STUDIES OF THE EFFECTS OF APHOLATE ON THE ALFALFA WEEVIL *HYPERA POSTICA* (GYLLENHAL)

by

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I. INTRODUCTION

The alfalfa weevil, Hypera postica (Gyllenhal), was first discovered in the United States in Utah in 1904 (Titus 1910). In 1951 the weevil was first discovered in the east in Maryland (Poos and Bissell 1953). By 1967 it had spread to 29 Eastern States (U.S.D.A. Coop. Econ. Ins. Rpt. 1967). Presently it is considered the most important pest of alfalfa in most infested areas (Cothran 1966).

During the early years control measures consisted of good farming practices coupled with methods to retard the development of the alfalfa weevil (Wakeland 1920, Newton 1933). Many inorganic chemicals, e.g. zinc arsenite, lead arsenite, calcium arsenite plus sulfur, were also applied to control the weevil (Hagan 1918). In recent years recommendations included: dieldrin, methoxychlor, endrin, and toxaphene (App 1954); and heptachlor especially in winter or early spring (Muka 1957). Evans (1959) reported in Virginia that granular heptachlor or heptachlor mixed with fertilizer applied in early spring gave satisfactory control. Pre-oviposition or fall insecticide application in the Eastern United States was described by Dorsey and Quinn (1962). Fall heptachlor treatment gave excellent control until the alfalfa weevil became resistant to heptachlor (Adler and Blickenstaff 1964, Bishop 1964). Spring foliar sprays of malathion, methyl parathion, parathion or special formulation of methoxychlor and diazinon have since been recommended to control the weevil in Virginia (Rowell 1966). However, these spring sprays are not so effective as was the fall heptachlor treatment (Pienkowski

personal communication). This stimulated the search for new approaches for controling the alfalfa weevil. These include flaming (Bennett and Luttrell 1965, Pienkowski et al. 1966), biological control (Coles and Puttler 1963), resistant varieties (Campbell and Dudley 1965), attractants and stimulants (Byrne 1966), and sterilization (Burgess and Bennett 1966). In spite of various control measures and quarantines the alfalfa weevil has continued to spread.

Sterilization, which involves the use of ionizing radiation or chemosterilants, is being developed as one of the promising methods in insect control. It interferes with the organism's normal reproductive cycle so that viable offspring are not produced. Smith et al. (1964) concluded that although it had not been recommended for the control of any insects, chemosterilants could be used in the control of some species by sterilizing reared insects for release in the natural population.

During the present development of sterilization technique, chemosterilization rather than irradiation, has been more emphasized. The reasons are: chemosterilants can be used more easily to treat large numbers of insects in the laboratory and field; the dosage can be adjusted more easily; chemosterilization is less expensive; chemosterilants cause less somatic damage and less loss of sexual vigor, e.g. gamma radiation was ineffective in the sterilization of male boll weevil since the sterilizing dose exceeded the lethal dose (Davich and Lindquist 1962) whereas the chemosterilant apholate induced more sterility with less mortality than did radiation (Lindquist et al. 1964, Hedin et al. 1964).

The need for more effective control of the alfalfa weevil and the existance of a study to find attractants for the weevil led to the present studies on the effects of apholate on the alfalfa weevil. Due to the effects of the known chemosterilants on mammals, attractants will probably have to be used with chemosterilants in practical control operations. Smith et al. (1964) state that the activity of chemosterilants is not restricted to insects, so their effects on other organisms and their chronic toxicity must be carefully evaluated and studied.

Apholate was selected for this study because it has been effective in very low concentrations in the sterilization of the male boll weevil (Hedin et al. 1964, Lindquist et al. 1964) and has shown effectiveness on a broad spectrum of organisms (Alexander 1960).

The objectives of this investigation were:

1. to determine which sex, stage and age of the weevil was most susceptible to sterilization;
2. to determine the most effective concentration, i.e. the concentration causing the greatest sterility and the least mortality;
3. to record the gross changes in the size and shape of the reproductive organs as caused by apholate;
4. to determine the effect of apholate on the ability to compete sexually, including mating and sperm competition.

II. REVIEW OF LITERATURE

A. The Alfalfa Weevil

Since its discovery in the United States in 1904 (Titus 1910), the alfalfa weevil is now distributed through 15 western states (Cothran 1966) and 29 eastern states (U.S.D.A. Coop. Econ. Ins. Rpt. 1967). The history, bionomics, and control of the alfalfa weevil have been studied extensively. Cothran (1966) has published a bibliography of literature on the weevil.

1. Economic Importance

The large numbers of larvae found in alfalfa fields during the spring cause the most severe damage by feeding on leaves, growing tips and buds. In severe infestations they destroy the first cutting of alfalfa and retard the second cutting. Adults upon emergence may also cause considerable damage to alfalfa by skeletonizing leaves and girdling stems.

There are many reports indicating that the alfalfa weevil causes great damage. Campbell et al. (1961) said for North Carolina "If alfalfa is to be grown in this state, control of the weevil is essential." Evans (1959) reported "Control measures cost the farmers of Virginia an estimated $237,600 a year, but without these measures the losses would be almost 2-1/2 million dollars." It is presently considered to be the most important pest of alfalfa in most areas where it occurs. Cothran (1966) stated "It is a serious pest in nearly 5 million of the 25 million acres of alfalfa grown in these states with 1.5 million acres being so severely infested as to

require control programs at costs estimated to approach $10,000,000 per annum."

2. Taxonomy of the Alfalfa Weevil

The classification of the alfalfa weevil is as follows: (Borrar and DeLong 1964)

Order Coleoptera

Suborder Polyphaga

Superfamily Curculionoidea

Family Curculionidae

Subfamily Curculioninae

Genus *Hypera*

Species *postica* (Gyllenhal)

B. Sterilization

Blakiston's new Gould Medical Dictionary (Hoerr and Osol 1956) defined sterilization as "any procedure which renders an individual incapable of reproduction." Sterilization can be accomplished by either irradiation or chemosterilization.

1. Irradiation and Chemosterilization

Irradiation involves the use of ionizing radiation to cause sexual sterility in organisms. Runner (1916) observed that the cigarette beetle, *Lasioderma serricorne* produced infertile eggs after exposure to X-rays. Muller (1927) found that radiation would induce mutation in *Drosophila*. In 1938 Knipling proposed a theory that insects might be reared, sterilized and released in sufficient

numbers (exceeding the natural population) so that upon interbreeding with the natural population sterile males will compete for natural females and produce infertile matings. The theory led to one of the outstanding achievements in applied entomology when Bushland and Hopkins (1951, 1953) induced sterility in the screw-worm fly, Cochliomyia hominivorax (Coquerel). This was followed by the successful eradication of this fly from the island of Curacao (Baumhover et al. 1955) and later from the southeastern United States (Lindquist 1959, Knipling 1960). The success of this program stimulated research for a method to replace radiation in order to obtain a more flexible and economical method of achieving sterility.

Certain chemicals have been shown to produce sterility similar to that caused by irradiation. Goldsmith and Frank (1952) first discovered that the folic acid antagonist, aminopterin (4-amino pteroylglutamic acid) induced sterility in the female fruit fly, Drosophila melanogaster (Meig). In 1959 Knipling listed several theoretical advantages of using chemicals to induce sterility in a large segment of the natural population. Alexander (1960) reported that certain compounds which caused an alkylation reaction produced biological effects similar to those caused by ionizing radiation. In 1961 LaBrecque showed that three aziridinyl derivatives--aphamide, tepa and apholate--caused sexual sterility in both male and female house flies. He designated these compounds as "chemosterilants." Hence chemosterilants can be defined as chemicals capable of causing sexual sterility, i.e. they prevent reproduction in insects or other organisms (Smith et al. 1964). Chemosterilants may act in one of

three ways: by causing a failure in the production of sperms or ova; by destruction of living sperms or ova; or by damaging the genetic material in the sperms or ova (lethal mutation) (Smith et al. 1964).

The actual mechanism by which irradiation or chemosterilization causes changes in living cells is not well known. Much research is being conducted to compare methods of irradiation with those of chemosterilization. The results indicate that sterilization by both appear to be similar.

A review of the effects of radiation on insects has been published by Grosch (1962). Smith et al. (1964) have reviewed the insect chemosterilants. In 1965 LaBrecque published a report about insect population control by the sterile-male technique. LaBrecque and Smith (1967) published a comprehensive volume, "Principles of Insect Chemosterilization", which covers all phases of chemosterilization at present time.

2. Sterilization in Coleoptera

Sexual-sterility research has been initiated in many species of insects, including Diptera, Lepidoptera, Coleoptera, Homoptera, Hemiptera and Orthoptera. Among Coleoptera several species have been studied. A very high degree of sterility of the boll weevil, Anthonomus grandis Boheman, was obtained by three dippings in 0.5, 1.0, or 2.0 per cent apholate (Lindquist et al. 1964). Sterilization was also accomplished by allowing boll weevils to feed on a diet containing from 0.001 to 0.020 per cent of apholate or on plants sprayed with 0.5 and 2.5 per cent solutions of apholate (Hedin et al. 1964). The adult male and female Mexican bean beetle, Epilachna

varivestis Mulsant, when dipped in an aqueous solution of 0.5 per cent apholate, or confined for 48 hours on foliage sprayed with the same concentration, were completely sterilized (Henneberry et al. 1964).

The following Coleoptera have also been studied, either by irradiation or chemosterilization:

plum curculio, Conotrachelus nenuphar (Herbst) (Hays and Cochran 1964, Roach and Buxton 1965)

Trogoderma glabrum Herbst (Tilton et al. 1966a)

banded cucumber beetle, Diabrotica balteata LeConte (Creighton et al. 1966)

Japanese beetle, Popillia japonica Newman (Ladd 1966)

alfalfa weevil, Hypera postica (Gyllenhal) (Burgess and Bennett 1966)

sweetpotato weevil, Cylas formicarius elegantulus (Summer) (Walker, 1966)

black vine weevil, Brachyrhinus sulcatus (F.) (Cram 1967)

cereal leaf beetle, Oulema melanopus (L.) (Ezueh and Hoopingarner 1967)

lesser grain borer, Rhyzopertha dominica (F.) (Tilton et al. 1966b)

confused flour beetle, Tribolium confusum Jacquelin duVal (Tilton et al. 1966b)

rice weevil, Sitophilus oryzae (L.) (Tilton et al. 1966b)

cigarette beetle, Lasioderma serricorne (F.) (Tilton et al. 1966b)

LaBrecque (1965) listed other five species in his report --

Colorado potato beetle, Leptinotarsa decemlineata (Say)

bean weevil, Acanthoscelides obtectus Say

pea weevil, Bruchus pisorum (Linnaeus)

weevil, Rhynchites cupreus var. auratus

granary weevil, Calandra granaria L.

3. Apholate Used as a Chemosterilant

Apholate has been one of the most widely used of the alkylating agents, since it is effective in very low concentrations (LaBrecque 1961, Hedin et al. 1964) and is effective on a broad spectrum of organisms. It causes mutations in organisms from bacteriophages to mammals (Alexander 1960). It has been reported to sterilize many Diptera (screw-worm fly -- Lindquist 1961; house fly -- LaBrecque 1961; fruit fly, melon fly and Mediterranean fruit fly -- Keiser et al. 1965), Lepidoptera (Cabbage looper -- Howland et al. 1965; gypsy moth -- Collier and Downey 1965; fall army worm -- Young and Cox 1965; tobacco budworm -- Soto and Graves 1967), greenhouse spider mites, Tetranychus telarius (L.) and T. cinnabarinus (Boisduval) (Smith et al. 1965), Coleoptera such as the Mexican bean beetle (Henneberry et al. 1964), the boll weevil (Hedin et al. 1964, Lindquist et al. 1964, Haynes 1966, etc.), the plum curculio (Roach and Buxton 1965), the Japanese beetle (Ladd 1966), the banded cucumber beetle (Creighton et al. 1966), the black vine weevil (Cram 1967), and the cereal leaf beetle (Ezueh and Hoopingarner 1967).

Apholate can be applied to insects in several experimental

methods. Hedin et al. (1964) sterilized male boll weevils by incorporating apholate into their diet or spraying it on cotton plants. Lindquist et al. (1964) sterilized male boll weevils with apholate by dipping them in a solution, by applying it topically, by including it in their diet, and by exposing them to residues on glass. It has been effective against both sexes (LaBrecque 1961, Lindquist 1961). In Coleoptera, however, most research was done by treating males only. Ladd (1966) reported that application of apholate to males and to both sexes of Japanese beetles resulted in a high degree of egg infertility, but application of the same compound solely to females did not appear to inhibit production of fertile eggs.

C. Development of the Sex Organs

1. The Alfalfa Weevil

The earliest work on the sexual development of the alfalfa weevil probably was done by Snow (1928). He described ovulation in the weevil throughout the year in Utah. Manglitz (1958) noted that no ovarian development occurred during estivation in the alfalfa weevil. Guerra and Bishop (1962) reported in considerable detail the effect of estivation on the sexual development in the female weevils from June to November. Tombes (1964) described more detail in a complementary account of the reproductive systems of both sexes of the weevil from fall to summer.

2. The Chemosterilant-treated Insects

There have been several reports on the influence of chemosterilants on the reproductive systems in insects. Morgan and

LaBrecque (1962) reported that when apholate was administered in the food of newly emerged adult female house flies at a 1.0 per cent concentration it inhibited, but did not eliminate ovarian development. In laboratory studies, Lindquist et al. (1964) described that the testes of the boll weevil were decreased in size after the weevils had been dipped once in a 1 per cent apholate solution. Henson (1966) sterilized 7-hr-old face flies with 2 per cent apholate in comparison with the check and found that while the untreated ovaries continued to develop to the point of oviposition, the treated ovaries did not develop normal eggs at the end of 7 days. He stated the treated flies eventually deposited the eggs but the larvae either did not hatch or if they did hatch, they did not live to form pupae. He also noted no significant differences in the sizes of the treated and untreated testes. Smittle et al. (1966) injected 5 ug of tepa into male German cockroaches which stopped spermatogenesis and caused the testes to atrophy. When 10 ug of tepa was injected into female German cockroaches, the oocytes were significantly smaller than the check females. Tepa also caused abnormal oocyte development and partial deterioration of the ovaries. In 1967 Hedin et al. reported that decreases in the size of the testes and changes in morphology and cytology occurred when the boll weevils were injected with 1 or 3 ug of tepa.

D. Mating Studies of Sterilized Insects

To insure success of control programs that utilize the sterile-male technique, sterile males must be able to compete with normal

males. Weidhaas and Schmidt (1963) described that males of Aedes aegypti sterilized by feeding on 1 per cent of apholate in honey solution successfully inseminated normal females; and when normal males and females were caged at various ratio with sterile males, the chemosterilized males mated as readily as the normal males and were equally competitive with them. Lindquist et al. (1964) reported that a ratio of treated to untreated male boll weevils of at least 19:1 would have been necessary to reduce the egg hatch to 10 per cent during the first 10 days after treatment. A higher ratio of treated and untreated males probably would be necessary at a later time since treated male weevils often regained fertility from 10 to 20 days after treatment. Chang (1965) showed full sterilization occurred in about 3-1/2 hr. by injecting 1 ug of tepa into male house flies. The male flies remained sterile for about one week. Partial recovery of male fertility occurred thereafter. Ouye et al. (1965) stated that the male pink boll worms, sterilized with 15 ug metepa, were fully competitive with normal males in mating with females at all ratios tested, but some reduction in ability to compete was exhibited when males were sterilized with 50 ug, the highest dosage investigated. Tilton et al. (1966a) irradiated virgin male Trogoderma glabrum Herbst with 25,000 rads of gamma irradiation. When an irradiated male and a nonirradiated male were placed with a virgin female, 56 per cent of the females produced eggs, as compared with 97 per cent for the females with a nonirradiated male only. Davich et al. (1965) in their preliminary field test with a sterile-male-release program concluded that the sterility principle could be applied for the

elimination of a boll weevil population if a high ratio of sterile insects was provided, even though the sterile males were low in mating competitiveness. Howland et al. (1966) described in cabbage looper the increasing sterile male release ratios required to decrease the number of larval progeny. At the highest ratio of 20 sterile males to one untreated male and female each, larval populations were reduced 97 per cent as compared with those in the check. Gilliland and Davich (1966) used virgin female boll weevils to mate alternately to apholate-treated and untreated males. They concluded that the initial mating in the sequence had little effect on over all egg hatch, while the last mating prior to oviposition was most influential on subsequent egg viability. Lindquist and House (1967) made similar studies and reported that considerable mixing of sperm occurred during the alternate mating of female boll weevils with normal and apholate-treated males. They also stated that the second mating was more effective than the first, and sperm from the last mating prior to egg deposition appeared to fertilize most of the eggs laid at that time.

