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The effects of synthetic juvenile hormone on the viability of eggs, metamorphosis of nymphs, and mating behavior of males and females in diapause was investigated by treating Draeculacephala crassicornis with ENT 33972a. The viability of eggs was reduced 14% by a small dose (0.001 μg) of synthetic juvenile hormone applied topically to the eggs; however 8% of the eggs were still viable after they had been treated with 2.0 μg of hormone. The age of the eggs did not affect their sensitivity to the hormone.

Third, IV, and V instars responded differently to topical treatments of synthetic juvenile hormone. A 5.0 μg dosage of hormone was more lethal to III instars than IV and V instars, but a larger dosage of hormone was required to produce morphological abnormalities in III instars than in older nymphs. The adult emergence of most IV instars was prevented by a hormone dosage of 0.50 μg.

Morphological abnormalities were produced when young V instars (less than 3 days) were treated with hormone but older V instars (greater than 8 days) molted to normal adults even when they were treated with large dosages of hormone. Hormone treatment of V instar females committed to enter diapause prevented the reproductive diapause of the subsequent adults, but treatment of older V instar females committed to develop ovaries had no effect on subsequent adult egg production and offspring survival.

The viability of eggs from gravid females which were treated topically with only 0.001 μg of hormone was reduced by 22%, but larger dosages (up to 5.0 μg) had little additional effect. In this study, the hormone produced no deferred effects on offspring from viable eggs.

Females in diapause were topically treated with a small dose (1.0 μg) of synthetic juvenile hormone to observe the effect of the hormone on survival and ovarial development. The survival of the females was not markedly affected and these females developed and oviposited a normal complement of eggs in autumn.

The activity of foliage sprays of seven synthetic juvenile hormones was compared. Three of these compounds, ENT 33972a, ENT 70119a, and ENT 70221 prevented the adult development of IV instars, and also terminated the reproductive diapause of females. The compounds were active against IV instars and females at the

respective spray concentrations of 0.10 and 1.0%. Comparable results were obtained in laboratory and field tests.

Laboratory tests were designed to study the effects of synthetic juvenile hormone on the mating activity of males and females in diapause. Females in diapause had undeveloped ovaries and were not attractive to males. When these females were treated with synthetic juvenile hormone, they mated after they had initiated ovarial development. Males in diapause did not mate readily unless they had been treated with synthetic juvenile hormone. Additional laboratory experiments showed that the hormone stimulated the mating behavior of males in diapause but had no effect on the growth of testes or the rate of increase of mature sperm bundles in the testes. However, mature sperm bundles appeared earlier in the testes of treated than untreated males.

Periodic sampling of a field population of males in diapause revealed that their low mating activity was not due to underdevelopment of the testes since the testes matured at the same rate as those of nondiapausing males in the field. Laboratory studies of males in diapause which were confined in controlled environments showed that their mating activity was stimulated by short days but not affected by temperature.

The Effects of Synthetic Juvenile Hormone on the Leafhopper, Draeculacephala crassicornis

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE EFFECTS OF SYNTHETIC JUVENILE HORMONE ON THE LEAFHOPPER, DRAECULACEPHALA CRASSICORNIS

INTRODUCTION

During the 1960's, several compounds with insect juvenile hormone activity were synthesized. Williams (1967) suggested that these substances could become a new generation of pesticides because they were active in extremely small amounts, nontoxic to other organisms, and insects would be unable to develop resistance to their own hormones. Although most of the compounds with juvenile hormone activity had a broad spectrum of activity, Juvabione, discovered by Slama and Williams (1965), was active against only one species of insects. Williams expressed hope that more compounds with juvenile hormone activity which could be used as selective insecticides would be discovered in the future.

The advantages of using compounds with juvenile hormone activity as insecticides have stimulated considerable research in the last several years. Although many new compounds with juvenile hormone activity have been synthesized, continued research will be necessary before any of these substances can be employed commercially. In addition to more field testing, the effects of these compounds should be evaluated on a wider range of insects including pests, predators, parasites and beneficial species. Additional

basic research on the effects of compounds with juvenile hormone activity on insect behavior and physiology may lead to new ways in which the materials can be employed as insect-control agents.

In the leafhoppers, an economically important family of Homoptera, there have been no detailed studies of the effects of compounds with juvenile hormone activity. This work is a description of the effects of synthetic juvenile hormone on one species of leafhopper, Draeculacephala crassicornis. One portion of the study was the evaluation of the effects of one synthetic juvenile hormone on various life stages of the leafhopper. This included an investigation of the sterilizing effects on eggs and gravid females, morphogenic effects on nymphs, and termination of the reproductive diapause in females. These experiments were done to compare the responses of the various life stages and determine which parts of the leafhopper's life cycle were particularly sensitive to the hormone. Another part of the study was testing the effectiveness of foliage sprays of synthetic juvenile hormones in causing morphogenic effects in nymphs, and terminating the reproductive diapause of females. These experiments were done to compare the sensitivity of the two stages to the various compounds both in the laboratory and in the field. The third portion of this study was the investigation of the effects of synthetic juvenile hormone on the mating activity of diapausing males and females.

REVIEW OF THE LITERATURE

Compounds which exhibit insect juvenile hormone activity have been called synthetic juvenile hormones (Kamm and Swenson, 1972), insect juvenile hormone analogs (Slama, 1971), juvenile hormone mimics (Briger, 1971), insect developmental inhibitors (Schaefer and Wilder, 1972), and materials with insect juvenile hormone activity (Slama, Romanuk, and Sorm, 1972). No clear definitions of the preceding terms have been proposed. Most of the compounds designated by these terms are synthetic materials which differ in chemical structure from the true juvenile hormone of cercropia (Roller et al., 1967), but exhibit juvenile hormone activity. However, the cercropia juvenile hormone has also been synthesized (Dahm, Roller, and Trost, 1967). Also, some naturally occurring materials such as Juvabione, which has been isolated from balsam fir, exhibit juvenile hormone activity (Bowers et al., 1966). In this study synthetic compounds which exhibit juvenile hormone activity will be called synthetic juvenile hormones.

The History of Compounds with Juvenile Hormone Activity

Wigglesworth (1934, 1936) demonstrated that a hormone responsible for maintaining larval characters was produced by the corpora allata in Rhodnius larvae. No progress was made in identifying this

material which was later called juvenile hormone until 20 years later. Williams (1956) discovered that the abdomens of male cercropia moths contained large amounts of active material, and isolated a crude extract. Roller, Bjerke, and McShan (1965) isolated a more purified fraction of juvenile hormone from cercropia moth abdomens, and two years later, the compound was identified (Roller et al., 1967). Roller and Dahm (1970) obtained juvenile hormone from in vitro culture of isolated neuroendocrine systems of Hyalaphora cecropia, confirming directly that the corpora allata produces the previously identified juvenile hormone.

Before the initial isolations of juvenile hormone in insects, researchers discovered that substances that would produce juvenile hormone effects in bioassays were widespread in nature. Compounds with juvenile hormone activity were found in other invertebrates (Schneiderman and Gilbert, 1958), vertebrate tissues and organs (Williams, Moorhead, and Pulis, 1959), and in plants and microorganisms (Schneiderman, Gilbert, and Weinstein, 1960). None of these materials were identified.

The first compounds with juvenile hormone activity to be identified, farnesol and its aldehyde farnesal, were isolated from mealworm feces by Schmailek (1961). Several other active farnesol derivatives were synthesized after this discovery (Schmailek, 1963; Karlson, 1963). One of the most active farnesol derivatives

methyl-10, 11, -epoxyfarnesoate was prepared by Bowers, Thompson, and Uebel (1965) and differed from the true juvenile hormone by only two carbons. Another farnesol derivative, prepared by bubbling HCL gas through farnesenic acid, was highly active against a wide range of insect orders. This mixture, because it was easily synthesized, was one of the first synthetic juvenile hormones to be considered for use as an insecticide (Law, Yuan, and Williams, 1966).

Juvabione, an ester isolated from balsam fir, was the first compound discovered to exhibit juvenile hormone activity for only one insect species, Pyrrhocoris apterus (Bowers et al., 1966). The high activity and specificity of this compound stimulated research to find other such compounds which could be used as selective insecticides. However, most presently known compounds with juvenile hormone activity are not specific, and are active against many insect species.

In the past several years many new compounds with juvenile hormone activity have been synthesized and several of these compounds are currently being considered for commercial use as insecticides. Some of the chemical structures and diversity of these compounds have been reviewed by Slama (1971).

Physiological Effects of Juvenile Hormone

The following description of the physiological effects of juvenile hormone has been summarized from Wigglesworth (1970). Juvenile hormone is secreted by the corpora allata and suppresses insect metamorphosis by preventing the expression of adult characteristics and favoring the retention of juvenile characteristics. Metamorphosis is controlled by the secretion of juvenile hormone during the larval stages. When the corpora allata secretion ceases, the adult transformation occurs. Removal of the corpora allata prior to the final metamorphosis causes the development of a premature, miniature adult, and the implantation of active corpora allata into late larval stages causes a supernumary molt to another immature rather than a normal adult. In adults of most insect species, juvenile hormone is necessary for female vitellogenesis and the normal development of male accessory glands. The hormone may also affect adult mating activity, either by directly affecting behavior or by influencing pheromone production and release.

Effects of Compounds with Juvenile Hormone Activity on Insect Eggs

In some species of insects, egg hatching can be prevented by exposing the eggs to vapors or direct topical applications of compounds with juvenile hormone activity. These compounds block

embryonic development, and embryonic morphogenesis can be arrested at any stage (Novak, 1969). The sensitivity of eggs to compounds with juvenile hormone activity, which is usually greatest immediately after oviposition, varies widely among insect species and is dependent upon the particular material used (Slama and Williams, 1965; Walker and Bowers, 1970).

Riddiford (1970) reported that treatment of eggs of P. apterus with synthetic juvenile hormone caused deferred effects of lower offspring survival and morphological abnormalities among surviving nymphs. Apparently these morphological abnormalities were due not to a carryover and persistence of the hormone applied to the eggs, but rather to an abnormal activity of the corpora allata during the last nymphal instar. The abnormal activity of the corpora allata was attributed to a disruption of normal programming in the embryo caused by the hormone treatment. Willis and Lawrence (1970) disputed this interpretation and suggested that the deferred effects noticed by Riddiford were due to the carryover and persistence of the hormone applied to the embryo. Deferred effects of compounds with juvenile hormone activity have been demonstrated only in P. apterus, Oncopeltus fasciatus, and H. cecropia, (Riddiford, 1970).

Effects of Compounds with Juvenile Hormone Activity on Immature Stages

In insects the stage immediately preceding the adult, the pupa in holometabolous insects or the last nymphal instar in hemimetabalous insects, is affected by compounds with juvenile hormone activity. Hormone treatments during these stages block normal metamorphosis to the adult and cause the insect to molt into another immature form. The degree of morphological abnormality produced is usually directly proportional to the dosage of hormone applied (Karlson and Nachtigall, 1961). The last immature stage is called the critical stage, since prior treatment has no effect until the insect attempts the final molt to an adult. Insects treated early in the critical stage are quite sensitive to compounds with juvenile hormone activity, but those treated just prior to adult metamorphosis are virtually insensitive and develop into normal adults (Slama and Williams, 1965; Critchley and Campion, 1971). Although insects treated late in the critical stage develop into normal adults, the subsequent adult development may be affected by the hormone. For example, male mosquitoes that developed from larvae treated with synthetic juvenile hormone were unable to rotate the genitalia for normal copulation (Spielman and Williams, 1966). Also, spruce budworm females which had been treated with synthetic juvenile hormone during the late pupal stage laid a reduced number of eggs

with a lower hatching rate than those from normal moths (Richmond, 1972).

In addition to producing morphological abnormalities, compounds with juvenile hormone activity may also cause rapid mortality if applied in high dosages to immature insects. Young immature stages are more sensitive to this effect than older immatures, especially young larvae of flies and mosquitoes (Srivastava and Gilbert, 1968; Craig, 1970).

Effects of Compounds with Juvenile Hormone Activity on Adult Insects

The embryonic development of insect eggs can be arrested by treating gravid females with compounds exhibiting juvenile hormone activity. The effects of the hormone on eggs within the female are similar to those previously described for a direct treatment of eggs. The sensitivity of gravid females to hormone sterilization varies greatly among insect species. Female P. apterus lay a high percentage of nonviable eggs after treatment with 1.0 μg of synthetic juvenile hormone (Masner, Slama, and Landa, 1968), while Benz (1971) was unable to affect egg viability by treating gravid females of Pieris brassicae or Galleria mellonella with high dosages of synthetic juvenile hormone.

