The effects of Altosid®, a juvenile hormone analogue, on the development of Tribolium castaneum, Tribolium confusum, Tribolium audax, and Tribolium madens

Clark Taylor

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THE EFFECTS OF ALTOSID\textsuperscript{R}, A JUVENILE HORMONE ANALOGUE, ON THE DEVELOPMENT OF TRIBOLIUM CASTANEUM, TRIBOLIUM CONFUSUM, TRIBOLIUM AUDAX, AND TRIBOLIUM MADENS

A Thesis

Presented to the

Faculty of

California State

College, San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

by

Clark Taylor

June 1976
THE EFFECTS OF ALTOSID®, A JUVENILE HORMONE ANALOGUE, ON THE DEVELOPMENT OF TRIBOLIUM CASTANEUM, TRIBOLIUM CONFUSUM, TRIBOLIUM AUDAX, AND TRIBOLIUM MADENS

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Approved by:

Chairman, Biology Department
Graduate Committee

[Signatures]

Committee Member

Committee Member

Major Professor

Representative of the Graduate Dean

2 June 1976
ABSTRACT

The juvenile hormone analogue, isopropyl-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (Altosid®), was included in varying amounts in the growth medium of four species of flour beetles, Tribolium castaneum, T. confusum, T. audax, and T. madens. First instar larvae of these beetles were placed into vials of treated growth medium and then observed periodically during their development until they had either successfully emerged as adults, or were dead. The lethal effect of Altosid on the development of the beetles was calculated at the LD₅₀, LD₅₀, and LD₉₅ levels by probit analysis.

It was found that three of the species, T. castaneum, T. audax, and T. madens, displayed similar susceptibility to Altosid with LD₅₀ values of 0.026, 0.029, and 0.043 parts per million respectively. T. confusum demonstrated a much higher tolerance level to the chemical with an LD₅₀ of 0.194 parts per million.

The results of this experiment show that the morphogenetic development of the four species of flour beetles tested is strongly affected by relatively minute concentrations of Altosid contained in their nutrient-growth medium. These data are compared to similar work by other investigators, and the significance of the difference in dosage response between T. confusum, and the other species tested is discussed.
ACKNOWLEDGEMENT

It would be difficult to acknowledge all of the people who have aided me during the course of my graduate studies. I owe an especially large debt of gratitude to my major professor, Alexander Sokoloff, for his continual guidance regarding my research and my career as a graduate student, and for his friendship. In addition, I would like to thank the entire C.S.C.S.B. biology staff for their friendship and encouragement.

I extend my appreciation to the U.S. Army Research Office (Grant #RDRD LP11790-LS) for their support, in part, of my research at C.S.C.S.B. Additionally, I am indebted to the Tribolium Stock Center at the California State College in San Bernardino, California, for providing all stocks of beetles and general supplies, and to the Zoecon Corporation of Palo Alto, California, for supplying a free sample of their juvenile hormone analogue, Altosid.
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THE EFFECTS OF ALTOSID® A JUVENILE HORMONE ANALOGUE, ON THE DEVELOPMENT OF TRIBOLIUM CASTANEUM, TRIBOLIUM CONFUSUM, TRIBOLIUM AUDAX, AND TRIBOLIUM MADENS

INTRODUCTION

The goal of this research project was to determine the effect which varying concentrations of the juvenile hormone analogue, isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (Altosid®), has on the development of four species of flour beetles, Tribolium castaneum, T. confusum, T. audax, and T. madens, when mixed in their nutrient-growth medium.

Altosid is one of the more potent juvenile hormone analogues to have been developed as of this date for use in controlling unwanted populations of insects (Cerf and Georghiou, 1972), and for this reason, was chosen for this project.

JUVENILE HORMONE BACKGROUND INFORMATION

Hormonal control of insect metamorphosis was first demonstrated in 1922 by Kopec (Kopec, 1922), and later substantiated by Wigglesworth (1933). Wigglesworth demonstrated that a humoral factor released from the corpora allata maintained the larval condition. By removing the corpora allata from early instar larvae, he was able to induce pupation. Conversely, transplanting active corpora allata to late-last instar larvae resulted in further
larval molts. Lastly, through ligation experiments, he proved that the juvenizing factor was carried in the haemolymph, and not transmitted by some other means.

