RELATIONSHIPS BETWEEN BRUCHID BEETLES (*Amblycerus robiniae*) AND HONEY LOCUST TREES (*Gleditsia triacanthos*).

by

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INTRODUCTION

Wickham (1895) was one of the first workers to associate the bruchid, Amblycerus robiniae, with the honey locust tree, Gleditsia triacanthos. Pierce (1908b) determined some parasites of Amblycerus, and Cushman (1911) worked on emergence times of this bruchid and its parasites, but since this early work little information has been published on Amblycerus. Craighead (1950) indicated that Amblycerus was distributed throughout the eastern part of the United States and that it is the only important bruchid associated with eastern forests.

This study concerns the relationships between the bruchid beetle Amblycerus robiniae and the honey locust tree Gleditsia triacanthos. Since Amblycerus concentrates specifically on Gleditsia, and on specific parts of this tree, it may influence plant density. Thus, such a phytophagous insect can effect and control the species composition and the structure of a plant community.

One of the most direct ways to determine the impact of insect populations on their host plants is to measure the amount and type of damage that the insects do to the host plant, and then infer what it would mean to the plant population not to sustain this damage. In this context the bruchid beetle vs. honey locust system is ideal for study, since the beetle damages the reproductive tissues (the seeds) of the host plant, and thereby has a direct influence on the number of offspring a honey locust tree is capable of producing. The influence of the plant on the insect population is in this case of a different sort, for the insect species is totally dependent on the plant. This insect-plant system is presumably a result of coevolution since while

the insects are evolving mechanisms to exploit the plants, the plants are evolving mechanisms to counteract the insect pressure.

Coevolutionary relationships between plants and herbivores have been studied by various authors (e.g. Janzen,1969 ; Ehrlich and Raven, 1965 ; Breedlove and Ehrlich, 1969). The present study is intended to provide details about the insect and plant involved in one presumed instance.

The family Bruchidae is thought to represent an adaptive radiation of a chrysomeloid group for feeding in seeds of Leguminosae (Bridwell, 1918). Most species lay their eggs on the developing seeds or pods of growing plants. The larva develops in the seed, eating the cotyledons ; in many species there is one bruchid per seed, while in others as many as 25 bruchids per seed are found or one larva eats several mature seeds (Johnson,1967 ; Howe and Currie,1964 ; Bridwell,1918).

Relatively few bruchids feed on plants other than Leguminosae ; these include members of about 20 families including Palmae and Compositae (Howe and Currie,1964). Bridwell (1952) indicates that bruchids infest the Anacardiaceae, affecting plant parts other than seeds. Notes on the host plants of bruchids and rearing procedures are given by Bottimer (1961).

The honey locust, Gleditsia triacanthos , grows naturally in the humid section of the eastern United States. It inhabits the borders of streams, bottom lands, and rocky hillsides, growing best on soils of limestone origin.

A seed crop is defined here as the total number of mature seeds produced by a single tree in one year. Samples of twenty-five pods

from each seed crop were collected from each tree except for within-tree comparisons where 225 pods were collected from each tree. These samples were collected from the ground if the pods had already fallen. As shown below, 14-100% of the seeds produced in one season by a honey locust tree are destroyed by the bruchid beetle. Comparable data on damage to other legumes by other bruchids has been reported. For example, Bruchus atrolineatus destroyed 11% of a seed crop of cowpea (Vigna unguiculata) (Prevett,1961) ; Mimosestes sallaei, 39.9% of Acacia farnesiana seeds (Hinckley,1960) ; and Bruchus baudoni, 61% of Acacia arabica seeds (Peake,1952). Janzen (1969) gives a substantial account of bruchid damage to seeds of Central American Leguminosae, showing 8 to 100% destruction of the crops of various species.

Locations of the experimental study areas used are in Leavenworth,Johnson,and Douglas Counties,Kansas, as follows : 1.) around Lone Star Lake,Lone Star ; 2.) 2 miles south of Lone Star ; 3.) Morris Farm, 1 mile southwest of Clinton ; 4.) 2 miles east of Lecompton ; 5.) Olathe ; 6.) the Sunflower Tract, 10 miles east of of Lawrence ; and 7.) the University of Kansas Natural History Reservation, 8 miles northeast of Lawrence. Local differences, e.g., in exposure, are discussed in the section that deals with assessing bruchid damage to honey locust seeds.

Biology Of The System

THE BRUCHID BEETLE , AMBLYCERUS ROBINIAE

Life Cycle

Adult : The adult Amblycerus is 7 to 8 mm in length, ovate, brown, with the pronotum and elytra conspicously but irregularly spotted with dark patches of hair. The adult emerges from a seed by cutting out a disc-shaped cap (which was started during its last larval instar) in the seed coat ; one bruchid develops per seed. Spring emergence in 1967 was about one month earlier than in 1969 (first week in April,1967, vs. first week in May, 1969). The warm spring of 1967 vs. the cold spring of 1969 appears to account for this difference.

Laboratory tests indicated that adults usually live from five to eight weeks. They eat pollen of honey locust trees and of other plants during spring flowering. They are active at night from dusk to daybreak (see below), flying among the honey locust trees, where the females oviposit on the pods.

Adults are sexually mature and ready to mate as soon as they emerge from seeds ; oviposition on immature or mature pods then follows within a day or two. Mating behavior includes a short period (two minutes) of the male brushing the female's dorsum with his front legs,plus continous tapping on the female's pronotum with his antennae. The male then mounts the female, and his antennae now continously tap the female's antennae. Copulation lasts from 30 seconds to two minutes. Females have been observed to copulate up to three times in the laboratory.

Of 1000 adults that emerged from seeds during fall emergence (1969) 474 were males and 526 females. This sex ratio is not significantly different from a one to one ratio. Males did emerge, on the average, one or two days before the females.

Seasonal History : The seasonal cycle is summarized in Figure 1. Adults emerge in the spring (April-June) from seeds that matured the previous summer and lay on the ground in pods through the winter. After mating many of them oviposit on mature pods lying on the ground in April and May (direct observation, and presence of freshly laid eggs on pods on the ground), and on green immature pods hanging in the trees in June. The next generation of adults from the dry mature pods emerges 5 to 7 weeks later (life cycle length was determined in the laboratory using 100 individuals) and again oviposits on mature pods on the ground and on green immature pods hanging in the trees. The third group of emerging adults then oviposits on the new mature pods hanging in the trees and on any available pods on the ground. The resulting individuals overwinter as larvae,pupae,or adults until apring when the cycle starts anew. There are three generations per year on mature pods. The relationship of the seasonal history of Amblycerus to that of the honey locust is shown in Figure 1.

The seasonal history in immature green pods, however, is somewhat different. Green pods appear in trees in early or mid June and some of the overwintered adults oviposit on them instead of on dry pods on the ground. Complete development to adulthood in green pods does not occur until the seeds are almost mature (late August). These adults then oviposit on mature pods hanging in the trees or on

pods on the ground. Thus, only two generations per year occur when eggs are laid on immature green pods in spring.

Eggs : Eggs are .4 to .5 mm wide by .9 to 1.0 mm long, yellow, and are enclosed in a sticky gelatinous outer membrane which adheres to the pod. The egg is fastened to the pod surface by a viscid fluid, which, upon drying, approximates the color of the egg. The shell of the hatched egg is clear, its white appearance being due to frass produced by the larva in boring into the wall of the pod.

Larvae : All five larval instars have a chestnut head, yellow to white body and functional legs. The relative number of individuals in each larval instar during June and July, 1969, is indicated in Figure 2.

In hatching, the first instar larva of Amblycerus chews its way through the egg where it is cemented to the pod surface. Once through the chorion, the larva, which is photonegative, bores straight on through the soft or hard tissues (immature or mature pods) into the pod cavity. As the larva enters the pod the tunnel behind becomes blocked with residues from the excavation ; the abandoned egg chorion also covers the entrance to the tunnel. If disturbed the larva will turn around in its tunnel and face the disturbance. It makes striking movements with its head, continously working its mandibles, and will bite anything within range.

Although in mature pods larvae ordinarily enter seeds directly, without much tunneling in the pods, in immature pods tunnels vary

from 1 to 8.5 cm long, the places of attack being indicated by sticky masses of pod fragments and larval feces. The larvae apparently range back and forth in their tunnels within the pod until they enter a seed. Quantitative documentation of larval damage to immature seeds is detailed in Table 11 and discussed in a later section (see page 47).

Once inside the pod, the larva attacks a seed, whether soft and green or hard, brown, and mature. It does not necessarily bore into the nearest seed, as in several instances a single larva was found inhabiting a seed up to 8 cm from its point of entry into the pod cavity, having passed up to six seeds before entering one. Upon entering the larva commences to feed on the parts of the cotyledons directly below the entrance hole. As many as 3 seeds in green pods were found completely destroyed by one larva. The damage was often progressive from one seed to the next in the series. One mature honey locust seed provides enough food for the complete development of one larva. Little if any mature seed remains after larval feeding has terminated.

The first instar is primarily a locomotory stage, and little feeding occurs until the larva molts ; the second, third, and fourth instars completely devour the seed embryo starting with the cotyledons. As in many species of bruchids, the last (fifth) Amblycerus larval instar gnaws half way through the tests (seed coat) before pupation, thus marking out the hole through which the adult finally emerges. In this position (facing the future emergence hole) the larva enters the pupal stage. After a short pupal period (4 to 10 days) the adult emerges and completes the oval incision in the seed coat. Having chewed through the tests, the adult pushes off the oval trap-door and emerges ; it then gnaws its way through the pod wall. Alternatively, the bruchid may escape

from the pod through a break or a hole made by an earlier adult.

Mortality Factors

Mature pods hanging in a honey locust tree were used for determining mortality factors. The study period was from September 1 to the end of October.

Mortality in the egg stage (31.6% , Table 1) is from three causes : 1.) eggs lost from pod, 21.1% 2.) eggs with no visible development, 4.1% and 3.) eggs with a dead embryo, 6.4% . The dropping of eggs from pods, regardless of their condition, is a major mortality factor. Adults brush some eggs off as they crawl around during oviposition ; other eggs appear to be weakly cemented to the pods and fall off shortly after oviposition. Plant movement, wind, rain, and resulting plant contact could brush off many eggs. Loss of eggs was determined by examining pods with a microscope for traces of missing eggs. The presence of the remnants of the dried and somewhat shiny adhesive substance indicates a lost egg. If a larval entrance hole appeared beneath such a finding the egg was not counted as lost.

Larval mortality (40.1%,Table 1) results from two causes : 1.) failure to gain entrance into a seed, and 2.) parasitism by wasps. The failure of a larva (first instar) to gain entrance into a seed may be a function of egg placement by the female or the way in which the larva tunnels into the pod. Some larvae tunnel directly into the pod, while others wander about between the outer and inner layers of the pod. Many tunnels up to 1 cm long were seen to end blindly, having

a dead larva at their end. The larvae appeared capable of entering a seed at any point where the seed was adjacent to some other structure. A point of purchase is seemingly necessary for a larva to gain entry into a seed ; that is , larvae appeared to gain entry where seeds were adjacent to one another or close to the walls of the pod.

Mortality of the later larval instars (second through fifth) was mainly due to several parasites (see below). This fall mortality was checked for early pod collection by examining a control group of pods picked up the next spring from under the same tree. No significant differences were observed.

Pupal mortality (.3%, Table 1) is slight and no causes can be assigned at this time ; adult mortality (.5%, Table 1) results from failure to escape from the seed.

Total mortality for bruchids in pods on trees is 72.5% from egg to emerged adult. This total mortality I feel is representative of the picture in mature pods on trees. Egg mortality is higher on immature pods hanging in trees during the summer, but larval mortality is less. Winter kill of larval, pupal, and adult stages in pods on the ground is another, unmeasured source of mortality.

An artificial crowding experiment was conducted with first instar larvae of Amblycerus , to determine what would happen should more than one larva attack a seed. Twenty adult females, enclosed with five honey locust pods, laid a total of 547 eggs. The number of eggs laid per female was 27.35. The number of eggs laid per pod ranged from 91 to 136, with a mean of 109.4. Five days later the pods were dissected. All but one of 95 seeds had more than one larval entrance (range = 1 to 6, mean = 4.07 entrances per seed). When these seeds were dissected it

was found that only one larva , feeding on the cotyledons, had survived in each. One to five dead shrivelled larvae per seed were found in the passages occupied by the remaining larva, with a mean of 3.07 per seed. Death apparently resulted from bites inflicted by the surviving larva.

Field observations of 10 to 15 eggs per pod , only rarely in 40 to 70 range (compared to 109.4 per pod in this experiment) perhaps indicate that the beetles usually lay one egg per seed, dispersing their eggs and avoiding crowding. The number of seeds per pod ranges from 8 to 23, with a mean of 17.

Oviposition Preference and Behavior

The eggs are usually glued singly to the outside of pods. They are frequent in the grooves between seeds, on concavities over seeds, in old adult exit holes, in cracks, or along the pod edge.

In the act of oviposition the beetle curves the end of the abdomen downward and deposition is accomplished by rhythmic motions of the body. After laying the female moves to another spot, or flies to another pod, and in this manner places the eggs, one at a time, upon the pods of the host plant.

The honey locust pod is a temporary microhabitat which occurs in the field year round (either on the ground or in the tree). The pod plays some role in stimulating the adult Amblycerus to oviposit because they will not normally do so in its absence. No eggs were ever found on other parts of the tree although loose seeds, stems, leaves and flowers were searched.

The first set of oviposition experiments conducted used 200 freshly emerged female Amblycerus (plus an equal number of males) and 1000 mature honey locust pods from the ground. The rate of oviposition on mature pods is shown in Table 2. Most oviposition occurred from the second to sixth day after emergence, while less than 2% of the eggs were laid during the first 24 hours. The average number of eggs laid per female ranged from 30 to 75, with a mean of 47.14. The lower rate of oviposition after the sixth day may indicate 1.) the egg-laying capacity of this bruchid or 2.) a response to egg-density (i.e. eggs already present may inhibit laying). The latter explanation is supported by the much lower egg productivity per female in the crowding experiment reported above.

Another set of experiments tested Amblycerus on other legume pods. Amblycerus in captivity lay eggs on immature Gymnocladus dioeca (Kentucky Coffee Tree) pods. Some larvae enter pods but no larval development occurs.

A choice experiment using a Latin Square Design was set up, the five choices being : 1) mature honey locust pods, 2) immature full grown but green honey locust pods, 3) immature Gymnocladus pods, 4) mature Phaseolus sp. pods, 5) mature Pisum sp. pods. The possibility of conditioning was tested by leaving beetles to be tested for a week with each of the five choices and then submitting them to the choice experiment. No conditioning effects were observed since a significant (.1% level) preference for mature honey locust pods was found in each of five replicates (range = 85 to 94% , mean = 91%). Most of the eggs were laid on mature honey locust pods (range = 85 to 94% , mean = 91%) ,

some on immature honey locust pods (range = 5 to 12% , mean = 8%), a few on immature Gymnocladus pods (range = 0 to 3% , mean = 1%), and none on bean or pea pods. Thus, Amblycerus prefers to oviposit on mature honey locust pods with immature honey locust pods as the second choice. This suggests that Amblycerus lays eggs on pods from the previous years crop already on the ground (mature pods) in preference to green immature pods hanging in the trees. Larvae that do develop in green pods have a long developmental period, two to three months vs. five to seven weeks in mature seeds, as noted above. This may indicate that certain necessary nutrients are present only at marginal concentrations in green pods or that inhibitors are present.

Bruchids that emerged from seeds of four different Gleditsia trees were tested with regard to oviposition preference between mature honey locust pods from the parental tree, different trees in the same area, and trees in other areas. No significant differences were found between the number of eggs laid on pods from these three sources. This indicates no highly specific responses to a bruchid's parental tree vs. other honey locust trees.

Parasites

Parasitization of Amblycerus by wasps is an important component of the overall interaction since the wasps help to determine bruchid abundance. Five species of parasites were found : two braconids (identified by Dr. Paul Marsh) , Heterospilus bruchi Vier. and Urosigalphus bruchi Cwfd. ; and three chalcidoids (identified by Dr. B.D. Burks), Eurytoma tylodermatis Ashm. (Eurytomidae), Eupelmus cyaniceps

Ashm. (Encyrtidae), and Horismenus missouriensis Ashm. (Eulophidae). All are primary parasites on bruchid larvae except for H. missouriensis which is a hyperparasite (secondary) on H. bruchi. E. tylodermatis , E. cyaniceps, and U. bruchi are reported as parasitizing other bruchids (Cushman, 1911) ; whereas H. bruchi appears to be species specific.

Bruchid larvae are infested by Heterospilus in the autumn (September, October) ; each parasitized larva contains 4 or 5 H. bruchi larvae which when mature are enclosed in small white cocoonc. Three to four black pupae of Horismenus , .2 mm long,were frequently found within an H. bruchi cocoon. The wasps probably overwinter as pupae or late instar larvae in the seeds and emerge in the spring. The other three parasites mature one to two adults per bruchid by the next spring ; their seasonal cycles are unknown.

During the summer parasites may switch to other hosts, or else estivate, for parasitization of Amblycerus was not observed.

Parasite emergence for the most common species (Heterospilus) and its hyperparasite (Horismenus) is shown in Figure 3 for the years 1967 and 1969, based on 2000 pods from the same trees each year. This pod removal probably had some effect on the next year's parasite population, but since the same trees did not produce pods in 1968 (alternate year pod production is common in honey locust) it was not possible to assess the effect. The results are similar in showing two annual peaks ; one is quite early in spring and the other later in the year. There is no summer of autumnal emergence. In 1967 the emergence was almost a month earlier than in 1969. This was probably due to the great difference in weather conditions between the two years.

In 1967 spring was early and warm temperatures held from March into summer, whereas in 1969 the spring was late and the first warm temperatures came at the end of April. The Amblycerus spring emergence was similarly altered. In 1969 the secondary parasite (Horismenus) population was much larger than in 1967, while the H. bruchi population was larger during the first peak in 1969, but less in the second.

With regard to the other three parasites : 1) in 1967 only a few individuals of each were obtained (2 to 3% of all parasites), and 2) in 1969 a larger number of individuals appeared, but the percentage of total parasite composition still only ranged from 1 to 5.5% It appears that this group of three parasites is not a major factor at this time.

The parasites emerge before the bruchids and all at once, whereas the bruchid emergence in staggered. This staggering or asynchrony may be a mechanism of parasite avoidance.

THE HONEY LOCUST, GLEDITSIA TRIACANTHOS

Gleditsia and Gymnocladus

There are 14 species in the genus Gleditsia found in North and South America, temperate and subtropical Asia, and the Malay Archipelago.

Gleditsia is most closely related to Gymnocladus. Neither forms root nodules, regarded by Burkart (1952) as an indication of the primitiveness and antiquity of both genera , a view strengthened by their disjunct distributions and abundant fossil histories. The chief differences between the two genera are the larger flowers, longer calyx tubes, and

thicker pods of Gymnocladus (Gordon, 1966).

Vegetative Morphology

G. triacanthos typically reaches heights of 20-25 meters and trunk diameters of 50 to 80 centimeters in northeastern Kansas, but it attains greatest size in the Wabash River Valley in Southern Indiana and Illinois (Gordon,1966). There, maximum height is about 50 meters with a trunk diameter of about 2 meters. The average growth in height of G. triacanthos in shelter belts in Kansas (Munns and Stoeckler,1946) was 45 cm per year for the first seven years, with annual growth decreasing after this period.

A noteworthy feature of the honey locust is the spines (abortive branches according to Gordon,1966) which are often abundant on the trunk and decrease in frequency to absence near the top of the tree. Sargent (1890) reported that trees grown under conditions of full light most frequently develop spines, while those in the forest in the shade of other trees are often thornless or have reduced thorn size and frequency. The present study supports both authors. Almost all honey locust trees observed during this study had greatest thorn density in the lower tree regions, and the most heavily armed trees were in open fields.

Reproductive Cycle of G. triacanthos

Flowering occurs in the spring (May-June) , pod formation and growth in summer (June,July,August), and pod and seed maturation in September. Most pods fall from the trees in October and November

(Figure 1), but some remain in trees until the following spring.

Flowering

The flowers are inconspicous, yellow-green to white. The male flowers are clustered on the axis of the inflorescence, while the female flowers are more widely spaced. The flowering period usually begins in mid-May and ends the first week of June (lasting for two or three weeks). Flowering periods varied little between areas studied, but within areas there was considerable variation between trees on different exposures. Trees on north-facing slopes were from 3 to 5 days delayed as compared to trees on south exposures, while trees on east and west facing slopes were intermediate.

Observations indicate that some trees produce seed crops one year and not the next (30 to 40 examples), while others have a few pods one year and large crops the next. Trees have only male flowers (45%), only female (35%), or both male and female flowers (20%).

Aborted Peduncles

Fifty peduncles (flower and pod bearing structures attached to twigs and brnaches) per tree were examined. Samples from 32 trees with pods around the circumference of Lone Star Lake, Lone Star, Kansas, were examined. Floral scars were counted to give the total number of female flowers which could have been produced on the inflorescence (potential production, Breedlove and Ehrlich,1968). Pod numbers give a measure of percentage success, since the floral scars remain on the

peduncle into fall.

The number of female flowers produced per peduncle ranged from 1 to 43. The average number of flowers per peduncle per tree near Lone Star Lake ranged from 10.4 to 28.7 (S.D. = 3.63 to 9.12) , and the average for all trees was 21.4 (S.D. = 6.74).

Two kinds of flower abortion may occur : 1) failure in pollination or fertilization, and 2) failure in late embryo development. Flower abortion for the 1969 crop for a given tree ranged from 15.45 to 93.90% , with a mean of 43.62%. The failure of pod production is undoubtedly an expression of unfavorable physiological and environmental factors.

Dispersal

The wide distribution of this species in America perhaps suggests an efficient method of seed dispersal, although much time and a weak dispersal mechanism could give the same results. Sargent (1890) tried to explain the mode of seed dispersal by the contraction of the pods. He thought the twisted pods roll like wheels, and being light, would blow for great distances over the frozen ground. The obstacles the pods encountered then would break them open and liberate the seeds. This may occur, but I think a more efficient means of dispersal is by animals. The fruits are eaten by cattle, cottontail rabbits, gray squirrels, fox squirrels, white-tailed deer, bobwhite, and snowshoe hare (Deam,1953 ; Bugbee and Riegel,1945 ; Dice,1945 ; and personal observations). According to Fowells (1965) passage of seeds through the

alimentary canal of animals apparently softens the tough seed coats (unless seeds are chewed and digested), thus increasing the chances for germination. This aspect of dispersal then can be expressed as the failure of seed predators (bruchids) to infest all the seeds before some are removed by dispersal agents (Janzen,1969).

Seed Germination and Seedling Success

Seed germination in G. triacanthos occurs after abrasive action, passage through the alimentary canals of animals, probably after fungal and microbial attack which may penetrate the outer hard seed coat, or after insect penetration (bruchid larval entrance holes). Insect penetration would result in germination only if the seed were otherwise not or little injured by the larva.

Seed germination tests were conducted in the laboratory. Seeds, with coats filed (seeds not filed did not germinate) to admit water were placed in a petri dish between layers of paper toweling kept moist with water. Germination in the laboratory ranged from 90 to 100% for 10 samples of 50 seeds each.

Field work consisted of planting five plots of 25 seeds each (all seeds were filed) in three habitat types : 1) open field , 2) edge ofopen field, and 3) forest. The experimental area used was 2 miles south of Lone Star, Kansas. Percentage germination varied from 6.3 to 61.2% (Table 3). The highest percentage germination occurred in plots on the edge of open fields (53.84%), the next highest in open fields (24.76%), and the lowest (8.28%) in forest plots. Sunlight and available nutrients may govern germination success.

Seedling survival for five months after germination in the 15 experimental plots used for the seed germination study varied from 43.1 to 71.8% (Table 3). The highest survival was in plots at the edge of open fields (65.82%), while the lowest was in forest plots (46.52%). Browsing (perhaps by mice,deer,etc.), and lack of sunlight, water, or nutrients may account for most of the mortality.

Adult *Amblycerus* Activity Patterns and Behavior

Introduction

Laboratory experiments were undertaken to determine the daily activity cycle of adult bruchid beetles. Simple color preference experiments were also made to see if color is important to adult bruchids in finding their host plant. The importance of olfaction was investigated with T-tube choice chambers.

The seasonal activity of bruchid beetles (*Amblycerus robiniae*) was noted in three types of field experimental situations : 1.) honey locust trees with pods, 2.) honey locust trees without pods, and 3.) control osage orange (*Maclura pomifera*) .

Materials and Methods

Beetles were tested under two different light regimes : 1.) 16 hours light and 8 hours dark (long day conditions), and 2.) 12 hours light and 12 hours dark (short day conditions).

The test chambers were petri dishes with floors of black, white, brown, and light and dark green paper, two colors for each dish, plus a honey locust pod fragment (one per dish). Adult bruchid beetles (10 males and 10 females) were released in the center of each test chamber in the daytime and their subsequent positions recorded, by day as well as at night, at varying time intervals.

The activity of the beetles after dark was observed with the use of a red light (flashlight covered with red cellophane). No recognizable reactions to the red light were noted during the experiment.

Color choice (as indicated by bruchid position) in the test chambers and activity (the number of bruchids out of the total number (20) that were moving) were recorded.

The olfactory experiment was set up as a T-tube design with air being drawn over 1.) a chamber with dry brown mature honey locust pods, and 2.) an empty chamber. Twenty beetles were released (10 males and 10 females) into the test chamber and subsequent movements were recorded.

Activity patterns and behavior in the field were studied at the Sunflower Tract and the Natural History Reservation, both field study areas of the University of Kansas. Within each of these areas two experimental locations were selected. At the Sunflower Tract the two locations were 500 m apart, while at the Natural History Reservation they were 1000 m apart. Each location included (1) a honey locust tree with pods, (2) a honey locust tree without pods, and (3) an osage orange tree (the control). Sticky traps were hung in these three types of trees in each location according to the following design :

At each location the three trees were 50 to 75 m apart, with intervening trees (honey locust and others). All were about 8 m tall with canopies approximately symmetrical around the trunks.

Brown linoleum strips 7.5 cm wide by 45 cm long were used for the sticky traps. These strips were covered on both sides with

tanglefoot to a thickness of 4 to 7 mm before hanging in the trees. The capture of grasshoppers, cicadas, and other large insects (plus laboratory tests with Amblycerus) indicated that this layer was thick enough to hold bruchids. Such a thick layer resisted drying and rain damage much better than a thin-layer and needed little retouching. However, retouching (adding more tanglefoot as necessary) was done every two weeks to insure continued trap effectiveness.

Twenty four traps were hung in the canopy of each experimental tree, twelve above the middle of the canopy and twelve below (Figure 5). At each of these levels four traps were placed within 1.5m of the trunk among the largely bare branches (core area) and eight were placed in the outer leafy canopy (canopy area). These positions were subdivided according to direction. Directions of the traps from the tree trunk are indicated in Figure 6. Trapping was carried out from May 26 to November 24, 1969 (Figure 6).

To test the frequency of beetle capture, tests of independence using a three-way table were used. The number of bruchid beetles caught on sticky traps was analyzed with respect to three factors : 1.) type of tree, 2.) trap direction, and 3.) trap position in the tree. The G-test was used to test independence. Since the overall G-value can be partitioned into components, each aspect of the independence of the three factors could be tested. A model I design (Sokal and Rohlf, 1969) was used since the marginal totals (rows and columns in the comparison tables) were not fixed by the experimenter ; they were free to vary and reflect population parameters.

RESULTS

Bruchids were active in the laboratory at night, with some tendency for early and late night peaks especially under short day conditions (Figure 4). Bruchids were placed in each experimental chamber along with a mature honey locust pod fragment. Most (90%) went directly to the pod and stayed there for the duration of the experiment in all replicates. The honey locust pods acted as an attractant and regardless of colors on which they were placed in other tests most of the bruchids were attracted to them. The darker background colors (brown,black,and dark green) were preferred in all tests (more than 80% of the beetles were on these colors). This result and the time of activity suggest that the bruchids find their host tree via olfactory cues rather than by color cues.

