

MATING BEHAVIOR OF THE ALFALFA WEEVIL,

HYPERA POSTICA (GYLLENHAL)

by

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I. GENERAL INTRODUCTION

It is preferable to know the normal behavior of an animal before experimenting on it. So insisted the pioneer ethologist, Charles O. Whitman in a Woods Hole lecture on animal behavior in 1898. This aim is indeed a noble one, but unfortunately one which is hard to achieve in the study of many animals. Such an animal is the alfalfa weevil, Hypera postica (Gyllenhal), (Coleoptera: Curculionidae), whose physical habitat alone makes it impossible to observe the insect's normal behavior. Consequently, such a basic and important aspect of its biology as its mating behavior has previously gone unstudied. In view of the great economic losses caused by this prolific pest, a need for a better understanding of its mating activities and indeed its whole reproductive biology made a comprehensive laboratory study essential.

The alfalfa weevil is the most important pest of this crop in the country today. Cothran (1966) cites the following alarming statistics. Since its discovery in the United States near Salt Lake City, Utah in 1904 (Titus, 1910a), it spread rapidly in the West to include 15 states. In 1951 it was discovered in Maryland, this being the first report of the weevil in the East (Poos and Bissell, 1953). Today it has become firmly established in the East and covers a 25 state area.^{1/} Despite quarantine and attempts at control, the insect is continuing to increase its boundaries. In the states where the alfalfa weevil occurs five of the 25 million acres of alfalfa undergo damage, and approximately 1.5

^{1/} More recent data reveal that the alfalfa weevil is now in Michigan (Dowdy, 1966) and Wisconsin (Conrad and Lovett, 1966).

million acres are so badly infested that they require control measures (Wagner, 1965) at costs approaching \$10,000,000 annually.

Since the spring of 1960, when heptachlor was removed from the list of materials acceptable to regulatory officials for use on forage crops, control of the alfalfa weevil has become increasingly difficult (Dorsey and Quinn, 1962). Insecticides available to replace heptachlor have not been markedly successful in controlling the pest. Cultural methods have also proven to be to a great extent unsuccessful or in some other way prohibitive. Thus the logical alternatives would be integrated control, biological control, resistant alfalfa varieties, or sterilization by either radiation or chemical means. For some of these methods to work, a better understanding of the mating habits and more specifically the reproductive biology of the alfalfa weevil must be attained. These aspects could best be explored in a laboratory study.

A method of rearing nondiapausing alfalfa weevils in the laboratory modified slightly from Huggans and Blickenstaff (1964) made available a ready supply of insects at all times and greatly facilitated the present study. It was soon established that the weevils would mate readily under laboratory conditions, even though they were out of their natural environment. While it is thought that the results of this study give an indication of mating potential rather than that which may actually occur in the field, it is believed that this information has shed light on many aspects of normal weevil mating behavior and pointed the way for further studies in the reproductive biology and behavioral ecology of the alfalfa weevil.

The specific objectives of the work were as follows:

1. to investigate the qualitative aspects of alfalfa weevil mating behavior, i.e. the specific movements and/or patterns of movements which are involved in their precopulatory, copulatory, and post-copulatory behavior.
2. to explore the quantitative aspects of their mating behavior: the percentage of time occupied by mating activities, the number of mountings and the average duration per mounting, and other related activities which could be expressed quantitatively.
3. to determine the relative importance of the stimuli which are involved in mating behavior.
4. to evaluate the effect of temperature on mating behavior and oviposition.

II. GENERAL MATERIALS AND METHODS

Alfalfa weevils utilized throughout this entire investigation were from two sources. The first was an alfalfa field (unsprayed in 1967) on Route 460, one mile west of Blacksburg, Virginia, where overwintered insects were collected. These weevils were brought to the laboratory between April 6 and April 23, 1967, a time when they were quite active and easily obtained. Because they were overwintered adults which had undergone diapause the previous summer, they were considered as postdiapausing adults and designated as such throughout this report. In the laboratory they were immediately sexed, the males and females separated and placed in gallon food cartons, and provided with food and water. The open tops of the cartons were covered with polyethylene (Eno, 1960), which is permeable to O_2 and CO_2 . An effort was made to maintain a temperature of $75 \pm 3^\circ$ F, and a daily photoperiod of 12 hours, though both of these may have fluctuated slightly.

The second source of insects was a colony of nondiapausing weevils established and maintained under strictly controlled conditions in a Sherer-Gillette growth chamber in the laboratory. The colony had been started and maintained for several generations under conditions set forth by Huggans and Blickenstaff (1964): 8 hours of light at 75° F and 16 hours of darkness at 65° F. The insects spent their entire life cycles under these conditions. After emergence, the adults were fed for one to three weeks in the growth chamber, and were placed in a Basal Oxygen Demand Incubator at 3° C. After three to five weeks, the weevils were removed, sexed, and separated into individual food

containers, where they were then held under the same conditions as the postdiapausing weevils.

Although different experiments necessitated certain modifications of the following techniques, a general method was adopted and used throughout the entire investigation. Plastic petri dishes, 90 x 23 mm, were utilized. The temperature within the dishes was $75 \pm 3^{\circ}$ C, with a relative humidity of 80 to 90%. Into each dish was placed a two-inch sprig of alfalfa with a small, wetted ball of cotton at the tip. This provided moisture for the weevils and served to keep the alfalfa fresh. A pair of alfalfa weevils, male and female, was introduced into the dish. Because preliminary tests indicated that pairs of alfalfa weevils usually mounted and always remained in copula for at least 15 minutes, observations were taken at 15-minute intervals for 12 hours daily. When this information is presented in the form of a table or figure, the number of 15-minute periods in which the pairs were mounted out of a maximum 48 periods per day has been given as a percentage of time spent in mating behavior. In most cases the insects were checked under lighted conditions of 25 foot-candles as determined by a Weston, Model 756, photometer. Where changes from the above procedures were made, they will be described in detail in connection with that specific experiment.

III. QUALITATIVE ASPECTS OF MATING BEHAVIOR

A. Introduction

The account presented in this section constitutes the least quantitative portion of the work. It includes general observations and descriptions of the morphological and behavioral differences between male and female alfalfa weevils. The major portion of this section, however, is concerned with their precopulatory, copulatory, and postcopulatory behavior. In short, it is a descriptive survey of both the normal and unique aspects of alfalfa weevil mating activity. It is the result of many hours of observing alfalfa weevils mate under laboratory conditions, and now an attempt will be made to fit this mating behavior into some sort of pattern.

In this section many aspects of weevil mating behavior will be only briefly discussed, since details covered in later sections are required to adequately describe them. Thus the intention here is to furnish a rather complete but general coverage of their mating behavior, so that from the onset the study will be placed in its proper perspective.

B. Literature Review of the Mating Behavior of Selected Insects

This section will provide a background for comparisons between the mating behavior of the alfalfa weevil and certain other insects. Since this study represents the first investigation of the mating behavior of the alfalfa weevil, no information from the literature of the alfalfa weevil is included here.

In the Orthoptera, mating behavior in some species resembles to a certain extent that found in the alfalfa weevil. Srivastava (1957) working on the tobacco grasshopper, Atractomorpha crenulata, noticed that, "When a male sights a female, he approaches her stealthily and after establishing himself in a convenient position jumps quickly onto the female who offers some resistance." The author continued to say that when the male had established his position, he repeatedly patted with his forelegs and antennae the pronotum and head of the female. The hold on the female was maintained by the first and second pairs of legs, while the third pair remained free. During copulation, the female sometimes moved about slowly, and occasionally kicked the male with her hind tibiae. Copulation lasted from 1½ to 3 hours.

Srivastava further noted that sometimes a male would remain on the female for 30 minutes attempting to mate without success. In these attempts he bent his abdomen to one side or the other in order to bring the genitalia in contact. Finally he brought his abdomen between the femora and abdomen of the female and gave it a spiral twist. At this time, the copulatory apparatus was inserted.

The female ceased to feed while in copula. Disturbance caused her to move and often resulted in the separation of the pair. After completion of copulation, the female would not mate for about half-an-hour. If the male attempted to mount again, she kicked him off. Sometimes the male remained mounted after completion of the sex act.

It was noticed that males which had copulated once preferred to copulate with the former mate who offered little resistance. Rivalry sometimes existed between males which disturbed and attempted to dis-

lodge mounted individuals. Females did not copulate more than two to four times per day.

Federov (1927), in studying Anacridium aegyptium L. (Acrididae), found mating behavior similar to that described in the tobacco grasshopper. However, Federov noted that copulation lasted from 18 to 60 hours. Ferkovich et al. (1967) also found that mating behavior of the big-headed grasshopper, Aulocara elliotti, closely resembled that described in the tobacco grasshopper. It was pointed out by Ferkovich et al. that caged males were normally attracted by the quick movements of other grasshoppers. A female big-headed grasshopper could avoid copulation by vibrating its hind femora or elevating the terminal portion of its abdomen to prevent the male from mounting or as in the case of a mounted male, to cause him to become disoriented and reverse his position.

Ferkovich et al. further discovered that the copulation frequency per male increased as the ratio of males to females decreased. This response may have been because of reduced competition among the males or due to the increased availability of females for mating. Males isolated from females occasionally exhibited homosexuality which in some instances was accompanied by aggressiveness.

Alexander (1961, 1962) found that certain crickets remained in pairs through several copulations. Some crickets also copulated several times in succession (Alexander, 1962). According to Alexander (1967), in certain species of both grasshoppers and crickets, the male remained mounted on the female without the genitalia engaged. This activity occurred either before or between copulations.

The mating behavior of the pear psylla, Psylla pyricola, (Homoptera: Psyllidae) has been extensively studied by Burts and Fischer (1967). Males were found to be the aggressors and had the capacity to mate about once a day through the first two weeks as adults. During mating activity females remained passive and did not resist the advances of the males. Copulations lasted from less than one minute to four hours but averaged 36 minutes. Cook (1963) gave a detailed account of the mating act itself, in which a male was observed to approach a female from her right, facing her, then turn to his right and back up so that he was beside her and facing the same direction. The male then moved the posterior portion of his abdomen to beneath the female's genital area and pushed up on her ventral valve with the tip of his abdomen. After several seconds, the female turned the tip of her abdomen downward. The male then bent the tip of his abdomen upward approximately 135° so that it was nearly inverted and appeared scorpion-like. At this time copulation took place.

The mating behavior of another Homopteran, Sogata orizicola (Delphacidae), was studied by McMillian (1963) who learned that neither sex mated until the adult was three days old. Prior to mating the male stimulated the female with abdominal vibrations. If the female was receptive she responded in a similar manner. He then approached the female, and together they fluttered their wings. Copulation soon took place and generally lasted for about three seconds. In an 8-hour period the male mated with as many as three females, each successive mating taking progressively longer. Once the female mated, she ceased to exhibit the characteristic virgin courtship behavior.

Acker (1966), reporting on reproductive behavior in Agulla species (Neuroptera: Raphidiidae), observed that mating behavior began about a week after the emergence of the adult female. Olfactory senses served to bring the sexes together before visual contact was established. Courtship took place when the male and female faced each other while the male carried out abdominal, mandibular, and antennal movements. The female responded with similar movements, but to a lesser degree. Copulation was achieved when, both facing the same direction, the female raised her abdomen and ovipositor allowing the male to crawl beneath her. He then turned with the abdomen upward and clasped her terminal segments. The female moved forward, turning the male upside down relative to her. Copulation lasted from 1½ minutes to 1½ hours.

Keller et al. (1964) presented evidence that the female boll weevil was attracted to the pheromone of the male. Cross and Mitchell (1966) substantiated this observation and mentioned that the female used elaborate tactile behavior in inducing the male to mate. Mayer and Brazzel (1963) found that when males and females were introduced into the same cage, they became excited. Their movements, however, appeared to be random with mating occurring upon chance collisions. The majority of male weevils mated from one to four times per 24 hour period, the duration of each copulation being from 15 to 30 minutes.

According to Mayer and Brazzel (1963), 90% of the females mated again 24 hours after the last copulation. Prior to this time, many females, if mounted by a male, resisted by (1) rapid swaying of the body (2) preventing insertion of the aedeagus by extension of the ovipositor or (3) moving the tip of the abdomen dorsally to obscure

the genital opening under the elytra.

The mating behavior and position of Boreus brumalis (Mecoptera) have been described by Crampton (1940). Initially the male ran up to the female, hooked his slender wings over her back, extended the end of his abdomen under hers and, provided she did not struggle too violently, succeeded in lifting her across his back. From this position, he inserted the intromittent organ. After the female became quiescent, the male gradually shifted her body along his back, forward to a position in which his wings clasped her front femora and her head extended downward between his wings.

The mating behavior of some Diptera has been studied. Provost and Haeger (1967) in reporting on the mating habits of the crab-hole mosquito, Deinocerites cancer, stated that the males spent considerable time on the water where they sought and attended pupae or examined all other mosquitoes near them. This behavior appeared to be the result of an olfactory stimulus. A male which remained with a pupa generally copulated with the helpless emerging female before she was entirely out of the pupal skin and often remained in copula for two hours. Males also readily attempted copulation with emerging males, and from all indications with either sex of almost any other emerging mosquito. Repulsion by hind tarsae, if already emerged, or malaccommodation of genitalia, if emerging, were apparently all that prevented interspecific mating.

Males walking on the surface of the water also became very excited on contact with a recently cast pupal skin. This reaction again points to an olfactory involvement. Males tried to copulate with the float-

ing skin and often formed fighting clusters over it. Females willingly mated only once. In comparing the mating of the New Zealand rock-pool mosquito, Opifex fuscus, the authors found similar behavior in regard to pupal attendance and males fighting among themselves over pupae and cast skins. In Opifex, however, the stimulus appeared to be visual rather than olfactory. Both species faced in the opposite direction while in copula. Supporting evidence for the mating activity described in the crab-hole mosquito was provided by Downes (1966).

In another mosquito, Aedea triseriatus, Wright et al. (1966) observed that mating took place only when the males were swarming. During swarming, a female flew from her resting position and passed near or through the swarm. Several males pursued, but only one contacted her. The female either dislodged the male or mating took place. In mating, the male, using claws of the first pair of legs, caught the female's second pair of legs and took a position beneath her. From this position their genitalia connected. This was a sternum to sternum position, with heads pointed in the same direction. With the genitalia locked, the legs released their hold, and the male swung downward and backward, coming to face in the opposite direction from the female, thus acquiring a tail to tail position. Although the male's wings continued to beat, the direction of flight was that of the female. If the female landed, the male's wings still beat, and his feet had no contact with the substrate. In blood-fed females, copulation averaged 55 seconds while 25 seconds was normal for those that had not had blood.

Finally, Roth (1948) noted that in Aedes aegypti, one virgin male placed in a cage with 16 virgin females copulated successfully 30 times and attempted 10 other unsuccessful matings during a 30-minute period. He further stated that 11 virgin males copulated successfully 50 times during one hour with a single virgin female.

Newkirk (1963) reported that in Leptogaster annulatus (Diptera: Asilidae), mating took place at night, or early morning with courtship occurring at dusk and copulation usually just before dawn. Prior to copulation, a male would pounce upon a female. In turn the female often bent her body in a C-shaped pattern, with her wings retaining their original position, and serving as a barrier to the male. At this point, other males often attempted to mate and were sometimes stacked three-high on the female. If the female was forced to straighten her back, one of the males often coupled with her, after which the male fell backward into a head down position, his sternum facing outward. The male hung in this position for two to ten minutes before separation of the pair. In this species, the male's response to the female was through a visual stimulus, despite the reduced light conditions.

A detailed study of the gall-former, Aciurina ferruginea (Diptera: Tephritidae) has been carried out by Tauber and Tauber (1967). They observed that adults of both sexes participated in a wing waving display and in this way communicated visually for the purpose of mating. The authors believed that these visual cues first aided in the recognition of the species, then the recognition and courtship of the opposite sex, and finally in the release of copulatory behavior.

When a male noticed a female, he turned toward her, then approached and mounted from the side and the rear, forcing his body between her wings. While initiating the mount, the male's mouthparts repeatedly contacted the abdomen of the female. At times the female attempted to reject the male by (1) kicking at his middle legs or striking against his head, neck, or thorax (2) not parting her wings or (3) depressing her abdomen and ovipositor against the substrate. In the copulatory position, the placing of the male's forelegs varied; generally only the foretarsi touched the female abdomen as the male was positioned quite far posterior. The mesotarsae and part of the mesotibia contacted the segment just anterior to the ovipositor. The hind legs either contacted the substratum or hung freely. During copulation, the male's mouthparts again touched the female's abdomen. Minimum time in copula was observed to be 59 minutes. Homosexual activity was exhibited by this fly.

In a detailed study of the mating behavior of the hen flea, Ceratophyllus gallinae, Humphries (1967) discovered that the two sexes did not appear to react to each other at a distance. Initiation of mating occurred only if they by chance came in contact. When they did contact, the maxillary palps of the male had to touch the female for mating activities to begin. However, sometimes such contact with other males also elicited sexual behavior.

After stimulation of his maxillary palps, the male ran forward with antennae erect, head lowered, and pushed the female which usually ran forward for a short distance. The male pursued, forced himself under the abdomen of the female and clasped her with his antennae.

He then lifted the tip of his abdomen so the distal ends of his genitalia were directed anteriorly over his back and met the female's genitalia. Copulation usually lasted about three hours, but varied from a few seconds to almost nine hours. Males would sometimes attempt to mate with dead females.

Although matings which had endured for an hour or more were almost invariably terminated by the male unlinking his genitalia, briefer matings often ended by the female rejecting the male. This she accomplished by depressing the posterior portion of her abdomen and running forward. She also often used her hind legs to thrust off the antennal hold or to push away the hind part of the male's abdomen.

Among the Hymenoptera, mating activity of Tetrastichus incertus (Hymenoptera: Eulophidae), a parasite of the alfalfa weevil, has been observed by Miller (1966). In mating which occurred immediately after emergence, the male did not appear to actively seek the female until she was within three to five millimeters. When he noticed her, he followed and attempted to mount in a male sternum to female dorsum position. After the copulatory position had been established the male alternately held and stroked the bases of the female's antennae with his foreleg tarsae, while striking her antennae with his antennae. If the female was disturbed, she often became active and dislodged the male. As many as three males were seen attempting to copulate with one female.

C. Materials and Methods

For the most part observations took place while the weevils were in petri dishes as described earlier. There are observations when only one pair of weevils was present per petri dish and also when mating was taking place under crowded conditions. Some descriptions were made when the insects were under less restricted conditions, as when mating took place in a large cardboard box (see section V E 1 a), the bottom of which was covered with moistened sand in which were inserted numerous sprigs of alfalfa. This provided considerable space for movement and represented an effort to simulate a natural alfalfa field habitat. Finally, the activities of the insects were also recorded when males and females were separated in individual gallon food cartons.

The information presented here is not so much from experiments specifically designed for the purpose of this section, but from a general compilation of observations recorded from all experiments.

D. Results and Discussion

1. Some general morphological and behavioral characteristics of male and female alfalfa weevils

Before discussing in detail the mating behavior of the alfalfa weevil, it may be appropriate here to point out some of the more apparent external, morphological differences between the sexes. Since no measurements have been made concerning these differences, they are presented in a brief, generalized form. The most significant and by far the most reliable distinguishing characteristic by which males

may be separated from females is by the position of the last tergite and the shape of the last sternite. In the male, the posterior-most tergite extends around the tip of the abdomen and may be easily viewed ventrally, while the last sternite terminates posteriorly in a broadly rounded, flattened projection which rests upon the tergite. In the female, the last tergite is not easily seen from beneath, because the hindmost sternite extends to the end of the abdomen and is very broadly rounded without a rounded projection or point (Figure 1).

There are other more subtle differences between male and female alfalfa weevils, which may not always hold true. The males were often slightly smaller and more narrow with a more nearly pointed abdomen, while the females had a blunter, more rounded abdomen, as shown in Figure 1. The size difference was also reported by Titus (1909, 1910a). Another slight difference was that the males generally had a darker stripe down the center of the pronotum and elytra than did the females. As the setae which give the insect its color rubbed off with age, however, this difference became less apparent and was of little value in distinguishing the sexes. Finally, it appeared that the males had slightly longer front legs.

Not all of the distinguishing characteristics between male and female alfalfa weevils were morphological. The males were more agile and moved rapidly about the container in which they were confined. Most of the time it was the males which rapidly climbed the sides in an apparent effort to escape. Their general movements seemed to be more uneven and at a faster pace than the females. When released in



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Figure 1. Comparison of ventral tips of the abdomen in male and female alfalfa weevils. Note the difference in the shape of the last sternite, which is broadly pointed in the male and rounded in the female.

a simulated alfalfa field environment, the males were very quick to disperse from the site of release. The males also seemed to feed less and spend considerably more time on the move than the females.

In contrast to males, females spent more time feeding and considerably less time moving about. Their oviposition activities may have influenced their behavior. Within the simulated environment they dispersed much slower than the males and showed less interest in climbing the sides of the container. Their movements were generally somewhat slower.