Despite this early knowledge of juvenile hormones, interest in them as a means of controlling unwanted insect populations was not aroused until the 1950's, when Williams (1956) demonstrated the strong morphogenetic activity of extracts from cecropia moths, _Hyatophora cecropia_ (L). Subsequent interest in juvenile hormones has led to the isolation and identification of juvenile hormones from many insect species, and later, to the synthesis of many juvenile hormone analogues (Mayer et al. 1973).

Juvenile hormones have been hailed as the new generation of pest control agents. According to Williams (1967), most insects on the earth are either neutral or beneficial to man, and only about 0.1% affect man adversely. These 3,000 or so species have caused man much grief and illness through the ages, and only recently has he learned to combat them chemically. In his categorization of these means of control, Williams (1967) has labeled chemicals such as kerosene (to coat ponds), arsenate of lead (to poison chewing insects), and nicotine and rotenone (to control sucking insects), as the first generation of pesticides. These chemicals are very general in their action, affecting many life forms adversely in addition to the insects they are intended to control.
The second generation of insect control agents began with the discovery of the insecticidal potential of DDT. With this find, many people felt that man's age old battle with the insect was conquered. Not only does DDT have a high toxicity against susceptible insects, but its mammalian toxicity is very low. But as with many good things, DDT was really a mixed blessing. In the environment, DDT is not easily degraded. This, coupled with its storage in fatty tissue, leads to environmental magnification of the substance as it moves up from one trophic level to another. In addition, insect resistance to DDT is quickly acquired, especially when it is used as the sole means of control. These problems led to a broadening of toxicological research to find new compounds with the combination of high insect toxicity, with lowered environmental impact. This search provided us with many useful organophosphates, carbamates, chlorinated hydrocarbons, pyrethrins, etc. that we have today. Some of these compounds are still very effective, but are increasingly plagued by the insects' ability to develop resistance to them. Also, a high degree of cross resistance occurs, even between such diverse compounds as DDT and some organophosphates. So, in a search for an ideal pest control substance, several workers began looking for the "Achilles' heel" of an insect's life and development to attack for successful control.
Williams (1967) states that the first and second generation methods of pest control have served their purposes and can still be used in integrated pest management, but points out that a new way is needed. Third generation control agents should not only be environmentally safe and specific in their effect, but should retain their effectiveness over an indefinite number of generations without any appreciable build up of resistance against them by the insect populations they are used to control.

During the late 1960's, it appeared, through studies of insect physiology, that the prime candidates for this class of control agents were the juvenile hormones. Williams (1967) reasoned that insects cannot readily evolve resistance to juvenile hormones, or an insensitivity to their own hormones without automatically committing suicide.

Relatively recently, however, a number of workers have found that a good deal of resistance to juvenile hormone analogues does occur (Dyte, 1972; Cerf and Georgiou, 1972; Piapp and Vinson, 1973). Apparently the same multifunction oxidase enzymes which are produced to degrade many of the second generation pesticides, also increase the rate of juvenile hormone metabolism or deactivation. This phenomenon is known as cross resistance, and has been reported for many organophosphate and carbamate strains of insects, such as the cross resistant strain of T. castaneum reported by Dyte (1972). Indeed, compounds such as piperonyl
butoxide and propyl 2-propylphenylphosphonate, which synergize organophosphate and carbamate insecticides by inhibiting the multi-function oxidase enzymes, have been shown to produce juvenile hormone effects on developing larvae (Slade and Wilkinson, 1973), and could possibly synergize the effects of juvenile hormone analogues (Cerf and Georghiou, 1972). The apparent juvenile hormone analogue activity of the synergist could occur because the degradative enzymes are inhibited from metabolizing the insect's own hormones at times when the titer of these hormones should be at a low level (Mayer et al. 1973; Terriere and Yu, 1973).