In one T-tube test, 16 of 20 bruchids released into the test chamber were in the honey locust pod arm the next morning, two were in the empty arm and two remained in the test chamber. Five replicates were run and all gave similar results (means : 16 in honey locust pod arm , 3 in empty arm, and 1 in test chamber). Single beetle releases gave similar results with more females (means : 80% in pod arm, 20% in empty arm) than males (means : 25% in pod arm, 25% in empty arm, 50% in test chamber) moving to the pods. When honey locust seeds alone were tested 20% of the beetles went to the seeds and 80% stayed in the test chamber. It appears that some compounds in the pods may act as orientation clues for the ovipositing female bruchids.

The numbers of Amblycerus caught (1298) on sticky traps over the

experimental period in each area are shown in Figure 6. The sex ratio was 1 : 1.15 (603 males : 695 females) which is quite similar to the sex ratio of emerging beetles (1 : 1.09). Seasonal peaks in activity occurred in early July and mid-October at the Sunflower Tract. At the Natural History Reservation the activity peaks were similar but a week later. The summer generations are not as large, lacking distinct peaks, although small peaks are present. This suggests that egg and larval mortality is much higher than in the fall (see pages 8-10).

The numbers of beetles caught in honey locust trees with pods, honey locust trees without pods, and in the controls are similar in the two areas (Table 4) and presumably indicate preference by the bruchids.

Tests of independence were made for each location in both experimental areas. All four locations gave similar results and location number one at the Sunflower Tract is used as a representative example.

There are significant differences (at .1% level) between the numbers of beetles caught in honey locust trees with pods, honey locust trees without pods, and the controls (see Table 5).

Apparently, the numbers of bruchids caught in different positions (factor C) were independent of the type of tree (factor A, Table 5). The numbers of bruchids caught at different compass directions (factor B) were not independent of the type of tree. Those caught in the west and south compass directions and those caught in the west,south, and southeast were tested against those caught in other directions. In each case, they were significantly different. Thus,

most bruchids were caught in the west, southwest,south, and southeast quadrants of the honey locust with pods (Figure 7). These differences existed and were significant in both upper and lower levels and core and canopy areas. Most bruchids were caught in the upper level and outer canopy area of the trees (Table 6). Comparable information for the honey locust without pods is given in Figure 8.

Since there is not independence among the three factors (type of tree, compass direction, position in tree, Table 5), the numbers of bruchids caught in a given trap depends on the interaction of these factors.

The results in location number one at the Natural History Reservation were similar to those at the Sunflower Tract except that most were caught in the noth,northeast,east,and southeast quadrants of both trees (Figures 9,10). Again, most captures were in the upper level and outer canopy area of these trees.

DISCUSSION

In addition to measuring seasonal activity of the bruchid beetle, the results give an estimate of bruchid density. By using the raw data, relative population sizes in the two areas can be estimated. Assuming sticky traps were equally efficient in each area, there were more bruchids per cubic meter of tree canopy at the Sunflower Tract ($.355/m^3$) than at the Natural History Reservation ($.261/m^3$).

Seasonal bruchid activity peaks (Figure 6) in both areas can be interpreted as follows. The first peak represents adults emerging

in the spring and early summer from overwintering larvae,pupae,and adults. This peak(end of June,first of July) comes when the honey locust pods are growing rapidly and this is when bruchids start ovipositing on them as well as on dead pods still on the ground from the previous year. The adults of the next generation also oviposit on mature pods on the ground and on pods hanging in the trees. The low population levels during the summer probably result from scarcity of mature pods and the slow growth rates in green pods. The adults that emerge from these, plus adults emerging from almost mature pods hanging in trees (both of these groups represent the second peak,Figure 7), then oviposit on the now mature pods in trees. These two peaks fit the seasonal history in green immature pods, but not in mature pods. Since traps were in trees near green pods this is probably accurate. Resulting individuals overwinter.

The capture of beetles in control trees during peak activity periods may simply indicate that there are more bruchids at such times and the chances of some straying into other trees are higher. Another possibility is that bruchids may visit other trees searching for pollen or other food and thereby get caught in the traps. Most bruchids were caught in the honey locust trees with pods, and there is evidence (see above) that pods act as attractants. Also, the relatively high number of beetles caught in honey locust trees without pods suggests that the honey locust tree itself is attracting beetles.

The data concerning directional preference requires explanation. As shown in Figure 7 and 9, directions of beetle catches are positively correlated with pod abundance ($r = .96$, significant at 1% level).

On the other hand, similar directionality in the locust trees without pods in each location shows that some other factor must also contribute to the directional findings. Since the directionality

persists throughout the season it is unlikely to be related to wind differences (unless prevailing winds were from same direction, recurring at certain time intervals) or other temporary local physical factors. It is more likely to be related to directions of bruchid movement associated with locations of the experimental tree in relation to other trees and beetle sources. Tree locations in relation to such features are shown in Figure 11. Bruchid movement from trees with pods to the southeast,south,southwest,and west may account for the capture pattern in location number one at the Sunflower Tract (top map, Figure 11). Movement from trees with pods to the north, northeast,east, and southeast may likewise explain the captures in location number one at the Natural History Reservation (bottom map, Figure 11). Directionality appears to be caused in podless trees by the proximity of trees with pods.

Another important source would be pods of previous crops on the ground. These pod locations initially depend in a minor way on wind direction and velocity during pod fall ; however, once on the ground seed predation, hoarding by squirrels, heavy rains,etc., are important. Thus, pod distribution on the ground might partially explain early season bruchid captures.

Most bruchids were caught in the upper and lower canopy area in both experimental areas. Possible hypotheses are 1.) the majority of honey locust pods and foliage are found in the outer regions, providing mating sites, oviposition substrate, and protection ; and 2.) bruchids probably search for pods in outer leafy canopy areas rather than near the core or trunk due to experience or innate

behavior patterns. Captures in the core areas may represent bruchid movement to and from outer areas via shortcuts through the tree center.

CONCLUSIONS

1.) Laboratory tests indicate that Amblycerus is nocturnal, showing little movement during the daylight hours.

2.) Activity under the two light regimes tested was similar ; under both long and short day conditions peak bruchid activity occurred shortly after the lights went out.

3.) Bruchids presumably use olfactory cues to locate host plants (honey locust). T-tube olfactory tests substantiate the positive response of bruchid beetles to honey locust pods.

4.) Seasonal activity peaks occurred in weeks six (last week in June) and twenty one (second week in October), or in a nearby area in weeks seven and twenty two.

5.) The numbers of bruchids caught in honey locust trees with pods was significantly greater (at .1% level) than those caught in honey locust trees without pods or in osage orange trees in both experimental areas and both locations within each area.

6.) Captures in particular compass directions within a tree depend on pod density, proximity to and directions of other honey locust trees, and presumably on pod distribution on the ground.

7.) Most bruchids were caught in the canopy areas of the trees. The higher density of pods and foliage per cubic meter of canopy in these areas, innate bruchid behavior patterns, or experience probably account for this difference.

Chemical Content of Honey Locust Seeds : Free Amino Acids as Bruchid Toxicants.

INTRODUCTION

The nutrients available to Amblycerus robiniae , a bruchid whose larvae feed only on these seeds during development, were assessed by analyzing the lipid, carbohydrate,protein, and water content of the seeds.

One set of experiments tested the hypothesis that the concentration or presence or absence of certain amino acids could account for the amount of bruchid damage to the seeds of different honey locust trees. The second set of experiments concerns Gymnocladus and Albizzia seeds which have no (or few) known insect pests; perhaps certain free amino acids are responsible for this lack. Cercis (infested by the bruchid, Gibbobruchus mimus Say) was used in this comparison as an example of another bruchid-infested legume.

MATERIALS AND METHODS

Water Content :

Water content of seeds was determined by two methods, freeze-drying and oven-drying. In the freeze-drying determinations two groups of seeds were used : 1.) intact seeds, and 2.) split seeds (seed coat physically split open). The purpose of using these two groups was to assess seed coat effect on water loss. Two replicates of 10 seeds

were run for each group in the freeze-dryer for 37 hours. At the end of this treatment, the seeds were subjected to oven-drying at 90°C for 113 hours to determine which method (freeze-dry or oven-dry) was the most reliable and repeatable.

In the oven-drying determinations five replicates of 10 seeds each for both intact and split seeds were subjected to 90°C without prior freeze-drying for equal time periods (137 hours).

In the third test seeds of different sizes were used to assess the effects of seed size (in mm^3 as determined by multiplying length x width x depth) on water loss. Fifteen intact and fifteen split seeds were weighed,measured and oven-dryed at 90°C.

Lipids :

Lipid extractions were done using the saxhlet distillation apparatus. Reagents used were absolute methane, and chloroform reagent grade. The seeds were homogenized in a Waring Blendor for two minutes with a mixture of 100 ml chloroform and 200 ml methanol. Chloroform (100 ml) was then added to the mixture and after blending for 30 seconds, 100 ml of distilled water was added and blending continued for another 30 seconds. The homogenate was filtered through Whatman No. 1 filter paper in a Buchner funnel with slight suction. The filtrate was transferred to a 500 ml graduated cylinder, and after allowing a few minutes for complete separation and clarification, the volume of the chloroform layer was recorded and the alcoholic layer removed by aspiration. A small volume of the choloroform layer

was also removed to insure complete removal of the top layer. The chloroform layer contains the lipid. The volumes of chloroform, methanol, and water, before and after dilution were kept in the proportions 1 : 2 : .8 and 2 : 2 : 1.8 , respectively.

A portion of the lipid extract was evaporated to dryness in a tared flask and the weight of the lipid residues determined. The dry weight of the residue was determined and subtracted from the initial weight. The lipid content of the sample was calculated as follows :

$$\text{Total lipid} = \frac{\text{Weight of lipid in aliquot} \times \text{volume of chloroform layer}}{\text{volume of aliquot}}$$

To assess which lipid classes were present, thin layer chromatography was used. Silica gel G was used as the thin layer, the solvent was diethyl ether : petroleum ether : acetic acid , and detection was by spraying with sulphuric acid and charring at 105°C. Known lipids were run with the samples to aid determinations ; they were cholesterol, B-sitosterol, and palmitic acid.

One of the ways to analyze lipids involves hydrolysis and subsequent isolation and characterization of the products. Hydrolysis of lipids is most often accomplished by hot alkali (saponification). The procedures of Clark (1964) were used to determine the saponification number of the lipids and to estimate the average molecular weight of the fatty acid fraction.

Carbohydrates :

The reducing sugar content was estimated using diestase to digest

the starch for one half hour at 40°C, and then analyzing with dinitrosalicylate. The reduction of the dinitrosalicylate by the sample was measured colorimetrically using the Bausch and Lomb Spectronic 20 spectrophotometer. Distilled water was used as a blank.

Proteins :

The Gelman Electrophoresis procedure was used to analyze proteins in seed and beetle homogenates. Cellulose polyacetate electrophoresis strips (Sepraphore III) were soaked in Gelman HR buffer until wet, then placed on an absorbent and blotted with another piece of absorbent. The absorbent strip was placed at the cathode end. The polyacetate strips were then placed on the rack and attached at the anode end. The sample was applied, the voltage was set at 3 milliamperes (1.5 milliamperes per strip), and the experiment was run for one half hour. Upon removal strips were placed in Ponceans stain for five minutes, washed four times for one minute in 5% acetic acid, and dehydrated in methanol. Strips were then dipped in 10% acetic acid in methanol and mounted on glass plates. They were dried at 60°C for 15 minutes.

For protein determination by the Folin-Ciocalteau method four stock solutions A,B,C, and D are needed. A = 2% Na_2CO_3 in .1 N NaOH ; B = 1% NaK Tartrate with .5% $CuSO_4$ - 5 H_2O ; C = .2 ml of B to 10 ml of A ; D = 1N Folin-Phenol Reagent (2N stock) diluted 1 : 2 with hydrochloric acid. Then to one test tube 1 ml of solution A plus 1 ml of the first supernatant was added, then 1 ml of saline solution was added to another tube for the reagent blank. Five ml of solution C

was then added to each tube, mixed, and incubated at room temperature for 10 minutes. Next, .5 ml of solution D was added to each tube, mixed thoroughly, and incubated at room temperature for 30 minutes. They were then read at 750 mu on the spectronic 20 spectrophotometer. The amount of protein in the sample was estimated from values obtained for the original solution and the first supernatant.

The quantitative Folin-Ciocalteau test for proteins has the advantage of applicability to dried material as well as to solutions. In addition, the method is sensitive ; samples containing as little as 5 micrograms of protein can be readily analyzed. The color formed by the Folin-Ciocalteau reagent is due to the reaction of protein with the alkaline copper in the reagent and to the reduction of the phosphomolybdate-phosphotungstate salts in the reagent by the tyrosine and tryptophan of proteins.

Amino Acids :

Ripe, dry seeds of the 1969 seed crops of all species were picked the same day while hanging in the trees and the analysis was started immediately. Gleditsia seeds were used from seven localities (10 total trees). The amount of bruchid damage per tree ranged from 14.5 to 97.2%. One seed sample each of Gymnocladus, Albizzia, and Cercis was analyzed.

The seeds were ground to fine particle size with a Waring Blendor. Five per cent trichloroacetic acid was added, the samples were cooled for one half hour and then extracted. Then they were centrifuged,

with the supernatant taken into another tube. Ether was added, the ether layer was removed and discarded ; this was repeated four times, and air was then run through the aquaeous layer. Samples were then dried over P_2O_5 in a vacuum.

A Beckman Model 120 Amino Acid Analyzer was employed, with chromatography of the acidic,neutral,and basic amino acids, using a lithium buffer system. Lithium citrate buffers were prepared according to the method of Benson, Gordon, and Patterson (1967). Columns of resin 56.0 x .9 cm were used to determine the neutral and acidic amino acids and 8.0 x .9 cm resin columns were used for the basic amino acid analysis. Duplicate samples were thus required, one for each column. A buffer flow rate of 70 ml/hr, a ninhydrin flow rate of 35 ml/hr, and a column temperature of 37°C were maintained throughout the analysis. The recorder chart speed was 6 in/hr with 1 dot/2 sec printing speed. The analysis time for the acidic and neutral amino acids was from 270 to 320 minutes, while for the basic amino acids it was 80 minutes.

The amino acids were identified by their order of elution over time as compared with a standard chromatogram. Concentrations were calculated by the method of triagulation with the net- and half-height distances measured in absorbency units.

The peaks on the effluent curves were calculated by multiplying the height of the peak by the width at half the height. The height of the peak in absorbance units was determined from the recorder chart and the base line was read to 0.001 absorbance unit. The height of the peak on the chart was measured, and the net height

obtained by correcting for the base line. The width of the peak at half-height was determined by taking half the net height and adding or subtracting the baseline correction. The method of width measurement involved counting the number of dots (in the peak) above the half height on the chart. For the final calculations the net height (H) of the peak was multiplied by the width at half-height (W). The constant C, by which H x W is divided to give micromoles of a given amino acid, was determined by calibrating the apparatus with a synthetic mixture of amino acids. The constant (C) is a function of the color yield of the amino acid under consideration and of the dimensions of the absorption cell. When once determined, the constant was valid for the particular instrumnet under a wide variety of conditions. Integration constants used were supplied by Mr. Tom Fuller, Department of Biochemistry, the University of Kansas. The concentration of unknown amino acids was calculated on the basis of the average of other constants (approximately 55.0) unless stated otherwise.

Tentatively identified amino acids (ones followed with a mark in Table 8) were evaluated by elution position and time compared to a standard chromatogram, but were not positively identified. A future paper will deal with the identification of these acids and the unknowns.

A two way analysis of variance without replication was used to test differences in concentration of 20 amino acids present in seeds from each of the 10 honey locust trees. The arcsine transformation of percentages was used to conform with the assumptions of anova. For testing "trees" we must assume interaction between trees and amino acids to be non-significant, so row and column mean squares are tested over error mean square (Sokal and Rohlf, 1969).

RESULTS

Water Content :

After freeze-drying for 37 hours, the intact seeds showed 4.07% water loss; however, the same seeds were then oven dried at 90°C and lost an additional 5.52% water respectively. The possibility exists that other compounds, probably lipids, may have been driven off by the 90°C temperature. These results are graphed in Figure 12 which shows a slow water loss at first during the freeze-drying, then a subsequent sharp drop when the samples were oven-dried. Figure 13 shows water loss of groups of seeds dried at 90°C.

The three tests are summarized in Table 7 ; in all three the percentage water loss was higher in the split seeds, indicating the importance of the seed coat in minimizing water loss. A total of 170 seeds, 85 intact and 85 split, was analyzed . The mean percentage water loss was 12.71% in intact and 15.38% in the split seeds. The seeds used in this experiment were in the dry dormant state ; the percentage of water in a seed increases greatly when germination occurs.

Lipids :

One sample of whole seeds (100) contained 7.54% lipid. A second sample of 100 seeds was tested to determine separately the lipid content of the seed coat and the cotyledons of the seed; this test showed 10.18% of the cotyledons of the seed to be lipid, while 5.03% of the seed coat was lipid.

To determine the major classes of lipids present, thin-layer chromatography was used. All the major classes of lipids were present in the seeds : phospholipids, sterols, free fatty acids, tryglycerides, and methyl esters (Figure 14). The classes actually used by the beetle were not determined.

The results of the saponification test indicate a positive test for glycerol. The saponification number obtained was 197, which is very close to other reported values for legume seeds ; the molecular weight of the tryglyceride was estimated at 846.3.

Carbohydrates :

To determine the reducing sugar content in honey locust seeds, known concentrations of dextrose solutions were used to set up a curve from which an unknown could be read. Using this method the percentage of reducing sugars present was estimated at 15.90%. This of course does not account for all the carbohydrates, carbohydrate-protein complexes, or carbohydrate-lipid complexes, but does give some idea of the amount of easily available sugar.

Proteins :

Protein content was determined qualitatively using electrophoresis. Distinct protein bands occurred 1.2 cm, 1.7 cm, 2.2 cm, and 2.6 cm from the starting point in all four replicates with honey locust seeds (Figure 15). The total number of bands varied from 5 to 7, with some variance as to position. Homogenates of adult Amblycerus were run and

distinct bands appeared at 1.2 cm and 2.1 cm from the starting point ; these bands (Figure 15) may represent the same or similar proteins to those in the seeds. Electrophoresis indicates there are at least seven proteins in honey locust seeds.

Using the Folin-Ciocalteau test the amount of protein present in honey locust seeds was determined quantitatively. Three runs were made and the mean results follow : 13.76% of each sample reacted in this test, indicating that this amount of the sample was protein. This information does not account for glyco-proteins or lipo-proteins, which are also probably present in the seeds.

Amino Acids :

Amino acids found in honey locust seeds are indicated in Table 8. The results show no significant difference among trees, but there are highly significant differences (at .1% level) in percentages of the different amino acids within each tree (Table 9). On the other hand, by taking certain amino acids alone into consideration some significant differences (1% level) among trees show up. The possibility of this relating to the amount of bruchid damage will be discussed later.

Amino acids and other compounds not used in the analysis of variance (see Table 8) which occur in some, but not all, Gleditsia seed samples are the following : glycerophosphoethanolamine (1 sample,.36%), phosphoserine (7 samples,.07 - .56%), phosphoethanolamine (7 samples, .13 - 5.12%), taurine (7 samples,trace - .61%), urea (7 samples,trace - .19%), hydroxyproline (7 samples,trace - .33%), glutamine (6 samples, trace - 6.08%), unknown no. 2 (6 samples,.02 - .48%), citrulline

(7 samples,trace - .36%), a-amino-n-butyric acid (5 samples,trace - .24%), unknown no. 3 (9 samples,trace - 2.03%), and tryptophan (9 samples, trace - .62%).

The four legume species show interesting differences in amino acid content (Table 10) ; Gymnocladus has high percentages of methionine sulfoxide, aspartic acid, hydroxyproline, proline, and sarcosine ; Cercis has high percentages of glutamic acid,glutamine, arginine, and homoserine ; Albizzia has high percentages of glutamic acid, glutamine, and unknown no. 1 ; and Gleditsia has high percentages of asparagine, alanine, and arginine.

Chromatograms of the acidic and neutral amino acids of these four legume species are represented in Figures 16-19. Figure 20 is a representative chromatogram of the basic amino acids present in these species : the amino acid concentrations present in each species may vary somewhat, but each acid is represented in all four species.

DISCUSSION

Sixty to 65% of the honey locust seed content is accounted for , even though the methods used ignore the polysaccharides, lipo-proteins, glyco-proteins, and perhaps some of the protein. Whether or not the percentages expressed here for the various groups are exact is not of prime importance, but the breakdown of the seed into its component parts indicates which nutrients are present in the seed.

Studies of water content indicate that the seed coat is important in water retention. It is also probably of great importance in

resisting microbial and fungal attack. In split seeds the percentage water loss was higher for large than small seeds but this was not evident for intact seeds.

A crude characterization of the tryglycerides in honey locust seed oil is provided. The molecular weight obtained (846.3) is the average molecular weights of the intact tryglyceride or the component fatty acids. Other legume oils have saponification numbers ranging from 183 to 207 ; the 197 value for the honey locust is within this range.

The hypothesis of possible relationships between amino acid concentration in honey locust seeds and bruchid damage has little support. Tree number one (see Table 8) had the lowest bruchid damage (14.5%) and number five had the highest (97.2%); number one has a high asparagine content and number five has high aspartic acid and low arginine concentrations. A second group (trees 3 and 9) with damage between 36-46%, and a third group (trees 2,4,6,7,8, and 10) with bruchid damage from 58-73% also show no distinctive patterns of amino acid concentrations. Of the substances which are not represented in all the honey locust seed samples, phosphoethanolamine has a high concentration in tree 3, glutamine in tree 2, and unknown number 3 in tree 5. Unknown number 3 does show a difference between the tree with low damage (tree 1, low concentration) and the tree with high damage (tree 5, high concentration), but no reliable patterns are indicated that are applicable to all trees tested. Thus, it appears that differences in amino acid concentrations cannot account for drastic differences in the amount of bruchid damage to seeds of different trees. However, oviposition rates on the individual trees

are not known and could certainly influence the observed variation in bruchid damage and thus obscure the possible effect of amino acid concentration in the seeds.

The four legume species used for this study show great variation in amino acid composition and concentration (Table 10). In seeds of the two species with no (or little) bruchid damage, certain amino acids are present which may act as toxicants. In Gymnocladus (Figure 17) high concentrations of methionine sulfoxide (11.51%), hydroxyproline (29.74%), and sarcosine (5.11%), may influence bruchid attack. Albizzia (Figure 18) affords a similar case with little if any insect attack in this area ; however, this tree is introduced and bruchids adapted to it may not be present here. It has one uncommon amino acid unknown no. I (5.47%) ; this compound may act as a toxicant and deter bruchid attack. Positive proof of insecticidal properties of any of these compounds awaits bio-assays involving rearing on artificial diets with varying percentages of certain amino acids and other compounds. The best candidates are methionine sulfoxide, hydroxyproline, sarcosine, and unknown no. 1.

The two species which are infested by bruchids, Cercis and Gleditsia (Figures 16 and 19) , have somewhat similar amino acid compositions. They both have high percentages of arginine, asparagine, and glutamic acid. Cercis has a greater concentration of glutamine and homoserine.

Similar work on other legumes includes that of Senevirarne and Fowden (1968) on the genus Acacia , that of Dunnill and Fowden (1967) on the genus Astragalus , and that of Bell and Tirimanna (1965). These studies

showed that most of the common amino acids were present plus a large number of rarely reported acids. Apparently, no evidence of phosphoserine, taurine, sarcosine, and citrulline were found in these genera ; however, since identification was not precise, the peaks in these studies may actually represent other compounds that overlie the positions of the amino acids mentioned. Amino acids such as methionine sulfoxide, hydroxyproline, homoserine, a-aminoadipic acid, and a-amino-n-butyric acid have been reported from these other genera (Senevirarne and Fowden, 1968 ; Dunnill and Fowden, 1967 ; and Bell and Tirimanna, 1965).

Free amino acids thus possibly occur with sufficient concentration and diversity in the seeds of four legume species to account for the diversity of host specificity. The advantage gained by the two species (Albizzia and Gymnocladus) with probable toxic compounds in seeds may be the basis of selective pressure resulting in the development of high concentrations (i.e. of several uncommon amino acids in these two species) of protective toxins. Other traits these seeds have that could confer protection include thick seed coats and a thick pod which may deter larval penetration.

Within a species polymorphism in toxicants in seeds may be an advantage to the plant in reducing the likelihood of bruchid adjustment to the toxins. However, within Gleditsia no support for this hypothesis was found.

CONCLUSIONS

1.) The mean percentage of water loss by oven drying was 12.71% in intact and 15.38% in split seeds. Thus, the seed coat is of importance in keeping water in the seed and thereby preventing dessication. Oven drying was a more repeatable and reliable method of determining dry weight of honey locust seeds than freeze-drying.

2.) Extractions showed that 10.18% of the inside of the seed and 5.03% of the seed coat was lipid. All major classes of lipids are present in the honey locust seed : phospholipids, sterols, free fatty acids, tryglycerides, and methyl esters. The saponification number was determined to be 197, which is close to that reported for other legumes ; the molecular weight of the tryglyceride mixture was estimated at 846.3.

3.) The reducing sugar content of the honey locust seed was estimated at 15.90%, thus giving some information with regard to the easily available sugar.

4.) Five to seven distinct protein bands occurred in electrophoretic runs done on honey locust seeds. Bruchid beetle homogenates gave two bands with similar positions (at 1.1 cm and 2.1 cm) to two of those found in the seeds. Folin-Ciocalteau tests indicate that 13.76% of the honey locust seed is protein.

5.) In summary, 60 to 65% of the honey locust seed is accounted for and the methods used ignore polysaccharides, lipo-proteins, glyco-proteins, and perhaps some of the protein. This then is a partial description of the microhabitat of the larval Amblycerus

robiniae with regard to its food source and living quarters for most of its life cycle.

6.) The amino acid compositions of Gleditsia triacanthos, Gymnocladus dioeca, Albizzia julibrissin, and Cercis canadensis seeds were determined. Thirty five different amino acids were found ; all but three were definitely or tentively identified.

7.) Differences in amino acid concentrations among honey locust trees cannot account for drastic differences in the amount of bruchid damage among trees, or between species attacked (Gleditsia,Cercis), and not attacked (Gymnocladus,Albizzia) in the study areas.

Assessment of Bruchid Damage to Honey Locust Seeds

INTRODUCTION

Bruchid damage to honey locust seeds (calculated as percentage of total seed crop destroyed) was analyzed from 1966 to 1969 in northeastern Kansas. Comparisons of damage within and between years, within and between areas, within and between habitats, within and between exposures, and within and between trees were undertaken.

This bruchid damage was analyzed with regard to several groups of parameters which will be outlined in detail later. The possibility that some of these parameters may vary with or determine the amount of bruchid damage is examined.

MATERIALS AND METHODS

Samples of honey locust seed crops were collected during the years 1966-1969 in northeastern Kansas. Each collection was made in autumn, after the pods had matured. Canopies of honey locust trees used in this study were separate from those of other individuals of this species.

Locations of the experimental areas (seeFigure 23) are as follows : total crops for 12 trees were collected from a farm 5 miles south of Lawrence,Kansas (Janzen Farm) in 1966. The 1967 crop (35 trees) was collected from two areas : 1.) 2 miles south of Lone Star,Kansas, and 2.) around Lone Star Lake,Lone Star,Kansas. The 1968 crop (23 trees) was collected from : 1.) the University of Kansas campus,Lawrence,Kansas, and 2.) 2 miles east of Lecompton,Kansas. In 1969 the crop (201 trees)

was collected from 7 areas (25 pod samples were collected,except for within tree comparisons where 225 pods were collected from each tree) : 1.) around Lone Star Lake,Lone Star,Kansas, 2.) 2 miles south of Lone Star,Kansas, 3.) Morris Farm, 1 mile southwest of Clinton,Kansas, 4.) 2 miles east of Lecompton,Kansas, 5.) Olathe,Kansas, 6.) Sunflower Tract, 10 miles east of Lawrence,Kansas, and 7.) the Natural History Reservation, 8 miles northeast of Lawrence,Kansas.