The reproductive diapause of adult females, which is thought

to be due to the inactivity of the corpora allata, can be terminated in many species of insects by the application of compounds exhibiting juvenile hormone activity (Bowers and Blickenstaff, 1967; Connin, Jantz, and Bowers, 1967; Kamm and Swenson, 1972). The dosage of material required to terminate the female diapause is relatively higher than the amount necessary to block embryonic or larval metamorphosis in most species. Generally, the amount of ovarial development produced in diapausing females is directly proportional to the amount of material applied.

The influence of the corpora allata on mating of insects varies. In Leucophaea females the mating behavior is controlled completely by the corpora allata; in the grasshopper Euthystria brachyptera the corpora allata partially controls the mating behavior of the females; while no influence of the corpora allata on mating behavior has been found in Galleria mellonella or Diploptera punctata (Engelmann, 1970).

Most experiments correlating mating activity and corpora allata secretion have been done with allatectomization and corpora allata implants, but presumably treatment of insects with compounds exhibiting juvenile hormone activity would have the same results as implanting active corpora allata. In some insect species the corpora allata influences mating in an indirect manner by controlling phermone production and release (Barth, 1965). Application of

synthetic juvenile hormone has been found to stimulate phermone production in the female mealworm adult, Tenebrio molitor (Menon, 1970) and the male bark beetle, Ips confusus (Borden, Nair, and Slater, 1969).

Structure and Activity Relationships for Compounds with Juvenile Hormone Activity

The varying response to different compounds with juvenile hormone activity among insect species demonstrates that compound structure-activity relationships vary widely among insect groups (Schwarz, Sonnet, and Wakabayashi, 1970; Brieger, 1971). Even within a single species there may be no obvious structural relationship among the active compounds (Schneiderman et al., 1965; Wigglesworth, 1969). The following reasons for differences in activity of compounds from species to species have been suggested by Slama (1971): factors determining integument permeability, extra and intracellular transport, and the ability of the synthetic juvenile hormone to react with receptor mechanisms. Reddy and Krishnakumaron (1972) have proposed that the most active materials for a given insect species are those compounds which cannot be quickly metabolically degraded.

Uses of Compounds with Juvenile Hormone Activity in Insect Pest Management

It has been suggested that compounds with juvenile hormone activity could be used in insect pest management in the following ways (Robbins, 1972): (1) To cause death and disrupt adult metamorphosis of immature stages. (2) To terminate adult female reproductive diapause, stimulating females to oviposit during unfavorable environmental conditions which could cause increased offspring mortality. (3) To sterilize insect eggs, either by direct application or treatment of gravid females. Compounds with juvenile hormone activity have several advantages over currently available pesticides. They are nontoxic to vertebrates, nonpersistent, and active in small amounts. Moreover, if additional synthetic materials with selective activity are discovered in the future, these compounds could be employed as selective insect control agents which would have no effect on populations of predators, parasites and beneficial insects.

Some disadvantages must also be considered if compounds with juvenile hormone activity are used as insect control agents. These compounds cannot be used to quickly reduce larval populations because they have no effect on many insect species until the adult metamorphosis. They may have to be applied repeatedly because of their nonpersistence, and the timing of application must be very precise if the compounds are to be used effectively. Finally,

because of economic considerations, many commercially produced compounds with juvenile hormone activity will probably have a broad spectrum of activity which will cause them to have undesirable effects on nontarget insect species.

MATERIALS AND METHODS

Biology of Draeculacephala crassicornis

D. crassicornis is widely distributed in Nearactic America and is found at various elevations, ranging from low meadows to high mountains. This species is a grass feeder and is not economically important although it has been shown to be a vector of Pierce's Disease in California (Neilson, 1968). D. crassicornis was chosen for this study because large numbers of insects could be easily collected in the field. Also, the larger size of this species relative to other leafhoppers facilitated the application of synthetic juvenile hormone and the evaluation of its effects. Finally, because both univoltine and bivoltine populations existed in close proximity to each other in the Corvallis, Oregon area; the response of both diapausing and nondiapausing adults could be compared.

Insects used in this study were collected from two areas near Corvallis, Oregon. The insects collected in each area were considered to be from different populations of D. crassicornis because of their differing biology. The biology of each of the two populations is summarized in the following paragraphs from unpublished data of Swenson and Kamm (1972).

Insects from the Corvallis population were collected from a

grassy field on the southwest edge of Corvallis, Oregon. This population is bivoltine, and overwinters in the egg stage. The eggs hatch in late March or early April and the insects become adults in early June. The adults mate and females lay nondiapausing eggs which hatch in mid-July. The second generation matures in the fall and females lay diapausing eggs on Bromus carinatus. The egg diapause is probably induced by cool temperatures and short photoperiods, and eggs require a chilling period before hatching will occur.

Insects from the McDonald Forest population were collected in McDonald Forest from a grassy meadow which was separated from the Corvallis population collecting site by 3.5 miles and 250 feet in elevation, the respective altitudes being 500 and 250 feet. The McDonald Forest population is univoltine and overwinters in the egg stage. The eggs hatch in mid to late April, and offspring become adults in early July. The adult females spend the summer in a reproductive diapause, characterized by a lack of ovarial development. The female diapause is terminated by short days and cool temperatures in the fall. After the females have begun ovarial development, mating occurs and the females oviposit in October and November on B. carinatus. The eggs overwinter in an embryonic diapause until the following spring. The McDonald Forest population, in contrast to the Corvallis population, undergoes both an embryonic and an adult female reproductive diapause.

During the remainder of this study insects from the Corvallis and McDonald Forest populations will be referred to by the respective abbreviations, C. and M. F.

The chemical names of the synthetic juvenile hormones used in this study, and the source from which they were obtained is shown in Table 1. ENT 33972a, a synthetic compound of mixed isomers of the juvenile hormone, was used to compare the response of various life stages of D. crassicornis, and also used as an activity standard in tests of the other synthetic juvenile hormones.

Greenhouse Techniques

Immature leafhoppers were reared in the greenhouse by confining them within gauze-covered glass lamp chimneys placed over barley seedlings planted in 10 cm diameter clay pots. Approximately six nymphs were confined in each lamp chimney. Leafhopper adults were maintained in the greenhouse by confining them in nylon screen cages placed over barley seedlings planted in 15 cm diameter clay pots. Approximately 20 adults were placed in each cage. The nymphs were transferred weekly to new barley seedlings, and adults were transferred weekly to new plants at two-week intervals. Insects were reared in the greenhouse under long day conditions, 16 hour photoperiod, and an approximate temperature of 23° C.

Table 1. Synthetic juvenile hormones which were applied to D. crassicornis.

USDA Entomology Number	Source	Chemical Name
33972a	Hoffman LaRoche	Methyl 10, 11-Epoxy-7-ethyl-3, 11-dimethyl-2-cis/trans-6-cis/trans 10-cis/trans-trideadienoate
34027	USDA	Carbamic acid (3, 7-dimethyl-6-octenyl)-ethyl ester
34038	USDA	Carbamic acid (3, 7-dimethyl-2, 6-octadienyl)-, ethyl ester (E)-
34233	USDA	Carbamic acid (6, 7-epoxy-3, 7-dimethylloctyl)-ethyl ester
34038-d	USDA	Carbamic acid (6, 7-epoxy-3, 7-dimethylloctyl)-phenyl ester
34375-a	USDA	2, 6-octadienylamine, N-(2, 5-dichlorophenyl)-3, 7-dimethyl-
34426	USDA	1-Nonene, 7, 8-epoxy-4, 8-dimethyl-1-phenyl-, (E)-
34455a	USDA	4-8-Tetradecadien-3-one, 12, 13-epoxy-5, 9, 13-trimethyl-, (mixed isomers)
34495	USDA	Carbamic acid, (3, 7-dimethyl-6-octenyl)-, 3, 4, -xylyl ester
34648	USDA	1-Nonene, 1-(o-chlorophenyl)-7, 8-epoxy-4, 8-dimethyl-
34650	USDA	1-Nonene, 1-(p-chlorophenyl)-7, 8-epoxy-4, 8-dimethyl-
34704	USDA	Crotonic acid, 3-[(3, 7-dimethyl-6-octenyl) = amino]-, methyl ester, (E)-
34708	USDA	Crotonic acid, 3-[(3, 7-dimethyl-6-octenyl) = amino]-, phenyl ester, (E)-
34710	USDA	Crotonic acid, 3-[(3, 7-dimethyl-6-octenyl) = methylamino]-, ethyl ester, (E)-
34716	USDA	Carbonic acid, 6, 7-epoxy-3, 7-dimethyloctyl methyl ester
34718	USDA	Carbonic acid, 6, 7-epoxy-3, 7-dimethyloctyl ethyl ester
34720	USDA	Carbonic acid, 6, 7-epoxy-3, 7-dimethyl octyl phenyl ester
34742	USDA	Nonane, 2, 3-epoxy-2, 6-dimethyl-9-phenyl-
34765	USDA	6-Octenylamine, N-(2, 4-dichlorophenyl)-3, 7-dimethyl-
34862	USDA	Nonane, 1-(p-chlorophenyl)-7, 8-epoxy-4, 8-dimethyl-

Table 1. Continued

USDA Entomology Number	Source	Chemical Name
70119a	Hoffman LaRoche	6, 7-epoxy-3, 7-dimethyl-1-[3, 4-(methylene-dioxy) phenoxy]-2-cis/trans-octene
70188	USDA	carbamic acid, (3, 7-dimethyl-6-octenyl) = methyl-, ethyl ester
70221	Stauffer	1-(4'-ethylphenoxy)-6, 7-epoxy-3, 7-dimethyl-2-octene
70348	Hoffman LaRoche	Ethyl 7, 11-Dichloro-3, 7, 11-trimethyl-2-trans-dodecenoate
70349	Hoffman LaRoche	Methyl p-[(1, 5-Dimethylhexyl)oxy]benzoate
70350	Hoffman LaRoche	Ethyl 10, 11-Epoxy-3, 7, 10, 11-tetramethyl-2-cis/trans-6-cis/trans dodecadienoate
70351	Hoffman LaRoche	6, 7-Epoxy-3, 7-dimethyl-1-(2-propynyloxy)-2-cis/trans-octene
70356	Hoffman LaRoche	6, 7-Epoxy-3, 7, diethyl-1-(p-chlorophenoxy)-2-cis/trans-octene
70357	Hoffman LaRoche	6, 7-Epoxy-3-methyl-7-ethyl-1-[3, 4-(methylene dioxy) phenoxy]-2-cis/trans-octene
70369	Hoffman LaRoche	6, 7-Epoxy-3-methyl-7-ethyl-1-(p-chlorophenoxy)-2 cis/trans-octene
70441	Hoffman LaRoche	10, 11-Epoxy-3, 7, 11-Trimethyl-1-(2-propynyloxy) -2, 6-Tridecadiene (cis/trans mixture)
70458	Zoecon	Submitted in confidence
70459	Zoecon	Ethyl 3, 7, 11-trimethyldodeca-2, 4-dienoate
70460	Zoecon	Isopropyl 11-methoxy-3, 7, 11-trimethyldodeca-2, 4-dienoate
70499	Zoecon	Submitted in confidence
70513	Zoecon	Submitted in confidence
(ZR-777)*	Zoecon	Submitted in confidence

*1. Zoecon Corporation designation, ENT number unknown.

Application of Synthetic Juvenile Hormone

A Hamilton glass syringe attached to a repeating dispenser was used for topical treatment of eggs, IV and V instars and adults. The desired dosage of hormone was dispensed in 0.5 microliter of acetone. Eggs were placed individually on a watch glass, the hormone solution was applied, allowed to evaporate, and the eggs were returned to a petri dish. The hormone solution was applied to the dorsum of the abdomen of IV and V instars, after they had been partially immobilized by chilling. Adults were immobilized between the thumb and first two fingers, and the hormone was applied to the venter of the abdomen. Synthetic juvenile hormone was applied topically to the dorsum of the abdomen of III instars with a Hamilton syringe equipped with a fine glass needle and a micrometer attached to the plunger. The desired hormone dosage was applied in 0.25 microliter of acetone.

In the greenhouse, foliage sprays of synthetic juvenile hormones were applied with a plastic DevilViss atomizer. Two types of plants in 15 cm pots were exposed to foliage sprays: (1) barley seedlings 10-15 cm high, 20 plants/pot, (2) Bromus carinatus, one clump approximately 10 cm in diameter/pot. Five ml of the appropriate hormone solution were applied to each pot of plants, and insects were caged on the plants after the hormone solution had dried.