III. MATERIALS

A. Test Insects

The alfalfa weevils used in these studies were collected from alfalfa fields near Blacksburg, Virginia and reared in the insectary. The field-collected weevils referred to hereafter as the diapaused weevils generally have one generation a year, followed by a diapause of 3 to 5 months. The laboratory-reared weevil, referred to hereafter as the nondiapausing weevils, can be continuously reared by maintaining the larvae under short daylengths. They will complete 6 generations within a year (Huggans 1964).

1. The Diapaused Adult Alfalfa Weevil

In Virginia the adult weevils emerge from cocoons in April and May, feed, then migrate to neighboring woods, or fields containing sod, for estivation for about 3 to 5 months, and reappear in the alfalfa fields again for oviposition in the early fall. The adults were collected in the late spring as well as early fall and placed in the insectary where field conditions were approximated. The spring-collected adults undergo diapause whereas the fall-collected adults have already completed their diapause.

2. The Larval Alfalfa Weevil

The majority of larvae and pupae were collected in the field in April and May. Some larvae, however, were also collected in the field during the summer. Larvae were reared to adults in the same

laboratory. Hence the age of newly-emerged adults could be accurately determined.

3. The Nondiapausing Adult Alfalfa Weevil

Most of the alfalfa weevils used in these studies were taken from the colony reared and maintained in the greenhouse under conditions modified from Huggans and Blickenstaff (1964). Laboratory colonies of the alfalfa weevils were started from larvae collected in the fall. The weevils were reared in the greenhouse on bouquets of alfalfa in quart ice-cream cartons with cheesecloth and polyethylene lids. Several colonies were reared to adults under a diurnal cycle of about 15 hours dark at 19-20°C and 9 hours light at 26-27°C.

In the rearing program the mating chambers contained 3 males and 7 females. Fresh alfalfa stems, which were cut from the greenhouse, with bases inserted through the chamber bottom into water, were supplied to the weevils weekly for food and oviposition sites. Old alfalfa stems were dissected to collect the eggs. Five hundred to 700 eggs were kept in a petri-dish on filter paper with a sponge underneath to keep both paper and eggs moist. First instar larvae were removed from the petri-dish and placed in quart ice-cream cartons and fed with fresh alfalfa. After one week, late instar larvae were transferred to gallon cartons, fed with fresh alfalfa every 3 days, and allowed to pupate and emerge. Newly-emerged adults were collected at 1-week intervals and reared in other gallon cartons with fresh alfalfa every 3 days. Three weeks later these

adults were removed to a cold cabinet (4°C) for storage. They were removed for 24 hours and fed with fresh alfalfa at 2-week intervals. All experiments using nondiapausing weevils were conducted in the diurnal cycled growth chamber. Moisture was available at all times. The life cycle of these non-diapausing weevils was as follows:

Eggs -- 7 - 12 days

Larvae -- 21 - 28 days

Pupae -- 7 - 10 days

Adults -- oviposition at 6 - 7 weeks.

These periods were approximate due to the variation among the weevils.

More than 8,000 nondiapausing adults were successfully reared in the laboratory.

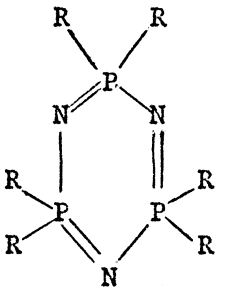
In tests in which virgin unmated weevils were required, newly-emerged adults (1-week old) were sexed to male and female groups before the treatment or experiment was initiated.

B. Apholate

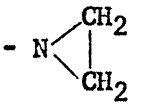
The official name, structure, chemical and physical properties of apholate were given in the Olin Mathieson Chemical Corporation technical data sheet "Experimental Insect Sterilant No. 2174 Apholate" (1964) as follows --

The official chemical name: 2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine.

Structural formula:



where R is -



Other designations: SQ 83388 or USDA ENT. No. 26,316.

Physical properties:

Form - White powder;

Melting point - approximately 150°C

Molecular formula - $C_{12}H_{24}NgP_3$

Approximate solubility (% weight) - water 33%, methylene chloride 33%; Chloroform 31%; methanol 14%; ethanol (70%) 15%, acetone, xylene, mineral oil and hexane less than 1%.

Stability - moisture, high temperature or low pH cause polymerization or inactive apholate.

Apholate used in these studies was manufactured by Olin Mathieson Chemical Corporation in two different samples from Squibb Institute for Medical Research through Dr. R. L. Pienkowski and Dr. J. A. Hair. Apholate was stored at 0°C.

IV. METHODS, RESULTS AND DISCUSSION

A. Sterilization of the Alfalfa Weevil

Some adult weevils were treated by topical application of apholate but larvae and most adults were treated by a dipping method.

1. Adult Topical Application Experiments

In the topical treatment 90 diapaused unmated males, reared from field collected larvae, were treated topically on the dorsum of the prothorax and mesothorax with 3.0 ug of apholate in 1.0 ul of benzene per insect as described by Lindquist et al. (1964). The males were divided into 3 groups and kept in petri dishes for 24 hours before the test was initiated. Ten treated males then were caged together with the same number of virgin diapaused females in a quart-carton and placed in the insectary as previously described. Treatments were replicated 3 times. The dead weevils were replaced whenever it was possible. From a total of 558 eggs collected weekly, at random, from stems in the three mating cartons over a 4-week period, 392 eggs hatched. Thus the egg viability was 70.25%, while 88.5% egg viability was observed in the check. The mortality of the treated males by the end of the 4th week was 75% while that of the untreated females, which mated with the treated males, was 30%. In the check the mortality of the normal males was 32%, while that of the females was 28%.

Although apholate was only partially effective in sterilizing the male weevils (by using topical application of 3.0 ug), the mortality of the treated individuals was fairly high.

2. Larval and Adult Dipping Experiments

In order to determine which stage of the weevil was most susceptible to sterilization, larvae (field-collected) and adults (both diapaused and nondiapausing) were sterilized by dipping in an aqueous-acetone-apholate solution (water: acetone = 1:1). A small amount of nontoxic detergent or Triton X 100 (Lindquist et al. 1964) was added as a wetting agent.

a. Larval Dipping Tests. - 100 4th-instar larvae each were dipped and swirled in 0.2% or 0.4% apholate solutions or aqueous-acetone solution (check) for about 30 seconds. The solution was poured off through cheesecloth, and the larvae were transferred to a petri dish and kept there for 6 hours before removal to a gallon-carton with fresh alfalfa. Adult emergence from the carton with 0.2% and 0.4% apholate-treated larvae was 70% and 36%, respectively. Adult survival to sexual maturity was 54% and 30%. Adult emergence in the check was 81% and survival to mature was 65%. They were divided into three replicates. Each replicate had 8 pairs of adults from the 0.2% larval treatment, 5 pairs of adults from the 0.4% larval treatment, and 10 pairs of adults in the check.

From a total of 674 eggs collected weekly at random from the three 0.2% replicates over a 4-week period, 80.1% hatched while 82.5% egg viability was observed in the check. The total mortality from larva to mature adult was 65.0% for the 0.2% apholate treated weevils and 40-50% for the check.

Of a total of 797 eggs collected weekly at random from the three 0.4% replicates over a 4-week period, 73.5% of the eggs hatched. The total mortality from larva to adult was 80%.

Larval dipping tests appeared to produce a high larval mortality, decrease emergence of adults and slightly reduce percent viability of eggs laid by the adults which emerged from apholate-treated larvae.

b. Adult Dipping Tests. - The sterilization procedure was the same in the various tests using adults treated by dipping. Weevils were anesthetized by chilling for 3 to 5 minutes and then dipped in a beaker containing an aqueous-acetone-apholate solution, swirled for about 30 seconds, and the solution was poured off through cheesecloth. Weevils on the cheesecloth were removed to paper toweling for a few seconds for absorption of excess solution. Following this they were transferred to a petri dish and kept there for 24 hours before being placed with males or females in a mating carton. Enough weevils were treated for each test so that the dead weevils during the test period could be adequately replaced (Lindquist et al. 1964). The initial numbers of males and females were maintained in the mating carton throughout the test period whenever possible.

1) In the test to determine the age of the weevil most susceptible to sterilization, diapausing and nondiapausing weevils were reared from field-collected larvae. These adults

were treated at three different ages (reproductively mature, in diapause, and newly-emerged from the pupa) by dipping in 0.25, 0.5, and 1.0% apholate solutions. The eggs for each test were collected weekly and the percent egg viability was recorded during a 4-week test period.

a) The results from the treatment of three replicates of adults (both sexes) are shown in Table 1. In these tests a single, simultaneous treatment of both sexes with either 0.5% or 1% apholate solution caused a decrease in egg laying for a few weeks, but a gradual recovery occurred. The percent egg viability was reduced by the apholate concentrations used.

b) When newly-emerged adults and adults in diapause were treated with different concentrations of apholate, results were inconclusive. Due to the fact that neither group was sexually mature at the time of treatment, and had been in diapause during the summer subsequent to treatment, the mortality was so high that it was not possible to determine the sterilization effects.

2) In a test to determine which sex was most susceptible to sterilization, or whether treatment of both sexes improved results, and which was the most effective concentration (causing the greatest sterility and the least adult mortality), several tests were conducted by dipping virgin mature (at least 2 months old) nondiapausing weevils in a 0.25, 0.5, 0.75, 1.0, 1.5 or 2.0% apholate solution. Three different

Table 1. Effects of apholate treatment of both sexes of diapaused reproductive adult alfalfa weevils on % egg viability.[1/]

Apholate Conc. (%)	% Adult Mortality		No. of Eggs Collected	% Egg Hatch
	Female	Male		
0.25	20	25	462	58
0.5	35	45	296	23
1.0	50	65	226	11
Check	10	10	610	89

[1/] The test was replicated 3 times using 10 pairs of weevils per replicate.

treatments were used: only females treated, only males treated, and both sexes treated. For each test 108 to 144 weevils of each sex were treated at each different concentration. Twelve pairs in each replicate were kept in quart mating-cartons. The same number of check (or control) weevils were treated with the aqueous-acetone solvent. The eggs were collected weekly from these cartons and the percent egg viability was recorded during a 10-week period. A total of 3 replicatesfor the 0.25 and 0.75% apholate treatments and 4 replicates for the 0.5, 1.0, 1.5 and 2.0% treatments were used. The results are shown in Tables 2, 3 and 4. When females alone had been treated with apholate concentrations ranging from 0.25 to 2.0%, the weevils continued to produce viable eggs close to the percent viability found in the check (Table 2, Figure 1). Thus apholate appeared to have little effect in sterilization of the female weevils. These results are similar to those obtained by Ladd (1966), who observed the effects of apholate on the female Japanese beetles.

In the apholate treatment of females, egg viability with concentration 0.5% and check were different from that observed with concentration 1.0 and 2.0% as shown in Appendix Table IA1. Egg viability in the first 2 weeks was significantly lower (1% level) than that observed later (Appendix Table IA2). The 0.25% and 0.75% concentrations were statistically analyzed separately from other concentrations since application was

Table 2. Effect of apholate treatment of female alfalfa weevils on % egg viability.[1/]

Apholate Conc.(%)	0.25		0.5		0.75		1.0		1.5		2.0		Check	
Week	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch
1	241	64.32	451	74.50	306	51.31	285	55.79	307	57.65	282	51.77	300	55.00
2	461	79.18	1036	89.38	616	68.02	416	78.85	280	84.29	220	80.91	973	71.74
3	423	86.29	1186	94.01	440	79.77	578	92.73	772	93.01	553	91.22	387	90.03
4	362	91.71	677	92.61	285	94.04	541	84.66	539	92.02	209	92.34	532	89.85
5	409	89.49	434	91.94	344	87.50	560	86.96	287	82.93	262	91.68	821	92.69
6	491	92.06	637	86.76	378	90.21	780	92.44	911	89.02	534	84.83	888	94.82
7	316	94.62	811	92.73	439	92.03	784	90.43	713	93.13	500	93.00	626	96.17
8	417	88.73	587	92.50	446	94.17	490	86.73	573	92.15	247	86.64	483	96.27
9	424	93.40	442	92.08	357	89.92	429	92.31	402	93.78	244	89.34	524	95.42
10	361	91.41	462	91.77	322	87.58	521	93.86	314	88.54	401	90.52	396	97.93

[1/]Tests using concentrations of .25% and .75% were replicated 3 times, all others were replicated 4 times.

Table 3. Effect of apholate treatment of male alfalfa weevils on % egg viability.[1/]

Apholate Conc.(%)	0.25		0.5		0.75		1.0		1.5		2.0		Check	
Week	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch
1	525	50.10	375	18.93	295	18.98	639	15.34	528	8.33	248	0	300	55.00
2	664	70.48	727	4.40	458	7.21	796	6.66	960	1.35	399	0.25	973	71.74
3	585	51.80	897	4.79	461	6.51	740	5.27	1052	0.57	872	0.34	381	90.03
4	640	66.41	491	17.52	315	11.11	582	8.25	572	0.70	651	1.54	532	89.85
5	678	69.17	434	29.95	414	20.05	341	15.84	560	0.54	556	0[2/]	821	92.69
6	475	70.01	474	36.29	640	20.00	476	22.06	583	0.17	423	0	888	94.82
7	600	67.50	592	39.36	392	38.27	376	27.39	578	0[2/]	449	0	626	96.17
8	424	74.76	496	52.82	322	45.03	653	32.62	381	0	125	0	483	96.27
9	602	79.90	539	64.20	308	56.49	503	36.98	344	0	272	0	524	95.42
10	415	85.06	595	72.77	489	64.21	492	47.56	243	0	280	0	396	97.93

[1/] Tests using concentrations of .25% and .75% were replicated 3 times, all others were replicated 4 times.

[2/] The mortality of males in mating chamber was extremely high (almost 100%) at 5 weeks after treatment.

Table 4. Effect of apholate treatment of both sexes of the alfalfa weevil on % egg viability.[1/]

Apholate Conc.(%)	0.25		0.5		0.75		1.0		1.5		2.0		Check	
Week	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch	No. of Eggs Collected	% Egg Hatch
1	396	47.22	265	13.60	365	14.80	494	12.55	301	4.98	152	0	300	55.00
2	527	54.65	399	4.10	434	9.45q	355	5.35	459	2.18	439	0	973	71.74
3	681	27.17	310	5.37	355	11.27	322	3.42	523	0.38	508	0.20	381	90.03
4	651	41.48	598	12.54	668	7.64	422	8.77	437	0.23	582	0	532	89.85
5	641	67.24	264	18.67	627	13.40	389	11.05	397	0.25	527	0.19	821	92.69
6	570	53.68	372	26.34	409	15.89	307	10.75	428	0[2/]	378	0[2/]	888	94.82
7	580	61.55	229	31.40	693	21.79	327	23.55	347	0	305	0	626	96.17
8	621	68.12	277	38.27	449	34.74	305	29.84	270	0	330	0	483	96.27
9	398	74.37	346	47.40	444	48.20	288	35.42	294	0	307	0	524	95.42
10	442	82.13	281	55.16	454	52.64	253	39.92	280	0	244	0	296	97.93

[1/] Tests using concentrations of .25% and .75% were replicated 3 times, all others were replicated 4 times.