Each pod was examined in the laboratory and the following information recorded : 1.) pod length, 2.) pod width, 3.) pod thickness, 4.) number of seeds, 5.) number of seemingly viable seeds, 6.) number of seeds with bruchid exit holes, 7.) number of parasitized bruchids as indicated by presence of parasitic larvae, cocoons, or adults, and 8.) number of seeds missing due to vertebrate predation. All apparently viable seeds were split to see if any had live bruchids ; if so, this number was added to the number of seeds with bruchids. A pod size index was computed as pod length (cm) x pod width (cm) x pod thickness (cm).

Field measurements were made as follows : 1.) canopy volume was estimated (based on canopy depth,diameter,and height) and used as an index of the photosynthetic activity of the plant (Janzen,1969) ; 2.) tree height was likewise estimated (several trees were felled to check the accuracy of this method and it was quite good (1-5% error); and 3.) diameter of tree trunks was measured at 1.5 m above the ground.

All honey locust pods beneath certain trees were picked up after pod fall in autumn. This was done to test the importance of the pod reservoir beneath a tree with regard to future bruchid success.

RESULTS

Tunneling damage by larvae to seeds in green pods.

An important aspect of the impact of the bruchid population on seed production is the damage done to immature seeds by larvae tunneling through immature pods. In the tunneling process the tunneled portion of the pod (which would have produced seeds otherwise) is destroyed. Usually one larva will tunnel half the length of a pod ; two or more larvae may destroy the whole pod. If a larva tunnels in the distal half of a pod the proximal part can still produce seeds ; however, if a larva tunnels in the proximal part the whole pod is destroyed.

The larval damage to immature honey locust seeds (actual percentage of seeds tunneled through) from the seven experimental areas in 1969 is summarized in Table 11. The highest frequency of immature seed damage occurred at Olathe (9.53%), the least at Lecompton (1.39%). Damage to seeds in a given tree ranged from .20% to 30.81%.

Dry mature seeds destroyed by larvae.

Within and between year comparisons : Individual trees are affected to differing degrees by bruchid seed predation, even in the same locality and year. The percentage bruchid damage to seed crops in 1966,1967, and 1968 ranged, for individual trees, from 31.39% to 100% (mean of 71.01%), and is detailed in the appendix for each tree (pp. 143-147).

There were significant differences (5% level) in bruchid damage between most seed crops during each of these years.

In a general study of samples from about 270 trees from all localities, seed crops were compared between years with regard to 1.) number of pods per tree, 2.) percentage bruchid damage, 3.) number of seeds per pod, 4.) mean dry weight of seeds, and 5.) pod size. The Student-Newman-Keuls test for multiple comparisons among means based on unequal sample sizes was used to test the data. Mean values for each year were used in these comparisons (Table 12). Seed crops from 1966-1969 were compared for number of pods per tree, bruchid damage, and mean number of seeds per pod ; while seed crops from 1967-1969 were compared for all five parameters.

The number of pods per tree varied significantly (1% level) between all four years. Bruchid damage (recorded as the percentage of the total seed crop destroyed) varied significantly (at 1% level) between years. The highest percentage of seeds destroyed (93.58%) was in 1967, with the lowest in 1968 (51.26%, Table 12). The number of seeds per pod was also significantly different (1% level) between years. Mean dry weight of seeds was significantly different (1% level) between 1967 and 1968 and between 1968 and 1969, but not between 1967 and 1969. Pod size likewise was significantly different (1% level) between years.

Samples of seed crops collected from selected trees, the same ones in 1967 as in 1969, were compared using Wilcoxon's signed ranks test for the same five parameters : 1.) number of pods per tree, 2.) percentage of bruchid damage, 3.) number of seeds per pod, 4.) mean dry

weight of seeds, and 5.) pod size (Table 13). Locations used were 1.) around Lone Star Lake, and 2.) 2 miles south of Lone Star. Seed crops of eight trees from each area for both years were tested.

There was significantly (at 5% level) more bruchid damage to the same trees in 1967 than in 1969 in both areas (Table 13). The number of seeds per pod was significantly greater (1% level) in 1967 than 1969 in locality 2 ; no significant differences occurred at the first location. Mean dry weight of seeds was significantly less (5% level) in 1967 than in 1969 in area 1, but not in area 2. Pod size, however, was greater (5% level) in 1967 than in 1969 in area 2, but not in area 1. The number of pods per tree was greater in 1967 than 1969 in area 1, but vice versa in area 2. All of these results confirm the findings of the general study , showing significant differences in these parameters for different years using different trees.

Within tree comparisons : Seed crops from 15 trees (1969 crop) were tested to see if position of pods in a tree influences the amount of bruchid damage and the mean number of seeds per pod. Each tree canopy was divided horizontally into three sections, each comprising one third of the canopy height, and vertically into four quadrants (Figure 21). The lines separating the quadrants were the compass directions, northeast,southeast, southwest, and northwest. The quadrants were designated A,B,C,D, clockwise starting with north. The top horizontal section of the tree was considered as a single unit, not divided into quadrants. One sample of 25 pods was taken from each quadrant in the bottom two sections and one such sample from the

top ; thus, 9 samples (25 pods each) were collected from each tree. Five different areas were sampled, three trees in each area.

Within tree variation was assessed with regard to 1.) bruchid damage , and 2.) the number of seeds per pod. Table 14 shows the comparisons for bruchid damage, Table 15, for seeds per pod. The Student-Newman-Keuls test for multiple comparisons of means was again used to test the data, with the 5% level of significance.

Maximal bruchid damage in these trees was in the upper north, top, and lower east quadrants (Table 14). Differences between bruchid damage in different parts of the trees are nonsignificant for three of the fifteen trees ; the other twelve trees had some significant differences between parts.

The highest number of seeds per pod was in the top, lower south, and upper north quadrants in these trees (Table 15). Within tree variation with regard to the number of seeds per pod is nonsignificant for eight of the fifteen trees.

Within and between area comparisons : Within each area the percentage of seeds destroyed varies significantly. The mean number of bruchid damaged seeds per pod for each 1969 seed crop within each of the seven experimental areas was determined.

The same crops were compared between areas. The following parameters were compared and are shown in Table 16 : tree canopy volume, height of tree, diameter of tree trunk at 1.5m, pod size, total number of pods per tree, total number of seeds per tree, mean number seeds per pod, mean dry weight of seeds, total dry weight of seed crop, percentage

bruchid damage, percentage seeds taken by vertebrate predators, dry weight of seeds per m^3 of canopy, number of seeds per m^3 of canopy, and number of pods per m^3 of canopy. Mean values of each parameter for each area (see appendix, p p. 148-169) were compared. The Student - Newman - Keuls test was used for the data, with the 5% level of significance. Each of the parameters will be discussed separately and summarized below.

Tree vegetative characters of general tree form and size in the seven areas vary. Significant differences in mean tree height between areas occur ; the tallest trees were in area 3 (Morris Farm), the shortest in area 1 (around Lone Star Lake). Mean trunk diameter (at 1.5m) also varied significantly between areas ; the largest trunks were in area 3 and the smallest in area 1. No significant differences in tree canopy volume were found between areas.

Mean pod size varies significantly between areas ; the largest pods are found in area 1 and the smallest in area 6 (Table 16,Sunflower Tract). The total number of pods produced per tree is significantly different between area 3 (Morris Farm) and the other areas, and between area 4 (2 miles east of Lecompton) and the rest. The greatest number of pods per tree was found in area 4 with the smallest in area 3.

The total number of seeds produced per tree is significantly greater in area 4 than in the rest. The greatest mean number of seeds per pod was in area 1, with the smallest in area 6 (Sunflower Tract). Mean dry weight of seeds was used to assess seed size ; the largest seeds were in area 1 (around Lone Star Lake) and the smallest in area 7 (Natural History Reservation).

Total dry weight of seed crops may be used to compare overall area and tree productivity. The greatest seed biomass was produced in area 4 (2 miles east of Lecompton) with the smallest in area 3 (Morris Farm).

The percentage of seeds taken by squirrels,rabbits,and other vertebrate predators before the time of pod collection varies significantly among all seven areas and probably reflects vertebrate predation pressure up to the time of pod collection.

The percentage of seeds destroyed by bruchids likewise is significantly different among all areas in 1969 (Figure 22). The greatest bruchid damage occurred at the Natural History Reservation (86.44%) with the least at Olathe (44.21%). These results are discussed below with regard to the other parameters.

Comparisons of habitats and exposures :- Seed crops (1969) were also examined as related to habitat and exposure. The three habitats considered are listed in Table 17 : 1.) open field, 2.) canopy member of forest, and 3.) edge of open field. The six parameters tested (Tables 17 and 18) are 1.) pod size index, 2.) total number of pods per tree, 3.) total number of seeds per tree, 4.) dry weight of seeds, 5.) number of seeds per m^3 of canopy, and 6.) percentage bruchid damaged seeds.

Effects of exposure within a given habitat type are shown in Table 17. In open field habitats no significant differences between trees on different exposures occurred in the number of pods, dry weight of seeds, and number of seeds per m^3 of canopy. Significant differences did occur in pod size, number of seeds per pod, and bruchid damage.

The number of pods per tree and number of seeds per m^3 of canopy did not vary significantly for canopy member trees or trees on the edge of open fields ; pod size, number of seeds per pod, dry weight of seeds, and bruchid damage did vary significantly within these two habitats.

The effects of habitat type examined for the various exposures are shown in Table 18. The number of pods per tree was not significantly different between habitats for any exposure ; the number of seeds per pod and percentage bruchid damage was significantly different between habitats. Pod size and number of seeds per m^3 of canopy was significantly different among habitats in trees on level ground, on north facing slopes, and on south facing slopes. Pod size varies significantly among trees on west facing slopes, while pod size, number of seeds per pod, and dry weight of seeds varies significantly among trees on east facing slopes.

There are significant relationships between the percentage of bruchid damage and some but not all of these parameters. Significant positive correlations exist between the amount of bruchid damage and canopy volume ($r = .755$, significant at 5% level), between bruchid damage and the number of seeds per pod ($r = .881$, significant at 1% level), between bruchid damage and mean dry weight of seeds ($r = .894$, significant at 1% level), and between bruchid damage and pod size ($r = .471$, significant at 5% level).

DISCUSSION

Larval damage via tunneling to immature seeds in green pods probably is a result of eggs being laid early (when seeds are not

mature) ; therefore, the larvae tunnel great distances in search of seeds. In mature pods this tunneling is much less provided seeds are not occupied by other larvae and in any event does no meaningful damage to the pod. Thus, larvae are responsible for considerable damage to honey locust seeds (up to 30.81% for a given tree) even as they are maturing.

The pattern of bruchid damage within honey locust trees is not clear, although the greatest amount of damage in areas 2,3,and 4 occurred in the upper north quadrant in two of three trees in each of the areas. Abrahamson and Kraft (1965) found a similar type of within tree variation in the distribution of cones in the jack pine, Pinus banksiana Lamb. The insect population (cone moth, Laspeyresia toreuta) was influenced by cone position within the tree crown ; the largest populations were in the lower ten feet of crown on the south side of the tree. Similarities between three experimental areas with respect to localization of damage in the upper north quadrant of honey locust trees probably result from L.) movement from trees with pods north of these trees, or 2.) movement from pods lying on the ground north of the trees.

An interesting pattern emerges when Tables 14 and 15 are compared. Trees with significant differences in bruchid damage also have significant differences in the number of seeds per pod. It appears that bruchid damage within a tree is positively correlated with (as shown prevously) the number of seeds per pod. Therefore, bruchids probably concentrate their efforts in areas of high seed density per pod and thereby more damage is done there than in regions with fewer seeds per pod within the same tree.

The mean number of seeds per pod is correlated (.1% level) with pod length and thereby fluctuates with pod size. This certainly is not unexpected, but relates to overall pod size and its probable role of attracting vertebrate predators. Pod length is correlated (10% level) with the number of seeds taken by squirrels,rabbits,etc. ; the greatest percentage of seeds taken by vertebrate seed eaters occurs in seed crops with the largest pods. This parameter (seeds per pod) is of importance when an ovipositing female approaches a pod. There should be selection for the female to lay one egg per seed ; a numerical mistake by the female may allow a seed to escape. This seems to be the situation with Amblycerus , as the female bruchid usually does lay one egg per seed. Possibly the more seeds there are per pod the greater the chance of a female missing one along the way. Of course, egg mortality factors are important and effectively reduce the initial, commonly one to one, relationship between seeds and bruchid eggs.

Also, the number of bruchid larvae per pod is correlated (.1% level) with pod length, and pod size, probably because of seed number.

The correlation between canopy volume and bruchid damage may relate to an optimum canopy size which affords protection, substrate for oviposition and development (pods and seeds), or provides attractant stimuli (i.e., a certain amount of foliage may be necessary to emit a threshold level of bruchid attractants).

There is also a significant correlation between bruchid damage and seed size (mean dry weight). Damage increase with an increase in seed size and decreases with size reduction. Seed size may be

important with regard to bruchid development (i.e., bruchids may not complete development in very small seeds), oviposition strategy of the beetle, and the reproductive success of the tree. There is some evidence to support Carlquist's hypothesis (Carlquist,1966b) of increased seed size (and loss of dispersibility) in forest areas vs. open areas with regard to honey locust seeds. Seeds from canopy members in the forest are significantly larger (5% level) than seeds from trees in open fields.

It has been suggested (Janzen, 1969) that the honey locust system operates via "predator satiation" with regard to escaping bruchid beetle attack. The system would work since the first group of Amblycerus does not utilize all the seeds due to low laying capacity (30 to 80 eggs) and removal of seeds by active dispersal agents before bruchids get them. Thus, there should be selection for traits that favor dispersal agents which remove seeds immediately after maturation. Apparently the honey locust fits this system since it matures all its seeds at one time, thereby both attracting dispersal agents and satiating the bruchids with large numbers of seeds which because of timing they cannot numerically respond too. The honey locust also drops most of its pods at the same time in fall and this plus the drastic color change from green to maroon may aid attraction of vertebrate dispersal agents. Honey locust pods or parts of pods are eaten by rabbits, squirrels, deer, wood rats, cattle,etc. The seeds then pass out after digestion (as evidenced in deer,rabbit,and cattle feces in which these seeds were found) and are likely to be a considerable distance from the parental tree and quite safe from bruchid attack.

Adult density was compared to the number of seeds available for larval development. In general larger seed crops are correlated with higher bruchid density per tree. Thus, the adult population of one year is in part determined by the number of pods present the previous year. In the "off " years the number of seeds produced per tree as well as the number of reproductive trees is reduced. Alternation of seed crops from year to year may be a mechanism for reducing bruchid destructive pressure. The adult bruchids which emerge following a seed crop year (" on " year) find pods on the ground (for reproduction in the summer), but few in the trees in the fall (since this now is a " off " year). Thus, a small population will overwinter into the next year which will again be an " on " year. The bruchids cannot destroy all the seeds present during this " on " year starting with a small population. By the end of the " on " year population levels of bruchids are high, but they will be reduced again in the " off " year. However, trees in a given area are not fully synchronized and in any year from 10 to 15% of the trees have some seed pods. But, since these trees only have a small number of pods the seed production in the " off " years is much less than 10 to 15% of that in " on " years (probably less than 5%).

It appears that high bruchid beetle densities should create strong selective pressures on the honey locust. The honey locust has apparently responded by 1.) alternating seed crops to avoid seed predation, 2.) increasing seed production to satiate the bruchids, and 3.) increasing the attractiveness of pods to dispersal agents thus dispersing some seeds before the bruchids destroy them all.

Large numbers of pods closely packed together may act as superattractant stimuli. These dense pod areas probably attract larger numbers of bruchids with the result that subsequent damage is higher. However, since the number of seeds is very high, increased bruchid destruction can occur, but a large number of viable seeds is still left. Low pod density areas then escape heavy bruchid destruction and this enhances future reproduction for the tree. This idea suggests that there must be a certain number of suitable host structures (pods) available before the bruchids are attracted to a tree. Other evidence along this line (Coulson and Franklin, 1968) indicates that shortleaf pine trees with fewer than 90 cones suffer very little attack.

Pod production and seed number and size is probably a function of available nutrients, sunlight, competitive conditions, and genetic composition. Generally, pods with the largest number of seeds occur at or near the tops of trees. This may indicate that available sunlight (maximum insolation) is an important variable that affects pod growth and size.

Total dry weight of seed crops probably is a measure of the amount of energy channeled into reproductive activities. An average honey locust tree 12 m tall produces a crop of 16,000 seeds every other year with a total dry weight of 3.2 kgs. (average seed dry weight = .1868 gm). A Kentucky Coffee Tree of the same height and size produces a seed crop each year of 600 seeds with a total dry weight of 1.2 kgs. (average seed dry weight = 2.005 gm). Thus, a honey locust tree may use a higher percentage of its total energy for seed production than the Kentucky Coffee Tree, and may have less competitive

ability (other things being equal) than the Kentucky Coffee Tree (which has no bruchid seed damage and produces a relatively small seed crop).

The reproductive output of a given tree appears to be on two levels : 1.) the support structure (pods), and 2.) the actual reproductive tissue (seeds). Pod size can again be mentioned in this context, since some honey locust trees partition more energy into the support structure than others (i.e., pod size differences between areas). Reasons for this may include 1.) increased attractiveness to vertebrate predators, and 2.) increased support may deter premature pod breakage and thus prevent seed loss during the immature stages.

Significantly different (.1% level) bruchid damage between areas (see Figure 22) most likely indicates different bruchid population sizes, differential bruchid reproductive success, fluctuation in intensity of bruchid mortality factors, different local physical environmental conditions, differences in seed crop size for consecutive years, and possibly individual tree defensive mechanisms perfected to a lesser or greater degree. Different individuals of Gleditsia probably vary in susceptibility to bruchid attack because of either inherited factors or environmental influences. Certain trees may be more prone to attack because they contain positive factors that induce it, or lack the chemical and physical impediments to it. Circumstantial factors such as proximity of other honey locust trees with pods, nearby pods on the ground from past years, or certain wind directions may be of primary importance.

The number of pods per tree and number of seeds per m^3 of canopy do not vary significantly within the three habitats tested. This can be explained if the numbers of pods and seeds are fixed genetically within narrow limits. On the other hand, pod size and seed size (mean dry weight of seeds) do vary significantly within these habitats and probably reflect local environmental conditions (different growth rates exist on north vs. south facing slopes for example).

Bruchid damage within these habitats does vary significantly. In open fields the highest percentage damage occurred in trees on level ground (76.81%) with the lowest in trees on west facing slopes (57.01%) ; in forest areas (i.e. canopy members) the greatest damage was again in trees on level ground (73.34%) with the lowest in trees on north facing slopes (58.22%) ; and in trees on the edges of open fields most damage occurred on west facing slopes (78.30%) with the least on north facing slopes (44.89%). In general then, the greatest bruchid damage occurs in trees on level areas and the least on north facing slopes. Reasons for this might include : 1.) wind directions on slopes perhaps limit bruchid access to trees, 2.) reduced insolation on north slopes, 3.) infestation from last year's pods would be easier from under a tree on a level, since pods may roll down a slope a considerable distance, and 4.) the search for other seed crops away from their own pods.

Comparisons between habitats having the same exposure show similar results. The number of pods and number of seeds per m^3 of canopy do not vary greatly between habitats, but pod size and seed size do. Bruchid damage is significantly different between habitats for all exposures.

On north facing slopes the greatest damage is in open fields (75.58%) with the lowest on the edges of open fields (44.89%) ; on south facing slopes the greatest damage is on the edges of open fields (78.30%) with the least in open fields (57.01%) ; on east facing slopes the most damage was in open fields (70.46%) and the least on the edges of open fields (54.08%) ; on west facing slopes the most damage was on the edges of open fields (69.18%) with the lowest in forests (63.41%) ; and on level areas the most damage was in open fields (76.81%) with the least on the edges of open fields (65.69%). Some repeatable patterns emerge with the most damage occurring in open fields or on the edges of open fields and the least in forested areas. Higher damage in one of these habitats versus another may depend on 1.) proximity of other honey locust trees , 2.) numbers of pods from past years that have not been dispersed, 3.) effects of rainfall, sunlight, and wind on bruchid egg mortality rates, and 4.) vertebrate seed predator populations (I would anticipate that forested areas would have more seed dispersal since populations of squirrels,rabbits,etc. appear higher there).

The possibility that parasitism is an important factor in determining bruchid density merits consideration. Five parasites have been identified (see section on parasites), two braconids and three chalcidoids. From 1 to 18 parasites of the various species develop per beetle. The percentage of bruchids parasitized in different trees over the four year study was from 9 to 45% (Table 19). Significant differences (5% level) occur among mean percentages of parasitism between each year. At these percentages parasites can

definitely influence bruchid populations and therefore aid survival of honey locust seeds.

CONCLUSIONS

1.) Amblycerus robiniae larval damage via tunneling in green pods of Gleditsia triacanthos is an important factor with up to 31% of the immature seeds being destroyed.

2.) Bruchid damage (computed as the percentage of total mature seed crop destroyed) was significantly different within and between years, within and between areas, within and between trees, within and between habitats, and within and between exposures during a four year (1966-1969) study in northeastern Kansas.

3.) The greatest percentage bruchid damage occurred at the Natural History Reservation (86.44%), with the least at Olathe (44.21%). Generally, the most damage occurred in the upper tree levels ; specifically, in the upper north quadrants of the trees.

4.) Significantly different bruchid damage within and between areas most likely indicates different bruchid population sizes due to differential bruchid reproductive success, fluctuation in intensity of bruchid mortality factors (both biotic and physical), tree reproduction, and individual tree differences.

5.) The number of pods per tree and number of seeds per m^3 of canopy did not vary significantly within or between habitats, but pod and seed size did. Probably pod and seed number is genetically determined, but pod and seed size may be influenced by local

environmental conditions.

6.) The most bruchid damage occurred in trees on level ground and the least in trees on north facing slopes. The most damage occurred in open field habitats and the least in forest.

7.) Amblycerus robiniae damage is correlated with the number of seeds per pod, pod size, seed size (mean dry weight), and m^3 of canopy.

SUMMARY

The bruchid beetle (Amblycerus robiniae) vs. honey locust (Gleditsia triacanthos) system has been examined from several viewpoints. Bruchid mortality factors such as egg droppage from pods, failure of larvae to gain entrance into seeds, parasitism by wasps, and intraspecific competition seem to be responsible for keeping the bruchids from destroying all the honey locust seeds.

Peak bruchid adult activity occurs from 9 p.m. to 6 a.m. ; thus Amblycerus is nocturnal. It is suggested that this bruchid does not use visual cues (color) when seeking host plants (honey locust), but relies on olfaction. The nocturnal nature of the beetles activity also indicates that color vision would be of little use.

During two years of weekly sampling, adult seasonal activity peaks occurred in early July and early October. Most bruchids frequent honey locust trees with pods vs. honey locust trees without pods and few are found on other species of trees. Captures (in tanglefoot traps) in particular regions within a tree depend on pod density, proximity of other honey locust trees, and pod distribution on the ground. Highest bruchid densities occurred in the upper and lower outside regions of the trees.

Chemical analysis of honey locust seeds accounted for sixty to sixty five per cent of the seed content. This study suggests which nutrients should be available to the bruchid beetle and thus provides a partial description of the microhabitat of Amblycerus larvae.

Thirty two different amino acids were found in honey locust seeds. Differences in amino acid concentrations among honey locust trees

cannot account for striking differences noted in the amount of bruchid damage to different trees, or between species attacked (Gleditsia, Cercis) and not attacked (Gymnocladus,Albizzia) in the study areas.

Amblycerus larval damage via tunneling in green pods is an important immature seed mortality factor (up to 31%). The bulk of the bruchid population, however, feeds on seeds in mature pods. Bruchid damage in such pods varies significantly between and within years, between and within areas, between and within habitats, between and within exposures, and between and within trees. Most damage occurs in the upper tree levels, specifically in the upper north quadrant. Most bruchid damage occurred in trees on level ground and the least in trees on north facing slopes. The most damage occurred in open field habitats and the least in the forests. Amblycerus damage is positively correlated with the number of seeds per pod, pod size, seed size, and m^3 of canopy volume.

It appears that high bruchid beetle densities should create strong selective pressures on the honey locust tree. The honey locust has apparently responded by 1.) alternating seed crops from year to year to avoid seed predation, 2.) increasing seed production to satiate the bruchids, and 3.) increasing the attractiveness of pods to dispersal agents in order to disperse some seeds before the bruchids destroy them all.

The regulation of bruchid population size appears to depend on : 1.) available food resources, 2.) environmental fluctuations, 3.) parasitism and other mortality factors, and 4.) on intraspecific larval competition.

Thus, the bruchid appears to have a significant effect on potential honey locust tree density, but probably has less effect on adult plant density now extant. In such a situation a case can be made for strong selective pressure on the honey locust favoring a genotype that contributes the highest proportion of new plants to later generations. This genotype would be maximized through selection for seed predator satiation, even though size of the plant population changes little.

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Table 1. Mortality table for Amblycerus robiniae.

Stage	Number	Mortality Factors	Number Dying	Per Cent Mortality D/I	Per Cent Mortality D/T
Eggs	2000	Eggs lost from pod	422	21.1	
		Eggs with no visible development	82	4.1	
		Embryo dead in egg	128	6.4	
		Mortality in egg stage			31.6
Larvae	1368	Failure of first instar to enter seed	209	15.3	
		Parasitism by wasps	339	24.8	
		Mortality in larval stages			40.1
Pupae	820	Found dead in seed	2	.3	
		Mortality in pupal stage			.3
Adults before emergence	818	Failure to escape from seed	4	.5	
		Mortality in adult stage			.5
Adults emerged	814				
Accumulated Mortality (egg to emerged adult)					72.5

D / I = the number dying divided by number in initial population

D / T = the number dying during a stage divided by total population for that stage

Table 2. Rate of oviposition by Amblycerus on mature honey locust pods (200 female beetles caged with 1000 pods).

Time interval from emergence (hrs.)	0-24	24-48	48-72	72-96	96-120	120-144	144-240	Total
Number of eggs laid	104	809	1886	1941	1701	1309	1677	9427
Percentage of total eggs laid	1.1	8.5	20.0	20.5	18.0	13.8	17.7	100.0

Table 3. Honey locust seed germination and seedling survival in three habitat types : 1.) open field, 2.) edge of open field, and 3.) forest (2 miles south of Lone Star,Kansas). Five plots of 25 seeds each were planted in each habitat type. Percentage seedling survival is the percentage of seedlings alive 5 months after germination.

Percentage Germination

Plot number	open field	edge of open field	forest
1	20.7	41.1	6.3
2	18.1	57.3	7.6
3	19.3	59.4	9.1
4	31.2	61.2	8.9
5	34.5	50.2	9.9
$\bar{Y}$ =	24.76	53.84	8.28

Percentage Seedling Survival

Plot number	open field	edge of open field	forest
1	46.3	63.4	46.1
2	51.9	71.8	43.1
3	43.4	65.1	47.3
4	47.8	69.1	51.2
5	52.1	59.7	44.9
$\bar{Y}$ =	48.30	65.82	46.52

Table 4. Percentage of bruchids caught in the three types of trees at two locations.

	type of tree			
	locust with pods	locust without pods	osage orange	total N
Sunflower	76.5	21.4	2.1	709
Natural History Reservation	75.5	21.2	3.3	589

Table 5. Comparisons of bruchid capture in three types of trees, direction, and position in the tree.

ARRANGEMENT OF DATA

Factor A	Factor B	Factor C
Type of tree	Compass direction	Position in tree
T_1 = honey locust with pods	D_1 = north	P_1 = upper outside
T_2 = honey locust without pods	D_2 = northwest	P_2 = upper core
T_3 = osage orange	D_3 = west	P_3 = lower outside
	D_4 = southwest	P_4 = lower core
	D_5 = south	
	D_6 = southeast	
	D_7 = east	
	D_8 = northeast	

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Table 5 (concl.).

