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Effects of Diflubenzuron When Fed to Adult Female Alfalfa Weevils

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EFFECTS OF DIFLUBENZURON WHEN FED TO ADULT FEMALE ALFALFA WEEVILS

by

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Mohamed M. Middib

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ABSTRACT

Effect of Diflubenzuron
When Fed to Female Alfalfa Weevils

By

Mohamed M. Middib, Master of Science

Utah State University, 1984

Major Professor: Dr. Donald W. Davis

Department: Biology

The purpose of this project was to evaluate diflubenzuron ovicidal effects on adult female alfalfa weevils Hypera postica in the laboratory. There was little relationship of weevil mortality to dosage or length of exposure of the adult to the chemical. An indirect result of diflubenzuron was an increase in muscardine fungus (Beauveria Sp.). Other effects of the chemical on the adults were yellow deposits and tissue extending from the tip of the abdomen.

The main effect of dilfubenzuron when fed to female alfalfa weevils was on the eggs. The effects were dosage related, especially on eggs viability. The viability was dropped from 99% in the control to 38.9% on the treated insects in ten days using the highest dosage. Another effect of diflubenzuron on alfalfa weevil eggs was on the shape of the eggs. The treated insects laid longer and lighter color eggs.

(70 pages)

INTRODUCTION

The alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae) first appeared in the United States at Salt Lake City, Utah, in 1904 (Titus, 1910). It then became a serious pest, causing an estimated 56 million dollars damage annually during the mid 1960's (Anon, 1967 and Anon, 1969).

In Utah, as well as in many other parts of the U.S.A., it has one generation a year. Three biotypes are known, which are very similar but biologically distinct. The eastern alfalfa weevil biotype spread through the eastern United States of the U.S.A.; western alfalfa weevil spread in the western United States, especially the mountain states; and the Egyptian weevil became established in the hot areas of southern California and Arizona. The biotype in Utah is the western alfalfa weevil. Adults spend the winter hiding in litter under trees near field margins, and when the alfalfa starts to grow, the adults become active again. The eggs deposited inside the stems of the alfalfa pltans, can number up to 800 per female (Davis and Knowlton, 1976). Based on temperature and humidity, hatching takes place in 4 days to 3 weeks.

Much research has been done to control this serious pest by cultural, chemical and biological methods (Miller, 1970; Neal, 1974; and Wakeland, 1924).

Diflubenzuron (Dimilin^{R}) is a growth regulator with a unique mode of action used experimentally to control many pests. It can be applied to the different insect stages. It has low mammalian toxicity

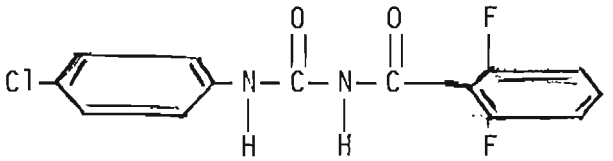
and does not accumulate in food chains. Therefore, diflubenzuron could be environmentally acceptable when used in insect management (Post and Muelder, 1974).

The objectives of this study were to:

1. Measure the effects of diflubenzuron fed to adult female alfalfa weevil, on egg numbers and viability.
2. Study the effects of diflubenzuron on egg morphology.
3. Study the effects of diflubenzuron on the treated adult weevils.

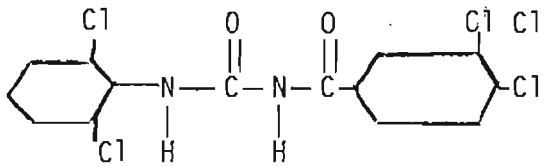
LITERATURE REVIEW

Chemical Structure, History and Properties



1-[4-chlorophenyl)-3- (2,6-difluorobenzoyl)-urea]

The first compound related to diflubenzuron studied as a chitin inhibitor of insect cuticle was Dulgill [1-(2,6-dichlorobenzoyl)-3-(3,1-dichlorophenyl)-urea] (Rockstein, 1978).



Dulgill

After the discovery of Dulgill, a large number of structural analogues were synthesized, one of which was Diflubenzuron.

Diflubenzuron was stable for no more than 6 days in water, but water temperature had no influence on the stability. It displayed a considerably higher stability in soil, where it remained unchanged and biologically active 3 months after application (Rabond, 1980).

Diflubenzuron showed very little absorption by plants. Bull (1980) reported that very little diflubenzuron is absorbed by cotton leaves when applied on cotton to control Anthonomus grandis Boh. The compound adhered well to the foliage and was highly resistant to photo degradation on foliar surface. It is not phytotoxic.

Ovicidal Effects

Diflubenzuron or Dimilin and its relatives were the first insecticides used which act as cuticle inhibitors (Muelder and Gijswift, 1973). The effect of diflubenzuron is connected with the disruption of the cuticular matrix formation, especially the chitin component (Muelder and Gijswift, 1973). It inhibited emergence of the progeny from the eggs by apparently disturbing cuticle formation for a period of up to 3 weeks (Moore and Taft, 1978).

Several workers have reported that diflubenzuron has an ovicidal effect. Adult female boll weevils, Anthonomus grandis Boh., laid eggs which did not hatch when they were treated with 0.42 and 2.25 mg diflubenzuron per insect (Don and Ivie, 1980; Bull and Ivie, 1980; and Graynard, Bradey, Boyd and Brazzel, 1977).

One-tenth percent diflubenzuron wettable powder had 100% ovicidal effect when fed to female Melolontha melolontha on beech leaves (Reuchi and Jossi, 1979). It also had an effect against larvae and eggs after feeding by either larvae or adults of Gastroidea viridula DeG. on sprayed leaves.

Randwan, Abo-Elghar and Ammar (1978); and Salma and Magd (1977) reported that diflubenzuron has a sterilizing effect causing reduction in egg production as well as egg viability of cotton leafworms, Spodoptera littoralis Boi. When eggs were dipped in 5 ppm diflubenzuron, 100% lethality of eggs resulted.

European corn borers (Ostrinia nubilalis Hub.) laid eggs which did not hatch when female larvae were fed corn leaves sprayed with 0.1% diflubenzuron (wettable powder 25%). Ninety-four percent of the eggs failed to hatch (Ruechi, 1978).

Diflubenzuron also has been applied agains rootknot nematodes. Veech (1978) indicated that treatment of cotton seeds with diflubenzuron reduced numbers of Meloidogyne incognita eggs and egg masses. When this chemical was applied to an apple orchard, it caused 100% mortality of apple leafminer eggs, Leucoptera scitella Gue. and Phyllonorycter blancardella Fab.

Kranovskaya and Chipischchuk (1978) reported that diflubenzuron reduced the percentage of viable eggs in the Colorado potato beetle, Leptinotarsa decemliniata . Other workers reported that 88% of the eggs of the stable fly, Stomoxys calcitrans L., did not hatch when the adults were exposed for 7 days to 7 day-old spray residues of diflubenzuron and that horn fly eggs did not hatch, though larvae developed within the eggs (Ivie and Wright, 1978; Wright, 1978; Wright and Harris, 1976). When adult horn flieds were exposed to the treated steer manure, hatchability was inhibited after 48 h exposures to 35 day-old spray residues (Kunz and Harris, 1978; and Kunz, et al., 1977).

When the pear psylla, Psylla pyri Foe., laid eggs on branches treated with diflubenzuron, 10-30% of the eggs did not hatch (van Busschback and Philips-Duphar, 1975). Simulium vittatum Zet. eggs did not hatch when insects were treated with Dimilin (Lacey and Mulla, 1977).

Usage of the Chemical

Different ways to test diflubenzuron effects on insect eggs have been used. Some workers have used diflubenzuron as contact against adult femals, some have fet it to the female parents, others have used it in water for mosquito control and others have treated eggs directly. Each method has its own merits.

When diflubenzuron is used in research related to egg viability evaluation, it is usually a suspension of wettable powder in water. It is sometimes used with some other material. Some have used feeding stimulants, others have used detergents to increase the sticking of the chemical to the plant or to the insect or its eggs. Taft and Hopkins (1975) used diflubenzuron 25% WP as a sprayable bait with type 50 invert sugar or a combination of type 50 invert sugar and molasses added as a feeding stimulant.

Radioactive tracers have also been used by some workers. Bull and Ivie (1980) used ^{14}C-labeled diflubenzuron. Metcalf, Lu and Bowlus (1975) used the same procedure (^{14}C) to study the degradation and environmental fate of diflubenzuron.

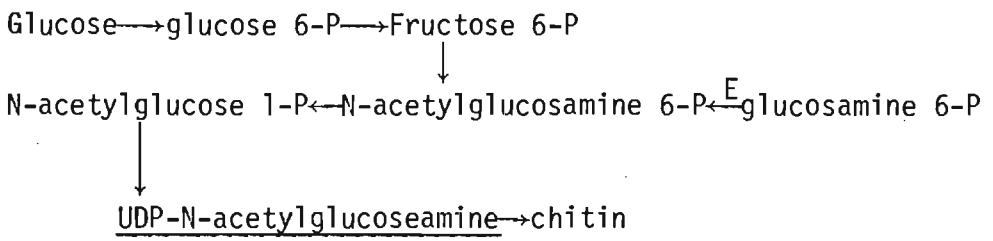
Still and Leopold (1978) used diflubenzuron by either spotting acetone solution topically or dipping boll weevils into acetone solutions. They also injected boll weevils with solutions in peanut oil. Elliott and Anderson (1982) used Tweenzo (polyoxyethylene sorbitan monolaurate) with diflubenzuron.

Hoying and Helmut (1980) used acetone as a solvent material in which diflubenzuron was dissolved and then added to distilled water.

Mode of Action

Diflubenzuron acts directly on the chitin synthetase (CS). This enzyme is necessary for the polymerization step in insect chitin formation (Rundall and Keuchington, 1973). It binds to non-CS sites which are important to chitin polymerization and fibrillogenesis. It also has an indirect action via active metabolites formed in the integument (Cohen and Casida, 1980).

The primary effect of diflubenzuron is to block the incorporation of uridine 5-diphospho-N-acetyl-glucosamine (UDP-GlcNAC)[3] into chitin (Deul, Jong and Kortonbach, 1978). Blockage occurs at the first step of chitin polymerization (see diagram below):



(Rockstein, 1978).

Another action of diflubenzuron, as discussed by Cohen and Casida (1980), involves differences in the organization and properties of the enzyme complexes. Enzymes seem to have many properties in common; for example, requirements for GlcNAC or Mg^{+3} depending on their location in the insect body. Also, there are differences in their organization and functioning. Example for that: chitin is associated with protein in the insect cuticle, and enzyme is involved in continuous secretion of the peritrophic membrane.

Both sugar and protein are affected by diflubenzuron. These components are found in the insect egg, especially the integument. The egg consists of the serosal cuticle consisting of a chitinous endocuticle, sometimes called the white cuticle (Chapman, 1982). When the chorion differentiates to form endocuticle and exocuticle, the latter contains tanned protein chorionin which resembles the cuticulin in the insect cuticle.

Diflubenzuron also stimulates the follicles, the cells which produce the chorion of the egg while the egg is in the ovary. Lineva

and Chunina (1980) reported that when Musca domestica L. was allowed to feed on diflubenzuron treated food they laid more eggs than in the control during the first 10 days of feeding.

Eck and Van (1979) reported that the main cause for unhatched eggs with diflubenzuron is that post embryonic development is disturbed in many insect species.

A complete inhibition of larval ecolosion from eggs deposited by treated Dendroctonus rufipennis Kby., was caused by diflubenzuron (Sahota and Ibarak, 1980).

The effect of diflubenzron on insect eggs depends on the age of the eggs when exposed. Ottens and Todd (1979) reported that direct application of diflubenzuron on Graphognathus peregrinus Kby. and G. leucoloma Buc. eggs caused substantial reduction in hatchability of young eggs 0-14 days old. They were more susceptible than eggs 15-38 days old. The same basic results were reported with Culex pipiens Say. eggs treated with diflubenzuron (Miura et al., 1976).