Based on what has been seen in the laboratory concerning the movement of both sexes, it is quite conceivable that in order to escape a declining habitat or to search for mates, the insects could travel considerable distances in a relatively short time. Similarly there is little doubt that males seeking mates can move far enough, even in a large alfalfa field with a small population of weevils, to make random encounter possible. Thus reproduction and survival is assured without a chemical attractant. Titus (1909, 1910a), Reeves et. al. (1916), Henderson (1919), Wakeland (1921), and Snow (1925) all agreed that in a given field the alfalfa weevil could disperse to a great extent by crawling.

2. Precopulatory, copulatory and postcopulatory behavior

Male alfalfa weevils displayed extraordinarily aggressive mating behavior toward females under controlled conditions in the laboratory. They mounted from any angle or direction, on or off the plants, and

maneuvered into a precise position for copulation. They have been observed to fall or jump off a plant and land beside or on a female below them. If they landed sideways or facing the wrong direction, they simply hung on tenaciously until an opportune time for them to maneuver themselves into copulating position. Often the female would run rapidly about but was seldom able to shake off the male. If her attempts were successful, however, she often ran into thick foliage as if trying to hide from the male.

Some females were observed to struggle violently with aggressive males, and even used their legs in an apparent effort to remove him or in some way prevent him from achieving copula. Analogous activity has been observed in the tobacco grasshopper by Srivastava (1957). Females were seen to run rapidly through alfalfa as if trying to dislodge the male. On one occasion, a male which had mounted from the front remained firmly mounted in this backward position while the female moved rapidly about. When she stopped, the male quickly reversed his position and inserted the aedeagus. Still another method was employed by female weevils which were reluctant to mate. Very commonly seen in laboratory reared, nondiapausing weevils was the mechanism where the females raised the abdomen up under their elytra, thereby covering the genital opening and preventing copulation. This behavior was most often observed in virgin females. Mechanisms similar to those described above have been reported for the female Anthonomus grandis (Curculionidae) (Mayer and Brazzel, 1963), Deinocerites cancer (Culicidae) (Provost and Haeger, 1967), Aciurina ferruginea (Tephritidae) (Tauber and Tauber, 1967), Aulocara elliotti (Acrididae) (Ferkovich

et al., 1967), and Ceratophyllus gallinae (Ceratophyllidae) (Humphries, 1967). No female alfalfa weevils from the field were seen to employ this method to repel males. Rather their methods were those of the running, kicking type which were described earlier. It was perhaps for these reasons that the males often attempted to mate with the females when they were feeding or boring stems for oviposition, and thus less able to avoid the males.

While the kind of activity just described did occur regularly, it is not meant to imply that the females always tried to escape the male and avoid mating. In fact there was evidence that females were often mutually attracted to other weevils, not necessarily only males, by a visual stimulus. The same is true of males which apparently responded readily to the motion of any other weevil. They seemed to visually perceive motion from at least seven inches, with heavy foliage or other types of obstructions reducing this distance. The apparent visual attraction of the males was not limited to other alfalfa weevils but was also seen with other species of curculionids, especially the white pine weevil, Pissodes strobi, the clover leaf weevil, Hypera punctata, both larger than the alfalfa weevil, and even the smaller clover root curculio, Sitona hispidula.

With the white pine weevil, male alfalfa weevils showed almost the same patterns of behavior as with other alfalfa weevils. They mounted the larger weevils readily and remained mounted for long periods of time. At times they dismounted, only to mount again a few seconds later. While there is little doubt that this was mating ac-

tivity, it must be said, that even though they aggressively mounted both male and female white pine weevils, they did not as a rule attempt to insert the aedeagus, which of course they would be unable to do because of morphological differences.

Male alfalfa weevils did however often vainly attempt to insert the aedeagus in other male alfalfa weevils, which they mounted very aggressively. This situation has also been found in Atractomorpha crenulata (Acrididae) (Srivastava, 1957), Aulocara ellioti (Acrididae) (Ferkovich et al., 1967), Deinocerites cancer (Culicidae) (Provost and Haeger, 1967), Aciurina ferruginea (Tephritidae) (Tauber and Tauber, 1967), Leptogaster annulatus (Asilidae) (Newkirk, 1963), and Tetrastichus incertus (Miller, 1966). Male weevils will do the same with sexually immature, newly emerged, female alfalfa weevils. In both of these examples the male sometimes remained mounted for hours with the aedeagus fully extended but, of course, not inserted (Figure 2). This kind of behavior occurred frequently even when sexually mature females, with which they could have copulated, were present. Thus the stimulus appears to be visual, rather than olfactory. If an orienting pheromone were involved, it would seem that the males could have readily determined the presence of the females. Since the alfalfa weevil has not been found to possess a stridulatory structure, nor to produce sound other than noises caused by their motion, it would seem that vision is the major stimulus for mating. It seems that males are the true initiators of copulation, but do not immediately recognize the sex partner, and even at close range cannot readily distinguish



Figure 2. Pair of alfalfa weevils mounted with aedeagus of male extended but not inserted.

the sexes. If there is a distinguishing mechanism, then it would seem to be a very subtle, tactile one.

It has been stated that both males and females appear to be mutually attracted to other weevils though not necessarily those of the opposite sex and perhaps not necessarily as a mating drive. When males came in contact with other weevils, their initial response was often an attempt to copulate. Thus they are probably the true initiators of mating, even though the females may have been mutually attracted by vision to them.

Another indication that visual stimuli play an important role is the fact that males appeared to be attracted more to other mating pairs than to lone individuals. Sometimes the males attempted to insert the aedeagus. This kind of activity often continued until there was a column of weevils seven or eight high, with one female on the bottom. Some of the males were even facing in the wrong direction, but as a rule many of them would have the aedeagus erected. This type of behavior, illustrated by Figure 3, has been noticed in other species of insects such as elaterid beetles (Lilly, 1959) and June beetles (Travis, 1939).

Though vision has been shown in many instances to play an important role in mating behavior, the fact still remains that in petri dishes, alfalfa weevils mated just as heavily in the dark as they did under lighted conditions. This may have been due to chance contacts within the limited area of the dish. It is also possible that the insect sees in the dark to some extent. Newkirk (1963) reported that a robber fly, Leptogaster annulatus, mated in the dark. Another pertinent



Figure 3. Aggressive mating behavior and disturbance activity by male alfalfa weevils.

observation is that weevils located the sex partner and mated equally well on either light or dark backgrounds. It might be suspected that mating activity would be reduced on darker backgrounds because of less contrast with the insects, but this has not been proven.

Observations in the laboratory have indicated that time of day does not affect the amount of mating activity. This may not be true under field conditions, because diurnal and nocturnal habits differ considerably there. It is generally true that alfalfa weevils, particularly in the warmer seasons, are more active at night when they are usually found upon plants. During the day they remain on the ground or at the base of the alfalfa plants (Titus, 1910a; Parks, 1913; Poinar and Gyrisco, 1960). What effect this diel cycle might have on their mating behavior has not been determined.

No elaborate courtship was observed in the alfalfa weevil. The males mounted quickly and were persistent in their attempts to achieve copulation. To successfully insert the aedeagus, the male had to be in a definite male sternum, female dorsum position. The front legs were fitted into the notch just behind the prothorax; the mesothoracic legs, which grasped the female most firmly, were positioned around the middle of the elytra, while the metathoracic legs generally performed no true holding function and usually trailed behind, as shown in Figure 4. The hind legs of the male, however, were necessary in mounting, for they had to be on a firm substratum for the male to mount successfully. If for instance, the tip of the abdomen of a female extended over a leaf which was very flexible, the male had



Figure 4. Pair of alfalfa weevils copulating.

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considerable difficulty in mounting. He could not place the rear pair of legs on a firm portion of the leaf to achieve sufficient momentum for mounting.

When mounted in the described position, the male bent the tip of his abdomen downward so that it formed a right angle with the substratum and came in close proximity with the genital opening (vulva) of the female (Figure 5). Once in this position the male needed only to extend the aedeagus and insert it. This situation is somewhat different than that found in many other insects, where the male must bend his abdomen far forward to reach the genital opening of the female.

While in copula the pair of alfalfa weevils usually remained in an upright position. Occasionally, however, they were seen in an inverted position, where the male was on his back holding the female, whose legs were projecting upwards and usually kicking. Finally, weevil pairs were not infrequently observed copulating while lying on their sides. There is little doubt that these two latter positions occurred by accident, as when an active female lost her footing, overturned, and was unable to regain an upright position because of the weight of the male. One final observation concerns the fact that it was not too unusual to see a male tightly clasping another weevil male or female, in a sternum to sternum position, with the aedeagus fully erected but, of course, not inserted. They sometimes remained this way for hours (Figure 6).

Initially after mounting, the male almost invariably stroked with his antennae the pronotum of the female. The stroking was gen-



Figure 5. Position of the aedeagus of the male and the genital opening of the female.



Figure 6. Male with aedeagus extended grasping another weevil in abnormal mating position. This pair remained in this position for 6 hours.

erally rapid at first but usually slowed after a few seconds and generally subsided altogether after a minute. However, if the female continued to struggle or ran about, the rapid stroking continued until the female became calm. Similar mechanisms have been reported for the males of Atractomorpha crenulata (Acrididae) (Srivastava, 1957), Tetrastichus incertus (Eulophidae) (Miller, 1966), and Aciurina ferruginea (Tephritidae) (Tauber and Tauber, 1967). Stroking might serve to calm the female to prevent her struggling or to prepare her for insertion of the aedeagus. This assumption may not be correct however, since stroking also occurred with those females which did not resist and even with dead females, with which the male was copulating. Perhaps the most reasonable explanation is that stroking is an outward manifestation of sexual excitement in the male. Any other reason for it is secondary, since males mate almost at a normal rate within a few days after both antennae and front tarsae have been clipped. Furthermore, these organs do not seem necessary for the male to maintain his balance. The fact that the insects mated even with these receptor bearing organs excised also supports the hypothesis that vision rather than a pheromone is the stimulus for mating.

When a pair was in copula, the female seemed to go about her normal daily activities. Of course she could not oviposit, but she often fed voraciously and moved about the container on and off the plants at will. Not infrequently she was observed walking upside down on the top of the plastic petri dish, with the male still maintaining his position. Mating seemed to take place equally well on either the plants or the surface of the dishes. There was, however, some evidence

that the presence of alfalfa served to stimulate mating (see section V. C.), and certainly the female weevil was decidedly more content in the presence of alfalfa as shown by the fact that she moved about less and spent a considerable portion of her time feeding and laying eggs. When the alfalfa was removed, the female became extremely restless.

Pairs of alfalfa weevils generally remained mounted for long periods of time. Sometimes during these extended periods, the male would never actually copulate, but just remain in position, often with the aedeagus extended but not inserted. At other times, as illustrated by Figure 7, the aedeagus was not even extended, and there were no overt signs of sexual stimulation other than the male being in the mounted position. It is important to note that duration of mounting does not necessarily denote time spent in actual copulation. The number of mountings, like duration, varied considerably. Some weevils were observed to remain in copula for 12 hours. Humphries (1967) stated that fleas sometimes copulated for up to nine hours, and Federov (1927) observed that Anacridium aegyptium (Acrididae) copulated from 18 to 60 hours. In Sogata orizicola (Homoptera: Delphacidae), however, the average mating was about three seconds (McMillian, 1963). Mayer and Brazzel (1963) found that boll weevils copulated from 15 to 30 minutes.

At certain times, a copulating pair of weevils were very difficult to separate. Often at the end of an experiment, it was almost impossible to separate mating individuals without injuring them. However, it was soon discovered that they could be separated rather easily within a minute or two by placing a ball of cotton saturated with water up to their genitalia. A flash of light from an electronic



Figure 7. Pair of alfalfa weevils mounted but not copulating.

flash often had the same effect. When a copulating pair was disturbed by one of these two methods, the male exhibited an unusual type of behavior. His front legs were withdrawn from the prothoracic notch and the front portion of his body was raised. This activity, displayed in Figure 8, was a definite indication that the male would soon leave the female.

The next step was the partial or complete release of the female by the second pair of legs, the pair with which the male held on so tenaciously. With this partial release, the male often appeared as if sitting up on the back of the female. In a complete release involving this pair of legs, the male was sometimes suddenly knocked backwards by the forward movement of the female which, under these stress conditions, was generally moving about. Consequently the male was dragged along on his back with the aedeagus still inserted. This activity apparently did not permanently harm either individual, for in nearly all cases they mated again that day or certainly by the following day.

Under normal circumstances, the usual procedure involved in dismounting was for the male to back up slightly to disengage the genital armature, and then to move forward again, on one side or the other of the female. This process is similar to that described for the hen flea (Humphries, 1967). Following this event, the released female usually ran rapidly away. The male, on the other hand, remained stationary and lifted his abdomen, sometimes at an angle exceeding 45° . Usually, a portion of the aedeagus was visible at these times, and on a few occasions, a white, viscous substance was seen at the end of the aedeagus, as shown in Figure 9. It is probable that this behavior



Figure 8. Disturbed male alfalfa weevil in the process of dismounting the female. Note the raised front legs of the male.



Figure 9. Male alfalfa weevil elevating tip of abdomen, presumably to facilitate retraction of the aedeagus after copulation. White substance at the end of the aedeagus might be sperm.

facilitated retraction of the aedeagus and that the substance was sperm, perhaps indicating an incomplete or lack of sperm transfer. This observation raises an important question as to what actually occurs in the process of sperm transfer in these insects which mate repeatedly for such long durations over long periods of time.

Of any given sample of alfalfa weevils, about 70% can be expected to mate. The remainder show no mating behavior whatsoever, and it appears that they may never mate. The reason these insects do not mate has not been determined. However some conjectures may be offered to explain why heavy mating among pairs of insects may suddenly stop. Either an injury to one or both of the insects, or imminent mortality after all the eggs have been laid, appears to be the answer. In overwintering adults which were collected in April, both mating activity and oviposition were heavy until about the first week in June, when both greatly declined. The amount of food ingested was also drastically reduced at this time. Although a few of these insects remained alive until the following November, no mating behavior or oviposition was observed throughout the summer and fall. Their food intake remained extremely low and their movement slow. The effects of aging appear to be the cause for this physiological decline. However, there is a possibility that this decrease in activity may be due to a second diapause.

Some other aspects of mating behavior were seen among mature insects at imminent or actual death of one of the sex partners. If the male were about to die, he would of course not initiate mating,

and the female was not observed to influence this in any way. On the other hand, a moribund female, lying on her back and barely moving, was often adeptly manipulated by the male into mating position whereby he succeeded in copulating with her. Not infrequently males did the same thing with dead females (Figure 10). These matings continued for up to two days after the female had died. During this time the male mated repeatedly, and went through the same behavioral patterns as when copulating with living females. After a brief manipulation, he would grasp the female in normal copulating position, initially stroking the prothorax of the dead weevil and simultaneously inserting the aedeagus. The longest observed, continuous period of copulation involving a dead female was $6\frac{1}{2}$ hours, at the end of which the male released her for 15 minutes and then copulated again. On the following day, the male continued to copulate with this dead female. In a few cases, the males spent considerable time clasping the dead females without actually inserting the aedeagus.

A particularly interesting example of a male mating with a dead female is presented here. The female of a pair of alfalfa weevils which had been mating heavily suddenly died. Shortly after her death, the male grasped the female, maneuvered her into mating position, and while resting on his back copulated with her. This behavior continued with occasional interruptions for two days, with the male resting on his back or his side, copulating with the female for long periods of time. By the end of the first day, because of the excessive handling by the male, portions of the female's legs were broken off. By the middle of the second day the entire head and prothorax had been com-



Figure 10. Mounted male alfalfa weevil after copulating with dead female.

pletely separated from the abdomen. Still the male continued to copulate with the abdomen of the female. Figure 11 shows the pair just seconds after he released the female. When a live female was supplied this male, he soon began to mate with her. This example should serve to illustrate just how strong the mating drive of a male alfalfa weevil can be. Humphries (1967) noted that male fleas attempted to copulate with dead females, and Provost and Haeger (1967) indicated that male crab-hole mosquitoes tried to mate with cast pupal skins.

While the preceding description, which was based on observations of individual pairs of alfalfa weevils in petri dishes, indicate extremely aggressive mating behavior, this may not always be true. In a large simulated alfalfa field habitat in the laboratory, where both male and female insects were under more dispersed conditions, the males did not appear to be quite as aggressive in their mating behavior. They spent a lot of time moving about, and if they encountered another weevil, they usually attempted to mate. The weevil which they mounted was sometimes a female, sometimes a male. Apparently they had some difficulty distinguishing the sexes. The point is that they often approached the other insect at a slower pace than was the case when they were in the petri dishes. Their attempts also appeared less determined, for if the other insect resisted or attempted to run away, the male would quite often not follow. The reason for this behavior is not entirely clear, but it seems that mating activity in petri dishes may be a measure of potential mating behavior, rather than an indication of what occurs under field conditions.

Although mating behavior under dispersed conditions appeared



Figure 11. Male alfalfa weevil directly after copulating with the posterior portion of female whose head and prothorax have been broken off.

considerably less intense than in petri dishes, it was noticed that mounted weevils stayed in copula for long periods of time. Quite often the male remained mounted for varying lengths of time between copulations. According to Alexander (1967) this kind of activity is also known among grasshoppers and crickets. Further, it seemed that when the pair was not mounted, they remained very close together, often side by side on the same plant. Under low population densities, this sort of activity may insure a sufficient number of matings of adequate duration to supply the female with a complement of sperm for the entire breeding season (see section IV. G.). Newton (1933) reported that females separated from males for periods as long as 81 days still continued to produce viable eggs. Such a mechanism would assure survival under low density conditions, where the chances of encountering a mate by visual stimuli would be greatly reduced. This apparent adaptation is evidence against a pheromone. When weevils were collected and brought to the laboratory, they exhibited very aggressive mating behavior, indicating that males and females may often be out of contact in the field.

Although some aspects of alfalfa weevil mating behavior can be adequately explained on the basis of current knowledge, there remain others about which little is known. One of these which was occasionally observed was the behavior in which males, their antennae held erect, approached other weevils, both males and females, and pushed the snout under the posterior end of the abdomen. A modification of this activity was seen, when they shoved the snout right up against a mounted pair of insects. The males performed this action as if testing for some

substance; however what substance if any they might have detected by doing this is not known. It would seem that such behavior might represent a way of distinguishing the sexes, or possibly indicate an olfactory sex stimulant which is produced by both sexes but which acts only on the males. Tschinkel et al. (1967) have reported such a pheromone in the mealworm beetle, Tenebrio molitor.

Another puzzling activity was also seen in male alfalfa weevils. On a very few occasions the male while mounted has been observed to spread the elytra horizontally so that they are at right angles with his body. It would seem that this would be an apparent attempt at flight, but the membranous underwings were never once extended or even unfolded. Also for a beetle of this sort to attempt flight while in copula is not easy to comprehend. Thus as yet no plausible explanation for such activity has been found.

Finally, on numerous occasions throughout the study, a male alfalfa weevil pounced upon a female, rapidly shuffled backwards and forwards, and suddenly left the female without inserting the aedeagus, which may or may not have been extended during this process. The male often repeated this activity several times with many other weevils. The duration of each encounter was seldom more than a minute. This behavior seemed to occur when the males were very anxious to mate and the females not immediately receptive. However, not all of the females mounted would be unreceptive. The reason for not achieving copula then appears to lie for the most part with the male. This behavior was also observed under conditions of high temperature, where the general activities of the insects were greatly accelerated. Seemingly

the male just did not remain in one position or place long enough to consummate a mating. Nevertheless, there may be considerably more involved to this behavioral pattern than is indicated by this simple explanation.

E. Summary and Conclusions

The mating activities described for the alfalfa weevil seem to be an integral part of their normal mating behavior. The apparent function of some of these behavioral patterns in those cases where it could be determined or even hypothesized was discussed when the specific activity was mentioned. In other cases it was more difficult even to express an opinion as to what the actual mechanism was and further what purpose if any the activity truly served. Such is the way with the behavior of any organism. Konrad Lorenz (1957) has aptly stated that the most significant thing about animal behavior is that it is not as simple as was once supposed.

Baerends (1959) listed several functions for the often elaborate behavioral patterns introducing copulation: (1) selection of a partner of the right species, sex, and physiological condition, (2) stimulation of the partners, (3) inhibition of such motivations as aggression or flight, (4) attraction and direction of a partner, and (5) discouraging competitors.

According to Alexander (1967), the usual pattern of events in sexual behavior are (1) pair formation, (2) courtship, (3) copulation or insemination, and (4) sometimes pair-maintenance and further inseminations. Of these, pair formation and copulation are the minimum

requirements for perpetuation of the species in many insects. Courtship and pair-maintenance which commonly play important roles in the successful mating of many insects are not essential requirements and may be omitted in some species.