Hypothesis tested	df	G
A x C independence	6	3.716
A x B independence	14	31.286 ***
T_1 vs T_2 vs T_3		
D_3+D_5 vs $D_1+D_2+D_4+D_6+D_7+D_8$	1	.506
$D_3+D_5+D_6$ vs $D_1+D_2+D_4+D_7+D_8$	1	.098
$D_3+D_4+D_5+D_6$ vs $D_1+D_2+D_7+D_8$	1	.624
B x C independence	21	73.138 ***
$D_3+D_4+D_5+D_6$ vs $D_1+D_2+D_7+D_8$		
$P_1 + P_2$ vs $P_3 + P_4$	1	.134
$P_1 + P_3$ vs $P_2 + P_4$	1	2.828
A x B x C interaction	42	24.614
A x B x C independence	83	132.754 ***

$\chi^2_{.005}(1) = 3.841$, $\chi^2_{.001}(1) = 6.635$, $\chi^2_{.001}(14) = 29.141$,

$\chi^2_{.001}(21) = 38.932$, $\chi^2_{.001}(83) = 115.88$, $\chi^2_{.005}(42) = 58.124$.

*** = significant at .1% level

Table 6. Percentages of bruchids caught in various positions in the trees in location number one at the Sunflower Tract.

	locust with pods	locust without pods	osage orange
upper outside	55.8	62.8	66.7
upper core	9.1	8.6	0.0
lower outside	30.4	24.3	22.2
lower core	4.7	4.3	11.1
N	276	70	9

Table 7. Water content of Gleditsia seed as determined by freeze-drying and oven-drying. Means are given for each of the three tests (described in text).

	INTACT SEEDS		SPLIT SEEDS	
	Total weight loss	% water	Total weight loss	% water
Test 1	.0160	9.41	.0141	9.78
	.0165	9.77	.0168	10.72
$\bar{Y}$ =	.0162	9.59	.0154	10.25
Test 2	.0152	9.36	.0233	14.84
	.0169	10.10	.0222	15.23
	.0140	9.20	.0211	13.74
	.0166	10.12	.0162	10.70
	.0132	8.45	.0129	9.79
$\bar{Y}$ =	.0152	9.45	.0191	12.86
Test 3	.0305	15.41	.0269	14.52
	.0274	14.84	.0367	20.98
	.0257	16.37	.0290	14.80
	.0156	12.76	.0249	14.34
	.0312	13.58	.0381	20.47
	.0238	11.93	.0231	16.76
	.0170	15.83	.0306	16.76
	.0255	19.98	.0271	16.09
	.0272	13.06	.0274	17.67
	.0239	12.59	.0190	18.16
	.0176	12.11	.0227	14.13
	.0218	12.48	.0228	15.17
	.0155	11.29	.0335	19.29
	.0266	15.04	.0185	14.55
	.0243	15.93	.0327	19.87
$\bar{Y}$ =	.0236	14.21	.0275	16.90

Table 8. Amino acid composition of honey locust seeds from various locations. The quantities are expressed as percentages of the total of all amino acids for a given sample as determined on the amino acid analyzer. Symbols : - indicates absence and t = trace in micromoles. Seed crops are from 7 locations.

Amino acid	Location number 1	2	3	4
Phosphoserine	.29	-	.42	.26
Glycerophospho-ethanolamine	.36	-	-	-
Phosphoethanolamine	.14	-	5.12	.13
Taurine	.15	-	t	.55
Urea	.19	-	t	.05
Unknown no. 1	-	-	-	-
Methionine sulfoxide	-	-	-	-
Aspartic acid	7.34	.30	7.70	3.17
Hydroxyproline	.16	-	-	.18
Threonine	1.48	2.35	1.51	1.76
Serine	1.71	2.03	3.92	4.12
Homoserine (?)	-	-	-	-
Asparagine	44.55	30.89	11.71	28.65
Glutamic Acid	3.63	16.97	11.09	5.28
Glutamine	-	6.08	-	2.24
Sarcosine (?)	.12	.08	.36	.06
a-Aminoadipic acid	.79	.13	.61	.24
Unknown no. 2	.20	.12	-	.10
Proline	3.51	2.23	5.14	8.04
Glycine	.97	.77	2.74	2.19
Alanine	5.42	5.86	9.18	12.74
Citrulline	.12	.11	-	.11
a-Amino-n-butyric acid	t	.24	-	.08
Valine	1.37	1.49	3.10	3.73
Half Cystine	1.56	5.14	8.98	2.31
Methionine	.39	.06	.38	.17
Isoleucine	.49	.70	1.34	1.56
Leucine	.29	.32	.93	1.62
Tyrosine	.23	.31	.97	.50
Phenylalanine	.24	.52	.98	.84
Unknown no. 3	.18	1.69	-	.90
Tryptophan	.09	.54	-	.24
Lysine	1.48	1.75	4.38	3.48
Histidine	1.25	.74	2.89	1.31
Arginine	21.32	18.53	16.54	13.38

(continued on p. 80)

Table 8(concl.).

Location number					
5	6	7	8	9	10
.56	.47	.07	-	.30	-
-	-	-	-	-	-
.51	.36	.20	-	.16	t
-	t	.61	-	t	t
-	t	.09	-	t	t
-	-	-	-	-	-
-	-	-	-	-	-
11.50	7.37	4.96	7.95	9.43	5.66
.33	t	.17	t	-	.18
2.29	1.32	2.30	2.12	1.84	3.45
5.56	2.72	3.32	4.16	3.76	5.91
-	-	-	-	-	-
20.98	22.34	24.42	14.29	7.34	2.08
6.33	6.49	5.89	4.37	.63	5.74
-	t	1.29	-	.32	.44
.26	.23	.05	.21	.42	.22
.83	1.61	.31	.56	1.88	.09
-	.16	.02	-	.48	-
6.23	4.60	14.47	4.06	6.02	7.02
2.50	1.57	2.14	2.12	1.65	4.06
10.37	7.82	10.59	7.04	8.35	16.32
t	.36	.03	-	.24	-
-	.19	-	-	.16	-
3.88	2.28	2.62	2.76	3.50	5.90
3.57	6.95	2.95	2.51	11.50	4.62
.06	.32	.12	.10	.60	.01
1.88	.71	.78	1.38	1.87	2.48
.92	.46	1.08	.82	.94	2.24
.57	.66	.45	.38	.80	1.10
.81	.53	.49	.57	.95	1.44
2.03	.10	.57	.15	.73	t
.37	t	.62	t	t	t
2.63	2.07	2.86	3.06	3.20	5.81
2.33	2.40	1.25	2.59	2.31	3.38
12.69	25.88	15.29	38.84	30.60	21.84

Table 9. Anova table testing honey locust trees vs. amino acids. No significant differences among trees occur, but there are highly significant differences (at .1% level) in percentages of different amino acids within each tree.

Source of variation	df	SS	MS	Fs
A (column ; trees)	9	75.8409	8.4268	.6413 ns
B (rows ; amino acids)	19	9008.5756	474.1356	36.0823 ***
Error (remainder ; discrepance)	171	2247.0026	13.1404	
Total	199	11331.4191		

$F_{.05\ (9,\infty)} = 1.88$, $F_{.001\ (20,\infty)} = 2.27$

Table 10. Amino acid composition of Gymnocladus dioeca, Cercis canadensis, Albizzia julibrissin, and Gleditsia triacanthos seeds. Values for Gleditsia triacanthos are means from 10 samples, others are from one sample each. The quantities are expressed as percentages of the total of all amino acids in micromoles as determined on the amino acid analyzer. The symbol - indicates absence and t indicates presence in trace amounts.

Amino acid	Gymnocladus dioeca	Cercis canadensis
Phosphoserine	.12	1.85
Glycerophosphoethanolamine	-	-
Phosphoethanolamine	.10	.06
Taurine	.33	.32
Urea	.04	.09
Unknown no. 1	-	-
Methionine sulfoxide	11.51	-
Aspartic acid	31.48	4.24
Hydroxyproline	29.74	-
Threonine	.30	1.02
Serine	.73	1.96
Homoserine (?)	-	2.90
Asparagine	2.11	8.72
Glutamic acid	1.60	12.15
Glutamine	.12	21.89
Sarcosine (?)	5.11	.38
a-Aminoadipic acid	2.36	.36
Unknown no. 2	-	-
Proline	8.05	5.75
Glycine	.22	1.55
Alanine	.86	5.14
Citrulline	-	-
a-amino-n-butyric acid	-	-
Valine	.53	2.16
Half cystine	.34	.91
Methionine	t	.09
Isoleucine	.10	.41
Leucine	.11	.49
Tyrosine	.23	.73
Phenyalanine	.04	.41
Unknown no. 3	.003	.16
Tryptophan	.09	.25
Lysine	.24	3.96
Histidine	.14	3.88
Arginine	3.39	18.16

(continued on p. 83)

Table 10 (concl.).

Albizzia julibrissin	Gleditsia triacanthos
.12	.24
.58	.04
2.00	.66
.26	.13
.12	.03
5.47	-
.56	-
2.66	6.54
.36	.10
.67	2.04
2.54	3.72
-	-
-	20.72
17.10	6.64
53.50	1.04
.17	.20
-	.70
-	.11
.98	6.13
.82	2.07
1.84	9.37
-	.10
-	.07
.73	3.06
.17	5.01
.13	.22
.41	1.32
.47	.96
.16	.60
.42	.74
t	.64
.15	.19
2.16	3.07
1.60	2.04
3.81	21.49

Table 11. *Amblycerus* larval damage to immature seeds in green pods by tunneling. Samples of 25 pods were collected from 10 trees in each of the seven areas. The mean percentage of seeds damaged for all areas is listed, with the range in parentheses.

Area	Number of Seeds Per Pod	Mean Percentage of Total Immature Seeds Damaged
Lecompton	17.5	1.39 (.40 - 6.92)
2 miles South of Lone Star	18.4	2.84 (.80 - 7.20)
Sunflower Tract	15.7	3.14 (1.32 - 6.50)
Around Lone Star Lake	18.6	1.76 (.20 -10.47)
Olathe	15.9	9.53 (1.00 -30.81)
Natural History Reservation	15.7	9.02 (1.60 -30.13)
Morris Farm	18.4	5.13 (.60 -11.62)

Table 12. Mean values of parameters used for between year comparisons of seed crops of Gleditsia.

Year	Number of Pods Per Tree	Percentage Bruchid Damaged Seeds	Numbers of Seeds Per pod	Mean Dry Weight of Seeds	Pod Size Index
1966	701.4	68.18	14.7	-	-
1967	591.7	93.58	19.2	.1836	11.105
1968	640.3	51.26	20.8	.2215	10.276
1969	852.9	66.74	17.2	.1895	8.736

Table 13. Comparisons of seed crops collected from the same trees of Gleditsia in 1967 and 1969. Samples of 25 pods were collected from each tree each year. Mean values for each of the five parameters are listed.

Area	Tree Number	Number of Pods Per Tree		Percentage Bruchid Damaged Seeds		Numbers of Seeds Per Pod		Mean Dry Weight of Seeds		Pod Size Index	
		1967	1969	1967	1969	1967	1969	1967	1969	1967	1969
Area 1											
	1	694	519	97.82	75.00	21.4	19.2	.1886	.1790	12.187	11.413
	2	891	1149	100.00	64.72	17.4	18.4	.1470	.2158	9.814	10.698
	3	210	360	92.38	46.87	22.0	16.9	.1953	.2550	9.547	10.267
	4	1301	1209	100.00	85.69	18.0	18.8	.1689	.2250	11.120	10.450
	5	318	137	98.66	82.22	21.2	20.2	.2306	.2041	9.538	8.217
	6	474	393	100.00	38.46	22.6	18.9	.2101	.2528	12.640	12.108
	7	231	182	100.00	38.18	15.6	18.4	.2087	.2400	10.567	10.941
	8	901	1032	98.21	52.40	19.8	17.8	.2395	.2306	9.833	8.681
	$\bar{Y}$ =	627.50	623.38	98.38	60.44	19.75	18.58	.1986	.2253	10.656	10.347
Area 2	1	701	915	85.71	69.09	20.0	21.0	.2031	.2093	10.942	11.206
	2	1682	1563	89.39	69.23	24.0	19.0	.1651	.1721	10.001	8.187
	3	247	171	98.41	61.11	22.0	15.8	.1911	.1551	14.049	6.358
	4	418	313	95.65	46.26	21.4	18.0	.1836	.2004	10.820	6.997
	5	643	370	81.57	66.67	21.0	18.4	.2312	.2078	10.126	11.507
	6	1521	1942	86.11	74.99	20.0	17.8	.1591	.1702	11.686	8.869
	7	492	310	95.31	57.14	21.0	20.2	.1388	.2193	11.902	10.444
	8	1013	1346	97.56	67.64	20.6	14.4	.1684	.2163	10.126	10.008
	$\bar{Y}$ =	839.62	866.25	91.21	64.02	21.25	18.07	.1800	.1938	11.206	9.197

Table 14. Within tree comparisons of bruchid damage to mature Gleditsia seeds. Set of means (mean number of bruchid -destroyed seeds/pod) that are not significantly different (5% level) are subtended by lines. Within each set the values increase from top to bottom.

Area	tree 1	tree 2	tree 3
1	1 5 2 3 4 9 8 6 7	3 7 1 6 8 4 9 5 2	8 6 2 7 4 1 5 3 9
2	9 8 7 4 1 6 3 5 2	1 3 5 2 4 8 6 7 9	9 8 1 6 5 7 4 3 2
3	5 4 6 8 3 1 9 7 2	4 3 1 7 5 8 9 6 2	7 8 5 2 1 4 3 9 6

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Table 14 (concl.).

area	tree 1	tree 2	tree 3
4	5 8 4 7 6 1 3 9 2	6 5 8 4 3 7 9 1 2	6 3 9 1 5 4 7 2 8
5	4 3 5 2 9 7 8 6 1	7 9 2 8 3 6 5 1 4	9 4 5 3 7 8 2 6 1

Key : 1 = top
2 = upper north
3 = upper south
4 = upper west
5 = upper east
6 = lower north
7 = lower south
8 = lower west
9 = lower east

Table 15. Comparisons of the number of seeds produced per pod in different tree regions. Set of means (mean number seeds per pod) that are not significantly different (5% level) are subtended by lines. Within each set the values increase from top to bottom.

Area	tree 1	tree 2	tree 3
1	1 5 2 3 4 9 8 6 7	1 3 9 2 5 4 8 6 7	8 7 5 2 6 3 4 9 1
2	9 8 7 4 1 6 3 5 2	7 6 9 2 5 3 2 8 1	9 8 1 6 5 7 4 3 2
3	5 4 6 8 3 1 9 7 2	4 2 1 6 3 8 9 7 5	7 1 8 4 3 5 6 2 9

(continued on p. 90)

Table 15 (concl.).

area	tree 1	tree 2	tree 3
4	8	6	9
	2	5	5
	5	8	6
	4	4	3
	7	3	4
	9	7	2
	1	9	8
	3	1	7
	6	2	1
5	4	9	9
	3	7	4
	5	2	5
	2	8	3
	9	3	7
	7	6	8
	8	5	2
	6	1	6
	1	4	1

Key : 1 = top
2 = upper north
3 = upper south
4 = upper west
5 = upper east
6 = lower north
7 = lower south
8 = lower west
9 = lower east

Table 16. Comparison of tree and seed crop parameters and bruchid and vertebrate damage for seven areas (1-7)(see text) in which collections were made in 1969. Sets of means that are not significantly different (5% level) are subtended by lines. Within each set the values increase from top to bottom.

Tree canopy volume	Tree height	Trunk diameter at 1.5m	Pod size
6	1	1	6
1	2	2	7
5	6	6	5
7	7	5	3
2	4	7	2
3	5	4	4
4	3	3	1

Total numbers of pods	Total numbers of seeds	Number seeds per pod	Mean dry weight of seeds
3	3	6	7
1	1	7	3
7	7	5	6
2	2	4	4
6	6	3	5
5	5	2	2
4	4	1	1

Total dry weight seed crop	Percentage taken by vertebrates	Percentage bruchid damage
3	5	5
7	1	4
1	7	1
5	2	6
2	3	2
6	6	3
4	4	7

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Table 16 (concl.).

Grams of seeds per m^3	Number of seeds per m^3	Number of pods per m^3
7	7	3
3	3	7
2	2	2
4	4	4
6	6	5
5	5	1
1	1	6

Table 17. Effects of exposure within a given area on seed crop parameters and percentage of seeds damaged by bruchids. Sets of means that are not significantly different (5% level) are subtended by lines. Within each set the values increase from top to bottom. 1 = north facing slope, 2 = south facing slope, 3 = west facing slope, 4 = east facing slope, and 5 = level.

Parameter	Habitat		
	open field	canopy member	edge of open field
Pod size	1 4 2 3 5	1 2 3 5 4	5 2 4 1 3
Number of pods	1 4 2 3 5	1 4 2 5 3	2 4 5 1 3
Seeds per pod	1 2 4 5 3	1 2 3 4 5	2 4 5 3 1
Dry weight of seeds	5 4 1 3 2	5 4 3 2 1	3 4 5 1 2
Seeds per m^3	1 5 3 4 2	5 1 3 4 2	5 1 2 3 4
Percentage bruchid damage	3 2 4 1 5	1 4 2 3 5	1 4 5 2 3

Table 18. Effects of habitat type for the various exposures on seed crop parameters. Sets of means that are not significantly different (5% level) are subtended by lines. Within each set the values increase from top to bottom.

Exposure	Pod size	Number of pods	Seeds/pod
North facing slope	1 2 3	1 2 3	2 1 3
South facing slope	3 1 2	3 1 2	3 2 1
West facing slope	3 2 1	1 2 3	2 1 3
East facing slope	1 3 2	3 1 2	3 2 1
Level	3 1 2	3 2 1	3 2 1

Key : 1 = open field
2 = canopy member of forest
3 = edge of open field

(continued on p. 95)

Table 18 (concl.).

Dry weight of seeds	Seeds per m^3	Percentage bruchid damage
3	1	3
1	3	2
2	2	1
3	3	2
1	1	1
2	2	3
3	1	1
1	3	2
2	2	3
3	1	3
2	2	2
1	3	1
2	3	3
3	2	2
1	1	1

Table 19. Mean percentage of parasitization of bruchid beetles in different trees (25 to 50 pods per tree were sampled). Twelve trees per year per area were sampled ; for the 1966 crop (one area), the 1967 crop (two areas), the 1968 crop (two areas), and the 1969 crop (seven areas). The total number of trees is thus 144.

Year	Mean	Range
1966	26	17 - 44
1967	21	11 - 40
1968	17	9 - 38
1969	24	14 - 45

Figure 1. Seasonal history of Amblycerus robiniae and Gleditsia triacanthos in northeastern Kansas in 1969. Solid lines in section on the Bruchid indicate when most individuals of a given stage are found ; dotted lines indicate that generations overlap or merge.

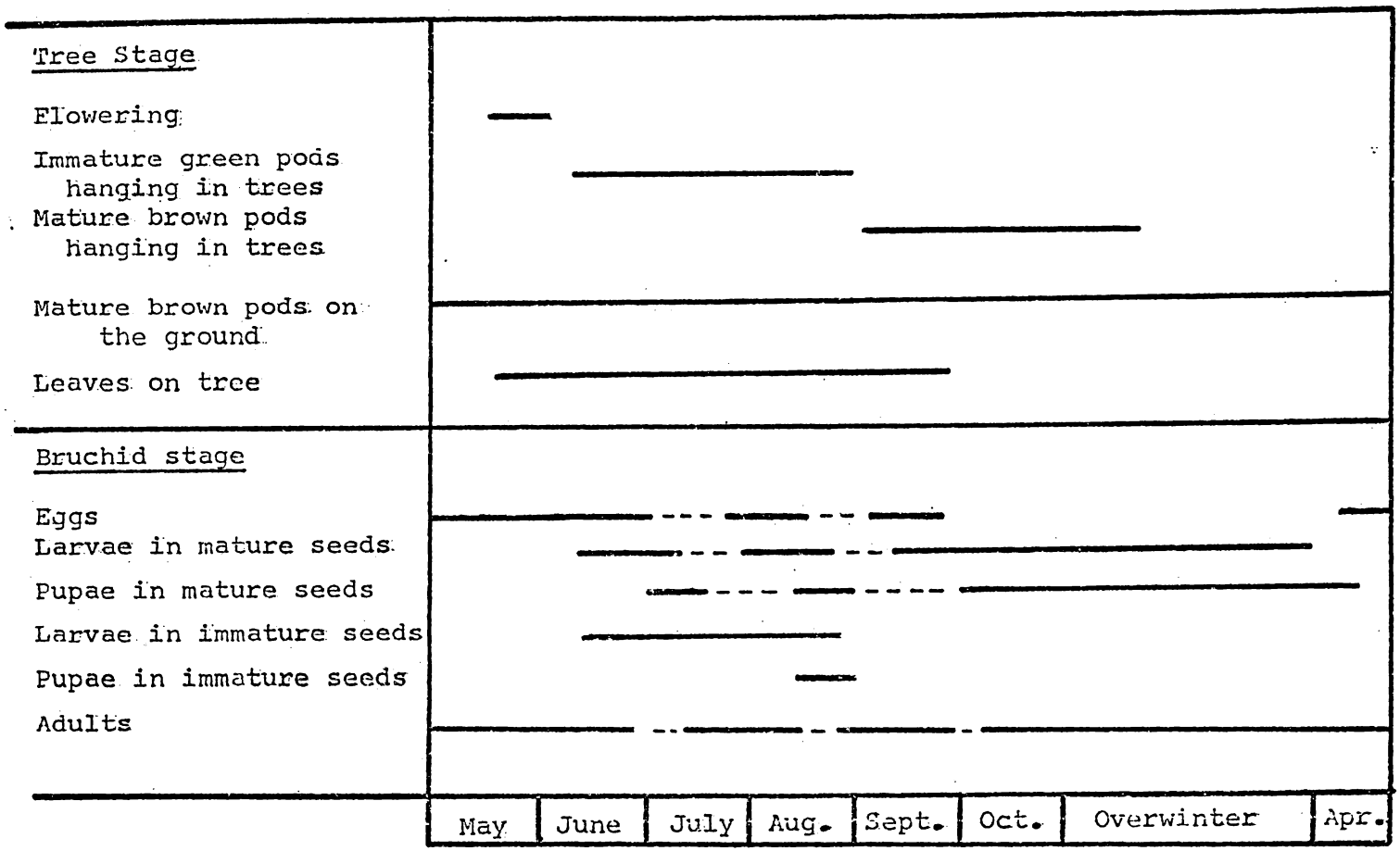

Tree Stage
Flowering
Immature green pods hanging in trees
Mature brown pods hanging in trees
Mature brown pods on the ground
Leaves on tree
Bruchid stage
Eggs
Larvae in mature seeds
Pupae in mature seeds
Larvae in immature seeds
Pupae in immature seeds
Adults
May
June
July
Aug.
Sept.
Oct.
Overwinter
Apr.

Figure 2. Frequency distribution of head capsule width classes (0.10 mm) for A. robiniae larva collected in 1969. Bottom graph summarizes the results.

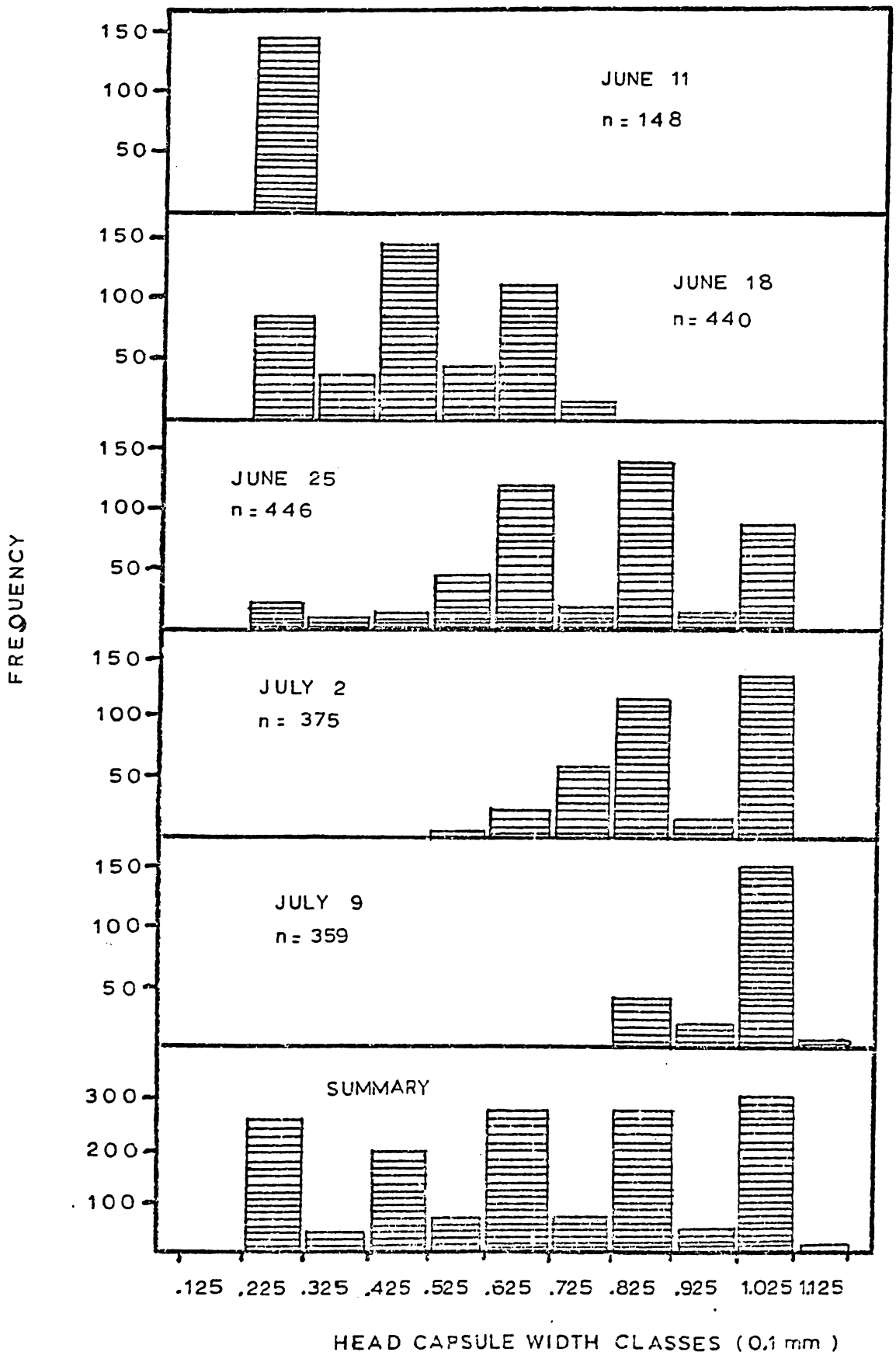

JUNE 11
n = 148
JUNE 18
n = 440
JUNE 25
n = 446
JULY 2
n = 375
JULY 9
n = 359
SUMMARY
FREQUENCY
150
100
50
300
200
100
.125 .225 .325 .425 .525 .625 .725 .825 .925 1.025 1.125
HEAD CAPSULE WIDTH CLASSES (0.1 mm)

Figure 3. Emergence dates of the two most common parasites (of Amblycerus) in 1967 and 1969. 1 = Horismenus missouriensis and 2 = Heterospilus bruchi. The number of parasites are recorded as the number that emerged per 2000 pods.