Foliage sprays of synthetic juvenile hormones were evaluated in the field on plots of Bromus carinatus planted on the entomology experiment station on the campus of Oregon State University. Each plot was a 0.914 m square and contained nine equidistant clumps of B. carinatus. Plots were separated by a 0.914 m border. A hand operated sprayer was used to apply 0.5 liter of the desired hormone spray to each plot. After the spray on the plants had dried, the plot was covered with a wire mesh cage and the desired number of insects were introduced into the cages.

Criteria Used to Evaluate Hormone Activity

Eggs

Viability was the only criterion used to evaluate the effects of synthetic juvenile hormone on eggs of D. crassicornis. Viability was estimated by recording the number of nymphs which emerged from each group of eggs. No attempt was made to determine the state of embryonic development in hormone treated eggs which failed to hatch.

Nymphs

The percentage of mortality and percentage of abnormal insects were recorded for nymphs treated with synthetic juvenile hormone in

this study. Normal D. crassicornis nymphs pass through five instars before becoming adults. Insects which retained any external nymphal morphological characteristics after the V instar molt or insects which molted into adults with abnormal external morphological characteristics were considered abnormal. The insects were examined visually, and only gross external morphology was observed. No distinction was made between the varying degrees of morphological abnormalities which occurred. The percent mortality recorded consisted of those insects which died prior to the V instar molt and any subsequent abnormal adults which died. Any morphologically abnormal insects which died after the V instar molt prior to observation were considered abnormal instead of being recorded as dead.

Adult Diapause

The amount of ovarial development was used as a criterion for testing the effects of synthetic juvenile hormone on D. crassicornis females in a reproductive diapause. The ovaries of females in diapause contain only very small white eggs. When diapause is terminated, vitellogenesis is initiated, the number and size of eggs in the ovaries increases, and the eggs become yellow. The females were dissected by the rapid dissection technique described by Kamm and Ritcher (1972) and the number of yellow eggs in the ovaries of treated females was recorded to measure the activity of synthetic juvenile

hormones. When diapausing females were brought into the greenhouse, the diapause was not terminated as long as the females were maintained under greenhouse conditions. If females from McDonald Forest were brought into the greenhouse as nymphs and reared to adults, the insects also remained in a reproductive diapause when they were maintained under greenhouse conditions.

RESULTS

I. The Effects of Synthetic Juvenile Hormone on Various Life Stages of D. crassicornis

Topical Treatment of Eggs with Synthetic Juvenile Hormone

To compare the sensitivity of D. crassicornis eggs of different ages to the sterilization effects of synthetic juvenile hormone, the eggs were topically treated with ENT 33972a. Eggs were obtained from ovipositing C. females confined in the greenhouse, which were transferred daily to new barley seedlings. Each day, the eggs were completely dissected from the barley seedling leaf tissue, and one-half of the day's egg production was treated immediately and the other half received identical treatments seven days later. The following treatments (μg/egg) were applied: acetone check, 0.001, 0.01, 0.05, 1.0, 2.0. In each age group, 45 eggs were given each hormone treatment. After treatment, eggs were incubated until they hatched in the greenhouse in glass petri dishes lined with moist filter paper.

No significant difference in response to ENT 33972a was found between eggs treated immediately after oviposition and those treated seven days after oviposition ($P > .05$), so the data was pooled. The percentage of eggs which hatched in the pooled treatments is shown in Figure 1. A linear decrease in viability of eggs occurred when

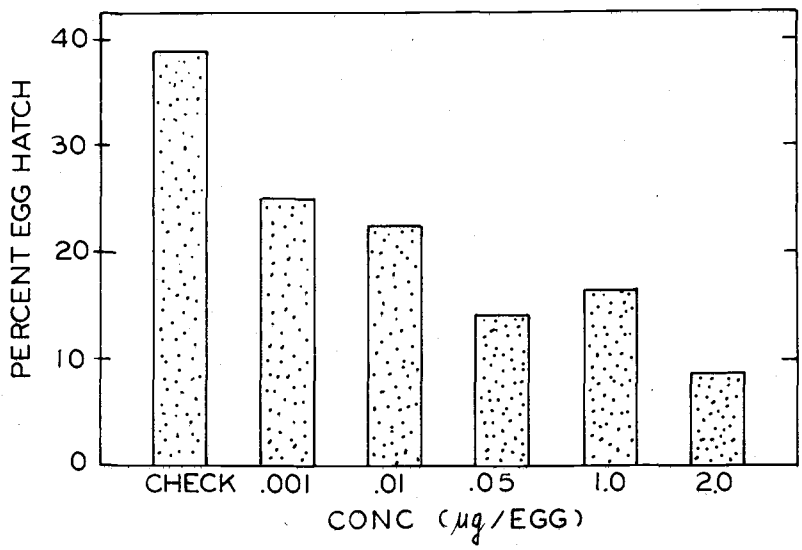


Figure 1. Viability of D. crassicornis eggs that had been topically treated with ENT 33972a.

they were treated with increasing amounts of hormone ($P < .05$), but the rate of decrease was quite small (-0.0086/0.001 μg of hormone). Even when the eggs were treated with the highest dose of hormone tested (2.0 μg), 8% of them were still viable. These results indicate that although egg viability decreases linearly when eggs are treated with increasing amounts of hormone, a large dosage of hormone would be required to completely sterilize the eggs.

Topical Treatment of III, IV, and V Instars

Third, IV and V instars of D. crassicornis were treated with ENT 33972a to compare the amount of hormone required to produce morphological abnormalities and cause mortality. Third instars from M. F. were topically treated with the following concentrations of hormone (μg/insect): 0.01, 0.05, 0.50, 1.5, 5.0. Eighteen nymphs were treated with each concentration of hormone. Fourth and V instar M. F. nymphs were topically treated with slightly larger dosages of hormone (μg/insect): 0.05, 0.50, 1.5, 5.0, 10.0. Six nymphs of each instar were treated with each concentration of hormone. Nymphs treated with acetone were used as checks in all treatments. Treated nymphs were observed periodically for morphological abnormalities and mortality.

No morphological abnormalities occurred in any of the treated nymphs until they attempted the final molt from V instars to adults.

The degree of morphological abnormality was variable, but fell predominantly into three classifications: (1) Supernymphs which were abnormally large VI or VII instars which resembled normal V instars (Figure 2). (2) Adultoids, which had an adult thorax and retained a nymphal abdomen (Figure 3). (3) Deformed adults which had underdeveloped external reproductive organs and twisted wings (Figure 4). Generally, the number of immature characters retained after the V instar molt was directly proportional to the amount of hormone applied. Abnormal insects eventually died, without reproducing, although the internal reproductive organs of those which survived for several weeks had begun to develop.

A comparison of the mortality and morphological abnormalities resulting from hormone treatment of the III, IV and V instars is shown in Figure 5. The III instars were more sensitive to rapid mortality produced by ENT 33972a than the IV and V instars. The smallest hormone dosage that produced morphological abnormalities in the III instars was 0.50 μg which was ten times higher than the amount of hormone required to produce similar effects in IV and V instars. Fourth instars were the most susceptible to morphological abnormalities produced by the hormone since only about 50% of the IV instars treated with 0.05 μg of hormone developed into normal adults, and the adult metamorphosis of IV instars was effectively blocked with a hormone treatment of 0.50 μg or more. Some normal adults

A

B

Figure 2. A) A normal V (final) instar, and B) supernymph produced by treating D. crassicornis nymphs with synthetic juvenile hormone.

A

B

Figure 3. A) Normal adult and, B) adultoid produced by treating D. crassicornis nymphs with synthetic juvenile hormone.

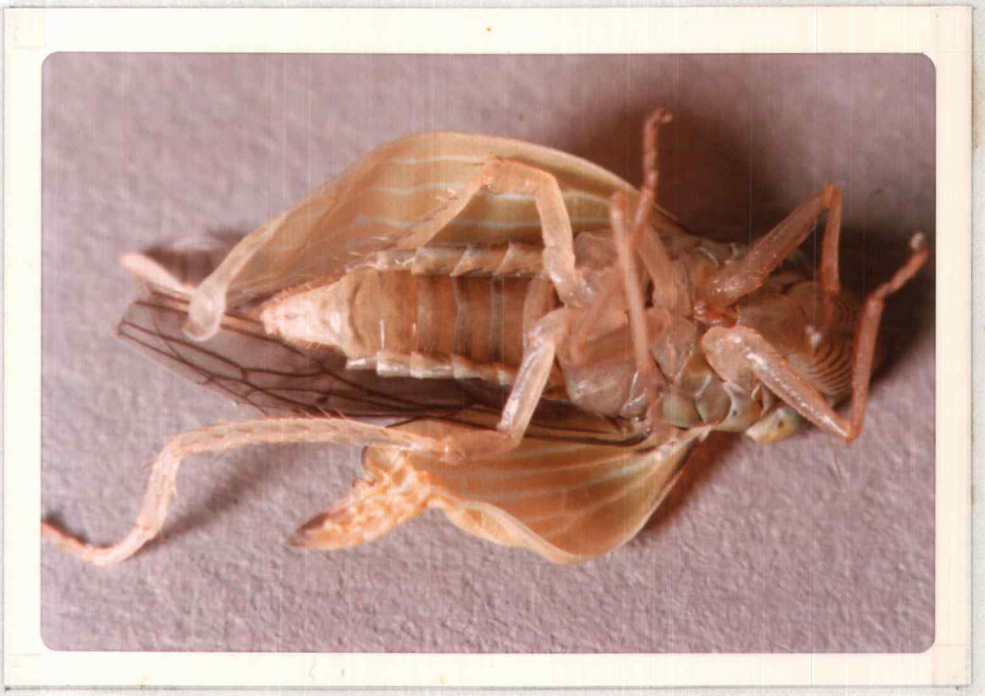


Figure 4. A deformed adult produced by treating a D. crassicornis nymph with synthetic juvenile hormone.

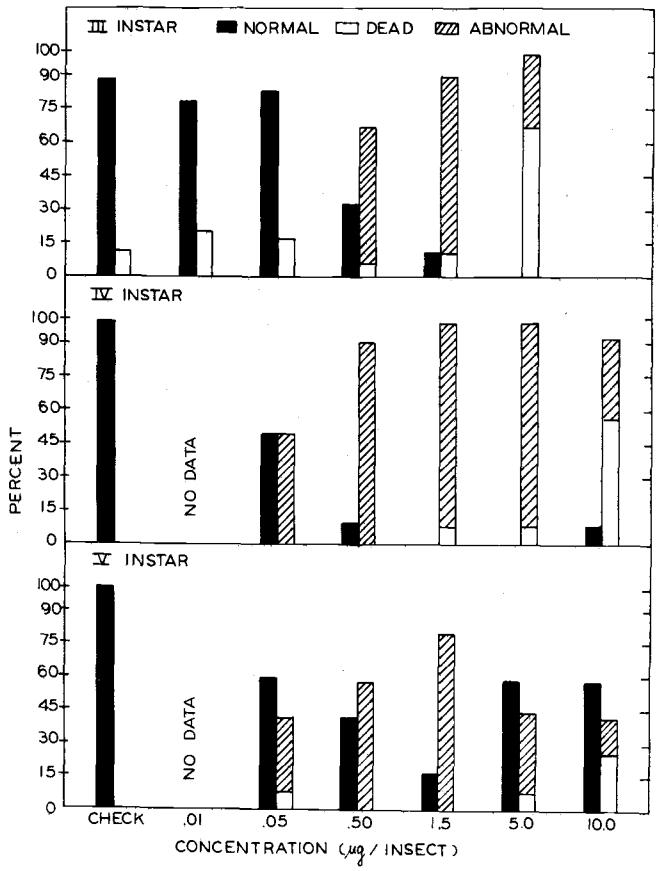


Figure 5. Effects produced by treating various nymphal instars of D. crassicornis with ENT 33972a.

developed in all hormone treatments of the V instar because both early and late V instars were treated; apparently the age of the V instars treated varied and some of the late V instars were already committed to molt to the adult stages. Therefore, the hormone was unable to interrupt metamorphosis. These results show that large dosages of synthetic juvenile hormone are more lethal to young nymphs than older nymphs. The adult emergence of treated IV instars can be disrupted with small amounts of hormone, but older V instars are virtually insensitive to synthetic juvenile hormone.

Deferred Effects on Offspring of Synthetic Juvenile Hormone Treated Nymphs

The previous experiment indicated that early and late V instars responded differently to synthetic juvenile hormone treatment. Since Slama and Williams (1966) reported that sensitivity to synthetic juvenile hormone in the final V instar of P. apterus was maximal during the first three days and declined until the older V instars became insensitive, tests were performed to see if a similar response to synthetic juvenile hormone occurred in D. crassicornis V instars. Two age groups of nymphs were tested, V instars less than three days old and V instars at least eight days old.