Although a considerable amount of work has been expended to elucidate the mode of action of juvenile hormones, the specific mechanism has not yet been worked out. Schneiderman (1972) has reviewed the actions of the three main insect morphogenetic hormones: the brain hormone, ecdysone, and juvenile hormone. He states that brain hormone is secreted from neurosecretory cells in the protocerebrum, and this activates the prothoracic glands to secrete either the actual molting hormone, ecdysone, or a precursor of it. The type of molt, either larval, pupal, or adult, is controlled by the titer of juvenile hormone in the haemolymph. The level of this hormone is controlled by its rate of secretion from the corpora allata, coupled to its rate of degradation by
juvenile hormone metabolizing enzymes.

The effects of juvenile hormone on the developing insect may be divided into three categories: morphogenetic, prothoracotrophic, and gonadotrophic (Judy, 1974). The morphogenetic effects include conservation of larval features and the suppression of metamorphosis at the time of molting. Thus, an excess of juvenile hormone will lead to extra (supernumerary) larval molts, or, when applied at just the right time, may lead to the formation of larval-pupal, or pupal-adult intermediates. Under normal circumstances, juvenile hormone is not present during embryogenesis, but when it is applied externally, the prothoracotrophic effects appear. Apparently juvenile hormone interferes with the genetic programming of tissues, thus upsetting normal developmental patterns, and resulting in either early embryonic death or the formation of freakish larvae. The gonadotrophic effects of juvenile hormone include stimulating protein synthesis in the yolk by the fat body, and stimulating protein uptake in the yolk by developing oocytes.

Williams and Kafatos (1972) have suggested that juvenile hormones participate in the corepression of three hypothetical "master regulatory genes". Each of these genes positively codes for a specific RNA polymerase for a specific regulator substance or sigma factor which in turn stimulates the action of a particular part of the
genome, be it larval, pupal, or adult. For this system to operate successfully, the larval master regulatory gene would need to function at any titer of juvenile hormone, the pupal master regulatory gene would need to function at a very low titer of juvenile hormone, and the adult master regulatory gene could only function when no juvenile hormone is present. These hormone levels correspond with those reported for each stage of morphogenetic development. As each part of the genome begins to function, it produces an inhibitor substance for the other two parts of the genome. Thus this model is one of positive control of successive gene sets by the master regulatory genes, which are themselves corepressed under juvenile hormone influenced negative control.

The mechanism by which juvenile hormones affect the genome is still under question. It has been shown that juvenile hormones interact with intracellular membranes, causing changes in their ionic charge balance. Baumann (1968) detected depolarizing effects on intracellular membranes when treated with juvenile hormones. Furthermore, Leuzzi and Gilbert (1969; 1970) showed the presence of a juvenile hormone specific chromosomal puff which responded to changes in the sodium ion concentration in the nuclear environment. Lemeunier (1973) has shown that various insect hormones induce chromosomal puffing, and that the quantity of protein in the region of the puff is
proportional to the size of the puff and to the degree of membrane depolarization. The case for membrane depolarization causing DNA transcriptional changes is strengthened by Cohen and Gilbert (1973), who have shown that polyribosomes which have been induced to produce protein in cells treated with ecdysone, will disaggregate and cease protein production upon the introduction of juvenile hormone. This phenomenon will not occur in cells whose nuclear membrane has been damaged and is leaky.

Whether the actual mechanism by which juvenile hormones affect the genome is by ionic messengers, or some other means, Miller and Collins (1975) have shown that the addition of Altosid, a juvenile hormone analogue, to differentiating larval cells causes a marked increase in the uptake of high molecular weight RNA precursors by the nucleolus. This effectively limits the rate at which mRNA can be formed, and therefore ultimately slows the rate of protein synthesis, upsetting the process of cell differentiation.

During an organism's development, the cells of various tissues often mature at different rates. It is possible that each cell limits the amount of juvenile hormone in its cytoplasm to control its own rate of maturation. Kramer et al. (1974) have found that juvenile hormone quickly complexes with a soluble binding protein in the insect's haemolymph. This binding is by a simple
thermodynamic equilibrium. The binding protein serves as a juvenile hormone carrier and inhibits the actions of juvenile hormone hydrolyzing enzymes. Ferkovich et al. (1975) have identified a similar carrier protein whose molecular weight is approximately $2.5 \times 10^4$ grams per mole. They also found that lipoproteins will bind excess juvenile hormone, but do not function as carriers. Apparently the carrier proteins are selectively bound to the plasmalemma of cells not undergoing differentiation. Schmialek et al. (1973) found that slowly differentiating cells attract a higher titer of juvenile hormone than rapidly differentiating cells. Since most cells are bathed in haemolymph containing approximately equal amounts of the hormone, a specific mechanism for juvenile hormone attraction to the cell membrane seems likely.