Based on the described observations of the mating behavior of the alfalfa weevil, it seems apparent that while pair formation, copulation, and pair-maintenance are readily evident and probably play vital roles, the presence of courtship behavior depends on the definition ascribed to it. If courtship behavior is considered to be specific mating activity occurring after pair formation but before mounting, then such behavior is not evident in the alfalfa weevil where the male simply approaches the female and attempts to mount. However if courtship is considered as continuing after the male has mounted and up to the actual time of insemination, then the activity in which the male strokes the female's prothorax with his antennae would definitely be a part of courtship behavior as well as play some role during copulation and pair-maintenance. In fact, as mentioned previously, if the female struggles, this stroking may be initiated at any stage of the mating process. Based on this last definition, which is supported by tenets 2 and 3 of Baerends (1959), male alfalfa weevils do indeed engage in courtship.

The mating behavior of Hypera postica consists of certain patterns and activities that seem peculiar and hard to understand, but this, as seen from examples cited in the literature review, is not unusual among the insects. In this section, my goal has not been to attribute

prescribed meanings to every mating activity but instead to explain in sufficient detail how reproductive behavior in the alfalfa weevil is accomplished.

IV. QUANTITATIVE ASPECTS OF MATING BEHAVIOR

A. Introduction

In this section an attempt has been made to treat quantitatively certain vital aspects of alfalfa weevil mating behavior. The work was carried out in the form of experiments specifically designed to yield information on certain comparisons. Each of these experiments is presented with its materials and methods placed next to its results and discussion to make the presentation more clear, concise, and meaningful.

The experiments reported in this section are as follows:

1. a comparison in a given sample of the number of pairs of postdiapausing and nondiapausing weevils exhibiting mating behavior.
2. a comparison under conditions of both light and dark of the percentage of time spent in mating behavior and the average number and duration of mountings by individual pairs of postdiapausing and nondiapausing weevils that were separated or non-separated during the 12 hours when not under observation.
3. a comparison of the percentage of time mounted and in copula in postdiapausing and nondiapausing weevils.
4. a comparison of the mating behavior of non virgin, male alfalfa weevils when given a choice between mating with virgin and non virgin females.
5. a comparison of the percentage of time spent in mating behavior, the number of mountings, and the decrease in mating behavior in sep-

arated and non-separated pairs of weevils over a 30-day period.

6. a comparison of the percentage of time spent in mating behavior and the number and duration of mountings in those pairs of insects exposed to large surface area (gallon cartons) as opposed to those maintained under conditions with less surface area (petri dishes).

7. a comparison of the percentage of time mounted and in copula in pairs of weevils maintained under various conditions of crowding by other weevils.

8. a comparison of the number of eggs laid by previously mated females kept individually in petri dishes with the number laid by females maintained in petri dishes with males, thus determining the effect of mating behavior on oviposition.

B. Percentage of Weevil Pairs of a Given Sample Exhibiting Mating Behavior

1. Materials and methods

The percentage of weevils that would mate was determined with postdiapausing and nondiapausing adult weevils. The insects were maintained under the conditions described in the section on general materials and methods. Petri dishes containing alfalfa and a wet cotton ball were used as a cage for each pair of weevils. Every 15 minutes over a 12-hour period, it was noted whether the pairs were exhibiting mating behavior. They were exposed to light for this period. When a pair was mounted, they were considered as displaying mating

behavior. Because more experiments involved nondiapausing than postdiapausing insects, the replicates were uneven and thus could not be statistically analyzed.

2. Results and discussion

Data presented in Table I indicate that there was little or no difference in the percentage of postdiapausing and nondiapausing alfalfa weevils which initiated mating behavior. Thus, this aspect of their mating behavior was quite similar.

C. Percentage of Time Spent in Mating Behavior, and the Number and Average Duration of Mountings

1. Materials and methods

This portion of the investigation was an attempt to compare the percentage of time spent in mating behavior as well as the number of mountings and average duration of mounting. Postdiapausing and nondiapausing alfalfa weevils were observed under conditions of both light and dark and under conditions in which the pairs were separated for 12 of each 24 hour period as opposed to those which remained together continually for several days.

Each pair of alfalfa weevils was placed in a petri dish with a sprig of alfalfa and a moistened cotton ball. All observations on mating behavior were taken at 15 minute intervals for 12 hours daily. The number of observation periods per daily maximum of 48 in which a pair was exhibiting mating behavior is expressed as a percentage of time spent in mating behavior (Table II). From the number of periods

Table I. Number and percentage of alfalfa weevil pairs exhibiting mating behavior.

	Total no. weevil pairs	No. pairs exhibiting mating behavior	%
Postdiapausing	90	68	75.56
Nondiapausing	145	105	72.41

in which a pair was mounted, the number and average duration per mounting per day was also easily obtained.

When observations on mating behavior were made under lighted conditions for 12 hours, the insects were placed in the dark for the 12 hour period when not under observation. In those cases where it was necessary to keep the pairs under essentially dark conditions during the 12 hour period in which observations were made, they then received 12 hours of light when not under observation, so as to maintain a uniform photoperiod in the two groups.

Several methods were utilized to make observations on those that were held in the dark during the 12 hour testing period. An infrared sniper scope was employed, but this method proved unsuccessful because it did not furnish enough illumination. Also the employment of the infrared scope was time consuming and the effect of infrared on the behavior of this insect was not known. Two alternative methods were used.

The first was the use of a light-tight box. The dishes containing the insects were placed in the bottom of the box. At 15-minute intervals, the box was opened in a light of approximately five foot-candles intensity for about one minute - the time necessary to observe the twenty pairs within the box.

In another technique, each petri dish was wrapped in aluminum foil. The dishes were then placed in a gallon food carton painted black, the top of which was covered with aluminum foil and masonite. Every 15 minutes each dish was opened in reduced light of about five foot-candles for a period of two seconds, to allow observations on mating behavior.

This second method was designed as a check on the first, and since no differences in mating behavior were seen with either method, results from both were combined.

Data presented in Tables II and III are based on the same experiments. The data have been compiled from different experiments conducted at different times. Since different numbers of insects were used in the several experiments, observed for different periods, it was not possible to analyze the results statistically. However, it is believed that by drawing data from these different portions of the investigation in which there was considerable replication that a meaningful picture of alfalfa weevil mating behavior is presented.

2. Results and discussion

Figures in Table II, calculated on the basis of only those weevils initiating mating behavior, indicate that in the light, the average percentages of time spent in mating behavior by nondiapausing and postdiapausing adults were remarkably similar. The same may be said for the field collected weevils held under conditions of light and dark. When the insects were not separated, but rather remained together continuously, there was as expected a decrease in the mating behavior of both the nondiapausing and the postdiapausing.

Data in Table III indicate certain basic differences in the mating behavior of the nondiapausing and postdiapausing weevils. When the nondiapausing insects were not separated, the average number of mountings increased while the average duration per mounting decreased markedly. In the non-separated postdiapausing insects, however, the

Table II. Average percentage of time spent in mating behavior per pair of alfalfa weevils per daily 12-hour period, calculated using only those pairs which mated.

	<u>Nondiapausing</u>		<u>Postdiapausing</u>	
	Light	Dark	Light	Dark
Separated	57.98	55.19	59.62	55.19
Non-separated	35.69	43.20	44.69	43.20

Table III. Average number and duration (minutes) of mountings per pair of alfalfa weevils per daily 12-hour period, calculated using only those pairs which mated.

	<u>Light</u>				<u>Dark</u>	
	<u>Nondiapausing</u>		<u>Postdiapausing</u>		<u>Postdiapausing</u>	
	<u>No.</u>	<u>Dur.</u>	<u>No.</u>	<u>Dur.</u>	<u>No.</u>	<u>Dur.</u>
Separated	2.51	166	4.11	104	3.91	102
Non-separated	3.17	81	3.46	93	3.01	103

average number of mountings decreased while the average duration changed very little.

Another difference between nondiapausing and postdiapausing weevils is that in the groups which were separated at night, the younger, laboratory reared insects mounted fewer times for a longer duration while the older, field collected ones mounted more often but for shorter periods of time. With the postdiapausing weevils, there was little difference in the number or duration of mountings in either the light or the dark.

D. Percentage of Time Mounted and in Copula

1. Materials and methods

In this experiment, 20 pairs of field collected, postdiapausing and 28 pairs of laboratory reared, nondiapausing alfalfa weevils were checked for one day under lighted conditions. The methods of maintenance and observation were like those described in the general materials and methods. At each observation it was noted whether a pair was mounted and if the male actually had the aedeagus inserted. Percentage of mounted time in copula were determined.

2. Results and discussion

There was no significant difference between postdiapausing and nondiapausing weevils as to time in mounted position (Table IV). This evidence supports the data in Table II.

Postdiapausing and nondiapausing weevils, however, differed con-

Table IV. Average percentage of time spent mounted and in copula per pair of alfalfa weevils per daily 12-hour period, calculated using only those pairs which mated.

	Mounted	In copula	% mounted time in copula
Postdiapausing	59.69	43.96	73.65
Nondiapausing	60.64	28.42	46.87

siderably in percentages of time in actual copulation (Table IV) (analysis of variance, $P = .05$). The true meaning of this difference may be more easily recognized when seen as the percentage of mounted time in copula. From these data it is clear that the nondiapausing males spent over half of their mounted time merely riding the females without actually copulating. The reason for such behavior probably involved reluctance to mate on the part of the young, nondiapausing female weevils which sometimes prevented the male from inserting the aedeagus.

In the postdiapausing insects, a greater portion of the mounted time was spent in actual copulation. As reported earlier in section III. D. 2. field collected females were seldom observed to successfully prevent the male from achieving copulation. Thus there is some indication here that prior mating experience increased the propensity to mate in the female weevil.

E. Male Mating Preference for Previously Mated Females Over Virgin Females

1. Materials and methods

An experiment was designed to give previously mated, adult males a choice of mating with either virgin or previously mated nondiapausing females. Only laboratory reared weevils were used. One male weevil and two females, one previously mated, the other virgin, were placed in each of 35 petri dishes. For three days, observations were taken at 15 minute intervals for 12 hours daily under lighted conditions.

At night the sexes were separated. Virgin and non-virgin females were distinguished by dusting one of them with Sun-Glo, orange fluorescent powder. To obviate the effect of marking the females, virgins were marked for the first two days and only non-virgins on the third. Whenever a virgin mated, she was removed and replaced by another virgin. For the entire three day period, the same males were used as well as the same previously mated females. All of the insects, both virgin and non-virgin, were the same age and had been maintained under identical conditions of food, light, and temperature. All percentages presented in Table V were determined on the basis of the total sample of weevils.

2. Results and discussion

Male alfalfa weevils mated with many more previously mated females than with virgins (Table V). Analysis of variance showed that virgin and non-virgin females differed significantly ($P = .01$) in time spent in mating and number of times mounted. The main reason for the differences is that virgin females, when first approached by males, often prevented them from inserting the aedeagus. The most common method of repelling the male was lifting the abdomen up under the elytra to conceal the genital opening. If a male mounted such a female and did not achieve copulation within a short time, he would usually then mount the non-virgin and immediately copulate.

Although repulsion of males by virgin females was a major influence, there were doubtless others which also came into play. For example, virgin weevils were more secretive than non-virgins. For the

Table V. Male alfalfa weevil preference for previously mated females over virgin females.

	Avg. % time spent in mating behavior	Avg. no. ♀♀ mounted	Avg. no. of mountings	Avg. duration/ mounting (min.)
Previously mated ♀♀	39.86	21.33	40.33	224.13
Virgin ♀♀	4.70	3.33	3.33	355.50

most part they stayed within the alfalfa foliage and moved little. Previously mated females, on the other hand, roamed the container, and this movement seemed to attract males. Regardless of how much or how little the non-virgin females moved, however, the males seemed more attracted to them.

The behavior just described indicates that prior mating experience in the female increases the likelihood that she will mate again. This hypothesis is supported by the observation that adult female weevils from the field, seldom prevented the male from copulating, presumably because of prior conditioning to mating. Further evidence involves the fact that on the first day of this experiment, only one male persisted in remaining mounted on a virgin female. This male remained mounted for 98 percent of the 12-hour observation period without once being observed with the aedeagus inserted, though during most of this time it was extended. On the second day, four males remained mounted on virgins, and not one of these males was observed to actually copulate. On the third day, however, five females were mounted. Of these five virgins, only the two which had been mounted the previous day allowed the males to insert the aedeagus. This information further indicates that males mounted on young, nondiapausing females spend less than half of their mounted time in copula (Table IV).

Even though most of the males that mounted virgins were unable to copulate, they nevertheless remained mounted longer than males which mounted previously mated females. Although the reason for such behavior is not clear, data presented here and in Table III indicate that in laboratory reared, nondiapausing alfalfa weevils, mountings are of

extremely long duration.

F. A 30-Day Comparison of Mating Behavior in Separated and Non-separated Weevils

1. Materials and methods

To obtain a clear comparison between the mating behavior of separated and non-separated, laboratory reared, alfalfa weevils, a 30-day experiment was conducted. Two groups of 20 pairs each were placed in individual petri dishes. In one group, males and females were separated for 12 hours each day. Observations on mating behavior were made every 15 minutes for 12 hours daily under lighted conditions. This time was long enough to accurately determine any change in mating behavior with time. Data in Figure 12 were calculated on the basis of the total sample of 20 pairs in each group.

2. Results and discussion

In the separated weevils, average percentage of time spent in mating behavior actually increased during the first two weeks before gradually declining, whereas in the non-separated pairs, mating decreased after the first week (Figure 12, Table VI). An analysis of variance of the frequency of 15-minute periods occupied by mating behavior was made for each group of insects to test the variability among the days on which observations were made. The results of this test indicate that the days were different at the .01 level of significance in the separated insects, but not significantly different in the non-separated group. Consequently Duncan's Multiple Range Test could be run only

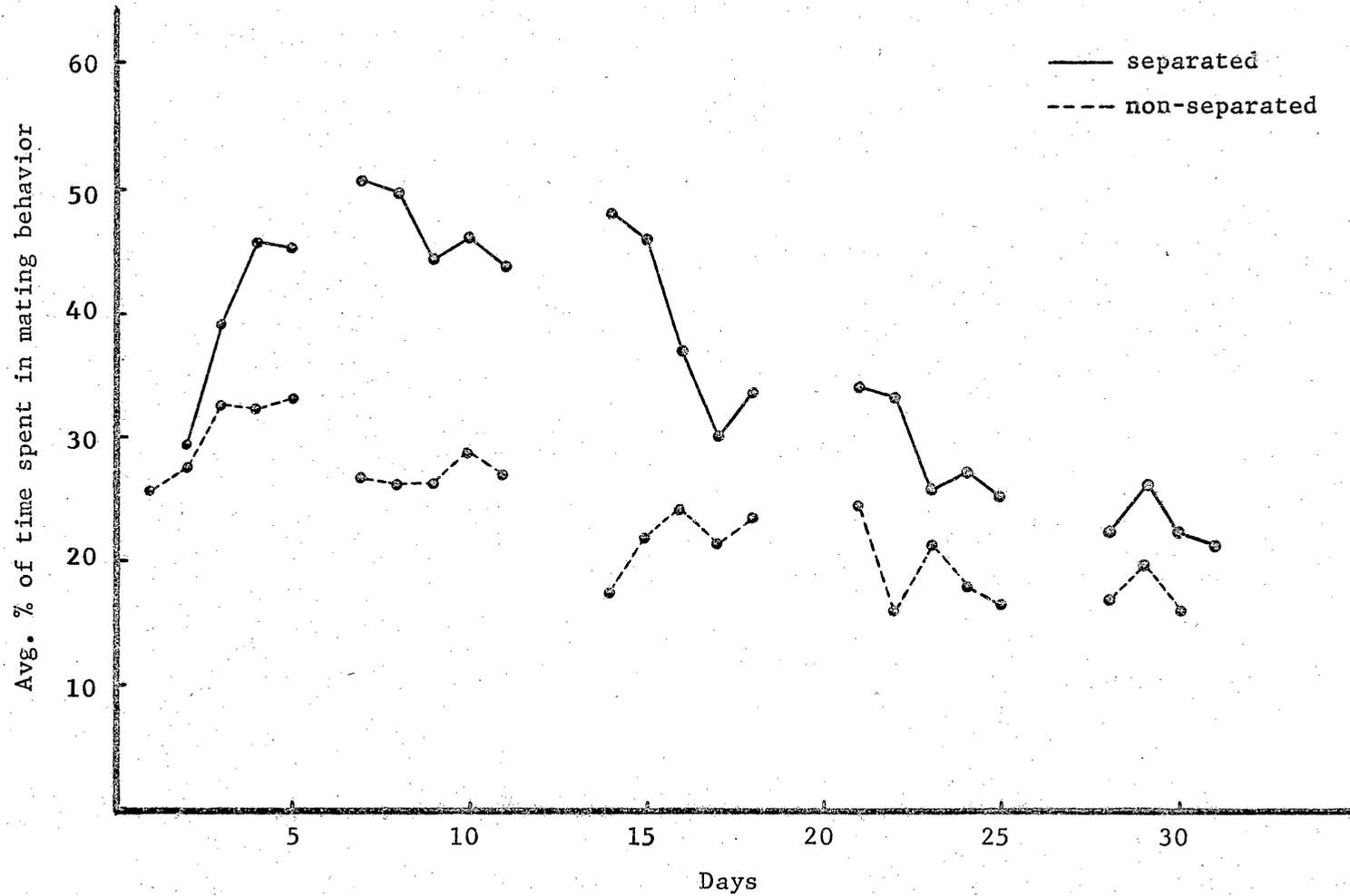


Figure 12. A comparison of mating behavior in separated and non-separated nondiapausing alfalfa weevils over a period of 30 days.

Table VI. Average percentage of time spent in mating behavior per pair of alfalfa weevils per daily 12-hour period, based on 23 days of observations over a 30-day period.

Separated			Non-separated	
	Mean (%)	Stat. sig. ^{1/}	Mean (%)	Stat. sig.
Day 1	29.90	c d e f	Day 1	25.62
2	39.38	a b c d	2	28.35
3	45.52	a b c	3	33.24
4	45.10	a b c d	4	32.60
Wk. mean	39.98		5	33.66
			Wk. mean	30.69
6	50.82	a		
7	49.90	a	7	27.20
8	44.38	a b c d	8	26.15
9	46.15	a b c	9	26.24
10	44.28	a b c d	10	28.96
Wk. mean	47.11		11	27.40
			Wk. mean	27.19
13	47.50	a b		
14	45.74	a b c	14	17.92
15	36.88	a b c d e	15	22.61
16	30.00	d e f	16	24.27
17	33.75	b c d e f	17	21.57
Wk. mean	38.77		18	23.03
			Wk. mean	21.88
20	34.06	b c d e f		
21	33.34	b c d e f	21	23.96
22	25.73	e f	22	16.68
23	26.98	e f	23	21.57
24	25.20	e f	24	18.76
Wk. mean	29.06		25	17.29
			Wk. mean	19.65
27	22.70	f		
28	25.94	e f	28	17.70
29	22.71	f	29	20.74
30	21.36	f	30	16.88
Wk. mean	23.18		Wk. mean	18.44

^{1/}

Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

for the separated insects (Table VI). The weekly means in the table are a convenient way of indicating decrease in mating as the experiment progressed.

An analysis of variance on the frequency of 15-minute periods in mating behavior by the separated and non-separated weevils indicated that the two groups were highly significantly different ($P = .01$), and that the separated insects spent more time in mating behavior. This is also clearly illustrated by Figure 12 and further supports data presented in Table II.

Within the separated and non-separated groups, the daily number of mountings were different at the .01 level of significance. Results of Duncan's Multiple Range Test on these data is given in Table VII. The weekly means again provide a convenient way of depicting the change in the number of mountings over a period of time. In both groups there was an increase until the end of the second week and a decrease thereafter.

The difference in the number of mountings per pair between the separated and non-separated groups was significant at the .01 level. The non-separated weevils mounted more times than the separated ones.

G. Crowding: Influence of Surface Area on Mating Behavior

1. Materials and methods

Petri dishes and gallon paper cartons were used to determine the effect of surface area on the mating behavior of field collected alfalfa weevils. The petri dishes contained alfalfa and a wetted ball of cotton.

Table VII. Average number of mountings per pair of alfalfa weevils per daily 12-hour period, based on 23 days of observations over a 30-day period.

