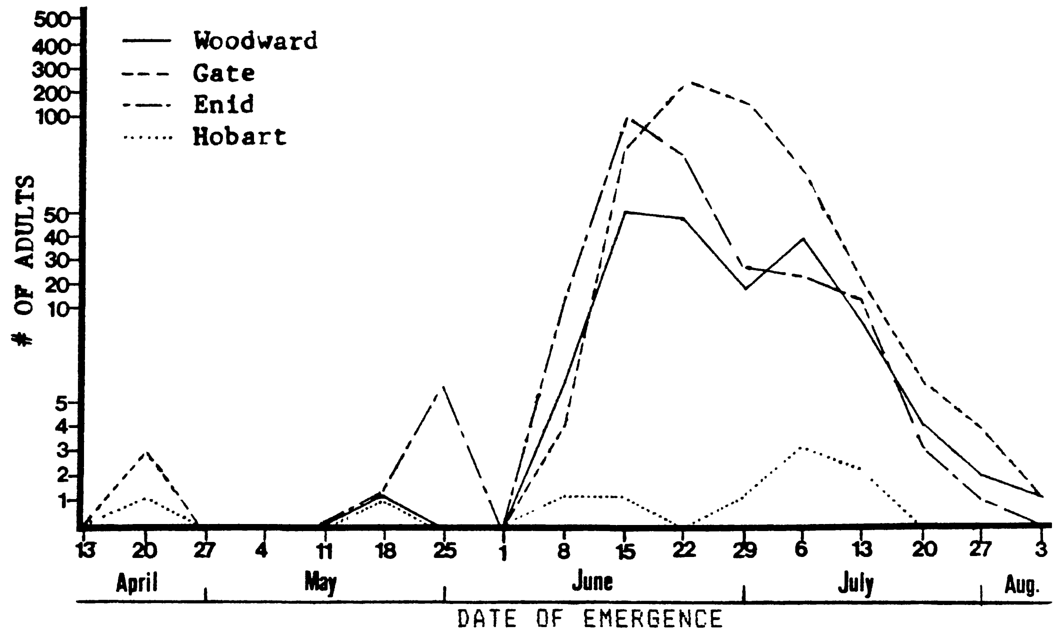

Figure 9. Numbers of Male L. perplexus Emerging Weekly from Four Seed Sources in 1984

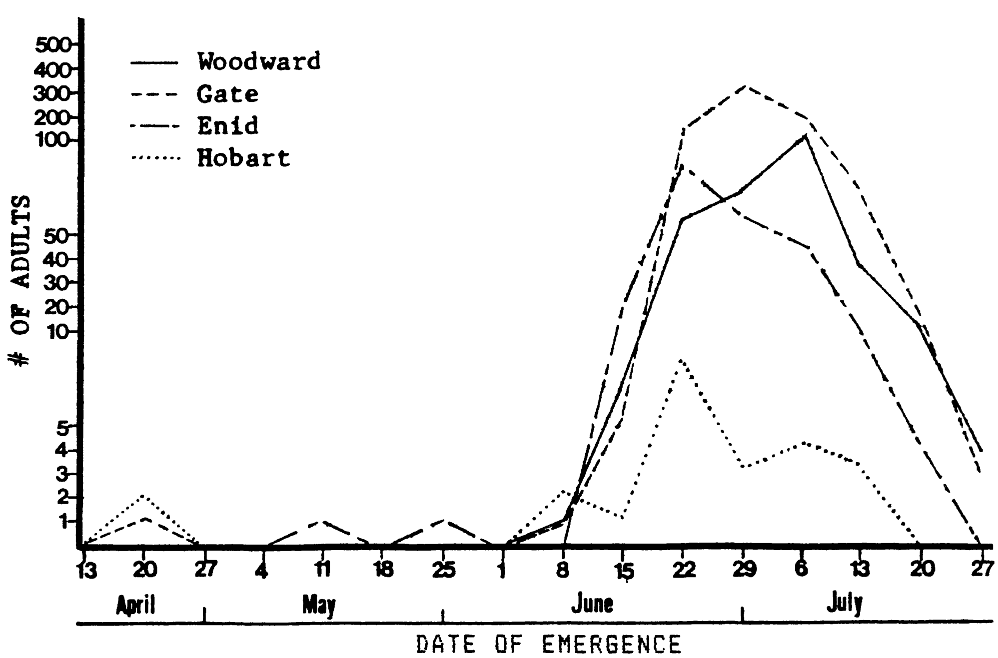

Figure 10. Numbers of Female L. perplexus Emerging Weekly from Four Seed Sources in 1984

TABLE VII

MEAN NUMBERS OF *L. PERPLEXUS* EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1984

	Location			
	Hobart	Enid	Gate	Woodward
Date	Mean 95%CI	Mean 95%CI	Mean 95%CI	Mean 95%CI
Apr. 20	0.3 ± 0.3	0 ± 0	0.4 ± 0.4	0 ± 0
27	0 ± 0	0 ± 0	0 ± 0	0 ± 0
May 4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
11	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0
18	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0
25	0 ± 0	0.7 ± 0.7	0 ± 0	0.1 ± 0.1
June 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		*	*	*
8	0.3 ± 0.3	1.5 ± 1.0	0.5 ± 0.4	0.7 ± 0.5
		*	*	*
15	0.2 ± 0.2	11.9 ± 5.6	8.9 ± 4.1	5.7 ± 2.4
			*	
22	0.1 ± 0.1	16.7 ± 4.2	33.0 ±18.1	10.1 ± 5.6
		*		
29	0.9 ± 0.8	8.0 ± 3.1	48.3 ±20.4	8.5 ± 4.2
July 6	0.6 ± 0.6	6.3 ± 3.4	26.8 ±16.0	14.1 ± 7.1
				*
13	0.6 ± 0.6	2.2 ± 1.6	8.8 ± 3.4	4.7 ± 2.2
			*	
20	0.3 ± 0.3	0.6 ± 0.6	2.2 ± 1.8	1.6 ± 1.3
27	0 ± 0	0.1 ± 0.1	0.7 ± 0.6	0.6 ± 0.6
Aug. 3	0 ± 0	0 ± 0	0.1 ± 0.1	0.1 ± 0.1

* Values above and below symbol are significantly different at $P \leq 0.05$

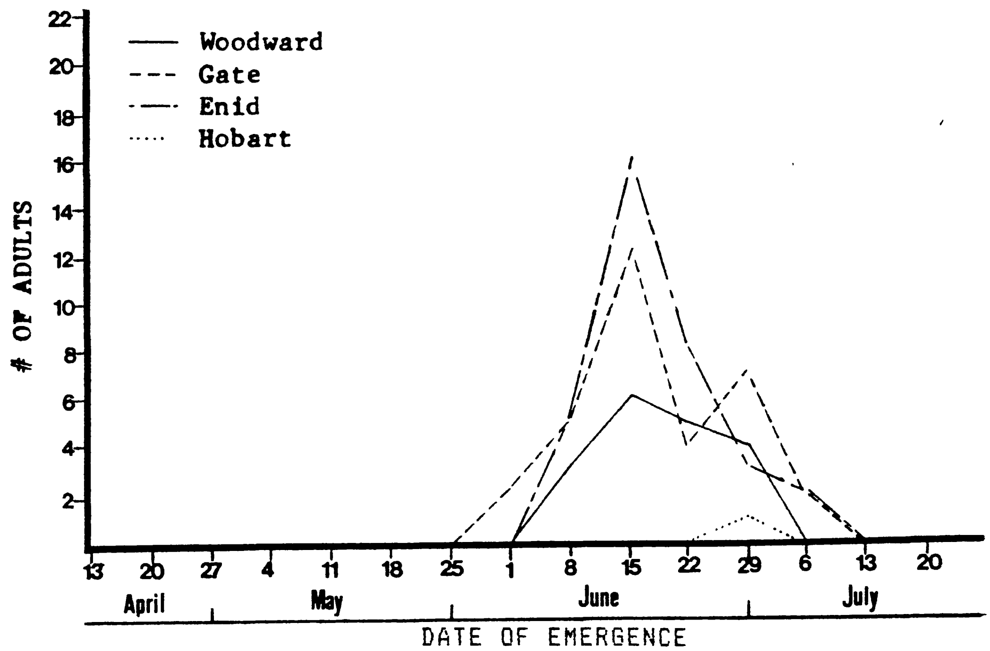

Figure 11. Numbers of Male T. bruchophagi Emerging Weekly from Four Seed Sources in 1984

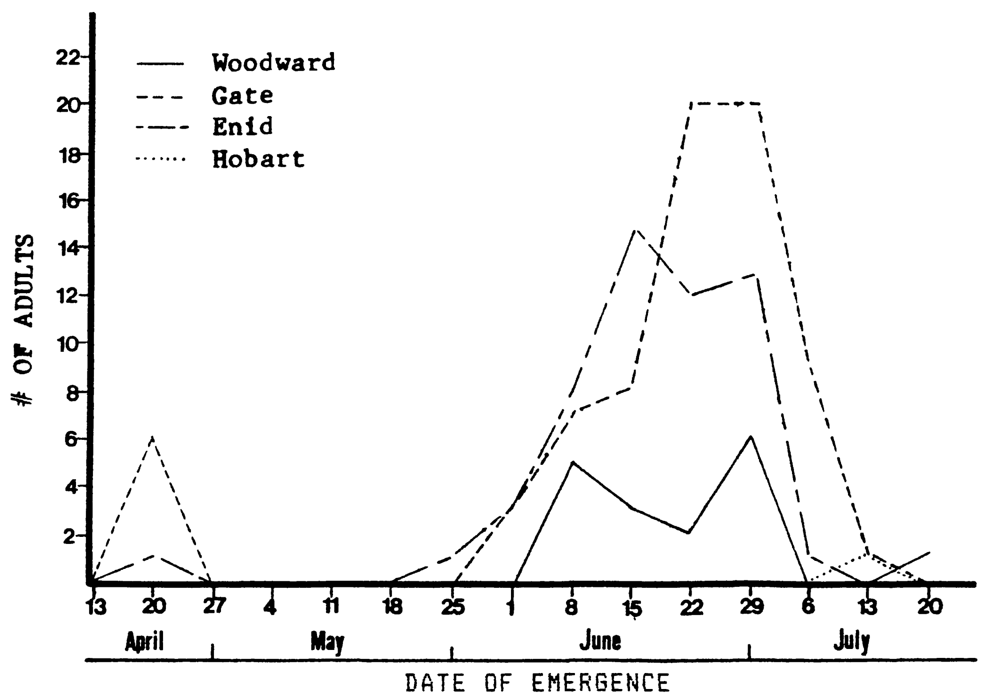

Figure 12. Numbers of Female T. bruchophagi Emerging Weekly from Four Seed Sources in 1984

TABLE VIII

PERCENTAGES OF L. PERPLEXUS AND T. BRUCHOPHAGI EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1984

Date	*L. perplexus*				*T. bruchophagi*			
	Hobart	Enid	Gate	Woodward	Hobart	Enid	Gate	Woodward
Apr. 20	8.8	0	0.2	0	0	1.1	5.6	0
27	0	0	0	0	0	0	0	0
May 4	0	0	0	0	0	0	0	0
11	0	0.2	0	0	0	0	0	0
18	3.0	0.2	0	0.2	0	0	0	0
25	0	1.5	0	0	0	1.1	0	0
June 1	0	0	0	0	0	3.4	4.7	0
8	8.8	3.1	0.4	1.5	0	14.8	11.2	22.9
15	5.9	24.7	6.9	12.3	0	36.3	18.7	25.7
22	3.0	34.6	25.4	21.9	0	22.7	22.4	20.0
29	26.5	16.6	37.2	18.4	50.0	18.2	25.2	28.6
July 6	17.6	13.1	20.7	30.5	0	3.4	10.4	0
13	17.6	4.6	6.8	10.2	0	0	0.9	0
20	8.8	1.2	1.7	3.5	0	0	0	2.8
27	0	0.2	0.5	1.3	50.0	0	0	0
Aug. 3	0	0	0.2	0.2	0	0	0.9	0

T. bruchophagi occurred from June 1 to 29 (Figure 11) and females emerged in highest numbers from June 8 to July 6 (Figure 12). About 89% of the *T. bruchophagi* emerged between June 1 and 29 (Table VIII). The sex ratio of male to female was 1:1 for Woodward, 1:2.3 for Gate, 1:1.6 for Enid and 1:1 for Hobart. Peak emergence of *T. bruchophagi* was one week later than the ASC peak emergence.

Total number of all adults (ASC and parasites combined) that emerged from seeds collected at each location in 1983 were; 5,749 from Woodward, 4,710 from Gate, 3,049 from Enid, and 132 from Hobart. Percentages of the above listed total numbers that were composed of ASC

TABLE IX

MEAN NUMBERS OF *T. BRUCHOPHAGI* EMERGING WEEKLY FROM FOUR SEED SOURCES IN 1984

	Location							
	Hobart		Enid		Gate		Woodward	
Date	Mean	95%CI	Mean	95%CI	Mean	95%CI	Mean	95%CI
Apr. 20	0	± 0	0.1	± 0.1	0.6	± 0.6	0	± 0
27	0	± 0	0	± 0	0	± 0	0	± 0
May 4	0	± 0	0	± 0	0	± 0	0	± 0
11	0	± 0	0	± 0	0	± 0	0	± 0
18	0	± 0	0	± 0	0	± 0	0	± 0
25	0	± 0	0.1	± 0.1	0	± 0	0	± 0
					*			
June 1	0	± 0	0.3	± 0.3	0.5	± 0.4	0	± 0
							*	
8	0	± 0	1.3	± 0.9	1.2	± 0.7	0.8	± 0.5
15	0	± 0	3.1	± 1.1	2.0	± 1.1	0.9	± 0.8
22	0	± 0	2.0	± 1.0	2.4	± 1.1	0.7	± 0.6
29	0.1	± 0.1	1.6	± 0.8	2.7	± 1.4	1.0	± 0.8
			*				*	
July 6	0	± 0	0.3	± 0.3	1.1	± 0.8	0	± 0
13	0	± 0	0	± 0	0.1	± 0.1	0	± 0
20	0	± 0	0	± 0	0	± 0	0.1	± 0.1
27	0.1	± 0.1	0	± 0	0	± 0	0.1	± 0.1
Aug. 3	0	± 0	0	± 0	0.1	± 0.1	0	± 0

* Values above and below symbol are significantly different at $P \leq 0.05$

parasites were: 30.7% from Gate, 28.4% from Hobart, 15.5% from Enid, and 8.7% from Woodward.

The second year (1984) emergence of ASC and / or parasites from seeds obtained in the fall of 1982 occurred from three locations: Enid, Woodward, and Gate. Emergence of ASC occurred from two locations (Woodward and Gate) and was sporadic with very low numbers (Table X). The ASC male to female ratio was 1.4:1 for Woodward and 1.5:1 for Gate. *Liodontomerus perplexus* was more numerous than any other species at all three sites (Table XI). Male *L. perplexus* were more numerous than females, with male to female sex ratios of 1.1:1 for Woodward, 2:1 for Gate, and 3.7:1 for Enid. *Tetrastichus bruchophagi* was more numerous than the ASC at Woodward (Table XII). Male to female sex ratios of *T. bruchophagi* were 1:1.4 for Woodward, 1:3.0 for Gate, and 1:1 for Enid.

TABLE X

SECOND YEAR EMERGENCE OF ASC FROM SEEDS COLLECTED IN 1982

		Location			
		Woodward		Gate	
Date		Male	Female	Male	Female
Apr. 27		1	2		
May 4		5	1		
11		5	2	1	
18		3	1		
25		1			
June 1					
8		2	3		
15		2	4	1	
22		1	1		1
29					
July 6				1	1
	Total	20	14	3	2

TABLE XI

SECOND YEAR EMERGENCE OF *L. PERPLEXUS* FROM SEED COLLECTED IN 1982

	Location					
	Woodward		Gate		Enid	
Date	Male	Female	Male	Female	Male	Female
June 8		1				
15	10	1	1			
22	39	29	4	1	8	2
29	20	17	2	4	3	
July 6	6	13	6	2		1
13	3	9	7	2		
20	2	2		2		
Total	80	72	20	11	11	3

TABLE XII

SECOND YEAR EMERGENCE OF *T. BRUCHOPHAGI* FROM SEED COLLECTED IN 1982

	Location					
	Woodward		Gate		Enid	
Date	Male	Female	Male	Female	Male	Female
June 1	3	5				
8	14	13				
15	8	10	1		1	
22	1	7		1	1	4
29				1	4	1
July 6		1		1		1
13		1				
Total	26	37	1	3	6	6

Discussion

In Oklahoma. ASC emerged from the middle of April through early August. Peak ASC emergence occurred during the month of June. ASC have been previously observed emerging in late April in Oklahoma (Ahring et al. 1984). The general pattern of ASC emergence exhibited during this study agrees with the dates presented by Nielson (1976), except that in his study ASC emergence ended in mid-June while in our study it continued until early August. Urbahns (1920) observed ASC emerging from samples held in the laboratory as late as September 14, and mentions that in dry "desert" conditions the diapausing stage may be prolonged. Batiste (1967b) worked with the closely related trefoil seed chalcid (*Bruchophagus kolobovae* Fedoseva) and reported that low humidity adversely affected emergence from the seeds. Thus the emergence observed during July and August in our study could be due to the dry conditions of the rearing cartons extending the emergence into these later months.