Three hormone treatments (μg/insect) were given to each age group: 0.05, 0.50 and 5.0. Nymphs treated with acetone were

used as a check and in each age group 20 insects were subjected to each treatment. The treated nymphs were observed until they had molted, and the number of normal adults was recorded. The number of normal adults emerging from nymphs treated as early V instars were 17, 4, 4, and 2, respectively for the check, 0.05, 0.50, and 5.0 μg treatments. The number of normal adults emerging from the same respective treatments of late V instars were 20, 17, 20 and 17. These results show that V instar nymphs which were at least eight days old, were able to molt to normal adults even though they had been treated with a large dosage of hormone

Although the previous experiment had shown that late V instar nymphs treated with synthetic juvenile hormone were able to molt to normal adults, it was not known if enough of the hormone persisted in the adults to affect the egg hatch of females and reduce offspring survival by producing deferred effects on offspring such as noted by Riddiford (1970). To investigate this possibility, Corvallis V instars at least eight days old were subjected to the following hormone treatments: 5.0, 0.50, and 0.05 μg/insect. Nymphs treated with acetone were used as checks, and 50 nymphs were given each treatment. All normal adults from each group of treated nymphs were paired and a sample of 50 eggs from females in each treatment was dissected from barley seedlings. The eggs were incubated in the greenhouse on moist filter paper in glass petri dishes. These egg

samples were used to estimate egg viability from females subjected to hormone treatment as late V instars. The remainder of the eggs in each treatment were removed from barley seedlings and were similarly placed in petri dishes. The nymphs which emerged from these eggs were collected daily from each treatment and reared on barley seedlings. These nymphs were observed weekly and mortality was recorded until all insects had emerged as adults.

The estimated egg viability and survival of offspring from normal adults which had received various amounts of ENT 33972a as late V instars is shown in Figure 6. Analysis of the data in Figure 6 revealed that there were no significant differences (Chi-square $P > .05$) in egg viability of offspring survival among any of the treatments. Furthermore, no morphological abnormalities were observed among any of the offspring. These results suggest that hormone treatment of late V instars had no effect on subsequent adult reproductive capability.

Deferred Effects on Offspring from Adult Females Treated with Synthetic Juvenile Hormone

The possibility of deferred effects on offspring survival as described by Riddiford (1970) was further investigated by treating gravid C. females with ENT 33972a. The females were collected in the field, brought into the greenhouse, and subjected to the following

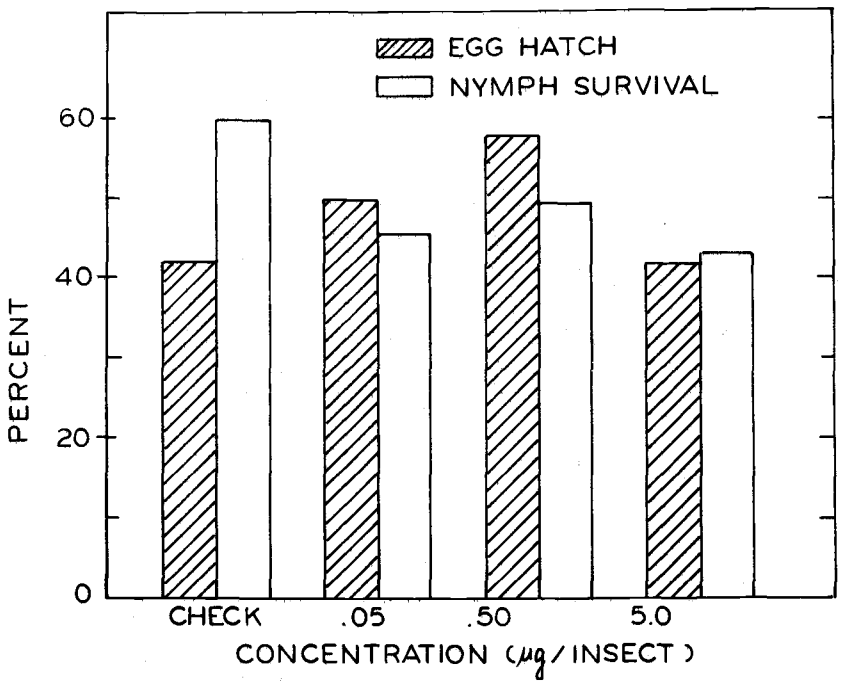


Figure 6. Viability of eggs and survival of offspring from D. crassicornis males and females treated with ENT 33972a when they were late V instars.

hormone treatments (μg/female): 5.0, 1.5, 0.05, 0.01, and 0.001. Twenty females were subjected to each hormone treatment and acetone treated females were used as checks. The viability of eggs and survival of offspring from females in each treatment was estimated in the same manner as described in the previous test of deferred effects on offspring of late V instars treated with synthetic juvenile hormone.

Figure 7 shows the viability of eggs and survival of offspring from gravid C. females treated with various amounts of ENT 33972a. The eggs of females that received the smallest dosage of hormone (0.001 μg) were significantly less viable than those from check females. However, the treatment of females with larger amounts of hormone caused no significant additional reduction in egg viability below that obtained by a 0.001 μg dosage (Chi-square $P > .05$). Also, females still produced some viable eggs at the highest hormone dosage tested (5.0 μg). The survival of offspring from the viable eggs produced by the hormone treated females was not significantly lower than that of offspring from check females, and no morphological abnormalities were observed among offspring produced by any of the females treated with hormone. These results indicate that although synthetic juvenile hormone treatments reduced egg viability, offspring survival from viable eggs of treated females was not affected.

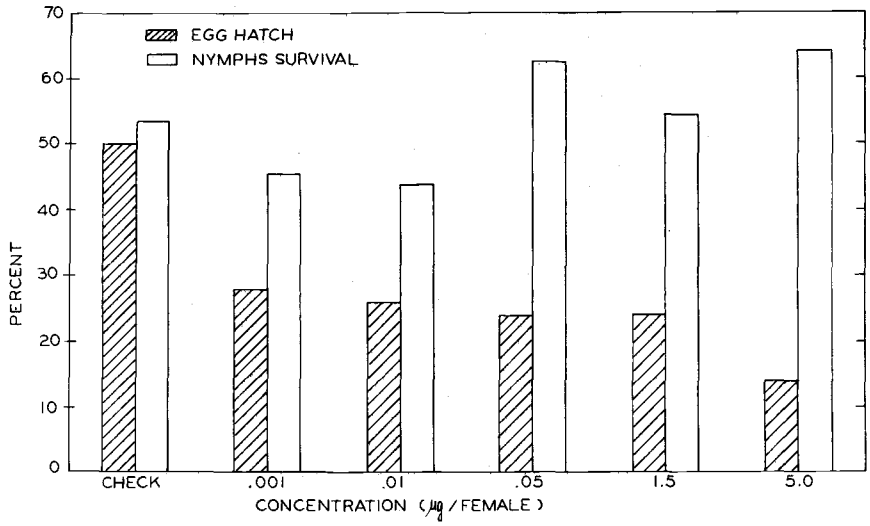


Figure 7. Viability of eggs and survival of offspring from gravid D. crassicornis females treated with ENT 33972a.

Prevention of Adult Female Reproductive Diapause by Treating V Instars with Synthetic Juvenile Hormone

Kamm and Swenson (1972) demonstrated that the adult female reproductive diapause of D. crassicornis could be terminated by treating females with ENT 33972a. This experiment was an attempt to obtain similar results by treating late V instar females that were committed to molt into diapausing adults. Fourth instars collected from the M. F. population were brought into the greenhouse. The nymphs received one of the following dosages of ENT 33972a eight days after they had molted into V instars: 15.0, 10.0, 5.0, 1.5 μg. Insects treated with acetone were used as a check. After the V instar molt, the adult females were confined on barley for two weeks and then dissected and the number of yellow eggs/female was recorded. The number of insects dissected in the check, 1.5, 5.0, 10.0 and 15.0 μg treatments were respectively, 15, 26, 26, 24 and 16 females.

The average number of yellow eggs/female produced by hormone treatment of late V instar M. F. females is shown in Figure 8. Females which developed from the acetone treated check V instars had an average of less than 1 egg/female when dissected, which indicated that these females were in a reproductive diapause. An analysis of regression showed that the relationship between amount of hormone applied to the V instar and subsequent adult egg production was linear with an estimated increase of 2.3 eggs/μg of hormone

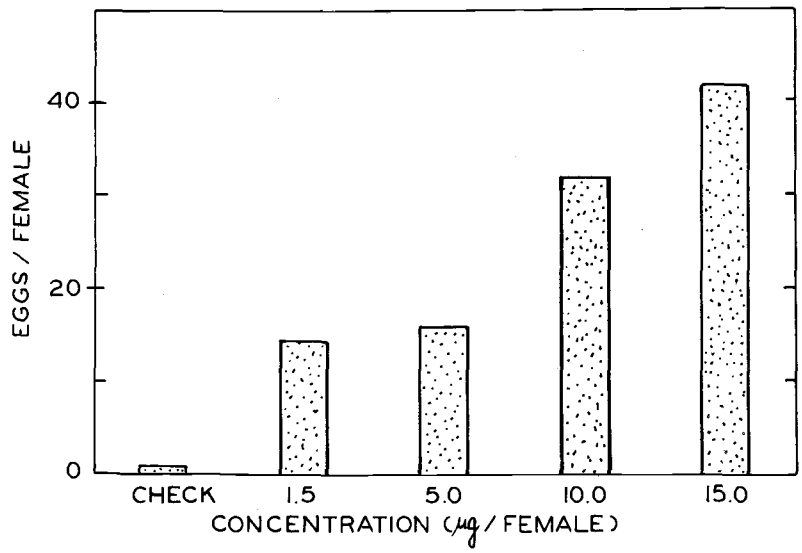


Figure 8. Average number of eggs/female produced by treating McDonald Forest V instar D. crassicornis nymphs with ENT 33972a.

applied to each female. These results clearly show that when synthetic juvenile hormone is applied to late V instar females which are committed to develop into diapausing adults, the diapause is prevented and the females begin to develop eggs. The prevention of diapause is probably due to the carryover and persistence of the synthetic juvenile hormone applied to the V instars.

Treatment of Diapausing McDonald Forest Females with a Low Dosage of Synthetic Juvenile Hormone

It has been suggested that terminating the reproductive diapause of adult females with synthetic juvenile hormone would cause the females to deposit eggs, and the eggs and offspring would suffer high mortality from unfavorable environmental conditions (Robbins, 1972). In order for this strategy to succeed, females would undoubtedly have to receive high dosages of hormone. This experiment was designed to determine if treating diapausing D. crassicornis females with a low dosage of synthetic juvenile hormone which would not produce enough eggs/female to cause premature oviposition, had any long term effects on survival of females during the summer months and ovarial development in the fall.

Males and females in reproductive diapause were collected from McDonald Forest on June 13 and 300 females were topically treated with 1.0 μg of ENT 33972a and 300 females were treated

with acetone as a control. After treatment, insects were caged on barley in the greenhouse (20 females and 5 males/cage) during the remainder of the summer. Ten females from each treatment were dissected at two-week intervals to determine if any absorption of eggs or ovarial degeneration had occurred, and the number of yellow eggs/female was recorded. After the insects had been confined in the greenhouse for 119 days, the remaining insects in both treatments were caged on B. carinatus and moved outdoors. Insects were moved outdoors because unpublished work by Swenson and Kamm has shown that insects kept in greenhouse constant temperature do not complete normal ovarial development and oviposition in the fall. Sixty treated females and 70 check females had survived the greenhouse confinement. After the insects had remained outdoors for two weeks, a final sample of 15 females from each treatment was dissected to record the number of yellow eggs/female. The remaining insects were left on the plants until oviposition was completed and the females had died. The average number of eggs laid/female in the check and hormone treatments was then determined by partially dissecting the eggs from the plant in order to count them.