[2/] The mortality of the treated weevils, especially the males, in the mating chamber was extremely high (almost 100%) at 5 weeks after treatment.

made at different times and only three replicates were used with these two concentrations. The 0.75% concentration was significantly (5% level) more effective than the 0.25% concentration. Egg viability in the first 3 weeks was significantly lower (1% level) (Appendix Table IB).

Tables 3 and 4 show that the apholate was effective in sterilization of the male weevils. The effective concentration in sterilizing males appeared to range from 0.5 to 1.0% apholate solution. Above 1.5% the mortality of the treated adults was extremely high, especially 3 to 4 weeks after treatment. There was little difference in egg viability caused by treating both sexes as compared with the use of treated males and untreated females. A gradual increase in egg viability occurred starting at about the 3rd week and continuing to the end of the test (Figures 2, 3).

When evaluating the eggs which were collected from 4 single-pair of weevils in petri dishes 8 weeks after treatment, the egg viability was apparently higher than that from quart cartons which contained 2 to 12 pairs of weevils. This was true in all three different treatments. The reason was probably that the stems in the petri dish were more moist than in the cartons.

In the apholate treatment of males egg viability in the check was statistically different from that observed in all other concentrations, as was egg viability in the 0.5% and 1.0% concentrations. The 0.75% concentration was highly

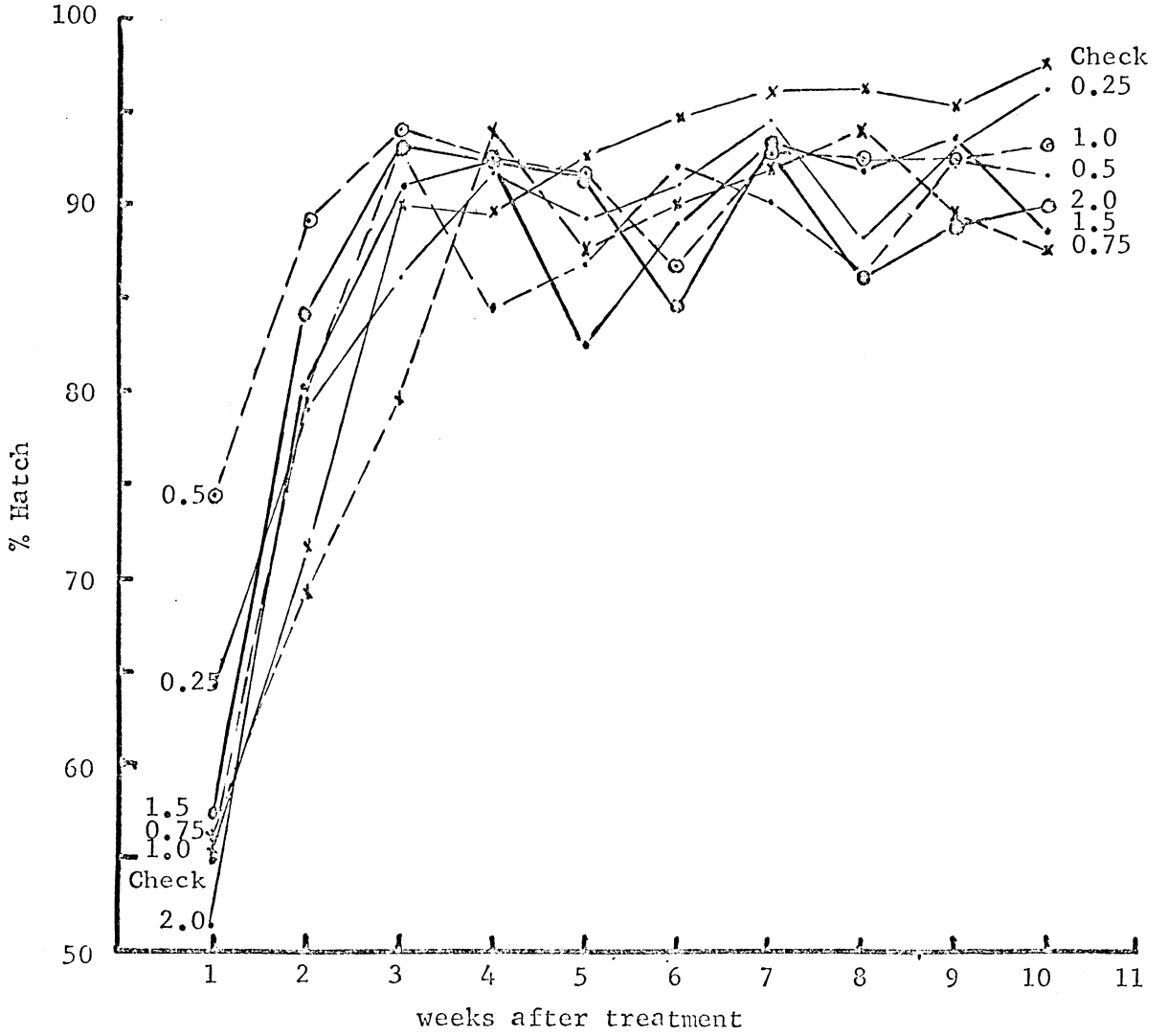


Figure 1. Effect of apholate treatment of female alfalfa weevils on % egg viability.

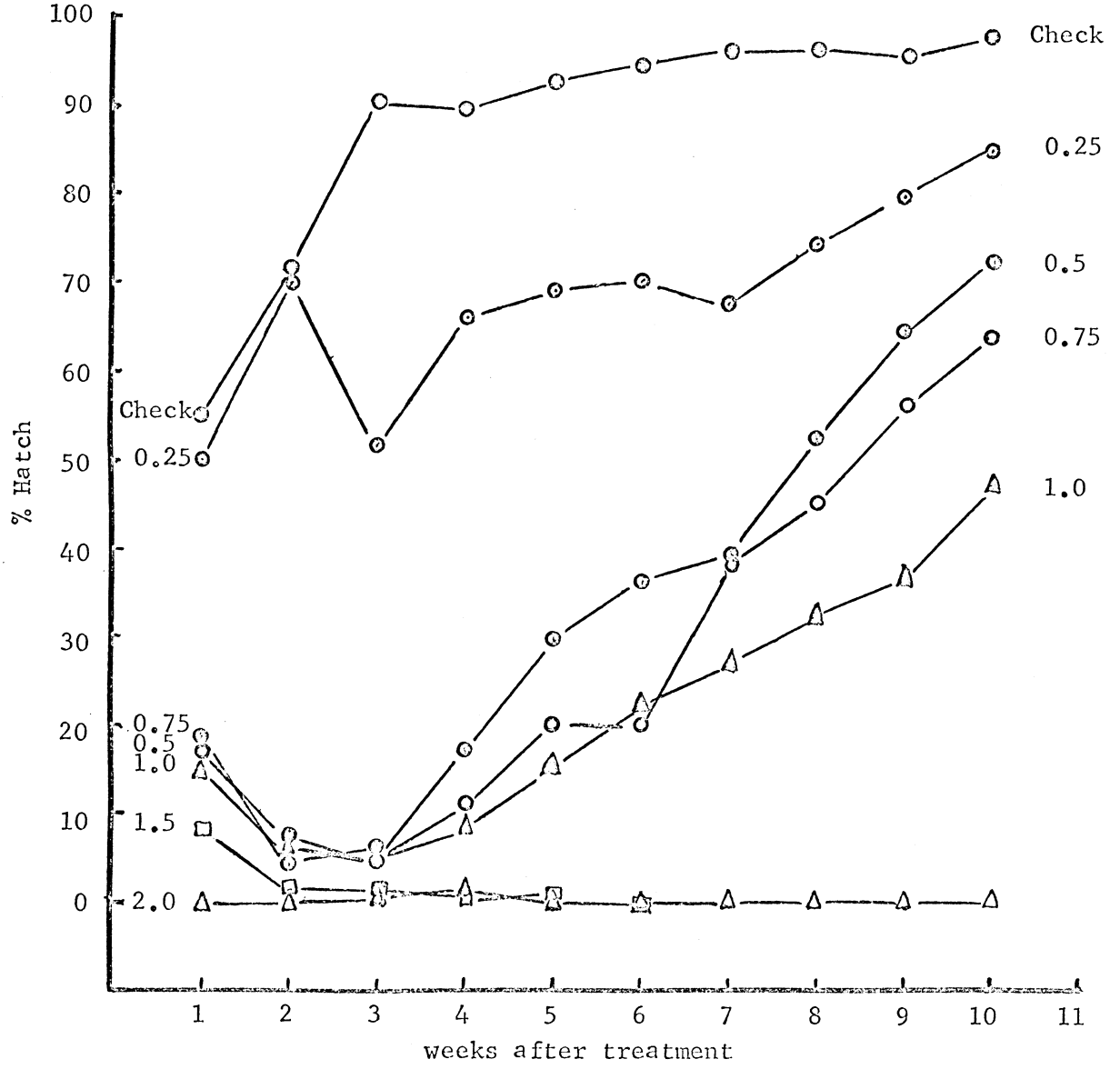


Figure 2. Effect of apholate treatment of male alfalfa weevils on % egg viability.

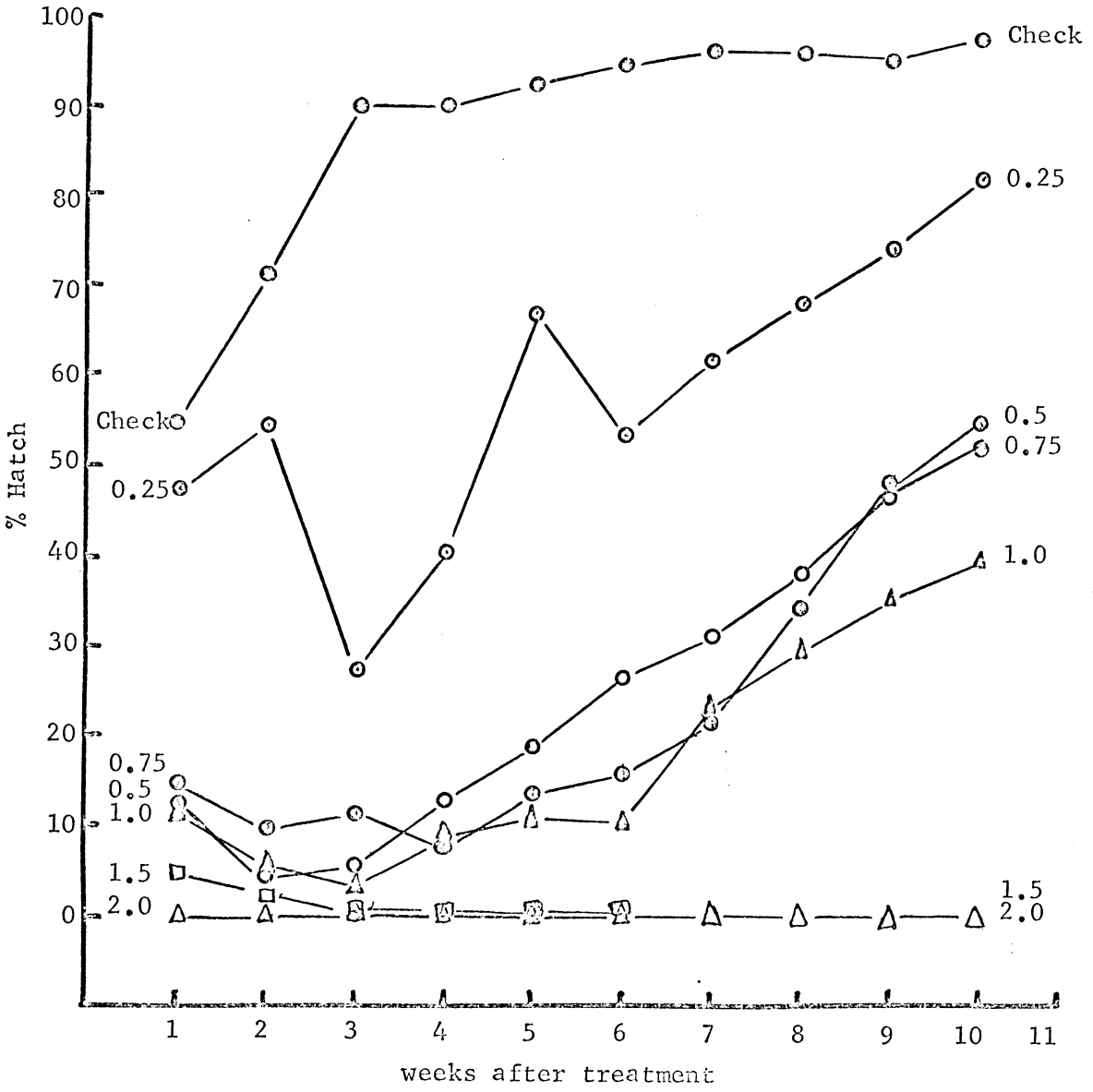


Figure 3. Effect of apholate treatment of both sexes of the alfalfa weevil on % egg viability.

significantly better than the 0.25% concentration when applied to males (Appendix Table IIA). There was a statistically significant increase in egg viability with time after treatment in both tests (Appendix Table IIA).

In the apholate treatment of both sexes egg viability in the check was statistically different from that observed in all treatments, and the viability was significantly lower in the 1.5% and 2.0% than in the 0.5% and 1.0% treatments. (Appendix Table IIIA). Egg viability again gradually but significantly increased with time after treatment (Appendix Table IIA). The 0.25 and 0.75% concentrations were also analyzed statistically (Appendix Table IIIB).

Total adult mortality of each sex at 10th week after different apholate treatment (only female treated, only male treated or both sexes treated) is given in Table 5. In general, the male mortality was higher.

With treated females and untreated males, female mortality produced by each concentration was statistically different from mortality produced in check female. Male mortality produced by the same treatment was not significantly different from check male mortality (Appendix Table IVA, B).

With untreated females and treated males, male mortality produced by each concentration was statistically different from mortality produced in check male. Female mortality produced by the same treatment was not different from check female mortality (Appendix Table VA, B).

Table 5. Total % adult mortality at 10 weeks after apholate treatment.[1/]

Treatment	Treated Females X Untreated Males		Untreated Females X Treated Males		Treated Males X Treated Females		Untreated Males X Untreated Fem.	
Apholate Conc.(%)	F	M	F	M	F	M	F	M
0.25	44.44	21.30	19.44	50.93	49.07	52.78	20.14	22.92
0.5	54.17	18.06	13.89	60.42	54.86	63.89		
0.75	63.89	19.44	17.59	68.52	60.19	69.44		
1.0	66.67	19.44	17.36	73.61	62.50	70.14		
1.5	69.44	20.14	17.36	80.56	70.83	79.17		
2.0	78.47	20.83	18.75	84.03	76.39	82.64		

[1/] 0.25 and 0.75% with 3 replicates, the rest with 4 replicates.

With treated females and treated males adult mortality produced by each concentration was statistically different from mortality produced in check adults (Appendix Table VI A, B).

Therefore, apholate does cause significant mortality with all treatments and concentrations used.

B. Studies of the Reproductive Organs

The general morphology of the internal reproductive organs in the nondiapausing alfalfa weevil and the effect of apholate on them was studied by dissecting adult weevils. The development and changes in the normal and apholate-treated reproductive organs was observed in immature and mature adults.