Side Effects

Diflubenzuron has altered the activity of hormone-metabolizing enzymes (Cohen and Casida, 1980). It also has inhibited furcular development of Collembola (Doppelreiter, 1979).

Lawrence (1981) reported that diflubenzuron not only affects chitin synthesis of insects, but also causes abnormally developed ovipositors of adult female of Anastrepha suspensa Loe. emerging from the treated eggs. Such females were unable to successfully locate hosts to parasitize.

Diflubenzuron when formulated with Savol (a paraffinic spray oil) was toxic to the parasites of Heliothis virescens Fab. eggs when applied to cotton fields (Ables et al., 1980).

Ables et al. (1980) reported that diflubenzuron affects several species of entomophagous arthropods associated with soybeans. In Texas it also reduced Geocoris punctipes Say. and Nabis spp. in cotton fields.

Not all arthropods are affected by diflubenzuron. McCoy (1978) reported that there was no effect of diflubenzuron to the egg stages of the citrus rust mite, Phyllocoptruta oleivora Ash. Also, there were no effects to the eggs of the carrot rust fly, Psila rosae Fab. when diflubenzuron was mixed with the food for the adult or applied directly on the eggs (Overbeck, 1979). Also, some natural enemies in cotton fields were not affected by diflubenzuron. This included Coleomegilla maculata De., Orius insidious Say, Chrysopa spp. and Araneida (Keever et al., 1977).

Muscardine Fungus

Muscardine has been known for a long time in France as muscardin (de ver soie) because insects infected with the fungus were transformed into white mummified specimens that resembled comfits (bonbons) and in Italy as calcino (calcium or white powder) (MacLeod, 1954).

The fungus which causes this disease belongs to the genus Beauveria. It is named in honor of Beauverie who, in 1911, had pointed out the characteristics of the group to which muscardine belongs.

The frequent association of members of the genus Beauveria with insects throughout the world suggests that they may be important in the natural control of many insect pests. This genus has a wide host range including insects, rodents, and humans. Rockwood (1951) reported this

wide host range in the Pacific Northwest. As early as 1916, he had reported it from the alfalfa weevil (Rockwood, 1916). It is also a major fungus affecting the European cornborer (Lefebvre, 1931).

The Fungus History

The name Sporotrichum globuliferum has been applied in America to the fungus which parasitizes the cinch bug (Blissus leucopterus). Two generic names were given to the fungus: 1) Botrytis was used more commonly by European mycologists, and 2) Sporotrichum was preferred by the Americans. The present generic name, Beauveria, was created in 1912. The name, Sporotrichum globuliferum, became Beauveria globulifera (Benham and Jose, 1953).

The fungus' importance

The fungus, Sporotrichum globuliferum, was reported the first time on the alfalfa weevil from near Salt Lake City, Utah, on March 14, 1914 (Rockwood, 1916). Most of the infected, dead weevils were under alfalfa plants covering an area of about 4 square feet on a high bank outside the irrigated area.

The optimum conditions for the fungus growth occurred in alfalfa fields during the early spring along the east bench of old Lake Bonneville. When the spring rains ceased, the fungus seemed to be restricted (Rockwood, 1951). There was another fungus species killing the alfalfa weevil, Beauveria bassiana, which produced long, thin, cottony mycelial growth with scattered spore clusters. B. bassiana produced flat, mealy, chalky, pulverulent growth. In contrast, B. globulifera produced a characteristically elevated,

cottony, loose, floccose myclial growth. Other differences between the two fungi was the color of the colony. After a period of time B. bassiana turned red due to a bacterial infection occurring at the same time B. globulifera remained white.

Rockwood (1951) reported that in some cases the B. globulifera growth became purple when it infected insects that continued to live or at least hibernate under or in the bark of trees, such as bark beetles. White B. globulifera was characteristic of insects that lived on the ground, trees, roots or fallen trees.

The Fungus

Beauveria globulifera is widely distributed and commonly found on several insects of orders reared in the dark at a temperature of 37°C. Insects become covered with a loose, cottony mycelial growth. In the outer areas of the fungus, balls of spores are developed. The balls are separated from each other by a short distance.

In well ventilated and lighted cages, the fungus forms a dense felt-like mass. The elytral, thoracic, and abdominal sutures become outlined by the white bands of mycelia (MacLeod, 1954). The old fungus growth assumes a well-defined cream color.

Microscopic Morphology

Beauveria globulifera produces fine branching, septate mycelia with flask-shaped sporophores or phialides. The conidiophores are borne along the hyphae. They occur singly, in pairs opposite each other, or in groups. They often occur in whorls or verticils (MacLeod, 1954).

A phialide becomes elongated into a filament upon which the spores are born basifugally. A spherical spore is born at the axis of the sporophore attached to a tiny sterigma. The sporophore then grows in the opposite direction producing a second spore followed by additional spores until a zigzag arrangement is obtained with the youngest spore at the tip. The infectious forms are both spores and fragmented hyphae.

Mode of Action

Death of an infected insect is the result of an accumulation of phenols which may be produced by either the fungus or the insect (MacLeod, 1954). The fungus forms a hard, compact sclerotium inside the body of the insect, the chitinous exoskeleton being the only part apparently unaffected.

Host Range

Both fungus species (B. globuliferum and B. bassiana) have a wide host range as shown in Table 1 (MacLeod, 1954).

Protein Assay

The nutrient of the egg, when it forms, is supplied either from the daily food of the insect (alfalfa in the case of alfalfa weevil, Hypera postica) or from the stored food in the body. Alfalfa contains 17% protein by dry weight. Not all that protein is metabolized by the insect but some goes without utilization to the fecal material and the remainder is utilized by the insect to meet the need of energy and tissue formation, including eggs (Chapman, 1982).

Table 1. Insect species from which Beauveria cultures have been studied (MacLeod, 1954).

Host	Family	Order	*B. bassiana*	*B. globulifera*
Melanoplus sp.	Locustidae	Orthop.	+*	−†
Camnula pellucida Scudd.	Acrididae	"	−	+
Sitona cylindricollis Fahr.	Curculionidae	Coleop.	+	−
Papillia japonica Newm.	Scarabaeidae	"	+	−
Scymnus impexus Muls.	Coccinellidae	"	−	+
Acanthocinus aedilis L.	Cerambycidae	"	−	+
Pyrausta nubilalis (Hbn.)	Pyralidae	Lepidop.	+	−
Crambus sp.	"	"	+	−
Mineola vaccinii (Riley)	"	"	−	+
Cacoecia murinana Hbn.	Tortricidae	"	+	−
Choristoneura fumiferana (Clem.)	"	"	+	+
Choristoneura pinus Free.	"	"	−	+
Archips fervidana Clem.	"	"	−	+
Archips cerasivorana (Fitch)	"	"	−	+
Unidentified. sp.	"	"	−	+
Bombyx mori L.	Bombycidae	"	+	−
Campaea perlata Gn.	Geometridae	"	+	−
Lambdina somniaria Hlst.	"	"	+	−
Nepytia canosaria Wlk.	"	"	+	+
Oporinia autumnata Gn.	"	"	+	−
Venusia cambrica Curt.	"	"	+	−
Enypia venata Grt.	"	"	−	+
Unidentified sp.	"	"	−	+
Malacosoma disstria Hbn.	Lasiocampidae	"	+	+
Malacosoma pluviale (Dyar)	"	"	−	+
Malacosoma americanum (F.)	"	"	−	+
Tolype sp.	"	"	+	−
Epicnaptera americana Harr.	Lasiocampidae	"	−	+
Carpocapsa pomonella (L.)	Oletlreutidae	"	+	+
Sciaphila duplex Wlshm.	"	"	+	−
Unidentified sp.	Phalaenidae	"	+	−
Zenobia pleonectusa Grt.	"	"	−	+
Cossus cossus L.	Cossidae	"	+	−
Unidentified sp.	Liparidae	"	−	+
Euphydryas chalcedona (Dbldy. and Hew.)	Nymphalidae	"	−	+
Nymphalis antiopa (L.)	"	"	−	+
Hyphantria textor Harr.	Arctiidae	"	−	+
Semasia rufimitrana H.-S.	Eucosmidae	"	−	+
Paraclemensia acerifoliella Fitch.	Incurvariidae	"	−	+
Monoctenus juniperinus MacG.	Diprionidae	Hymenop.	+	−
Neodiprion abietis (Harr.)	"	"	+	−
Neodiprion banksianae Rob.	"	"	+	−
Neodiprion lecontei (Fitch)	"	"	−	+
Neodiprion sertifer (Geoff.)	"	"	+	+
Neodiprion swainei Midd.	"	"	−	+
Neodiprion tsugae Midd.	"	"	+	−
Neodiprion virginiana Roh.	"	"	+	+
Diprion hercyniae (Htg.)	"	"	−	+
Hemichroa crocea (Fourc.)	Tenthredinidae	"	+	−
Pikonema alaskensis (Roh.)	"	"	+	−
Pristiphora erichsonii (Htg.)	"	"	+	+
Unidentified sp.	"	"	+	−
Trichiosoma triangulum Kby.	Cimbicidae	"	−	+

Egg Formation

The egg is formed in an egg tube, the middle section of an ovariole (Snodgrass, 1935). It is specifically formed at the germarium. The maturation of the egg does not take place until the egg is laid. At this time (while still in the oocyte stage) the egg is completely enclosed in the follicular egg chamber and is prevented from escaping prematurely into the oviduct. As discussed earlier, Dimilin when fed to insects stimulates the follicle cells. The insects start to lay premature eggs, or eggs with only partial shell coverings. When a normal egg is fully formed, the epithelium of the chamber begins a secretive activity producing a substance which is discharged upon the egg which then hardens to form the egg shell. This shell is the chorion. The chorion in appearance resembles the harder parts of the insect cuticle, but it is nonchitinous (Chapman, 1982). Therefore, the action of diflubenzuron is probably on the follicle cells not the chorion.

Dimilin, as a chitin inhibitor, could affect the median oviduct which is ectodermal, not part of the primitive genital system. It is a secondary exit structure formed from a series of invaginations of the body wall. The common oviduct has a cuticular chitinous lining continuous with the cuticula of the body wall. The epithelial tube is surrounded by a strong, muscular sheath consisting of circular and longitudinal fibers.

Egg Nutrition

Nutrition for the egg is supplied from either the daily food of the insect absorbed into the blood, or from the food reserves stored

in the insect body principally in the fat tissue. With insects that do not feed in the imaginal stage, all of the egg material must be drawn from the latter source. An alfalfa weevil adult feeds on alfalfa containing about 17% protein by dry weight. Material passes by diffusion directly through the walls of the ovarioles, especially by way of the epithelial layer of the follicle cells. Food material is absorbed from the blood and incorporated in the follicle cell cytoplasm. The highly nutritious plasma of the follicle cells is then absorbed by the egg cells.

METHODS

Chemical Preparation

Four grams of 25% WP diflubenzuron were added to 400 ml distilled water as a stock solution (0.01 g/ml) to equal 2.5 x 10^{-3} g/ml active ingredient. Fresh stock solution was prepared every 7 to 10 days. Four dilutions were prepared:

1. One ml from the stock solution was mixed with 499 ml distilled water to prepare 2 x 10^{-5} g/ml (5 x 10^{-6} g/ml active ingredient).
2. Ten ml from the stock solution were added to 490 ml water to get 2 x 10^{-4} g/ml (5 x 10^{-5} g/ml active ingredient).
3. One hundred ml from the stock solution were added to 400 ml water to get 2 x 10^{-3} g/ml (5 x 10^{-4} active ingredient).
4. Ten grams of 25% WP diflubenzuron were added to 400 ml water to get 0.025 g/ml (6.3 x 10^{-3} g/ml active ingredient). Abbott's formula for the chemical preparation was followed (Abbott, 1925).

Insect Rearing

Alfalfa weevils were collected from a North Logan alfalfa field by sweeping during early May 1982 and brought to the laboratory, where they were separated according to sex. Only females were used in the experiments.