Sorenson (1930) reported that the earliest emerging ASC are males. Data from our study indicate that males did emerge earlier than females from two of the locations and males did emerge in greater numbers at all locations up to early June. Female ASC outnumbered the males in overall numbers. Apparently, the males emerge first in order to be present and ready to mate when the females emerge.

A total of five species of ASC parasites were found in Oklahoma. Butler and Hansen (1958) reported the presence of *L. perplexus*, *T. bruchopagi*, and *L. insuetus* in Oklahoma. The other two species, *T. maculatus* and *E. allynii* have not been previously reported from Oklahoma, but Brewer and Horber (1982) reported both in Kansas.

Parasites in Oklahoma were principally of two species; *L. perplexus* and *T. bruchophagi* which were the same major parasites reported in Kansas by Brewer and Horber (1982). These two species emerged during the same time period as the ASC, but the emergence was not constant through April and May. The scattered emergence that occurred could indicate that a small percentage of the parasite population of each species overwinters in a slighty more advanced prepupae than the rest of the population. Peak emergence of *L. perplexus* was two weeks later than the ASC peak emergence. This agrees with the findings of Saunders and Hsiao (1970) who reported that late emergence of the parasite synchronized its life cycle with the ASC in order to assure the presence of ASC larvae suitable for parasitization. Peak emergence for *T. bruchophagi* was one week later than the ASC peak emergence. The ASC life stage that *T. bruchophagi* parasitizes occurs roughly seven days before the stage that *L. perplexus* parasitizes (Butler et al. 1968). Thus *T. bruchophagi* has also synchronized its life cycle with the ASC similar to that exhibited by *L. perplexus*. Peak emergence of males of both species occurred before the females, to allow the males to be present for mating when the females emerged. Percentages of the emergence that was composed of parasitic species (all species combined) ranged from 3.8% to 81.1% over both years. This agrees with ranges of parasitism reported by Sorenson (1930, 1934) and Butler and Hansen (1958). The high percentages of parasitism found in the study seem to indicate that parasites may be an important regulatory factor on ASC populations.

Urbahns (1920) reported emergence of ASC from seeds held two year in the laboratory. Second year emergence of ASC was observed in our study from seed gathered from two locations. Sorenson (1930) reported that 1.91% of the total ASC that emerged from alfalfa seed held in the laboratory resulted from second year emergence. This is higher than the 0.2% from Woodward and 0.1% from Gate found in our study. No previous study has mentioned second year emergence of any of the parasites. So it was suprising to find the second year emergence was predominantly made up of ASC parasites. *Liodontomerus perplexus* was the most numerous of all the species that emerged, and *T bruchophagi* also emerged. There was also a higher concentration of males in the ASC and *L. perplexus* that emerged.

CHAPTER IV

POPULATION SURVEY OF ADULT ASC AND ASC PARASITES

Introduction

In spring of each year, adult alfalfa seed chalcids (ASC), *Bruchophagus roddi* Gussakovskii, and ASC parasites begin to emerge from alfalfa (*Medicago sativa* L.) seeds in which they overwintered as diapausing prepupae. Soon after, adult ASC can be found where alfalfa plants are setting seed. Field populations of ASC adults are sampled by several methods, but collecting ASC with a sweep net is the fastest, easiest, and least costly method. Parvez (1968) used a sweep net to sample adult ASC field populations in Oklahoma; he collected ASC from May 14 to September 14 and reported a range of three to 101 ASC per 100 sweeps. He reported that ASC in Oklahoma appeared to have three generations. ASC parasite species occur in different concentrations, compositions, and times of the season in different areas across the United States (Bacon et al. 1968) and field populations of parasites are sampled with a sweep net (Urbahns 1916).

In 1983, the objectives of this study were to determine seasonal occurrence of the adult ASC and parasite populations in alfalfa. In 1984, the objectives included those of 1983 as well as species determination of ASC parasites and computing male and female composition of ASC and ASC parasite populations.

Methods and Materials

Adult populations of ASC and parasites were sampled using a sweep net in 1983 and 1984. Sweep samples were taken twice a week during the 1983 season at field 1 (near Stillwater in Payne county) and field 2 (near Perry in Noble county). Management of both fields in 1983 consisted of fencing off 0.5 hectares in spring and allowing alfalfa to bloom and produce seed throughout the season. Neither field was cut during the study and no insecticide was applied. The alfalfa vegetation changed during the season depending on plant maturity; from mainly leaves and blooms on upright plants to mainly stems on lodged plants. At field 1, height of first growth alfalfa was 40 cm in late April and blooms appeared during the first week of May. Plant height reached 90-125 cm by mid-July and blooming had ceased. Second growth from the crown reached a height (35-40 cm) above the lodged first growth in mid-July and the second growth also grew to lodging plants but only attained a stem length of 90-95 cm by the end of August. At field 2 the first growth of the season was infested with blue alfalfa aphids, *Acyrthosiphon kondoi* Shinji, to the extent that growth was stunted and virtually no blooms occurred, second growth of the season occurred at the same time as in field 1, and grew in virtually the same manner. In 1983, weekly means of number of ASC and ASC parasites were calculated by adding the counts from two samples taken within each week.

Sweep samples were taken weekly during the 1984 season at fields 3 and 4: field 3 was near Woodward and field 4 was on the South Central Research Station at Chickasha. Field 3 was six hectares in size (divided into 24 equal plots) and field 4 was 0.5 hectares (divided into nine equal plots). Management at both fields in 1984 consisted of staggering harvest dates in order to provide blooming alfalfa throughout the season. At field 3, toxaphene insecticide was applied at a rate of 1 kg per hectare on July 23 to control grasshoppers (Melanoplus spp.). No insecticides were applied during the season at field 4. Sweep samples were taken from the area that had the newest blooming alfalfa (i.e. samples were taken from one plot until the time the alfalfa in a second plot started to bloom and the sampling was switched to the second plot, etc.). Alfalfa height was more consistent in this type of management, ranging from 40 - 60 cm at the time when the samples were taken.

Each sample consisted of 100 pendulum sweeps. Length of each sweep through vegetation was one meter. Sweeps were kept at a level so that half of the net was in contact with vegetation during the motion. Diameter of the net was 38 cm. Total vegetation covered in 100 sweeps was estimated to be about 30 square meters. Net mesh of 1 mm was used to catch the tiny ASC. Net contents were transferred to a kill jar containing ethyl acetate and taken to the laboratory for identification. Sampling started on April 15 in 1983 and on May 1 in 1984. No samples were collected during the week of June 23 in 1984. Sweep samples ended on September 29 in 1983 and September 27 in 1984. Data recorded were number of ASC, and ASC parasites in 1983. In 1984, samples were futher divided by sex of adult and species of parasite.

Butler et al. (1968) reported a regression equation for predicting the development time for egg to adult in the ASC. This equation was used to calculate the threshold temperature for ASC development of 43 F and the degree day accumulation needed for generation development of

760 dd. Weather data were obtained from the research station nearest each field for the months of May through September. Starting on May 1, the daily high and low temperatures were used to calculate the degree day accumulations.

Results

In 1983, the first adult ASC was collected on May 18 and ASC were collected on every sampling date in fields 1 and 2 until sampling ceased on September 29. In 1984, adult ASC were collected from June 5 to September 25 at field 3 and from May 17 through September 27 at field 4.

At field 1, highest population density of 28 adult ASC per 100 sweeps occurred during the week of August 11 (Figure 13). There were six ASC peaks during the season separated by differing time intervals; 29 days from peak 1 to 2, 28 days from peak 2 to 3, 20 days from peak 3 to 4, 20 days from peak 4 to 5, and 21 days from peak 5 to 6. Highest population density of 27 and 28 parasites per 100 sweeps occurred during the weeks of September 1 and 8 (respectively). There were three ASC parasite peaks, each occurred one week after the ASC peaks number 3, 4, and 5. Parasites were more numerous than the ASC from August 18 to the end of the season, making up 64.7% of the collected adults. In 20 weeks of sampling, 514 ASC and 368 parasites were collected.

At field 2, highest population density of 13 adult ASC per 100 sweeps occurred during the week of September 15 (Figure 14). There were three ASC peaks separated by intervals of 20 days from peak 1 to 2, and 27 days from peak 2 to 3. Highest population density of eight parasites per 100 sweeps occurred during the week of August 18. The

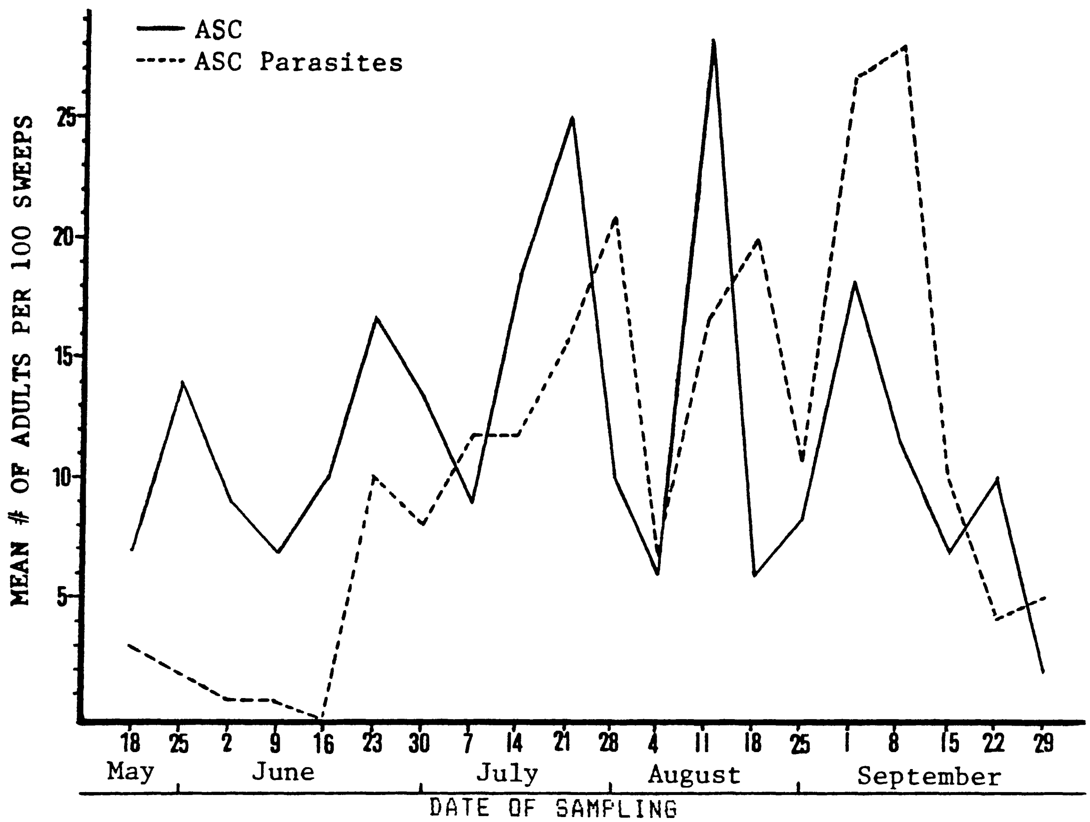

Figure 13. Sweep Sample Collections of ASC and Parasites at Stillwater (Field 1), 1983.

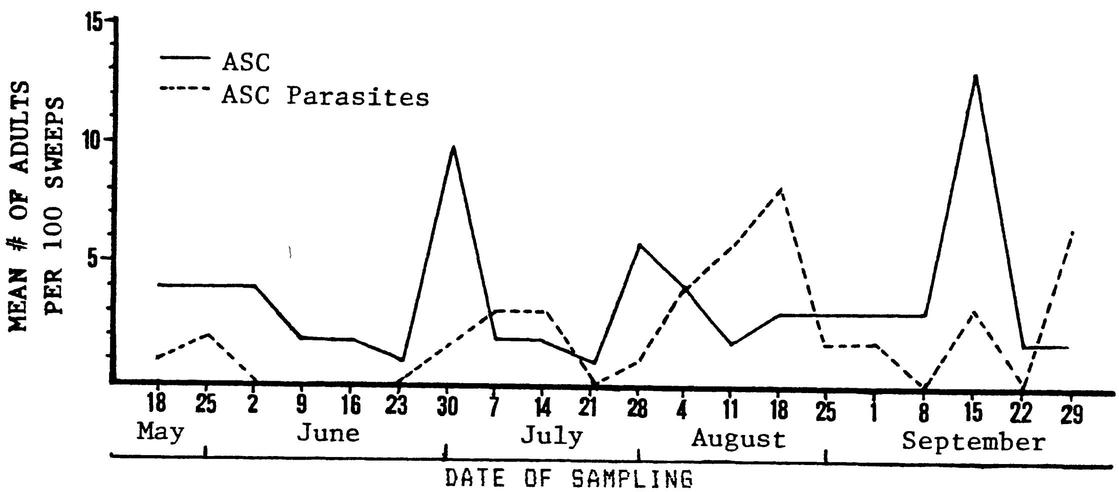

Figure 14. Sweep Sample Collections of ASC and Parasites at Perry (Field 2), 1983.

parasite peaks are not as closely related to the ASC peaks as was found in Field 1. Parasites were more numerous than ASC during the month of August, when they comprised 64.1% of the adults collected. In 20 weeks of sampling, 102 ASC and 72 parasites were collected.

In 1984, at field 3, highest population density of 11 adult ASC per 100 sweeps occurred during the week of August 7 (Figure 15). There were three ASC peaks separated by intervals of 29 days from peak 1 to 2, and 28 days from peak 2 to 3. Females outnumbered males in every sample, except for July 17 when identical numbers were collected, and on August 7 when males outnumbered females (Table XIII). The highest population density of 27 parasites occurred during the week of August 14, which was one week later than the highest ASC population. Parasites were more numerous than the ASC during the month of August, when they comprised 68.2 percent of the adults collected. Female ASC parasites outnumbered males in every sample. In 19 weeks of sampling, 60 ASC (20 male, 40 female), 73 L. perplexus (11 male, 62 female), 4 T. bruchophagi (1 male, 3 female), and 1 T. maculatus (female) were collected.

At field 4, highest population density of 52 adult ASC per 100 sweeps occurred during the week of August 9 (Figure 16). There were four ASC peaks separated by intervals of 12 days from peak 1 to 2, 35 days from peak 2 to 3, and 21 days from peak 3 to 4. Females outnumbered males in 14 out of 19 samples (Table XIV). The highest population density of 55 parasites per 100 sweeps occurred during the week of August 16, one week later than the highest ASC population. ASC were the most numerous species from May 17 to July 19 (76.0 percent of the collected adults) and parasites were more numerous than ASC from July

25 to September 20 (58.7 percent of the collected adults). Female parasites outnumbered males in every sample. In 19 weeks of sampling, 278 ASC (96 males, 182 females), 247 L. perplexus (52 males, 195 females), 40 T. bruchophagi (5 male, 30 female), 2 T. maculatus (both female), and 1 Eupelmus allynii (female) were collected.

Degree day accumulation for fields 1 and 2 showed that a new generation could have developed by June 4, June 29, July 21, August 8, August 26, and September 16. At field 3, a new generation could have developed by June 2, June 24, July 15, August 4, August 24, and September 13. At field 4, a new generation could have developed May 27, June 19, July 8, July 27, August 16, and September 7.