In the studies of the gross changes in the general size and shape of the reproductive organs as caused by apholate, two tests were conducted.

1. Sterilization Procedures

a. Tests using immature nondiapausing adults: 700 two-week old adults were collected, and placed in 2 gallon cartons. One carton contained 500 weevils (250 males and 250 females) which were treated by dipping in a 1% apholate solution as described above. The other contained 200 weevils (100 males and 100 females) and served as an untreated check. Both cartons were kept in a growth chamber programmed for a diurnal light-temperature cycle as described previously.

b. Tests using mature nondiapausing adults: 300 weevils (150 males and 150 females) were treated by dipping in a 1% apholate solution, and 30 weevils (15 males and 15 females) served as an untreated check.

2. Dissection Procedures

Fifteen treated and 15 untreated weevils of each sex were removed from the cartons at intervals of one week commencing 7 days after treatment. Subsequently, these weevils were placed in small vials filled with preservative (4:1:1 parts by volume of ethanol, acetic acid, and chloroform as used by Guerra and Bishop (1962)) and kept at 0°C. A minimum number of 6 weevils of each sex in each group was dissected each week. More than 144 weevils in each treatment were dissected. Sketches were made by using a camera lucida and photographs were taken of typical reproductive organs for each group.

Dissection was accomplished under a stereoscopic dissecting microscope using watchmaker's forceps and probes made from minutin pins. Dissecting dishes were made by filling petri-dishes or watch glasses half full of mineral wax. Weevils were embedded dorsal side up in the wax for dissection. Saline (NaCl 9.0, KCl 0.2, $CaCl_2$ 0.2, $NaHCO_3$ 0.2, and Glucose 2.5-5.0 grams in 1 liter water) (Roeder 1953) was added to the dish after embedding. All measurements and observations were thus performed under physiological saline with a dissecting microscope. The elytra and hind wings were first removed by carefully pressing on the suture behind the prothorax and the suture between the two elytra, and then pulling the wings loose. The

abdominal tergites were carefully stripped off using forceps. The digestive tract, fat bodies, muscular tissues and other internal organs were also carefully removed until only the reproductive organs were left in the abdominal cavity. It was found that if the weevils were decapitated before placing the insects in the preservative, fat bodies and tracheae were sufficiently hardened for easy removal, while the reproductive organs and muscles remained pliable enough to withstand considerable manipulation without breaking.

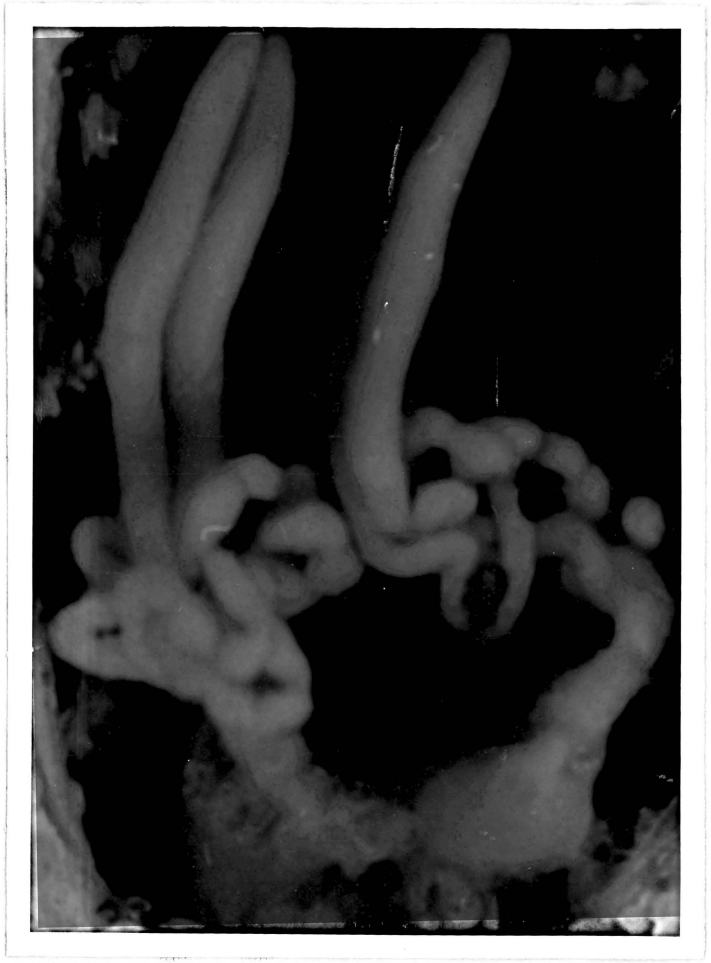
3. Gross Studies of the Reproductive Organs

The gross studies of the reproductive organs involved measuring the length of the left ovary and oviduct or the diameter of a testes of each weevil and recording these measurements for treated and untreated weevils at 1-week intervals for a period of 5 weeks. More than 60 organs in each treatment of each sex were measured with an ocular micrometer and then converted into millimeters by calibration with a stage micrometer. Photographs were taken at a magnification of 12X on Kodak Plus-X film in a Nikon M-35 camera which connected with a phototube and attached to a Wild-Heerbrugg M5 research microscope.

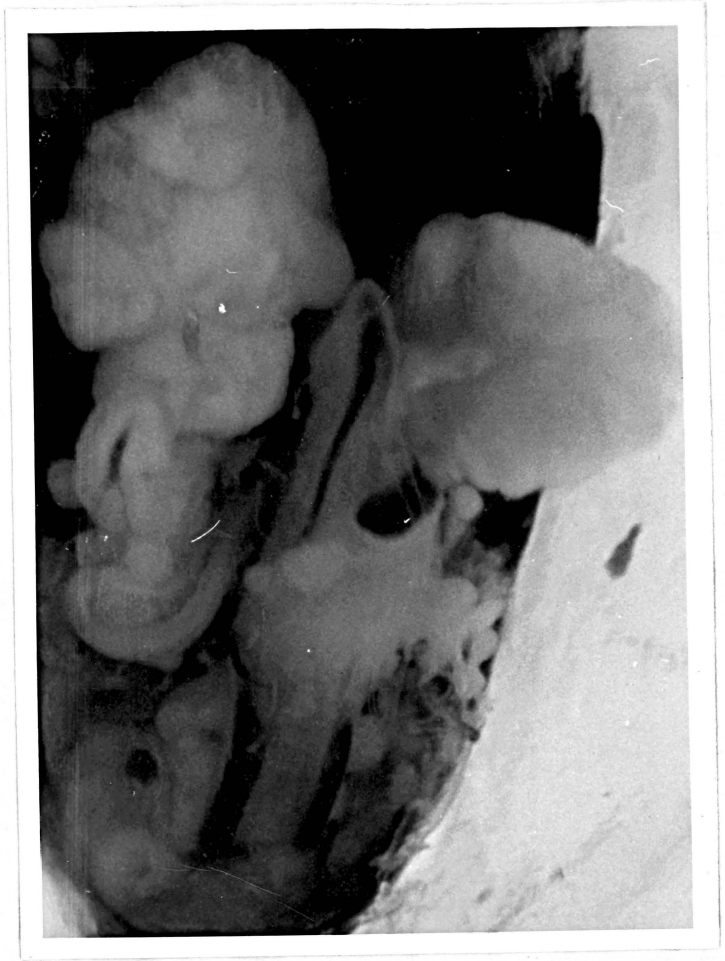
a. Normal Development of the Female Sex Organs. The general morphology of the reproductive organs of the alfalfa weevil (Figure 4 A, B) is similar to that of the boll weevil as given by Burke (1957), so it will not be described here.

Snow (1928) reported on the year-round ovulation sequence of H. postica as it occurred in Utah. He recognized three

major stages of ovarian development, naming them nondeveloped, fully developed, and spent. Tombes (1964) classified the female reproductive system only as fully developed or as non-developed. The time required for the main generation adults to attain sexual maturity has been stated to range from 3 to 5 months. However, with photoperiod control, the alfalfa weevil attains sexual maturity within 6 to 7 weeks as previously described. Measurements of the oviducts and ovaries from 2-week-old to 7-week-old weevils are summarized in Table 6. The sex organs from the 2-week-old to the 4-week-old female (Figures 5B, 6B) appeared little developed. From the data shown in Table 6 and Figure 10, there was only a slight increase in the size and length of the ovaries and oviducts during that period. These stages are similar to the "diapaused stages" described by Guerra and Bishop (1962). It was not until the weevil reached 5 weeks of age (Figure 7B) that the organs grew rapidly, especially in the ovaries. The systems of the 5-week-old females gradually took on the characteristics of fully developed weevils. There was a considerable increase in the length and width of the ovaries but oviducts remained short. This stage corresponds to the stage at the end of diapause described by Guerra and Bishop (1962), and Snow's (1928) "medium stage". In the 6-week-old weevil (Figure 8B) the ovaries were somewhat enlarged and the oviducts had considerably increased in length and started to form a loop as they extended. This stage may correspond to Snow's (1928)

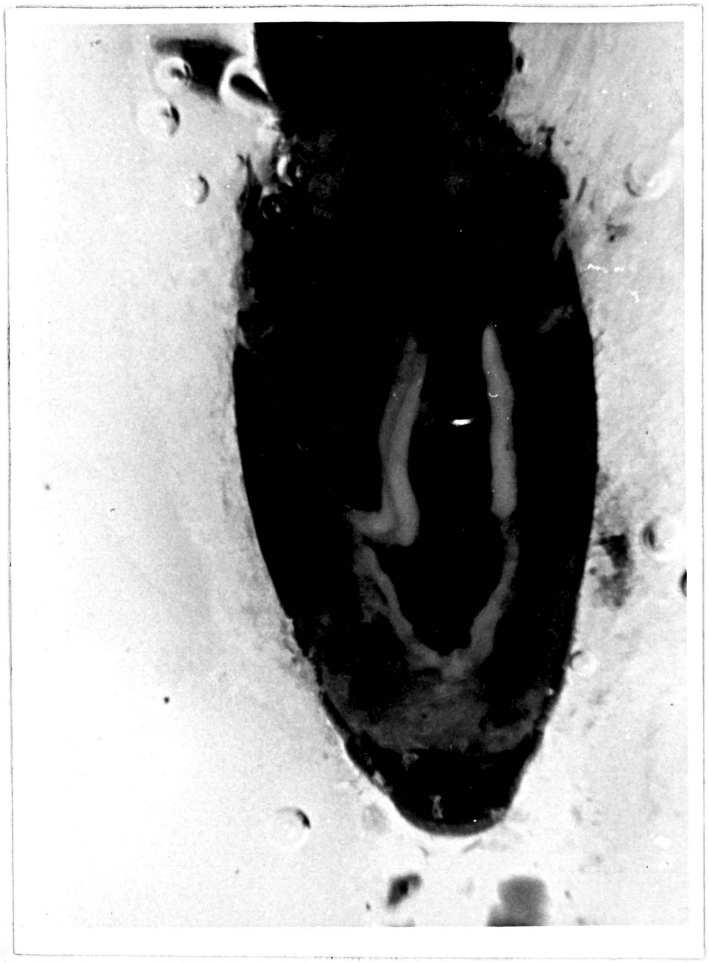


A. Mature ♀

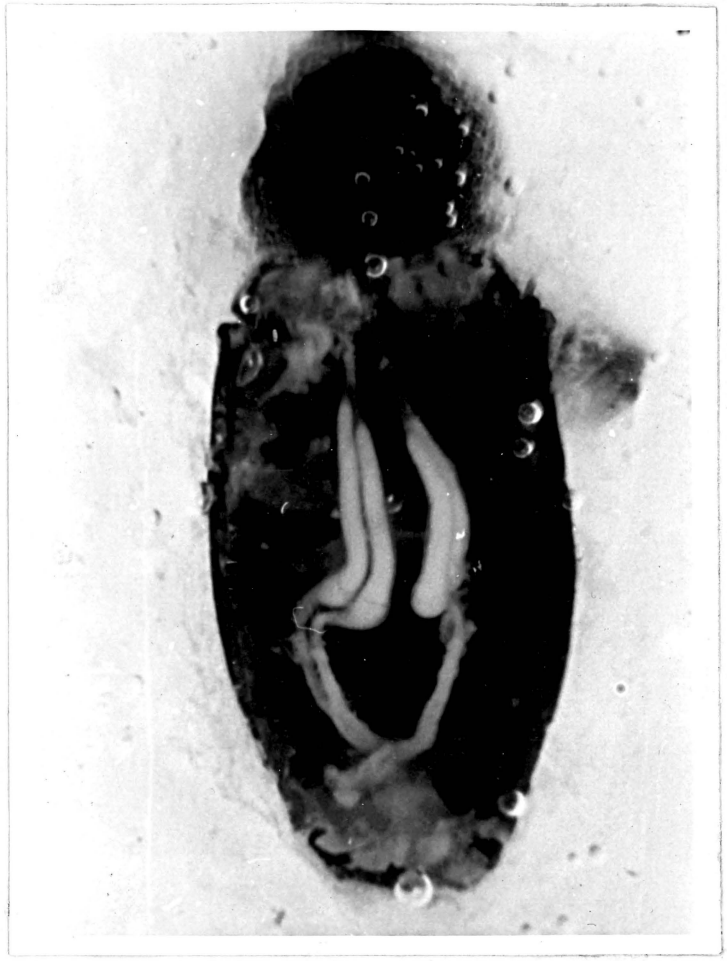


B. Mature ♂

Figure 4. The mature sex organs of the alfalfa weevil.