Alfalfa (4 stems) was dipped in each concentration listed above, plus a distilled water control, then air-dried. It was then placed in plastic vials (7 x 3 cm) with distilled water within a plastic

jar (10 x 4 inch). Twenty insects were placed in each jar. The total jars in each series was 20, 4 cages for each of 5 treatments.

The plants were changed each 48 hr. In the first series of experiments, the fresh replacement plants were not treated with diflubenzuron. In the second series of experiments, the fresh food was dipped to allow continuous exposure for 22 days.

The number of eggs was determined by using a dissecting microscope. Eggs laid each 48 hr were recorded. At the same time, dead insects were collected and placed on moist filter paper in petri dishes for 3 days to determine whether death was due to the fungus.

To determine egg viability, the eggs (100 whenever possible) were dissected from the stems in each treatment. They were kept in petri dishes on moistened filter paper and incubated at room temperature, 22°C.

After one week, all petri dishes were examined and the number of unhatched eggs was subtracted from 100 (the original number) and percent hatchability determined.

The shape of the eggs was determined from random samples of eggs selected from the controls and from the treated insects. Some measurements were also made of eggs inside the plants. The length and width of each egg was measured using a lens micrometer.

Muscardine Studies

Adult alfalfa weevils were collected, using sweeping nets, from North Logan alfalfa fields on September 15, 1982. They were fed Dimilin

treated alfalfa foliage (6.3×10^{-3} g/ml) continuously for 2 weeks. A concurrent series of weevils were fed untreated foliage. They were placed in 9 plastic cages containing 12 insects each. Each cage contained four alfalfa stems in (7 x 3 cm) plastic vials containing water.

Both diflubenzuron and controls were divided into two series. One series was treated with muscardine fungus spores (the weevils were exposed to fungus spores in petri dishes for 48 hr). The other series did not involve an intentional muscardine contamination. All experiments were replicated three times.

All cages were covered by plastic in order to increase the humidity inside the cages to about 75% at 25°C.

Every 48 hr, cages were checked and results recorded. All dead insects were collected and placed in petri dishes at 25°C. and 65% relative humidity.

Protein Assay

The purpose of this experiment was to test the protein content of the fecal material by weight by the colorimetric method using the spectrophotometer (Zeiss PMQII, single beam spectrophotometer) (Fritz, 1979).

This protein assay is applied by the Coomassie Brilliant Blue G-250 dye. The reason for using this test, and not other tests, is that this assay is very rapid, with the dye binding process virtually complete in approximately 2 min, with good color stability for 1 hr. There is also little or no interference from cations, such as sodium or potassium, nor from carbohydrates, such as sucrose (Bradford, 1975).

Many weevils which were fed alfalfa treated with diflubenzuron showed a yellow deposit on the ventral side of the abdomen. Protein analysis was used to partially clarify the nature of the deposit.

Sample preparation

Insects were collected from the field and kept in containers with alfalfa as food. Some were fed alfalfa treated with diflubenzuron 6.3×10^{-3} g/ml. After 15 days they were removed and the container washed with distilled water. The volume was then completed to 100 ml by 100 ml volumetric flask.

Optical density readings

Five test tubes containing 0.5 ml, 0.4 ml, 0.3 ml, 0.2 ml and 0.1 ml of the stock solution, respectively, were prepared. Water was added to each test tube, except the first, to bring the volume to 0.5 ml. After that, 5 ml of Coomassie Brilliant Blue G-250 dye was added to each test tube (filtration for the dye was done). Then optical density readings, using Zeiss PMG-II spectrophotometer at 595 nm, were increased and the absorptions recorded.

Sample weight

Three test tubes were selected, dried quickly, then cooled and weighed. After that, 10 ml from the original solution was placed in each test tube and dried at 102°C for 18 hr, then weighed again. The weight before heating was subtracted from the latter weight. This difference represented the amount of the sample in the 100 ml which represented the original solution.

For the statistical analysis, I followed the two-way analysis of variance. For presenting the data, I followed a regression plot fit.

RESULTS

Mortality of Adults

Direct mortality of adult weevils following feeding on foliage treated with diflubenzuron was minimal. Differences were not significant at the 5% level.

From one time feeding

The highest percent initial mortality occurred with 5×10^{-6} g/ml dose of diflubenzuron, the lowest dose used in the experiment (Table 2, Fig. 1). There was extreme variation between replicates. Mortality was due mostly to handling, weevil age, and muscardine fungus. All of the dead insects which were collected and held for 3 days became covered by the fungus.

From continuous feedings

The highest mortality at the end was associated with treatment 5×10^{-4} g/ml (47.65%), but the differences were not significant (Table 3 and Fig. 2).

Table 2. The mean percent mortality of female alfalfa weevils fed for 48 hours on foliage dipped in different concentrations of diflubenzuron.

Dimilin concentration	Mean mortality at 48 hr.											
	May 4	May 6	May 8	May 10	May 12	May 14	May 16	May 18	May 20	May 22	May 24	$\bar{X}$
0 g/ml	6.43	13.54	15.32	18.43	25.37	26.76	31.17	32.55	32.55	33.94	36.72	24.8
5×10^{-6} g/ml	18.15	22.34	34.93	34.93	34.93	38.22	40.50	43.67	58.82	57.14	60.99	40.42
5×10^{-5} g/ml	10.62	15.62	20.61	23.75	28.75	30.82	37.30	50.24	55.13	56.80	60.12	35.43
5×10^{-4} g/ml	9.69	16.18	33.20	43.72	45.28	46.84	41.18	47.26	58.92	58.93	58.92	42.74
6.3×10^{-3} g/ml	8.44	12.02	18.74	19.99	25.08	25.08	29.66	32.89	33.89	33.89	33.89	24.87

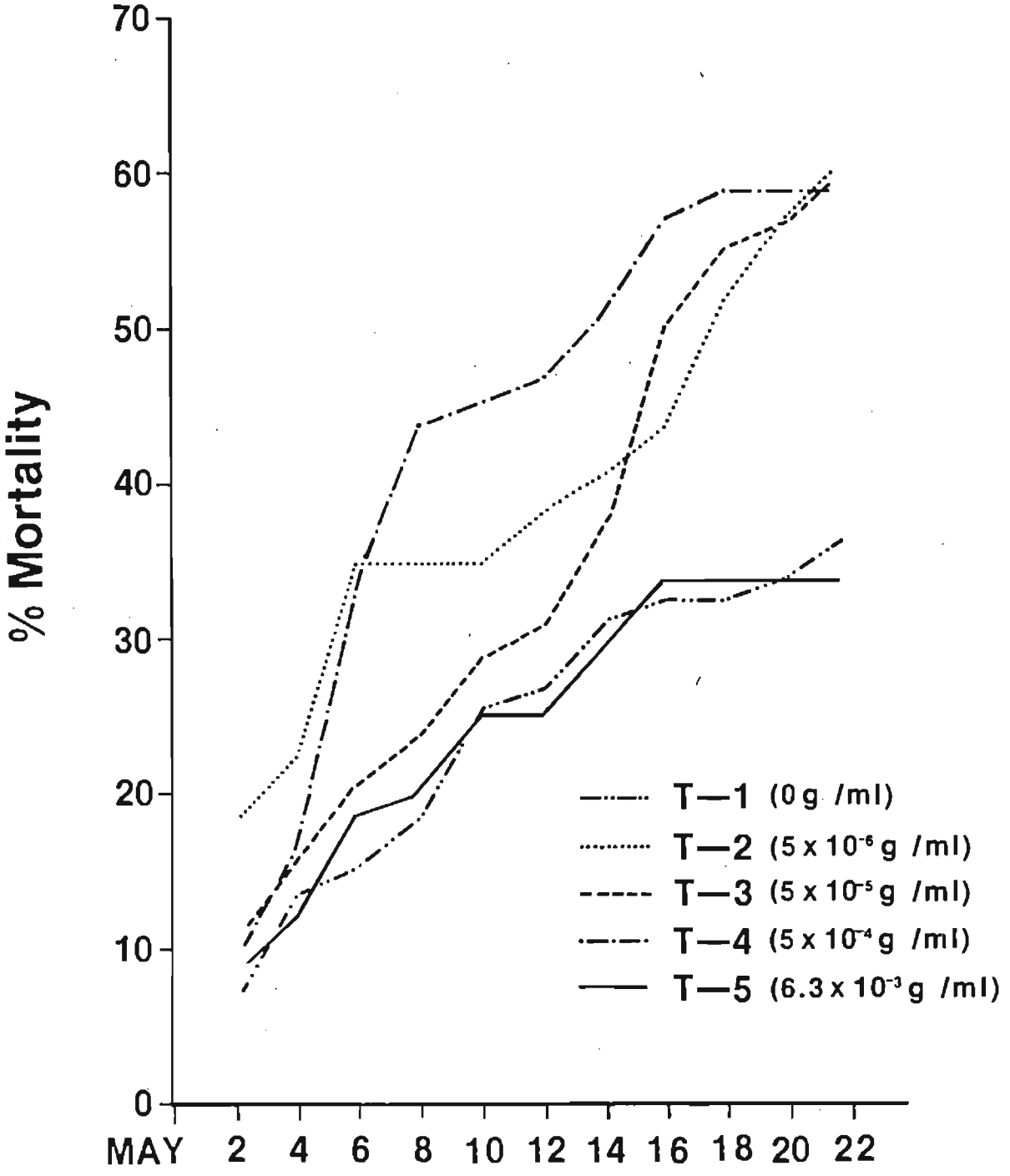


Fig. 1. The percent mortality of female alfalfa weevils when the insects were fed for 48 hr with different doses of diflubenzuron (Dimilin) (one time feeding).

Table 3. The mean mortality for alfalfa weevil adults fed continuously starting June 21 on foliage dipped in four concentrations of diflubenzuron.

Diflubenzuron Concentration	Percent mortality								
	June 23	June 25	June 27	June 29	July 1	July 3	July 5	July 7	$\bar{X}$
0 g/ml	0	16.53	14.03	16.81	16.81	16.81	19.93	23.06	17.71
5×10^{-6} g/ml	0	5.00	8.13	14.03	17.15	17.15	30.56	33.33	17.91
5×10^{-5} g/ml	0	5.00	10.28	12.78	12.78	15.56	15.56	18.06	12.86
5×10^{-4} g/ml	0	0	15.28	26.11	29.86	41.11	41.11	46.65	27.16
6.3×10^{-3} g/ml	0	6.7	6.7	19.48	13.05	31.99	35.55	35.55	21.29