The average number of eggs/female when the treated females were confined in the greenhouse during the summer is shown in Figure 9. The average number of eggs/female remained relatively constant during the 12 weeks after treatment except for the four and

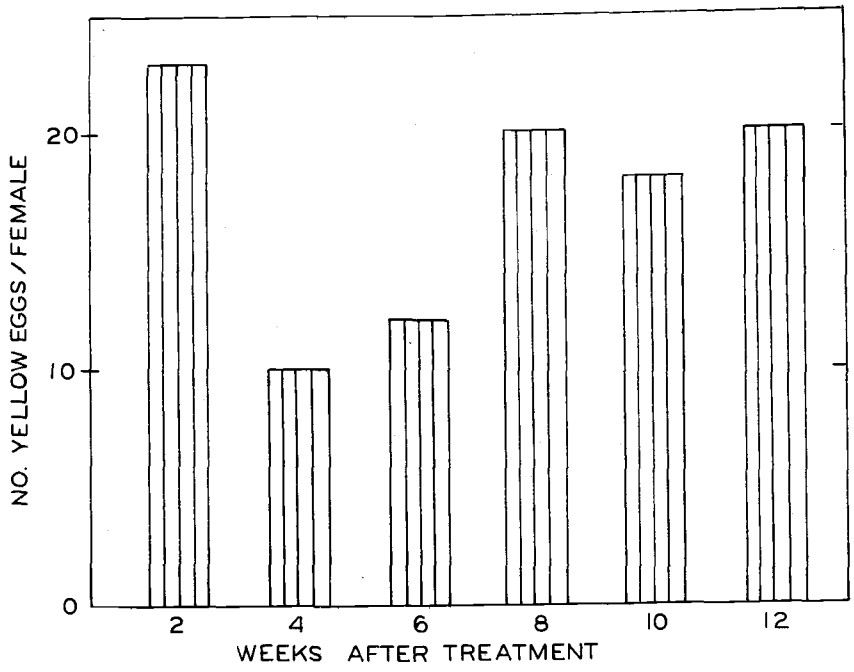


Figure 9. Average number of eggs/female when McDonald Forest D. crassicornis females in diapause were treated with 1.0 μg of ENT 33972a and confined in the greenhouse.

six week samples. Possibly, the decrease in number of eggs during this period was due to egg reabsorption by the females. However, it is also possible that these decreases were mainly due to cage differences among groups of females sampled. Presumably, if the decrease noted at four to six weeks was due to egg reabsorption, the decreasing trend would have continued during succeeding weeks in the greenhouse because the females would eventually have reabsorbed all eggs and returned to a diapausing state under greenhouse conditions. None of the treated females oviposited on the barley plants on which they were reared. At the end of the greenhouse confinement, the cumulative mortality of treated females was only slightly higher than that of the checks, indicating that female survival was not decreased by the hormone treatment. The check females did not initiate any ovarial development while they were confined in the greenhouse. However, in autumn when survivors of both groups of females were caged outdoors on the natural oviposition host, B. carinatus, both treated and control insects completed a normal reproductive cycle with an average of 16.7 and 11.9 eggs/female deposited on the plants respectively by females in the two treatments. Thus, the survival and reproductive capacity of M. F. females in diapause was not affected by a low dosage of ENT 33972a.

Gonadotropic Activity of Various Synthetic Juvenile Hormones Applied Topically to Females in Diapause

The ovarial development of M. F. females in diapause was used as a bioassay criterion to determine if various synthetic juvenile hormones were as effective in terminating the diapause as ENT 33972a.

Females in diapause were collected from the M. F. population and brought into the greenhouse. Each of 27 hormones was evaluated at concentrations of 10.0 and 1.0 μg and 16 females were given each treatment. Females treated with acetone were used as controls. After treatment, the females were confined on barley seedlings within glass lamp chimneys. Ten females from each treatment were dissected nineteen days after treatment to determine the number of yellow eggs/female (Table 2).

Several of these compounds, ENT 70460, ENT 70458, and ENT 70513, were active in terminating the diapause of the females although none of these was as active at the 10.0 μg level as ENT 33972a. No compound, except ENT 33972a, was active at the 1.0 μg level. Since the three compounds which were active in this test have also shown activity against other insect species (Zoecon, Unpublished) further testing of these compounds may be desirable. These results also suggest that M. F. females in diapause could serve as reliable

Table 2. Gonadotropic effects of various synthetic juvenile hormones applied topically to McDonald Forest D. crassicornis females in diapause.

Compound		Hormone Concentration	
Source	ENT Number	1.0 ug Avg. # Eggs/Female	10.0 ug Avg. # Eggs/Female[1]
Hoffman LaRoche	33972a	18.4	35.0
Zoecon	70460	0.1	31.2
Zoecon	70458	0.7	27.5
Zoecon	70513	0.2	25.9
USDA	34308	3.3	18.7
Zoecon (777)	?	1.8	19.0
USDA	34862	0.4	16.5
Zoecon	70459	0.7	15.2
USDA	34650	0.1	9.1
Zoecon	70499	0	7.2
USDA	34742	0	7.1
USDA	34495	0	0.5
USDA	34710	0	0.3
USDA	34027	0	0.2
USDA	34455a	0	0.2
USDA	34038	0	0.1
USDA	34426	0	0.1
USDA	34233	0	0.1
USDA	34765	0	0
USDA	34648	0	0
USDA	34708	0	0
USDA	34701	0	0
USDA	70188	0	0
USDA	34716	0	0
USDA	34718	0	0
USDA	34720	0	0

[1] Average consists of eggs/female at time of dissection + average number of eggs in plant (total number of eggs in plant/initial 16 females in treatment)

test insects for comparing the activity of various synthetic juvenile hormones.

Effect of Confining Synthetic Juvenile Hormone Treated Females in Diapause on Different Host Plants

Both the quantity and quality of food influence total egg production and oviposition of a given species (Englemann, 1969). This experiment was designed to compare the ovarial development of females in diapause which had received identical dosages of synthetic juvenile hormone, and were then provided with two different nutritional sources, barley seedlings or B. carinatus. Females in diapause collected from McDonald Forest were brought into the greenhouse and treated topically with either 15.0 or 2.0 μg of ENT 33972a. Acetone treated females were used as a control. Each treatment consisted of 60 females (10 females/cage). One-half of the females in each treatment were confined on B. carinatus and the other half was confined on barley seedlings. Fourteen days after treatment, 15 females of each treatment on each of the two species of host plants were dissected to determine the number of yellow eggs/female (Table 3).

At both hormone dosages the average number of eggs/female was greater for females confined on barley than those confined on B. carinatus, but the differences between the averages is significant

only in the 2.0 μg treatment (Duncan's NMRT, $P < .05$). This suggests that, particularly at smaller hormone dosages, the gonadotropic effects of the hormone on females in diapause may vary considerably, depending upon the species of host plant on which the females feed. In this experiment, differences in ovarial development were probably related to differences in the quality or quantity of nutrients furnished by the two host plants. Apparently, barley seedlings are a better nutritional source for females developing eggs than is B. carinatus although B. carinatus is the normal field oviposition host plant.

Table 3. Ovarial development of synthetic juvenile hormone treated D. crassicornis females in diapause which were confined on different host plants.

	Hormone Treatment ug/Female		
Host Plant	15 Avg. # Eggs/Female	2 Avg. # Eggs/Female	Check Avg. # Eggs/Female
Bromus carinatus	23.1	16.7	0
Barley seedlings	29.3	27.4	0

II. A Study of the Effects of Foliage Sprays of Synthetic Juvenile Hormone

Many studies of the effects of synthetic juvenile hormones on insect species have shown the effects of injecting or topically applying the hormones. Few studies have evaluated the effects of

exposing insects to foliage sprayed with formulations of synthetic juvenile hormone. Since future use of synthetic juvenile hormones to control insects will undoubtedly utilize foliage sprays of these compounds, additional laboratory and field studies of the effectiveness of spray formulations of these compounds is essential. This section of the study describes the effects of foliage sprays of various synthetic juvenile hormones on D. crassicornis.

Phytotoxicity Effects of Foliage Sprays of Synthetic Juvenile Hormones

The phytotoxicity produced by spraying barley seedlings and B. carinatus with synthetic juvenile hormone was investigated because severe phytotoxicity symptoms were observed in preliminary tests when barley seedlings were sprayed with synthetic juvenile hormones. Foliage sprays of the following synthetic juvenile hormones were tested for phytotoxicity on barley seedlings and B. carinatus: ENT 70348, ENT 70349, ENT 70350, ENT 70351, ENT 70119a, ENT 70221, and ENT 33972a. Each compound was formulated as a 25% emulsifiable concentrate solution, except ENT 70221 which was a 73% solution of technical material. All of the emulsifiable concentrate formulations were diluted with distilled water to obtain 1.0% and 0.10% (weight actual ingredient) spray solutions. The ENT 70221 solution was diluted to the same spray concentration with acetone. A solution of distilled water and xylene was sprayed on control

plants. One clay pot (15 cm diameter) containing the plants was treated with each spray concentration of each compound as previously described in the Materials and Methods section, Application of Synthetic Hormones. The plants were observed periodically after treatment for symptoms of phytotoxicity.

All of the synthetic juvenile hormones tested as 1.0% concentration foliage sprays in the greenhouse were highly toxic to barley seedlings. Phytotoxicity was expressed by the rapid, severe wilt of the seedlings, followed by a general leaf necrosis and death of the plants. Barley seedlings treated with 0.10% sprays of the same compounds developed slight phytotoxicity symptoms which consisted of small necrotic spots on the leaf surface. In contrast to the barley seedlings, B. carinatus grown both in the greenhouse and in the field, was only slightly affected by either 1.0% or 0.10% sprays of the same compounds, although some isolated necrotic spots were produced on the leaves by the 1.0% sprays. Most of the compounds tested in the greenhouse were formulated as an emulsifiable concentrate with an xylene base. Because no phytotoxicity symptoms were observed on any of the control plants sprayed with a xylene solution, the phytotoxicity observed was probably due to the hormones rather than to the formulating agents. Apparently, high spray concentrations of the synthetic juvenile hormones can produce quite severe phytotoxicity symptoms in young plants with pliable leaf tissue such

as barley seedlings, but do not affect older plants with tougher leaf tissue such as B. carinatus.

Effects of ENT 33972a Applied as a Foliar Spray to Bromus carinatus which Contained D. crassicornis Eggs

Since previous tests showed that the viability of D. crassicornis eggs could be lowered by a topical application of ENT 33972a, an attempt was made to produce similar effects by spraying ENT 33972a on B. carinatus leaf tissue which contained D. crassicornis eggs.

Gravid females from the Corvallis population were caged on B. carinatus in the greenhouse (15 females/plant). The females were allowed to oviposit for three days on the plants, and then at two successive three-day intervals were transferred to new plants of B. carinatus. Immediately after the females were removed, the plants were sprayed with one of the following solutions of ENT 33972a; 0.10%, 0.01%, (weight actual ingredient). Plants sprayed with water were used as a control. The spray solutions were applied to the plants as previously described. Each treatment had been applied to three pots of B. carinatus which contained eggs at the conclusion of the nine day female oviposition period. Ten days after treatment, the eggs were dissected from the plant tissue and placed in glass petri dishes lined with moist filter paper. The viability of eggs in each treatment was determined by counting the number of eggs

which hatched.

The viability of eggs dissected from B. carinatus sprayed with a 0.10% and 0.01% solution of ENT 33972a, and the controls sprayed with only water was 70, 63, and 61% respectively for the three groups. Clearly, the viability of eggs in the foliage was not lowered by either of the hormone treatments.

Effects of Exposing IV Instars to Foliage Sprayed with Synthetic Juvenile Hormone

Previous experiments had shown that a small dosage of ENT 33972a which was applied topically would prevent the adult metamorphosis of IV instars. Both laboratory and field tests in which nymphs were exposed to foliage treated with synthetic juvenile hormones were done to determine if foliage sprays of these compounds could also prevent the adult metamorphosis of IV instars.

All of the synthetic juvenile hormones shown in Table 4 were tested in the greenhouse during the summer of 1971 except ENT 70459 which was tested during the summer of 1972. These compounds were formulated as 25% emulsifiable concentrates except ENT 70459 which was formulated as a 50% emulsifiable concentrate. Each of these compounds was sprayed at three concentrations, 0.10%, 0.01%, 0.001%, and plants sprayed with water were used as a check. After the spray on the plants had dried, IV instars were caged on these

plants (25 nymphs/cage) for two weeks. The nymphs were then transferred to glass lamp chimneys and reared on untreated barley until they died or emerged as adults.

Immediately after the laboratory tests, the three most active compounds, ENT 33972a, ENT 70119a and ENT 70459, were evaluated in the field on B. carinatus test plots. Foliar sprays of each compound were applied at the 0.10% and 0.01% concentration and plots sprayed with water were used as a check. One hundred IV instars from McDonald Forest were introduced into cages placed on each plot. Each treatment was replicated twice. Fourteen days after treatment, 50 nymphs were removed from each treatment and immediately transferred to untreated barley plants in the greenhouse. These nymphs were periodically observed until they died or became adult.

The results shown in Table 4 indicate that three of the compounds, ENT 70119a, ENT 33972a, and ENT 70459, were effective in preventing the emergence of normal adults both in laboratory and field tests when applied as 0.10% sprays. None of these three compounds was effective when applied as a 0.01% spray, particularly when tested in the field.

Table 4. Effects of synthetic juvenile hormone on adult emergence produced when D. crassicornis IV instars were exposed to treated foliage.