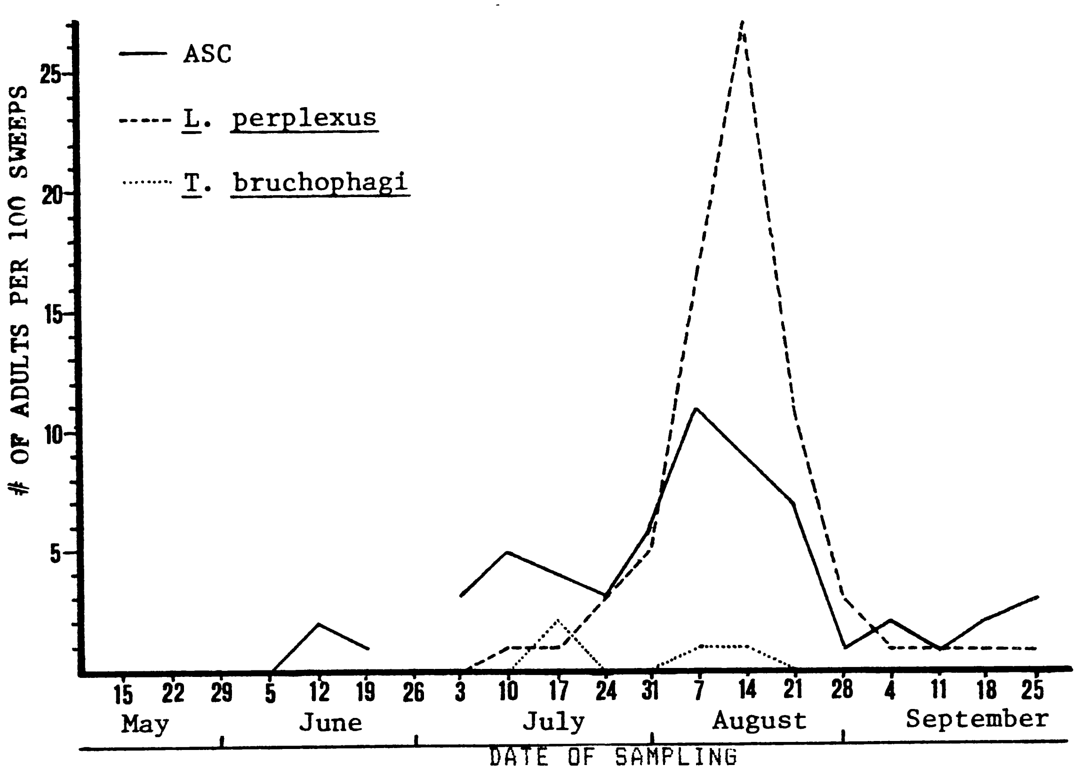

Figure 15. Sweep Sample Collections of ASC and Parasites at Woodward (Field 3), 1984.

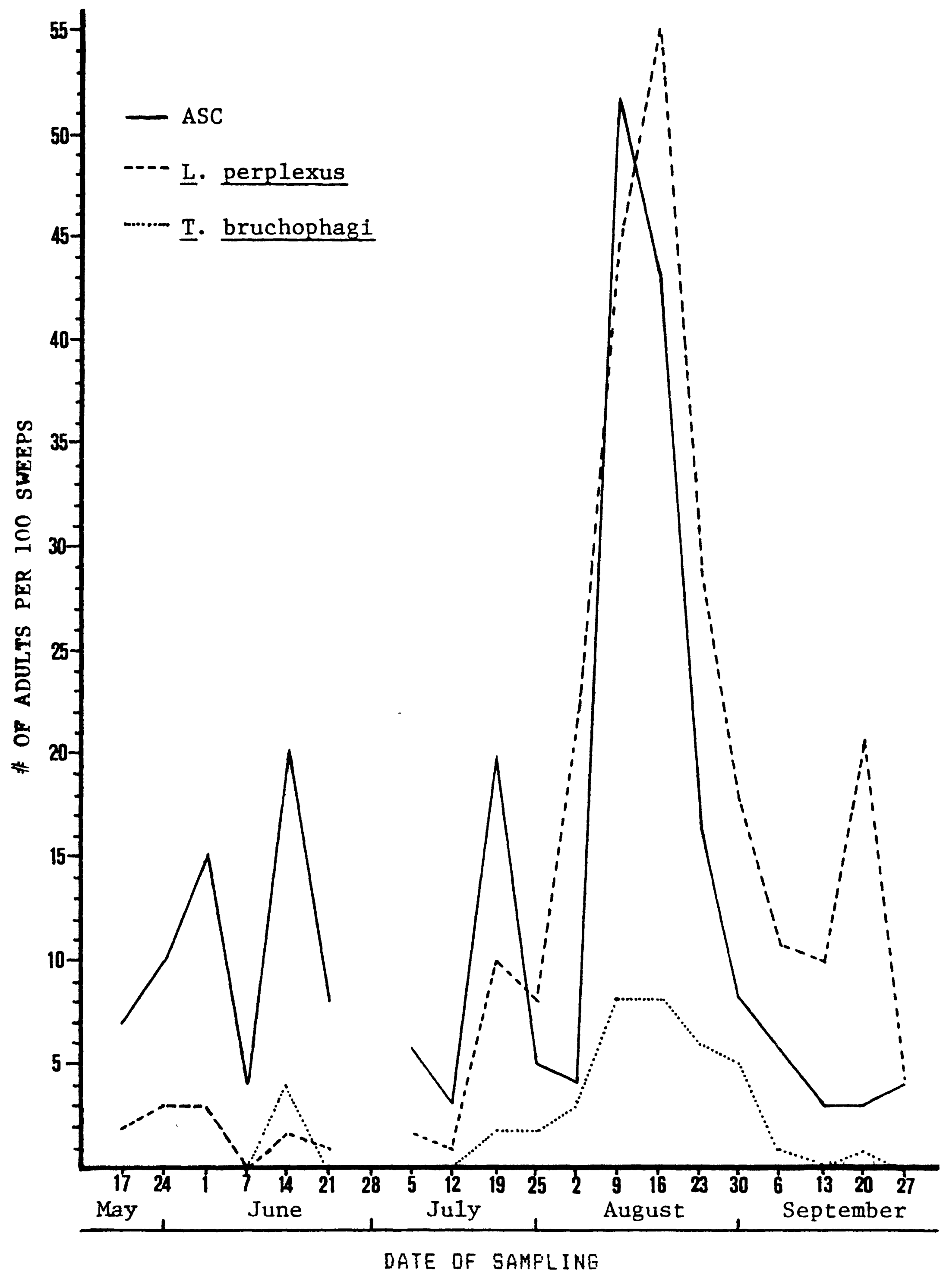

Figure 16. Sweep Sample Collections of ASC and Parasites at Chickasha (Field 4), 1984.

TABLE XIII

NUMBER OF ASC AND PARASITES COLLECTED IN SWEEP NET SAMPLES AT WOODWARD (FIELD 3), 1984

	Species Collected						
	ASC		*L. perplexus*		*T. bruchophagus*		Other Parasites
Date	Male	Female	Male	Female	Male	Female	Sex as Listed
May 15	0						
22	0						
29	0						
June 5	0						
12		2					
19		1					
28	No Sample Taken						
July 3	1	2					
10	1	4	1				
17	2	2		1	1	1	
24	1	2		3			
31	2	4		5			
Aug. 7	6	5	2	15		1	
14	4	5	5	22		1	
21	3	4	1	10			
28		1	1	2			
Sept 4		2		1			
11		1		1			*T. maculatus* (1 F)
18		2		1			
25		3		1			
	20	40	11	62	1	3	

TABLE XIV

NUMBERS OF ASC AND PARASITES COLLECTED IN SWEEP NET SAMPLES AT CHICKASHA (FIELD 4), 1984

	Species Collected						
	ASC		*L. perplexus*		*T. bruchophagus*		Other Parasites
Date	Male	Female	Male	Female	Male	Female	Sex as Listed
May 17	2	5		2			
24	3	7		3			
June 2	3	12		3			
7		4					
14	2	18		2		4	
21	3	5	1				
28	No Sample Taken						
July 5	4	2	1	1			
12		3		1			
19	4	16	1	9		2	
25	2	3		8		2	
Aug. 2	2	2		22		3	*E. allynii* (1 F)
9	27	25	7	38	1	7	*T. maculatus* (2 F)
16	20	23	10	45	2	6	
23	12	5	9	20	1	5	
30	7	1	6	12	1	4	
Sept 6	3	3	4	7		1	
13	1	2	5	5			
20	1	2	7	14		1	
27		4	1	3			
	96	182	52	195	5	35	

Discussion

At field 1 in 1983, as well as fields 3 and 4 in 1984 the highest population density of ASC adults occurred during the month of August, which agrees with the findings of Urbahns (1920) and Sorenson (1930). At field 2 in 1983, the highest population density of adults was in mid-September. The adult population could have had delayed development due to the fact that there were no seeds produced in that field until mid-July, or the method of sampling for some reason was not successful in collecting adults when the highest numbers were expected in August. Females were more numerous than males in nearly every sample. This does not agree with the findings of Sorenson (1930) who reported that males were more numerous throughout the season, but does agree with Batiste (1967a) who reported that females were more numerous in field collections of the trefoil seed chalcid, Bruchophagus kolobovae Fedoseeva. It is more logical to find high numbers of females in the field because that is where the oviposition sites are located. The alternative is that the sampling method used in the study could have had a bias towards collecting females.

Parasites were more numerous than ASC in late season samples and high population densities of parasites occurred one week later than high ASC densities at fields 1, 3, and 4. The numbers of parasites built up slowly in early summer and high population densities appeared to be dependent on numbers of ASC, which agrees with the findings of Batiste (1967a) working on the trefoil seed chalcid. At field 2, very few parasites were collected which makes it difficult to show a valid seasonal pattern. In 1984, females were more numerous than males in every sample which also agrees with the findings of Batiste (1967a).

In Oklahoma, the first ASC adults were collected in mid-May and the highest population density of ASC did not occur until later in the season, usually in August. Female ASC were present in the alfalfa fields in greater numbers than the male. ASC parasites were more numerous than the ASC in collections taken during the later part of the season and high densities of parasites occurred one week later than high densities of ASC.

CHAPTER V

SEASONAL INFESTATION OF THE ASC IN OKLAHOMA

Introduction

Once alfalfa (*Medicago sativa* L.) initiates spring bloom and seed set, alfalfa seed chalcids (ASC), *Bruchophagus roddi* Gussakovskii, move into the area and infest developing alfalfa seeds. Urbahns (1920) found ASC infestation whenever newly formed alfalfa seeds were present and diapausing ASC starting in mid-July in alfalfa managed for seed production. He also harvested 88 alfalfa seed samples from 33 seed producing areas across the United States and reported some extremely high infestations, such as; 80% and 83% in Kansas, 82% and 91% in Arizona, and 85% in California. Sorenson (1930) collected seed pods from alfalfa fields just before seed harvest and reported a range of infestations from less than 1% to 62%.

Two important factors to be determined in this study were the seasonal infestation of alfalfa seeds by the ASC in Oklahoma, and the occurrence throughout the season of ASC that entered the diapausing stage in Oklahoma.

Materials and Methods

Field studies were conducted in 1983 and 1984 to determine ASC infestation levels in Oklahoma. This study included a total of four fields. Fields 1 and 2 were used in 1983: field 1 was near Stillwater in Payne county and field 2 was near Perry in Noble county. Fields 3 and 4 were used in 1984: field 3 was near Woodward in Woodward county and field 4 was on the South Central Research Station at Chickasha.

In 1983, at fields 1 and 2, 0.5 hectares of alfalfa was allowed to bloom and produce seeds throughout the growing season. Neither of the fields was cut during the study and no insecticide was applied. Newly blooming alfalfa racemes were tagged at random with date and field location marked. Tagging was begun at field 1 on June 6, continued at weekly intervals for 11 weeks until August 13. Blooming of alfalfa was delayed at field 2 due to an infestation of pea aphids, *Acyrthosiphon kondoi* Shinji. Tagging started at field 2 on July 15, continued at weekly intervals for 6 weeks, until August 20. Sample size at both fields was 100 racemes. A total of 105 racemes were tagged each week at each field to insure recovery of at least 100 stems bearing tagged racemes. Both fields were pollinated by naturally occurring pollinators and set seed under natural field conditions. Stems with a tagged raceme were harvested five weeks after tagging. Two samples were taken from each stem. The first sample contained 100 of the tagged racemes, while the second sample consisted of the 100 racemes closest to the tagged racemes. Each sample was placed in a 0.5 liter jar. The jar was covered with a close knit material, and labelled as to field, week, and sample number. Samples were stored at room temperature until adult ASC and parasite emergence ceased and seeds were dried. Each sample was processed with a light abrasive action using a rub-board to thresh the seed pods and a Dakota seed blower to separate seeds from threshings. Care was taken not to crush any parasitized seeds. Seeds were examined using a stereomicroscope and placed in one of three categories: 1) Seeds with emergence holes (Figure 17), 2) Seeds with immature chalcids; those with living or dead larvae or pupae (Figure 18) especially noting those with diapausing chalcids (Figure 19), and 3) uninfested seeds (Figure 20). Percentage of ASC damaged seeds was calculated by dividing total number of ASC damaged seeds by total number of seeds in sample. Percentage of ASC that entered diapause was calculated by dividing number of seeds containing diapausing chalcids by total number of ASC damaged seeds. Weekly means were calculated by totaling both samples taken from a field during a week and dividing by two.

In 1984, at field 3, the experiment was arranged in a randomized complete block design with six replications (Figure 21). Field size was six hectares divided into 24 plots of 0.25 hectares each. Five treatments were studied: 1) Uncut primary spring growth allowed to mature seed, 2) Alfalfa cut at bud stage (May 10), 3) Alfalfa cut at the 10% bloom (May 23), 4) Alfalfa cut at the 50% bloom (May 27), and 5) Second seed crop from the uncut treatment. The second seed crop treatment is a seed crop produced from the alfalfa that grows back after seed has been harvested from the uncut treatment. Four colonies of honeybees (*Apis mellifera* L.) per hectare were used for pollination throughout the study on field 3. Toxaphene insecticide was applied at the rate of 1 kg per hectare on July 23 to control grasshoppers (*Melanoplus* spp.). Racemes were tagged in the same manner as in 1983. Tagging started in each treatment when the alfalfa began to bloom. Racemes were tagged in each treatment once a week for a duration of four weeks. The tagging dates for the five treatments were: Treatment