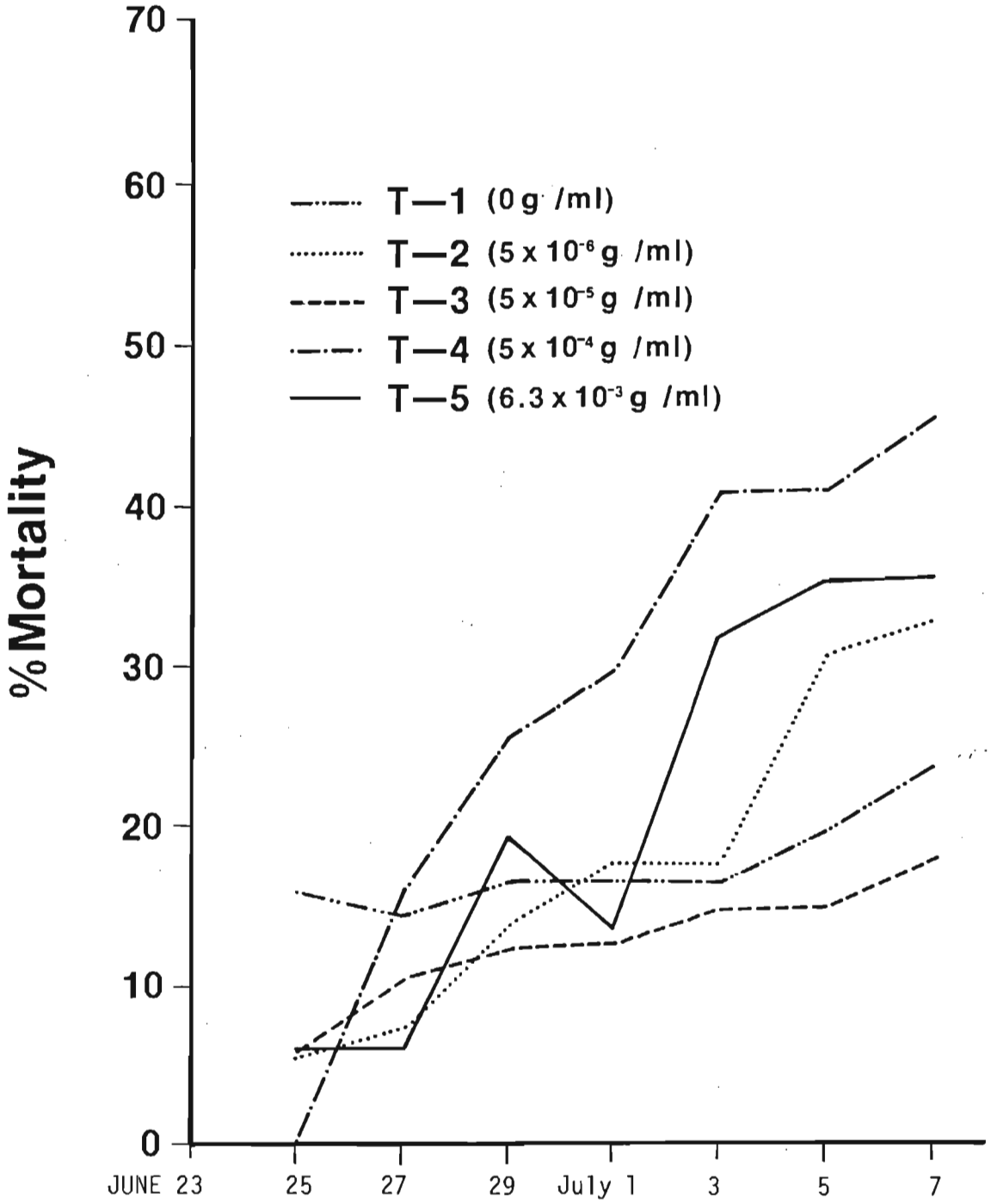


Fig. 2. The percent mortality of the female alfalfa weevils when the insects were fed continuously on alfalfa with different doses of diflubenzuron (Dimilin).

Egg Numbers

Alfalfa weevils in northern Utah return to alfalfa fields during April. About late April or early May each adult female lays about 25 eggs per day. By late May the daily oviposition rate is less than half that of early May. The oviposition then levels off at approximately 5 eggs per day until late July when most old adult weevils die off.

Egg numbers related to single 48 hour feedings

The decline in daily oviposition from May 4 to 24 is typical of alfalfa weevils (Fig. 5 and Table 4). All comparisons should be related to feeding on the same date. There were no significant differences in feeding rates until May 8, or 6 days after the start of the experiments on May 2. From May 8 to May 20, the heaviest feeding rate showed a significant (5%) reduction in the number of eggs laid. There were no differences between the other treatments and the control.

Egg numbers related to continuous feeding

This second series of feeding tests was made later in the season when the daily oviposition was less than that in the first series. On three dates (June 29, July 1 and July 5) there were more eggs laid per female in the most diluted diflurobenzuron treatement than in the controls (Table 5 and Fig. 4). Reasons for these increases are unknown. Late in the experiments there were fewer eggs laid per female in the heaviest treatment. This was significantly lower than two of the treatments, but not the controls.

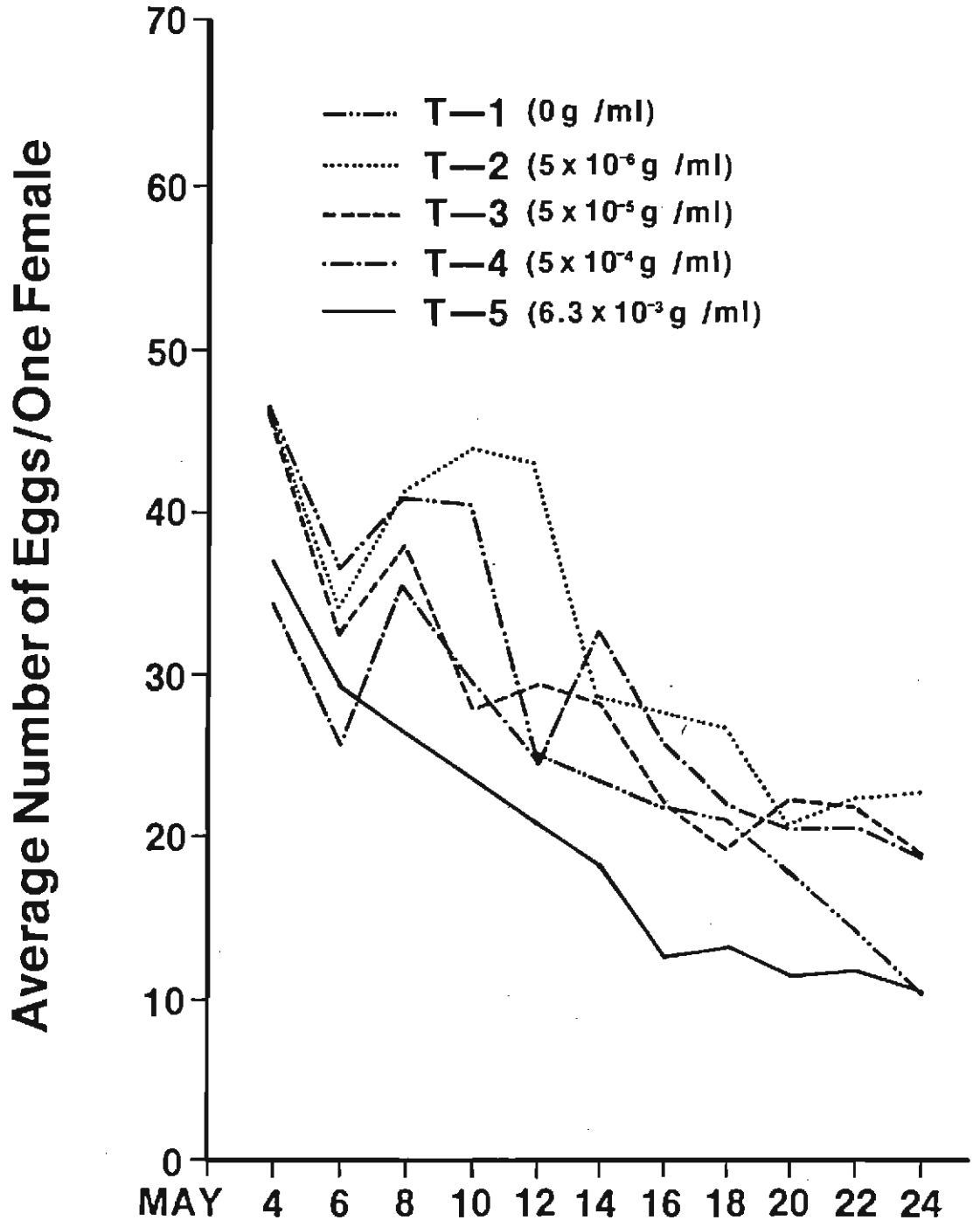


Fig. 3. The average number of eggs laid per female alfalfa weevil, when they were fed alfalfa treated with different doses of diflubenzuron for 48 hours starting May 2.

Table 4. The average number of eggs laid per female alfalfa weevil fed for 48 hours starting May 2 on foliage dipped in different concentrations of diflubenzuron.

Diflubenzuron Concentration	Average number of eggs												
	May 4	May 6	May 8	May 10	May 12	May 14	May 16	May 18	May 20	May 22	May 24	Total	$\bar{X}$
0 g/ml	46.91	36.46	40.83	40.37	25.25	23.50	22.33	21.91	17.65	14.65	10.50	300.35	25.03
5×10^{-6} g/ml	46.81	34.06	41.1	43.78	43.28	28.42	27.40	26.56	20.69	22.53	22.98	356.56	29.80
5×10^{-5} g/ml	45.83	32.88	37.74	27.41	29.53	28.13	22.68	29.03	22.88	22.23	18.75	307.09	25.59
5×10^{-4} g/ml	34.21	25.81	35.07	29.79	24.53	32.88	25.73	22.28	20.47	20.57	18.35	289.69	24.14
6.3×10^{-3} g/ml	36.99	29.15	26.48	32.92	20.90	17.34	13.03	13.67	11.52	11.78	10.7	224.48	18.71

$\bar{X} = 22.99$

Table 5. The average number of eggs laid per female alfalfa weevil fed continuously on foliage dipped in four concentrations of diflubenzuron.

Diflubenzuron Concentration	Average number of eggs per female								
	May 2	May 4	May 6	May 8	May 10	May 12	May 14	Total	$\bar{X}$
0 g/ml	5.01	6.27	8.17	5.02	10.92	6.65	6.25	48.29	6.9
5×10^{-6} g/ml	9.56	14.46	14.75	7.95	18.47	11.74	10.81	87.74	12.53
5×10^{-5} g/ml	8.45	7.6	11.89	8.28	9.13	6.92	4.35	56.62	8.09
5×10^{-4} g/ml	6.91	9.7	7.35	6.15	4.72	16.06	3.17	54.06	7.72
6.3×10^{-3} g/ml	3.12	7.36	9.13	7.48	8.49	2.01	2.13	39.72	5.67

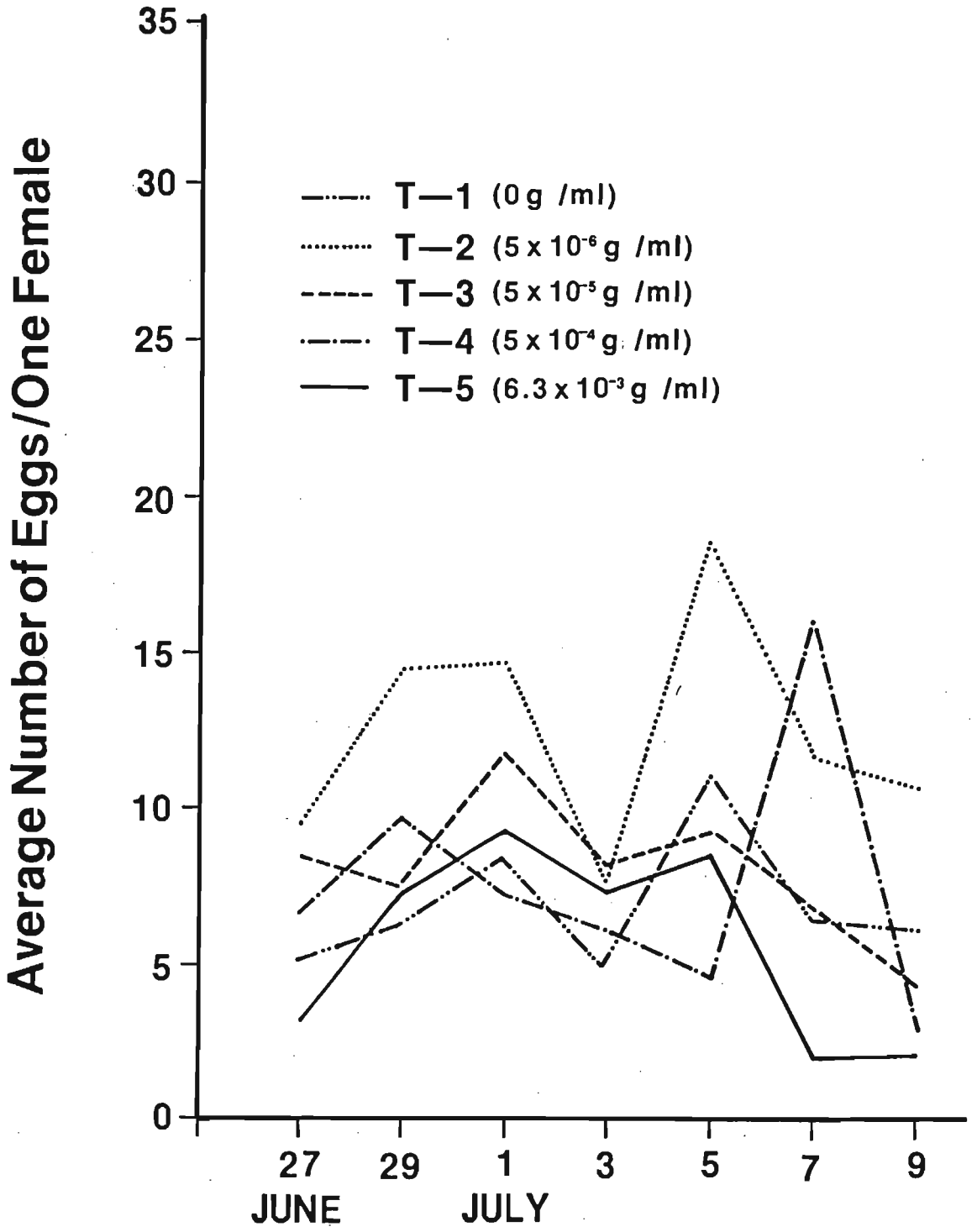


Fig. 4. The average number of eggs laid per female alfalfa weevil collected at 48 h intervals when fed (continuously) with different doses of diflubenzuron (Dimilin).