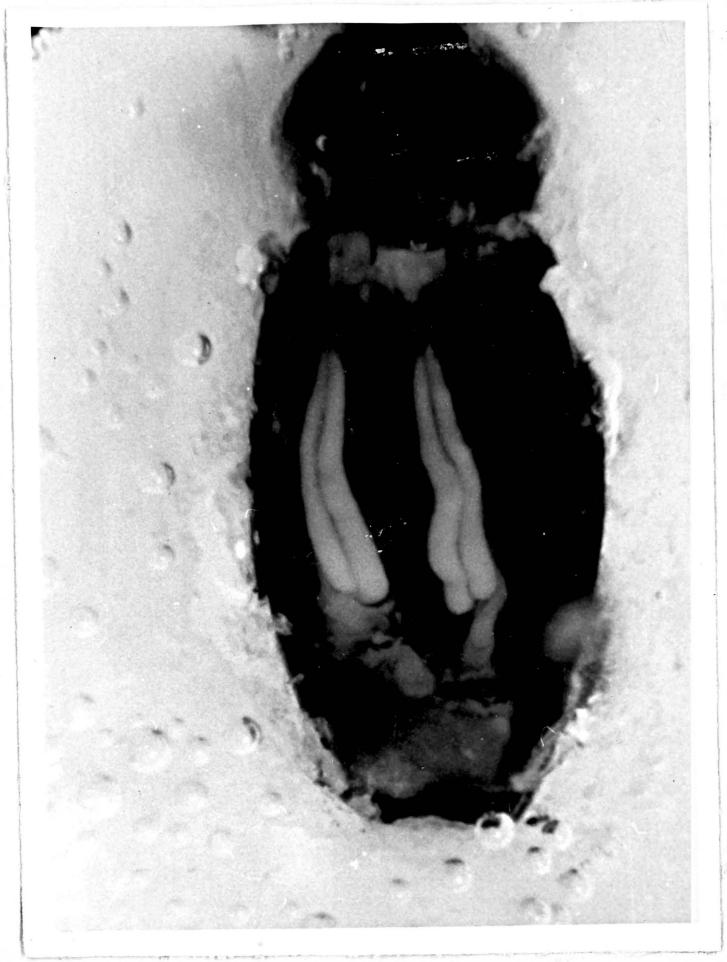
A. 3-week old treated ♀



B. 3-week old untreated ♀

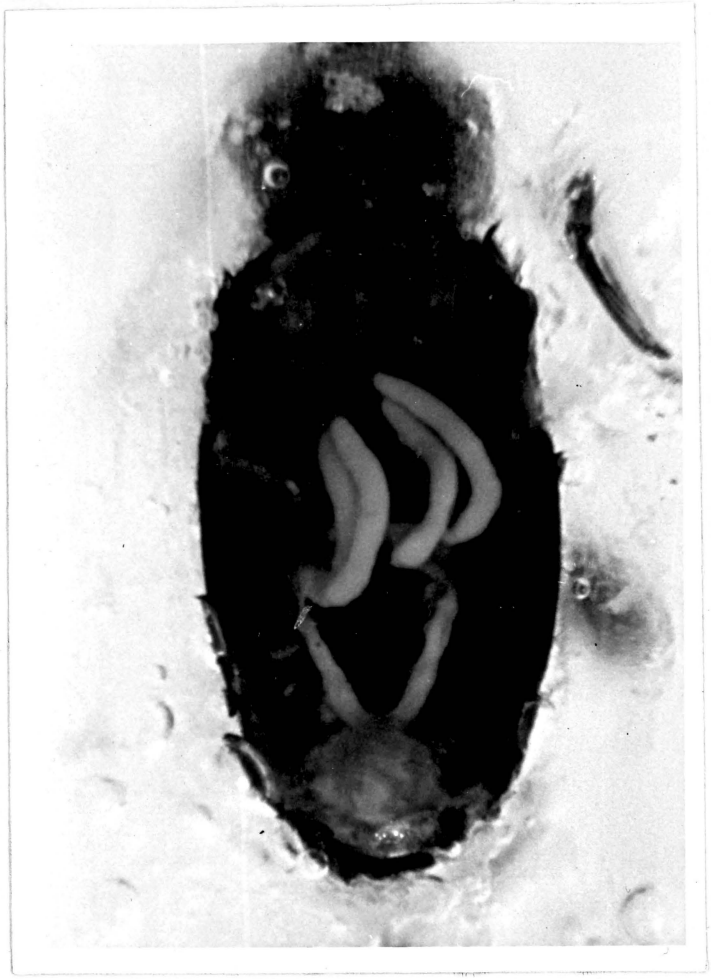
Figure 5. The sex organs of the apholate-treated and untreated female alfalfa weevil at the age of 3 weeks.

A. 4-week old treated ♀

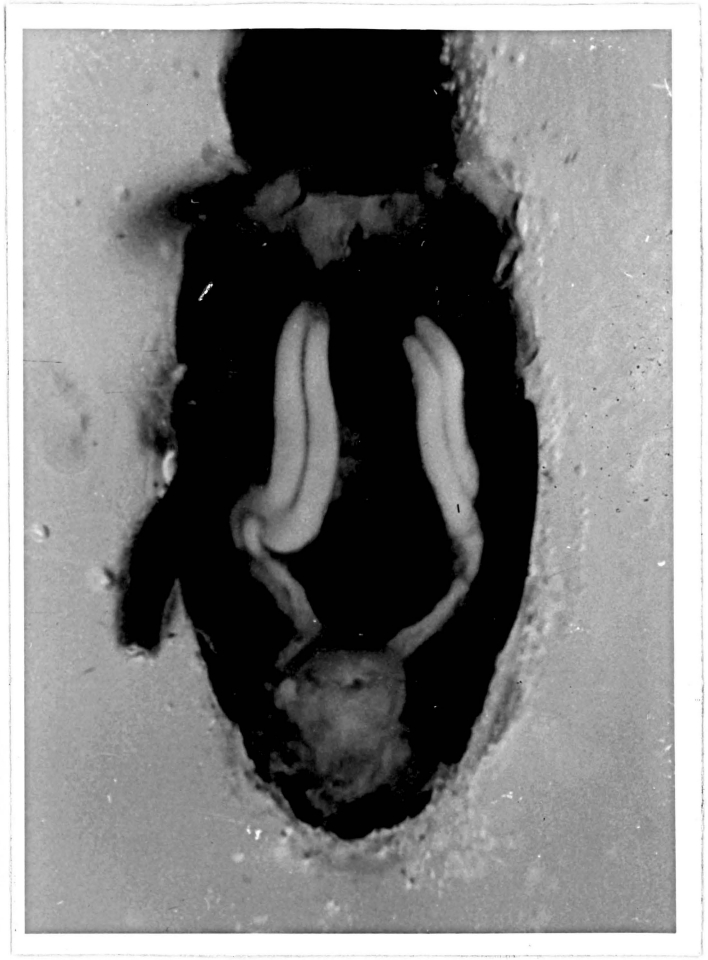


B. 4-week old untreated ♀

Figure 6. The sex organs of the apholate-treated and untreated female alfalfa weevil at the age of 4 weeks.

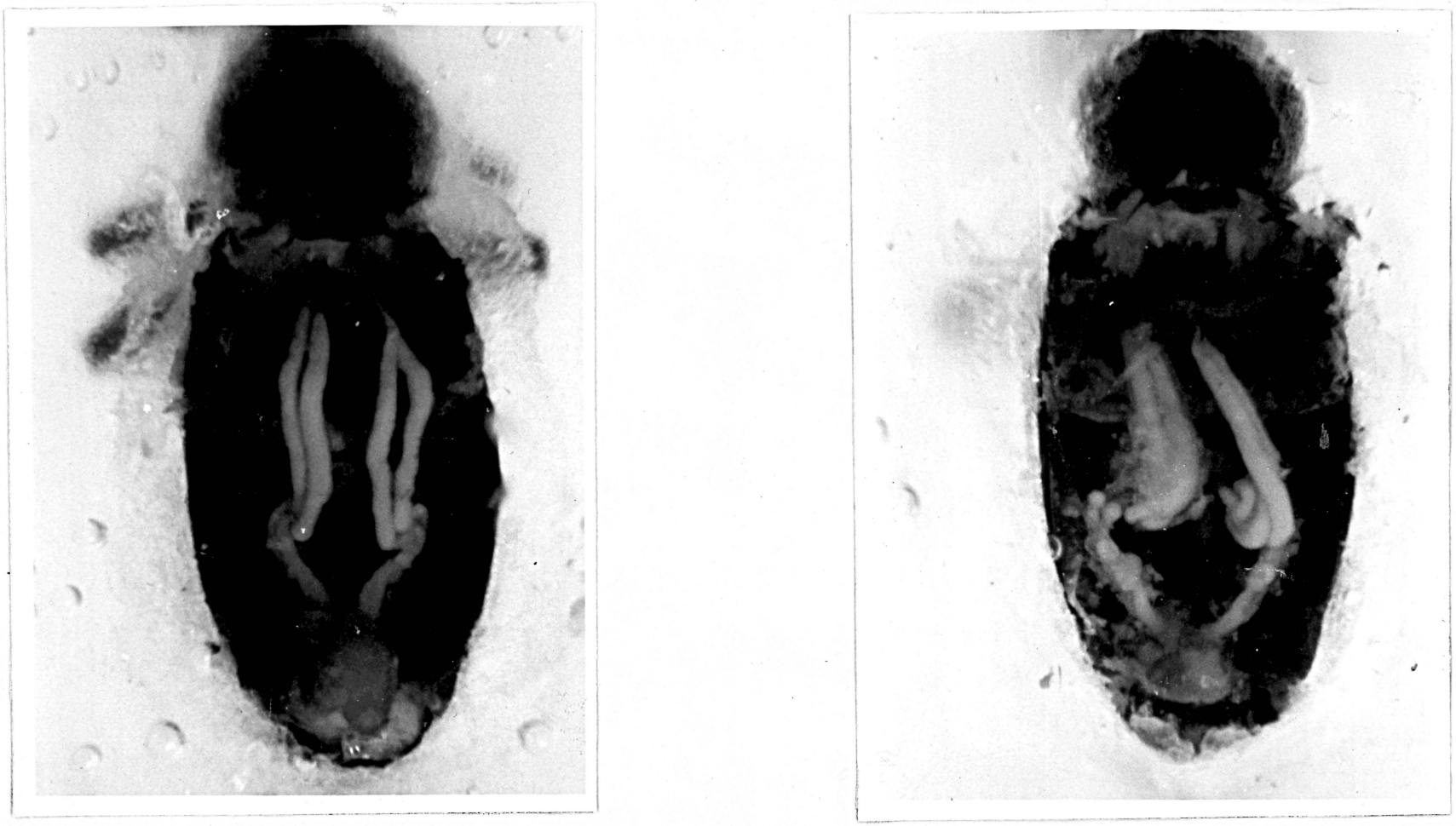


A. 5-week old treated ♀



B. 5-week old untreated ♀

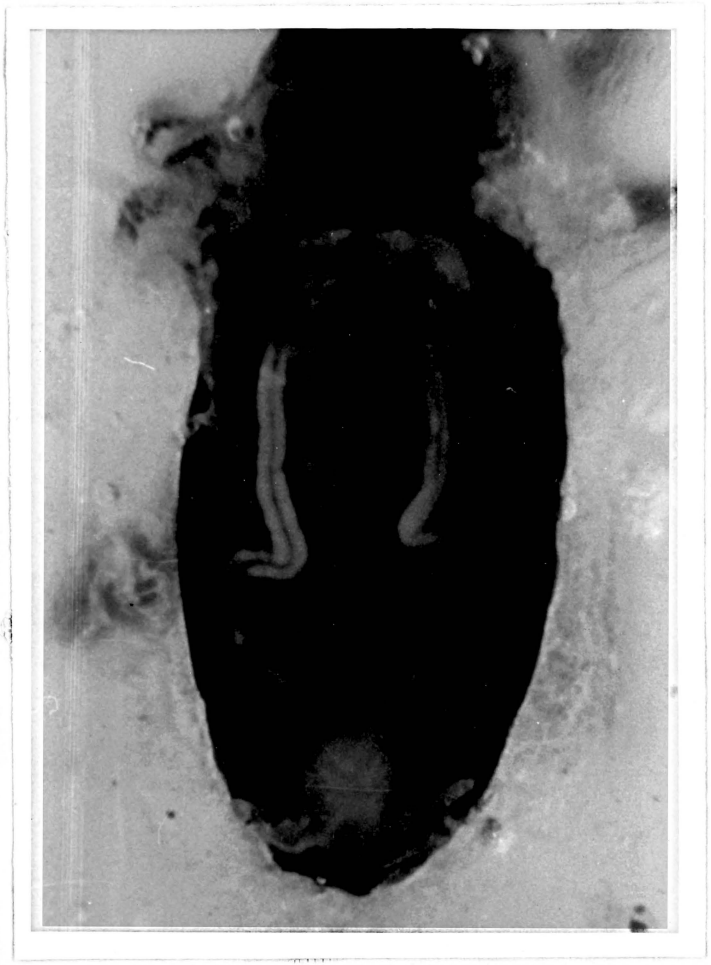
Figure 7. The sex organs of the apholate-treated and untreated female alfalfa weevil at the age of 5 weeks.



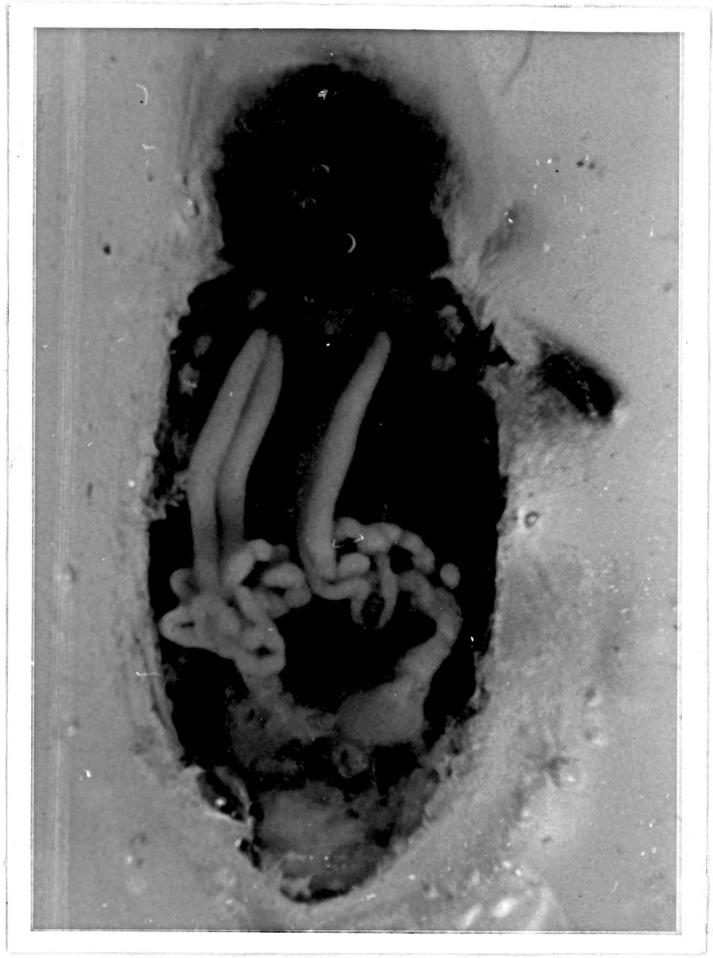
A. 6-week old treated ♀

B. 6-week old untreated ♀

Figure 8. The sex organs of the apholate-treated and untreated female alfalfa weevil at the age of 6 weeks.



A. 7-week old treated ♀



B. 7-week old untreated ♀

Figure 9. The sex organs of the apholate-treated and untreated female alfalfa weevil at the age of 7 weeks.

"segmented stages". In the 7-week-old weevil the reproductive organs were fully developed (Figure 9B). The oviducts were elongated, broadened, and distended, with eggs visible in various stages of development within their canals. Their more distal portion, proximal to the ovaries, clearly showed segmentation, and several eggs ready to be deposited. This stage corresponds to the 6-month-old weevils reported by Guerra and Bishop (1962) and to the "large stage" described by Snow (1928).

The vagina, spermatheca and spermathecal gland remained unchanged from the 2nd to the 7th week.

b. Effects of Apholate on the Size of the Female Sex Organs. Morgan and LaBrecque (1962) reported that apholate inhibited but did not eliminate ovarian development in the house fly. After treating the face fly with apholate, Henson (1966) reported that there were very highly significant differences in size of apholate-treated and -untreated ovaries on the fourth day. Eggs did not develop in the treated ovaries. Smittle et al. (1966) reported that the oocytes were significantly smaller in tepa-treated female German cockroaches than the check females. Table 6 shows that in immature female adult weevils there were no distinct differences between the treated and untreated weevils during the first two weeks (Figures 5A, 5B, 6A, 6B) after treatment. Beginning with the third week post-treatment (5-week-old weevils in Table 6 and Figures 7A, 7B) there was a considerable difference in size

of the ovaries and a great difference in length of the oviduct, particularly during the 4th and 5th week. Figures 8A and 9A reveal that the 6 and 7-week-old treated reproductive organs exhibited little or no convolution, and that the oviducts showed only slight evidence of segmentation and were rather small while untreated sex organs had reached almost full development. In the untreated ovaries and oviducts there was a continuous increase in size until the 5th week (7-week-old weevil). The treated ovaries and oviducts decrease in length during the 3rd week and increase during the 4th and 5th week. When ovaries and oviducts of the treated females were compared in length with those of the untreated females, it appeared that apholate inhibited and decreased or delayed oviduct and ovarian development. Furthermore, the treated weevils did not oviposit while oviposition had occurred in the untreated weevils since the 4th week (over 6 weeks old) of the experiment.