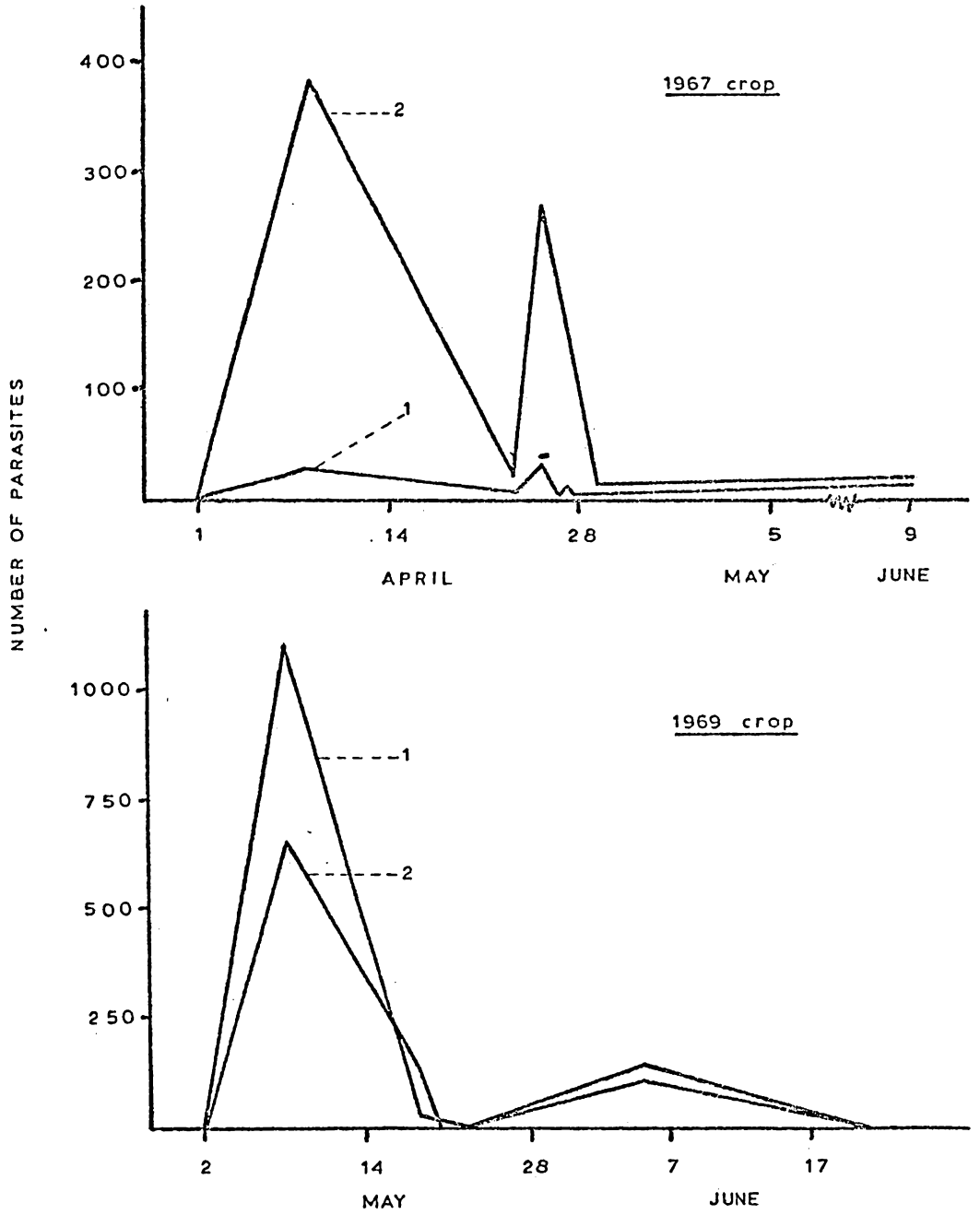

NUMBER OF PARASITES
400
300
200
100
1967 crop
2
1
1
14
28
5
9
APRIL
MAY
JUNE
1000
750
500
250
1969 crop
1
2
2
14
28
7
17
MAY
JUNE

Figure 4. Daily activity patterns of bruchid beetles under long and short day conditions.

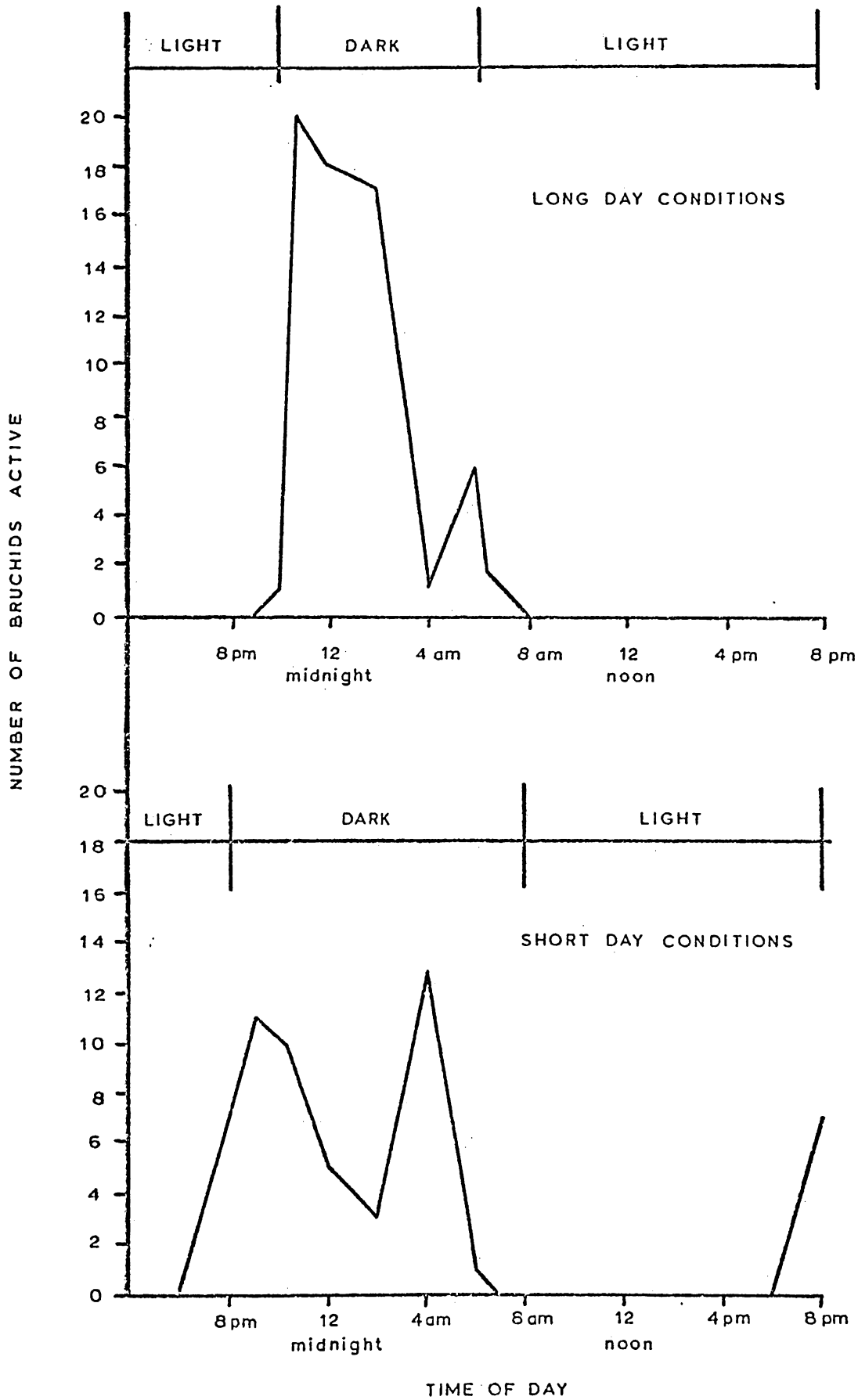

LIGHT
DARK
LIGHT
LONG DAY CONDITIONS
20
18
16
14
12
10
8
6
4
2
0
8 pm
12
midnight
4 am
8 am
12
noon
4 pm
8 pm
LIGHT
DARK
LIGHT
SHORT DAY CONDITIONS
20
18
16
14
12
10
8
6
4
2
0
8 pm
12
midnight
4 am
8 am
12
noon
4 pm
8 pm
NUMBER OF BRUCHIDS ACTIVE
TIME OF DAY

Figure 5. Design for sticky trap placement in experimental trees.

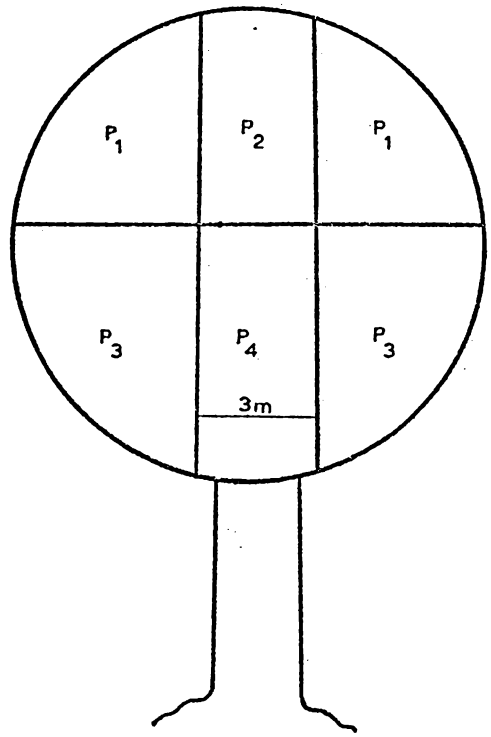
POSITION IN TREE

P_1 = upper outside

P_2 = upper core

P_3 = lower outside

P_4 = lower core



COMPASS DIRECTION

D_1 = north

D_2 = northwest

D_3 = west

D_4 = southwest

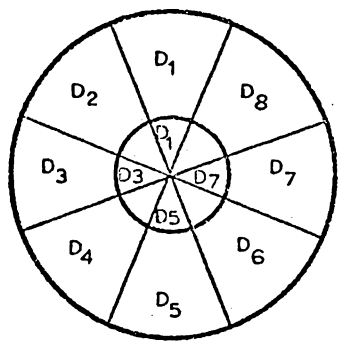
D_5 = south

D_6 = southeast

D_7 = east

D_8 = northeast

LOWER LEVEL



UPPER LEVEL

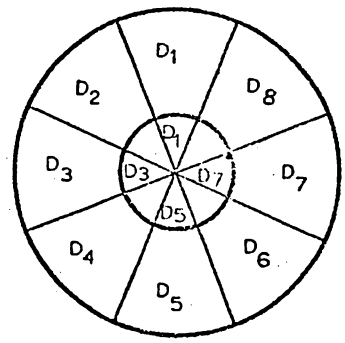


Figure 6. Bruchid beetles caught in honey locust trees with pods (I), honey locust trees without pods (II), and osage orange trees (III).

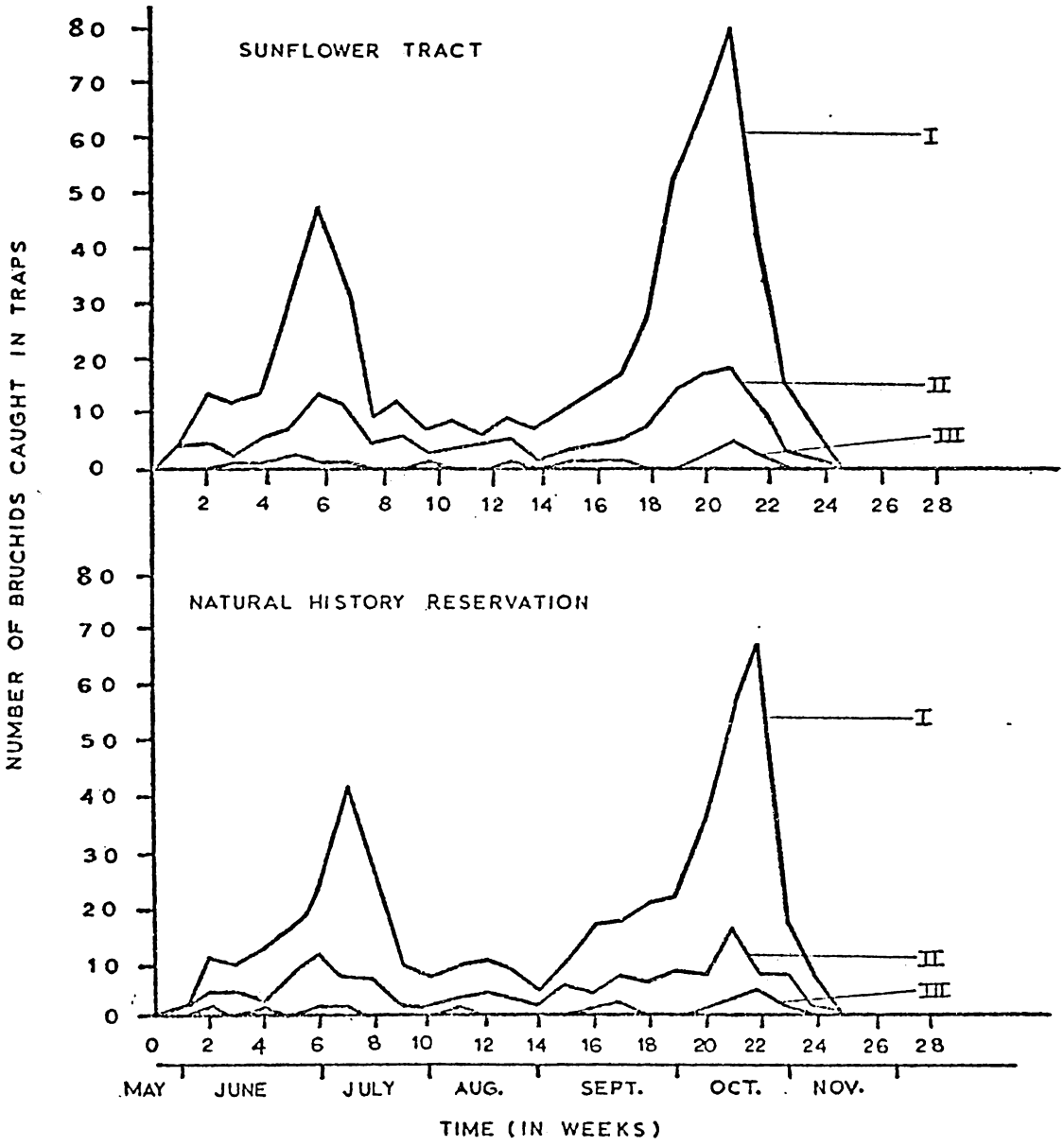
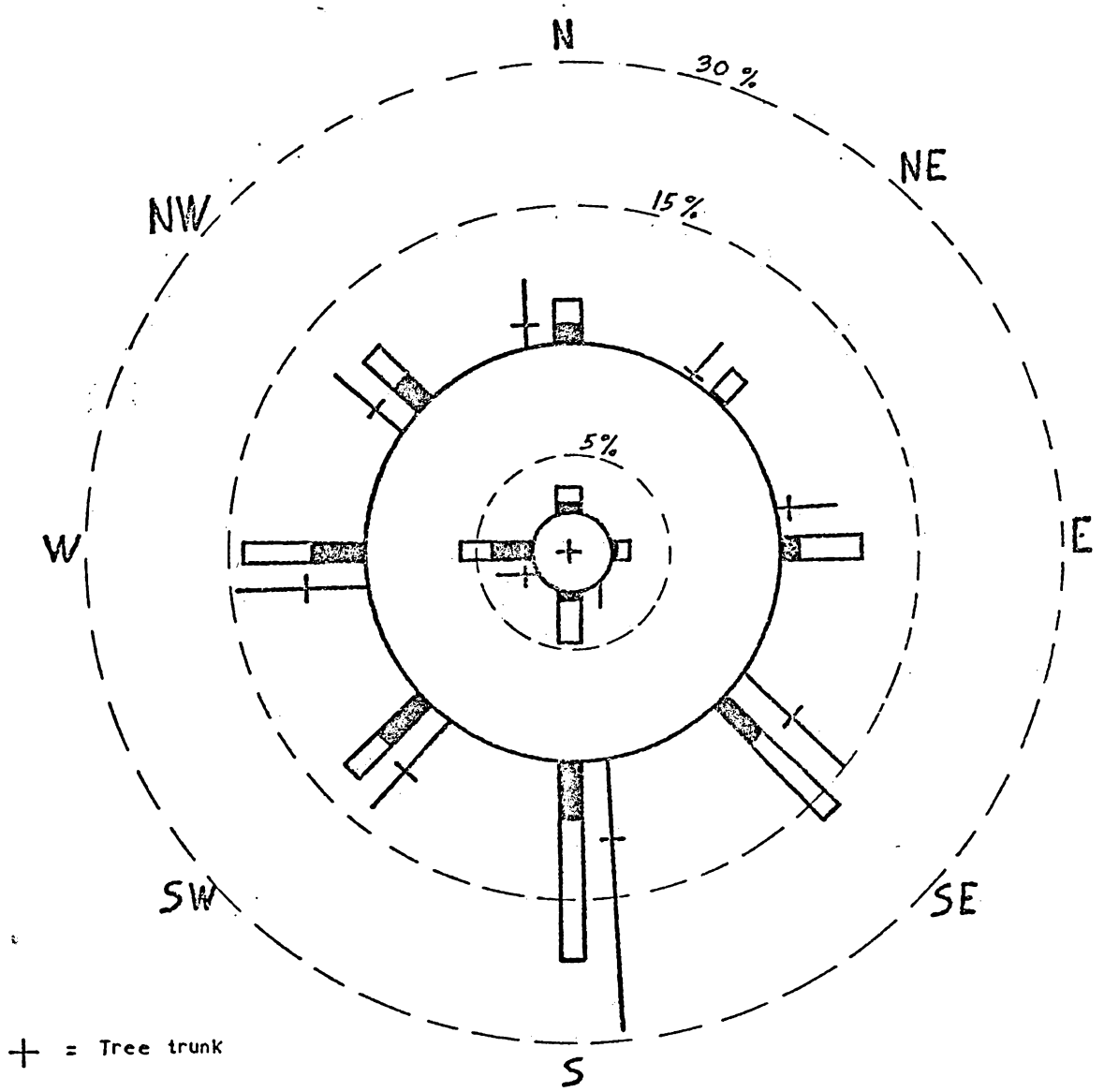

SUNFLOWER TRACT
NATURAL HISTORY RESERVATION
I
II
III
NUMBER OF BRUCHIDS CAUGHT IN TRAPS
0
10
20
30
40
50
60
70
80
2
4
6
8
10
12
14
16
18
20
22
24
26
28
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
TIME (IN WEEKS)

Figure 7. Relationship of bruchid position within a tree to the density of seed pods (Sunflower Tract - location one). Small center heavy line ring is core area, outer heavy line ring is canopy area.



+ = Tree trunk

= Percentage of total bruchids caught in lower half of tree

= Percentage of total bruchids caught in upper half of tree

= Percentage of total pods in lower half of tree

= Percentage of total pods in upper half of tree

Figure 8. Bruchid positions within a tree without pods (Sunflower Tract - location one). Small center heavy line ring is core area, outer heavy line ring is canopy area.

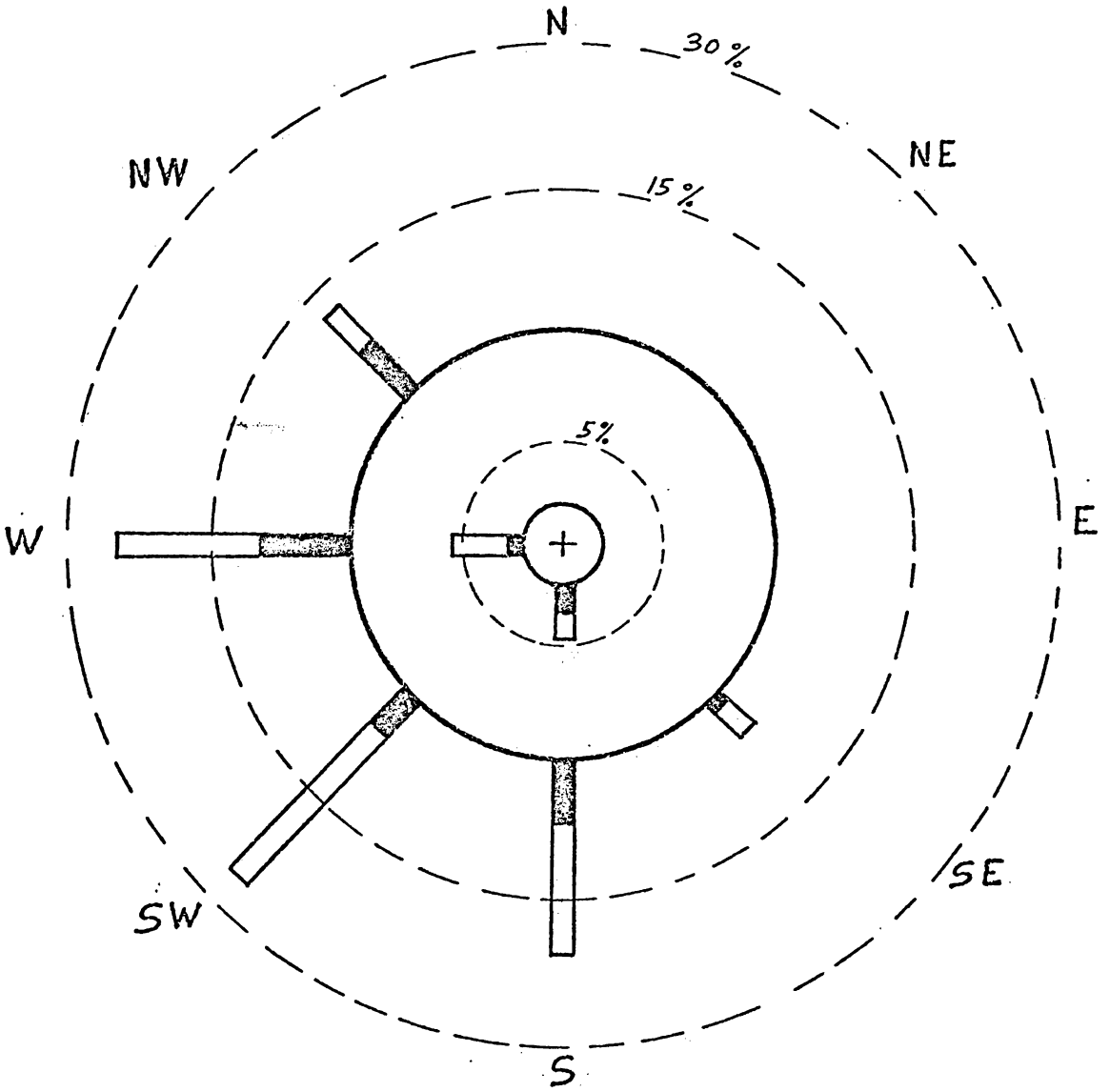

N
30%
NE
NW
15%
5%
W
E
SW
SE
S

Figure 9. Relationship of bruchid position within a tree to the density of seed pods (Natural History Reservation - location one). Small center heavy line ring is core area, outer heavy line ring is canopy area.

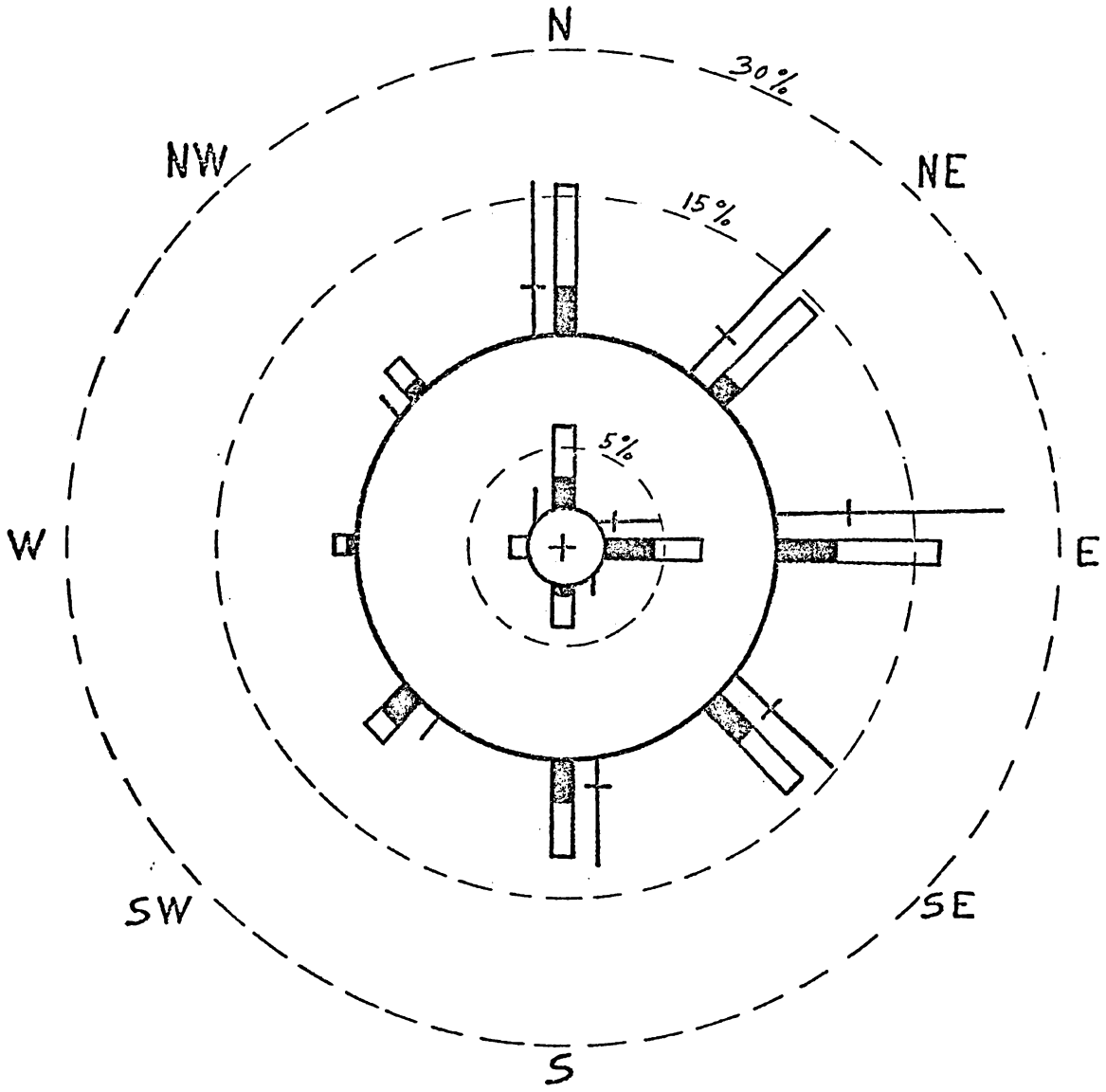

N
NE
E
SE
S
SW
W
NW
30%
15%
5%

Figure 10. Bruchid positions within a tree without pods (Natural History Reservation - location one). Small center heavy line ring is core area, outer heavy line ring is canopy area.