Separated			Non-separated		
	Mean (No)	Stat. sig. ^{1/}		Mean (No)	Stat. sig.
Day 1	1.45	c d e f g	Day 1	1.05	e
2	1.50	b c d e f g	2	2.00	a b c d e
3	2.05	a b c	3	1.80	b c d e
4	2.55	a b	4	2.50	a b c d
Wk. mean	1.89		5	3.35	a
			Wk. mean	2.14	
6	1.75	a b c d e	7	2.85	a b c
7	1.85	a b c d e f	8	2.85	a b c
8	2.25	a b c d	9	3.15	a b
9	2.65	a	10	3.25	a
10	2.10	a b c d	11	2.65	a b c
Wk. mean	2.12		Wk. mean	2.95	
13	1.40	c d e f g	14	2.00	a b c d e
14	1.85	a b c d e f	15	2.10	a b c d e
15	2.20	a b c d e	16	2.40	a b c d
16	2.15	a b c d e f	17	2.85	a b c
17	1.35	c d e f g	18	1.65	b c d e
Wk. mean	1.79		Wk. mean	2.20	
20	1.40	c d e f g	21	2.05	a b c d e
21	1.70	a b c d e f g	22	2.15	a b c d e
22	1.15	d e f g	23	2.30	a b c d e
23	1.60	c d e f g	24	2.25	a b c d e
24	1.50	c d e f g	25	1.45	d e
Wk. mean	1.47		Wk. mean	2.04	
27	1.10	f g	28	1.65	c d e
28	1.00	g	29	2.30	a b c d e
29	1.20	e f g	30	1.90	b c d e
30	1.40	c d e f g	Wk. mean	1.95	
Wk. mean	1.18				

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

The gallon cartons each contained two 6 to 8 inch, densely foliated sprigs of alfalfa. The basal ends of the alfalfa protruded through a small hole in the center of the bottom of the carton into a jar of water. The sprigs leaned away from each other in opposite directions. Saran Wrap covered the top of the carton. A pair of weevils was placed in each container. As usual, observations were taken every 15 minutes for 12 hours per day under conditions of light. The same individuals were paired throughout the experiment. For the first three days, each group, consisting of 10 pairs of weevils, was confined to the same type of container, either petri dish or gallon carton. These insects were designated as Test 1. For the next three days, those which had been in the dishes were placed in the gallon cartons and vice versa. These were designated Test 2. The percentage of time in mating behavior and the number of mountings were determined on the basis of the total sample of 10 pairs (Tables VIII and IX). The average duration per mounting was based only on the pairs initiating mating behavior (Table X).

2. Results and discussion

In Test 1, the average percentage of time spent in mating behavior by the weevil pairs in petri dishes decreased significantly on the third day, but there was a significant gradual increase in mating for the pairs in gallon cartons (Table VIII). The trend in Test 2 is similar to the trend in Test 1.

The best explanation for such behavior is that these postdiapausing pairs were collected from the field where a considerable amount of mating may have taken place for an extended period. When placed

Table VIII. Average percentage of time spent in mating behavior per pair of alfalfa weevils per daily 12-hour period when tested under conditions of varying surface areas.

Day	Test 1				Test 2			
	Petri Dishes	Stat. Sig. ^{1/}	Gallon Cartons	Stat. Sig.	Petri Dishes	Stat. Sig.	Gallon Cartons	Stat. Sig.
1	56.66	a	24.18	b	46.26	-	12.09	b
2	60.83	a	31.87	a b	41.25	-	40.01	a
3	38.55	b	39.79	a	30.85	-	43.12	a

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

together in the petri dish, they were unable to continue mating heavily for all three days, thus a decrease on the third day. The reason mating behavior increased for weevils tested in the cartons was probably because they did not encounter each other until late on the first day. However, it was noticed that once a pair had come together, they remained together throughout the remainder of the test. Not once after coming together were they observed to be separated by more than two centimeters. Titus (1910a) supported this observation by stating that males accompanied females during oviposition and repeated periods of mating and egg laying occurred. Titus (1910b) also said that male alfalfa weevils had the same habit as those of Phytonomus punctatus (= Hypera punctata) in that they followed the females around and mated a number of times.

On the first day of the experiment, the percentage of time spent in mating behavior by pairs in the gallon containers and the petri dishes were significantly different at the .05 level for Test 1 and at the .01 level for Test 2. On the second and third day for each test, there was no significant difference in the mating behavior between those in the dishes and those in the cartons.

Related to the observation that pairs in gallon cartons remained close together is the fact that they mounted fewer times for longer durations than those pairs in petri dishes. An analysis of variance indicated that for both Tests 1 and 2, the difference in the number of mountings was significant at the .01 level (Table IX). Although an analysis of variance was not run on the duration per mounting, it would seem that in both tests, pairs in the containers with the larger

Table IX. Average number of mountings per pair of alfalfa weevils per daily 12-hour period when tested under conditions of varying surface areas.

Day	Test 1				Test 2			
	Petri Dishes	Stat. Sig. ^{1/}	Gallon Cartons	Stat. Sig.	Petri Dishes	Stat. Sig.	Gallon Cartons	Stat. Sig.
1	2.70	-	1.10	b	2.30	-	0.50	b
2	3.60	-	0.70	a	3.20	-	1.60	a
3	2.40	-	2.10	b	2.20	-	2.10	a

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

surface mounted for longer durations (Table X).

It would indeed seem reasonable that under dispersed conditions in their natural habitat, pairs which had a tendency to remain close together and mate for longer durations could possibly enjoy a selective advantage over those which mated only once and then separated. This behavior could also point to a need for repeat matings of relatively long duration, although this has not yet been demonstrated.

H. Crowding: Influence of Other Weevils on Mating Behavior

1. Materials and methods

To elucidate the effect of crowding by other weevils on mating behavior, nondiapausing alfalfa weevils were tested in petri dishes. Observations were taken at 15-minute intervals for 12 hours daily under lighted conditions. The time spent mounted and the time with the aedeagus inserted were noted. On the first day of the test, 40 pairs of insects were tested in 40 petri dishes, one pair per dish. That night the pairs were separated. The next day the same weevils were utilized, and 20 pairs were placed in each of two petri dishes. The tops of the dishes were perforated for ventilization. After two days of separation, the same 40 pairs were placed in a single ventilated petri dish. All percentages presented in Table XI were calculated on the basis of the total sample of weevils.

2. Results and discussion

The percentage of time spent mounted would appear to indicate

Table X. Average duration (minutes) per mounting per pair of alfalfa weevils per daily 12-hour period when tested under conditions of varying surface areas.

Day	Test 1		Test 2	
	Petri Dishes	Gallon Cartons	Petri Dishes	Gallon Cartons
1	151.11	158.18	144.78	174.00
2	121.67	327.86	92.81	180.00
3	115.62	136.43	100.91	147.86

that crowding slightly stimulated mating behavior (Table XI). If this were true, the stimulation would probably be the result of males being attracted to mounted pairs and immediately mounting the females as soon as the mounted males released them. However, this is only a hypothesis, since these data were not found to be significantly different.

A Chi Square Test indicated that crowding reduced the time in actual copulation ($P = .01$). The probable reason is that under crowded conditions, males disturbed pairs of copulating weevils, thus markedly reducing the percentage of mounted time in copula. Under crowded conditions, males spent over 70% of their mounted time without the aedeagus inserted. Therefore it would seem that mating efficiency under conditions of extremely high population densities might be reduced appreciably. However, adequate sperm transfer probably occurs and reproduction is probably not reduced.

I. Effect of Mating Behavior on Oviposition

1. Materials and methods

An experiment was designed to test whether the extensive amount of time occupied by mating behavior could limit oviposition in the restricted area of a petri dish. Twenty pairs of field collected alfalfa weevils were placed individually into petri dishes with food and water. Previously mated female weevils were placed in another set of dishes. The experiment was conducted for 14 days. Every 24 hours the stems of alfalfa were changed and the eggs were counted.

Table XI. Average percentage of time spent mounted and in copula per pair of alfalfa weevils per daily 12-hour period when maintained under varying conditions of crowding by other weevils.

No. weevil pairs per petri dish	% of time mounted	% of time in copula	% mounted time in copula
1/dish	42.45	19.89	46.87
20/dish	43.49	10.16	23.35
40/dish	45.94	13.33	29.02

On the seventh through tenth day of the experiment, temperatures dropped below the normal $75 \pm 3^{\circ}$ F in the laboratory. This resulted in a reduced number of eggs during that period.

2. Results and discussion

Previously mated females maintained alone in individual petri dishes laid more eggs than the females which remained in dishes with males (Table XII). The number of females laying and the average daily number of eggs laid for each group were found to be significantly different at the .01 level. The average numbers of eggs per day per ovipositing female for the two groups of weevils were significantly different at the .05 level.

The probable explanation for this difference in oviposition is that the aggressive mating activities of the male in the restricted area of a petri dish occupied such a great portion of the female's time, that her available time for boring stems and egg laying was somewhat limited. Unpublished data on the effects of temperature on oviposition showed that in a favorable temperature range of $59 - 77^{\circ}$ F, lone females in a petri dish laid more eggs than those kept with males. Other supporting evidence is furnished by Table II, which indicates that non-separated, postdiapausing alfalfa weevils in petri dishes spent about 44% of their time in mating behavior.

Further evidence is found in Figure 12 which shows only a slight decrease in mating activity for the non-separated weevils for the first two weeks. It is doubtful that under field conditions the males would ever play such a role as results in decreased oviposition.

Table XII. A comparison of oviposition in lone, previously mated females and mating females maintained with males.

	Avg. no. of ovipositing ♀♀ per day	Avg. no. of eggs per group per day	Avg. no. eggs per ovipositing ♀ per day
Mating ♂♂ ♀♀	11.79	268.64	22.79
Previously mated ♀♀	14.79	411.93	27.86

Nevertheless, the findings herein presented may possibly have value in a laboratory rearing program. Assuming the eggs produced by the lone, previously mated females are viable (Newton, 1933), a greater output of eggs might be obtained by using fewer males or even omitting them altogether from the laying chambers.

J. Summary and Conclusions

Results of experiments on the quantitative aspects of alfalfa weevil mating behavior may be summarized as follows:

1. Little or no difference existed between the percentage of field collected, postdiapausing and laboratory reared, nondiapausing insects that initiated mating behavior.

2. Postdiapausing and nondiapausing weevils, under lighted conditions, spent about the same percentage of time in mating behavior. In both groups the separated weevils spent a greater percentage of time in mating behavior than the non-separated ones. With postdiapausing insects, there was little difference in the time spent in mating behavior under conditions of light and dark.

3. In the light, postdiapausing pairs mounted a greater number of times for shorter duration than nondiapausing weevils. Postdiapausing insects differed little in either number or duration of mountings in the light or the dark. Separated, nondiapausing weevils mounted fewer times for greater durations than the non-separated, nondiapausing weevils. In both the light and the dark, separated, postdiapausing pairs mounted more often than non-separated pairs, while the durations of mountings differed very little.

4. Postdiapausing pairs spent a considerably greater percentage of mounted time in copula than nondiapausing pairs.

5. Males, when given a choice, overwhelmingly mated more with non-virgin than with virgin females.

6. Over a period of 30 days, mating behavior decreased in both separated and non-separated nondiapausing weevils. Separated pairs spent a greater percentage of time in mating behavior, but the non-separated pairs mounted more often.

7. Once males and females maintained in containers of large surface area located each other, they spent as much time in mating behavior as those pairs confined to containers of lesser surface area. Pairs in large containers had a tendency to maintain close contact, and mount fewer times for longer duration than pairs in small containers.

8. The percentage of time spent mounted was little affected by conditions of crowding by other weevils, while the percentage of time in copula was greatly reduced.

9. Within a petri dish, aggressive mating behavior by males reduced the rate of oviposition in females.

V. STIMULUS FOR LOCATING AND MATING

A. Introduction

Throughout this investigation, the question of how male and female alfalfa weevils located each other for the purpose of mating, continually presented itself. The stimuli considered were sound, olfaction, and vision. Laboratory experiments were designed to test the relative importance of these factors. Mating behavior was checked in both the presence and absence of alfalfa to determine if the presence of the natural food plant served to promote mating. The probable importance of tactile behavior, once the sexes have been brought together by other means was conjectured, and the possibility of random contact and mating explored.

B. Literature Review

1. General

As aptly stated by Wallace and Srb (1961), it is generally taken for granted in the case of both plants and animals that not only does "like beget like" but also that "like mates with like." Seldom does it occur to us that things should be otherwise. According to Carthy (1965) every aspect of arthropod courtship behavior, which has been investigated, has shown complex mechanisms permitting only intraspecific mating.

It is usually considered that three prominent types of stimuli are involved in this selectivity. These may be sound production as

in the case of characteristic songs, chemical cues as exemplified by pheromones, or visual releasers such as stimuli caused by visually perceived motion. Carthy (1965, 1966) believed that these signals employed in locating and identifying a mate act as barriers to intra-specific mating and may in part be responsible for the great amount of speciation among the arthropods.

A review of the literature of the alfalfa weevil has furnished little insight into which of these mechanisms; sound, odor, or sight; might play the major role in the mating behavior of this beetle. Therefore, in an attempt to compare and correlate the results of experiments designed to determine the relative importance of these three stimuli in Hypera postica, with those found in other insects, a limited literature review was undertaken, and the findings are herein presented.

2. Sound

It has been known since antiquity that insects produce sounds, but only more recently has it been found that they can also perceive sounds. Today it is generally known that sound production and reception occurs widely in insects and that many vital aspects of their behavior are determined by sounds (Frings and Frings, 1958).

Sound in insects may be produced in two basic ways; either as a by-product of other movements, such as locomotion or chewing, or as a result of special movements solely for the purpose of sound making. Sounds in the first category probably have little or no behavioral significance, whereas those in the second most often do play an

important role in certain behavioral patterns (Carthy, 1965).

Carthy (1965) considered courtship, defense against possible predators, and social communication to be the most important aspects of behavior involving sound. Of these, sounds involving courtship and mating behavior have received the most attention and it is here that most emphasis will be placed in the following discussion.

Alexander (1967) recognized three types of acoustical communication concerned with reproductive behavior in insects. He considered these to be calling signals (pair-forming signals), courtship signals, and postcopulatory signals.

In the Orthoptera, sound production by stridulation, and sound reception were quite commonly found to play a vital role in pair formation. This type of signal calling was most often seen when a responsive female was readily attracted to the calling male. These responses have been clearly demonstrated in the families of Tettigoniidae (Duijm and Van Oyen, 1948) and Gryllidae (Alexander, 1960 and Walker, 1957). Similar responses have also been demonstrated in certain Hemiptera, Cicadidae (Alexander, 1958), and Corixidae (Leston, 1955). Finally, in the family Trypetidae (Diptera), sounds produced by the male's wings attracted females (Monro, 1953).

In other cases of calling signals, males were attracted to frequencies of sound produced by the wings of acoustical females. This type of behavior occurred in the following Diptera: Culicidae (Roth, 1948; Wishart and Riordan, 1959), Ceratopogonidae (Downes, 1955), and Chironomidae (Gibson, 1945). Alexander et al. (1963) believed that beetles

of the family Trogidae also exhibited such responses. There are still other insects in which males and females mutually responded to the other's sound - Acridinae (Haskell, 1958) and Tettigonidae (Allard, 1928; Fulton, 1933; Spooner, 1964) among the Orthoptera and in the Coleoptera the plum curculio, Conotrachelus nenuphar, (Mampe and Neunzig, 1966).

Courtship signals occur after pair formation and culminate in copulation or insemination (Alexander, 1967). They may have three possible levels of significance: 1. to serve as a reproductive isolating mechanism in cases where the possibility of accidental pair formation is great; 2. to be responsible for timing inseminations by informing individuals in a pair when both are sexually responsive; 3. to facilitate insemination by moving the pair into copulatory position. Incorporated in the above functions are such purposes as creating a sexually responsive state in the female or allowing a female to recognize a male of her own species.

Alexander (1967) recognized acoustical courtship signals in males of Acridinae and Oedipodinae (Orthoptera: Acrididae), Gryllidae, and Cicadidae. Hartman and Roth (1967a, 1967b) discovered courtship stridulation in the male cockroach, Nauphoeta cinerea. It seemed to occur in certain Hemiptera (Hungerford, 1924) and possibly in Drosophila (Waldron, 1964). Based on personal communications with Laura Berkeley, Alexander (1967) also indicated that courtship signals played a role in the mating activities of the Passalidae (Coleoptera).

According to Alexander (1967) the third major type of sound pro-

duction involved in mating behavior was that encountered when the pairs were copulating or after copulation. In some members of Acrididae (Haskell, 1961) the male stridulated during copulation, apparently because of the female's movements or because copulation was threatened by another individual. This type of behavior was also found in certain members of Gryllidae, its function being the maintenance of the pair until the female had been inseminated.

Both crickets and grasshoppers produced sounds which resembled aggressive stridulations whenever courtship was interrupted. These sounds most likely served to call back departing females, or were produced as an external manifestation of aggressiveness toward the intruding males which may have caused the interruption. In many crickets sound production occurred after each copulation or between copulations. The probable functions involved keeping the female with the male until he had produced another spermatophore or preventing the female from removing the spermatophore before the completion of insemination (Alexander, 1967).

3. Olfaction

The possibility of a pheromone in the alfalfa weevil has been explored, and the results of the experiments discussed later in this section. Although no evidence for a sex attractant was found in this weevil, it would still seem appropriate to briefly discuss insect pheromones, with particular reference to certain species of Coleoptera.

Currently insect pheromones comprise a very active subject of biological and chemical research. Jacobson (1965) listed nine orders in which they were known to occur: Orthoptera, Isoptera, Hemiptera, Mecoptera, Neuroptera, Coleoptera, Lepidoptera, Diptera, Hymenoptera. Lepidoptera, however, contained by far the most representatives possessing sex attractants, while other orders such as Coleoptera contained relatively few.

Within the Coleoptera, there were certain representatives in which the male was attracted to the pheromone of the female. Valentine (1931) found that in Tenebrio molitor, the yellow meal worm, live females when disturbed were capable of releasing a potent odor which was attractive to males. A yellow liquid produced in the female's abdomen was discovered to be the source of attractancy. Further evidence for a pheromone in this beetle was furnished by Tschinkel et al. (1967) who found that while both sexes produced the substance, only the males responded to it and that it appeared to serve more as a mating stimulant than an attractant. Travis (1939) observed that crushed females of the June Beetle, Phyllophaga lanceolata, lured males from as far as 30 to 40 feet. He further noticed that when females extruded their genitalia, males were attracted. Soo Hoo and Roberts (1965) reported that in Rhopaea magnicornis (Scarabaeidae), a pheromone from the abdomen of the female attracted males from 30 yards away. Pheromones are also known from other species of the genus, Rhopaea.

Certain female wireworms (Elateridae) were also found to possess

a pheromone. In Limonis californicus, the sugar-beet wireworm, it was discovered that newly emerged females were attractive to males as was 70% ethyl alcohol extracts of virgin females (Shirck, 1942). Supporting evidence was provided by Lilly (1959). Doane (1961) was the first to suggest a pheromone in Ctenicera destructor, while Chapman (1964) believed a sex attractant to be active in Hemicrepidius morio. Lilly and McGinnis (1965) demonstrated sex attractants in extracts of virgin females from four species, representing three genera of Elateridae. More recently Hayes and Wheeler (1968) presented observational evidence of a pheromone in females of another elaterid, Hemi-crepidius decoloratus.

Cuthbert and Reid (1964) found in females of the banded cucumber beetle, Diabrotica balteata (LeConte) a natural sex attractant which lured males from as far as 49 feet. Unmated females remained attractive for as long as 79 days, however after one mating most ceased to be attractive and none were attractive after two matings.

Evidence of sex pheromones in females of several species of Dermestidae has been reported by Burkholder and Dicke (1966). Those species found to produce attractants were: the black carpet beetle, Attagenus piceus; Trogoderma inclusum; and Trogoderma glabrum. The males of these species were attracted soon after the emergence of the females.

In most of the Coleoptera which exhibit a sex pheromone, it is the female which produces the sex attractant and the male which responds to it. Nevertheless there is one well known case where this

situation is reversed. In the boll weevil, Anthonomus grandis, the male secretes a pheromone to which the female responds. Keller et al. (1964) reported females traveling from 2 to 30 feet to plants in which males were situated. Females also responded to chloroform extracts of activated charcoal containing odors from male boll weevils, by exhibiting rapid walking and standing high on their front legs, with heads high and antennae held high and forward. Supporting evidence was provided by Cross and Mitchell (1966) who in a detailed field study of boll weevil mating behavior found that males did not respond to females over distances greater than one or two inches, while females often located males from as far as 30 feet. Hardee et al. (1967a, 1967b) further confirmed the fact that boll weevil females sought the pheromone-emitting males.

In certain Coleoptera, one sex may produce an attractant which under special conditions causes both sexes to assemble for mating. In some species of the genus Dendroctonus sexually mature, virgin females produced a substance, to which both males and females responded in flight (Rudinsky, 1963; Vite and Gara, 1962; Vite et al., 1964).

Another example of the female producing an attractant to which both sexes responded, occurred in the ambrosia beetle, Trypodendron lineatum, (Rudinsky and Daterman, 1964). Both fertilized and unmated females were capable of producing the volatile substance.