**METHODS AND MATERIALS**

In this experiment, four species of flour beetles, *Tribolium castaneum*, *T. confusum*, *T. audax*, and *T. madens*, were tested for changes in their morphogenetic development when exposed to the juvenile hormone analogue, isopropyl-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (Altosid®). The original stocks of beetles, supplied by the *Tribolium* Stock Center at the California State College in San Bernardino, were not selected for any resistance to insecticides or juvenile hormone analogues. All *Tribolium*
growth medium (19 parts whole wheat flour : 1 part brewers' yeast, by weight) was supplied by the same stock center.

Treatment of Growth Medium

In order to test the effects of Altosid on the beetles' development, the beetles were raised in growth medium containing various amounts of the juvenile hormone analogue. Acetone was used as a solvent to dilute the Altosid so that one milliliter of solution would contain enough Altosid to treat fifty grams of growth medium, yielding a treated growth medium with a specific Altosid concentration.

The mixing of each Altosid-acetone solution with the growth medium was accomplished by spreading fifty grams of growth medium a quarter inch deep on aluminum foil, and depositing, dropwise over the entire growth medium surface, one milliliter of the appropriate Altosid-acetone solution. After a ten minute drying period to allow the acetone to evaporate from the growth medium, the medium was mixed, first with a spatula and then by rolling in a glass container for ten minutes.

Effective Altosid concentrations for use against *T. castaneum* and *T. confusum* have been roughly determined by Taylor (1975). The concentrations tested in this project were chosen using that previous work as a guide. For *T. castaneum*, the concentrations tested were: 0.030, 0.035, 0.040, 0.045, 0.050, 0.055, 0.060, 0.070, 0.080, 0.090, and 0.100 parts per million. For *T. confusum*, they were: 0.100, 0.150, 0.200, 0.250, 0.300, 0.400, 0.600, 0.800,
1,000, 1,500, and 2,000 parts per million. The range of Altosid concentrations tested on *T. audax* and *T. madens* extended over the combined ranges of *T. castaneum* and *T. confusum*: 0.030, 0.040, 0.050, 0.060, 0.080, 0.100, 0.150, 0.200, 0.400, 0.800, and 1.500 parts per million.

Controls for all four species included growth medium treated with acetone only and growth medium with no treatment at all. Very little difference in effect on the beetles' development was noted between these two controls.

Treatment of all growth medium was done in one day. After treatment and mixing, the medium was added to one dram vials, approximately one and one-half grams per vial. These vials were stoppered with perforated plastic caps (to allow respiratory gas exchange) and were stored in the dark at 90 degrees Fahrenheit and 60 to 70 per-cent humidity until use.

**Rearing Experiment**

Ten replications were run per species, each starting on successive alternate days following the day of growth medium treatment. Ten first instar larvae were placed into each vial. Collection of the first instar larvae from stock cultures was accomplished by means of an aspirator into a glass vial after the larvae had been separated from their medium by sifting.

After a two week growth period, every vial was
inspected to determine the number of larvae which had survived the initial handling. This count established the number of organisms per vial to be used in future calculations. At this point, the treated medium did not appear to have adversely affected the growth or viability of the beetles. Thereafter, every vial was periodically checked at two to three week intervals and the total numbers of living larvae, pupae and adults were noted. This procedure was continued until all of the organisms in a given vial had either emerged as adults or were dead. The last readings were taken after ten weeks of developmental time. Throughout the experiment, all vials were maintained in the dark at 90 degrees Fahrenheit and 60 to 70 per-cent humidity.