Compound	% Normal Adults Concentration % 0.001	0.01	0.10
Laboratory Tests			
ENT 70348	87	76	96
ENT 70349	80	76	80
ENT 70350	96	92	60
ENT 70351	80	72	88
ENT 70221	88	84	48
ENT 70119a	80	36	0
ENT 33972a	68	40	0
ENT 70459	64	40	20
Check	88	--	--
Field Tests			
ENT 33972a		78	12
ENT 70119a		90	16
ENT 70459		96	16
Check	100		

Effects of Exposing Adult Diapausing Females to Foliage Sprayed with Synthetic Juvenile Hormone

Previous work by Kamm and Swenson (1972) has shown that the adult reproductive diapause of female D. crassicornis can be terminated by a topical application of various synthetic juvenile hormones. Females in diapause were exposed to B. carinatus, which had been sprayed with synthetic juvenile hormone, to determine if the foliar sprays of synthetic juvenile hormones were also effective in terminating the female diapause. The synthetic juvenile hormones which were tested in the greenhouse are shown in Table 5. All of these compounds were formulated as 25% emulsifiable concentrates except ENT 70221 which was a 73% technical acetone solution. Each compound was sprayed on B. carinatus as 1.0% and 0.10% concentrations and then 12 M. F. females in diapause were caged on each of the treated B. carinatus plants. Females placed on plants sprayed with water were used as controls. After 14 days, all surviving females in each treatment were dissected to determine the number of yellow eggs/female.

The three compounds ENT 33972a, ENT 70119a and ENT 70221 which were the most active in laboratory tests in terminating female reproductive diapause were then tested in the field. In addition, ENT 70357, which was structurally similar to ENT 70119a, and ENT 70441,

Table 5. Effects of synthetic juvenile hormone on ovarial development of D. crassicornis females in diapause which were exposed to treated foliage.

Compound		Avg. # Eggs/♀ (0.10% Spray)	Avg. # Eggs/♀ (1.0% Spray)
Laboratory Studies			
		(S.E.)	(S.E.)
ENT 70348		0	0.2 ± .55
ENT 70349		0	0.7 ± 2.1
ENT 70350		0	3.3 ± 1.91
ENT 70351		0	0.4 ± 0
ENT 33972a		0	14.0 ± 7.5
ENT 70119a		0	17.7 ± 3.4
ENT 70221		0	10.7 ± 3.4
Check		0	0
Field Studies			
ENT 33972a	oil	11.2 ± 1.7	28.8 ±8.9
ENT 33972a	xylene	14.2 ± 2.2	35.4 ± 9.9
ENT 33972a	oil + summer oil	21.2 ± 2.4	--
ENT 70119a	xylene	8.9 ± 11.7	32.1 ± 3.8
*ENT 70357	oil	1.0 ± 2.0	6.5 ± 4.9
ENT 70357	oil + summer oil	2.0 ± 2.6	--
ENT 70221	xylene	4.3 ± 4.4	14.3 ± 8.8
ENT 70221	xylene + summer oil	0.2 ± 0.7	--
ENT 70441	oil	5.5 ± 2.8	13.5 ±3.5
ENT 70441	oil + summer oil	1.2 ± 2.5	--
Check	water	0.8 ± 2.3	--
Check	water + summer oil	1.2 ± 2.2	--

*Differs from ENT 70119a only in having one less methyl group on the number 3 carbon
(S.E.) = Standard Error

which had shown activity for other species of Homoptera, were also tested in the field. Various formulations of each compound were also tested to determine the effect on activity.

Spray Formulations

1.0% or 0.10% spray, formed by diluting a 25% emulsifiable concentrate xylene formulation with water

1.0% or 0.10% spray, formed by diluting a 50% emulsifiable concentrate oil formulation with water

0.10% spray, formed by diluting an oil or xylene emulsifiable concentrate with water which contained a 2.0% summer oil carrier.

Field plots of B. carinatus were randomly assigned treatments and each treatment was replicated twice. After the plots were sprayed, M. F. females in diapause were caged on each plot. Fourteen days later 30 females from each treatment were dissected to determine the number of yellow eggs/female.

Table 5 shows that none of the compounds tested in the laboratory were effective in terminating female diapause when applied as 0.10% sprays. The compounds ENT 33972a, ENT 70119a and ENT 70221 which were active in preventing normal nymphal development were also the most successful in terminating the reproductive diapause of females when applied at the 1.0% spray level in both

laboratory and field tests. ENT 33972a was the only compound which showed any activity against the females in field tests when applied as a 0.10% spray. The different formulations of the same compound which were tested in the field had no consistent effect on the activity of these compounds.

ENT 70347 tested in the field as a 1.0% spray was about 20% as active as ENT 70119a; this compound differed from ENT 70119a structurally only by the absence of one methyl group on the number three carbon. This indicates that some very specific structure-activity relationships must exist for those compounds affecting the reproductive diapause of D. crassicornis females.

III. Effects of Synthetic Juvenile Hormone on Mating of McDonald Forest Adult D. crassicornis

When the diapause of P. apterus was terminated by implanting corpora allata into the females, both male and female mating activity increased (Zdarek, 1968). Synthetic juvenile hormone treatments of both male and female cereal leaf beetles which were in diapause terminated the diapause of females and normal post diapause mating activity began (Connin et al., 1967). This research suggested that the mating activity of some species of insects in diapause could be affected by synthetic juvenile hormone. The following work was done to determine if synthetic juvenile hormone affected the mating

behavior of D. crassicornis in diapause.

Effects of Synthetic Juvenile Hormone on the Frequency of Mating of Combinations of McDonald Forest and Corvallis Adults

Although it was known that females collected from McDonald Forest were in a reproductive diapause which consisted of a lack of ovarial development, the reproductive condition of M. F. males was not known. To compare the mating activity of hormone treated and nontreated M. F. males and females paired with each other and nondiapausing C. males and females, various mating combinations of these insects were set up in the greenhouse. To synchronize the development of the C. and M. F. populations, III and IV instars were collected from McDonald Forest in mid-May, brought into the greenhouse and were reared under conditions of 23° C and 16 hour photoperiods to increase the rate of development. Two weeks later V instars from the C. population were brought into the greenhouse and reared in the same conditions. The V instars of both populations were sexed (Pollard, 1962) and males and females of each group were then reared separately until they emerged as adults. Then, 15 pairs of each of the combinations shown in Table 6 were caged on barley in groups of three males and females per cage.

After five weeks, the remaining females in each combination were dissected to determine if they had mated. The criterion used

Table 6. Effects of synthetic juvenile hormone on the frequency of mating of diapausing (McDonald Forest) and nondiapausing (Corvallis) D. crassicornis.

Mating Combination					Proportion mated[b]	% ♀ mated	Off-spring
Males			Females				
Treated[a]	Nontreated	x	Treated[a]	Nontreated	♀		
	C.	x		C.	11/11	100	yes
	C.	x	C.		6/6	100	yes
	C.	x		M. F.	1/11	9	no
	C.	x	M. F.		9/10	90	no
	M. F.	x		M. F.	0/16	0	no
	M. F.	x		C.	2/10	20	yes
	M. F.	x	M. F.		8/11	73	no
M. F.		x		M. F.	2/14	14	no
M. F.		x		C	7/7	100	yes
M. F.		x	M. F.		9/11	82	no

[a] Treatment = 5.0 ug ENT 33972a

[b] Numerator = Number mated
Denominator = Number surviving females at the time of dissection

to confirm mating was the presence of sperm in the spermatheca. Eggs obtained prior to the dissection of the females in any of the treatments were removed from the plant tissue and incubated on moist filter paper in glass petri dishes. The number of offspring from each combination was recorded to confirm the fertility of the females in each group. A very low percentage of M. F. nontreated females in diapause mated with C. males (Table 6). However, a high percentage of hormone-treated M. F. females which had terminated diapause and developed ovaries mated with C. males. This suggests that females must have actively developing ovaries to incite males to mate, and the M. F. females which had been stimulated by synthetic juvenile hormone to develop eggs, were just as sexually attractive and receptive to C. males as were the C. females which had developed ovaries naturally.

Only 20% of the C. females paired with untreated M. F. males mated, while all C. females had mated which were paired with M. F. males treated with hormone. This suggested that M. F. males did not mate readily even when paired with a sexually attractive female unless the males were treated with hormone.

When untreated M. F. males were paired with M. F. females treated with hormone, a high proportion (73%) of the females mated, indicating that there was considerable mating activity by untreated M. F. males in this combination. The high mating frequency of these

males was quite different from the low mating frequency (20%) of untreated M. F. males paired with C. females. Possibly, the untreated M. F. males received enough hormone from the treated M. F. females, either by vapor absorption or body contact to stimulate these males to mate with the females.

No offspring were obtained from M. F. females treated with hormone because the eggs were in an embryonic diapause and do not hatch unless exposed to cool temperatures for a period of time. However, offspring were obtained from C. females mated with either treated or untreated M. F. males or C. males. This indicated that treated males were fertile and capable of interpopulation mating.

The previous experiment showed that treating M. F. males with synthetic juvenile hormone stimulated their mating behavior, but gave no indication of the time required for this stimulation since females were not dissected to determine mating until five weeks after the beginning of the experiment. Therefore, another test was designed to compare the weekly mating activity of treated and untreated M. F. males and C. males. The development of M. F. males and C. males was synchronized by the early field collection of M. F. nymphs as described in the previous experiment. Fifteen virgin males and females were paired individually in the mating combinations shown in Table 7. Each of the treated M. F. males received a 5.0 μg dosage of ENT 33972a. Corvallis insects were not treated with

Table 7. Mating behavior of Corvallis males and synthetic juvenile hormone treated and nontreated McDonald Forest D. crassicornis males, when all groups were paired with Corvallis females.

Males[a]	Cumulative Number and % Males Mated					
	1 Week		2 Weeks		3 Weeks	
	No.	%	No.	%	No.	%
Corvallis (nontreated)	--	--	13	87	15	100
McDonald Forest (nontreated)	1	7	1	7	1	17
McDonald Forest (5 μg ENT 33972a)	2	13	9	53	13	87

[a]Each treatment contained 15 pairs

hormone. Each pair was confined separately in a glass lamp chimney on barley. Both the males and females were of known age. Each week, females were dissected to determine whether or not the males had mated, and these females were replaced with virgin females of a similar age. The experiment was continued for three weeks. Males which died during the experiment were also replaced with males of a similar age. When the experiment was concluded after three weeks, the males in each treatment were dissected and the testes measured.

After three weeks, all of the C. males and most of the M. F. males that were treated with hormone had mated but only one of the 15 untreated M. F. males had mated (Table 7). These results substantiate the previous experiment by again demonstrating that M. F. males did not mate readily with C. females unless the males had been treated with hormone. Also, under greenhouse conditions, most of the M. F. males treated with hormone and C. males had mated three weeks after emergence.

The testes were large in all three groups of males at the end of the experiment. No significant difference was found between the size of the testes of the C. and hormone treated M. F. males, but the testes of the untreated M. F. males were significantly larger than those of the other two groups (LSD, $P < .05$). Enough sperm may be ejaculated when males mate to reduce the size of the testes.

Thus, the larger size of the testes in untreated M. F. males may have been caused by their low mating frequency. The large testes size of the untreated M. F. males showed that their low mating activity was not due to underdevelopment of the testes, and suggested that the hormone stimulated the M. F. males to mate by affecting their behavior rather than testes development.

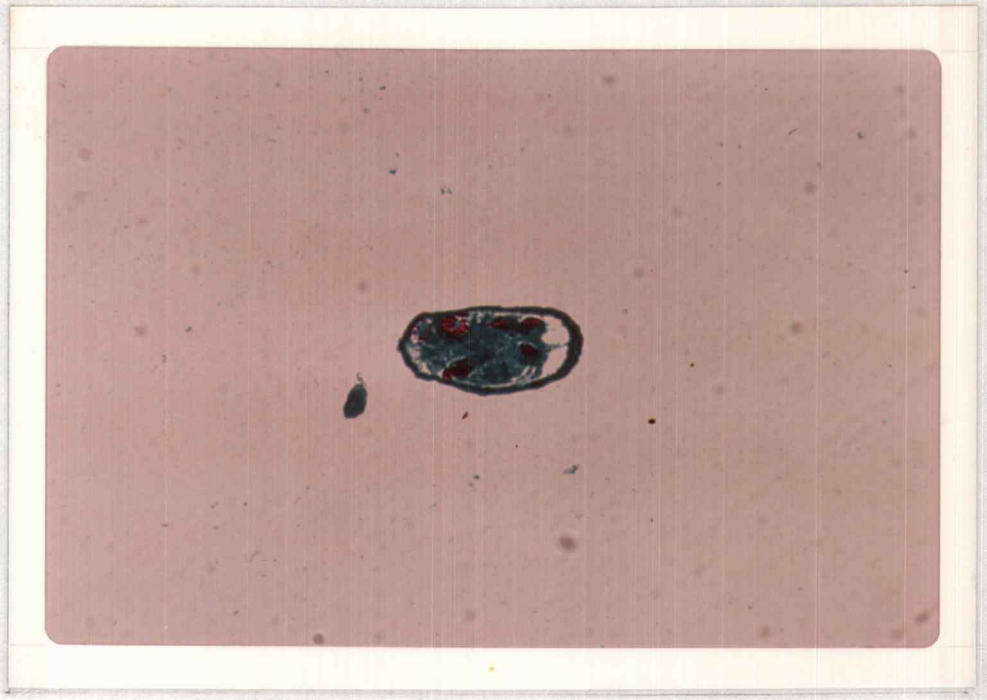
Testes Development of Field Populations of Corvallis and McDonald Forest Males

Since previous experiments had indicated that M. F. and C. males confined in the greenhouse developed mature testes in about three weeks, field populations were sampled periodically to determine the rate of testes development under normal environmental conditions. Sampling began when approximately 20% of the populations were adult. Ten males were collected from each population at three-day intervals. Sampling of both populations was terminated when the testes were mature.