In contrast to the two previously described situations where the females were responsible for the assembling, it is the males in the genus Ips which possess a volatile substance in the hindgut, resulting in the attractancy of both males and females to this substance, and

to the frass. This activity has been recorded for a number of species in this genus (Vite and Gara, 1962; Vite et al., 1963; Vite et al., 1964; Wood and Vite, 1961).

4. Vision

There are many examples in entomological literature, regarding the role of vision in reproductive behavior. A limited number of these are herein recounted.

Burnette and Eisner (1964) claimed that since many insects have good vision, sex recognition, at least in diurnal forms, may be primarily on the basis of sight. Male dragonflies, Platycnemis pennipes, used color and pattern to identify the females (Buchholtz, 1956).

In some Orthoptera, vision may be the primary stimulus causing males to initiate mating behavior. Srivastava (1957) found that when a male tobacco grasshopper, Atractomorpha crenulata, sighted a female, he approached and then readily mounted. Ferkovich et al. (1967) indicated that in the big-headed grasshopper, Aulocara elliotti, males were attracted to the quick movements of other grasshoppers.

In Coleoptera, female fireflies of the genus Photinus flashed their lights in such a pattern as to attract males (Lloyd, 1966). Among the Lepidoptera are some of the better known examples of vision playing an important role in courtship. The mating activities for Eumenis (Satyrus) semele, the grayling butterfly, have been described in detail by Tinbergen et al. (1942) who discovered that the way in which a model simulating the female was moved through the air, determined the response of the male. Thus color and wing pattern were

relatively unimportant in this reaction.

In another butterfly, the silver washed fritillary, Argynnis paphia, the male responded to the color and pattern of the wings of the female. In fact they responded more readily to a supernormal stimulus, that is a model whose wings were totally orange-yellow in color rather than an actual female, the wings of which were essentially this color but mottled (Mangus, 1954).

With the aggressive males of the mosquito, Opifex fuscus, vision mediated the search for female pupae (Provost and Haeger, 1967). Newkirk (1963) presented data on the mating behavior of the robberfly, Leptogaster annulatus, which indicated that even under extremely reduced light conditions, the males responded visually to the females.

According to Tauber and Tauber (1967) the adult gall-formers, Aciurina ferruginea, employed their wings as visual cues for mating communication. The male thus stimulated, approached the female and mounted. Manning (1959) reported that in Drosophila melanogaster, the initial sexual attraction to a mate was visual, with the males approaching and touching with their forelegs any other insect of approximately the same size. In poor light, however, courtship was initiated when the insects blundered into one another. Miller (1966) presented evidence that at close range, males of Tetrastichus incertus (Hymenoptera: Eulophidae) were attracted visually to the females.

C. Alfalfa as a Stimulus for Mating Behavior

1. Materials and methods

An experiment to test whether the presence of alfalfa might serve as a stimulus to mating behavior was conducted. Twenty pairs of non-diapausing alfalfa weevils were used. Observations on mating were made every 15 minutes for 12-hour periods in the light; at night the pairs were separated. The insects were tested for two consecutive days with each of the following food plants: Trifolium pratense, red clover; Medicago sativa, alfalfa; Trifolium repens, ladino clover; and Melilotus alba, sweet clover. Finally, they were tested for two days in the absence of food. Water was provided in a soaked cotton ball in all tests. Since the insects which were observed in the absence of food had been fed alfalfa until the beginning of each daily test, it would seem that hunger would not have been a limiting factor.

There was a lapse of two days in which the pairs were separated between each two-day testing period. Because of these periods of separation, mating behavior should not have been reduced toward the end of the experiment. Earlier evidence (Figure 12) has shown that there is no reduction in mating behavior over a period of time equal to or greater than that required for this experiment as long as the pairs of weevils are separated for at least a portion of the time.

2. Results and discussion

More pairs initiated mating behavior and maintained it for a greater percentage of time in the presence of alfalfa (Table XIII),

Table XIII. Comparison of alfalfa weevil mating behavior in the presence of selected food plants.

Condition.	Food				
	Red Clover	Alfalfa	Ladino Clover	Sweet Clover	No food plant
% time in mating behavior, per pair, based on total sample of 20 pairs	27.92	46.93	34.95	37.88	23.08
% time in mating behavior, per pair, based on only those exhibiting mating behavior	42.95	64.73	55.92	58.27	48.58
Avg. no. pairs mating per day	13.00	14.50	12.50	13.00	9.50
Avg. duration per mounting	90.34	159.00	109.40	158.04	108.93

although an analysis of variance based on the total sample of 20 pairs indicated no significant difference. This apparent preference for alfalfa, however, could be expected, since it is their main host plant. The weevils also fed well on ladino clover and sweet clover. On red clover, feeding was moderately heavy on the leaves, but extremely light on the hirsute stems. In the absence of food, the insects seemed uneasy, and mating behavior was reduced. Hunger is not thought to have been a factor in this reduction of mating behavior.

D. Importance of Sound in Mating Behavior

1. Materials and methods

To test the possibility that sounds produced by alfalfa weevils may play a role in mating behavior, an experiment was set up in the sonic laboratory at Virginia Polytechnic Institute. A well-insulated, sound-proofed room provided the space for the actual testing and a B & K $\frac{1}{4}$ inch calibrated condenser microphone with a B & K microphone amplifier were utilized. Mr. Glenn Kranzler of the Department of Agricultural Engineering assisted with the testing. Field collected, non-virgin insects were placed into a petri dish. The tip of the microphone was placed through a small hole cut in the polyethylene covering the dish. Two pairs in the dish were copulating before testing began and throughout the test. Other males disturbed these mating pairs, and some mounting activity was also evident in other pairs of weevils. If sound were a factor in their mating behavior, it should have been produced at this time. No food or water was provided during

the 10 minutes of testing, and the temperature within the room was approximately 100° F.

2. Results and discussion

Alfalfa weevils in the petri dish produced no detectable sound other than background noise. Much of the background noise apparently emanated from one of the weevils which throughout the test continually attempted to fly. The background sound pressure level (SPL) was 61-67 decibels (re 2×10^{-4} μ bars). Thus unlike certain other curculionids, such as the plum curculio, Conotrachelus nenuphar (Mampe and Neunzig, 1966), sound probably does not play a role in the mating behavior of Hypera postica.

E. The Relative Importance of Olfaction and Vision in Mating Behavior

1. Test with simulated alfalfa field habitat

a. Materials and methods

Forty pairs of laboratory reared, virgin alfalfa weevils were used to test for a pheromone. One week after emergence in the growth chamber, they were stored at 3° C. After three weeks, they were sexed, separated, and maintained in the growth chamber at 75 ± 3 ° F with a photoperiod of 12 hours. Prior to the testing period, five pairs of weevils, which underwent the same treatment as those 40 pairs to be tested, were found to mate readily. Thus it would seem that if a pheromone existed, it would be evident at this time.

A cardboard box, 26 x 10½ x 13 inches, covered with polyethylene screening, served as a simulated field habitat. The box was filled to a depth of 1½ inches with wet sand. Six to 12 inch pieces of alfalfa were placed in the sand. A small cage constructed of the top of a pint food carton and polyethylene screening was situated about two inches from one end of the box. The center of the top of the food carton had been removed, leaving a cardboard ring. Fastened to this ring by paper clips was the polyethylene screening rolled into the form of a cylinder and pinched together at the top with staples. With the paper ring forced into the sand the height of the cage was about six inches, the diameter of the base 3½ inches.

The virgin female weevils were placed in the cage. Forty virgin males were released at the other end of the box. Hourly observations on the number of males crawling upon the cage were made for 12 hours each day on three consecutive days. On the next three days, the males were placed in the cage and the females outside the cage. On the first day of each three-day period, a revolving electric fan was positioned level with the top of the box and six feet from it. The fan blew air over the weevils in the cage toward the end of the box where weevils of the opposite sex were released (Figure 13).

b. Results and discussion

Neither sex responded positively to the opposite sex (Table XIV). Had there been a pheromone or any attractive odor emanating from the cage, it would seem that weevils of the opposite sex would have aggregated around or on the cage. This, they did not do. In fact the



Figure 13. Simulated alfalfa field habitat.

Table XIV. Alfalfa weevils attracted by olfaction to the opposite sex in a simulated alfalfa field habitat.

Hours of observation	Females in cage			Males in cage		
	No. of males attracted			No. of females attracted		
	Days			Days		
	1	2	3	1	2	3
1	0	0	0	0	0	0
2	0	1	0	0	1	0
3	0	0	0	0	0	0
4	1	2	0	2	0	0
5	0	0	0	1	1	0
6	0	0	1	0	0	0
7	0	0	2	0	0	0
8	0	0	1	0	0	0
9	1	0	0	1	0	0
10	2	0	0	0	0	0
11	0	0	1	0	0	0
12	0	0	0	0	0	0
Total	4	3	5	4	2	0

insects moving about freely in the box seemed oblivious to the presence of other insects in the cage. Perhaps this was because they could not visually perceive the weevils in the cage due to the polyethylene screening. The males roaming free in the box did seem attracted to other males, and on several occasions showed aggressive mating tendencies by mounting other males. Males seemed particularly attracted to any male moving within a few inches of them. Once it was observed that when a male which had been resting upon an alfalfa leaf was knocked to the sand, it was, upon trying to turn over, aggressively mounted by another male. The second male was $1\frac{1}{2}$ inches away, but upon perceiving the movement immediately turned at an angle of 90° and rapidly traversed the distance to the other male and attempted copulation. There can be little doubt that at short distances, visually perceived motion plays a relatively important role in alfalfa weevil mating behavior.

Released males were exceedingly active, while the females moved about considerably less and showed no mating activity when segregated. The males, on the other hand, seemed to employ a random trial and error method of mate selection. They mounted with equal vigor any male weevil that passed at a close range. Being unable to insert the aedeagus, they sometimes dismounted and moved on. At other times they remained mounted for long periods. Occasionally a male was noticed holding on to another male tenaciously, even though they were not in a normal mating position. Similar though slightly less aggressive mating behavior was seen under these more dispersed conditions than in the petri dishes.

The aggressive mating activity of the males points to the fact that they were physiologically ready to mate, but that no odor attracted them to the females. Visual attraction to the females was severely limited by the screening. Because the males responded visually to each other, it would appear that vision is more important than olfaction in alfalfa weevil mating behavior.

2. Test with circular olfactometer

a. Materials and methods

A circular olfactometer was designed to furnish information on the presence or absence of a pheromone in the alfalfa weevil. The sides of a gallon food container were cut down until only $1\frac{1}{2}$ inches remained as the inside depth. Around the circumference of this reduced carton were bored 12 equidistant holes. Into these holes were inserted $1\frac{1}{2}$ inch lengths of $\frac{1}{2}$ inch diameter vinyl tubing. About one half of each piece of tubing extended inside the carton. Between these lengths of tubing were inserted pieces of cardboard $1\frac{3}{4}$ inches long and $1\frac{1}{2}$ inches high. These divided the carton into 12 equal sections. In the center of the bottom of the carton was bored a small hole into which was placed a piece of $\frac{1}{4}$ inch diameter vinyl tubing. This was connected to a suction pump (Fisher Scientific, Neptune Dyna-Pump, Model 2) (Figure 14). When the top of the olfactometer was tightly covered with Saran Wrap and the air pump turned on, air was drawn through the lengths of tubing and out the opening in the bottom of the carton, the point at which its rate was determined as 500 feet per minute by a Model G,



Figure 14. Circular choice olfactometer with air pump.

Hastings Air-Meter with a Type D-7 probe.

Four field collected female alfalfa weevils were placed into each of four sections of the $\frac{1}{2}$ inch tubing. Another four sections each contained four males, while the remaining four each housed two pairs of copulating insects. The sections of tubing were alternated, so that no two sections containing the same combination of weevils were placed next to each other. Cheesecloth covered the ends of the tubing (Figure 15).

Five groups of 15 field collected weevils each were utilized. Each group was released three times within the olfactometer for 10 minute periods. The number of weevils in each section was recorded each minute as attracted to that section. Both male and female weevils were tested. For every reading, 15 insects were present and capable of responding; however, frequently many of them remained in the center of the olfactometer and not in the sections with the tubes.

b. Results and discussion

A pheromone serving as a sex attractant does not appear to exist in the alfalfa weevil (Table XV). The males responded to the tubes containing males more than to tubes containing females. Similarly, more released females were attracted to other females than to males. The cheesecloth covering the plastic tubing obscured the weevils inside from the insects released in the olfactometer. Thus any positive selection response had to be an olfactory one. Released males were very aggressive in mounting each other. This behavior indicated a physiological readiness to mate and also accentuated the importance

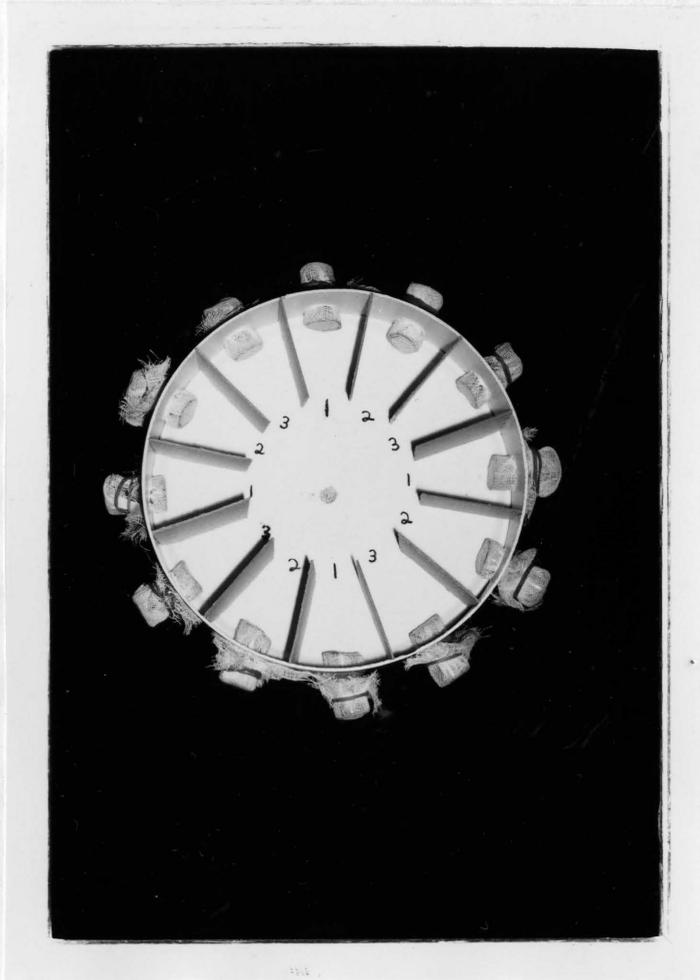


Figure 15. Circular choice olfactometer viewed from the top.

Table XV. Number of male and female alfalfa weevils attracted by olfaction to ♀♀, ♂♂ and ♀♀, and ♂♂ in a circular choice olfactometer.

	♀♀	♂♂ & ♀♀	♂♂
♂♂ responding	566	538	597
♀♀ responding	680	640	675

of vision in mating behavior.

Males are usually attracted to a copulating pair of weevils. However, Table XV indicates a lower response to pairs in copula than to either lone males or females. These data indicate that the attractancy of males to a mating pair is the result of a visual and not an olfactory stimulus. In conclusion, neither males nor females showed any olfactory preference to males, females, or copulating pairs (Table XV). These data then seem to refute the possibility of a pheromone acting as a sex attractant in the alfalfa weevil and imply that at close range, vision may be the primary stimulus bringing the sexes together for mating.

3. Test with moving air olfactometer

a. Materials and methods

A modified suction type of olfactometer designed by Mr. R. N. Waghray was employed to further test for a pheromone in the alfalfa weevil. The apparatus was enclosed in a temperature controlled chamber fitted with a masonite door with a window to facilitate observation (Figure 16). Four 6-inch pieces of vinyl tubing, each $\frac{1}{2}$ inches in diameter were fastened parallel to each other on the testing platform. The tubes were situated about $1\frac{1}{2}$ inches apart. At each end of the olfactometer, the ends of the tubes were connected to Y-tubes, which terminated in a stoppered vial three inches long and $\frac{7}{8}$ inches in diameter. In the middle of each of the 6-inch lengths of tubing were opposing $\frac{1}{4}$ inch holes in the top and bottom. Y-tubes inserted



Figure 16. Moving air olfactometer in Basal Oxygen Demand Incubator.

in these holes connected the tubing to a suction pump drawing air at the rate of 500 feet per minute.

Twenty weevils were placed in one of the stoppered vials (treated), while the other vial remained empty (control). Five insects of the opposite sex were released in each of the four tubes. When the suction pump was turned on, air moved over the weevils in the vial, through one half the length of the tubes and out the hole in the center, while the other half of the tubes contained air flowing from the empty vial. If the insects responded to a pheromone, they would aggregate at the ends of the tubes containing the air coming from the vial with weevils of the opposite sex. Both non-virgin and virgin nondiapausing alfalfa weevils were utilized, and in each group responses of both males and females were checked by readings each minute for 30 minutes.

b. Results and discussion

Neither virgins nor non-virgins of either sex responded positively to the stream of air flowing across weevils of the opposite sex (Table XVI). With the exception of the non-virgin females, all seemed equally attracted to both the treated and control ends of the olfactometer. Since the non-virgin females responded more to the control end, however, no significance was attributed to this behavior. These data probably indicate absence of a sex attractant in the alfalfa weevil.

Table XVI. Percentage of alfalfa weevils responding by olfaction to the opposite sex as compared to those responding to control in a moving air olfactometer.

	Virgin		Non-virgin	
	$\frac{\text{♀♀}}{\text{♂♂}}$ in vial ♂♂ responding	$\frac{\text{♂♂}}{\text{♀♀}}$ in vial ♀♀ responding	$\frac{\text{♀♀}}{\text{♂♂}}$ in vial ♂♂ responding	$\frac{\text{♂♂}}{\text{♀♀}}$ in vial ♀♀ responding
Treated	47.17	44.33	54.00	39.50
Control	52.83	55.67	46.00	60.50

4. Test with still air olfactometer

a. Materials and methods

A modification of an olfactometer designed by Pielou (1954) was used to test the relative importance of olfaction and vision in alfalfa weevil mating behavior. The olfactometer, 7 7/8 inches x 12 inches, was constructed of plexiglass and consisted of an upper and lower half (Figure 17). In the lower half was a 1/8 inch deep circular depression, 7 inches in diameter. A narrow, plexiglass partition divided this depression in half. Activated charcoal filled one half. At the far end of the other half was a small depression, which contained a perforated, No. 2 gelatin capsule.

The whole circular cavity was covered with a piece of 8 inch diameter filter paper, which had small, pinpoint perforations over the capsule. The upper half of the apparatus was clamped tightly on the lower half. In this top section at the end above the activated charcoal was a small hole in the plexiglass which permitted entrance of the insect. The hole was then covered tightly with a piece of tape. Finally, the top of the plexiglass was marked off in concentric arcs, each one inch in radius and numbered from 1 to 4 with 4 being the target area above the capsule. The remainder of the circular area was designated as 0 (Figure 17).

The actual testing involved both previously mated and virgin, nondiapausing alfalfa weevils. A female was placed in the perforated gelatin capsule, which was then situated under the filter paper as described. A male was introduced and allowed to move freely about

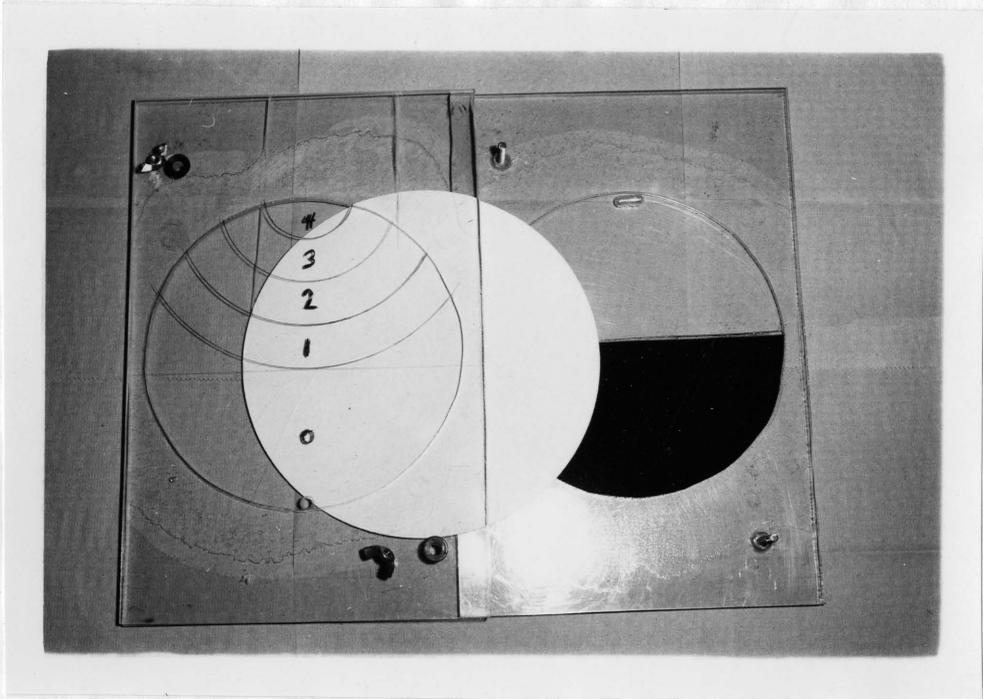


Figure 17. Still air olfactometer disassembled to show parts.

the circular area. The observation period was 10 minutes for each pair. Readings were taken each minute as to the numbered area in which the male was located at the time of the observation; also a map was made of the male's movements throughout the 10 minute period. This type of test was repeated 20 times, 10 with previously mated insects and 10 with virgin weevils. Between each testing period, the olfactometer was cleaned and the capsule and filter paper changed. The second phase of the experiment, which also consisted of 20 repetitions, was performed in a similar manner but with males in the capsule, while readings were taken on the females.