RESULTS AND CALCULATIONS

The results of this project are summarized in Table I. The LD$_5$, LD$_{50}$, and LD$_{95}$ values given for each species are in parts per million of Altosid contained in the growth medium of the flour beetles. A composite of the calculated dosage-mortality plots is given in Figure I. Table II gives the fiducial limits in probits at the 0.05 probability level for each of the dosage-mortality values given in Table I. For each species, the fiducial limits at the LD$_{50}$ level are below 1.00, thus indicating good accuracy at this most important point.
Calculation of the dosage-mortality points and of the fiducial limits were accomplished by using a procedure outlined by Finney (1952). Tables III through VI show a summary of the data collected in this project and the calculations used in the probit analysis. Starting from the left, the first column shows the dosage of Altosid in parts per million contained in the beetles' growth medium. The second column gives the observed kill over the total sample size for each dosage. The total sample size was determined during the first inspection after two weeks of larval development. The per-cent kill is determined by dividing the observed kill by the total sample and multiplying by one-hundred. The net per-cent kill was determined by using Abbott's formula (1925):

\[ \text{Net \% Kill} = \left( \frac{\% \text{ mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \right) \times 100 \]

The log of dosage was determined so that all values were positive. To achieve this, the dosages were first multiplied by either 10 or 100. The empirical probit values corresponding to the values for net per-cent kill are recorded from a table of probits by Finney (1947). Only doses providing between twenty and ninety-five per-cent mortality were used from here on in the calculations.

At this point, the experimental plots shown in Figures II, III, IV, and V were drawn, with the empirical
probits as the ordinate, plotted against the logarithms as the abscissa. Units were chosen for each graph in order to use as much of the graph sheet as possible. From the points available, eye-fitted lines were drawn, with the greatest weight being given to points nearest probit five. From these provisional lines, the provisional probits were determined and entered in the seventh column.

Column eight shows weighting coefficients. These were determined from the provisional probits by using a table given in Finney (1947). These figures represent the certainty with which probit values can be determined at the different mortality levels when equal sample sizes are used. Since the certainty of a given point increases with the sample size, and is directly proportional to it, column nine shows sample factors for each point. These factors were obtained by dividing all sample sizes by the smallest sample in each set of data. Column ten shows the weights which are the products of the weighting coefficients and the sample factors. Columns eleven and twelve show the products of the weights and log dosage and the weights and empirical probits.

The second page of Tables III, IV, V, and VI shows most of the calculations involved in probit analysis leading to the determination of a calculated dosage-mortality line. \( \bar{x} \) and \( \bar{y} \) are the means of concentration and mortality respectively. The slope of the calculated dosage-mortality
curve, \( b \), is equal to the number of mortality units per unit of concentration increase. \( Y \) equals the mortality, in probits, on the calculated dosage-mortality curve corresponding to any chosen value of \( x \) (dose). These values of \( x \) and \( y \) were then used to determine the slope and position of the calculated dosage-mortality plots shown in Figures II, III, IV and V.

An experimental \( \chi^2 \) was then calculated for the data by using the equation shown. Normally, a line has as many degrees of freedom as there are points upon which it depends. However, in this case, two degrees of freedom are lost in the process of fixing the slope and position of the calculated line, so the degrees of freedom equal \( n - 2 \). This figure was then used at the 0.05 probability level to determine a theoretical \( \chi^2 \) value from the table given by Finney (1947). The theoretical \( \chi^2 \) value represents the value which could be expected from chance variation in a homogeneous population. So, as long as the theoretical value is greater than the calculated experimental value, the data may be considered homogeneous with the calculated line.

Next, the variance (\( V \)) of \( a \) (position) and \( b \) (slope) were calculated using the calculated \( \chi^2 \) value and the equations shown (\( n = \text{Degrees of Freedom} \)).