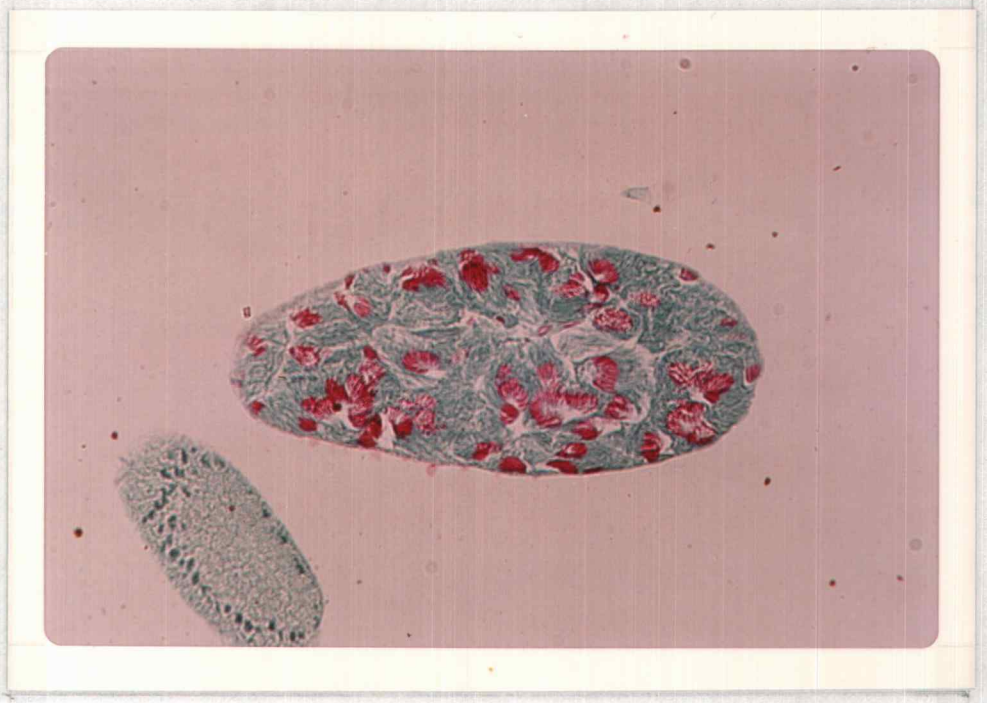
Males were dissected using the technique of Kamm and Ritcher (1972). After dissection, the testes were measured with an ocular micrometer to determine the maximum width and length of the testes to the point of attachment of the accessory gland. Then, testes were removed, dehydrated by the dioxan method, fixed in Bouin's solution, and embedded in Paraplast. Serial sections (5 μ) of each testes were prepared, and the sections were stained with the Fuelgen

technique and counterstained with a 0.10% alcoholic fast green solution. The sperm bundles that were mature absorbed Fuelgen stain whereas immature bundles did not. An estimate of the maximum number of mature sperm bundles for each testes was obtained by selecting the section on the side with a maximum number of bundles, counting the bundles in this section and the section on either side of it and then calculating the average for the three sections. The difference in the number of mature sperm bundles in the testes of a young (10 days) and an older (21 days) D. crassicornis male is shown in Figure 10.

The size of testes increases linearly with increasing age of the males in both populations (Figure 11, part A). The respective correlation coefficients between testes length and width and age in C. males was .907 and .928, and .970 for both length and width in M. F. males. An analysis of variance showed that no significant differences existed in the testes growth rate in the two populations, but the testes of C. males were significantly larger than those of M. F. males when young adults were sampled ($P < .05$). Although sampling began in each population when 20% of the insects were adults, sampling of C. males was completed in June while sampling of M. F. males was not begun until July. The cool evening temperatures in McDonald Forest may have caused the testes in the V instar M. F. males to develop more slowly than those of C. V instar males, resulting in a smaller size of the testes in newly emerged M. F.



A



B

Figure 10. Longitudinal section of a testes of an adult male D. crassicornis. A) 10 days old, and B) 21 days old.

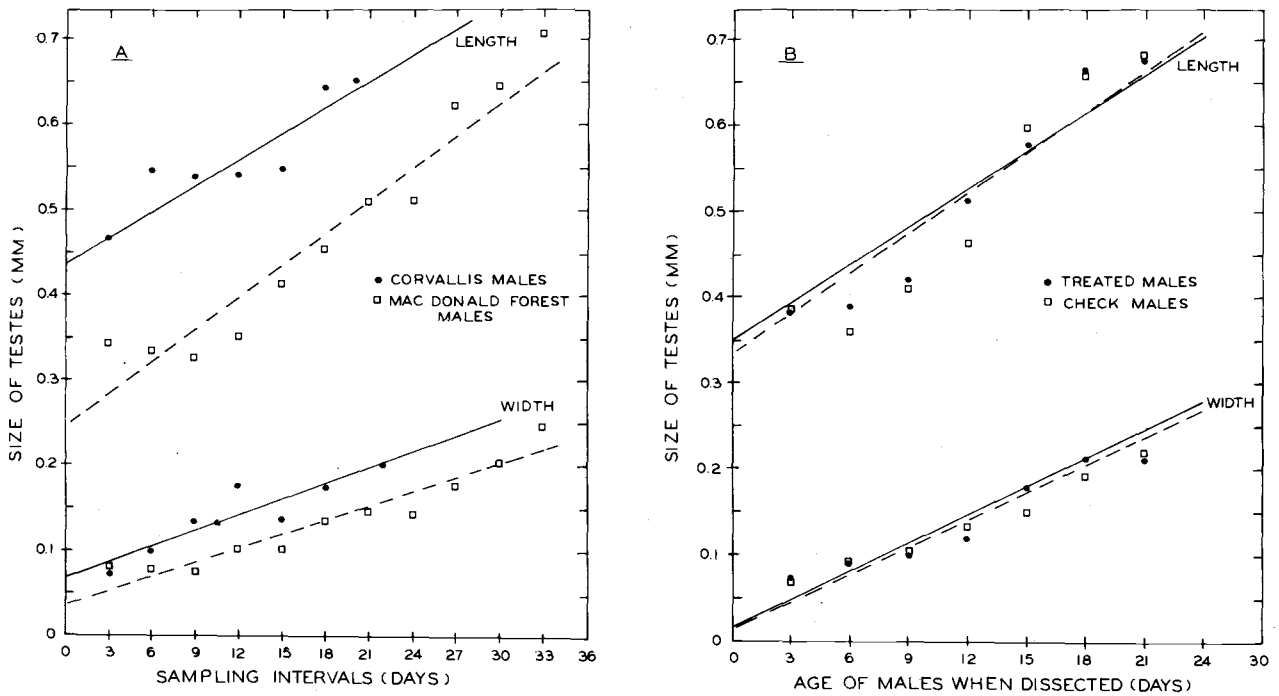


Figure 11. A) Progressive growth of testes of male D. crassicornis in periodic samples collected from Corvallis and McDonald Forest. B) Progressive growth of testes of treated (5.0 ug ENT 33972a) and untreated McDonald Forest males confined in the greenhouse.

males.

A comparison of the increase in mature sperm bundle density in testes of field populations of C. and M. F. males is shown in Figure 12, part A. In both populations, the sperm bundle density increases linearly with increasing age, with a linear correlation coefficient of .982 for C. males and .977 for M. F. males. An analysis of variance of the regression lines of the two populations revealed that no significant difference occurred ($P > .05$) in the rate of increase of sperm bundle density in the two populations. The elevation of the regression line for increase in the density of sperm bundles was significantly higher for C. males than M. F. males because of a lower density of mature sperm bundles in young M. F. males. The data points plotted around the regression line also show that the density of sperm bundles in testes of older M. F. males in late summer exceeds that found in mature males of the first generation of the Corvallis population.

Effects of Synthetic Juvenile Hormone on the Development of Testes of McDonald Forest Males

Previous tests showed that the frequency of mating of M. F. males was increased by treating them with synthetic juvenile hormone, and suggested that the hormone affected behavior rather than testes development. To determine the effects of synthetic juvenile hormone

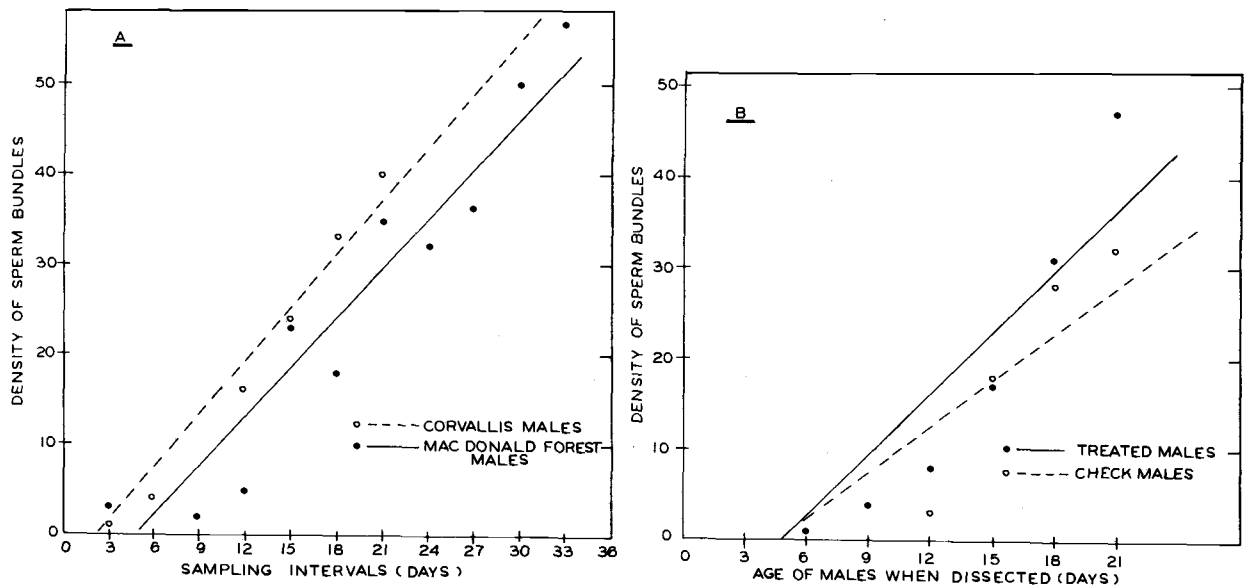


Figure 12. A) Increase in sperm bundle density in testes of male D. crassicornis in periodic samples collected from Corvallis and McDonald Forest. B) Increase in sperm bundle density in treated (5.0 ug ENT 33972a) and untreated McDonald Forest males confined in the greenhouse.

on testes development of M. F. males, V instars were brought into the greenhouse and kept on barley until adults emerged. Adult males less than 24 hours old were divided into two groups: one group received a dosage of 5.0 μg of ENT 33972a, and the other group was treated with acetone and used as a control. After treatment, ten leafhoppers of each group were dissected at three-day intervals, the testes were measured, sectioned, and the maximum density of mature sperm bundles was determined as described in the previous experiment. The experiment was terminated after three weeks when the testes of the insects were mature.

In both groups of males, the size of the testes increased linearly with increasing age (Figure 11, part B). The respective correlation coefficients between age and length and width of the testes of treated M. F. males were .924 and .960, and the respective correlation coefficients for the testes of untreated M. F. males were .890 and .967. When the regression lines for increasing length and width of the testes were tested with an analysis of variance, the growth rate of the testes of the males in the two treatments was not significantly different ($P > .05$). Therefore, synthetic juvenile hormone had no effect on testes growth.