The final phases of this experiment tested the importance of vision without the presence of odor from the opposite sex. This was accomplished by simply using a non-perforated, tightly fitting gelatin capsule, which was placed on top rather than under the filter paper in the target area, 4. The rest of the experiment was performed precisely as described above in the tests for an olfactory stimulus.

b. Results and discussion

Attraction of the sexes to each other was much greater when the weevils inside the capsule were visible to those outside, than when they were not visible (Table XVII). When attracted by vision, both males and females often touched and climbed upon the capsule and sometimes remained in the target area for several minutes. Since as stated previously the males seem to be the real initiator of mating and on many occasions have been noted to be attracted to other weevils

Table XVII. A comparison of the percentages of time spent by alfalfa weevils in each olfactometer zone with special reference to target zone 4.

Condition	Zone				
	0	1	2	3	4
<u>NON VIRGINS</u>					
<u>Olfaction</u>					
♀♀ in capsule	63.00	10.00	14.00	9.00	4.00
♂♂ in capsule	49.00	15.00	8.00	19.00	9.00
<u>Vision</u>					
♀♀ in capsule	36.00	6.00	13.00	10.00	35.00
♂♂ in capsule	55.00	7.00	9.00	12.00	17.00
<u>VIRGINS</u>					
<u>Olfaction</u>					
♀♀ in capsule	64.00	10.00	19.00	7.00	0.00
♂♂ in capsule	62.00	15.00	13.00	6.00	4.00
<u>Vision</u>					
♀♀ in capsule	27.00	27.00	19.00	6.00	21.00
♂♂ in capsule	43.00	14.00	10.00	11.00	22.00

especially those which were moving, it seems a little peculiar that the females showed such a positive response. It appears that there may be a mutual visual attractance bringing the sexes together, with the males then initiating mating. Whether this mutual visual response is purely for the purpose of mating cannot be definitely ascertained at this time.

Alfalfa weevils showed no response or orientation to the odor from the opposite sex. Maps drawn during the experiment, but which have not been presented, readily support this conclusion. Similarly, maps from a preliminary, undescribed test in which males were placed on filter paper where females had walked and been mapped, showed that male alfalfa weevils did not follow the trails of the females. There can be little doubt that vision does play a comparatively more important role in bringing the insects in contact than does olfaction.

5. Test with transparent and opaque cylinders

a. Materials and methods

To test the relative importance of vision and olfaction in mating behavior, a simple experiment consisting of two parts was designed. In part one a gallon paper food carton, an air pump, and a 5 cm. x 2.5 cm. open plastic cylinder were the principal constituents of the apparatus. With the food container sitting upright, a round hole with a diameter equal to that of the cylinder was cut in the side of the carton as close as possible to the bottom. Into this hole was inserted the rim of the cylinder so that the remainder extended outside the carton. On the other side of the carton, directly opposite the

cylinder, was a $1\frac{1}{2}$ inch square opening covered with two layers of cheesecloth. Outside the carton, an air pump of the type described earlier was positioned so that it pumped an air current at the rate of 500 feet per minute through the vial, into the carton, and out the window on the other side. The top of the food container was covered with Saran Wrap.

Thirty pairs of postdiapausing, field collected alfalfa weevils were used. The males were released in the carton. The cylinder, the ends of which were covered by two layers of cheesecloth (translucent), remained empty while air was pumped through it for 15 minutes. The insects were under constant observation, and whenever one crawled on or touched the cheesecloth covering the end of the cylinder, it was recorded. The test was repeated without the current of air. As controls, both of these tests were repeated with females.

In another pair of tests the females were placed in the vial and the males in the carton. For the first 15-minute period, the air pump was operating; for the last period it was turned off. These tests were repeated with the positions of the males and females reversed. Finally, in the two final phases of the experiment, transparent Saran Wrap replaced the cheesecloth. The pump was turned off during these tests. In the first, males were in the vial and females in the carton. In the second, females were in the vial and males in the carton.

All tests lasted for 15 minutes, and the insects were observed constantly throughout these periods. Any time a weevil came in contact with the cheesecloth or the Saran Wrap, it was considered as attracted.

When weevils responded to the cheesecloth-covered ends while other insects were in the vial, the response was considered an olfactory one. When they responded to the Saran Wrap, it was considered a visual response. Responses to the empty vials served as a control. The cartons were changed between each phase of the experiment, and the vial cleaned thoroughly with soap and water, and rinsed with 100% ethyl alcohol.

In part two, a plastic cylinder was also used. The sides of the cylinder were wrapped with white cotton cloth, which prevented the 18 male weevils on the outside from seeing the 18 females inside. The cylinder was placed horizontally in the center of a gallon food carton, which was covered by Saran Wrap. All of the weevils were field collected insects. For the first 30 minutes, the ends of the cylinder were covered with cheesecloth, allowing air to circulate while obscuring the females within from the males free in the carton. For the last 30 minutes, the cheesecloth was replaced with Saran Wrap. The insects were observed continually and the number of male weevils coming in contact with the cheesecloth and the Saran Wrap were recorded.

b. Results and discussion

Both sexes were attracted more to insects in the vials covered with saran wrap than to those in the vials covered with cheesecloth (Table XVIII). This behavior is further evidence that alfalfa weevils are attracted to other weevils much more by vision than by olfaction. Whether the air pump was on or off did not affect the low response

Table XVIII. A comparison of the number of alfalfa weevils attracted to weevils of the opposite sex in translucent and transparent vials.

Conditions					
Weevils attracting	Weevils attracted	Air	Vial covering	Attraction stimulus	# weevils attracted
Empty	♂♂	on	translucent	olfaction	2
Empty	♂♂	off	translucent	olfaction	4
Empty	♀♀	on	translucent	olfaction	4
Empty	♀♀	off	translucent	olfaction	4
♀♀	♂♂	on	translucent	olfaction	3
♀♀	♂♂	off	translucent	olfaction	1
♂♂	♀♀	on	translucent	olfaction	9
♂♂	♀♀	off	translucent	olfaction	4
♂♂	♀♀	---	transparent	vision	32
♀♀	♂♂	---	transparent	vision	22

to the vial covered with cheesecloth. It is likely that the insects which came in contact with the cheesecloth did so accidentally and probably were not attracted to the weevils within the cylinder, since they also responded to empty cylinders. More females than males were attracted to the transparent cylinders. This fact was at first surprising in view of the observation that males appear to be the initiator of mating behavior. This observation, however, supports the fact that both males and females are mutually attracted to other weevils by visual stimuli, especially if the other weevils are moving (Table XVII).

Regarding the overall low response in this experiment, the 30 alfalfa weevils in the carton were attracted more to each other than to the weevils inside the cylinder. This activity, particularly in the males which continued to aggressively mount each other, may partially explain why fewer males than females responded to the Saran-covered cylinder containing females.

In part two of this experiment, only one male responded to the females in the cylinder when the ends were covered with cheesecloth, while 17 responded when the ends were covered with Saran Wrap. It is once again clear that the response to the visually perceived motion of other insects is the primary factor involved in attracting alfalfa weevils to each other. This activity doubtless plays a vital role in their mating behavior. In this phase of the experiment, as in the first, the probable reason for the low overall attractancy to insects in the vials was that the males in the carton took so much interest in each other. In a 10-minute period of observation, males were observed to mount other males 69 times.

F. Final Discussion

Since there is no positive evidence that a pheromone acts as a sex attractant in alfalfa weevils, several questions are brought to mind: (1) how does density of a population increase greatly within one year? (2) how do these insects, which supposedly pass the summer in aestivation away from the alfalfa field, reassemble in the fall when they have become sexually mature and are ready to mate? It would appear that the weevils must have some potent means of bringing the sexes together for mating, and similarly, it would seem probable that a pheromone would be an effective way of accomplishing this.

The answer to question 1 may involve several factors. In the first place, it is sometimes difficult to accurately determine population densities of alfalfa weevils. Unless sampling is carefully done at night (Poinar and Gyrisco, 1960; Parks, 1913; Titus, 1910a), it is hard to say how large a population actually is. Consequently the population might be considerably larger than it appears. If we consider, however, that in reality the population is very small, the alfalfa weevil seems to have certain evolved mechanisms which allow not only survival at low population densities, but also apparently permit a large rate of increase from these limited numbers.

Data presented in this thesis indicate that males are very active, mate repeatedly over a long period of time, and thus probably inseminate many females. Evans (1959) indicated that alfalfa weevil females laid presumably viable eggs two months after the last mating. Newton (1933) found that the period of fertility of isolated females varied

from 20 to 81 days. Finally, the alfalfa weevil female has great fecundity. Laboratory studies by Evans (1959) indicate that a female lays from 113 to 1102 eggs. Other data by Titus (1910a, 1913), Parks (1913, 1914), Hagan (1919), and Newton (1933) indicated 200 to 1500 eggs per female, with 600 to 800 as the average. Based on my own unpublished data, there is little doubt that many females are capable of laying over 1000 eggs. It is quite conceivable that from a small initial population, a considerably larger population could be built up within a reasonably short period.

There are ways that a small population may increase greatly other than by its own fecundity. Immigration may have a profound effect on the population density. Immigration may also to a great extent answer question 2, because in the fall, alfalfa weevils leave their summer aestivating sites and fly back to alfalfa fields where they become sexually mature, mate and reproduce. There is a possibility that the field to which they return may not be the same field they left the previous spring. Prokopy et al. (1967) stated that from the rapidity with which the alfalfa weevil has infested new counties and states in the Eastern U. S., it is likely that some weevils remain airborne for 30 miles or more before landing.

Perhaps of more importance, however, is the fact that this movement into the alfalfa field at the time the insects become sexually mature effectively serves to bring the sexes into close proximity and in this way greatly facilitates mating. Since the host plant is a mutual attractant to males and females, a pheromone would not be essential to assemble the sexes for mating.

When the probable sites of olfactory reception, the antennae and front tarsi, were removed, male alfalfa weevils still readily copulated with female weevils. Normal weevils attempted to copulate with other male alfalfa weevils, and at least three other species of curculionids. This occurred when male alfalfa weevils were isolated with these groups and also when sexually mature, female alfalfa weevils were present. In these instances, aggressive male weevils seemed to mount the first passing insect of appropriate size and did not appear to be able to readily distinguish the sexes or even to immediately recognize its own species. Smith (1930) found similar mating behavior in males of Dibrachoides dynastes, a parasite of the alfalfa weevil, which mounted other males and did not quickly recognize the sexes.

The type of behavior described does not indicate the presence of a sex attractant, since most are species-specific and cause the insect to respond directly to the source of the attractant. It would seem that male alfalfa weevils would not respond sexually to any substances produced by other males, though Tschinkel et al. (1967) has shown that this situation exists in Tenebrio molitor. Nor would it seem that male alfalfa weevils would be attracted to sexually immature females. It appears that a sex attractant in the alfalfa weevil is an extremely remote possibility.

As previously mentioned, most young adults of this insect move in the spring from the alfalfa fields to ditch banks, woods, and other protected areas where they undergo a summer diapause. These weevils do not become sexually mature until they return to alfalfa fields in the fall (Snow, 1928; Guerra and Bishop, 1962). If a pheromone were

present, it probably would not become active until the fall, when the insects are approximately six months old.

Jacobson (1965) indicated that by far, most of the insects which have been shown to possess a pheromone are attractive soon after emergence. The attracted sex usually responds directly in an obvious manner. Mating often occurs only once or twice, and seldom more than a few times, the attractancy diminishes rapidly thereafter. An exception to this condition is found in Tenebrio molitor, where a pheromone serves more as an excitant than an attractant (Tschinkel et al., 1967). In this species mating occurs throughout their two-month life span (Cotton and St. George, 1929). Many of the insects that have sex pheromones have a very short period in which to mate and oviposit before death. Time is a critical factor, and there is need for a mechanism such as a pheromone to insure mating and survival of the species. The same is true for species in which individuals are few and the sexes are widely scattered.

With the alfalfa weevil none of these conditions is true. When they are widely scattered, they are not sexually mature. When they become sexually mature, the males and females are on the host plants and thus in reasonably close contact. Further, this beetle lives for a number of months after becoming sexually mature and continues to mate and reproduce profusely through most of this period. It seems very improbable that a pheromone would be produced for such a long time. Other than Tenebrio molitor, I know of no insect, possessing a pheromone, which even remotely approaches the type of reproductive behavior found in the alfalfa weevil. Finally, important behavioral

differences exist between the alfalfa weevil and the yellow mealworm adults. The boll weevil, in which the males release a sex attractant, is in the same family (Curculionidae) as the alfalfa weevil, but its reproductive habits differ considerably.

In most Coleoptera, as with most other insects which possess a sex attractant, flight is very often involved in the response to the pheromone. The flight to the opposite sex is frequently very obvious. In the alfalfa weevil, however, most flight occurs with migration to and from alfalfa fields in the spring and fall (Pamanes and Pienkowski, 1965; Poinar and Gyrisco, 1962; Prokopy and Gyrisco, 1963, 1965; and Prokopy et al., 1967). Generally the weevils participating in these flights are not sexually mature. For those which are sexually mature, it is very doubtful that these flights are specifically involved in attraction to the opposite sex. Though the alfalfa weevil may fly to a small extent in the alfalfa field, I have seen no reference connecting this flying to mating behavior. There has been no mention of large numbers of one sex being attracted to the opposite sex in the field. For the most part, alfalfa weevils stay on the plants or on the ground, and it would seem that a pheromone in an animal with such habits would be more limited in its effectiveness than in one in which flight is more predominant.

Evidence against participation of sound or chemical sex attractants in alfalfa weevil mating behavior has been presented. Now I will discuss what I believe to be the major factors involved in bringing males and females together for mating. At the time the insects are capable of mating, they are brought together by attractancy to the

host plant. Then a visual response results in contact and subsequent copulation. These two steps compose a unique mechanism for bringing insects together for mating. Nevertheless, there is some question as to the essentiality of vision in mating behavior. Pairs in the dark showed little or no reduction in mating behavior over those in the light. This could involve several factors. As mentioned in section III. D. 2., the sexes may encounter each other by chance within the petri dish; or the eyes of the alfalfa weevil are adapted to seeing in highly reduced light conditions as Newkirk (1963) reported for the robberfly. Another possibility is the presence of subtle olfactory or tactile stimuli which may act in connection with or in the absence of vision to stimulate mating activity in the male and alert him to the proximity of another weevil. To my knowledge, Tschinkel et al. (1967), working on Tenebrio molitor, was the first to report such an olfactory sexual excitant which served only as a very weak attractant.

Vision is probably the final step in the pairing of alfalfa weevils. Males through this visual response will mount either sex or perhaps any similarly sized weevil of another species. Thus their selection would appear to be by trial and error. It should be pointed out, however, that there must be certain factors which prevent interspecific breeding or even excessive time spent with males mounted other males. No doubt there is morphological distinctness but probably also significant, as Carthy (1965) pointed out, there may be subtle olfactory or tactile cues which prevent such activities from becoming detrimental to the population. As Carthy (1965) further stated,

little evidence for such has been elucidated. Certainly, in the alfalfa weevil, it has not been possible to isolate the factors and mechanisms involved.

G. Summary and Conclusions

The results of experiments to determine the relative importance of certain stimuli in alfalfa weevil mating behavior may be summarized as follows:

1. The presence of alfalfa stimulated mating behavior in the alfalfa weevil by (a) causing more pairs to initiate mating, (b) increasing the average duration per mating and (c) bringing about a greater percentage of time in mating behavior as a result of the two previous effects.
2. A sound test with sensitive sonic equipment indicated that the alfalfa weevil produced no sound by stridulation or other means which could play a role in its mating behavior.
3. No evidence of a pheromone as a sex attractant was found in the alfalfa weevil.
4. Vision seemed to be the primary mechanism by which the sexes were attracted to each other at distances of a few inches.

VI. EFFECTS OF TEMPERATURE ON MATING BEHAVIOR AND OVIPOSITION

A. Introduction

Ross (1965) stated, "In the lives of insects temperature is one of the most critical factors." Smith (1966) commented, "All living organisms apparently have a temperature outside of which they fail to grow or reproduce." Odum (1959) concluded, "Temperature, therefore, is universally important and is very often a limiting factor." Because temperature is a vital factor in biological systems and because in poikilothermic animals such as insects it has particularly great influence on physiological processes, it was deemed necessary to study mating and oviposition in the alfalfa weevil under varying temperature conditions in the laboratory. The experiments were designed to determine the limiting and optimal temperatures for mating and oviposition, and to discover whether the response of alfalfa weevils to temperature was immediate or whether antecedent temperatures played the more important role.

B. Literature Review

1. Mating behavior

Webster (1912) observed that with the first warm weather in the spring, alfalfa weevils became active, spread over the field, and paired. Parks (1914) indicated that in an experiment where weevils were maintained under fluctuating temperature conditions, copulation occurred repeatedly during the entire period. List and Wakeland (1919)

noted that on warm days in winter, the beetles were found active in fields where the snow had melted.

In Utah, Reeves (1927) found that activities were greatest at night during extremely hot weather. Sweetman (1929) observed that weevils became active when the temperature rose to about 10 - 12° C. Sweetman (1932) noted that on warm nights in the middle of June, movement and mating continued as during the daylight hours, whenever temperatures were high enough. According to Essig and Michelbacher (1933) alfalfa weevils have been observed to mate throughout the spring, summer, and fall.

Michelbacher and Leighly (1940) stated that high temperatures could check the activity of adult weevils and eventually inhibit it. Michelbacher (1943) indicated that alfalfa weevils were most active at temperatures of 70 - 75° F. Evans (1959) believed that mating took place in the fall and spring. According to Quinton (1959) mating pairs were commonly observed in early spring and late fall. Finally, Campbell et al. (1961) commented that copulating adults were collected under four inches of snow in the Piedmont region of North Carolina in the winter of 1960.

2. Oviposition

Titus (1913) stated that warm, dry, spring weather appeared to stimulate rapid egg laying in the alfalfa weevil, while a cold spring caused them to lay over a longer period of time. Based on a field experiment, Parks (1914) presented data indicating that the numbers of eggs laid varied directly with the temperatures of the days on

which they were laid. He further noted that there was very little oviposition until the mean daily temperature reached about 50° F. Reeves (1927) reported that females taken from a frozen field into warmer surroundings would feed immediately and begin laying eggs a few hours later.

Sweetman (1929) found that the number of eggs increased during warm periods, citing as evidence a warm week in May in which there was far greater oviposition than any other week during the course of his experiments. He further indicated that on a day which had a mean temperature of 4° C during a period of time when egg production had been heavy, thirteen females laid no eggs, while the remaining two beetles in the test laid only 15 eggs. On the days preceeding and following this cold day, however, over 100 eggs were laid by these same 15 weevils. According to Sweetman (1929) oviposition in the alfalfa weevil began when the temperature reached 10 - 12° C.

Sweetman and Widemeyer (1933) found that a temperature of 32° C was much less favorable for oviposition than 27° C, as the weevils discontinued laying after the second week at the higher temperature. When alfalfa weevils were exposed alternately to 27° and 32° C, egg production was far below that found under conditions with a constant temperature of 27° C. The authors interpreted this phenomenon as indicating a detrimental effect of the higher temperature. At 37° C, females ceased oviposition within a few days, and at 42° C no eggs were laid.

Hamlin et al. (1949) concluded that the most abundant egg laying occurred when the temperature oscillated daily around 50° F. Based

on unpublished data from F. H. Harries, the authors further stated that the rate of egg deposition at 80° - 90° F exceeded that at all lower temperatures, but that oviposition was not long continued and ceased abruptly. According to the authors, temperatures considerably below 50° F were little suited for oviposition. The oviposition of any one week was affected by the antecedent conditions, particularly the temperatures of the preceding week. A week totaling more than 65 day-degrees (base 50° F) favored egg laying, when it followed a week having low temperatures, while this temperature if sustained for 3 or 4 weeks greatly reduced oviposition.