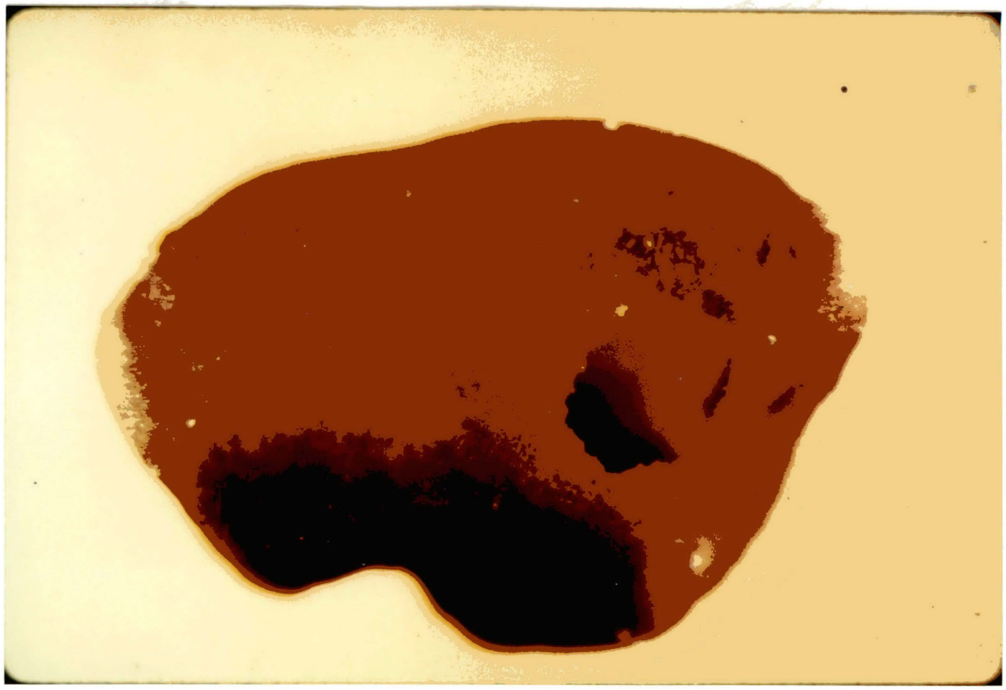

Figure 17. Alfalfa Seed with Chalcid Emergence Hole

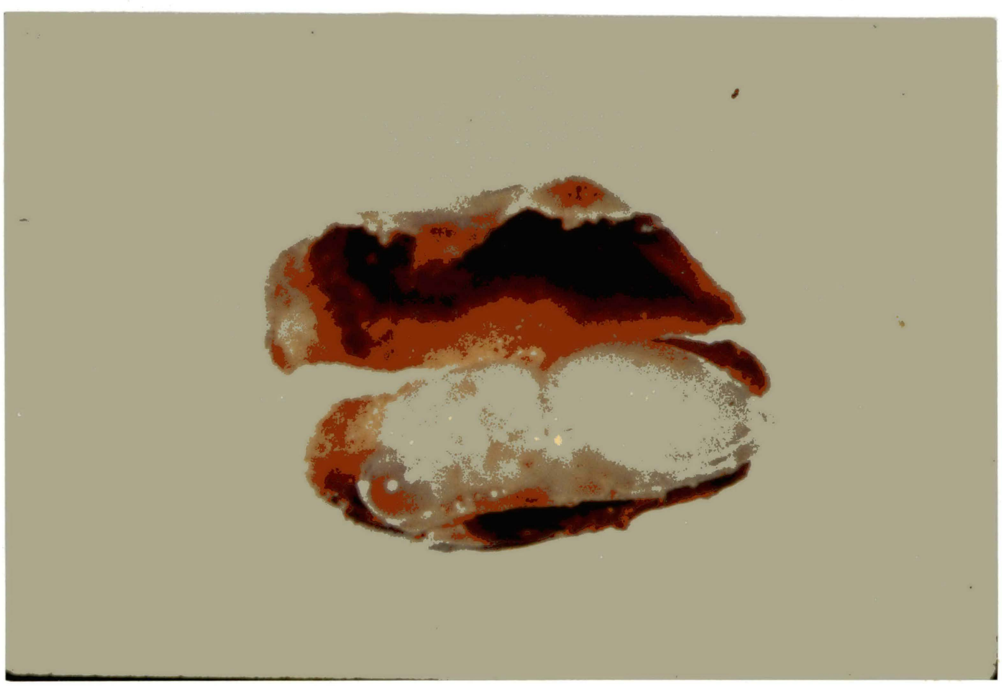

Figure 18. Alfalfa Seed Containing a Chalcid Pupa

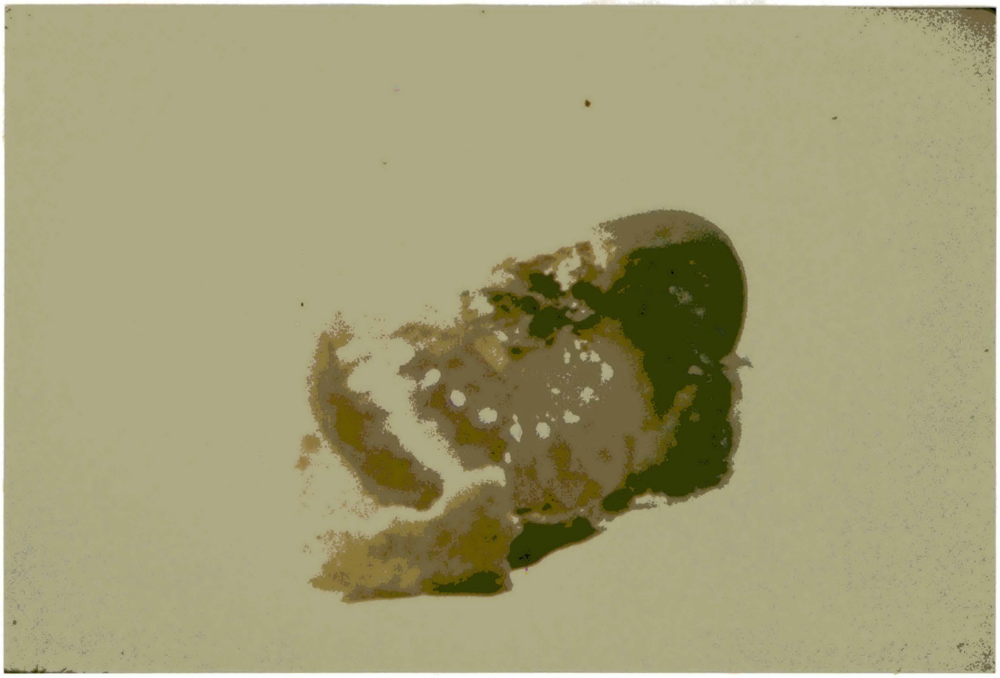

Figure 19. Alfalfa Seed Containing a Diapausing Chalcid Prepupa

Figure 20. UnInfested Alfalfa Seed (Left) and Two Chalcid Infested Seeds

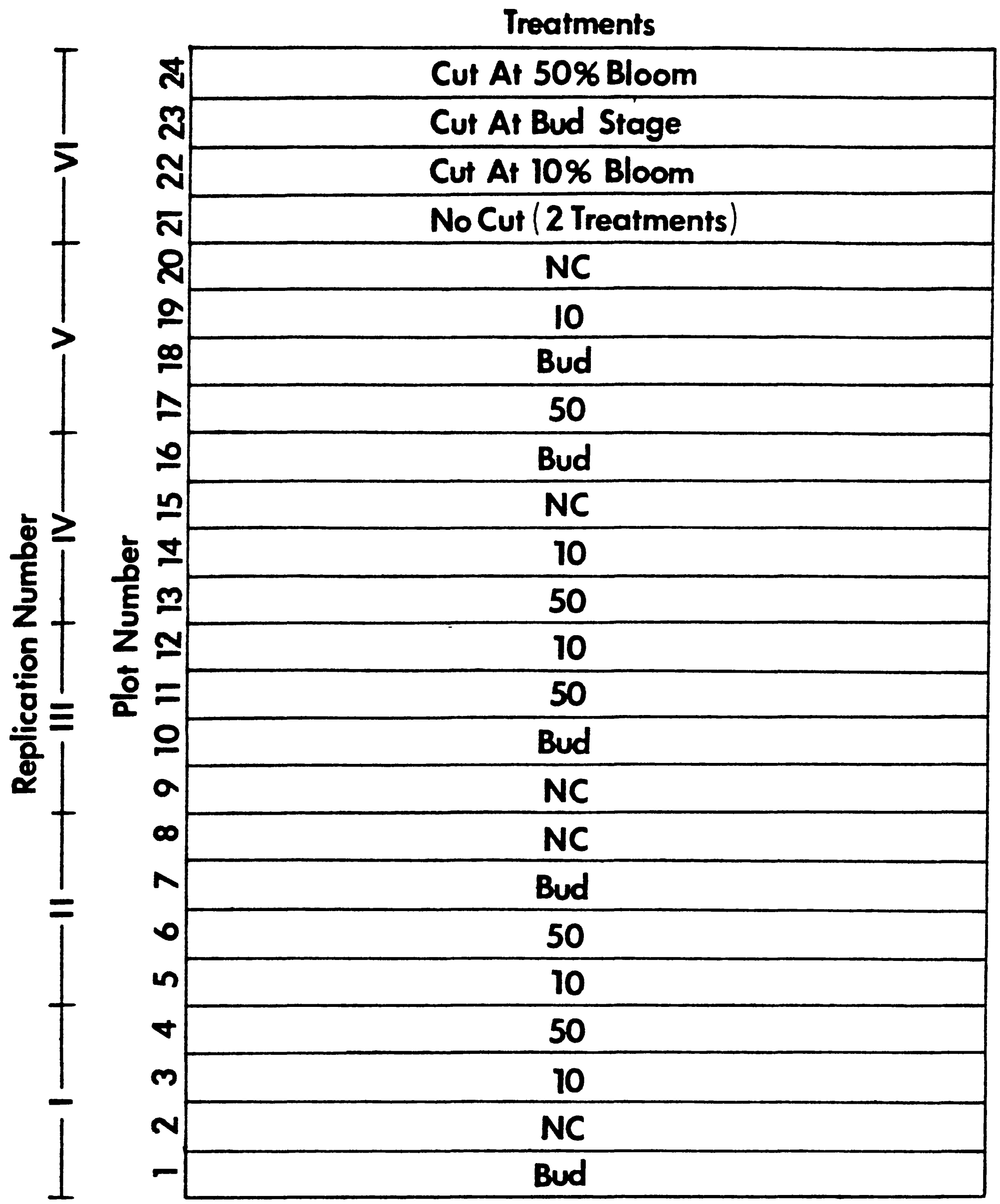

Figure 21. Randomized Complete Block Design for Field 3 (Woodward), 1984.

1) May 22 to June 12; Treatment 2) June 24 to July 15; Treatment 3) July 1 to July 22; Treatment 4) July 8 to July 29; and Treatment 5) August 19 to September 9. Overlapping of tagging dates occurred during bud cut, 10% bloom cut, and 50% bloom cut treatments, so a total of 14 weeks of tagging occurred. There was a two week period between 50% bloom cut and fall regrowth treatments where no racemes were tagged; the reason was that the harvest date of 50% bloom cut treatment was set by the field owner for August 20 which did not allow enough time for mature seeds and the fall regrowth treatment had not begun bloom. A total of 102 racemes (17 per replicate) were tagged in each treatment each week. Three samples were taken in each replication each week: sample A consisted of the 17 tagged racemes, sample B consisted of 17 racemes located apically next to the tagged racemes, and sample C consisted of 17 racemes located basally next to the tagged racemes. Thus a total of 18 samples were taken each week at field 3. Samples were processed in the same manner as in 1983.

At field 4, a 3x3 latin square design with three replications was used (Figure 22). Field size was 0.5 hectares and was divided into nine equally sized plots. Four treatments were studied: 1) Uncut, 2) Cut with first cutting of alfalfa for hay (May 8), 3) Cut with first and second cuttings of alfalfa for hay (June 18), and 4) Second seed crop from the uncut growth after a summer seed crop was harvested (July 19). Flowers were pollinated by naturally occurring pollinators. No insecticide was applied during the study. Tagging was done in the same manner as discribed for field 3. Weekly tagging dates for the four treatments were: Treatment 1) May 17 to June 7; Treatment 2) June 14 to July 5; Treatment 3) July 12 to August 2; and Treatment 4) August 9 to

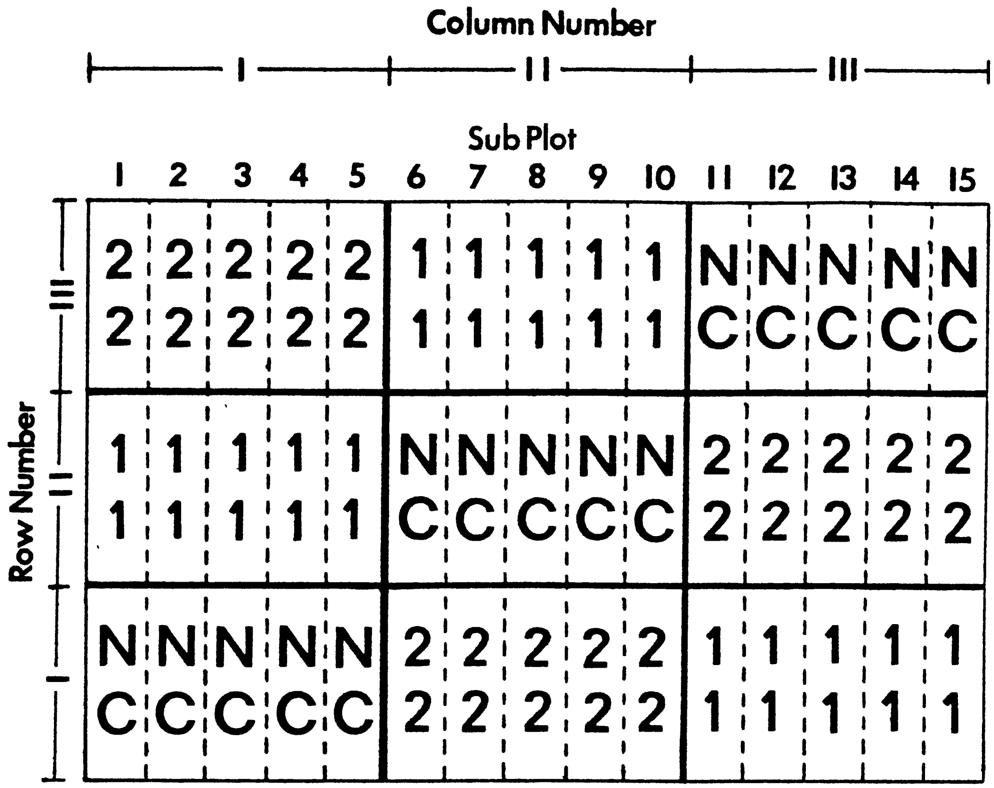

Figure 22. Latin Square Design for Field 4 (Chickasha), 1984.

August 30. No overlapping of tagging dates occurred. A total of 105 racemes (35 per replicate) were tagged each week in each treatment. Each replication was divided into five equally sized subplots and seven racemes were tagged in each subplot each week. The stems bearing the tagged racemes were harvested five weeks after the tagging occurred. Three samples were taken in each sampling plot each week; sample A consisted of the seven tagged racemes, sample B consisted of seven racemes located apically next to the tagged racemes, and sample C consisted of seven racemes located basally next to the tagged racemes. Thus a total of 15 samples were taken in each replication each week, making a total of 45 samples taken in each treatment each week. Samples were processed in the same manner as in 1983.

In 1984, the seeds were divided into five categories: 1) Seeds with emergence holes, 2) Seeds with diapausing chalcids, 3) Seeds with other chalcid damage (containing chalcid stages other than diapausing), 4) Sound seed, and 5) Other seeds (shrunken, not fully formed, or damaged seeds, with no sign of chalcid damage). Total number of ASC damaged seeds was calculated by adding together the numbers in categories 1 - 3. Total number of seeds produced was calculated by adding together the numbers in categories 1 - 5. Percentage of ASC damaged seeds was calculated by dividing total number of ASC damaged seeds by total number of seeds produced. Percentage of ASC that entered diapause was calculated by dividing number of seeds containing diapausing chalcids by total number of ASC damaged seeds.

Seed data from each field were analyzed using the appropriate test dictated by the field arrangement. Weekly means were calculated by totaling all samples taken during a week and dividing by number of samples taken during that week. A 95% confidence interval was calculated for each weekly mean. Treatment means were calculated by totaling all samples taken during that treatment and dividing by number of samples taken during that treatment. Treatment means were compared using the Least Significant Difference (LSD) analysis.

Results

In 1983, at field 1 (Stillwater) the range of numbers of alfalfa seeds per 100 racemes (Figure 23) was from 1143 seeds from racemes tagged on June 20 to a peak of 2124 seeds from racemes tagged on July

29, and at field 2 (Perry) the range was from 1559 from racemes tagged on August 20 to a peak of 2742 seeds from racemes tagged on August 6. Numbers of seeds not damaged by ASC per 100 racemes (Figure 24) ranged from 1037 seeds from racemes tagged on June 20 to a peak of 1900 seeds on July 29 and August 6 at field 1, and from 957 seeds from racemes tagged on August 20 to a peak of 2337 seeds from racemes tagged on August 6 at field 2. Percentage of ASC damaged seeds (Figure 25) ranged from 7.9% from racemes tagged on August 6 to a peak of 19.9% from racemes tagged on June 6 at field 1, and 5.4% from racemes tagged on July 15 to a peak of 38.1% from racemes tagged on August 20 at field 2. Percentage of ASC that entered diapause (Figure 26) ranged from

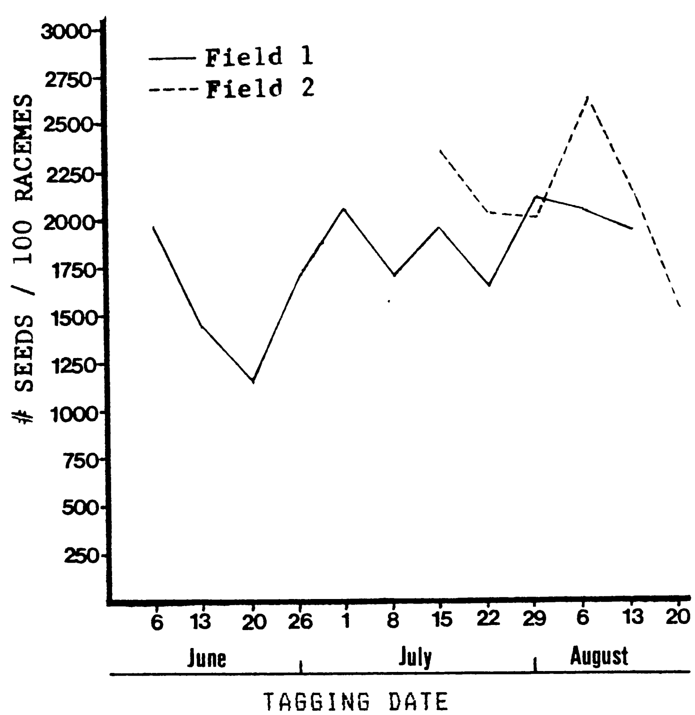

Figure 23. Mean Numbers of Alfalfa Seeds at Field 1 (Stillwater) and Field 2 (Perry), 1983.