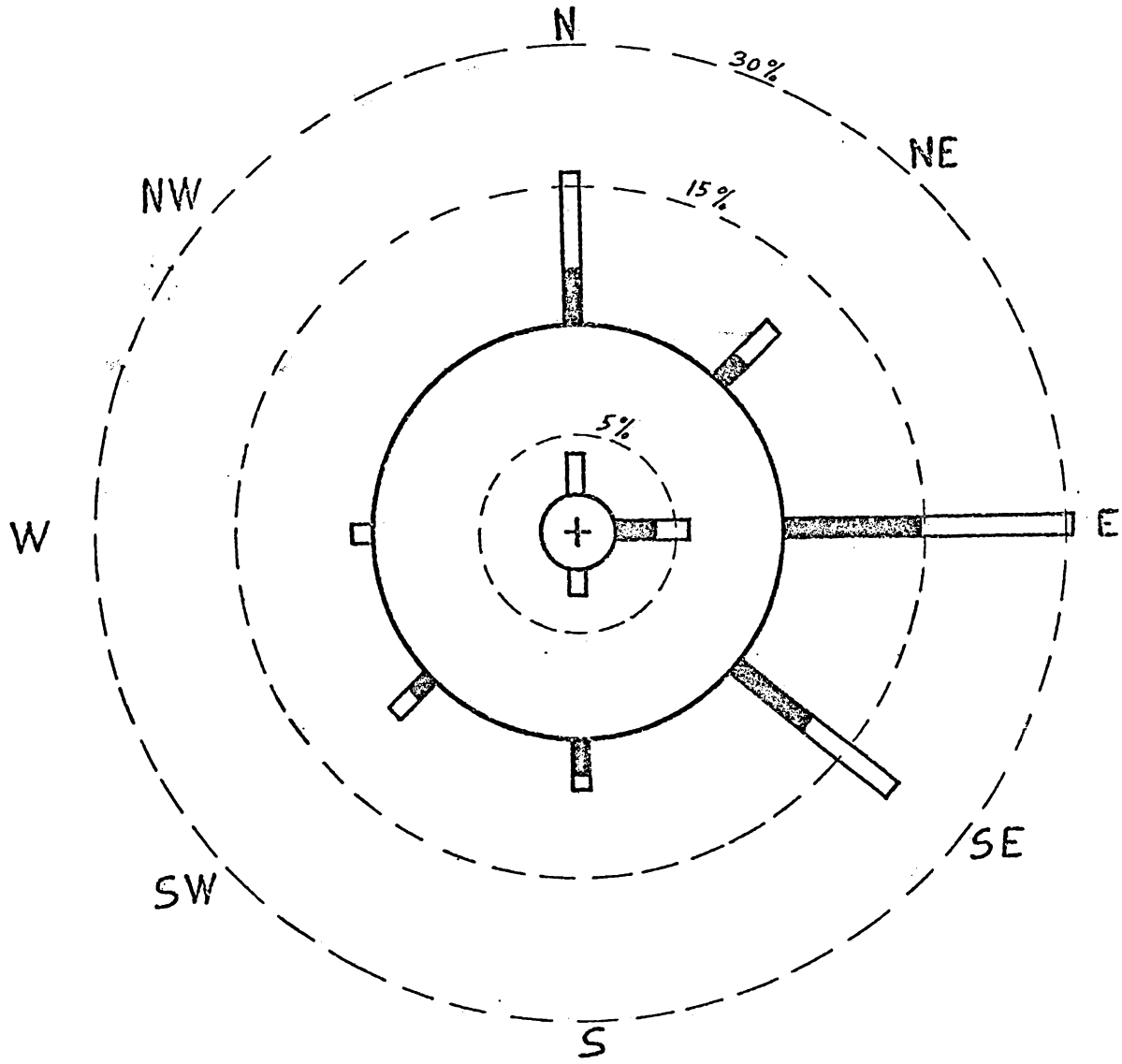
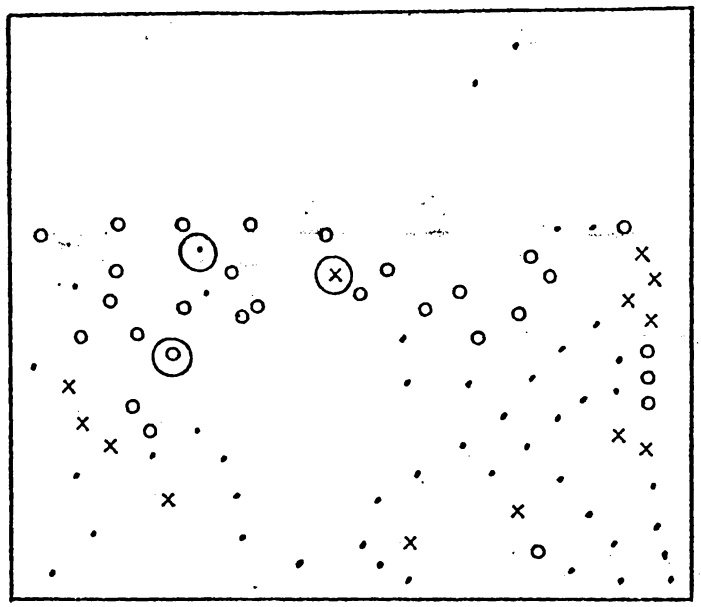
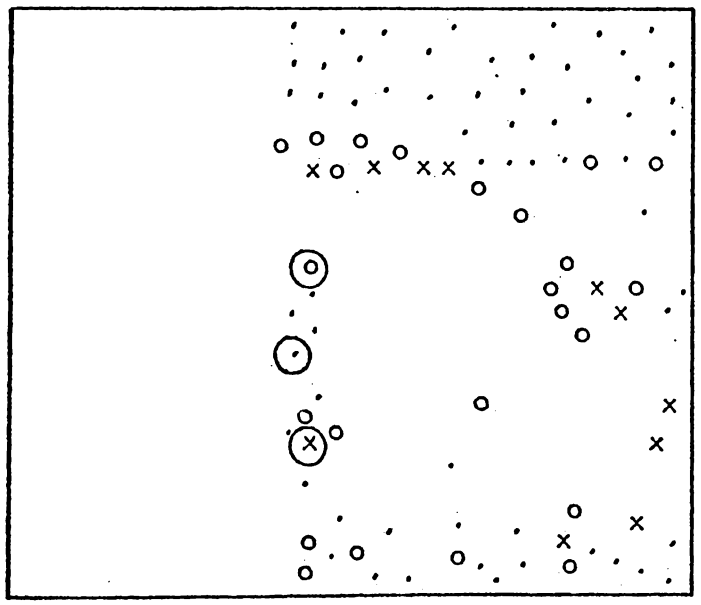

N
30%
NE
NW
15%
5%
W
E
SW
SE
S

Figure 11. Maps showing tree distribution at the Sunflower Tract and the Natural History Reservation. Circled trees are experimental ones.

Sunflower Tract



Natural History Reservation



1 cm = 25m

x = honey locust with pods

o = honey locust without pods

• = trees of other species

Figure 12. Water loss in honey locust seeds comparing freeze-dry method *vs.* oven-dry method (90°C).

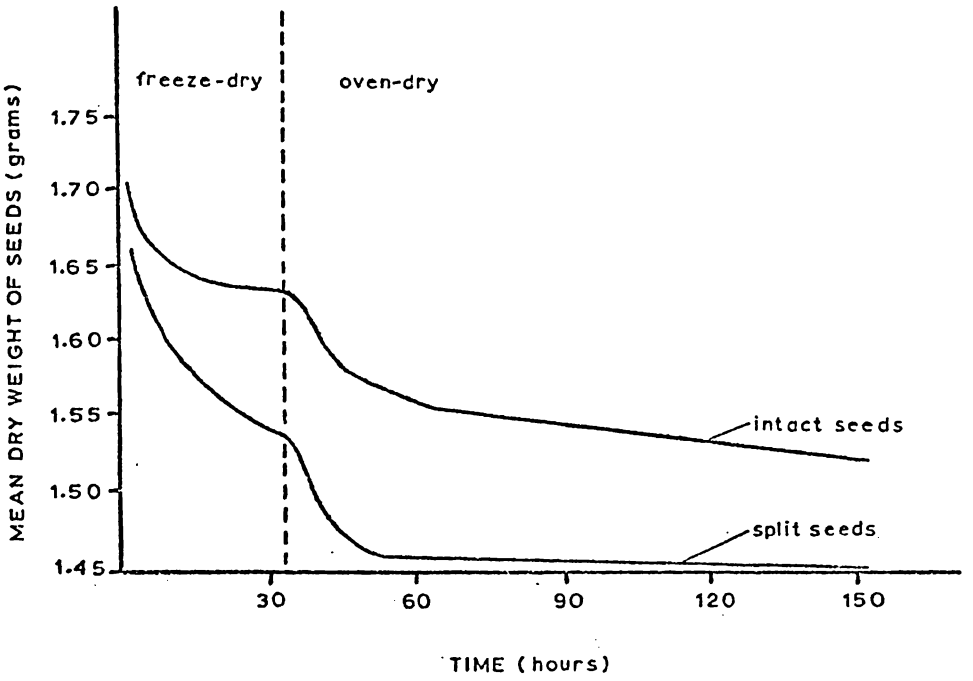

freeze-dry
oven-dry
1.75
1.70
1.65
1.60
1.55
1.50
1.45
MEAN DRY WEIGHT OF SEEDS (grams)
intact seeds
split seeds
30
60
90
120
150
TIME (hours)

Figure 13. Water loss in honey locust seeds (ten seeds of each type) oven-dryed at 90°C.

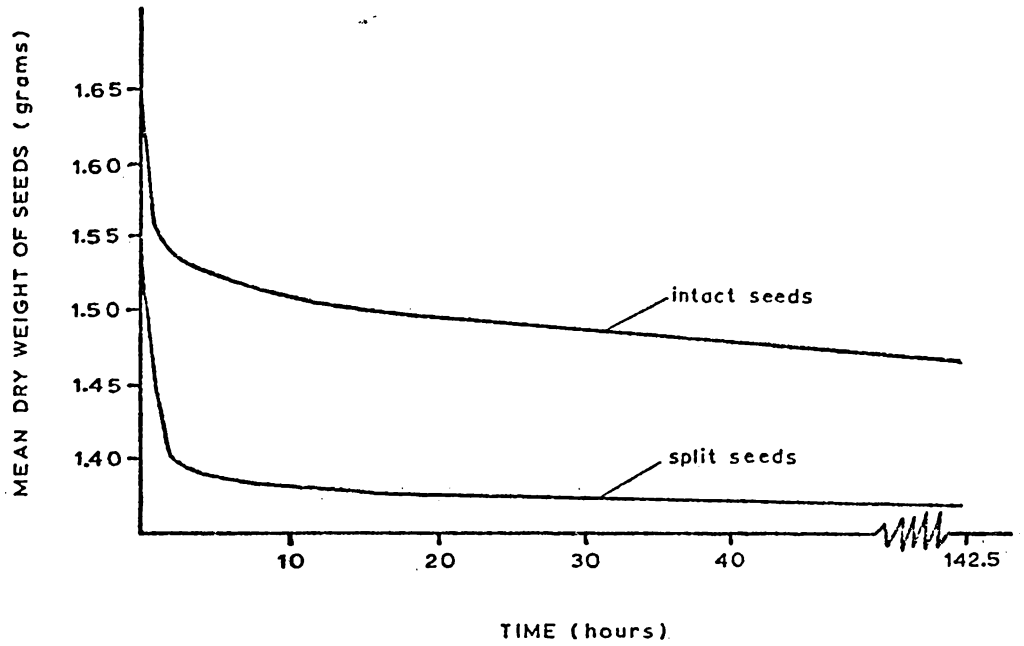

MEAN DRY WEIGHT OF SEEDS (grams)
1.65
1.60
1.55
1.50
1.45
1.40
intact seeds
split seeds
10
20
30
40
142.5
TIME (hours)

Figure 14. Representative thin-layer chromatography plate showing classes of lipids present in honey locust seeds.

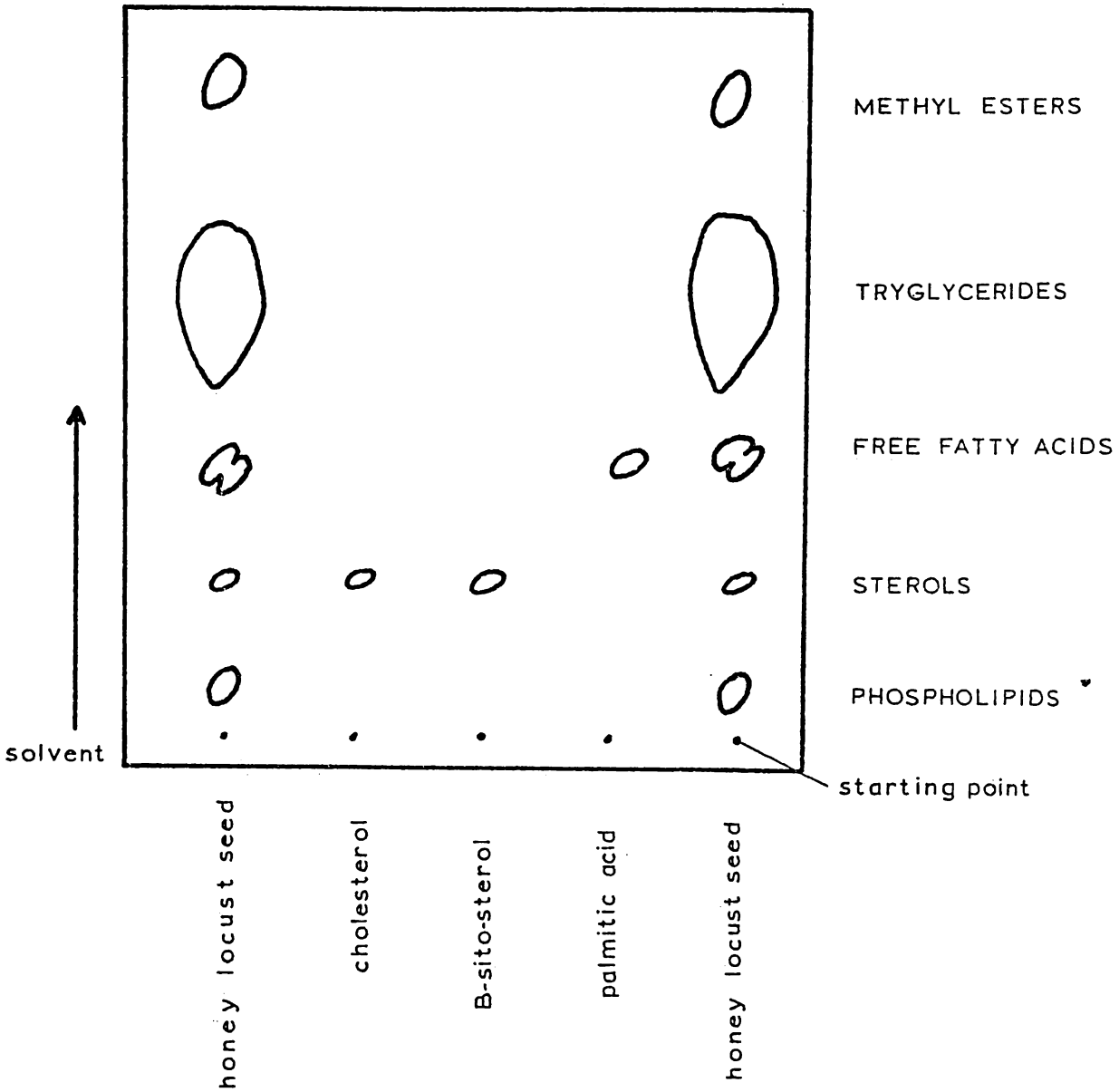
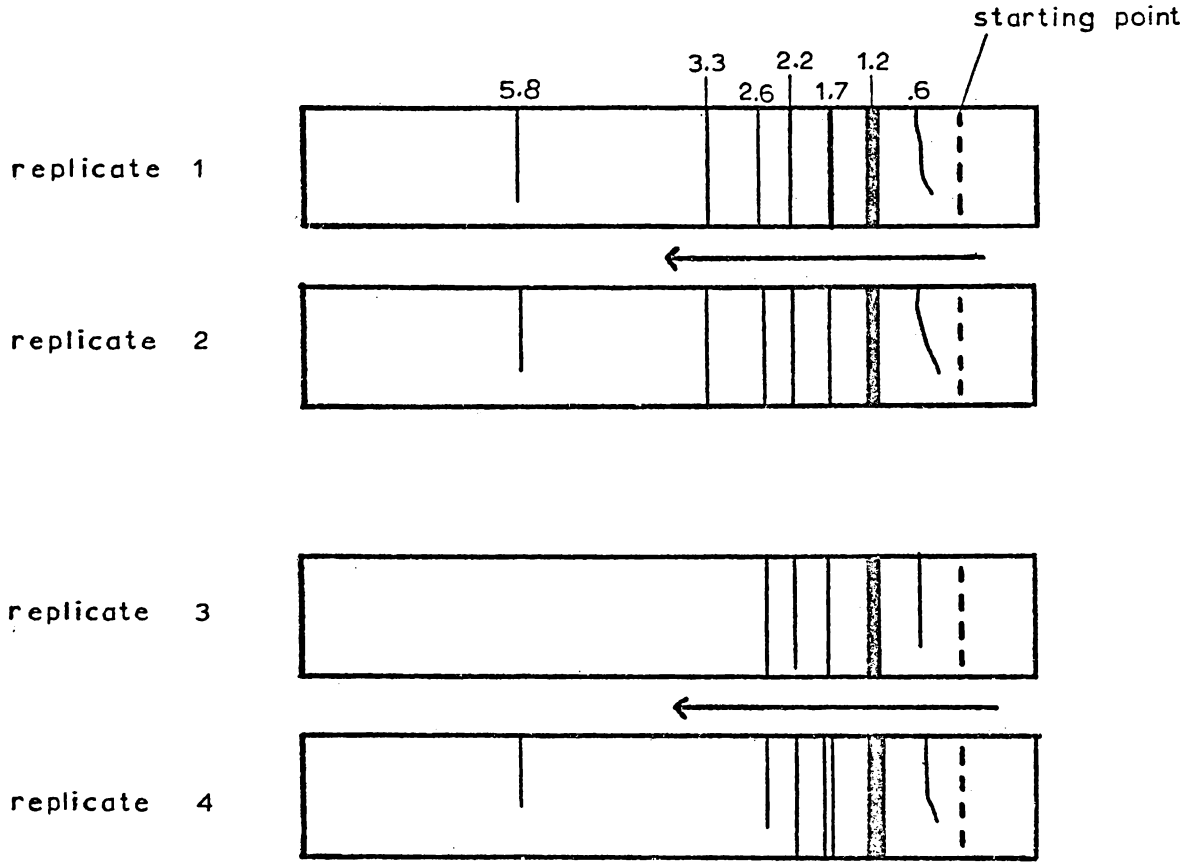

METHYL ESTERS
TRYGLYCERIDES
FREE FATTY ACIDS
STEROLS
PHOSPHOLIPIDS
solvent
starting point
honey locust seed
cholesterol
B-sito-sterol
palmitic acid
honey locust seed

Figure 15. Comparison of electrophoretic results of honey locust seed and bruchid beetle homogenates. Distance from starting point is in cms. Bands at the same points may represent similar or identical proteins in both seeds and bruchids.

HONEY LOCUST SEED HOMOGENATES



BRUCHID BEETLE HOMOGENATES

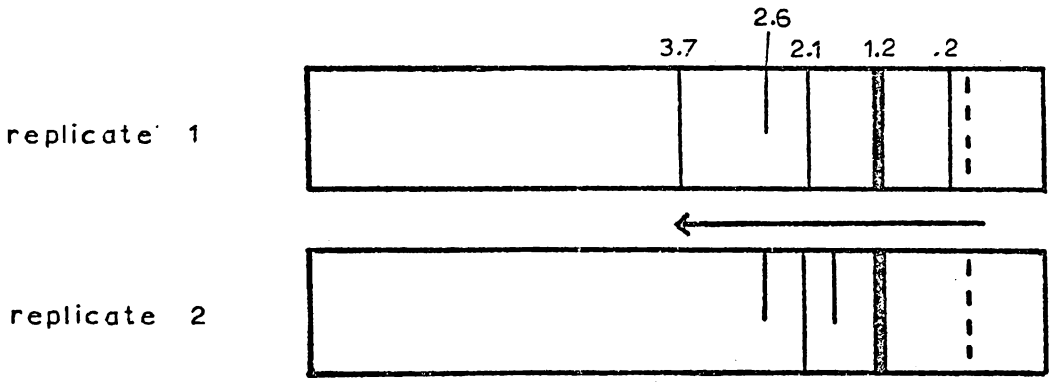


Figure 16. Elution sequence of the acidic and neutral amino acids from Gymnocladus dioeca seeds.

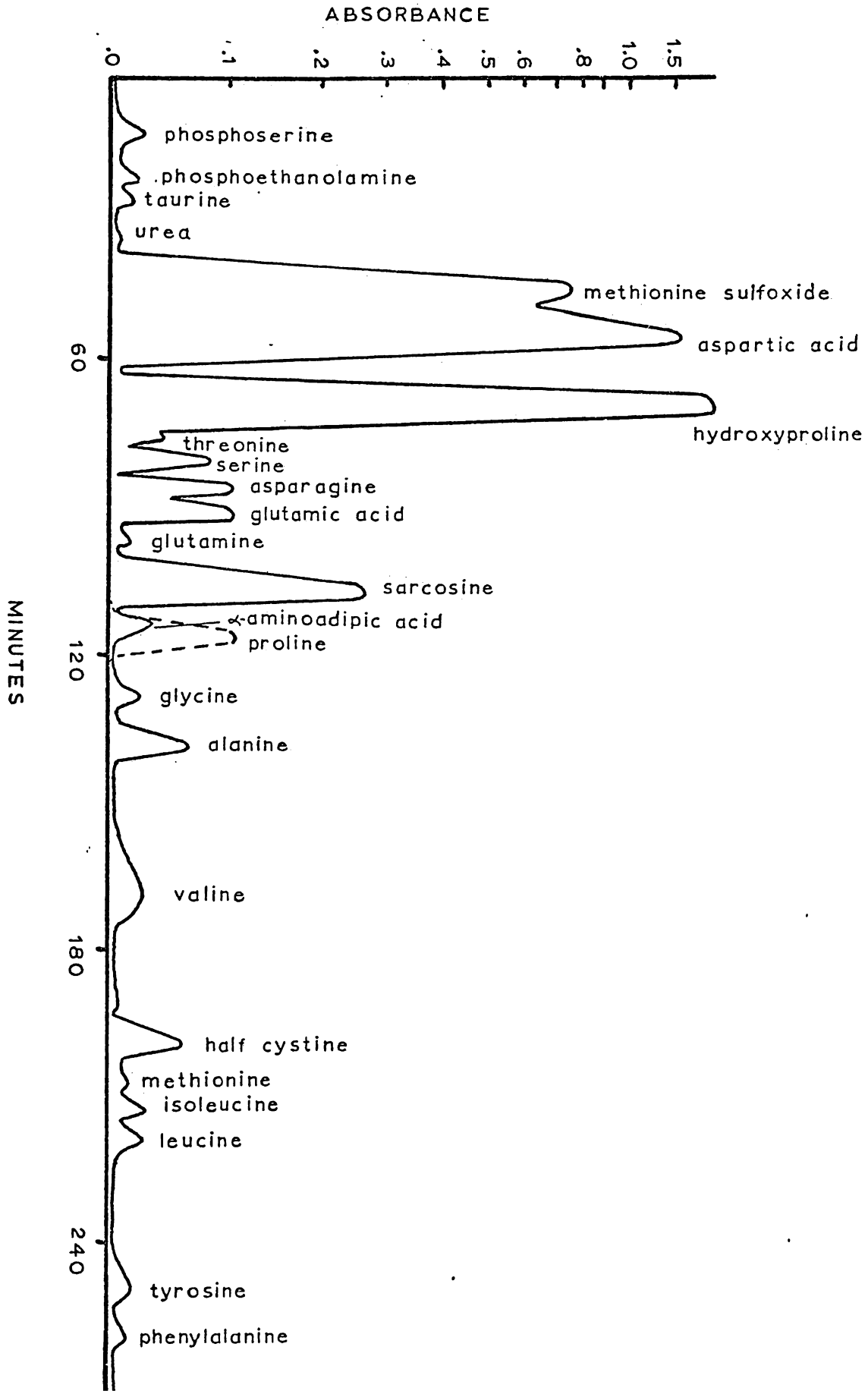

ABSORBANCE
.0
.1
.2
.3
.4
.5
.6
.8
1.0
1.5
MINUTES
60
120
180
240
phosphoserine
phosphoethanolamine
taurine
urea
methionine sulfoxide
aspartic acid
hydroxyproline
threonine
serine
asparagine
glutamic acid
glutamine
sarcosine
α-aminoadipic acid
proline
glycine
alanine
valine
half cystine
methionine
isoleucine
leucine
tyrosine
phenylalanine

Figure 17. Elution sequence of the acidic and neutral amino acids from Cercis canadensis seeds.

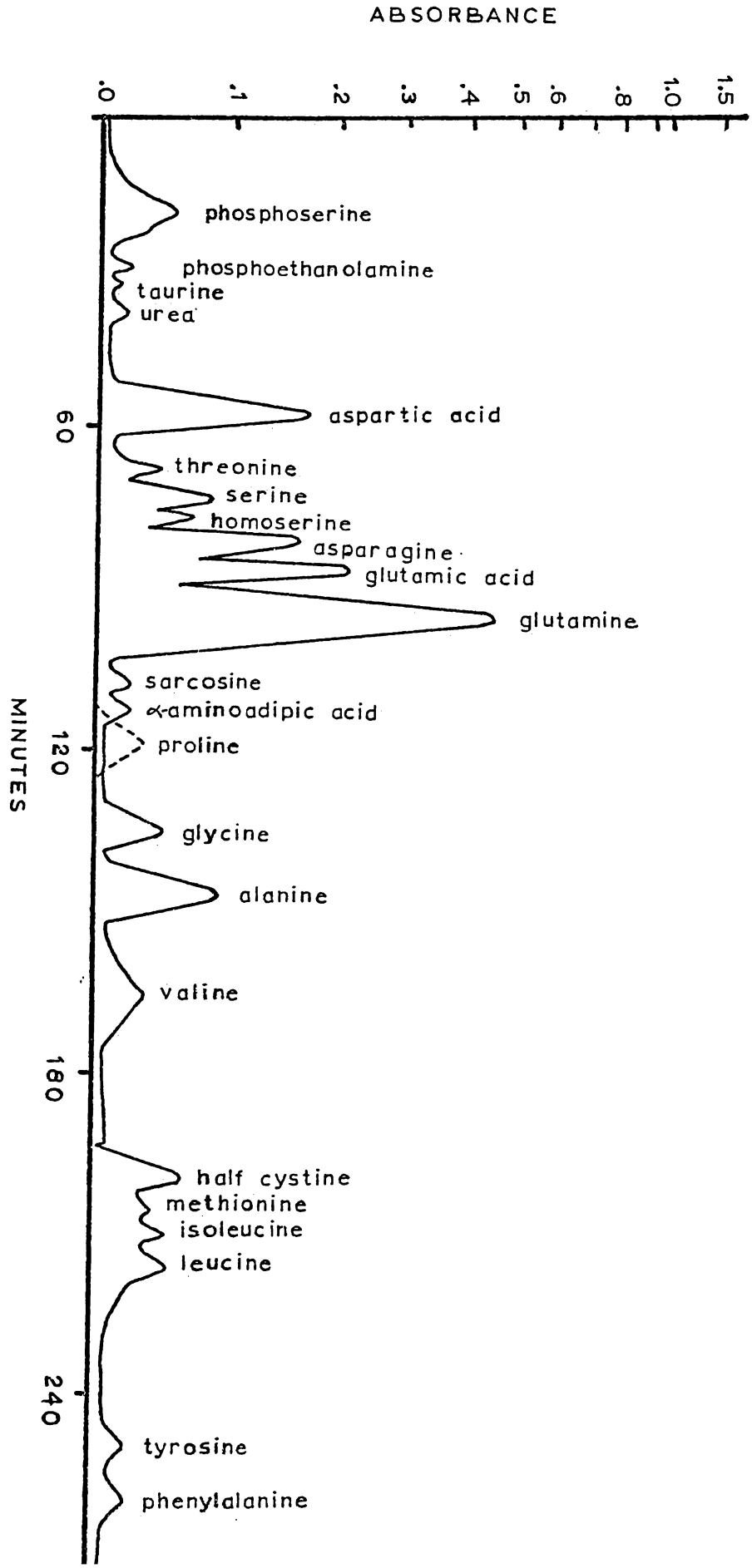

ABSORBANCE
.0
.1
.2
.3
.4
.5
.6
.8
1.0
1.5
MINUTES
60
120
180
240
phosphoserine
phosphoethanolamine
taurine
urea
aspartic acid
threonine
serine
homoserine
asparagine
glutamic acid
glutamine
sarcosine
α-aminoadipic acid
proline
glycine
alanine
valine
half cystine
methionine
isoleucine
leucine
tyrosine
phenylalanine

Figure 18. Elution sequence of the acidic and neutral amino acids from Albizzia julibrissin seeds.