Manglitz and App (1957) and Evans (1959) both indicated that egg deposition was interrupted by cold weather and resumed in the spring. Evans also concluded that females could oviposit for no more than one day at 85° F and that 77° F was the maximum temperature for oviposition. He found that weevils laid 6.5 times faster at 70° F than at 50° F, and while some oviposition did occur below 50° F, he believed that a lower limit of 45° F seemed probable. In connection with these data, he revealed that in Virginia, a mean temperature of 45° F and above occurs from March to November.

Quinton (1959) noticed that warm days fostered abundant egg laying and that cool, wet weather greatly extended the period of oviposition. When brought into the greenhouse during cold weather, alfalfa weevils mated and laid an abundance of eggs within 48 hours. The author concluded that this behavior indicated a physiological readiness whenever environmental conditions became suitable. Bass (1967) indicated that in mid winter on warmer days between cold spells, consid-

erable oviposition occurred. Finally, Melamed-Madjar (1962) calculated the threshold of oviposition to be 11° C.

C. Effect of Temperature on Mating Behavior

1. Materials and methods

To determine mating behavior in the alfalfa weevil when exposed to a range of temperatures, both postdiapausing and nondiapausing insects were utilized. Twenty pairs of each group were individually placed in petri dishes with alfalfa and a moistened ball of cotton. Each group was held for a 12-hour period in a Basal Oxygen Demand Incubator, fitted with a masonite door and glass panel to facilitate observation (Figure 18). For each of the daily 12-hour periods the insects were exposed to one of the following temperatures: 5° , 10° , 15° , 20° , 25° , 30° , 35° , 40° , 45° C. Observations were made at 15 minute intervals. Each night the pairs were separated and held at $75 \pm 3^{\circ}$ F. Because of the wide range of temperatures involved, no attempt was made to acclimate the insects.

When pairs were placed in the chamber at 10° C or below and 35° C or above, it took about 15 minutes for the temperature within the dishes to become equal to that of the cabinet. Thus, many pairs initiated mating behavior before the temperature in the dishes reached the intended level. This condition was probably not a limiting factor in initiation of mating at the higher temperatures. However, at 5° and 10° C where the insects were less active, it may well have been an important



Figure 18. Basal Oxygen Demand Incubator equipped with special masonite and glass observation door.

factor. At these low temperatures, figures representing the percentage of time spent in mating behavior possibly say little about initiation of mating. Therefore it was necessary to conduct tests on the initiation of mating behavior.

In the tests on initiation of mating, only the 20 pairs of non-diapausing insects were utilized. The insects were placed into pre-cooled dishes at 0° C. At two hour intervals the temperature was raised by 5° C, until a maximum temperature of 25° C was attained. The number of pairs mounted during each of these periods represent those which had initiated mating behavior. Since the cabinet would only provide a minimum temperature of 3° C, the pairs held at 0° C were placed in dishes floating in an ice bath.

2. Results and discussion

Data based on the total sample of 20 pairs, indicate that the percentages of time spent in mating behavior in both nondiapausing and postdiapausing weevils decreased as the temperature increased (Table XIX). An analysis of variance revealed that the percentages at the different temperatures were significantly different at the .01 level. Both groups spent a greater percentage of time in mating behavior at 10° C than at any other temperature. An analysis of variance between nondiapausing and postdiapausing pairs indicated that the percentages of time spent in mating behavior at the various temperatures were significantly different at the .01 level.

Based on the total sample, the number of mountings generally increased with the temperature up to 35° C for nondiapausing and 40° C

Table XIX. Average percentage of time spent in mating behavior per pair of alfalfa weevils per daily 12-hour period at varying temperatures.

Temp. C	Nondiapausing		Postdiapausing	
	Mean (%)	Stat. sig.	Mean (%)	Stat. sig.
5 ^o	60.42	a ^{1/}	57.81	b
10 ^o	64.06	a	83.54	a
15 ^o	60.32	a	75.22	a
20 ^o	51.57	a b	77.40	a
25 ^o	54.16	a b	56.04	b
30 ^o	45.84	b	48.43	b c
35 ^o	39.18	b	43.86	c
40 ^o	14.06	c	37.50	c
*45 ^o	7.82	c	3.44	d

* Based only on 6 hours of observation due to threat of mortality.

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

for postdiapausing weevils (Table XX). Within both groups the number of mountings at the various temperatures were different at the .01 level of probability. An analysis of variance between the number of mountings at the different temperatures for the two groups of insects revealed that they were highly significantly different. The fact that the postdiapausing pairs mounted more often than the nondiapausing supports data presented in Tables III and VII. The behavioral difference between the two groups may be the result of age differences since the nondiapausing insects were younger than the postdiapausing.

The average duration per mounting in both nondiapausing and postdiapausing weevils decreased as the temperature increased (Table XXI). The nondiapausing pairs remained mounted for longer durations than the postdiapausing pairs at most temperatures. These data support evidence in Table III, which indicate that nondiapausing weevils remain mounted longer than postdiapausing weevils. There is some indication that duration, like the number of mountings, may be a result of the age difference between nondiapausing and postdiapausing alfalfa weevils. The younger, laboratory reared insects throughout the investigation mounted fewer times for longer duration than the older, field collected ones.

Only one pair of weevils was able to mount at 0° C, when most of the weevils were immobile (Figure 19). This pair, however, did not achieve copula. Mating activity in the alfalfa weevil is greatly limited at freezing temperatures. At 5° C the weevil's movements were extremely slow, although three pairs initiated mating behavior. At 10° C mounting activity increased markedly, and by the end of the two-

Table XX. Average number of mountings per pair of alfalfa weevils per daily 12-hour period at varying temperatures.

Temp. C	Nondiapausing		Postdiapausing	
	Mean (%)	Stat. sig.	Mean (%)	Stat. sig.
5°	0.85	a b ^{1/}	1.95	b c
10°	0.90	a b c	1.80	b
15°	1.00	a b c	2.65	b c d
20°	1.70	c d	2.85	c d
25°	1.65	b c d	2.95	c d
30°	1.95	d	3.60	d
35°	3.00	e	2.95	c d
40°	1.95	d	3.25	d
*45°	0.45	a	0.50	a

* Based only on 6 hours of observation due to threat of mortality.

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

Table XXI. Average duration (minutes) per mounting per pair of alfalfa weevils per daily 12-hour period at varying temperatures.

Temp. C	Nondiapausing	Postdiapausing
5°	511.76	213.46
10°	512.50	334.17
15°	434.25	204.34
20°	218.38	195.53
25°	236.36	136.78
30°	169.23	96.87
35°	94.00	107.03
40°	51.92	83.08
*45°	43.33	49.50

* Based only on 6 hours of observation due to threat of mortality.

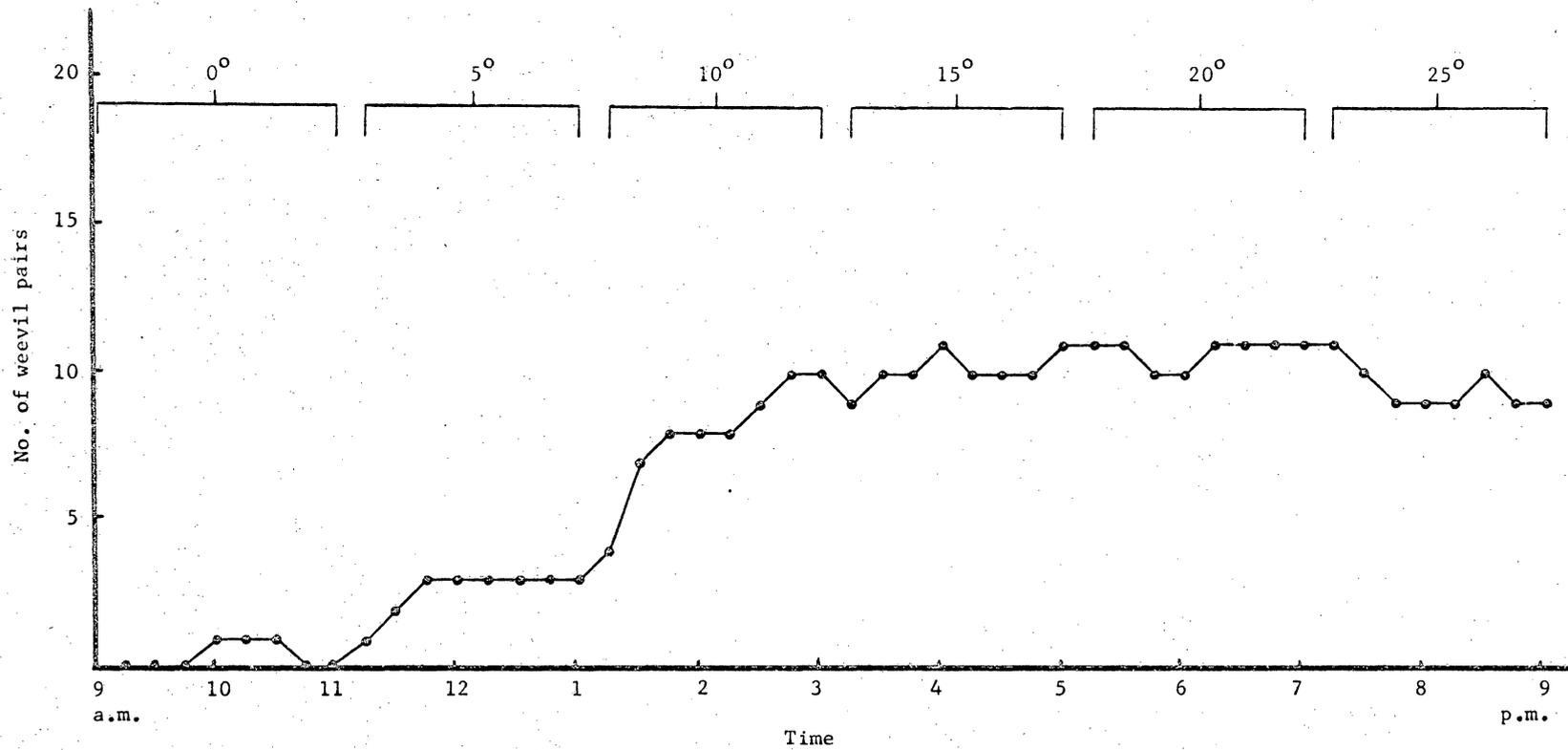


Figure 19. Number of pairs of alfalfa weevils exhibiting mating behavior at 15 minute intervals under varying temperatures ($^{\circ}$ C).

hour period many of the pairs had mounted.

In the 12 hour test at 5° C some of the initiation of mating may have occurred during the first 15 minutes before the temperature in the dishes had been reduced to that level. However at 10° C, it is quite probable that most pairs mounted throughout the 12 hour testing period. The capability to readily initiate mating behavior at 10° C, coupled with the fact that at this temperature, the average duration per mounting was 512.5 minutes, are the reasons why the greatest percentage of time spent in mating behavior was found at this temperature.

D. Effect of Temperature on Oviposition

1. Materials and methods

Forty field collected, alfalfa weevil females were used to determine the effect of temperature on the rate of oviposition. These insects had been employed earlier in a previously described experiment testing the effect of mating behavior on oviposition. All dishes contained a horizontally placed sprig of alfalfa for feeding and oviposition and also a wetted ball of cotton for moisture. The photoperiod was 12 hours. The stems were changed and the eggs counted every 24 hours.

The temperatures were varied daily in a Basal Oxygen Demand Incubator in the following sequence: 15°, 25°, 10°, 30°, 5°, 35°, 5°, 30°, 10°, 25°, 15° C. The temperature sequence provided considerable daily fluctuation to better determine if the temperature on the day of oviposition or the temperature of the preceding day affected more the rate of egg laying.

2. Results and discussion

More females laid eggs as the temperature was increased from 5° to 35° C (Table XXII). These females quickly responded to the temperature of each 24 hour testing period, as illustrated by the number that laid on days 5, 6, and 7, where a day with a temperature of 35° C fell between days having temperatures of 5° C. The total number of eggs laid daily and the average daily number of eggs per ovipositing female also increased with the temperature. Figure 20 depicting the average number of eggs laid daily per ovipositing female graphically illustrates the female alfalfa weevil's prompt response to oscillating daily temperatures.

E. Summary and Conclusions

At temperatures ranging from 5° to 45° C, the percentages of time spent in mating behavior by both nondiapausing and postdiapausing alfalfa weevils generally decreased significantly with increasing temperatures. Both groups of insects exhibited maximum mating behavior at 10° C. As the temperature increased, the duration per mounting generally decreased for both groups, with the greatest duration occurring at 10° C.

The number of mountings generally increased significantly with increased temperatures up to 35° C in nondiapausing and up to 40° C in postdiapausing pairs. As in previous experiments, the younger nondiapausing insects mounted fewer times for a greater duration per mounting than the postdiapausing weevils. Lower temperatures fostered

Table XXII. The effects of temperature on oviposition in the alfalfa weevil.

Day	Temp. C	# ♀♀ ovipositing	Stat. sig.	Total # eggs	Stat. sig.	Avg. # eggs per ovipositing ♀	Stat. sig.
7	5°	9	a ^{1/}	91	a	10.11	a
5	5°	19	b	221	ab	11.63	a
9	10°	18	b	200	ab	11.11	a
3	10°	21	bc	287	abc	13.67	a
1	15°	24	bcd	439	bc	18.29	a
11	15°	28	cde	488	c	17.43	a
10	25°	29	cde	885	d	30.52	b
2	25°	32	de	1038	de	32.44	b
4	30°	34	e	1137	de	33.44	b
8	30°	29	cde	1163	e	40.10	bc
6	35°	32	e	1416	f	44.25	c

^{1/} Means in a given column with similar letters are not significantly different according to Duncan's Multiple Range Test (P = .01).

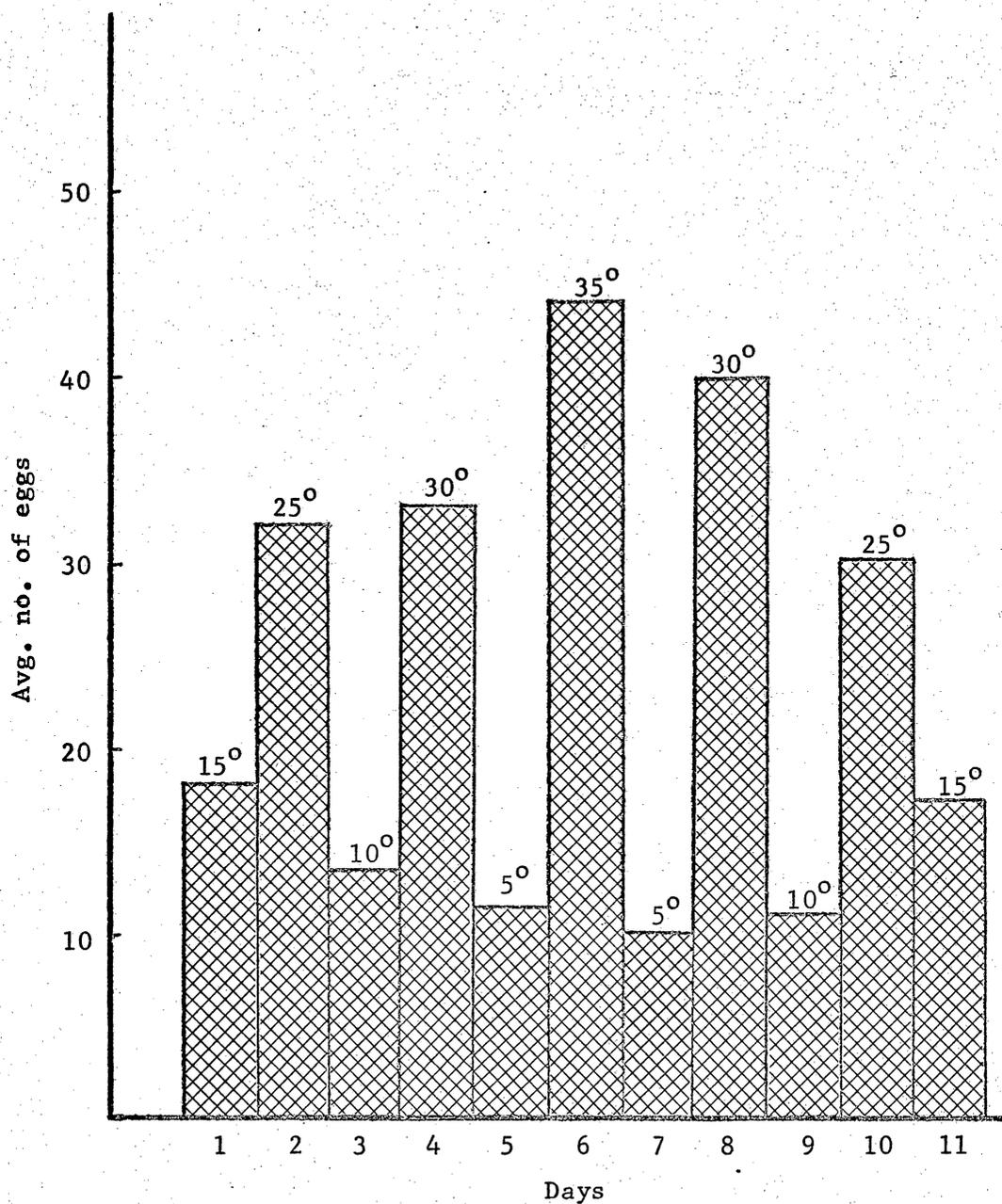


Figure 20. Average number of eggs per ovipositing female under fluctuating temperatures ($^{\circ}$ C).

greater percentages of time spent in mating behavior because of greatly increased durations per mounting. The prolonged period of copulation may indicate some inefficiency in the mating process at lower temperatures. At 0° C, only one of 20 pairs initiated mating behavior. At 5° C initiation was reduced, while at 10° C, it did not appear to be inhibited, even though movement was noticeably slowed.

The number of laying females, the total number of eggs deposited, and the average number of eggs per ovipositing female per 24 hour period, all increased significantly as the temperatures were increased from 5° to 35° C. The daily response to oscillating temperatures was prompt, and the temperature of the day on which the eggs were deposited affected oviposition more than temperatures of the preceding day.

LITERATURE CITED

- Acker, T. S. 1966. Courtship and mating behavior in Agulla species (Neuroptera: Raphidiidae). Ann. Entomol. Soc. Amer. 59 (1): 1-6.
- Alexander, R. D. 1960. Sound communication in Orthoptera and Cicadidae. IN: Animal sounds and communication, Chap. 3, p. 38-92. W. E. Lanyon and W. N. Tavolga (Eds.). Amer. Inst. Biol. Sci. Publ. No. 7. Washington, D. C. 443 p.
- Alexander, R. D. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets. Behaviour 17: 130-223.
- Alexander, R. D. 1962. The role of behavioral study in cricket classification. System. Zool. 11: 53-72.
- Alexander, R. D. 1967. Acoustical communication in arthropods. Ann. Rev. Entomol. 12: 495-526.
- Alexander, R. D., and T. E. Moore. 1958. Studies on the acoustical behavior of seventeen-year cicadas. Ohio J. Sci. 58: 107-127.
- Alexander, R. D., T. E. Moore, and R. E. Woodruff. 1963. The evolutionary differentiation of stridulatory signals in beetles. An. Behav. 11: 111-115.
- Allard, H. A. 1928. Our insect instrumentalists and their musical technique. Ann. Rept. Smithsonian Inst.: 563-591.
- Baerends, G. P. 1959. Ethological studies of insect behavior. Ann. Rev. Entomol. 4: 207-234.
- Bass, M. H. 1967. Notes on the biology of the alfalfa weevil, Hypera postica, in Alabama. Ann. Entomol. Soc. Amer. 60 (2): 295-298.
- Buchholtz, C. 1956. Eine Analyse des Paarungsverhaltens und der dabei wirkenden auslöser bei den Libellen Platycnemis pennipes Pall. und Pl. dealbata Klug. Z. f. Tierpsychologie 13: 13-25.
- Burkholder, W. E., and R. J. Dicke. 1966. Evidence of sex pheromones in females of several species of Dermestidae. J. Econ. Entomol. 59 (3): 540-543.
- Burnett, A. L., and T. Eisner. 1964. Animal adaptation. New York: Holt, Rinehart and Winston, p. 39-40.
- Burts, E. C., and W. R. Fischer. 1967. Mating behavior, egg production, and egg fertility in the pear psylla. J. Econ. Entomol. 60 (5): 1297-1300.