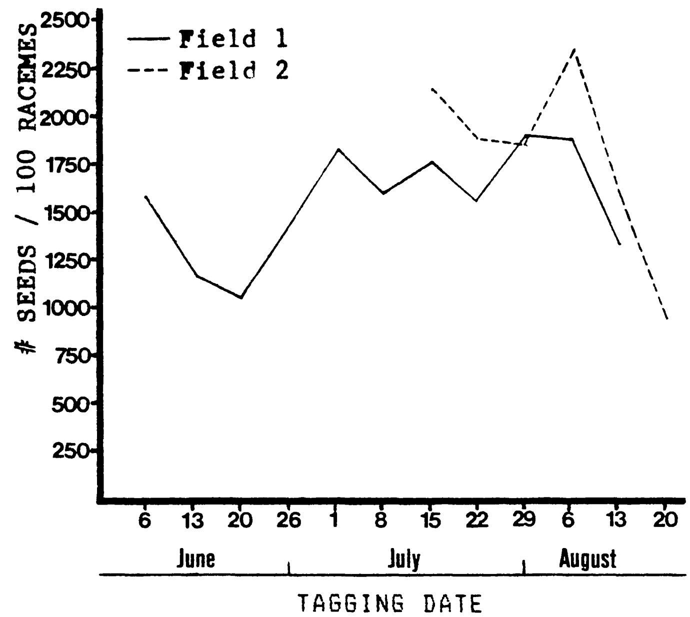

Figure 24. Mean Numbers of Alfalfa Seeds without ASC Damage at Field 1 (Stillwater) and Field 2 (Perry), 1983.

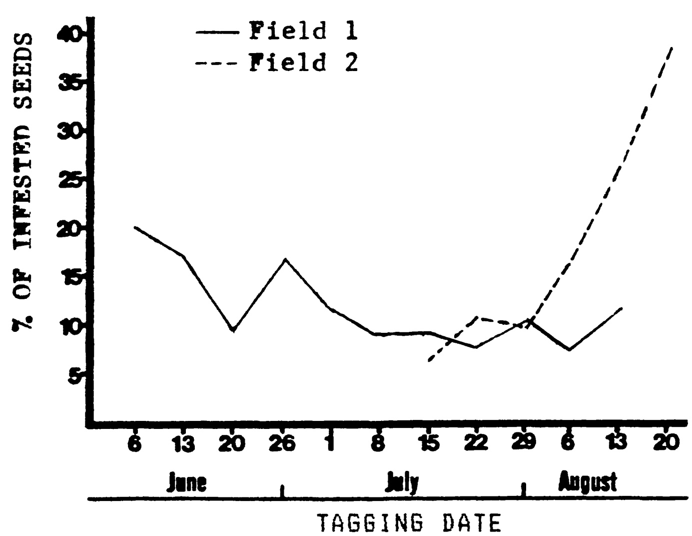

Figure 25. Percentages of ASC Damaged Alfalfa Seeds at Field 1 (Stillwater) and Field 2 (Perry), 1983

Figure 26. Percentages of ASC that Entered Diapause at Field 1 (Stillwater) and Field 2 (Perry), 1983

4.5% from racemes tagged on June 6-13 to a peak of 58.0% from racemes tagged on August 6-13 at field 1. and 20.3% from racemes tagged on July 8-15 to a peak of 72.0% from racemes tagged on August 13-20 at field 2.

In 1984, at field 3, the weekly means of the total numbers of alfalfa seeds produced in 17 racemes (Figure 27) showed similar patterns across time among the bud, 10% bloom, and 50% bloom cut treatments (a general inverted U shape), but dissimilar patterns from the uncut treatment (rising numbers) and second seed crop treatment (decreasing numbers). The uncut treatment produced significantly more seed than all the other treatments (Table XV), while the second seed crop and bud cut treatment produced significantly less seed (but not significantly less than each other).

Weekly means for numbers of sound alfalfa seeds in 17 racemes (Figure 28) closely followed the same pattern across time as described above for the total numbers of alfalfa seeds. The uncut treatment produced significantly more seed than all the other treatment means (Table XV), while the second seed crop and bud cut treatments produced significantly less seed (but not significantly less than each other).

Weekly means for total numbers of alfalfa seeds damaged by ASC (Figure 29) did not follow the same pattern as the total numbers of alfalfa seeds and numbers of sound seeds. Therefore the number of ASC damaged seeds appears to have been independent of the number of seed available. The 50% bloom cut treatment had significantly more ASC damage than any other treatment, while the bud cut treatment had significantly less (Table XV). The other three treatments did not have significantly different amounts of ASC damage from each other.

Weekly means for percentages of alfalfa seeds damaged by ASC (Figure 30) followed the same pattern as total numbers of alfalfa seeds damaged by the ASC. The 50% bloom cut treatment had significantly higher percentage of ASC damage than all the other treatments (Table XV). The No cut treatment had a significantly lower percentage of ASC damage than the 50% bloom and second seed crop treatments.

Weekly means for number of alfalfa seeds with emergence holes (Figure 31) closely followed the pattern of the total numbers of alfalfa seeds damaged by ASC. The 50% bloom cut treatment had significantly

TABLE XV

EFFECT OF PRODUCTION SCHEDULE ON SEED YIELD AND DAMAGE BY ASC AT FIELD 3 (WOODWARD) IN 1984 *

# Seeds / 17 Racemes	Uncut	Plant Stage At Hay Harvest			Second Seed Crop	LSD (0.05)
		Bud	10% Bloom	50% Bloom		
Total Seeds	431.6	301.6	390.3	381.5	319.1	32.7
Sound Seeds	376.5	265.3	338.2	302.2	278.8	22.3
Damaged by ASC	31.5	23.0	31.7	51.7	30.4	5.3
% ASC Damage	7.3	8.4	8.4	13.2	9.4	1.5
With Emergence Holes	26.5	20.4	26.7	44.6	7.2	7.8
With ASC in Diapause	1.9	0.3	1.5	3.4	21.8	0.8
% of ASC in in Diapause	5.8	0.7	3.8	6.9	68.2	1.4
With ASC Stage not in Diapause	3.1	2.3	3.5	3.7	1.4	1.1
Damaged By Agent Other than ASC	23.6	13.3	20.4	27.6	9.9	3.9

* Each value is a mean of 18 samples / week over a four week period

Seed produced from regrowth after first seed harvest of uncut alfalfa

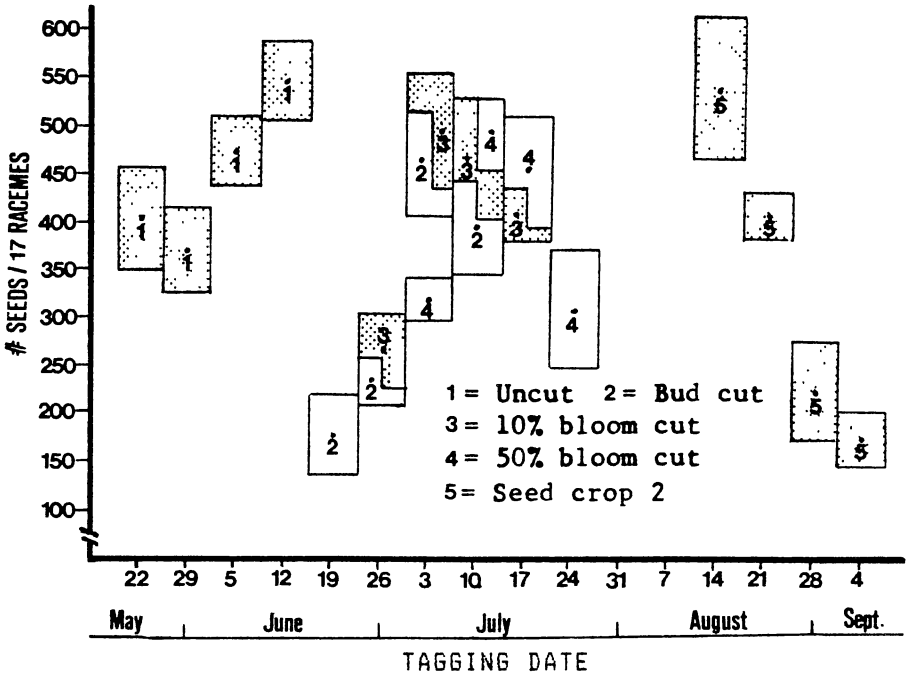

Figure 27. Total Numbers of Alfalfa Seeds in 17 Racemes at Field 3 (Woodward), 1984

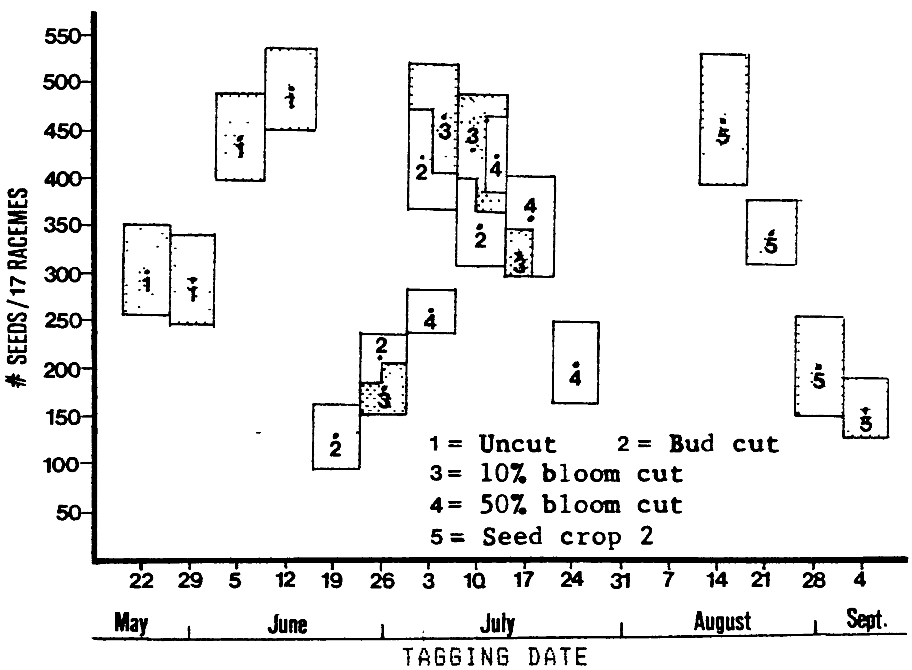

Figure 28. Numbers of Sound Alfalfa Seeds in 17 Racemes at Field 3 (Woodward), 1984

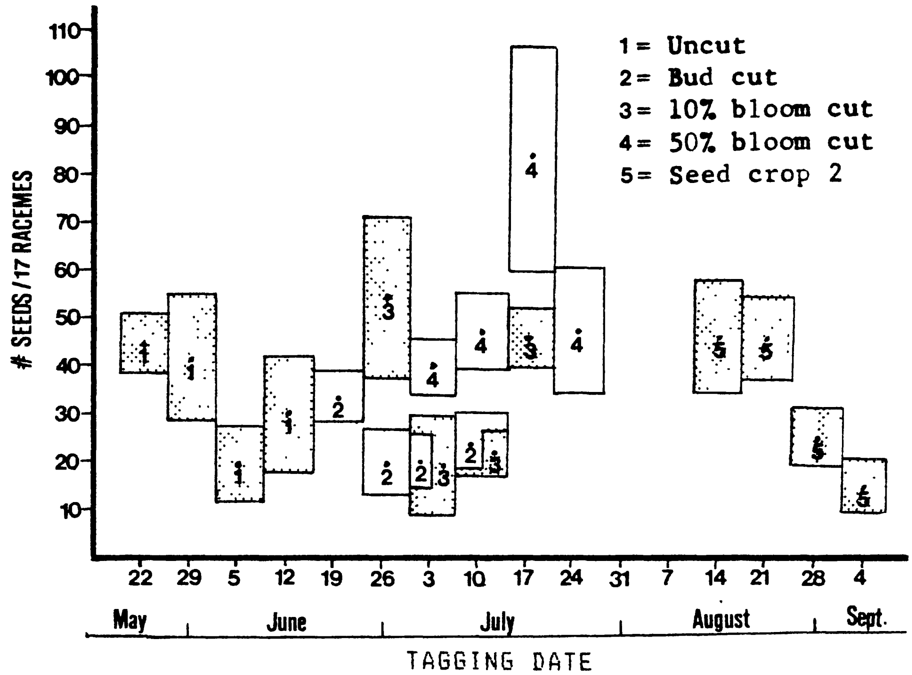

Figure 29. Total Numbers of Alfalfa Seeds Damaged by the ASC at Field 3 (Woodward), 1984

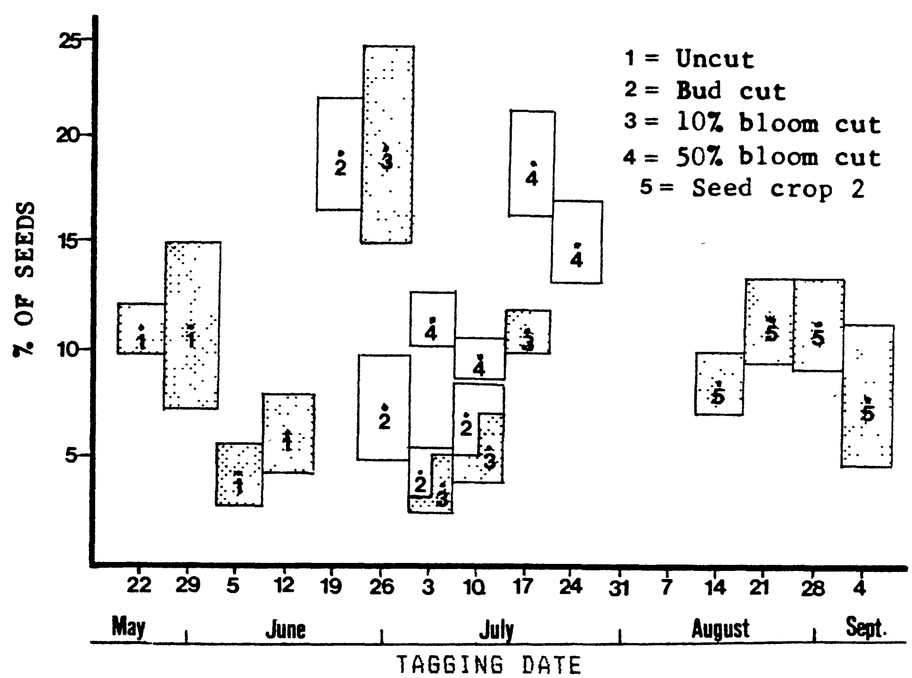

Figure 30. Percentages of Alfalfa Seeds Damaged by the ASC at Field 3 (Woodward), 1984