Differences in ovarial lengths between treated and untreated immature adults were not consistently significant, even towards the end of the experiment (Table 6). The lengths of the oviducts became significantly different in the 6th and 7th week of the test (Figure 10). The fact that the treated oviducts were significantly longer in the 3rd week is probably an artifact.

In another test, using mature weevils treated as in the preceding experiment, the ovaries decreased in length through the 5th week after apholate treatment. The oviducts from treated females showed considerable variation in length during

a given week, but were apparently shorter than the untreated check (Table 7).

For mature female adults the ovary length was significantly reduced beginning with the second week after treatment from that observed in untreated weevils; the oviduct length was also reduced, but beginning with the first week after treatment when compared to the check (Table 7).

d. Normal Development of the Male Sex Organs. - Data on the male reproductive organs are summarized in Table 8. It appears that the growth rate (Figure 11) of untreated testes is constant from 2 to 7 weeks (Figures 11, 12B, 13B, 14B, 15B, 16B). This growth differs greatly from that of the female organs (Figure 10). Tombes (1964) reported that the testis diameter averaged 0.3 mm from the spring on into the period of estivation, but increased to 0.75 mm when estivation was ended. The diameter of the testes of the 2-week-old weevils averaged 0.41 mm, and reached 0.76 mm when weevils were mature. As the testes reach maturity, the testicular follicles become distended with sperm and are not as distinct as those of the young weevil.

e. Effects of Apholate on the Size of the Male Sex Organs. - Lindquist et al. (1964) showed in their studies on sterilization of the mature boll weevil with apholate that the testes decreased in size after dipping weevils once in 1% apholate. Hedin et al. (1967) also found that decreases in the size of the testes occurred when boll weevils were injected with 1 or

Table 6. Length (millimeters) of ovaries and oviducts, comparing apholate treated and untreated immature alfalfa weevils. Results of analysis of variance are shown.

Age in Weeks	Ovaries Treated Range	Mean	Untreated Range	Mean	F
2	--	--	1.038 - 1.164	1.101	
3	1.164 - 1.289	1.208	1.164 - 1.307	1.241	1.18
4	1.164 - 1.289	1.238	1.110 - 1.325	1.244	--
5	0.895 - 1.432	1.202	1.253 - 1.790	1.500	7.32*
6	1.253 - 1.611	1.438	1.343 - 1.880	1.611	3.10
7	1.164 - 1.665	1.364	1.450 - 1.880	1.700	10.66**

Age in Weeks	Ovaries Treated Range	Mean	Untreated Range	Mean	F
2	--	--	0.734 - 0.931	0.836	
3	1.146 - 1.360	1.268	1.038 - 1.217	1.152	8.95*
4	0.716 - 1.253	1.034	1.092 - 1.217	1.160	1.45
5	0.573 - 1.343	0.991	0.895 - 1.325	1.182	1.93
6	1.074 - 1.880	1.552	1.701 - 2.685	2.193	14.94**
7	0.841 - 1.969	1.239	1.790 - 3.670	2.820	22.39**

Table 7. Length (millimeters) of ovaries and oviducts in mature females and size of testes in mature male alfalfa weevils at designated week after apholate treatment.[1/]

Week after treatment	Female				Male	
	Ovaries		Oviducts		Testes	
	Range	Mean	Range	Mean	Range	Mean
0(check)	1.522 - 1.880	1.683 a	2.685 - 3.401	3.058 a	0.698 - 0.806	0.755 a
1	1.343 - 1.701	1.525 ab	1.611 - 2.685	2.029 b	0.662 - 0.787	0.707 ab
2	1.253 - 1.665	1.379 bc	1.593 - 3.043	2.297 b	0.627 - 0.787	0.683 abc
3	1.164 - 1.611	1.322 bc	1.701 - 2.864	2.254 b	0.555 - 0.752	0.636 bc
4	1.164 - 1.593	1.358 bc	1.522 - 3.312	2.316 b	0.448 - 0.787	0.576 cd
5	1.074 - 1.522	1.298 c	1.289 - 3.401	2.011 b	0.358 - 0.716	0.516 d

[1/] Means with same letter in a given column are not significantly different according to Duncan's Multiple Range Test. Level of probability: ovaries F = 4.99** (P = .01), oviducts F = 2.85* (P = .05), and testes F = 5.51** (P = .01).

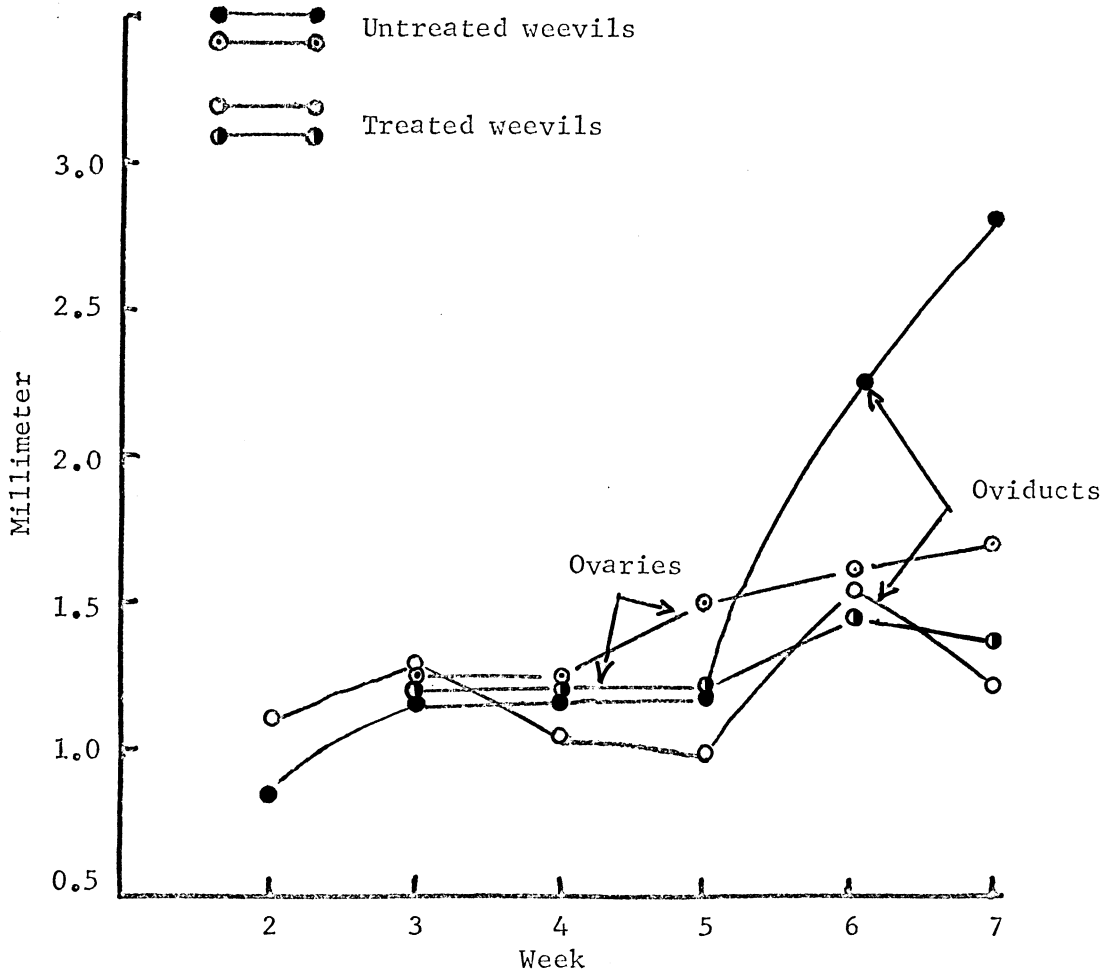


Figure 10. Growth rates of ovaries and oviducts in the treated and untreated immature alfalfa weevil.

Table 8. Diameter (millimeter) of testes from treated and untreated immature male alfalfa weevils. Results of analysis of variance are shown.

Age in Weeks	Treated Testes Range	Treated Testes Mean	Untreated Testes Range	Untreated Testes Mean	F
2	--	--	0.340 - 0.483	0.412	
3	0.483 - 0.573	0.517	0.483 - 0.573	0.528	
4	0.465 - 0.573	0.513	0.537 - 0.770	0.618	8.21*
5	0.430 - 0.609	0.513	0.627 - 0.752	0.689	32.62**
6	0.465 - 0.716	0.576	0.698 - 0.770	0.740	14.83**
7	0.358 - 0.734	0.567	0.716 - 0.806	0.761	9.97*

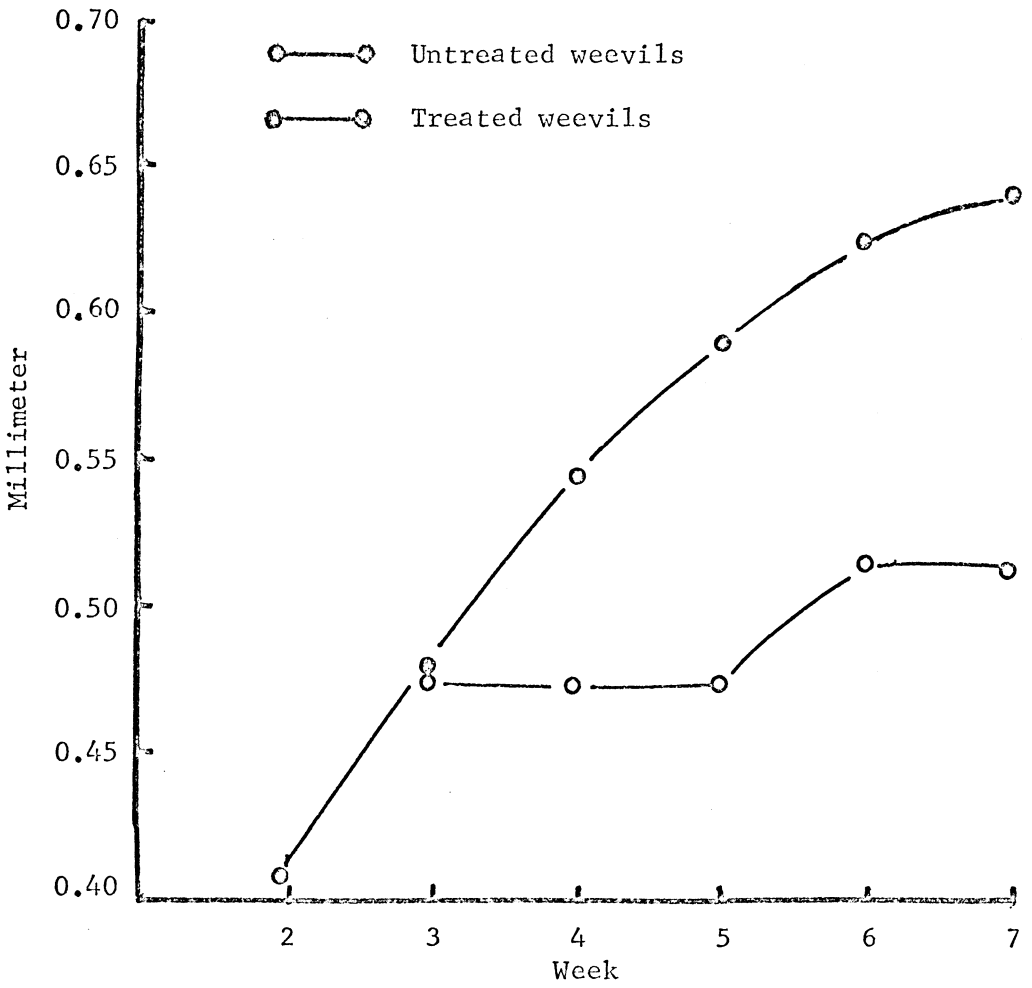
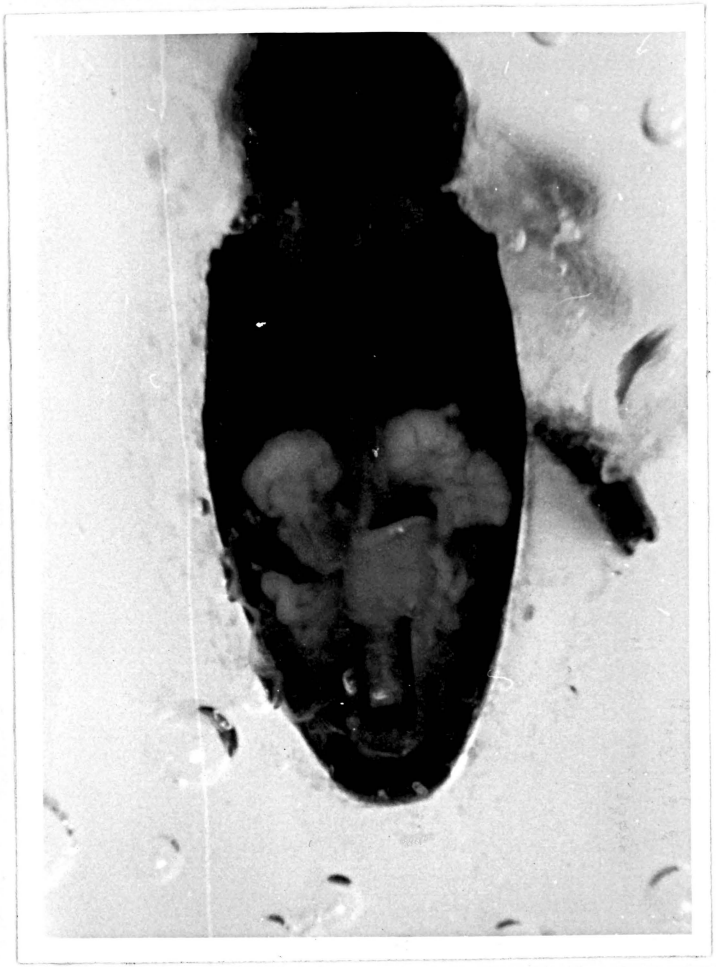
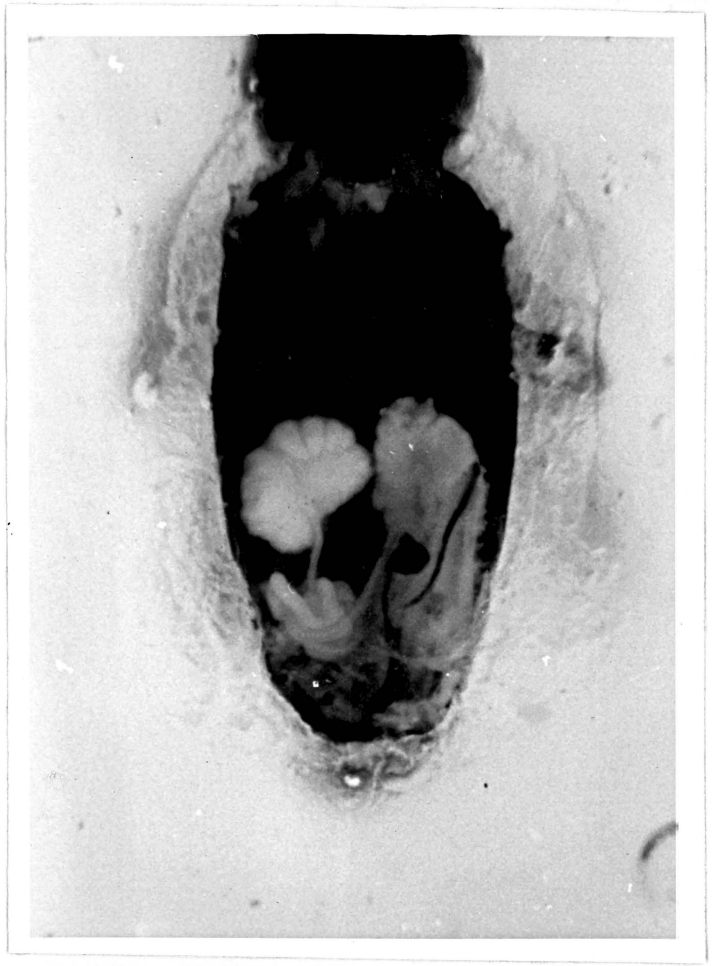


Figure 11. Growth rates of testes in the treated and untreated immature alfalfa weevils.