The increase in the mature sperm bundle density in the testes in both treatments of M. F. males is shown in Figure 12, part B. The relationship between sperm bundle density and increasing age

of the males in both treatments is linear, as shown by the respective correlation coefficients for treated and nontreated M. F. males of .906 and .894. An analysis of variance of the regression lines for the two populations reveals no difference in the rate of increase of mature sperm bundle density in testes of the treated and untreated M. F. males. However, the data points show that mature sperm bundles were present in testes of treated males which were six days old, but did not occur in testes of untreated males until they were 12 days old. This suggests that the synthetic juvenile hormone hastened sperm maturation in M. F. males.

In summary, this work has shown that synthetic juvenile hormone did not affect the growth or rate of increase in mature sperm bundle density in testes of M. F. males, but may have hastened sperm maturation.

Effects of Temperature and Photoperiod on the Mating of McDonald Forest males

In the locust, Oedipoda miniata, males remain in a behavioral diapause during the summer months which is characterized by a low mating activity. This diapause is controlled by the corpora allata and is terminated by the autumn environmental conditions (Broza and Pener, 1972). Since this work indicated that D. crassicornis males also spend the summer months in a behavioral diapause, the effect of autumn environmental conditions (short days and cool

temperatures) on mating activity was investigated. Newly emerged adults were collected from McDonald Forest in early July. Males and females were separated and confined on barley in lamp chimneys and were placed in three controlled environment chambers set for the following regimens:

Temperature	Photoperiod	Number of Males	Number of Females
21 °C	16 hours	80	0
21 °C	10 hours	80	0
15 °C	10 hours	80	240

After eight weeks the females which were all confined in short days and cool temperatures had terminated diapause and initiated ovarial development. Then, these females were paired with males from all three environmental regimens. Each chamber then contained 60 pairs of leafhoppers, with three pairs of leafhoppers confined in each glass lamp chimney. One week later 30 females from each of the environmental regimens were removed and dissected to determine the percentage of females which mated in each regimen. Fifteen males were also removed from each environmental regimen and dissected to determine the development of the testes.

The percent of mated females in each environmental regimen (Figure 13) was used to estimate the mating frequency of males in the treatments. The percent of mated females in the three environmental regimens was compared by Chi-Square tests. At a

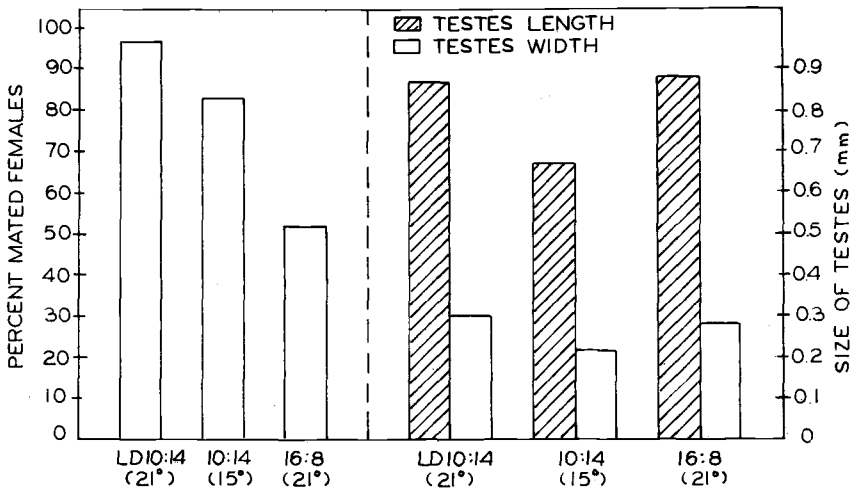


Figure 13. Percent of McDonald Forest females that mated and the size of testes of McDonald Forest D. crassicornis males when the insects were confined in controlled environmental regimens.

temperature of 21 ° C, the male mating activity was significantly greater ($P < .05$) under short days (10 hours daylength) than long days (16 hours daylength). In short days, the mating activity at 21 ° C was not significantly different ($P > .05$) than that occurring at 15 ° C. These results suggest that male mating activity is stimulated primarily by short days and temperature exerts little influence. However, since mating occurred in all environments, this suggests that environmental conditions do not completely control male mating activity. The testes of all groups of males were well developed (Figure 13), but the testes of males reared in the short-day cool temperature regimen were significantly smaller (Duncan's NMRT, $P < .05$) than those of males in the other environments.

The male mating activity in the short-day cool temperatures was significantly greater than that of males under 21 ° C, LD 16:8, ($P < .05$) but was not significantly different from that of males in 21 ° C LD 10:14 ($P > .05$). Therefore, in this test, no relationship existed between testes size and mating activity.

DISCUSSION AND CONCLUSIONS

The eggs of D. crassicornis were not highly sensitive to sterilization by synthetic juvenile hormone either by direct topical application or treatment of gravid females. Egg viability was reduced slightly by low dosages of hormone, but higher dosages had little additional effect. Some eggs were still viable even when treated directly with 2.0 μg of ENT 33972a or when females had received a 5.0 μg treatment. These dosages were quite high when compared to the amount of ENT 33972a, 0.05 μg, necessary to prevent adult metamorphosis of most IV instars. Younger eggs of D. crassicornis were no more sensitive to hormone sterilization than older eggs. In P. apterus and certain Coleoptera species freshly oviposited eggs are much more sensitive to hormone sterilization than older eggs (Slama and Williams, 1966; Walker and Bowers, 1970).

The insensitivity of D. crassicornis eggs to sterilization by synthetic juvenile hormone is quite similar to the response of some species of Lepidoptera. Both eggs and females of the spruce budworm, the cabbage butterfly, and wax moth, are quite resistant to the sterilizing effects of synthetic juvenile hormones (Retnakaran, 1970; Benz, 1971). It is not known if varying responses of eggs to synthetic juvenile hormones are due to differences in chorion permeability, or differences in embryonic response after the hormone has

entered the egg.

In general, the nymphal stage of D. crassicornis was quite sensitive to ENT 33972a, but the response of the different instars varied a great deal. A larger dosage of hormone was required to produce morphological abnormalities in nymphs treated as III instars than those treated as IV or V instars. Presumably, more of the hormone was metabolized when younger nymphs were treated because the hormone remained in the body for a long time period before affecting the final nymphal metamorphosis.

The III instars were more sensitive to the direct toxicity of large dosages of synthetic juvenile hormone than the older nymphs. Fourth instars were the most sensitive to morphogenic effects produced by ENT 33972a. The response of V instars was quite variable, since young V instars were very sensitive to ENT 33972a, but older V instars were virtually insensitive to the hormone.

The variable response of the nymphal instars illustrates some of the difficulties involved in using synthetic juvenile hormones to control immature insects. For D. crassicornis, low hormone dosages applied when insects were in the IV instar would be the most effective in blocking metamorphosis of subsequent adults. However, if a rapid reduction of nymphs was desired, high hormone dosages should be applied to III instars. If synthetic juvenile hormones were applied to late V instars, most of the insects would molt normally

to adults and reproduce normally unless extremely high dosages were applied.

No deferred effects such as low survival of offspring and morphological abnormalities (Riddiford, 1970) occurred in D. crassicornis when late V instars or adult females were treated with ENT 33972a. Since the hormone treatments caused no reduction in survival of offspring, the effectiveness of the hormone in reducing numbers of progeny from treated adults in this species would be limited to reductions in viability of the eggs. Although the deferred effects phenomenon could be a significant advantage in using synthetic juvenile hormones in insect control, at present it has only been demonstrated in three insect species (Riddiford, 1970).

When late V instar M. F. females committed to an adult diapause were treated with ENT 33972a, sufficient amounts of the hormone even at the lowest treatments (1.5 and 5.0 μg) persisted in the bodies of the adult females to stimulate ovarial development. After a two-week period, the average number of eggs/female for a given amount of ENT 33972a, was quite similar to the results obtained by Kamm and Swenson (1972) when they treated diapausing adult female D. crassicornis with ENT 33972a. This suggests that most of the hormone applied to the V instars of the M. F. females carried over into the adults and persisted for at least the early part of the reproductive cycle. In contrast, when nondiapausing late V

instar C. females were treated with 5.0 μg, apparently not enough of the hormone remained in the bodies of the adult females to reduce the viability of the developing eggs. This suggests that the amount of hormone carryover from treated V instars and the persistence of hormone in the early stages of a reproductive cycle is quite different in the C. and M. F. females. It is possible that the ability to metabolize or otherwise reduce concentrations of synthetic juvenile hormone differs in females of the two populations because the M. F. females are preparing to enter diapause while the C. females are programmed for continuous development. It would be interesting to investigate this possibility in the future.

Although spraying foliage containing D. crassicornis eggs with ENT 33972a did not reduce egg viability, foliage sprays of some synthetic juvenile hormone compounds were effective in terminating the adult reproductive diapause of M. F. females. In general, the same compounds were active against both nymphs and adults. Also, compounds which were effective in laboratory tests were also effective in the field. Even though some compounds were effective against nymphs as 0.10% sprays, which was ten times less than the concentration needed to terminate female diapause, it would probably not be economically feasible to commercially apply these compounds in the field even at the 0.10% level.

This study has shown that M. F. females in diapause which

received a low dosage of hormone shortly after adult emergence rapidly developed mature eggs which they carried until fall when they completed normal ovarial development, and oviposited a normal complement of eggs. Survival of females during the summer was not markedly affected by the hormone treatment. These results suggest that attempting to treat these females in diapause with synthetic juvenile hormone to disrupt their normal reproductive cycle and force them to lay eggs prematurely would be unsuccessful unless the females had received high dosages of hormone.

During mating experiments, M. F. males did not mate readily with receptive females unless the males were treated with ENT 33972a. Laboratory studies showed that the hormone affected behavior, but not testes growth or the rate of spermatogenesis, although mature sperm bundles appeared slightly earlier in testes of treated males than in testes of untreated males. There has been little other research on the effects of synthetic juvenile hormone on the testes and spermatogenesis in other insect species. Masner (1967) showed that synthetic juvenile hormone applied to V instar P. apterus had no effect on sperm differentiation in supernumary VI instar nymphs. Also, Landa (1972) noted that the effects of synthetic juvenile hormone on the male reproductive system vary widely among insect species. The hormones had no effect on the testes of some species, retarded testes development in others, and caused testes and sperm

degeneration in other species. There have been no reports of positive effects of synthetic juvenile hormone on testes growth or spermatogenesis.

In contrast to M. F. females which spend the summer months in reproductive diapause characterized by undeveloped ovaries, the testes of M. F. males increase in size and spermatogenesis proceeds throughout the summer. The diapause of M. F. males consists of a behavioral reluctance to mate, rather than a cessation of reproductive system development. In other insect species, growth of testes and spermatogenesis during male diapause has also been observed. Hodek and Landa (1971) observed that spermatogenesis continued in diapausing coccinellids except under extremely cold conditions. In the grasshopper, Oedipoda miniata, the males behave quite similarly to McDonald Forest D. crassicornis males. Although the male grasshoppers have a fully developed reproductive system, they remain in a behavioral diapause which consists of a reluctance to mate during the warm summer months (Pener, 1970).

The reproductive diapause in McDonald Forest D. crassicornis females is probably controlled by the corpora allata (Kamm and Swenson, 1972). Since diapause in both males and females can be terminated by synthetic juvenile hormone, it is also likely that the behavioral diapause of the males is influenced by the corpora allata. In acridids, the female reproductive diapause and a male behavioral

diapause were shown to be controlled by the corpora allata (Pener, 1970).

Swenson and Kamm (unpublished data) have shown that the McDonald Forest D. crassicornis female diapause can be terminated by short days and cool temperatures. Presumably, in autumn these conditions terminate the female diapause by reactivating the corpora allata. If the diapause of both sexes is controlled by the corpora allata, the male diapause should also be terminated by short days and cool temperatures to synchronize the reproductive cycle of males and females with the environment. I have shown that male mating activity was stimulated by short days, but temperature differences had only a slight effect. Although male mating activity was greater under short day conditions, some mating also occurred under long day conditions. Since the males were held in each environmental regimen for eight weeks prior to being exposed to receptive females, their mating pattern may have been similar to that observed in some diapausing male acridids. In acridids, short days caused a rapid increase in male mating behavior within two weeks. Males held under long day conditions gradually became more sexually active until the ninth or tenth week when their mating activity approached that of males held in short days for two weeks. These effects of daylength were thought to be caused by a gradual activation of the male corpora allata under long days and an abrupt activation under 12 hour days (Perez et al., 1971).

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