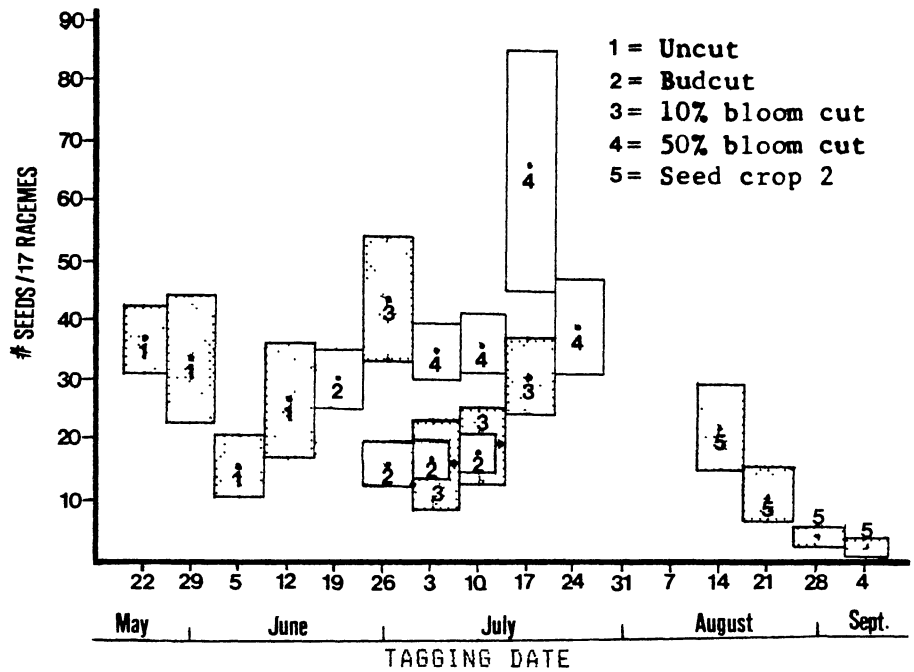

Figure 31. Numbers of Alfalfa Seeds with Emergence Holes at Field 3 (Woodward), 1984

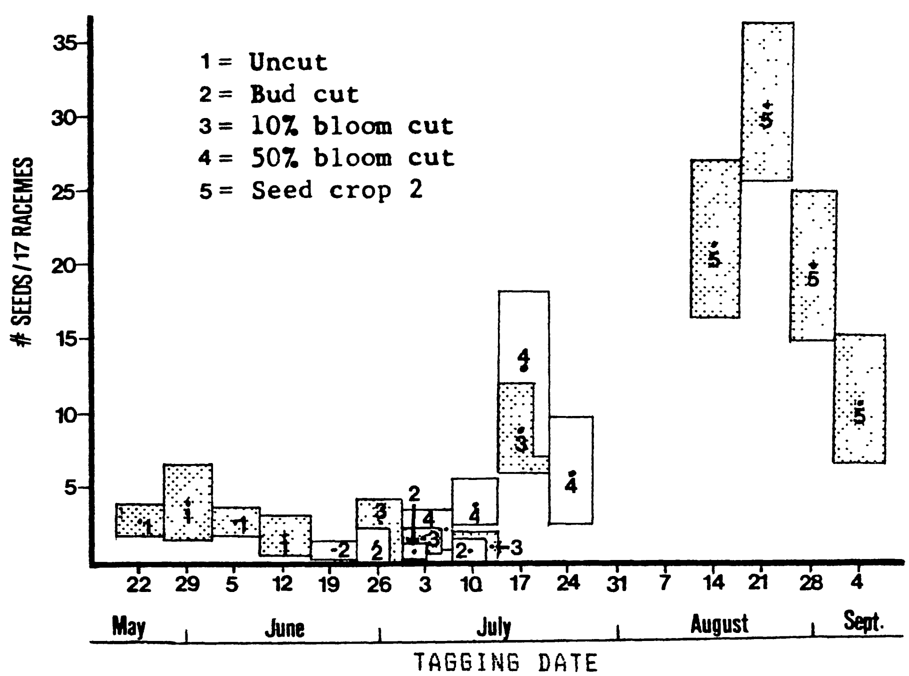

Figure 32. Numbers of Alfalfa Seeds Containing Diapausing ASC at Field 3 (Woodward), 1984

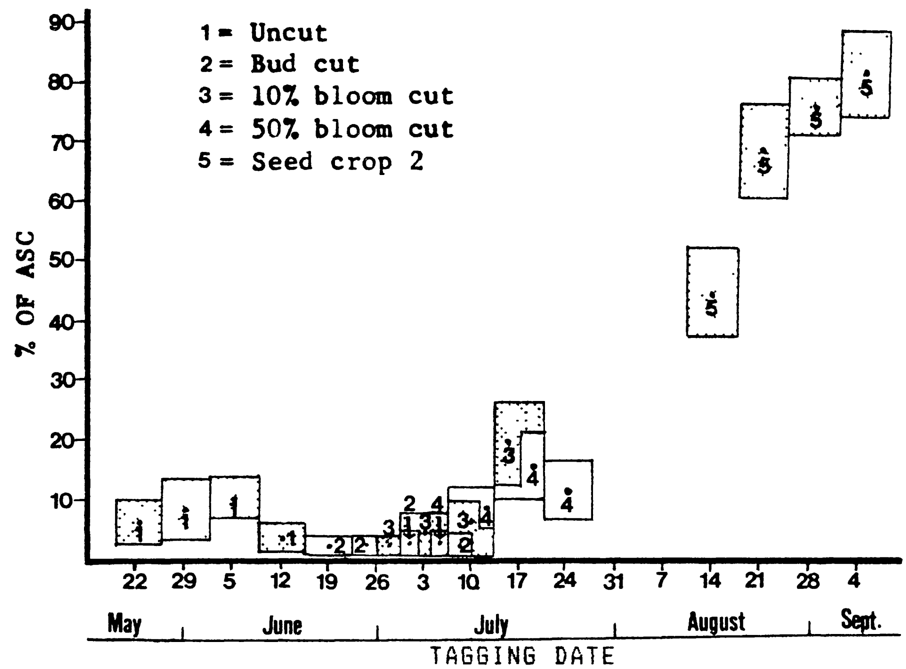

Figure 33. Percentages of ASC that Entered Diapause at Field 3 (Woodward), 1984

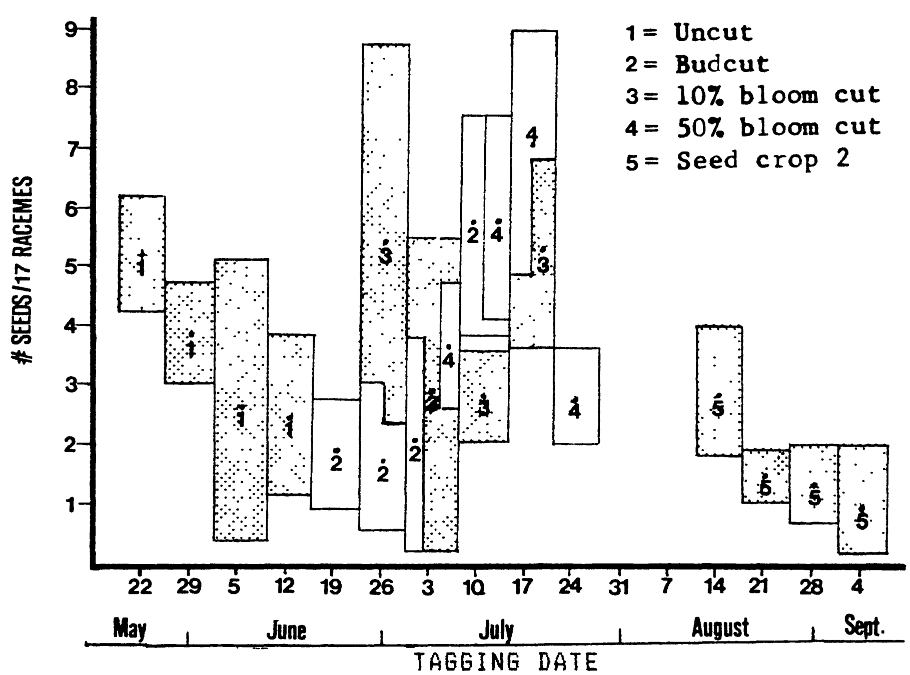

Figure 34. Numbers of Alfalfa Seeds Containing ASC Stages other than Diapausing Prepupae at Field 3 (Woodward), 1984

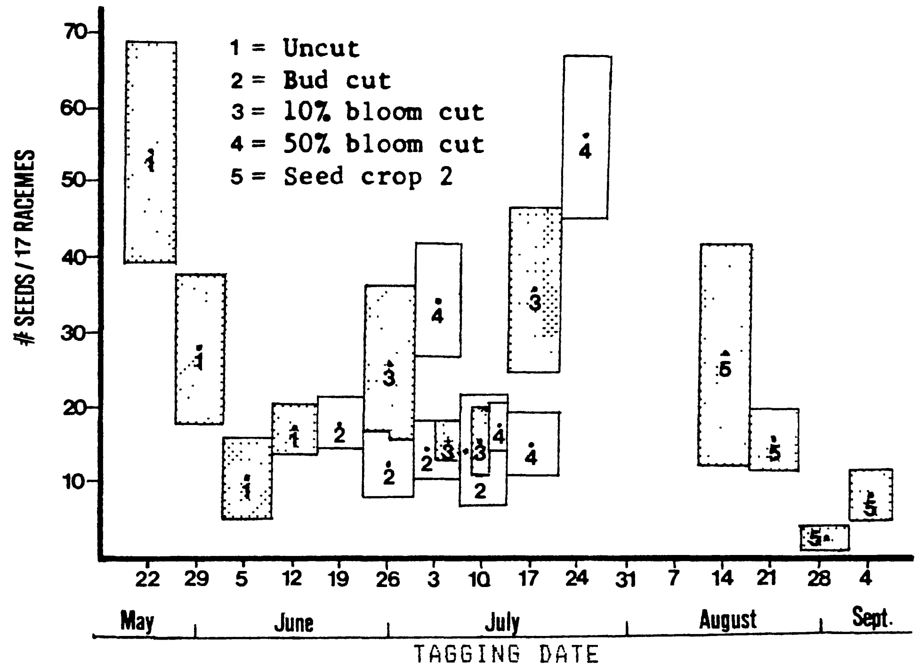

Figure 35. Numbers of Alfalfa Seeds Damaged by Agents other than ASC at Field 3 (Woodward), 1984

more seeds with emergence holes than any other treatment, while the second seed crop treatment had significantly less (Table XV). The other three treatments did not have significantly different numbers of seeds with emergence holes.

Weekly means for number of alfalfa seeds containing diapausing ASC (Figure 32) were very low until after mid-July and the pattern across time was not related to management. The second seed crop treatment had significantly more diapausing ASC than all other treatments, and the bud cut treatment had significantly less (Table XV).

Weekly means for percentages of ASC that entered diapause (Figure 33) followed the same pattern as the numbers of alfalfa seeds containing diapausing ASC. The second seed crop treatment had a significantly higher percentage of ASC in diapause than any other treatment, and the bud cut treatment had a significantly lower percentage (Table XV).

Weekly means for number of alfalfa seeds containing ASC stages other than diapausing prepupae (Figure 34) mainly followed the same pattern as the total numbers of alfalfa seeds damaged by the ASC, but the numbers were more variable. The 50% bloom cut and 10% bloom cut treatments had significantly more seeds containing ASC stages other than diapausing prepupae than the bud cut and second seed crop treatments (Table XV).

Weekly means for numbers of alfalfa seeds damaged by agents other than ASC (Figure 35) had such wide variation within a treatment that the management system did not appear to have an effect. The 50% bloom treatment had significantly more damage than all but the no cut treatment (Table XV). The bud cut and second seed crop treatments had significantly less damage than all the other treatments (but were not significantly different from each other).

At field 4, weekly means for total numbers of alfalfa seeds produced in seven racemes (Figure 36) show a seasonal pattern caused by time of year and no consistent pattern between any of the treatments. Each treatment mean was significantly different (Table XVI), with the two hay harvests treatment producing significantly more seed and the one hay harvest treatment producing significantly less.

Weekly means for numbers of sound alfalfa seeds (Figure 37) closely followed the pattern of the total numbers of alfalfa seeds. Each treatment mean was significantly different (Table XVI), with the

TABLE XVI

EFFECT OF PRODUCTION SCHEDULE ON SEED YIELD AND DAMAGE BY ASC AT FIELD 4 (CHICKASHA) IN 1984 *

# Seeds / Seven Racemes	Uncut	Number of Hay Harvests: One	Number of Hay Harvests: Two	Second Seed Crop	LSD (0.05)
Total Seeds	71.9	44.0	168.6	116.7	15.9
Sound Seeds	58.0	26.5	114.9	83.5	12.7
Damaged by ASC	5.4	7.3	40.9	26.9	5.3
% ASC Damage	7.7	18.1	24.6	21.7	4.6
With Emergence Holes	3.7	5.5	33.4	16.8	3.1
With ASC in Diapause	0.02	0.1	1.2	5.3	1.0
% of ASC in Diapause	0.2	0.8	2.9	29.1	1.3
With ASC Stage not in Diapause	1.6	1.7	6.4	4.9	2.5
Damaged by Agent other than ASC	8.6	10.3	12.7	5.5	2.7

* Each value is a mean of 45 samples / week over a four week period

Seed produced from regrowth after first seed harvest of uncut alfalfa

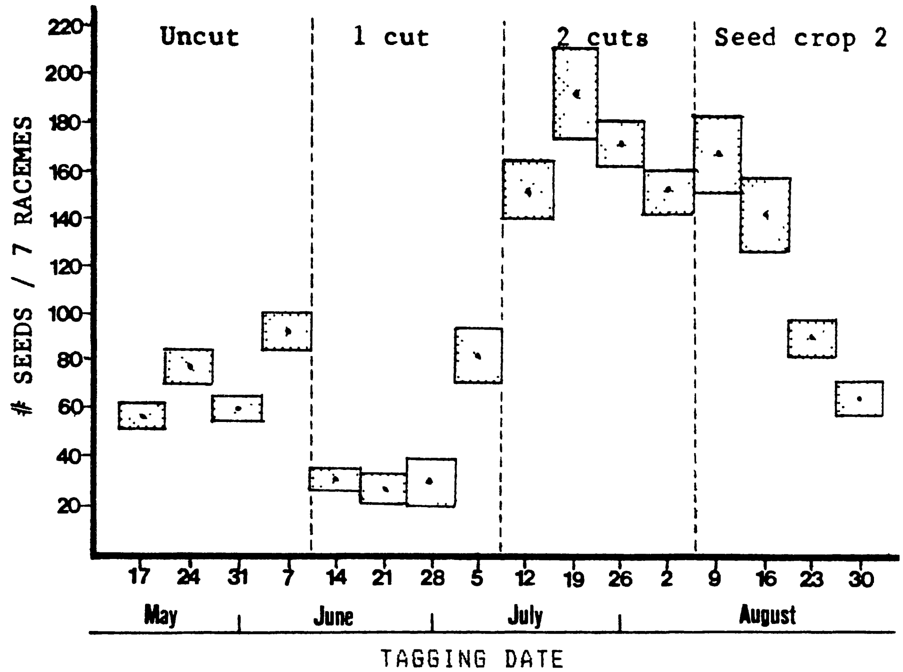

Figure 36. Total Numbers of Alfalfa Seeds in Seven Racemes at Field 4 (Chickasha), 1984

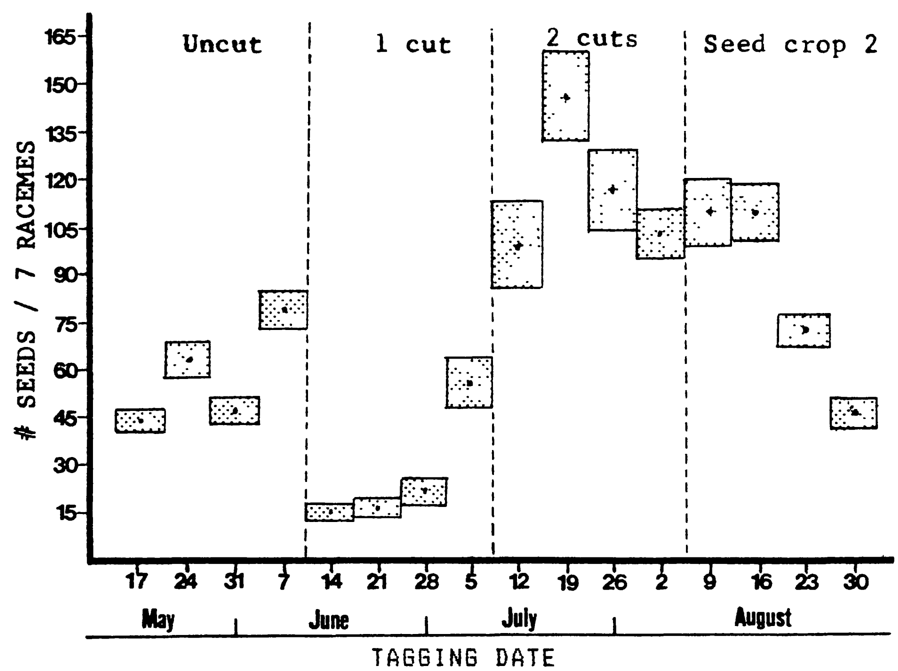

Figure 37. Numbers of Sound Alfalfa Seeds in Seven Racemes at Field 4 (Chickasha). 1984