Egg Hatchability

In contrast with other data, egg hatchability was affected dramatically by diflubenzuron. Also, the hatchability in the controls was consistently near 90%.

One time feeding

Diflubenzuron at 6.3×10^{-3} g/ml caused the greatest reduction in egg hatchability. The percent hatchability reached 38.9%, 8 days after feeding, it increased again reaching 66.9% by the end of the experiment (Table 6 and Fig. 5). Significant reduction was first noted within 2 days at the heaviest dosage but only after 6 days with the lowest rate.

The minimum dose of 5×10^{-6} g/ml also affected egg hatchability as shown in Fig. 5. The effects increased with each dose. All the treatments shown, except the control, showed a decrease in hatchability then an increase. Within the time limits of the experiments, there was never a complete recovery.

Continuous feeding

As with the 48 hour feeding experiments, the lowest percent hatchability was associated with the highest dose of diflubenzuron and

Table 6. The percent hatchability of eggs laid by female alfalfa weevils fed for 48 hours starting May 2 on foliage dipped in four concentrations of diflubenzuron.

Diflubenzuron Concentration	Percent hatched from eggs laid on each date						
	May 4	May 6	May 8	May 10	May 12	May 14	May 16
0 g/ml	91.2	92.4	91.4	89.6	91.2	92.1	90.9
5×10^{-6} g/ml	88.3	84.5	81.6	82.7	89.6	88.9	83.4
5×10^{-5} g/ml	89.6	75.7	71.0	71.4	73.9	75.7	76.9
5×10^{-4} g/ml	85.1	73.8	62.8	64.1	66.2	65.4	72.5
6.3×10^{-3} g/ml	81.0	73.1	45.1	38.9	54.2	51.2	66.5

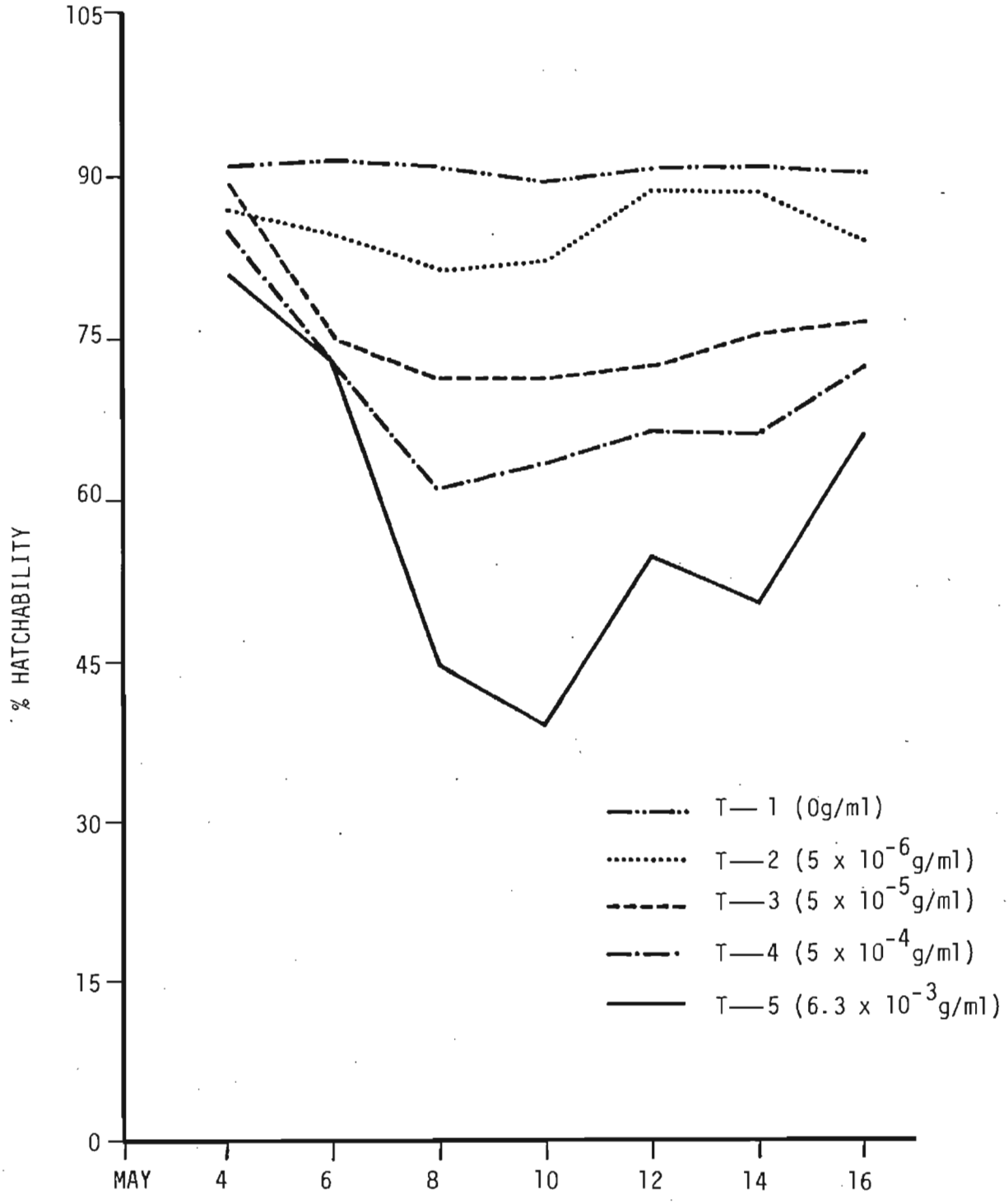


Fig. 5. The percent hatchability of eggs laid by female alfalfa weevils fed for 48 hours starting May 2 on foliage dipped in four concentrations of diflubenzuron

the minimum effects with the lowest doses. However, the decrease in hatchability was permanent and did not show recovery with time (Table 7 and Fig. 6).

Muscardine Fungus

Most of the weevil mortality was apparently caused by the fungus, Beauveria sp. Insects fed diflubenzuron treated alfalfa were noted to have most fungus infections during the experiments in the laboratory.

The highest mortality of 63.8% was associated with the treatment using both fungus spores and insects fed diflubenzuron (6.3×10^{-3} g/ml). The mortality in the control was 6.08% and 58.85% with insects exposed to the muscardine fungus spores only.

The total muscardine infections were not significantly different between insects fed diflubenzuron and treated with spores only. However, the patterns of infection were distinctly different. These differences are shown in Figs. 7 and 8. Note the sparse feathery fungal growth compared to the dense tufted growth. Note also in Table 8 and Fig. 9 that the disease appeared sooner in insects treated with both spores and diflubenzuron.

Effects of diflubenzuron on egg shape

Eggs laid by diflubenzuron treated weevils were elongated, easily broken and a lighter color. The abnormal elongation of the eggs compared to the normal shape of the treated insect eggs with diflubenzuron are shown in Fig. 13a and b. The example in 10b came from the highest dose level.

Table 7. The percent hatchability of eggs laid by female alfalfa weevils fed continuously on foliage dipped in four concentrations of diflubenzuron

Diflubenzuron doses	Average percent hatched[1]		
	June 27	June 29	July 1
0 g/ml	90	90.83	89.33
5×10^{-6} g/ml	90.77	80.67	76.67
5×10^{-5} g/ml	85.68	68.50	74.08
5×10^{-4} g/ml	83.75	74.33	50.42
6.3×10^{-3} g/ml	79.97	48.30	18.00

[1]After July 1 the total eggs laid in the diflubenzuron treatments was extremely low and erratic. The data could not be analyzed statistically.

Table 8. Average percent mortality of female alfalfa weevils when treated by the muscardine spores and/or diflubenzuron for 48 hours.

	Mortality at 48 hr intervals					
	2	4	6	8	10	$\bar{X}$
T- I*	2.76	2.76	5.53	5.53	13.83	6.08
T- II**	8.3	33.6	61.03	91.52	99.82	58.85
T-III***	22.2	41.6	69.36	86.00	99.83	63.8

*T- I: insects fed alfalfa treated with diflubenzuron 6.3 x 10^{-3} g/ml.