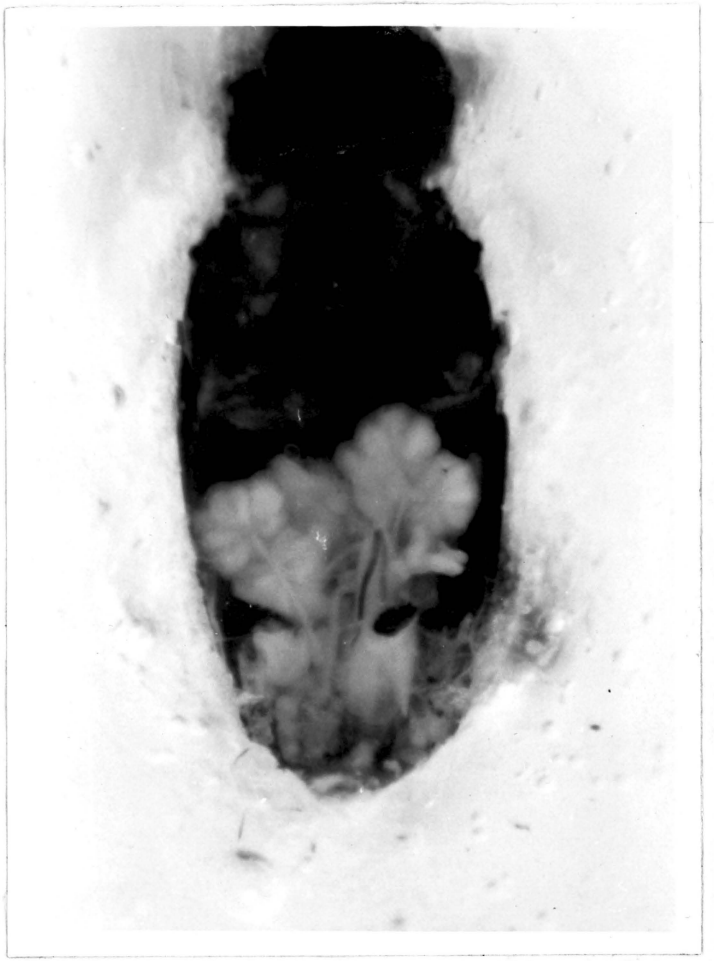


A. 3-week-old treated ♂

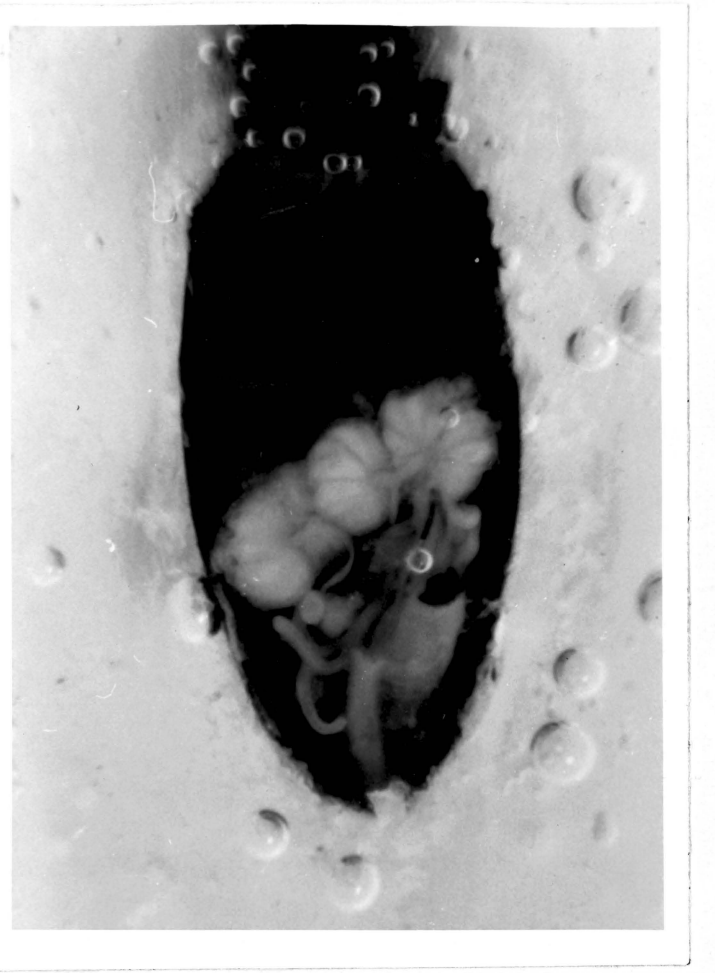


B. 3-week-old untreated ♂

Figure 12. The sex organs of the apholate-treated and untreated male alfalfa weevil at the age of 3 weeks.

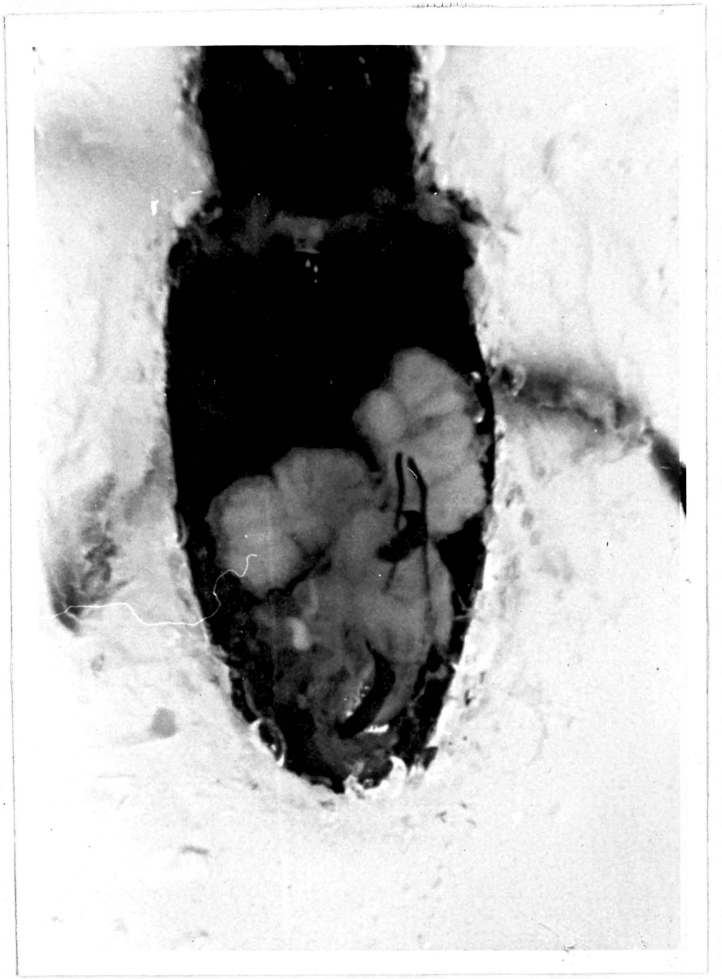


A. 4-week-old treated ♂

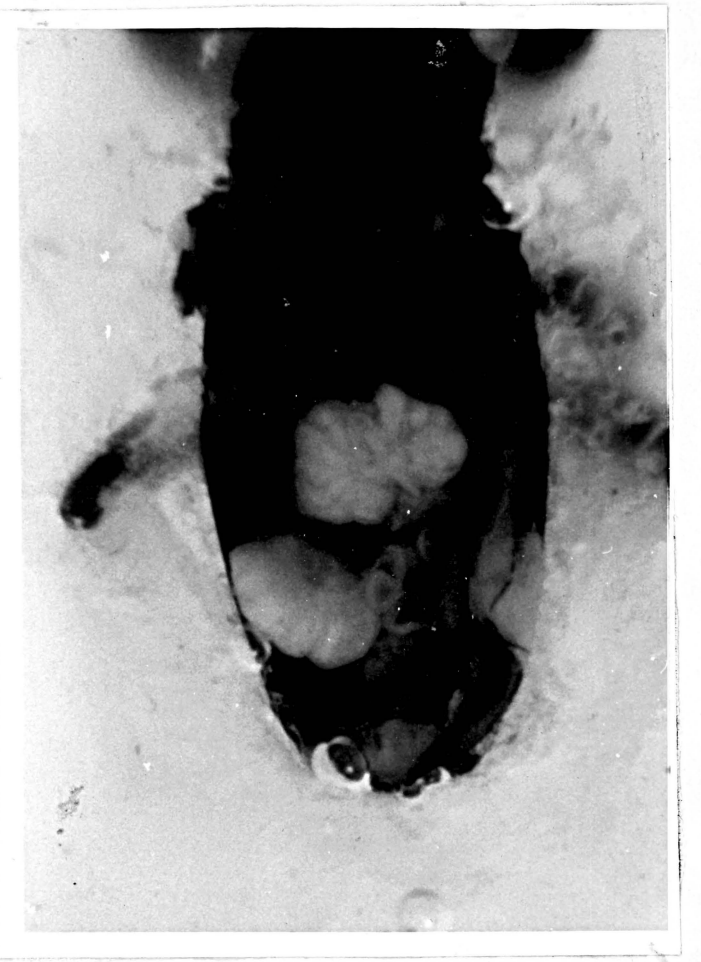


B. 4-week-old untreated ♂

Figure 13. The sex organs of the apholate-treated and untreated male alfalfa weevil at the age of 4 weeks.



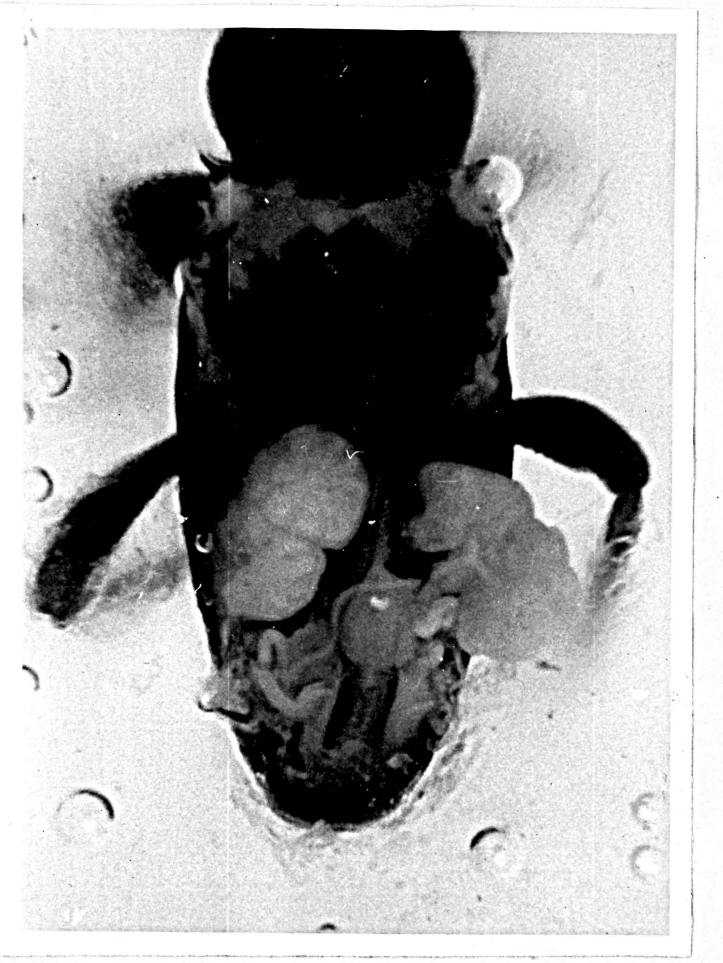
A. 5-week-old treated ♂



B. 5-week-old untreated ♂

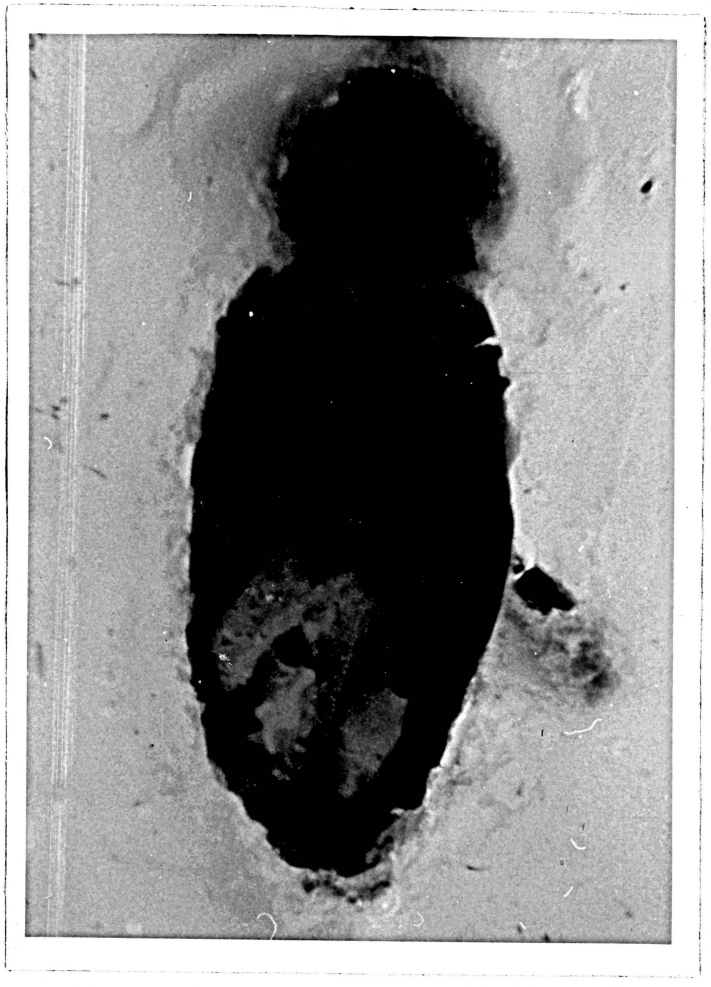
Figure 14. The sex organs of the apholate-treated and untreated male alfalfa weevil at the age of 5 weeks.

A. 6-week-old treated ♂



B. 6-week-old untreated ♂

Figure 15. The sex organs of the apholate-treated and untreated male alfalfa weevil at the age of 6 weeks.



A. 7-week-old treated ♂

B. 7-week-old untreated ♂

Figure 16. The sex organs of the apholate-treated and untreated male alfalfa weevil at the age of 7 weeks.

3 ug of tepa. Smittle et al. (1966) reported that 5 ug of tepa when injected into male German cockroaches stopped spermatogenesis and caused the testes to atrophy. In the immature alfalfa weevil, it appeared to have certain variations as is shown in Table 8 and Figure 11. The average diameter of testes of 3-week-old weevils is 0.519 mm. They remained essentially the same size until the sixth week when a moderate increase occurred in some individuals. Therefore, it appeared that apholate did not decrease the testes size when treated prior to maturation. It did inhibit and delay testicular development. In addition some red-spotted pigments were found in treated testes, but the significance is not known.

In the mature male adult weevils, the testes were increasingly reduced in size each week for 5 weeks after apholate treatment (Table 7), when compared to the untreated check.

For mature male adults the testis size was significantly reduced beginning with the third week after treatment.