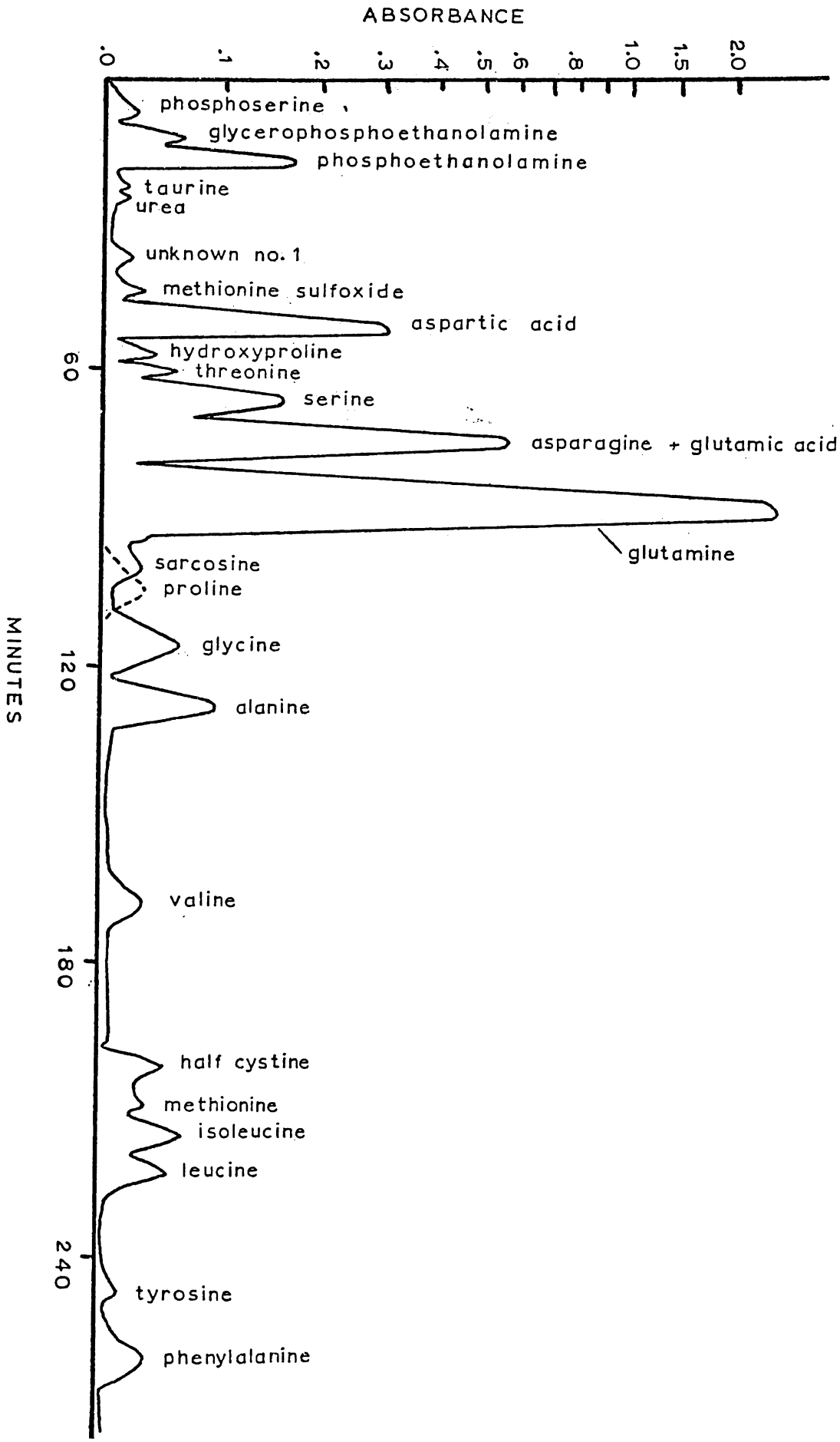

ABSORBANCE
.0
.1
.2
.3
.4
.5
.6
.8
1.0
1.5
2.0
MINUTES
60
120
180
240
phosphoserine
glycerophosphoethanolamine
phosphoethanolamine
taurine
urea
unknown no. 1
methionine sulfoxide
aspartic acid
hydroxyproline
threonine
serine
asparagine + glutamic acid
glutamine
sarcosine
proline
glycine
alanine
valine
half cystine
methionine
isoleucine
leucine
tyrosine
phenylalanine

Figure 19. Elution sequence of the acidic and neutral amino acids from Gleditsia triacanthos seeds.

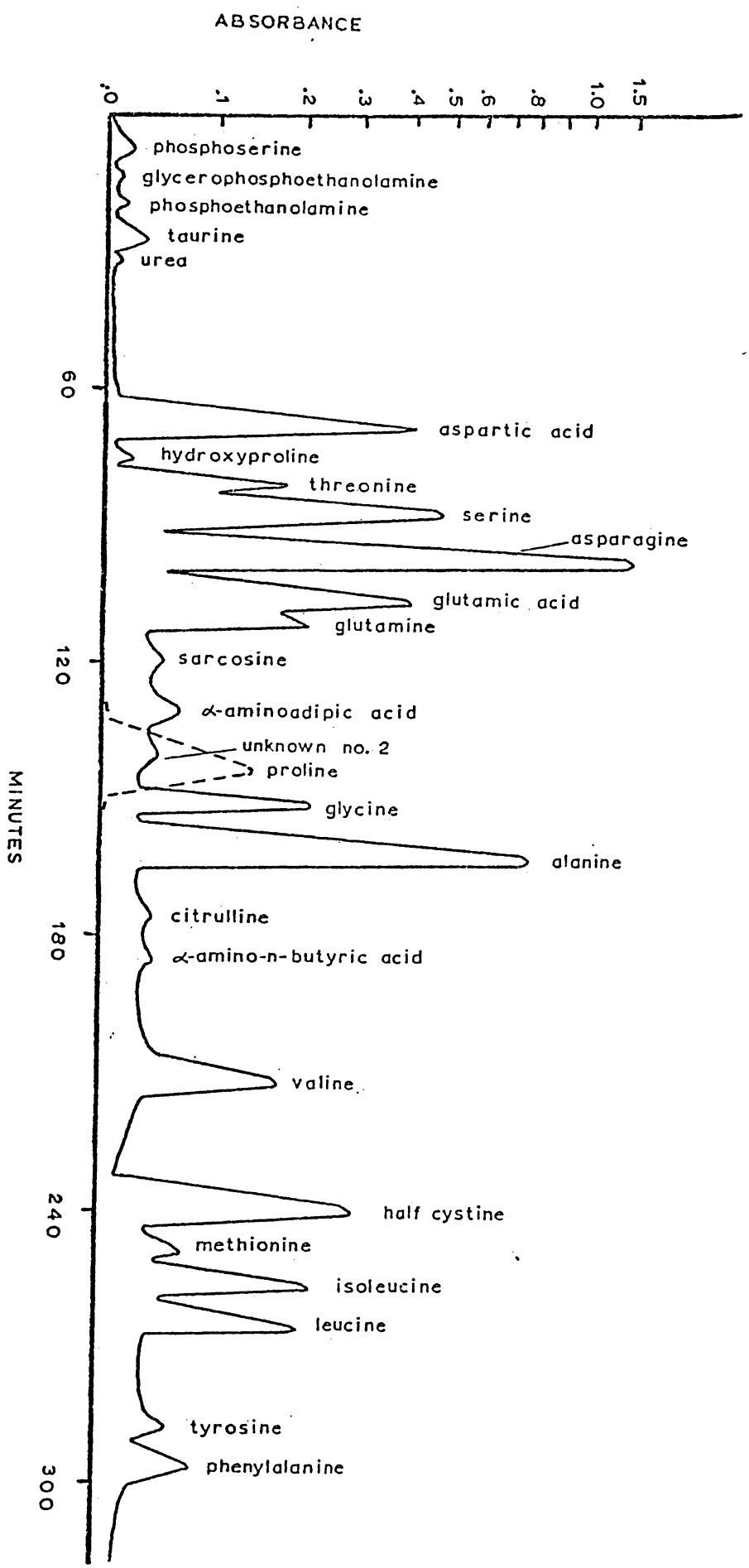

ABSORBANCE
.0
.1
.2
.3
.4
.5
.6
.8
1.0
1.5
MINUTES
60
120
180
240
300
phosphoserine
glycerophosphoethanolamine
phosphoethanolamine
taurine
urea
aspartic acid
hydroxyproline
threonine
serine
asparagine
glutamic acid
glutamine
sarcosine
α-aminoadipic acid
unknown no. 2
proline
glycine
alanine
citrulline
α-amino-n-butyric acid
valine
half cystine
methionine
isoleucine
leucine
tyrosine
phenylalanine

Figure 20. Representative elution sequence of the basic amino acids for all legume species considered in this study.

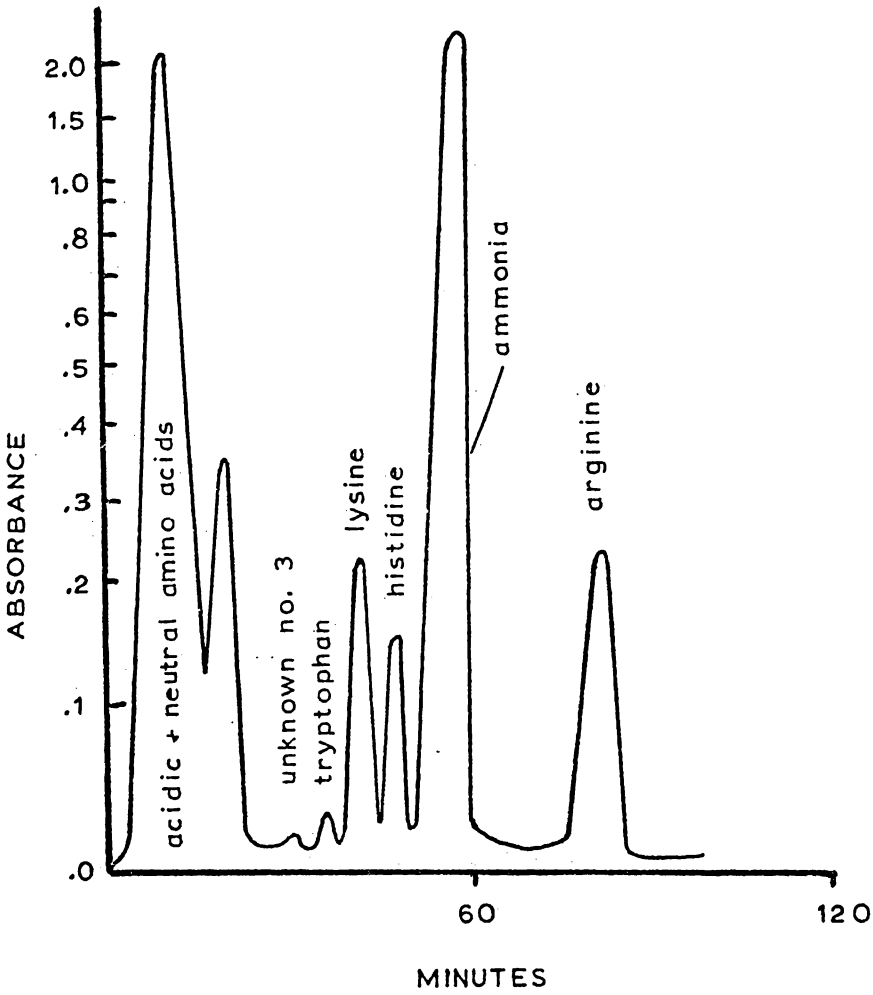
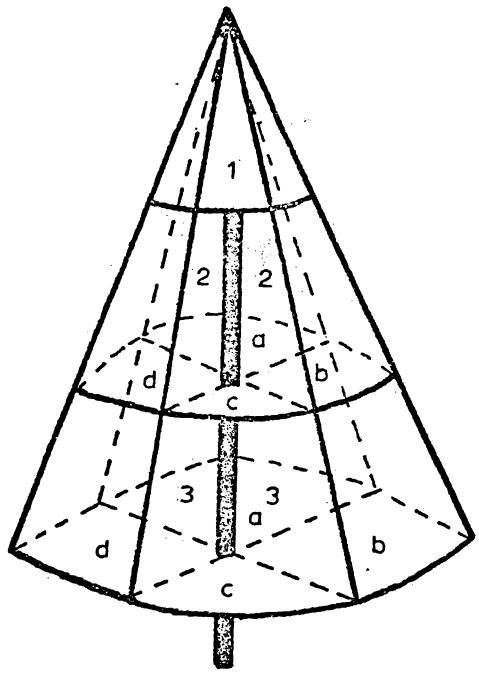

ABSORBANCE
2.0
1.5
1.0
.8
.6
.5
.4
.3
.2
.1
.0
acidic + neutral amino acids
unknown no. 3
tryptophan
lysine
histidine
ammonia
arginine
60
120
MINUTES

Figure 21. Schematic division of honey locust tree showing units used for pod sampling. Apical section not divided, but sampled as a single unit and referred to as the top. Twenty-five pods were sampled from each of the 9 regions (i.e. 25 pods from 1; 2a,2b,2c,2d; 3a,3b,3c,3d).



Key

1 = TOP SECTION
2 = MIDDLE SECTION
3 = BOTTOM SECTION

a = NORTH FACING QUADRANT
b = EAST " "
c = SOUTH " "
d = WEST " "

Figure 22. Graph showing mean percentage bruchid damage and range of damage variation among different trees. Vertical lines represent ranges and horizontal lines are the means. All means are significantly different at the 5% level.

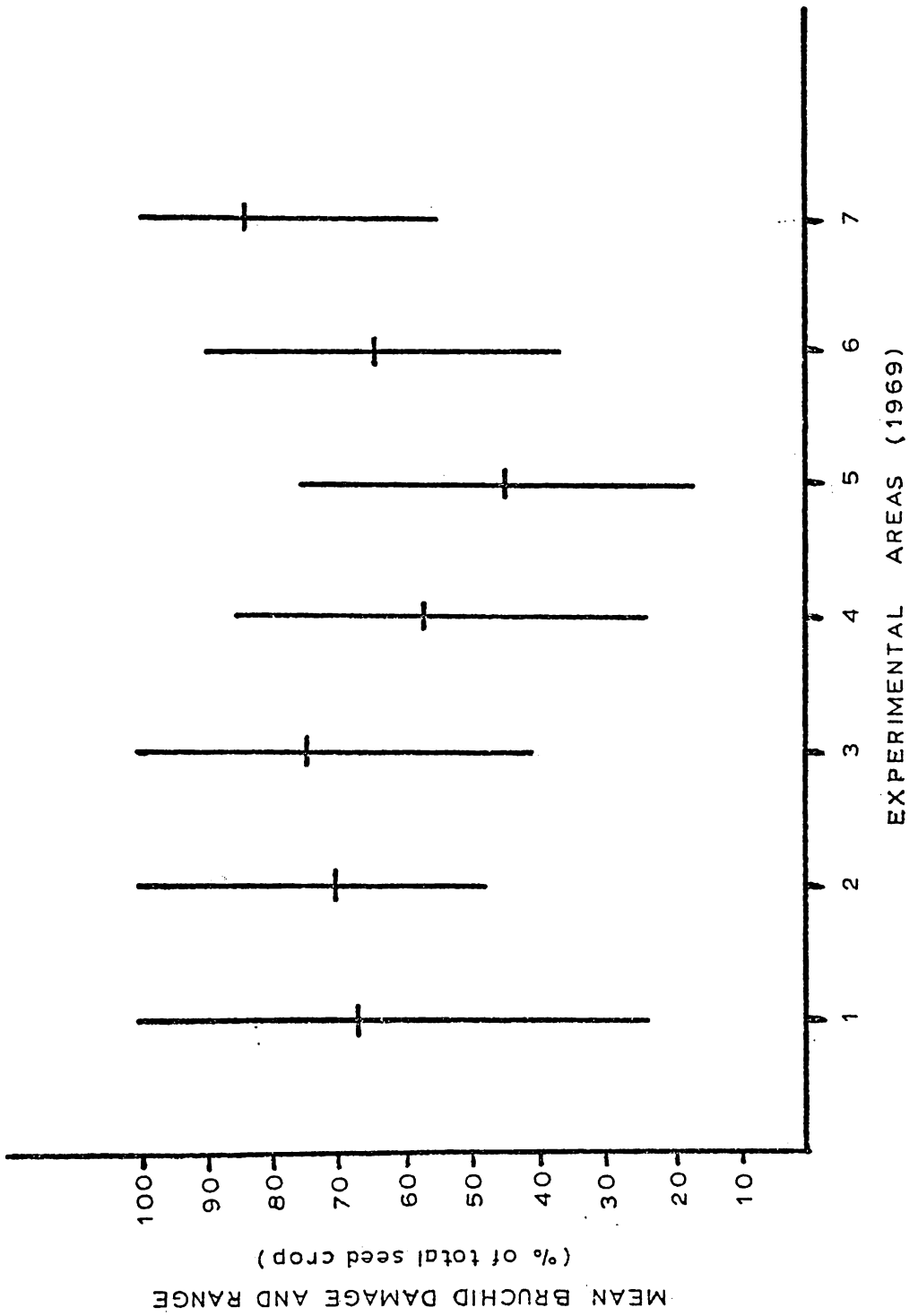

MEAN BRUCHID DAMAGE AND RANGE
(% of total seed crop)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
EXPERIMENTAL AREAS (1969)

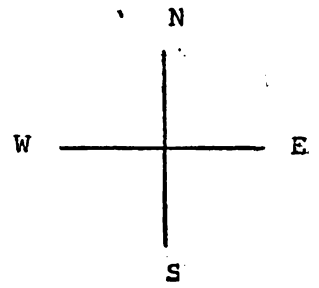
Figure 23. Map showing experimental areas. Years of collection and the number of honey locust trees sampled in each area are listed below.

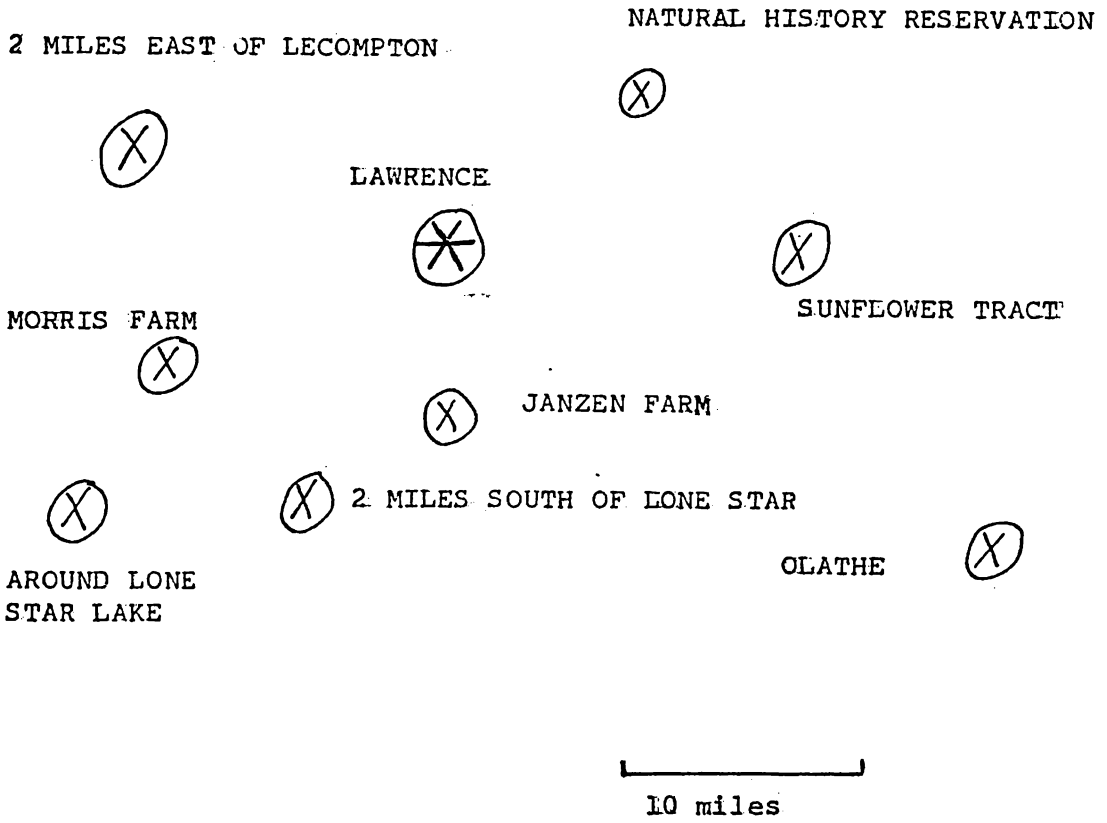
1966 Janzen Farm - 12 trees

1967 Around Lone Star Lake - 18 trees
2 Miles South of Lone Star - 17 trees

1968 University of Kansas Campus - 16 trees
2 Miles East of Lecompton - 19 trees

1969 2 Miles East of Lecompton - 19 trees
Natural History Reservation -27 trees
Sunflower Tract - 25 trees
Olathe - 18 trees
2 Miles South of Lone Star - 22 trees
Around Lone Star Lake - 32 trees
Morris Farm - 58 trees


N
W
E
S


NATURAL HISTORY RESERVATION
2 MILES EAST OF LECOMPTON
LAWRENCE
SUNFLOWER TRACT
MORRIS FARM
JANZEN FARM
2 MILES SOUTH OF LONE STAR
OLATHE
AROUND LONE
STAR LAKE
10 miles

Appendix : Tabulation of seed crop parameters.

Year	area	tree no.	seeds/pod	% bruchid damage
1966	Janzen Farm	1	12.3	75.36
		2	15.7	74.05
		3	15.8	60.90
		4	15.7	61.77
		5	15.1	78.85
		6	16.9	68.02
		7	13.8	67.85
		8	14.9	68.20
		9	15.3	58.11
		10	13.2	74.59
		11	14.2	81.55
		12	13.8	48.94
$n = 12$		$\bar{Y} =$	14.7	68.18
		$S^2 =$	1.8100	89.6618

Year	area	tree no.	pod size index	seeds/pod	avg. dry wt. seeds(gms)
1967	Around	47 or.	11.989	16.4	.1789
	Lone	91 pink	9.130	16.2	.1637
	Star	97 pink	10.188	16.8	.1983
	Lake	49 or.	11.042	21.6	.1876
		102 pink	10.942	20.0	.2031
	and	58 red	10.001	24.0	.1651
		103 pink	14.049	22.0	.1911
	2 miles	104 pink	14.395	21.0	.2041
	South	84 pink	12.732	16.0	.1967
	of	71 red	12.187	21.4	.1886
	Lone	92 pink	17.043	20.3	.1849
	Star	61 red	10.820	21.4	.1836
		60 red	10.126	21.0	.2312
		50 or.	11.253	21.4	.1987
		98 pink	10.567	15.6	.2087
		82 red	9.814	17.4	.1470
		88 pink	9.493	16.0	.1107
		99 pink	11.684	20.0	.1691
		101 pink	11.902	21.0	.1380
		93 pink	5.381	23.0	.2119
		72 red	9.547	22.0	.1953
		UN - 1	7.994	18.2	.1389
		83 pink	9.760	18.2	.1709
		70 red	11.423	22.6	.2049
		81 red	11.120	18.0	.1689
		69 red	8.731	20.2	.1601
		87 pink	9.536	21.2	.2306
		94 pink	12.640	22.6	.2101
		52 blue	7.992	15.6	.1905
		80 red	9.833	19.8	.2395
		59 red	10.610	17.8	.1746
		100 pink	17.663	23.4	.2017
		57 red	12.116	25.2	.1244
		29 red	10.126	20.6	.1684
		105 pink	10.947	23.6	.1561
$n = 35$		$\bar{Y} =$	11.105	19.8	.1836
		$S^2 =$	4.5933	2.8185	.008079

(continued on p. 145)

% bruchid damage	% taken by vertebrate predators
100.00	24.39
77.50	3.70
96.67	4.76
92.30	-
65.71	-
89.36	-
98.41	-
98.18	-
100.00	1.25
97.82	8.41
98.24	13.46
95.65	-
81.57	-
98.39	-
100.00	1.28
100.00	1.14
92.59	7.50
86.11	-
95.31	-
93.50	-
92.38	1.61
98.07	-
93.61	2.19
70.96	3.53
100.00	-
90.12	-
98.67	-
100.00	.68
100.00	-
98.21	4.04
100.00	25.84
100.00	-
88.15	2.38
97.56	-
60.30	-
93.58	2.87
252.3663	38.2815

Year	area	tree no.	pod size index	number seeds/pod
1968	2 miles	80	11.775	19.8
	East of	98	9.060	18.8
	Lecompton	97	7.701	16.4
		96	7.100	16.4
		94	10.931	18.0
		99	11.294	20.4
	Kansas	100	11.275	22.2
	University	90	9.926	20.4
	Campus	83	10.155	19.4
		59	7.982	15.2
		10	10.648	23.4
		14	9.145	22.0
		15	11.094	22.6
		35	10.613	22.8
		33	9.952	22.8
		34	10.479	20.6
		21	9.753	21.6
		8	10.223	20.8
		43	11.209	22.6
		39	11.021	23.4
		17	10.972	23.6
		40	9.603	22.0
		41	14.427	24.2
$n = 23$			$\bar{Y} =$ 10.276	20.8
			$S^2 =$ 2.2965	6.2763

(continued on p. 147)

Average dry weight seeds	% bruchid damage	% taken by vertebrate predators
.1554	66.67	-
.2135	61.29	4.25
.1829	82.75	3.65
.1763	55.55	2.43
.2281	49.25	-
.2249	43.13	-
.2325	47.16	1.80
.2336	92.30	.98
.2218	72.50	1.03
.2264	45.00	2.63
.2365	53.57	-
.2423	47.05	-
.2389	31.39	-
.2457	43.42	-
.2141	39.18	-
.2396	39.70	-
.2308	50.67	-
.2420	40.57	-
.2132	44.30	-
.2439	60.76	-
.2093	41.66	-
.2178	37.31	-
.2249	33.75	-
.2215	51.26	.73
.005227	237.0558	1.6928

Year	area	tree no.	est. m^3 canopy	ht (m)	diameter at 1.5m (cm)	pod size index
1969	Around	81 red	640	13	18.73	10.450
	Lone	75	80	7	11.43	11.043
	Star	80	490	13	14.76	11.772
	Lake	81	128	12	28.98	13.047
		82	160	12	15.87	6.874
		80 red	175	12	14.60	8.681
		68 blue	27	5	6.98	12.326
		52 blue	80	6	9.68	12.124
		71 red	294	9	23.17	11.413
		72	27	5	11.27	8.493
		72 red	100	7	15.87	10.267
		80 blue	8	4	8.57	10.398
		77	64	7	12.38	12.818
		76	64	7	12.06	10.722
		82 red	768	14	22.22	10.698
		89	252	13	19.05	6.612
		88	512	16	31.27	5.174
		133	486	16	21.59	11.740
		134	294	12	17.14	11.707
		135	28	8	6.67	14.359
		136	96	8	7.93	8.607
		137	11250	22	57.15	6.835
		138	36	9	15.55	14.412
		139	150	10	25.71	12.845
		140	8	8	7.93	11.370
		52 or.	720	14	31.11	6.857
		96 pink	27	8	10.79	10.941
		94 pink	216	10	12.06	12.108
		95 pink	288	16	14.12	11.996
		141	343	14	13.33	10.844
		89 pink	54	11	10.79	9.647
		87 pink	125	8	11.74	8.217
$n = 32$		$\bar{Y} =$	562.2	10.5	16.89	10.606
		$S^2 =$	910,423	16.00	90.4954	2.7867

(continued on p. 149)

total no. pods	total no. seeds	no. seeds per pod	avg. dry wt. seeds	dry wt seeds/m^3	total no. seeds/m^3
1209	22,729	18.8	.2250	7.9906	35.51
548	10,522	19.2	.1957	25.7395	131.52
877	15,436	17.6	.0994	3.1311	31.50
1381	29.968	21.7	.1921	25.5687	234.12
1149	17,924	15.6	.2240	25.0936	112.02
1032	18,370	17.8	.2306	24.2064	104.91
254	5,791	22.8	.2164	46.4140	214.48
274	5,809	21.2	.2045	14.8492	72.61
519	9,965	19.2	.1790	6.0671	33.89
347	6,107	17.6	.1981	44.8074	226.18
360	6.084	16.9	.2550	15.5142	60.84
44	823	18.7	.2676	27.5288	102.88
317	6,720	21.2	.2552	26.7959	105.00
254	5,232	20.6	.2499	20.4294	81.75
731	13,450	18.4	.2158	3.7793	17.51
412	7,416	18.0	.1684	4.9558	29.43
461	7,469	16.2	.2065	3.0120	14.58
536	9,541	17.8	.2479	4.8667	19.63
1041	16,031	15.4	.1930	10.5237	54.53
76	1,459	19.2	.1985	10.3432	52.11
86	1,531	17.8	.1982	3.1608	15.95
2131	35,373	16.6	.1738	.5465	3.14
40	728	18.2	.2044	4.1333	20.22
131	2,306	17.6	.2415	3.7127	15.37
233	4,171	17.9	.2472	128.8838	521.38
1931	32,055	16.6	.2114	9.4117	44.52
182	3,349	18.4	.2400	29.7689	124.04
393	7,428	18.9	.2528	8.6935	34.39
326	5,542	17.0	.2230	4.2912	19.24
806	18,538	23.0	.1878	10.1500	54.05
477	9,158	19.2	.2652	44.9759	169.59
137	2,767	20.2	.2041	4.5180	22.14
584.2	10,615.4	18.6	.2148	18.8522	86.84
2,116,214	810,072	3.6364	.001194	1,173.9903	10,433.67