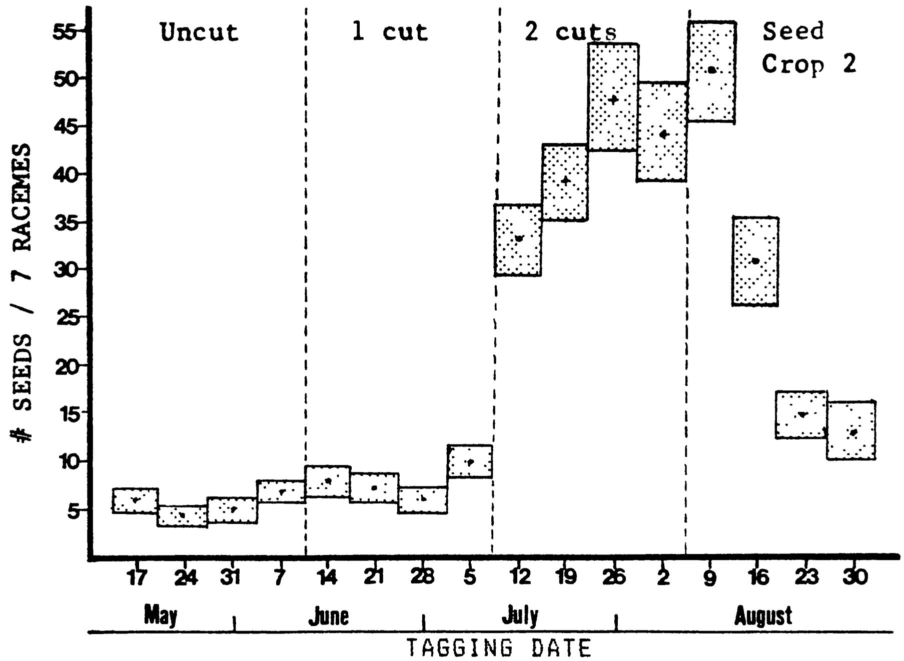

Figure 38. Total Numbers of Alfalfa Seeds Damaged by the ASC at Field 4 (Chickasha), 1984

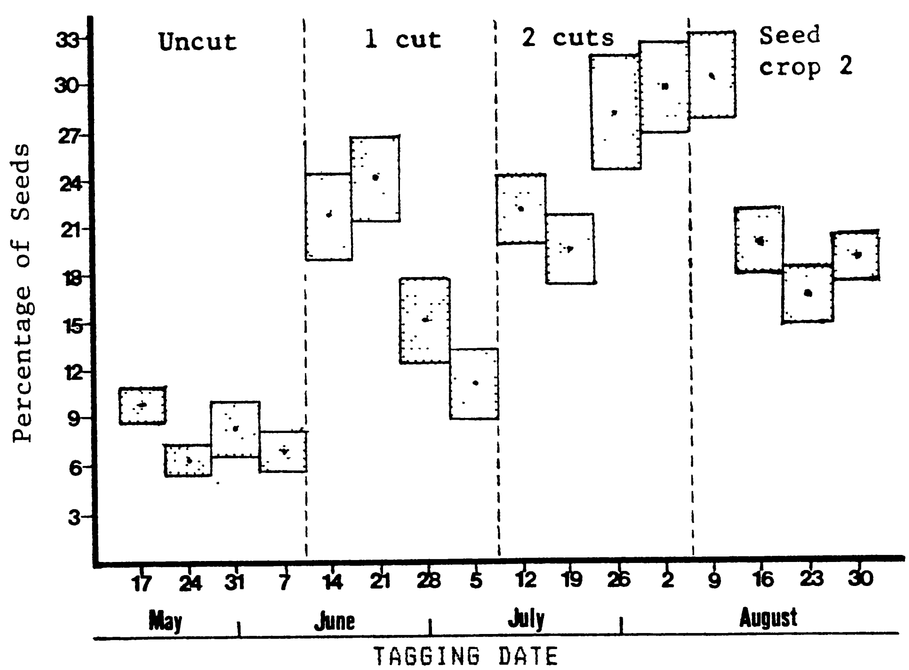

Figure 39. Percentages of Alfalfa Seeds Damaged by the ASC at Field 4 (Chickasha), 1984

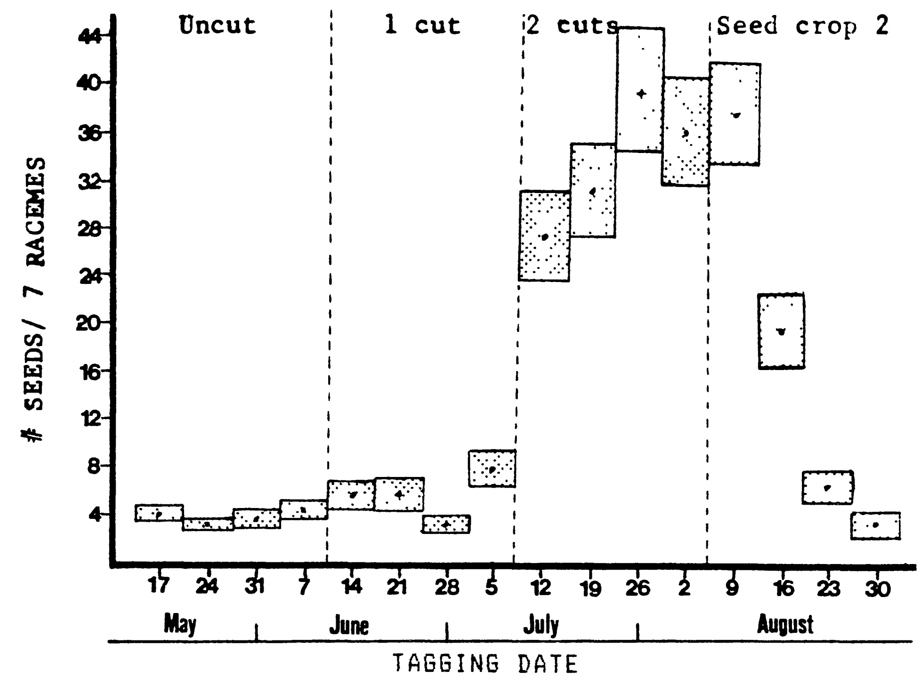

Figure 40. Numbers of Alfalfa Seeds with Emergence Holes at Field 4 (Chickasha), 1984

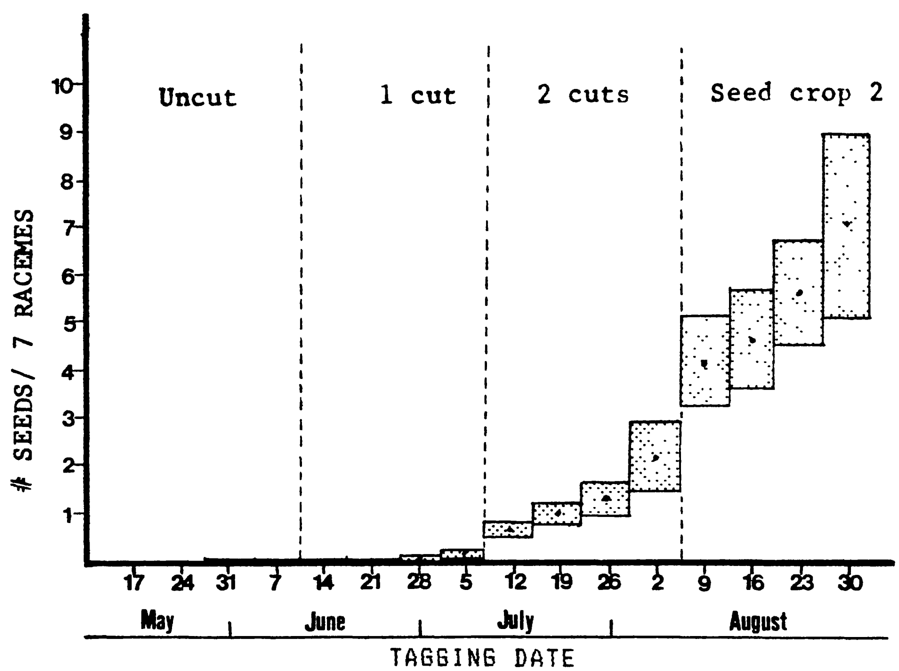

Figure 41. Numbers of Alfalfa Seeds Containing Diapausing ASC at Field 4 (Chickasha), 1984

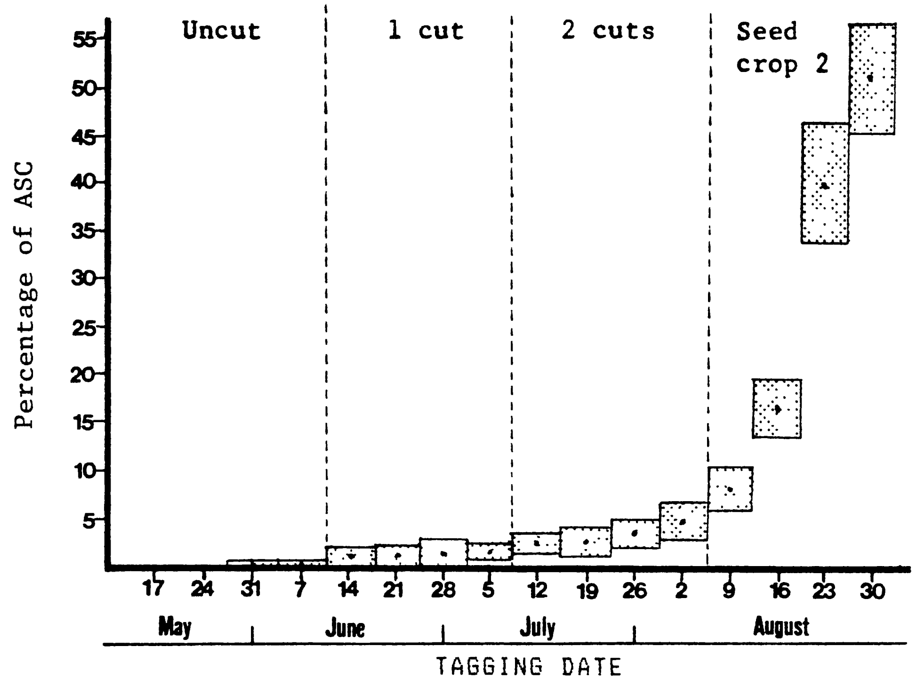

Figure 42. Percentages of ASC that Entered Diapause at Field 4 (Chickasha), 1984

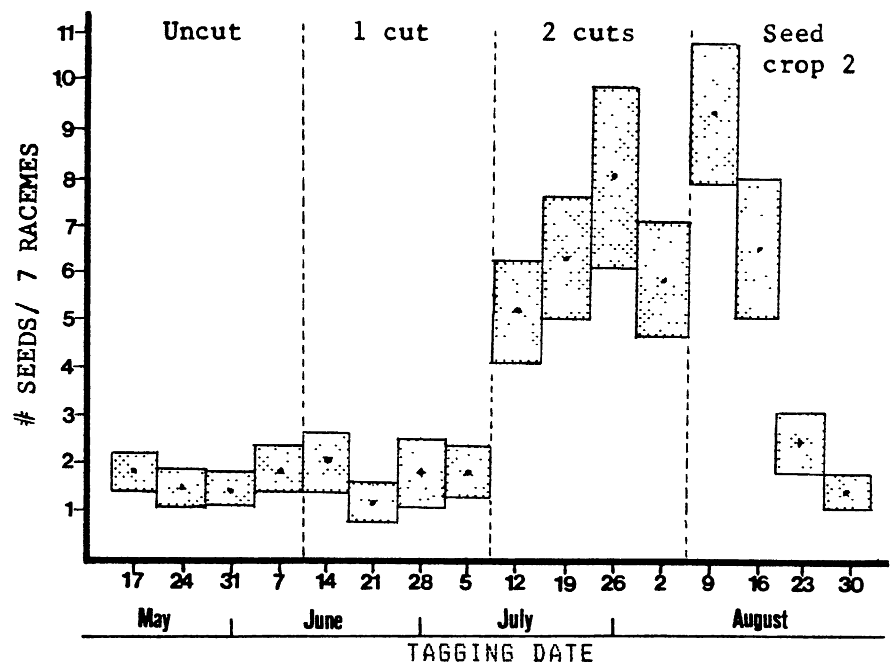

Figure 43. Numbers of Alfalfa Seeds Containing ASC Stages other than Diapausing Prepupae at Field 4 (Chickasha), 1984

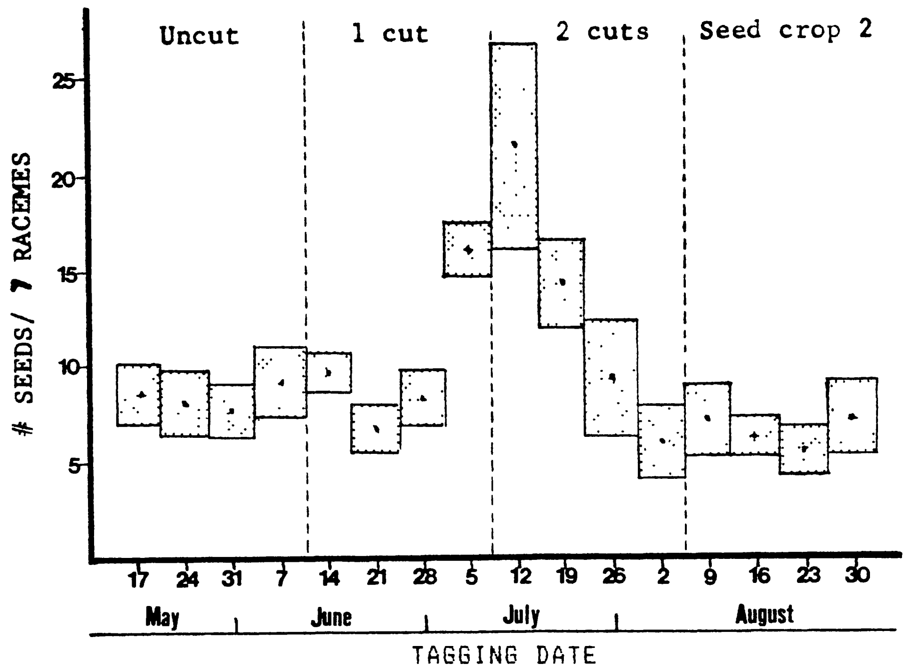

Figure 44. Numbers of Alfalfa Seeds Damaged by Agents other than ASC at Field 4 (Chickasha), 1984

two hay harvests treatment producing significantly more seed and the one hay harvest treatment producing significantly less.

Weekly means for total numbers of alfalfa seeds damaged by the ASC (Figure 38) followed the pattern of the total numbers of alfalfa seeds. The two hay harvests treatment had significantly more ASC damaged seeds than all other treatments (Table XVI). The one hay harvest and uncut treatments had significantly less ASC damage than the other treatments, but not significantly different from each other.

Weekly means for percentages of alfalfa seeds damaged by the ASC (Figure 39) had a pattern that was basically similar to the total number of seeds damaged by the ASC, except for several weeks with higher values. The two hay harvests treatment had a significantly higher percentage of ASC damaged seeds than the one hay harvest and uncut treatments, and the uncut treatment had a significantly lower percentage than any other treatment mean (Table XVI).

Weekly means for numbers of alfalfa seeds with emergence holes (Figure 40) closely followed the same pattern as the total number of ASC damaged seed. The two hay harvest treatment had significantly more seeds with emergence holes than all other treatments (Table XVI). The uncut and one hay harvest treatments had significantly less seeds with emergence holes than the other treatment means, but were not significantly different from each other.