The surviving adults of both sexes, which were dipped once in a 1% apholate at the age of two weeks, were caged together to determine the rate of recovery from the treatment. Only two replicates were used, each with 7 males and 9 females. Eggs were collected at 10 weeks after treatment. From the 10th to the 14th week egg viability ranged from 6 to 16%, in the 15th week it was 29%, and during the 16th to

21st week ranged from 44 to 68%. Recovery from the treatment was various. During this test egg viability in the control ranged from 85 to 96%.

C. Sexual Competition Experiments

Several tests were conducted on the effect of apholate treatment on mating competition and sperm competition in laboratory-reared non-diapausing weevils. Virgin mature females were used in these tests. Petri dishes, with two 3-inch long fresh alfalfa stems moistened by a small piece of cotton, were used as the mating cage. The alfalfa stem with leaves on it served as food and oviposition site.

Two tests were conducted prior to the sexual competition experiments. The first test was to determine whether virgin mature females will produce viable eggs. The results showed that the four replicates of two virgin females each in petri dishes over six days produced 153 nonviable eggs and four replicates of ten virgin females each in quart cartons over three days produced 232 nonviable eggs. No viable eggs were produced by virgin females. The second test was to check the decline in egg viability after removal of the male. The results showed that the four replicates of two mated females each in petri dish produced 95.7%, 91.0%, and 88.5% viable eggs during first, second and third week of isolation. This was within the range of normal egg viability.

1. Sterilization Procedures

One hundred male adults were sterilized by dipping the weevils in a 1% apholate solution as previously described.

2. Mating Competition Tests

In a limited test using virgin adults, 16 individual females were exposed to a treated and a marked untreated male. Observations were made to determine whether a treated or an untreated male mounted and mated with the female first. The first to mate was removed so the other would also mate. The result showed that in 13 of 16 cases the untreated males mated first. The mating period for the first mating ranged from 15 to 30 minutes.

3. Sperm Competition Tests

a. In the first test individual virgin females were allowed to mate only once each with a treated male, followed by an untreated male or vice versa as described under mating competition above. From a total of 582 eggs collected weekly from the female mated first with an untreated male over a 3-week period, 222 eggs hatched. Thus the egg viability was 38.1%. This was probably not a valid test of sperm competition since egg production per female for the 3 weeks was fairly low (averaging 36.4 eggs/female). The average duration of the first mating out of 18 cases was less than 30 minutes, as compared to the much longer normal duration of mating of 166 minutes (LeCato 1968). Also, under normal check conditions the first weeks eggs are much less viable than later in an experiment. Therefore, the initial matings of a virgin female may be less effective either due to the short duration of first mating or insufficient transmission of sperm during

first copulation. The egg viability for the female when mated with a treated male first was 16.9% (24 eggs hatched out of 142 eggs collected). Again this was perhaps a doubtful result as the test was limited to only a small number of insects.

b. In the next test individual virgin females were exposed to a male, either treated or untreated, for 3 days, isolated for 4 days, then exposed to a male with the opposite treatment for 3 days, and isolated again for 4 days. This was replicated 8 times. Eggs were collected and the percent egg viability was recorded during the 2-week duration of the test. The result is shown in Table 9.

The low % egg hatch in first week with a normal male is probably due to reduced viability of eggs and mating behavior of virgin females. The viability of eggs produced in a week when an untreated male was present was significantly higher ($P = .01$), therefore the effect of the available male appeared to be greatest.

c. In a similar alternate mating study the female was allowed to mate for 7 days, with either a treated or an untreated male. The males were alternated at weekly intervals. Eight replicates for each were used. The eggs were collected weekly and the percent egg viability was recorded for the 5-week duration of the test. The results are shown in Table 10.

The data shows decreasing oscillations in viability tending toward a uniform viability level between 65 and 75%.

Table 9. Average % egg viability from female alfalfa weevils mated with treated and untreated males.

Treatment Week	Male[1/]	No. of Eggs Collected	% Egg Hatch	Male	No. of Eggs Collected	% Egg Hatch
1	N	128	29.69	T	280	7.86
2	T	232	24.14	N	360	70.56

[1/] T = treated, N = untreated or normal.

Table 10. Average % egg viability from female alfalfa weevils mated with treated and untreated males.

Treatment Week	Male[1/]	No. of Eggs Collected	% Egg Hatch	Male	No. of Eggs Collected	% Egg Hatch
1	N	265	87.93	T	63	9.52
2	T	762	71.00	N	465	95.05
3	N	652	84.20	T	656	75.76
4	T	515	72.04	N	513	80.70
5	N	261	72.41	T	332	62.05

[1/] T = treated, N = untreated or normal.

Statistical analysis shows that the egg viability from females mated with untreated males was significantly greater than that of the females mated with treated males ($P = .05$). The competitive ability of sperm from the treated males appears to have been reduced, possibly due to: 1) damaged genetic materials in the sperm; 2) decreased sperm viability or activity; 3) decreased numbers of sperm transferred in copulation.

d. In another test, individual virgin females were caged in petri dishes in the presence of two males, one treated and one untreated. In this test repeated free choice mating was permitted to check both mating competition and sperm competition. A total of 8 replicates were used. The eggs were also collected weekly, and the percent egg viability was recorded for the 5-week duration of the test. The results are shown in Table 11.

In the mating competition tests described previously, it was found that treated males did not mate as readily as did untreated males. However, from the egg viability obtained in this test, it appears that the treated males can compete sexually, although not equally, with untreated males, and can cause a decreased egg viability.

Table 11. Average % egg viability from female alfalfa weevils mated with treated and untreated males.

Treatment Week	No. of Eggs Collected	% Egg Hatch
1	-	-
2	267	55.06
3	416	31.67
4	405	62.96
5	223	65.92

V. SUMMARY AND CONCLUSION

Although it has not been recommended for the control of any insects, the chemosterilant concept is receiving widespread acceptance as a promixing approach to control (LaBrecque 1965). This investigation is a primary study of the susceptibility of the alfalfa weevil to sterilization by apholate, the manner in which apholate affects the weevil, and the effect of apholate on the ability to compete sexually.

In determination of the susceptibility of the weevil to apholate an adult topical application experiment showed that apholate was effective in the sterilization of male weevils, but caused a high adult mortality. The larval dipping test produced a high larval mortality, a decreased emergence of adults and a slightly reduced percent viability of eggs laid by the adults which emerged from the apholate-treated larvae. The adult dipping experiment suggested a handy and effective method of application. The best adult age for dipping treatment appeared to be the reproductively mature virgin adults. The treatment of females had little practical effect since the weevils continued to produce normal viable eggs. The apholate treatment of male weevils showed the greatest effectiveness in sterilization. The most effective concentration for sterilizing males appeared to range from 0.5% to 1.0% apholate solution. There was little difference in egg viability caused by treating both sexes as compared with the use of treated males. A gradual increase in egg

viability occurred in the groups where either males or both sexes were treated with apholate, starting at about the 3rd week and continuing to the end of the test.

In tests on the gross changes in the size and shape of reproductive organs caused by apholate, immature and mature adults, after treatment with apholate, were dissected and their reproductive organs were measured. In untreated ovaries of the immature nondiapausing weevil there was a continuous increase in size until oviposition began. The normal oviducts were more than 3 times as long in the 7-week-old weevils as they were in the 2-week-old weevils. The treated immature ovaries and oviducts exhibited a lack of growth up to the 5th week and a retarded growth rate through the 7th week. There is a direct relationship between full development of the reproductive organs and occurrence of oviposition. The ovaries and oviducts of treated mature females were reduced in size and length following treatment, when compared with the normal mature ovaries and oviducts.

For normal testes of the immature nondiapausing weevil there was a constant increase in size from the first week until the 5th week when weevils reached maturity. The testes of treated immature weevils remained relatively constant through the 5th week and slowly increased in the next 2 weeks. Apholate appeared to inhibit, or delay the testicular development. The treated mature testes was reduced in size constantly from the first to 5th week after treatment.

Many reports, described previously, regarding the application of apholate to insects indicate that treated insects may regain fertility

after a certain time following treatment. This also occurs in the alfalfa weevil. In the immature adults, it is possible that some of the less susceptible weevils, in which testicular development has not been so markedly retarded, could reach sexual maturity, and thereby cause a "regained fertility" effect. In the mature adults, however, it is not known whether the "regained fertility" is due to the recovery of sperm activity, a partial resistance to reduction in size of the testis, or a more rapid recovery. It is not known whether complete recovery may occur over a longer period than was tested in these experiments.

In the mating competition test between the apholate-treated and -untreated males it seemed that the treated males did not mate as readily as did untreated males at the beginning of a test. The available male seemed to exert the greatest effect in the sperm competition test. The first weeks eggs laid by newly fertilized females are much less viable than those produced later. Therefore, the initial matings of virgin females may be less effective, either due to the mating behavior (short duration) or the quantity and quality of the sperm transferred during copulation. The reduced competitive ability of the treated males may be due to damaged genetic materials in the sperm, decreased sperm viability or activity, or decreased numbers of sperm transferred in copulation. The treated males seemed to mate more frequently in the later test period as a considerable mixing of sperm occurred during the mating of female weevil with the apholate-treated and -untreated males. Hence the treated males may compete sexually,

although not equally, with untreated males, and cause decreased egg viability.

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VII. APPENDIX

Appendix Table I. Results of analysis of variance for the effect of apholate treatment of female alfalfa weevils on % egg viability.

A. 4 concentrations with 4 replicates

1. Concentrations F = 4.24*

Apholate Conc.(%)	Mean	Sig.[1/]
2.0	66.49	a
1.0	67.33	a
1.5	69.04	ab
0.5	72.00	b
Check	71.86	b

2. Weeks F = 27.16**

Week	Mean	Sig.[1/]
1	46.18	a
2	63.97	b
3	73.75	cd
4	73.34	cd
5	69.32	c
6	72.34	cd
7	75.28	d
8	72.67	cd
9	74.19	cd
10	73.93	cd

B. 2 concentrations (0.25 and 0.75%) with 3 replicates

1. Concentration F = 3.04*

2. Weeks F = 37.83**

Week	Mean	Sig.[1/]
1	49.99	a
2	59.30	b
3	65.86	c
4	74.98	ef
5	69.98	d
6	72.82	def
7	75.80	f
8	73.60	def
9	73.28	def
10	70.96	de

[1/] Means with the same letter are not significantly different according to Duncan's Multiple Range Test.

Appendix Table II. Results of analysis of variance for the effect of apholate treatment of male alfalfa weevils on % egg viability.

A. 4 concentrations with 4 replicates

1. Concentrations F = 100.36** 2. Weeks F = 28.13**

Apholate Conc. (%)	Mean	Sig.[1/]
2.0	3.82	a
1.5	5.10	a
1.0	24.89	b
0.5	34.11	c
Check	71.87	d

Week	Mean	Sig.[1/]
1	22.36	ab
4	24.52	bc
5	27.45	cd
6	29.23	d
7	30.60	de
8	33.40	e
9	34.85	f
10	37.11	f

B. 2 concentrations (0.25 and 0.75%) with 3 replicates

1. Concentrations F = 44.24** 2. Weeks F = 81.48**

Week	Mean	Sig.[1/]
1	34.97	b
2	36.41	b
3	30.28	a
4	37.23	b
5	40.63	c
6	41.48	c
7	46.54	d
8	50.37	e
9	55.66	f
10	59.43	g

[1/] Means with the same letter are not significantly different according to Duncan's Multiple Range Test.

Appendix Table III. Results of analysis of variance for the effect of apholate treatment of both sexes of the alfalfa weevil on % egg viability.

A. 4 concentrations with 4 replicates

1. Concentrations F = 319.34** 2. Weeks F = 80.67**

Apholate Conc.(%)	Mean	Sig.[1/]
2.0	3.31	a
1.5	4.63	a
1.0	23.71	b
0.5	27.94	b
Check	71.87	c

Week	Mean	Sig.[1/]
1	20.64	a
2	19.49	a
3	20.42	a
4	23.33	b
5	24.60	b
6	26.41	c
7	29.11	d
8	31.63	e
9	32.83	ef
10	34.48	f

B. 2 concentrations (0.25 and 0.75%) with 3 replicates

1. Concentrations F = 40.70* 2. Weeks F = 50.52**

Week	Mean	Sig.[1/]
1	32.45	bc
2	32.95	c
3	25.11	a
4	28.65	ab
5	39.05	d
6	35.60	cd
7	39.66	d
8	45.69	e
9	51.36	f
10	55.78	g

[1/] Means with the same letters are not significantly different according to Duncan's Multiple Range Test.

Appendix Table IV. Results of analysis of variance for adult mortality at 10 weeks after treatment of apholate-treated female x untreated male alfalfa weevils.

A. Female mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

F = 25.64**

Apholate Conc.(%)	Mean	Sig.[1/]
Check	26.43	a
0.5	47.41	b
1.0	54.85	bc
1.5	56.63	c
2.0	62.93	c

2. At concentrations of 0.25, 0.75%

F = 13.17*

B. Male mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

F = N.S.

2. At concentrations of 0.25, 0.75%

F = N.S.

[1/] Means with the same letter are not significantly different according to Duncan's Multiple Range Test.

Appendix Table V. Results of analysis of variance for adult mortality of 10 weeks after treatment of apholate-treated male x untreated female alfalfa weevils.

A. Male mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

F = 33.68**

Apholate Conc.(%)	Mean	Sig.[1/]
Check	28.56	a
0.5	51.06	b
1.0	59.20	c
1.5	64.36	c
2.0	67.22	c

2. At concentrations of 0.25, 0.75%

F = 9.50*

B. Female mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

F = N.S.

2. At concentrations of 0.25, 0.75%

F = N.S.

[1/] Means with the same letter are not significantly different according to Duncan's Multiple Range Test.

Appendix Table VI. Results of analysis of variance for adult mortality at 10 weeks after treatment of apholate-treated male x treated female alfalfa weevils.

A. Male mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

   F = 21.19**

2. At concentrations of 0.25, 0.75%

   F = 52.69**

Apholate Conc.(%)	Mean	Sig.[1]
Check	28.56	a
0.5	53.24	b
1.0	57.06	bc
1.5	63.61	c
2.0	66.06	c

B. Female mortality

1. At concentrations of 0.5, 1.0, 1.5, 2.0%

   F = 29.96**

2. At concentrations of 0.25, 0.75%

   F = 4.47*

Apholate Conc.(%)	Mean	Sig.[1]
Check	26.41	a
0.5	47.82	b
1.0	52.36	bc
1.5	57.47	cd
2.0	60.62	d

[1] Means with the same letter are not significantly different according to Duncan's Multiple Range Test.

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STUDIES OF THE EFFECTS OF APHOLATE ON THE ALFALFA WEEVIL *HYPERA POSTICA* (GYLLENHAL)

Feng-kuo Hsieh

ABSTRACT

The effects of apholate on diapausing and nondiapausing alfalfa weevil, *Hypera postica* (Gyllenhal), were studied. Different stages, sexes, and ages of the weevil were treated with aqueous-apholate solution. Adult topical application and larval and adult dipping methods were used in the experiment. Both showed the effectiveness of sterilization, and caused high mortality to the treated insects. Adult dipping was an acceptable method of treatment. The best age for treatment seemed to be reproductively mature virgin adults. Egg viability was similar to the check when only the females were treated with apholate. When only males or when both sexes were treated the egg viability was decreased depending on the apholate concentration used. The most effective apholate concentration for sterilizing male alfalfa weevils ranged from 0.5 to 1.0%. A gradual increase in egg viability occurred starting about the 3rd week after treatment.

Dissection of the immature adults showed that gonadal development was delayed by apholate. The reproductive organs of apholate-treated mature adults were reduced in size.

The treated males do not compete sexually on an equal basis with the normal males, although considerable mixing of sperm occurred during the mating of female weevils with the apholate-treated and -untreated males.