Lastly, the fiducial limits at the \( \text{LD}_5 \), \( \text{LD}_{50} \), and \( \text{LD}_{95} \) levels were determined using the formula shown at the
top of Table II. The values for $t$ were chosen from a table of values given by Finney (1947).
Table I. Effective dosages of Altosid *

<table>
<thead>
<tr>
<th>Species</th>
<th>LD&lt;sub&gt;5&lt;/sub&gt;</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>LD&lt;sub&gt;95&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. castaneum</td>
<td>0.011</td>
<td>0.026</td>
<td>0.061</td>
</tr>
<tr>
<td>T. confusum</td>
<td>0.043</td>
<td>0.194</td>
<td>0.873</td>
</tr>
<tr>
<td>T. audax</td>
<td>0.010</td>
<td>0.029</td>
<td>0.084</td>
</tr>
<tr>
<td>T. madens</td>
<td>0.020</td>
<td>0.043</td>
<td>0.091</td>
</tr>
</tbody>
</table>

* Concentrations in parts per million of Altosid contained in nutrient-growth medium
Table II. Determination of fiducial limits at LD₅, LD₅₀, and LD₉₅ levels

\[ Y = \bar{y} + b(X-\bar{x}) \pm t\sqrt{Va + (X-\bar{x})^2Vb}, \quad p = 0.05 \]
\[ x = \log \text{Dosage (X 100)} \]

<table>
<thead>
<tr>
<th>Species</th>
<th>LD₉₅ @ y = 6.64, x = 0.787</th>
<th>y = 6.64 ± 0.428</th>
<th>LD₅₀ @ y = 5.00, x = 0.411</th>
<th>y = 5.00 ± 0.477</th>
<th>LD₅ @ y = 3.36, x = 0.035</th>
<th>y = 3.36 ± 1.245</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. castaneum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. confusum</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>T. audax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. madens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LD₉₅ @ y = 6.64, x = 0.924, y = 6.64 ± 0.802
LD₅₀ @ y = 5.00, x = 0.465, y = 5.00 ± 0.625
LD₅ @ y = 3.36, x = 0.005, y = 3.36 ± 1.777

LD₉₅ @ y = 6.64, x = 0.961, y = 6.64 ± 1.199
LD₅₀ @ y = 5.00, x = 0.631, y = 5.00 ± 0.616
LD₅ @ y = 3.36, x = 0.300, y = 3.36 ± 1.602
Figure I. Composite of calculated dosage-mortality plots

T. castaneum

T. confusum

T. audax

T. madens
<table>
<thead>
<tr>
<th>Dosage (µm)</th>
<th>Observed</th>
<th>% Kill</th>
<th>% Net</th>
<th>Log of Dosage (X 100)</th>
<th>Probits</th>
<th>Probits</th>
<th>Weighting</th>
<th>Sample Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Total)</td>
<td>Kill</td>
<td>Kill</td>
<td>(x)</td>
<td>(y)</td>
<td>Probits</td>
<td>Coefficients</td>
<td>Factor</td>
</tr>
<tr>
<td>Pure</td>
<td>31/91</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone (0.000)</td>
<td>35/88</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0.030</td>
<td>65/88</td>
<td>72</td>
<td>53.3</td>
<td>0.477</td>
<td>5.083</td>
<td>5.25</td>
<td>0.622</td>
<td>1.023</td>
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<tr>
<td>0.035</td>
<td>73/86</td>
<td>86</td>
<td>75.0</td>
<td>0.544</td>
<td>5.675</td>
<td>5.55</td>
<td>0.570</td>
<td>1.000</td>
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<tr>
<td>0.040</td>
<td>81/87</td>
<td>93</td>
<td>88.3</td>
<td>0.602</td>
<td>6.190</td>
<td>5.80</td>
<td>0.503</td>
<td>1.012</td>
</tr>
<tr>
<td>0.045</td>
<td>80/31</td>
<td>88</td>
<td>80.0</td>
<td>0.653</td>
<td>5.842</td>
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<td>88/92</td>
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<td>93.3</td>
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<td>70/86</td>
<td>91</td>
<td>88.0</td>
<td>0.741</td>
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<td>6.43</td>
<td>0.292</td>
<td>1.000</td>
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<tr>
<td>0.060</td>
<td>87/90</td>
<td>97</td>
<td>95.0</td>
<td>0.778</td>
<td>6.645</td>
<td>6.59</td>
<td>0.241</td>
<td>1.050</td>
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<td>81/82</td>
<td>98</td>
<td>98.3</td>
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<td>92/92</td>
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<td>0.903</td>
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<tr>
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<td>83/83</td>
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<td>0.100</td>
<td>86/86</td>
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<td>100.0</td>
<td>1.000</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table III. continued