**T- II: insects sprinkled with muscardine fungus sproes.

***T-III: insects treated with diflubenzuron and muscardine fungus spores.

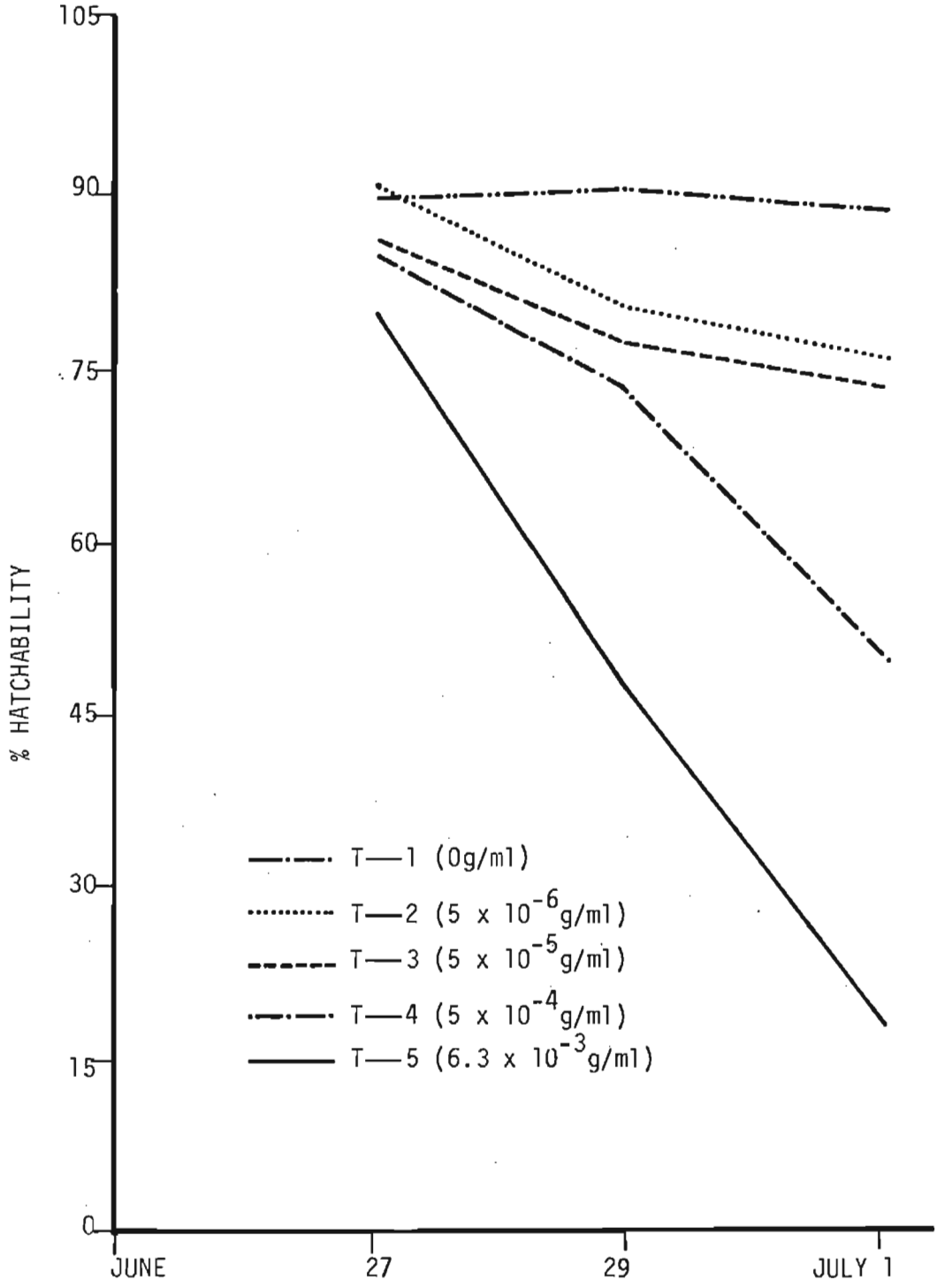


Fig. 6. The percent hatchability of eggs laid by female alfalfa weevils fed continuously on foliage dipped in four concentrations of diflubenzuron.

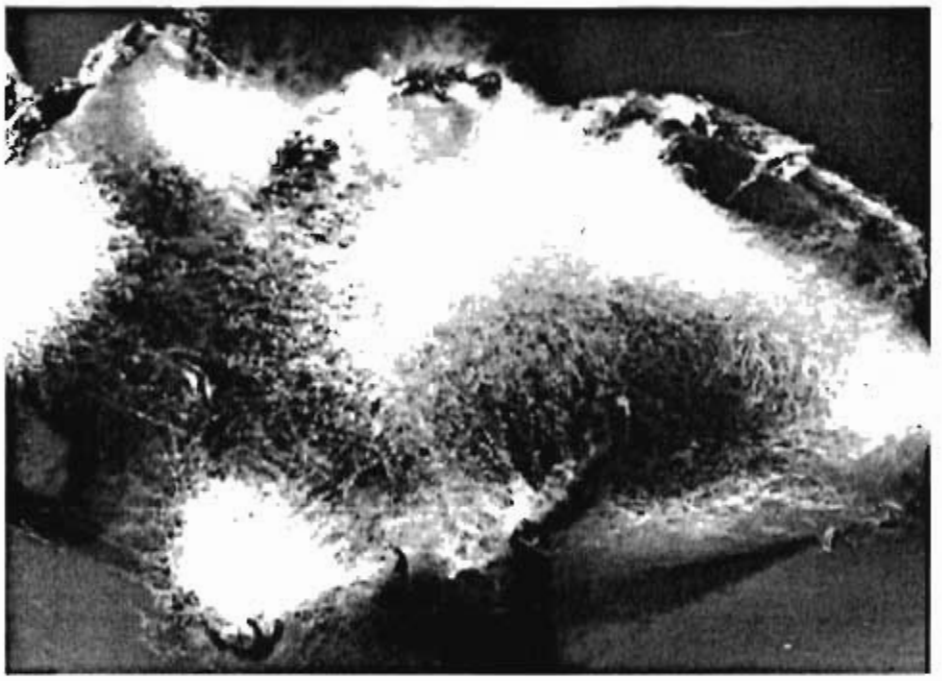


Fig. 7. The sparse coverage by muscardine fungus in specimens fed on untreated alfalfa.

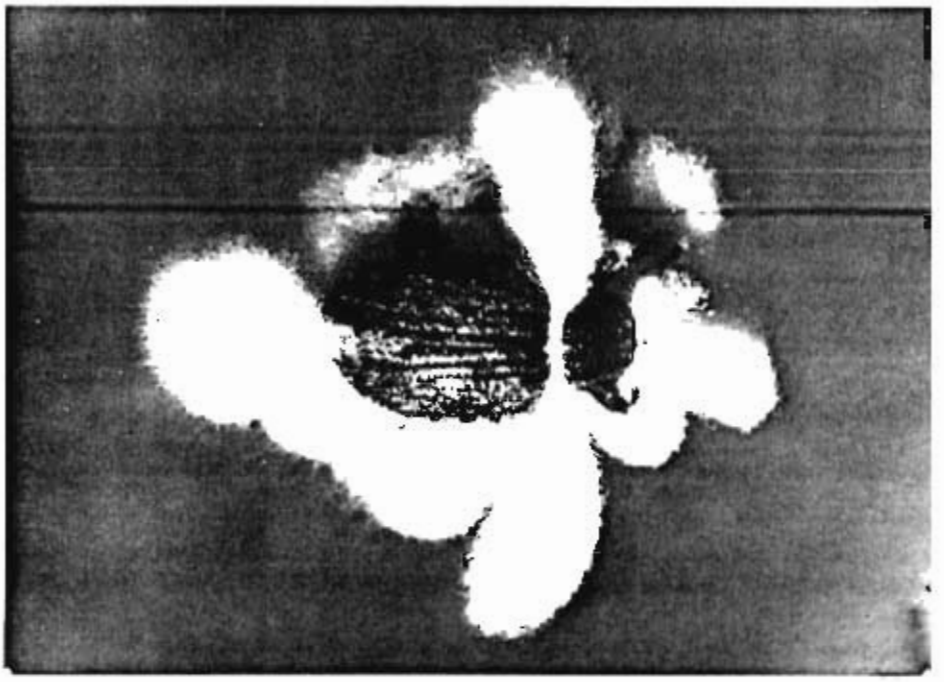


Fig. 8. The dense tufted coverage by muscardine fungus on insects fed on treated foliage by 6.3×10^{-3} g/ml diflubenzuron.

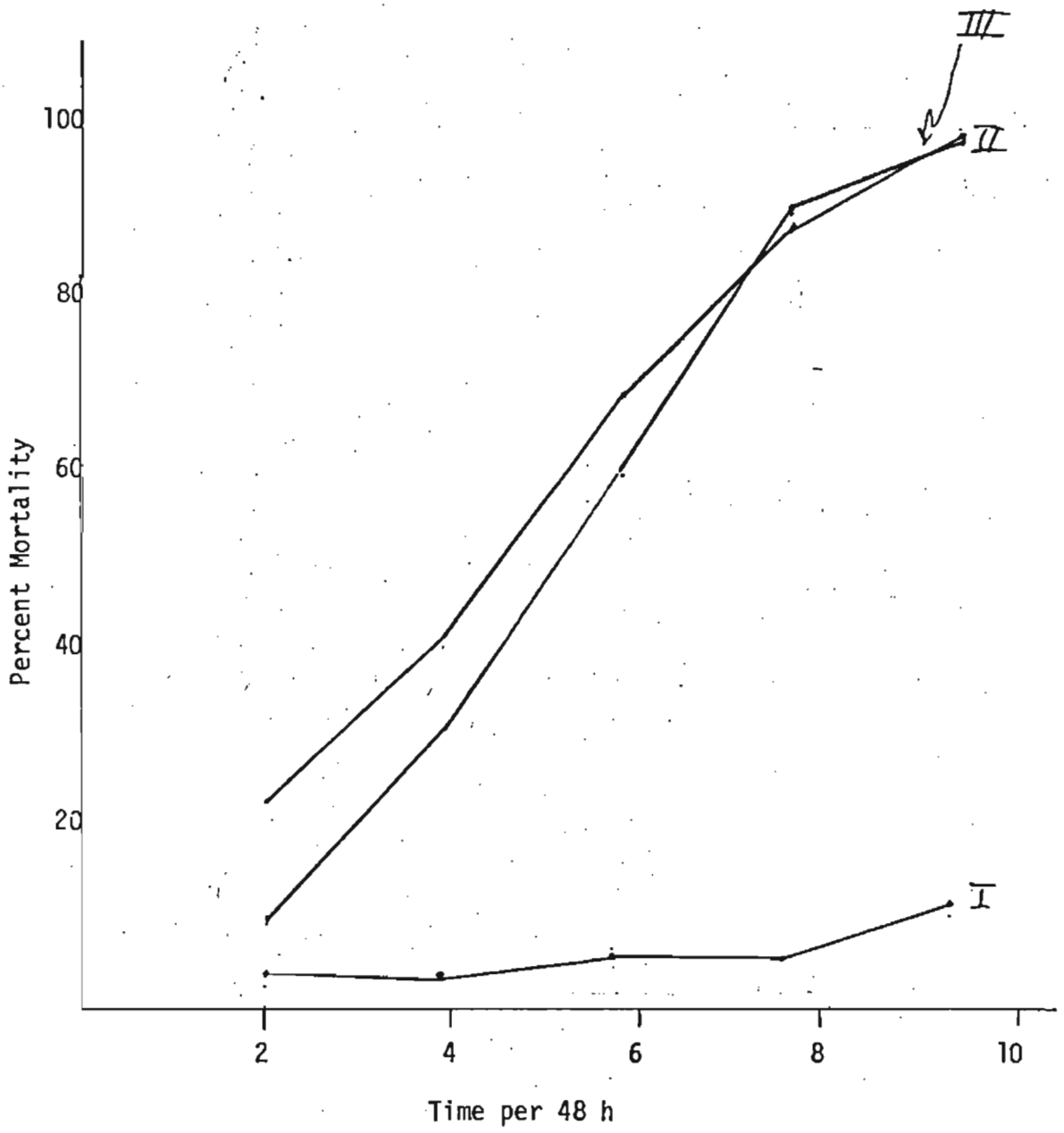
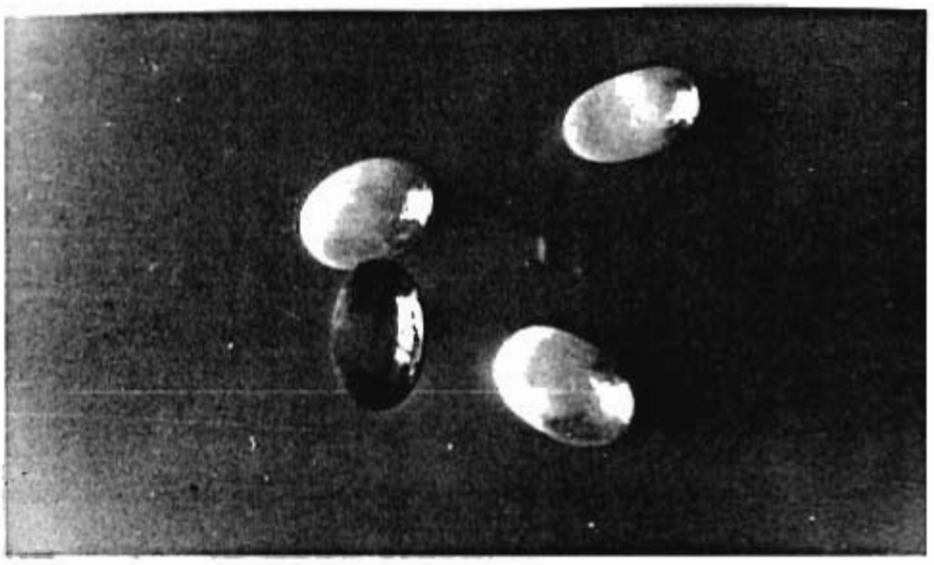
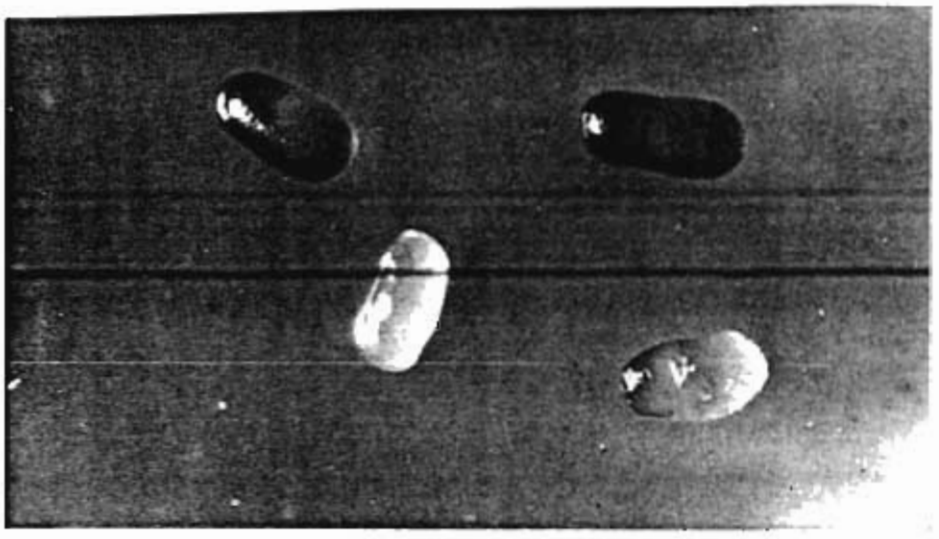