Weekly means for numbers of alfalfa seeds containing diapausing ASC (Figure 41) were very low until after mid-July and the pattern across time was not related to management. The second seed crop treatment had significantly more seeds containing diapausing ASC than any other treatment (Table XVI). The one hay harvest and uncut treatments had significantly less diapausing ASC than the other treatments, but were not significantly different from each other.

Weekly means for percentages of ASC that entered diapause (Figure 42) closely followed the same pattern as the numbers of seeds containing diapausing ASC. The second seed crop treatment had a significantly higher percentage of diapausing ASC than any other treatment (Table XVI). The one hay harvest and uncut treatments had significantly lower percentages of diapausing ASC than the other treatments, but not significantly different from each other.

Weekly means for numbers of alfalfa seeds containing ASC stages other than diapausing prepupae (Figure 43) closely follows the same pattern as total number of alfalfa seed damaged by the ASC. The two hay harvest and second seed crop treatments had significantly more seed containing other stages of the ASC than the one hay harvest and uncut treatments (Table XVI).

Weekly means for numbers of alfalfa seeds damaged by agents other than ASC (Figure 44) does not show a pattern across time that was related to management. The two hay harvest treatment had significantly more seeds damaged by other agents than the uncut and second seed crop treatments (Table XVI). The second seed crop treatment had significantly less seeds damaged by other agents than any other treatment.

Discussion

In Oklahoma, production of alfalfa seed has been considered a by product of the hay production operation. The usual procedure is to harvest one or two hay crops from a field and then let the regrowth mature into a seed crop in late summer. The timing and amount of rainfall are major factors in determining if and when a producer decides to produce a seed crop during a particular season. If moisture is limited, and the prospects of a good hay crop are poor, then the stand is allowed to mature into a seed crop (Sholar et al. 1982).

In 1983 the highest numbers of alfalfa seeds without chalcid damage were produced from plants flowering in late July and early August. During this same time period the percentage of ASC infested seeds was relatively low indicating that the ASC did not attack these seeds to a great extent. The percentage of infested seeds remained low at field 1 throughout the season while at field 2 the percentage increased sharply in late August. There are several explanations for the low infestation at field. Erdelyi et al. (1981) reported that damage by ASC increased according to how erect the plant was, and the plants in Field 1 were lodging plants 90-125 cm long at the time the samples were taken. Strong et al. (1963) studied ASC dispersal from fields with deteriorating conditions and reported that most ASC flew upwards until a breeze caught them and carried them away, so the ASC may have left the field due to its poor condition. Field 2 was in much better condition than field 1 because it had only one layer of lodged growth which could explain why the infestation increased at that field.

In 1984, at field 3, the field management involved cutting the alfalfa at various stages of bloom and letting the regrowth mature into a seed crop. Results showed the alfalfa that was not cut, but allowed to produce a mature seed crop by July was significantly higher in total number of alfalfa seeds produced and total number of sound seeds than other treatments. Honeybees were used for pollination and could be one of the reasons the most productive seed crop occurred early. The actual yield from this field was obtained from the producer and the kg / ha production of seed from each management system followed the same pattern that was obtained in this study except the second seed crop had the lowest production. Thus the sampling method of taking seeds / raceme was a good estimate of yield at this field. This early seed crop also had significantly lower percentage of ASC damaged seed than two produced later. The mean percentage of ASC damaged seed in the no cut seed crop was 7.3%, which is slightly lower than the range of 10% to 30% ASC damage in early season seed crops reported by Urbahns (1914). The pattern of numbers of ASC damaged seeds appeared to be independent of the pattern of number of alfalfa seeds produced.

At field 3, a significantly higher percentage of ASC damaged seeds occurred in the alfalfa that was cut in the 50% stage of bloom, with the regrowth flowering during July, and the resulting seed crop maturing in August. This agrees with Bacon et al. (1959), and Sorenson (1930) who reported higher ASC damage in late season seed. Mean percentage of ASC damaged seed in the 50% bloom cut treatment was 13.2% which is slightly lower than the range of 20% to 70% for late season seed crops reported by Urbahns (1914).

Bacon et al. (1964) reported that the ASC may enter diapause throughout the summer, this agrees with the data collected from all four fields in both years, where the ASC was found entering the diapausing stage throughout the sampling period of June 6 to August 20 in 1983 and May 17 to September 4 in 1984. The seasonal trend of percentage of ASC entering diapause was increasing percentages as the season progressed agrees with the findings of Sorenson (1930) and Antonova and Bazyleva (1974) who reported most diapausing ASC came from the late seed crop.

Field 4 was managed to produce a seed crop from the regrowth corresponding to each of the normal hay harvests. Results for the different cutting times showed that the seed crop produced after two hay harvests had significantly higher total numbers of alfalfa seeds and number of sound seeds. These results appear to support the traditional method of seed production in Oklahoma as mentioned above, but since total seed production of the field was not sampled this may be an artifact of the sampling method used. The pattern of numbers of ASC damaged seeds followed the pattern of the numbers of alfalfa seed produced. The field was pollinated by natural pollinators (mainly species of wild bees) which frequently have inadequate population numbers for pollination until late summer. The presence of adequate pollinators could be a reason why more seeds were produced after the second hay harvest instead of during the earlier treatments.

The results also showed that late season timing for seed production in field 4 also had significantly higher percentages of ASC damaged seed with a mean of 24.6% from the seed crop after two hay harvests and 21.7% in the fall regrowth seed crop. In one particular sample of August 2, 49.3% of the seeds were damaged. These figures agree with the 20% to 70% range of ASC damaged seeds for late season seed crops reported by Urbahns (1914).

The results showed that in Oklahoma, a late season alfalfa seed crop had significantly higher percentage of ASC damaged seed and percentage of ASC that entered diapause than other seed crops. Another potential problem is that the populations of native pollinators are not constant from year to year and may be inadequate during a given year. Despite the problems it appears that the traditional method of harvesting one or two hay crops and then letting the regrowth mature a seed crop in late summer produced significantly higher numbers of sound seed. If the producer has the resources to provide pollinators such as honeybees it appears that the significantly higher numbers of sound seed can be produced in early summer which had the significantly lower percentages of ASC damaged seed during this study.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

In Oklahoma, the ASC spring emergence started between the middle of April and early May. Peak ASC emergence occurred during the month of June, with most of the emergence concentrated near the middle of the month. The emergence ended in early August. The male ASC emerged in greater numbers during the early weeks, and the number of female ASC dominated later. The overall male to female ASC ratio was 1:1.5.

The first adult ASC were collected from alfalfa fields in mid-May. High numbers of the ASC generally occurred during the month of August. Female ASC were collected in greater numbers than the males. The ASC had from four to six generations in Oklahoma.

A total of five species of ASC parasites emerged in Oklahoma; *L. perplexus*, *L. insuetus*, *E. allynii*, *T. bruchophagi*, and *T. maculatus*. The most common parasitic species were *L. perplexus* and *T. bruchophagi*. The peak emergence of parasites was very concentrated and occurred one to two weeks after the greatest emergence of the ASC. The male to female sex ratio for *L. perplexus* was 1:1.25 and for *T. bruchophagi* was 1:1.75. Several high percentages of parasitism were found which seems to indicate that parasites may be an important regulatory factor on ASC populations.

The second year emergence from the alfalfa seeds was predominantly ASC parasites. *L. perplexus* was the dominant species. A higher concentration of males occurred in the ASC and *L. perplexus* that emerged.

Populations of parasites were not large until late in the season, when they frequently became more numerous than the ASC. Female parasites were collected in greater numbers than males. The dominant species of ASC parasite in the field was *L. perplexus*.

The results showed that a late season alfalfa seed crop had significantly high percentages of ASC damaged seeds and percentages of ASC that entered diapause. Despite these problems, the traditional method of producing alfalfa seed in Oklahoma (combined hay and seed management) produced significantly higher total numbers of seeds, and numbers of sound seeds. Early season seed crops had significantly lower ASC damage and percentages of ASC that entered diapause. When pollinators were present the early season seed crop also had significantly high numbers of sound seeds.

Conclusions

1. ASC spring emergence started near the end of April and peaked in mid-June. Male to female sex ratio was 1:1.5.

2. Adult ASC were collected in the field from mid-May through the end of September with high population densities occurring during the month of August. Females were collected in greater numbers than males. The ASC had four to six generations in Oklahoma.

3. Five species of parasites were found in Oklahoma. *Liodontomerus perplexus* was the dominant parasitic species and *T. bruchophagi*

was also common. The other species of parasites occurred very rarely.

4. Second year emergence from seeds was predominantly parasites, with *L. perplexus* the most numerous species.

5. Several high percentages of parasitism were found which indicates that parasites may be an important regulatory factor on ASC populations.

6. The highest densities of parasites were collected in late season field samples and *L. perplexus* was the dominant parasitic species. Female parasites were collected in greater numbers than males.

7. Early season alfalfa seed crop production was shown to have significantly lower percentage of ASC damaged seed and percentage of ASC in diapause. Late season seed crops had significantly higher percentages of ASC damaged seed and percentage of ASC in diapause.

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APPENDIX

PICTURE KEY TO THE ALFALFA SEED CHALCID AND ITS PARASITE SPECIES IN OKLAHOMA

This key is designed to help identify the species of parasitic Hymenoptera that emerge from alfalfa seeds. The sources for the descriptions used in the key are Butler and Hansen (1958) and Butler et al. (1968).

1. Body black, thoracic notum with thimble like projections. Antennae simple in female (Figure 45) or with flagellar segments quadrate and bearing long, erect setae in the male (Figure 46).
   Family Eurytomidae..................*Bruchophagus roddi* Gussakovskii

   Body metallic, thoracic notum withiut thimble like projections 2

2. Mid tarsus with a ventral comb of short black spines (Figure 47).
   Family Eupelmidae........................*Eupelmus allynii* (French)

   Midtarsus without a ventral comb of short black spines.......... 3

3. Ovipositor long, projecting for a distance at least 1/2 as great as length of abdomen... 4

   Ovipositor projecting only slightly............................ 5

4. Head and thorax bronze-green, abdomen bronze. Female (Figure 48) has long ovipositor, with sheaths 1/2 to 2/3 as long as abdomen, apical segments split on ventral side. Male (Figure 49) has a short projection at apex of abdomen, and apical segments not split.
   Family Torymidae......................*Liodontomerus perplexus* Gahan

   As above, but with sheaths longer than the abdomen in the female (Figure 50). Males of this species unknown.
   Family Torymidae......................*Liodontomerus insuetus* Gahan

   Ovipositor length comparison between *L. perplexus* and *L. insuetus* in Figure 51.

5. Mesonotum with a median groove, scuttellum with four parallel grooves (Figure 52). Body metallic green. Abdomen evenly tapered to tip with apical segments split on the ventral side in female (Figure 39) or more truncate, with a short median projection and apical segments not split in male.
Family Eulophidae....................*Tetrastichus bruchophagi* Gahan

Mesonotum and scutellum without grooves........................ 6

6. Head end thorax black to blue with four metallic blue spots anteriorly (Figure 53) apical segments of the abdomen split on the ventral side in the female (Figure 54) or with a short projection at the apex of abdomen and apical segments not split in male.
Family Pteromalidae...................*Trimeromicrus maculatus* Gahan

Figure 45. Female *Bruchophagus roddi*

Figure 46. Male *Bruchophagus roddi*

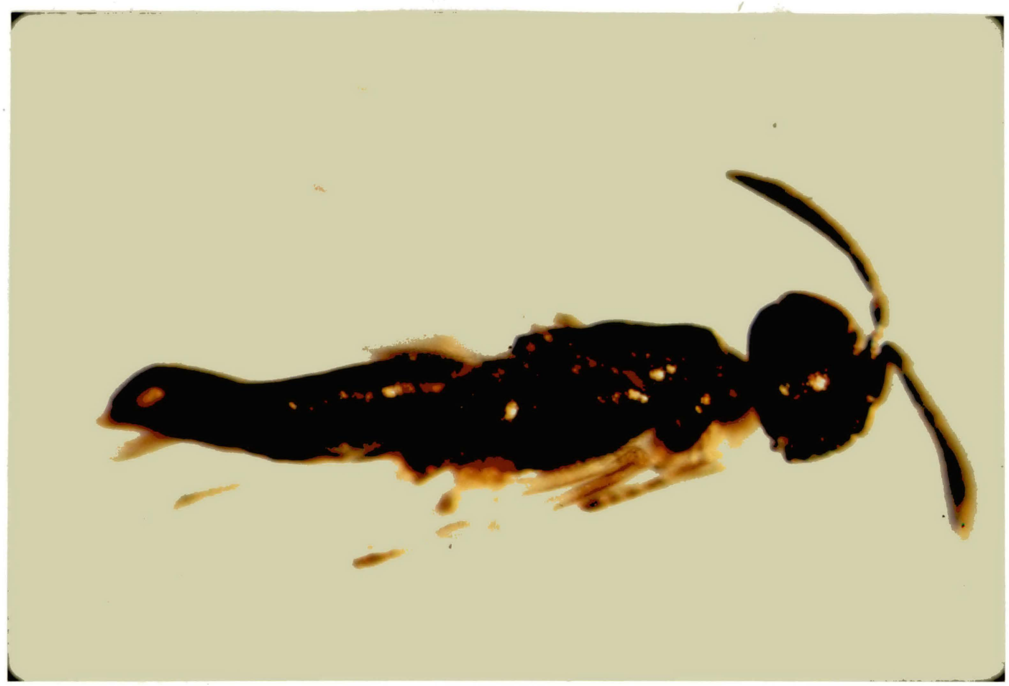

Figure 47. *Eupelmus allynii*

Figure 48. Female _Liodontomerus perplexus_

Figure 49. Male _Liodontomerus perplexus_

Figure 50. Female *Liodontomerus insuetus*

Figure 51. Ovipositor Length Comparison between *L. perplexus* (bottom) and *L. insuetus* (top)

Figure 52. Dorsal View of T. bruchophagi to show the Grooves on the Thorax

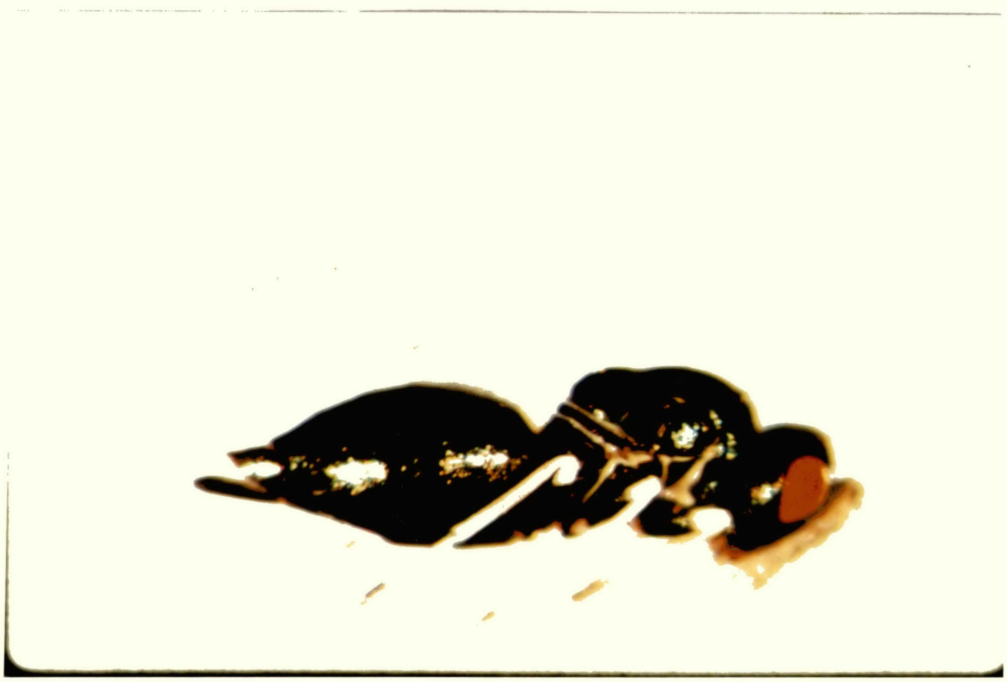

Figure 53. Female Tetrastichus bruchophagi

Figure 54. Dorsal view of _T. maculatus_ to Show the Blue Spots on the Thorax

Figure 55. Female _Trimeromicrus maculatus_

VITA

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