(continued on p. 150)

total no. pods/m3	total dry weight seed crop	% bruchid damage	% taken by vertebrate predators
1.889	5.114	65.69	-
6.850	2.059	43.93	-
1.790	1.543	94.44	-
10.789	3.272	60.19	.59
7.181	4.014	64.72	-
5.897	4.236	52.40	.81
9.407	1.253	100.00	-
3.425	1.187	100.00	-
1.765	1.783	75.00	-
12.852	1.209	70.90	-
3.600	1.551	46.87	.59
5.500	.220	46.67	1.06
4.693	1.714	87.17	-
3.969	1.307	68.75	-
.952	2.902	64.00	-
1.635	1.246	90.00	-
.900	1.542	88.23	1.23
1.103	2.365	22.34	19.83
3.541	3.093	67.30	-
2.714	.289	84.37	-
.696	.303	29.70	.56
.189	6.148	67.50	-
1.111	.148	67.44	.54
.673	.556	92.50	-
29.125	1.031	64.86	-
2.682	6.776	63.02	3.13
6.741	.803	38.18	1.08
1.819	1.877	38.46	-
1.132	1.235	54.54	1.17
2.350	3.481	30.58	-
8.833	2.428	69.69	-
1.096	.564	82.22	-

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5m	pod size index
1969	2	99 pink	448	8	19.20	8.869
	Miles	92	1152	20	31.11	7.598
	South	103 pink	54	10	11.11	6.728
	of	102 pink	252	9	18.09	11.206
	Lone	130	600	7	19.68	6.045
	Star	60 red	648	9	18.41	11.507
		61 red	640	11	16.09	6.997
		81 blue	324	11	13.33	8.319
		129	98	4	7.77	9.025
		128	45	10	12.06	7.855
		127	12	11	7.30	8.387
		no. or.	1000	12	23.81	9.492
		101	2475	12	33.33	8.948
		131	3584	18	32.38	7.870
		132	2250	12	18.41	5.748
		no. blue	36	6	10.79	8.888
		146	384	7	12.06	10.225
		147	13750	25	56.51	10.436
		58 red	448	10	25.71	8.187
		101 pink	200	11	14.12	10.444
		74 blue	80	6	8.25	7.878
		29 red	470	11	26.61	10.008
n = 22		$\bar{Y}$ =	1,316	10.9	19.82	8.667
		S^2 =	8,548,445	22.85	129.5390	2.3804

(continued on p. 152)

total no. pods	total no. seeds	number of seeds/pod	avg. dry weight seeds	dry weight seeds/m^3
1942	34,568	17.8	.1702	13.1327
835	15,865	19.0	.1747	2.4060
171	2,702	15.8	.1551	7.7607
915	19,215	21.0	.2093	15.9591
804	12,864	16.0	.2344	5.0255
370	6,808	18.4	.2078	2.1832
313	5,634	18.8	.2004	1.7641
199	3,741	18.0	.1628	1.8797
28	498	17.8	.2401	1.2201
210	3,906	18.6	.2091	18.1498
35	672	19.2	.2468	13.8208
1213	27,171	22.4	.2031	5.5184
2667	46,139	17.3	.2040	3.8030
593	9,488	16.0	.2294	.6073
119	1,975	16.6	.1489	.1307
215	4,042	18.8	.1727	19.3903
532	11,810	22.2	.1116	3.4323
4981	67,667	17.6	.2111	1.3459
1563	29,697	19.0	.1721	11.4081
310	6,262	20.2	.2193	6.8663
207	4,223	20.4	.1943	10.2566
1346	19,382	14.4	.2163	8.9198
888.5	15,187	18.4	.1952	7.0446
1,296,059	28,695,157	4.1142	.001108	36.0440

(continued on p. 153)

no. seeds per m^3	no. pods per m^3	total dry wt. seed crop	% bruchid damage	% taken by vert. predators
77.16	4.335	5.883	74.99	-
13.77	.725	2.771	50.00	1.05
50.04	3.167	.419	61.11	16.45
76.25	3.631	4.021	69.09	-
21.44	1.340	3.015	53.55	.13
10.51	.571	1.414	66.67	-
8.80	.489	1.129	46.26	-
11.55	.614	.609	73.52	11.70
5.08	.286	.119	86.04	-
86.80	4.667	.816	67.27	-
56.00	2.917	.165	70.58	-
27.17	1.213	5.518	54.16	-
18.64	1.078	5.412	75.29	.06
2.65	.229	2.176	96.89	-
.88	.053	.294	54.28	8.43
112.28	5.972	.698	81.25	10.63
30.76	1.385	1.318	89.18	-
6.38	.362	18.506	79.31	-
66.29	3.489	5.110	69.23	-
31.31	1.550	1.373	57.14	-
52.79	2.588	.820	100.00	1.61
41.24	2.864	4.192	67.64	-
36.72	1.978	3.1717	70.16	2.28
991.7852	2.8290	17.4072	215.6011	22.9219

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5m	pod size index
1969	Morris	102	1024	25	43.81	5.585
		103	1280	28	47.91	7.542
	Farm	104	1900	23	31.11	6.186
		105	160	18	39.68	5.829
		106	4500	30	68.89	8.068
		107	288	11	23.49	7.216
		108	1440	16	38.41	7.273
		109	1008	17	34.60	7.602
		110	539	12	28.09	7.604
		111	16875	30	58.10	6.904
		112	3375	20	52.36	7.009
		113	3825	20	53.97	7.097
		114	3757	15	48.57	8.067
		115	960	17	32.70	7.523
		116	125	7	18.41	8.376
		117	150	14	27.30	13.399
		118	9200	25	67.94	6.334
		119	1200	16	32.06	8.710
		120	4050	22	40.95	8.507
		121	640	11	19.68	3.380
		149	288	15	39.37	7.680
		150	175	10	16.19	9.864
		151	288	10	16.82	9.671
		152	6480	25	67.62	7.715
		153	6400	18	53.97	11.035
		154	810	12	30.79	12.500
		155	2250	20	44.13	11.847
		156	294	8	30.79	8.384
		157	180	10	20.14	8.097
		241	144	7	12.08	9.290
		242	512	10	38.73	7.174
		243	896	16	30.79	8.640
		244	125	12	29.84	7.189
		245	27	5	8.90	9.688
		246	128	12	20.63	10.275
		247	112	13	20.63	7.969
		248	360	12	21.90	8.617
		249	324	12	20.14	11.189
		250	112	13	23.49	12.673
		251	45	6	9.20	9.762
		252	96	11	12.38	10.056
		253	700	17	31.11	9.291
		254	48	13	13.65	8.692
		255	112	18	26.98	7.753

(continued on p. 155)

	256	1100	20	56.51	7.625
	257	1210	22	68.89	8.358
	258	27	4	14.12	7.598
	259	288	14	42.86	8.671
	260	432	20	36.83	8.506
	201	392	13	46.35	8.780
	202	2016	22	47.62	7.586
	203	588	18	24.44	7.142
	204	432	28	28.25	7.761
	205	768	25	44.45	9.237
	206	1000	30	38.73	9.733
	207	360	18	26.35	6.226
	208	27	5	16.51	7.766
	209	36	6	14.60	7.960
n = 58	$\bar{Y}$ =	1,480.6	16.0	33.70	8.381
	S^2 =	7,577,691.56	46.19	254.0966	3.1440

(continued on p. 156)

total no. pods	total no. seeds	avg. no. seeds/pod	avg. dry wt. seeds	avg. dry wt. seeds/m^3
53	696	13.1	.1890	.1284
48	806	16.8	.1708	.1075
33	620	18.8	.1241	.0405
36	608	16.8	.1620	.6156
67	1,273	19.0	.1435	.0406
103	1,295	18.4	.1136	.7475
143	2,145	15.0	.1783	.2656
94	1,153	16.1	.2302	.2633
137	2,041	14.9	.2095	.7933
1637	23,245	14.2	.1403	.1933
107	1,605	15.0	.1857	.0883
277	4,404	15.9	.1997	.2299
721	12,978	18.0	.1976	.6826
94	1,532	16.3	.2422	.3865
304	5,472	18.0	.2130	9.3243
501	10,972	21.9	.1893	13.8467
433	9,613	22.2	.1754	.1833
1329	23,125	17.4	.2019	3.8908
1007	18,327	18.2	.1576	.7132
84	756	9.0	.1332	.1573
114	2,588	22.7	.1463	1.3146
94	1,993	21.2	.1746	1.9884
164	3,575	21.8	.1640	2.0358
1031	17,939	17.4	.1885	.5218
1237	26,224	21.2	.2278	.9334
341	6,752	19.8	.1893	1.5780
1873	33,714	18.0	.2185	3.2740
253	5,465	21.6	.1351	2.5113
461	8,851	19.2	.1930	9.4902
203	3,857	19.0	.1566	4.1945
1030	17,304	16.8	.1416	4.7856
1747	31,970	18.3	.2042	7.2896
163	2,608	16.0	.1477	3.0816
147	2,837	19.3	.1986	20.8678
894	18,490	21.4	.1672	24.1526
269	4,896	18.2	.1821	7.9604
1053	18,322	17.4	.1853	9.4308
303	7,595	25.0	.1965	4.5941
309	6,860	22.2	.2204	13.4995
201	4.141	20.6	.1616	14.8707
94	2,331	24.8	.1812	4.3998
1647	29,811	18.1	.1610	6.8565
327	6,017	18.4	.1725	21.6235
273	4,914	18.0	.1630	7.1516

(continued on p. 157)

	789	14,360	18.2	.1551	1.0675
	416	7,571	18.2	.1861	1.1644
	247	4,644	18.8	.1975	33.9700
	1704	34,421	20.2	.1594	19.0511
	1133	16,768	14.8	.1584	6.1483
	54	907	16.8	.2061	.4769
	53	1,007	19.0	.1716	.0857
	316	5,309	16.8	.2198	1.9846
	346	6,159	17.8	.1781	2.5392
	973	19,752	20.3	.1761	4.5291
	213	3,791	17.8	.2128	.8067
	403	6,851	17.0	.2126	4.0459
	193	3,667	19.0	.2016	27.3804
	71	1,548	21.8	.1884	8.1011
$\bar{Y}$ =	489	8,939	18.4	.1803	5.5596
s^2 =	265,851.57	87,130,850	7.7619	.000710	58.4062

(continued on p. 158)

no. seeds per m^3	no. pods per m^3	total dry wt. seed crop (kgs)	% bruchid damage	% taken by vert. predators
.68	.052	.131	100.00	43.45
.63	.036	.137	100.00	32.14
.33	.017	.076	100.00	-
3.80	.225	.098	100.00	4.44
.28	.015	.182	100.00	9.47
6.58	.358	.215	97.43	1.63
1.49	.099	.382	85.29	8.00
1.14	.093	.265	46.75	-
3.79	.254	.427	81.81	2.67
1.38	.097	3.261	92.06	2.11
.48	.032	.298	80.00	.67
1.15	.072	.879	76.78	-
3.45	.192	2.564	53.84	1.67
1.60	.098	.371	80.26	.61
43.78	2.432	1.165	69.49	-
73.15	3.340	2.077	86.20	-
1.04	.047	1.686	80.00	=
19.27	1.108	4.668	75.67	-
4.52	.249	2.888	83.33	8.79
1.18	.131	.100	66.67	-
8.99	.396	.378	51.68	.88
11.39	.537	.347	68.35	10.37
12.41	.569	.586	63.04	5.50
2.77	.159	3.381	65.85	2.29
4.10	.193	5.973	54.28	-
8.34	.421	1.278	89.13	3.03
14.98	.832	7.366	77.12	.30
18.59	.860	.738	57.14	-
49.17	2.561	1.708	53.04	.52
26.78	1.410	.604	41.48	.52
33.80	2.012	2.450	52.17	14.88
35.68	1.950	6.531	56.94	.21
20.86	1.304	.385	58.33	3.75
105.07	5.444	.563	85.71	9.32
144.45	6.985	3.091	90.56	2.80
43.71	2.402	.891	86.67	2.19
50.89	2.925	3.395	74.54	-
23.30	.935	1.488	79.10	-
61.25	2.759	1.511	64.15	4.50
92.02	4.467	.669	67.02	-
24.28	.979	.422	83.13	-
42.59	2.853	4.799	89.28	.55
125.35	6.812	1.037	95.00	1.28
43.68	2.438	.800	66.33	.55

(continued on p. 159)

	13.05	.717	1.174	95.58	-
	6.26	.344	1.408	70.21	2.74
	172.00	9.148	.917	68.00	3.19
	119.52	5.917	5.486	94.73	-
	38.81	2.623	2.656	41.71	.12
	2.31	1138	.186	69.84	5.35
	.50	.026	.172	70.00	-
	9.03	.537	1.167	73.33	-
	14.26	.801	1.096	84.84	-
	25.72	1.267	3.478	57.89	-
	3.79	.213	.006	82.85	-
	19.03	1.119	1.456	66.67	18.82
	135.81	7.148	.739	60.00	-
	43.00	1.972	.291	82.35	-
$\overline{Y}$ =	30.64	1.576	1.607	74.89	3.61
S^2 =	1,652.8200	4.4607	3.0612	253.9318	58.6948

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5 m	pod size index
1969	2 miles	121	2904	27	52.07	11.993
		122	6000	20	54.77	12.444
	east	123	640	13	31.75	14.061
		124	1764	10	32.12	12.244
	of	125	8820	23	56.83	7.896
		164	375	17	26.35	8.142
	Lecompton	165	2925	16	61.59	10.700
		166	490	16	31.43	8.807
		167	1584	12	38.89	8.938
		168	5600	16	54.16	10.967
		169	2025	10	28.09	7.490
		170	27	6	12.06	7.826
		171	3179	12	24.13	9.580
		172	1584	12	31.43	9.380
		173	20	6	8.25	7.244
		174	900	10	30.79	7.798
		175	196	5	15.87	8.500
		176	108	4	14.28	4.825
		177	288	14	24.13	11.659
n = 19		$\bar{Y}$ =	2,075	13	33.10	9.500
		S^2 =	5,799,373	36.7667	257.6578	5.2437

total no. pods	total no seeds	avg. no. seeds/pod	avg. dry wt. of seeds	dry wt. seeds/m^3
1581	33,201	21.0	.2017	2.3060
1999	41,179	20.6	.1691	1.1606
378	8,694	23.0	.1349	1.8325
6017	108,306	18.0	.1506	1.8493
1941	36,491	18.8	.1998	4.1332
43	714	16.6	.1912	.3640
572	9,953	17.4	.1879	.6394
934	14,010	15.0	.1808	5.1694
5231	107,759	20.6	.2001	13.6127
1592	32,158	20.2	.1340	.7695
1320	21,384	16.2	.2273	2.4003
433	6,755	15.8	.1881	47.0600
1203	20,932	17.4	.2598	1.7106
301	4,876	16.2	.2031	.6252
234	3,662	15.6	.1951	35.7230
1742	29,266	16.8	.2041	6.6369
543	7,059	13.0	.1986	7.1527
83	1,162	14.0	.1507	1.6214
66	1,241	18.8	.2039	.8786
$\bar{Y}$ = 1,380	25,726	17.5	.1874	7.1392
S^2 = 2,547,803	1,010,539,343	6.9083	.009677	157.4517

(continued on p. 161)

	no. seeds per m^3	no. pods per m^3	total dry wt. seed crop (kgs)	% bruchid damage	% taken by vertebrates
	11.43	.544	6.697	68.18	-
	6.86	.333	6.963	40.90	-
	13.58	.591	1.173	66.67	-
	20.69	1.100	7.291	40.32	1.84
	12.28	.682	16.311	27.67	-
	1.90	.115	.136	85.45	1.20
	3.40	.196	1.870	52.30	6.89
	28.59	1.906	2.533	70.00	5.33
	68.03	3.302	21.563	62.28	.15
	5.74	.284	4.309	75.51	-
	10.56	.652	4.861	67.69	8.64
	250.18	16.037	1.271	76.19	11.54
	6.58	.378	5.438	54.54	2.29
	3.08	.190	.990	64.86	-
	103.10	11.700	.714	39.07	7.28
	32.52	1.936	5.973	31.88	2.38
	36.02	2.770	1.402	55.17	5.38
	10.76	.768	.175	41.67	25.00
	4.31	.229	.253	71.42	2.12
$\bar{Y}$ =	37.35	1.985	4.722	57.46	4.21
s^2 =	4,370.9681	10.7101	32.0566	274.0332	37.4287

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5m	pod size index
1969	Olathe	96	2000	24	33.65	8.027
		163	392	10	15.55	6.964
		162	800	20	33.17	7.568
		161	1152	17	40.00	12.030
		95	324	10	18.57	6.861
		99	180	9	17.93	10.455
		102 Or.	150	9	18.57	6.861
		107 Or.	12	4	5.87	10.216
		93	125	6	6.82	5.874
		113 r-o	36	7	12.54	10.277
		98	3136	18	28.89	7.295
		100	81	10	14.12	8.795
		97	3380	25	28.57	8.843
		AA	1120	14	38.94	8.377
		BB	672	16	42.48	8.231
		CC	600	12	28.32	6.236
		DD	720	12	26.55	5.257
		EE	504	14	29.38	5.302
n = 18		$\bar{Y}$ =	854.7	13.2	24.33	8.190
		S^2 =	1,014,904	61.0910	123.5657	3.9199

total no. pods	total no. seeds	avg. no. seeds/pod	avg. dry wt. seeds	dry wt. seeds per m^3
1128	19,176	17.0	.1620	1.5532
2337	33,185	14.2	.1609	13.6211
1432	23,198	16.2	.1657	4.8049
3203	55,732	17.4	.1944	9.4048
774	10,062	13.0	.1917	5.9533
1406	24,886	17.7	.1962	27.2157
2144	32,160	15.0	.1978	42.4083
110	1,936	17.6	.1905	30,7342
80	1,056	13.2	.1720	1.4530
627	11,411	18.2	.1944	61.6194
1051	20,179	19.2	.1945	1.2515
217	3,429	15.8	.2384	10.0922
1380	28,428	20.6	.1825	1.5349
445	7,387	16.6	.1871	1.2340
301	5,117	17.0	.2202	1.6767
705	10,152	14.4	.1965	3.3248
631	7,446	11.8	.1547	1.5999
817	9,477	11.6	.2186	4.1104
1,043.8	16,912	15.9	.1899	12.4218
701,468.11	200,639,518.8	6.2711	.000478	292.9963

(continued on p. 163)

no. seeds per m^3	no. pods per m^3	total dry wt. seed crop(kgs)	% bruchid damage	% taken by vertebrates
9.59	.564	3.106	75.86	-
84.66	5.962	5.339	24.39	-
20.99	1.790	3.843	73.46	-
56.38	2.780	10.834	52.96	-
31.06	2.389	1.928	71.42	-
138.26	7.811	4.882	25.50	.18
214.40	14.293	6.361	19.39	.12
161.33	9.167	.368	25.68	-
8.45	.640	.181	69.23	-
316.97	17.417	2.218	14.51	-
6.43	.335	3.924	60.86	-
42.33	2.679	.817	35.59	-
8.41	.408	5.188	44.89	.97
6.60	.397	1.382	29.23	-
7.61	.447	1.126	30.00	-
16.92	1.175	1.994	44.44	2.77
10.34	.876	1.151	50.00	-
18.80	1.621	2.071	53.33	3.44
64.42	3.931	3.151	44.21	.41
7,124.3540	25.8879	7.0858	309.4633	1.0227

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5m	pod size index
1969	Sunflower	108 r-o	567	8	23.65	9.072
		157 pink	512	10	21.26	7.701
	Tract	109 r-o	700	8	20.64	8.428
		110 pink	864	8	32.38	8.495
		114 pink	96	8	13.49	7.818
		111 r-o	1000	12	19.21	7.038
		55 or.	392	14	25.72	6.713
		135 pink	112	13	15.08	6.223
		180 pink	45	10	14.29	10.515
		182 pink	1872	15	31.75	6.223
		165 pink	360	12	20.00	6.978
		71 or.	1296	12	7.50	8.234
		76 or.	576	11	15.30	5.674
		73 or.	275	14	26.67	6.420
		79 or.	200	13	19.39	8.379
		74 Or.	225	13	23.18	9.241
		72 or.	64	13	21.91	8.201
		70 or.	128	13	33.34	7.022
		69 or.	81	13	24.13	6.168
		97 or.	1100	13	21.59	6.220
		115 pink	275	14	17.14	9.156
		62 or.	384	10	22.54	7.570
		141 pink	125	10	20.00	7.529
		113 r-o	150	10	15.88	7.203
		126 pink	441	11	19.68	8.516
n = 25		$\bar{Y}$ =	473.6	11.5	21.06	7.629
		S^2 =	203,937.83	4.5080	365.818	1.4311

(continued on p. 165)

total no. pods	total no. seeds	avg. no. seeds/pod	avg. dry wt. seeds	dry wt. seeds/ m^3
4257	85,991	20.2	.2153	32.6470
2257	40,175	17.8	.1937	15.1992
401	6.416	16.0	.2114	1.9376
723	12,146	16.8	.1952	2.7441
167	2,839	17.0	.1904	5.6306
1253	19,422	15.5	.1721	3.3425
246	3,985	16.2	.2037	2.0708
109	1,373	12.6	.1560	1.9124
149	1,997	13.4	.1711	7.5931
72	878	12.2	.1896	.0998
81	1,215	15.0	.2056	.6830
893	16,610	18.6	.2002	2.5658
57	912	16.0	.1473	.2332
711	10,238	14.4	.1794	6.6789
531	8,708	16.4	.1701	7.4062
326	5,868	18.0	.1774	4.6266
123	2,140	17.4	.2017	6.7444
1372	20,306	14.8	.1762	27.9525
1531	21,740	14.2	.1727	46.3518
5103	84,710	16.6	.2008	15.4634
2781	45,052	16.2	.1644	26.9329
613	9,808	16.0	.1995	5.0956
301	3,793	12.6	.1982	6.0142
213	2,726	12.8	.1670	3.0349
374	5,610	15.0	.2110	2.6841
985.8	16,586	15.7	.1868	9.4258
1,736.371	5,610,456.24	4.0475	.000344	139.2428

(continued on p. 166)

no. seeds per m^3	no. pods per m^3	total dry wt. seed crop (kgs)	% bruchid damage	% taken by vertebrates
151.65	7.508	18.510	87.59	2.47
78.46	4.408	7.781	65.45	-
9.16	.573	1.356	63.26	12.50
14.05	.837	2.370	83.33	5.95
29.57	1.740	.540	89.47	1.17
19.42	1.253	3.342	68.47	-
10.16	.628	.811	67.39	-
12.25	.973	.214	76.67	3.17
44.37	3.311	.341	76.92	4.47
.46	.038	.166	48.27	3.27
3.37	.225	.249	74.28	5.33
12.81	.689	3.325	77.77	3.22
1.58	.099	.134	57.77	-
37.22	2.585	1.836	67.56	2.77
43.54	2.655	1.481	39.58	-
26.00	1.449	1.040	60.34	4.44
33.43	1.922	.431	75.00	2.29
158.64	10.719	3.577	59.67	19.59
268.39	18.901	3.754	50.00	18.67
77.00	4.639	1.709	50.00	4.92
163.82	10.113	7.406	70.00	-
25.54	1.596	1.956	67.44	6.25
30.34	2.408	.751	62.50	-
18.17	1.420	.455	77.41	-
12.72	.881	1.183	70.00	-
51.29	8.157	3.201	67.44	4.02
4,351.4407	18.9467	19.3647	151.8771	28.5909

Year	area	tree no.	est. m^3 canopy	ht(m)	dia. at 1.5m	pod size index
1969	Natural	35	108	7	15.55	6.978
		154 pink	3328	17	61.91	9.876
	History	34	1100	13	24.13	4.700
		46	16	5	12.38	7.535
	Reservation	47	320	6	13.33	7.276
		65	4536	17	62.23	6.959
		51	320	7	15.55	10.652
		45	125	7	18.09	8.647
		43	216	7	13.65	8.679
		22	2352	18	63.81	9.097
		16	1152	12	29.21	9.551
		13	2744	18	65.67	7.868
		101 or.	1728	20	53.97	6.424
		43 red	112	9	14.28	4.542
		64 red	324	13	24.76	9.109
		48	18	3	5.39	7.012
		160	1575	14	31.11	10.733
		159	1163	15	36.19	12.884
		156	288	9	14.60	5.526
		142	700	8	25.71	7.176
		143	300	6	16.19	5.942
		145	288	12	16.51	6.529
		29	1700	22	35.87	12.407
		32	2160	23	33.65	9.248
		33	2352	18	28.41	9.832
		17	1470	19	37.94	5.929
		144	1152	20	52.07	9.813
n = 27		$\bar{Y}$ =	1,172	12.8	30.43	8.182
		S^2 =	2,040,905.11	34.26	330.7319	4.6540

(continued on p. 168)

total no. pods	total no. seeds	avg. no. seeds/pod	avg. dry wt. seeds	dry wt. seeds/m^3
55	894	16.3	.1689	1.3981
2682	44,521	16.6	.1194	1.5973
263	3,156	12.0	.1246	.3575
429	7,036	16.4	.1691	74.3691
667	10,405	15.6	.2215	7.2022
35	480	13.7	.1949	.0206
82	1,066	13.0	.1534	.5110
33	482	14.6	.1674	.6455
16	312	17.3	.1401	.2024
263	5,733	21.8	.1461	.3561
151	3,352	22.2	.1543	.4490
418	6,604	15.8	.1793	.4315
25	305	12.2	.2285	.0403
57	467	8.2	.1930	.8047
137	2,603	19.0	.1353	1.0870
136	1,809	13.3	.1839	18.4822
331	5,584	16.9	.1873	.6640
1234	24,860	20.0	.1965	4.0994
116	1,822	15.7	.0872	.5517
391	6,167	15.6	.1375	1.1982
67	925	13.8	.1849	.5701
133	1,623	12.2	.1576	.8893
2361	48,330	20.5	.2294	.7846
1531	30,008	19.6	.2110	5.9986
3007	61,553	18.1	.1895	2.6326
1256	13,992	11.1	.1921	5.0274
294	3,940	13.4	.1973	1.8780
599	10,659	15.7	.1722	4.8981
729,621.11	2,732,197	11.5869	.001204	203.0431

(continued on p. 169)

no. seeds per m^3	no. pods per m^3	total dry wt. seed crop(kgs)	% bruchid damage	% taken by vertebrates
8.28	.509	.151	71.15	3.07
13.38	.806	5.315	97.27	-
2.87	.239	.393	76.47	-
439.75	26.812	1.189	97.22	1.21
32.52	2.084	2.304	90.24	-
.45	.007	.093	100.00	11.67
3.33	.256	.163	94.11	1.53
3.67	.264	.080	64.70	5.47
1.44	.083	.043	63.98	21.17
2.44	.112	.837	100.00	-
2.91	.131	.517	100.00	-
2.41	.152	1.184	100.00	-
.18	.014	.069	69.09	-
4.17	.509	.090	55.55	-
8.03	.423	.352	85.71	-
100.50	7.536	.332	85.10	-
3.54	.210	1.048	95.17	.39
20.86	1.043	4.849	96.38	.50
6.33	.403	.158	94.20	-
8.71	.558	.838	100.00	-
3.08	.223	.171	79.41	-
5.64	.462	.256	81.81	-
3.42	.255	.903	95.83	1.49
28.43	1.389	10.197	90.47	.60
13.89	.709	5.606	72.72	-
26.17	1.278	11.824	77.27	1.10
9.52	.854	2.760	100.00	-
28.00	1.753	1.918	86.44	1.78
7,159.3543	27.1402	9.5541	179.2557	20.9867