Fig. 9. Average percent mortality of: I, female alfalfa weevils fed on foliage dipped in diflubenzuron (6.3×10^{-3} g/ml). II, female alfalfa weevils fed on untreated foliage and muscardine spores. III, alfalfa weevils fed on diflubenzuron treated foliage and muscardine spores.



a. Untreated



b. Treated

Fig. 10. The effect of diflubenzuron on the egg shape.

The average length of 30 eggs selected from the treated insects with diflubenzuron, 6.3×10^{-3} g/ml was 0.68 mm and the average width was 0.34 mm compared to the average length and width of the controls 0.65 mm, 0.47 mm, respectively. Treated weevils tended to lay more in leaf petioles than weevils from the controls.

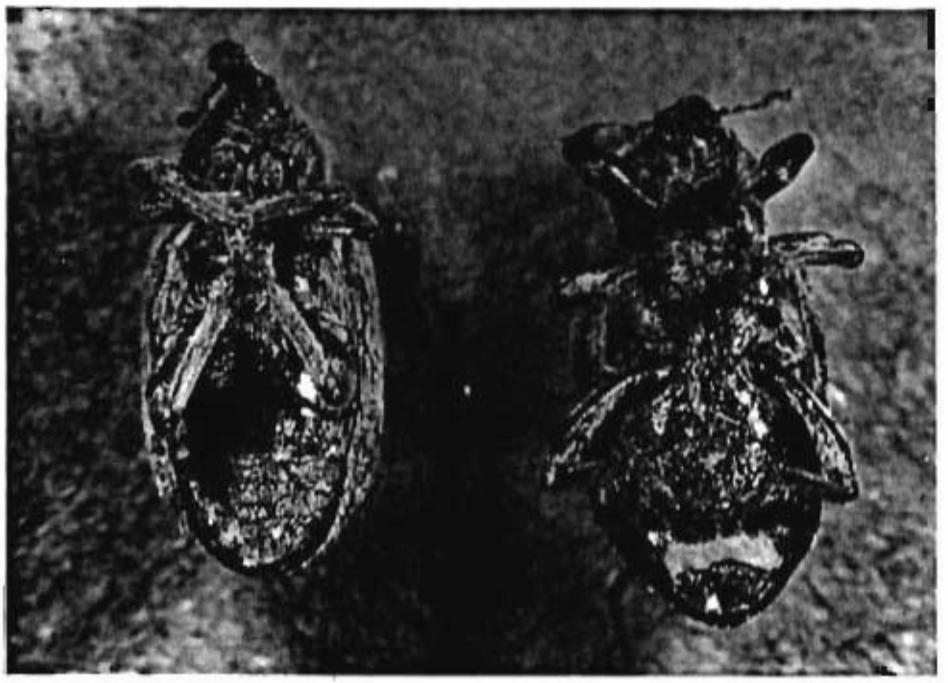
Effects on the terminal end of the insect

A yellow spot near the tip of the abdomens of insects fed diflubenzuron was usually present. A comparison of the abdomens of treated and untreated weevils is shown in Fig 11.

Also, Fig 12 shows an extended structure commonly observed on insects treated with diflubenzuron.

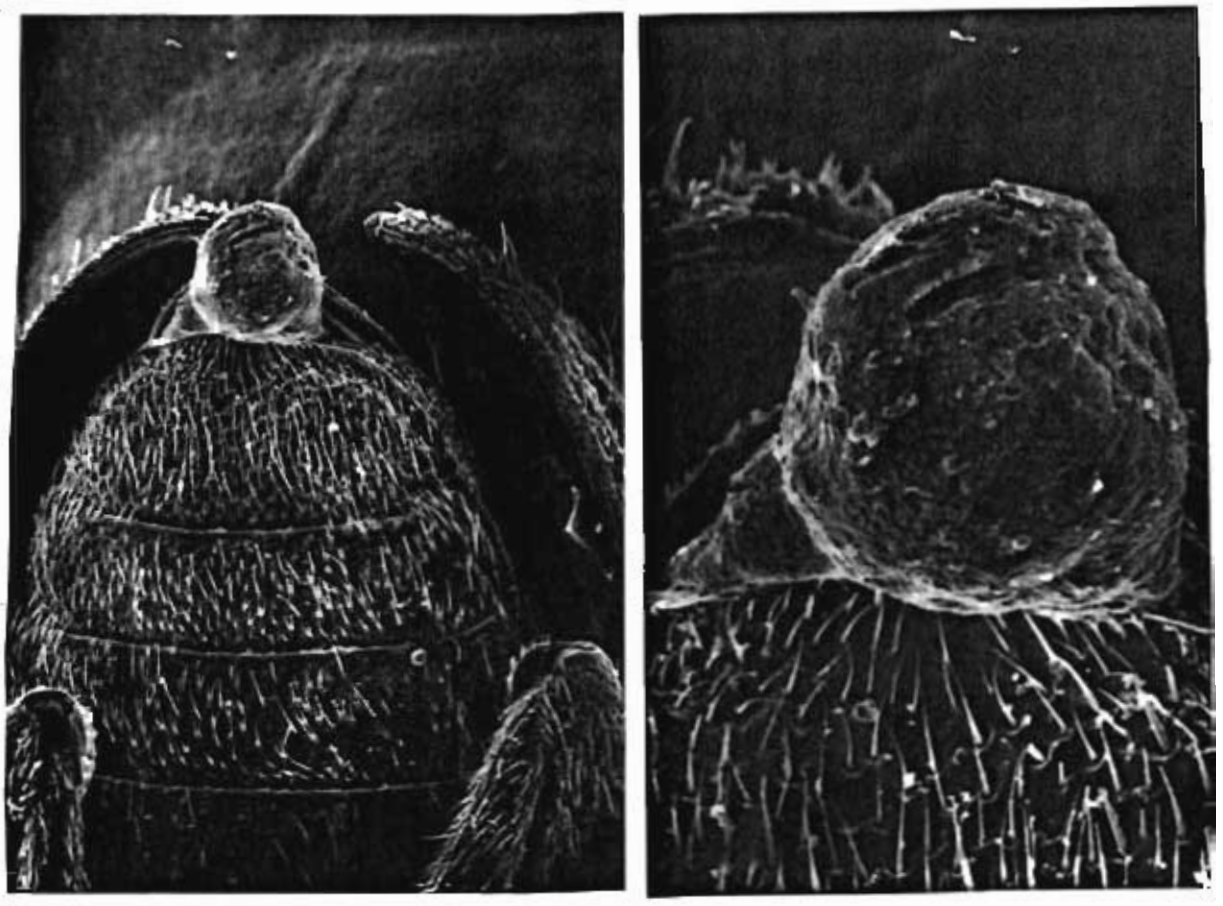
Protein assay

Protein analyses of the yellow spots at the end of the treated insects and of the fecal material in both control and treated insects were made. The protein percent of the fecal material of alfalfa weevil was 7.27% in general.



a. untreated b. treated

Fig. 11. Effect of diflubenzuron (6.3 x 10-3 g/ml) on the terminal end of the female alfalfa weevil.

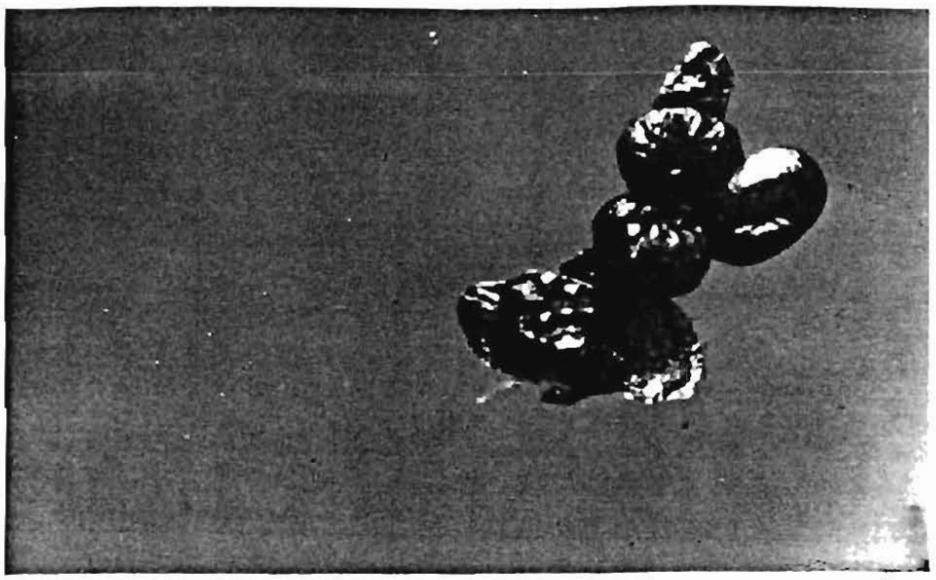


*100 times magnification

500 times magnification

Fig. 12. Extrusion of the alimentary canal of the weevils due to diflubenzuron when fed to the insect continuously (6.3×10^{-3} g/ml) (by scanning electron microscope).

a.*



b.*

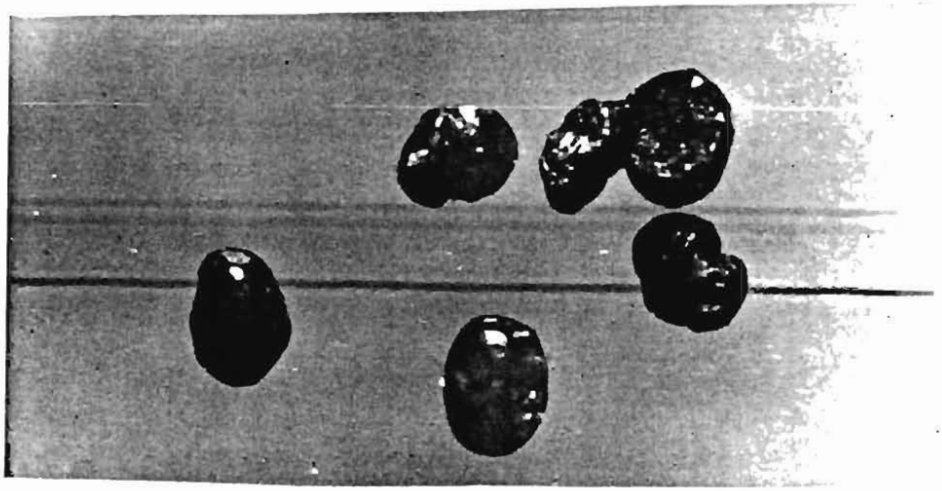


Fig. 13. The effect of diflubenzuron on the eggs of alfalfa weevils when fed treated alfalfa for 48 hours.