- Campbell, W. V., T. G. Bowery, and K. G. Jester. 1961. Seasonal history and control of the alfalfa weevil in North Carolina. *J. Econ. Entomol.* 54 (4): 743-747.
- Carthy, J. D. 1965. The behavior of arthropods. San Francisco: W. H. Freeman, p. 56-76.
- Carthy, J. D. 1966. The study of behavior. New York: St. Martin's Press, p. 13-20.
- Chapman, J. A. 1964. Evidence for a sex attractant in the elaterid beetle, Hemicrepidius morio (LeConte). *Canad. Entomol.* 96 (6): 909.
- Conrad, M. S., and O. L. Lovett. 1966. First report of the alfalfa weevil in Wisconsin. USDA, Plant Pest Control Division, Coop. Econ. Insect Rept. 16: 1079.
- Cook, P. P., Jr. 1963. Mating behavior of Psylla pyricola Forster (Homoptera: Psyllidae). *Pan-Pacific Entomol.* 39 (3): 175.
- Cothran, W. R. 1966. A bibliography of the alfalfa weevil, Hypera postica (Gyllenhal). *Bull. Entomol. Soc. Amer.* 12 (2): 151-160.
- Cotton, R. T., and R. A. St. George. 1929. The mealworms. USDA Tech. Bull. No. 95.
- Crampton, G. C. 1940. The mating habits of the winter mecopteran, Boreus brumalis Fitch. *Psyche* 48 (4): 125-128.
- Cross, W. H., and H. C. Mitchell. 1966. Mating behavior of the female boll weevil. *J. Econ. Entomol.* 59 (6): 1503-1507.
- Cuthbert, F. P., Jr., and W. J. Reid, Jr. 1964. Studies of sex attractant of banded cucumber beetles. *J. Econ. Entomol.* 57 (2): 247-250.
- Doane, J. 1961. Movement on the soil surface, of adult Ctenicera acripennis destructor (Brown) and Hypolithus bicolor Esch. (Coleoptera: Elateridae), as indicated by funnel pitfall traps, with notes on captures of other arthropods. *Canad. Entomol.* 93 (8): 636.
- Dorsey, C. K., and D. O. Quinn. 1962. Alfalfa weevil control studies in West Virginia. *J. Econ. Entomol.* 55 (3): 365-368.
- Dowdy, A. C. 1966. First report of the alfalfa weevil in Michigan. USDA, Plant Pest Control Division, Coop. Econ. Insect Rept. 16: 540.

- Downes, J. A. 1955. Observations on the swarming flight and mating of *Culicoides*. *Trans. Roy. Entomol. Soc. (London)* 106: 213-236.
- Downes, J. A. 1966. Observations on the mating behavior of the crab hole mosquito, *Deinocerites cancer* (Diptera: Culicidae). *Canad. Entomol.* 98 (11): 1169-1177.
- Duijm, M., and T. van Oyen. 1948. Het sjirpen van de zadelsprinkhaan. *Levende Natuur* 51: 81-87.
- Eno, F. F. 1960. Nitrate production in field by incubating the soil in polyethylene bag. *Soil Sci. Soc. Amer. Proc.* 24 (4): 277-279.
- Essig, E. O., and A. E. Michelbacher. 1933. The alfalfa weevil. *Calif. Agr. Exp. Sta. Bull.* 567. 99 p.
- Evans, W. G. 1959. The biology and control of the alfalfa weevil in Virginia. *Va. Agr. Exp. Sta. Bull.* 502. 28 p.
- Federov, S. M. 1927. Studies in the copulation and oviposition of *Anacridium aegyptium* L. (Orthoptera: Acrididae). *Trans. Roy. Entomol. Soc. (London)* 75: 53-60.
- Ferkovich, S. M., S. G. Wellso and W. F. Wilson. 1967. Mating behavior of the big-headed grasshopper, *Aulocara elliotti* (Orthoptera: Acrididae), under caged conditions in the greenhouse and outdoors. *Ann. Entomol. Soc. Amer.* 60 (5): 972-975.
- Frings, H., and M. Frings. 1958. Uses of sounds by insects. *Ann. Rev. Entomol.* 3: 87-106.
- Fulton, B. B. 1933. Stridulating organs of female Tettigoniidae. *Entomol. News* 44: 270-275.
- Gibson, N. H. E. 1945. On the mating swarms of certain Chironomidae (Diptera). *Trans. Roy. Entomol. Soc. (London)* 95: 263-294.
- Guerra, A. A., and J. L. Bishop. 1962. The effect of aestivation in sexual maturation in the female alfalfa weevil (*Hypera postica*). *J. Econ. Entomol.* 55 (5): 747-749.
- Hagan, H. R. 1919. Alfalfa weevil control in Utah. *Calif. Dept. Agr. Mon. Bull.* 8: 469-477.
- Hamlin, J. C., F. V. Lieberman, R. W. Bunn, W. C. McDuffie, R. C. Newton, and L. J. Jones. 1949. Field studies of the alfalfa weevil and its environment. *USDA Tech. Bull.* 975. 84 p.
- Hardee, D. D., E. B. Mitchell, and P. M. Huddleston. 1967a. Procedure for bioassaying the sex attractant of the boll weevil. *J. Econ. Entomol.* 60 (1): 169-171.

- Hardee, D. D., E. B. Mitchell, and P. M. Huddleston. 1967b. Laboratory studies of sex attraction in the boll weevil. *J. Econ. Entomol.* 60 (5): 1221-1224.
- Hartman, H. B., and L. M. Roth. 1967a. Stridulation by a cockroach during courtship behaviour. *Nature* 213 (5082): 1243-1244.
- Hartman, H. B., and L. M. Roth. 1967b. Stridulation by the cockroach, Nauphoeta cinerea, during courtship behaviour. *J. Insect Physiol.* 13: 579-586.
- Haskell, P. T. 1958. Stridulation and associated behaviour in certain Orthoptera. II. Stridulation of females and their behaviour with males. *Brit. J. An. Behav.* 6: 27-42.
- Haskell, P. T. 1961. *Insect sounds*. Chicago: Quadrangle Books, Inc., 189 p.
- Hayes, J. T., and A. G. Wheeler, Jr. 1968. Evidence for a sex attractant in Hemicrepidius decoloratus (Coleoptera: Elateridae). *Canad. Entomol.* 100 (2): 207-208.
- Henderson, W. W. 1919. Interstate quarantine on alfalfa weevil. *Calif. Dept. Agr. Mon. Bull.* 8: 461-469.
- Huggans, J. L., and C. C. Blickenstaff. 1964. Effects of photoperiod on sexual development in the alfalfa weevil. *J. Econ. Entomol.* 57 (1): 167-168.
- Humphries, D. A. 1967. The mating behaviour of the hen flea Ceratomyphillus gallinae (Schrank) (Siphonaptera: Insecta). *An. Behav.* 15: 82-90.
- Hungerford, H. B. 1924. Stridulation of Buenoa limnocastoris Hungerford and systematic notes on the Buenoa of the Douglas Lake Region of Michigan, with the description of a new form. *Ann. Entomol. Soc. Amer.* 17 (2): 223-226.
- Jacobson, M. 1965. *Insect sex attractants*. New York: John Wiley & Sons, Inc., 154 p.
- Keller, J. C., E. B. Mitchell, G. McKibben, and T. B. Davich. 1964. A sex attractant for female boll weevils from males. *J. Econ. Entomol.* 57 (4): 609-610.
- Leston, D. 1955. *Entomologists' Monthly Mag.* 91: 92-95. Cited in H. Frings and M. Frings. 1958. Uses of sounds by insects. *Ann. Rev. Entomol.* 3: 87-106. Original article not seen.

- Lilly, C. E. 1959. Response of males of Limenius californicus (Mann) (Coleoptera: Elateridae) to a sex attractant separable by paper chromatography. *Canad. Entomol.* 91 (3): 145-146.
- Lilly, C. E., and A. J. McGinnis. 1965. Reactions of male click beetles in the laboratory to olfactory pheromones. *Canad. Entomol.* 97 (3): 317-321.
- List, G. M., and C. C. Wakeland. 1919. Alfalfa weevil (Phytonomus posticus Gyll.). In: 10th Ann. Rept. (1918) Colo. State Entomol., Office of State Entomol. Circ. 27, p. 13-37.
- Lloyd, J. E. 1966. Studies on the flash communication system in Photinus fireflies. Univ. Mich. Museum Zool. Misc. Publ. No. 130.
- Lorenz, K. Z. 1957. The past twelve years in the comparative study of behavior. In: *Instinctive behavior*. C. H. Schiller. p. 288-310. London: Methuen & Co., 328 p.
- Mampe, C. D., and H. H. Neunzig. 1966. Function of the stridulating organs of Conotrachelus nenuphar. *Ann. Entomol. Soc. Amer.* 59 (3): 614-615.
- Manglitz, G. R., and B. A. App. 1957. Biology and seasonal development of the alfalfa weevil in Maryland. *J. Econ. Entomol.* 50 (6): 810-813.
- Mangus, D. 1954. Zur Problem der 'uberoptimalen' Schlüsselreize *Verk. dtsh. Zool. Ges., Tubingen, 1954: 317-325. Cited in: J. D. Carthy. 1965. The behavior of arthropods. San Francisco: W. H. Freeman, p. 56-76.*
- Manning, A. 1959. The sexual isolation between Drosophila melanogaster and Drosophila simulans. *An. Behav.* 7: 60-65.
- Mayer, M. S., and J. R. Brazzel. 1963. The mating behavior of the boll weevil, Anthonomus grandis. *J. Econ. Entomol.* 56 (5): 605-609.
- McMillian, W. W. 1963. Reproductive system and mating behavior of Sogata orizicola (Homoptera: Delphacidae). *Ann. Entomol. Soc. Amer.* 56 (3): 330-334.
- Melamed-Madjar, V. 1962. Bionomics of the alfalfa weevil (Hypera variabilis Hbst.) in Israel. *Israel J. Agr. Res.* 12: 29-38.
- Michelbacher, A. E. 1943. The present status of the alfalfa weevil in California. *Calif. Agr. Exp. Sta. Bull.* 677. 24 p.

- Michelbacher, A. E., and J. Leighly. 1940. The apparent climatic limitations of the alfalfa weevil in California. *Hilgardia* 13: 103-139.
- Miller, M. C. 1966. Emergence and mating of Tetrastichus incertus, a parasite of the alfalfa weevil. *J. Econ. Entomol.* 59 (6): 1532-1533.
- Monro, J. 1953. *Aust. J. Sci.* 16: 60-62. Cited in: P. T. Haskell. 1961. *Insect sounds*. Chicago: Quadrangle Books, Inc., 189 p. Original article not seen.
- Newkirk, M. R. 1963. The feeding and mating of Leptogaster annulatus (Diptera: Asilidae). *Ann. Entomol. Soc. Amer.* 56 (2): 234-236.
- Newton, J. H. 1933. The alfalfa weevil in Colorado. *Colorado Agr. Exp. Sta. Bull.* 399. 19 p.
- Odum, E. P. 1959. *Fundamentals of ecology*. 2nd ed. Philadelphia: W. B. Saunders Co. p. 105.
- Pamanes, G. A. and R. L. Pienkowski. 1965. Dispersal of the alfalfa weevil in Virginia. *Ann. Entomol. Soc. Amer.* 58 (2): 230-233.
- Parks, T. H. 1913. The alfalfa weevil. *Univ. Idaho Agr. Ext. Bull.* 7. 22 p.
- Parks, T. H. 1914. Effect of temperature upon the oviposition of the alfalfa weevil, Phytonomus posticus Gyll. *J. Econ. Entomol.* 7 (6): 417-421.
- Pielou, D. P. 1954. Apparatus for studying behavior responses of insects to olfactory stimuli in still air. *Canad. J. Zool.* 32 (3): 209-214.
- Poinar, G. O., and G. G. Gyrisco. 1960. Effects of light, temperature, and relative humidity on the diel behavior of the alfalfa weevil, Hypera postica. *J. Econ. Entomol.* 53 (4): 675-677.
- Poinar, G. O., and G. G. Gyrisco. 1962. Flight habits of the alfalfa weevil in New York. *J. Econ. Entomol.* 55: 625-626.
- Poos, F. W., and T. L. Bissell. 1953. The alfalfa weevil in Maryland. *J. Econ. Entomol.* 46 (1): 178-179.
- Prokopy, R. J., and G. G. Gyrisco. 1963. A fall flight period of the alfalfa weevil in New York. *J. Econ. Entomol.* 56 (2): 241.
- Prokopy, R. J., and G. G. Gyrisco. 1965. Summer migration of the alfalfa weevil, Hypera postica (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Amer.* 58 (5): 630-641.

- Prokopy, R. J., Edward Armbrust, W. R. Cothran, and G. C. Gyrisco. 1967. Migration of the alfalfa weevil, Hypera postica (Coleoptera: Curculionidae), to and from estivation sites. Ann. Entomol. Soc. Amer. 60 (1): 26-31.
- Provost, M. W., and J. S. Haeger. 1967. Mating and pupal attendance in Deinocerites cancer and comparisons with Opifex fuscus (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 60 (3): 565-574.
- Quinton, R. J. 1959. The alfalfa weevil. Conn. Agr. Exp. Sta. Bull. 621. 16 p.
- Reeves, G. I. 1927. The control of the alfalfa weevil. USDA Farmers' Bull. 1528. 22 p.
- Reeves, G. I., P. B. Miles, T. R. Chamberlin, S. J. Snow, and L. J. Bower. 1916. The alfalfa weevil and methods of controlling it. USDA Farmers' Bull. 741. 16 p.
- Ross, H. H. 1965. A textbook of entomology. 3rd Ed. New York: John Wiley & Sons, p. 442.
- Roth, L. M. 1948. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of Aedes aegypti (Linnaeus). Amer. Midland Naturalist 40: 265-352.
- Rudinsky, J. A. 1963. Response of Dendroctonus pseudosugae Hopkins to volatile attractants. Contrib. Boyce Thompson Inst. 22: 23.
- Rudinsky, J. A., and G. E. Daterman. 1964. Field studies on flight patterns and olfactory responses of ambrosia beetles in Douglas-fir forests of western Oregon. Canad. Entomol. 96 (10): 1339.
- Shrick, F. H. 1942. The flight of sugar-beet wireworm adults in southwestern Idaho. J. Econ. Entomol. 35 (3): 423.
- Smith, H. D. 1930. The bionomics of Dibrachoides dynastes (Foerster), a parasite of the alfalfa weevil. Ann. Entomol. Soc. Amer. 23 (3): 577-593.
- Smith, R. L. 1966. Ecology and field biology. New York: Harper & Row, p. 66.
- Snow, S. J. 1925. The alfalfa weevil in Nevada and its control by spraying. Nevada Agr. Exp. Sta. Bull. 108. 22 p.
- Snow, S. J. 1928. Effect of ovulation upon seasonal history in the alfalfa weevil. J. Econ. Entomol. 21 (5): 752-761.

- Soo Hoo, C. F., and R. J. Roberts. 1965. Sex attractants in Rhopaea (Coleoptera: Scarabaeidae). *Nature* 205 (4972): 724.
- Spooner, J. D. 1964. The Texas bush katydid - its sounds and their significance. *An. Behav.* 12: 235-244.
- Srivastava, P. D. 1957. Observations on the breeding habits of Atractomorpha crenulata (F.) the tobacco grasshopper (Orthoptera: Acrididae). *Ann. Entomol. Soc. Amer.* 50 (1): 15-20.
- Sweetman, H. L. 1929. Field studies of the physical ecology of the alfalfa weevil. *Wyo. Agr. Exp. Sta. Bull.* 167. 31 p.
- Sweetman, H. L. 1932. Further studies on the physical ecology of the alfalfa weevil. *J. Econ. Entomol.* 25 (3): 681-693.
- Sweetman, H. L., and J. Wedemeyer. 1933. Further studies of the physical ecology of the alfalfa weevil, Hypera postica (Gyllenhal). *Ecology* 14: 46-60.
- Tauber, M. J., and C. A. Tauber. 1967. Reproductive behavior and biology of the gall-former, Aciurina ferruginea (Doane) (Diptera: Tephritidae). *Canad. J. Zool.* 45 (6): 907-913.
- Tinbergen, N., B. J. D. Mecuse, and W. W. Varossieau. 1942. Die balz des samtfalters, Eumenis (= Satyrus) semele. *Z. Tierpsychol* 5: 182-226. Cited in: J. D. Carthy. 1965. The behavior of arthropods. San Francisco: W. H. Freeman, p. 56-76. Original article not seen.
- Titus, E. G. 1909. The alfalfa weevil. *J. Econ. Entomol.* 2 (2): 148-154.
- Titus, E. G. 1910a. The alfalfa leaf-weevil. *Utah Exp. Sta. Bull.* 110. 72 p.
- Titus, E. G. 1910b. On the life history of the alfalfa leaf-weevil. *J. Econ. Entomol.* 3 (6): 459-470.
- Titus, E. G. 1913. The control of the alfalfa weevil. *Utah Agr. Exp. Sta. Circ.* 10: 105-120.
- Travis, B. V. 1939. Habits of the June Beetle, Phyllophaga lanceolata (Say) in Iowa. *J. Econ. Entomol.* 32 (5): 690-693.
- Tschinkel, W., C. Willson, and H. A. Bern. 1967. Sex pheromone of the mealworm beetle (Tenebrio molitor). *J. Exp. Zool.* 164 (1): 81-85.
- Valentine, J. M. 1931. The olfactory sense of the adult mealworm beetle Tenebrio molitor (Linn.). *J. Exp. Zool.* 58: 165.

- Vite, J. P., and R. I. Gara. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). Contrib. Boyce Thompson Inst. 21: 251.
- Vite, J. P., R. E. Gara, and R. A. Kliefoth. 1963. Collection and bioassay of a volatile fraction attractive to Ips confusus (Lec.) (Coleoptera: Scolytidae). Contrib. Boyce Thompson Inst. 22: 39.
- Vite, J. P., R. I. Gara, and H. D. von Scheller. 1964. Field observations on the response to attractants of bark beetles infesting southern pines. Contrib. Boyce Thompson Inst. 22: 461.
- Wagner, R. E. 1965. The challenge and outlook for alfalfa. Better Crops and Plant Food (Maga. Amer. Potash Inst.) 49 (1): 44-51.
- Wakeland, C. 1921. Fighting alfalfa weevil. Idaho Agr. Exp. Sta. Ext. Bull. 50. 27 p.
- Waldron, I. 1964. Courtship sound production in two sympatric sibling Drosophila species. Science 144: 191-193.
- Walker, T. J. 1957. Specificity in the response of female tree crickets to calling songs of the males. Ann. Entomol. Soc. Amer. 50: 626-636.
- Wallace, B., and A. M. Srb. 1961. Adaptation. Englewood Cliffs, N. J.: Prentice-Hall., p. 77.
- Webster, F. M. 1912. Preliminary report on the alfalfa weevil. USDA Bull. 112. 47 p.
- Whitman, C. O. 1898. Animal behavior. Sixteenth Lecture. Woods Hole, Mass. Marine Biological Laboratory Biological Lectures 6: 285-338.
- Wishart, G., and D. F. Riordan. 1959. Flight responses to various sounds by adult males of Aedes aegypti (L.). Canad. Entomol. 91: 181-191.
- Wood, D. L., and J. P. Vite. 1961. Studies on the host selection behavior of Ips confusus (LeConte) (Coleoptera: Scolytidae) attacking Pinus ponderosa. Contrib. Boyce Thompson Inst. 21: 79.
- Wright, J. E., K. D. Kappus, and C. E. Venard. 1966. Swarming and mating behavior in laboratory colonies of Aedes triseriatus (Diptera: Culicidae). Ann. Entomol. Soc. Amer. 59 (6): 1110-1112.

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MATING BEHAVIOR OF THE ALFALFA WEEVIL,
HYPERA POSTICA (GYLLENHAL)

George Leonard LeCato, III

ABSTRACT

The qualitative and quantitative aspects of mating behavior, the stimulus for locating and mating, and the effects of varying temperatures on mating behavior and oviposition were studied in the laboratory.

Postdiapausing weevils, collected in April, and nondiapausing weevils, reared in the laboratory, were utilized. In both groups, the males aggressively mounted the females from any angle or position with little or no indication of prior courtship.

In petri dishes at $75 \pm 3^{\circ}$ F, both groups spent about 59% of their total time in mating behavior. Mounted time spent in actual copulation (aedeagus inserted) was 47% for nondiapausing pairs and 74% for postdiapausing pairs. This difference in behavior was probably caused by the younger, nondiapausing females which often prevented copulation by tucking the genital opening under the elytra. Males, when given a choice between virgin and non-virgin females, mated with non-virgins in a ratio of 7:1. Nondiapausing pairs mounted fewer times for longer durations per mounting than the postdiapausing. Crowding by other weevils appeared to reduce mating efficiency, but not mating initiation.

Sight was found to play a role in attracting weevils to each other over distances of a few inches. No evidence of sound production was discovered, nor was there found to be a pheromone serving as a sex attractant. Since males mounted other males as well as other species

of curculionids, there is apparently no mechanism for recognition of sex or species.

Higher temperatures resulted in a greater number of mountings of shorter duration. At lower temperatures, pairs mounted fewer times and not infrequently copulated for as long as 12 hours. The number of ovipositing females and the average number of eggs per female increased with the temperature.