*a. 5×10^{-4} g/ml

b. 6.3×10^{-3} g/ml

Table 9. Two-way analysis of variance of the hatchability of the eggs of alfalfa weevils when fed for 48 hours on foliage dipped in different doses of diflubenzuron.

Source of variance	df	ss	MS	F ratio
Dose effect	4	20,114.757143	5028.689289	51.326874
Time effect	6	5,032.171429	838.695238	8.560403
Dose time interaction	24	4,931.042857	205.460119	2.097092
Error	105	10,287.25	97.973810	
Total		11,391.08		

Dose F tabulated value = 2.78 $\alpha = 0.05$

Time F tabulated value = 2.51

Dose time interaction F tabulated value = 1.7

Since calculated F value exceeds the tabulated value, we can conclude that both dose of diflubenzuron and the time have significant effects on the hatchability of the eggs. Also, the interaction between the dose and time is significant.

Table 10. Pairwise differences of estimated means for the dose effect on the hatchability of alfalfa weevil when fed on alfalfa foliage dipped in different concentrations of diflubenzuron for 48 hr.

Dose applied	LSD 0.950 Confidence interval	Sorted estimated means**
C(1) - C(2)*	0.578 11.065	C_1 : 91.14286
C(1) - C(3)	9.828 20.315	C_2 : 85.23143
C(1) - C(4)	16.221 26.708	C_3 : 76.07143
C(1) - C(5)	28.935 39.422	C_4 : 69.67857
C(2) - C(3)	4.007 14.493	C_5 : 56.96429
C(2) - C(4)	10.399 20.886	
C(2) - C(5)	23.114 33.601	
C(3) - C(4)	1.149 11.636	
C(3) - C(5)	13.864 24.351	
C(4) - C(5)	7.471 17.958	

*C_1---C_5 are the concentrations of the chemical used in experiments (0 g/ml, 5×10^{-6} g/ml, 5×10^{-5} g/ml, 5×10^{-4} g/ml and 6.3×10^{-3} g/ml, respectively).

**The consequence of the doses which have the effect on the hatchability of alfalfa weevil eggs. Sorted from the control (C_1) to the highest effect (C_5, 6.3×10^{-3} g/ml).

Table 11. Pairwise differences of the estimated means for the time effect on the hatchability of eggs of alfalfa weevils when fed on alfalfa foliage dipped in different concentrations of diflubenzuron for 48 h.

Time effect*	LSD 0.950 Confidence interval	Sorted estimated mean**
T(1) - T(2)	0.996 13.404	T_1 : 86.9
T(1) - T(3)	12.746 25.154	T_2 : 79.7 a
T(1) - T(4)	11.646 24.054	T_7 : 77.75 ab
T(1) - T(5)	5.896 18.304	T_5 : 74.8 abc
T(1) - T(6)	5.996 18.404	T_6 : 74.7 abcd
T(1) - T(7)	2.946 15.354	T_4 : 69.05 bcde
T(2) - T(3)	5.546 17.954	T_3 : 67.95 bcde
T(2) - T(4)	4.446 16.854	
T(2) - T(5)	-1.304 11.104	
T(2) - T(6)	-1.204 11.204	
T(2) - T(7)	-4.254 8.154	
T(3) - T(4)	-7.304 5.104	
T(3) - T(5)	-13.054 -0.646	
T(3) - T(6)	-12.954 -0.546	
T(3) - T(7)	-16.004 -3.596	
T(4) - T(5)	-11.594 0.454	
T(4) - T(6)	-11.854 0.554	
T(4) - T(7)	-14.904 -2.496	

Table 11 (continued).

Time effect*	LSD 0.950 Confidence interval	Sorted estimated mean**
T(5) - T(6)	-6.104 6.304	
T(5) - T(7)	-9.154 3.254	
T(6) - T(7)	-9.254 3.154	

*T_1---T_7 are the notations for time at which the eggs collected from the treated foliage with different concentrations of diflubenzuron (at period of 48 hr).

**Sorted mean of the time effect from highest to lowest significance effect on the hatchability of the alfalfa weevil eggs.

Table 12. Two-way analysis of variance for the hatchability of the eggs of alfalfa weevils when fed continuously on foliage dipped in different diflubenzuron concentrations.

Source of variance	df	ss	MS	F ratio
Dose effect	4	13,970.553086	3492.638272	31.996889
Time effect	2	3,619.511111	1809.75556	16.579601
Dose time interaction	8	4,460.488889	557.561111	5.7950
Error	30	3,274.666667		
Total		5,747.89		

Dose F tabulated value = 3.84 $\alpha = 0.05$

Time F tabulated value = 4.46

Dose time interaction F tabulated value =

Since calculated F value exceeds the tabulated value, we can conclude that both dose of diflubenzuron and the time have significant effects on the hatchability of the eggs. Also, the interaction between the time and the dose is significant.

Table 13. Pairwise differences of the estimated means for the dose effect on hatchability of the eggs of alfalfa weevil when fed on foliage dipped in different concentrations of diflubenzuron continuously.

Dose applied*	LSD 0.950 Confidence interval	Sorted estimated mean**
C(1) - C(2)	-2.833 17.277	C_1 : 89.8889 a
C(1) - C(3)	0.500 20.611	C_2 : 88.66 ab
C(1) - C(4)	4.389 24.500	C_3 : 79.3334 bc
C(1) - C(5)	38.612 58.722	C_4 : 75.44 bc
C(2) - C(3)	-6.722 13.388	C_5 : 41.22
C(2) - C(4)	-2,833 17.277	
C(2) - C(5)	31.389 51.500	
C(3) - C(4)	-6.166 13.944	
C(3) - C(5)	28.056 48.166	
C(4) - C(5)	24.166 44.277	

*C_1---C_5 are the concentrations of the chemical used in experiments (0 g/ml, 5 x 10^{-6} g/ml, 5 x 10^{-5} g/ml, t x 10^{-4} g/ml and 6.3 x 10^{-3} g/ml, respectively).

**The consequence of the doses which have the effect on the hatchability of alfalfa weevil eggs. Sorted from the highest effect 6.3 x 10^{-3} g/ml (C_5) to the control (C_1).

Table 14. Pairwise differences of the estimated means of the time effect on the hatchability of the eggs of alfalfa weevil when fed continuously on foliage dipped in different concentrations of diflubenzuron.

Time effect*	LSD 0.950 Confidence Interval	Sorted estimated mean**
T(1) - T(2)	6.745 22.322	T_1 : 85.73 a
T(1) - T(3)	13.745 29.322	T_2 : 71.2 b
T(2) - T(3)	-0.789	T_3 : 64.2 b

*T_1---T_3 are the notations for time at which the eggs collected from the treated foliage with different concentrations of diflubenzuron (at period of 48 hr).

**Sorted mean of the time effect from highest to lowest significance effect on the hatchability of the alfalfa weevil eggs.

DISCUSSION

The studies involved feeding alfalfa foliage which had been dipped in four different concentrations of diflubenzuron in water to adult female alfalfa weevils. One set of experiments was limited to a single feeding period of 48 hours. The other approach was to use repeated feedings of treated foliage for a period of about 3 weeks.

Adult Effects

Mortality of adult weevils was minimal and erratic. Mortality was difficult to relate to the direct effects of diflubenzuron. Some mortality was caused by handling the weevils and weevils normally died soon after oviposition was completed. There were some indications of direct mortality (Tables 2 and 12 and Figs. 1 and 2), but there was little relationship to dosage or length of exposure. This was certainly not a clear-cut relationship and the statistical analysis does not show any usefulness.

An indirect result of diflubenzuron feeding was an increase in muscardine fungus (Beauveria sp.) infections. About 63% of the newly dead weevils from the controls, compared to 75% in the diflubenzuron treatments developed muscardine fungus growth. Not only was there a difference in total infections, but there were distinct differences in the fungus growth patterns.

Typical Beauveria infections (Fig. 8) developed in adult weevils from the treated insects. the fungus growth was in distinct tufts as described

by MacLeod (1954). These tufts originated from the natural insect openings. In the control treatment weevils hyphal growth was spread nearly evenly, was less dense, and did not show the distinct tufts (Fig. 7).

Two other effects were noted on the adult weevils following diflubenzuron treatments. Yellow deposits occurred on the underside of the abdomens (Fig. 11). Two origins of these deposits were considered to be possible explanations: broken eggs and fecal matter. A series of protein analyses of the fecal material was made to compare with protein found in eggs and fecal matter.

In addition to the yellow deposits, most female weevils from the diflubenzuron treatments showed tissue extending from the tip of the abdomen (Fig. 12). The nature of this tissue was not determined.

Egg Effects

The major purposes of this study centered on the effects to alfalfa weevil eggs following the adult feeding on diflubenzuron treated foliage. Two effects were studied most intensively.

Based on the research of other workers using different insects, a reduction in the number of eggs laid was expected (Table 4 and Fig. 3), Two variables caused difficulties in the analysis of total egg deposition. There was a seasonal pattern of egg deposition with with a peak daily number laid soon after egg laying starts. There

was then a rather rapid decline in daily numbers followed by a leveling off. Some eggs can be laid two or more months after the cycle starts. This seasonal pattern made the comparison between egg laying patterns very difficult. The other variable was the great difference in egg laying by different females.

An analysis of the total eggs laid either daily or within the study periods, did not show a consistent reduction due to diflubenzuron. As shown in Table 3 and 6 and Figs. 3 and 4, it is clear that if there was a reduction in egg numbers it was not consistent nor very great in alfalfa weevils. So, statistical analysis was not possible.

In contrast to the minimal effects on total eggs, the effects on egg hatchability were very dramatic. Regardless of total eggs laid, there was a percent viability in the untreated controls close to 90%. So this base line for comparison is a reliable figure.

In the 48 hour feeding trials (Fig. 5 and Table 6) note the drop in viability with all diflubenzuron treatments starting about 4 days after the feeding started. The effects were only temporary, with a recovery taking place about a week later. The effects were dosage related, with the viability dropping to 38.9% in the highest dosage on day 10. There is a significant difference at $\alpha = 0.05$ (Tables 9, 10 and 11) in both variables (dose and time effect).

In the continuous feeding trials (Fig. 6 and Table 7) the same pattern of drop in viability occurred, as shown in the 48 hr feeding, except that the viability remained low and did not recover. In the heaviest dosage, the viability dropped to zero. Due to the fact that

these continuous feeding trials were conducted late in the oviposition cycle, the total eggs laid in the diflubenzuron treatments also dropped to very low levels. This made statistical analysis impossible after day 8 but before day 8 there were also significant differences at α = 0.5 for the concentration effect and also time effect (Tables 12, 13 and 14).

Additional observations were made regarding alfalfa weevil eggs. The eggs from diflubenzuron treated weevils were distinctly longer than those in controls (Fig. 10), they were also a lighter color and tended to rupture more easily (Fig. 13). A higher proportion of eggs laid by treated weevils was laid on the surface rather than being inserted.

Meaning of These Results in Terms of Field Usage

While it is dangerous to draw too many applied conclusions from laboratory studies, two ideas are probably appropriate.

The effects on egg viability are likely to be ignored in traditional field testing of pesticides. When working with growth inhibitors we should adjust our evaluation techniques to measure factors other than direct mortality.

When considering field applications of diflubenzuron against the alfalfa weevil, the type of exposure would be more similar to the 48 hr than to the continuous feeding tests. Alfalfa grows rapidly and the weevils prefer the terminals. After a few days, the terminals outgrow the treatment.

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