\[ \Sigma (w) = 3.099 \]
\[ \Sigma (wx) = 1.897 \]
\[ \bar{x} = \frac{(wx)}{(w)} = 0.612 \]
\[ \Sigma (wy) = 18.217 \]
\[ \bar{y} = \frac{(wy)}{(w)} = 5.878 \]
\[ \Sigma (wx^2) = 1.192 \]
\[ A = (wx^2) - \bar{x} (wx) = 0.031 \]
\[ \Sigma (wxy) = 11.284 \]
\[ b = \frac{(wxy) - \bar{x} (wy)}{A} = 4.361 \]
\[ Y = \bar{y} + b(X-\bar{x}) = 3.209 + 4.361x \]
\[ @ x = 0.5, \ y = 5.390 \]
\[ @ x = 0.6, \ y = 5.826 \]
\[ @ x = 1.0, \ y = 7.570 \]
\[ @ x = 0.8, \ y = 6.698 \]
\[ \Sigma (wy^2) = 107.862 \]
\[ \chi^2 = (wy^2) - \bar{y} (wy) (wxy) - \bar{x} (wy) \]
\[ \chi^2 = 0.106 \text{ (experimental)} \]
\[ \chi^2 = 11.07 \text{ (theoretical), } p = 0.05, \text{ df } = 5 \]
\[ V(a) = \frac{\chi^2}{n (w)} = 0.00684 \]
\[ V(b) = \frac{\chi^2}{nA} = 0.684 \]
\[ t = 2.57 \quad \text{df } = 5 \]
Figure II. *T. castaneum*: dosage-mortality plot

Experimental

Calculated
### Table IV.

**T. confusum**  
Dosage-Mortality Calculation Worksheet

<table>
<thead>
<tr>
<th>Dosage (ppm)</th>
<th>Observed Kill</th>
<th>% Kill</th>
<th>Net Log of Dosage (x10)</th>
<th>Empirical Probits (x)</th>
<th>Provisonal Probits (y)</th>
<th>Weighting Coefficients (w)</th>
<th>Sample Factor</th>
<th>Total Weight (wx)</th>
<th>(wy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Medium</td>
<td>11/95</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone (0.000)</td>
<td>15/92</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100</td>
<td>21/95</td>
<td>22</td>
<td>7.1</td>
<td>0.000</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.150</td>
<td>26/98</td>
<td>27</td>
<td>13.1</td>
<td>0.176</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.200</td>
<td>49/96</td>
<td>51</td>
<td>41.7</td>
<td>0.301</td>
<td>4.790</td>
<td>4.75</td>
<td>0.622</td>
<td>1.032</td>
<td>0.193</td>
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</tr>
<tr>
<td>0.250</td>
<td>58/95</td>
<td>61</td>
<td>53.6</td>
<td>0.398</td>
<td>5.090</td>
<td>5.17</td>
<td>0.630</td>
<td>1.022</td>
<td>0.256</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.300</td>
<td>73/98</td>
<td>74</td>
<td>69.0</td>
<td>0.477</td>
<td>5.496</td>
<td>5.50</td>
<td>0.581</td>
<td>1.054</td>
<td>0.292</td>
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<td></td>
</tr>
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<td>78/98</td>
<td>80</td>
<td>76.2</td>
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<td>5.713</td>
<td>6.06</td>
<td>0.418</td>
<td>1.054</td>
<td>0.265</td>
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<td></td>
</tr>
<tr>
<td>0.500</td>
<td>91/93</td>
<td>98</td>
<td>97.6</td>
<td>0.778</td>
<td>6.977</td>
<td>6.78</td>
<td>0.185</td>
<td>1.000</td>
<td>0.144</td>
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<tr>
<td>1.500</td>
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<td>95</td>
<td>94.0</td>
<td>1.176</td>
<td></td>
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<td>1.301</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25
Table IV. continued

\[ \xi (w) = 2.524 \]
\[ \xi (wx) = 1.150 \]
\[ \bar{x} = \frac{\langle (\text{wx}) \rangle}{\langle (w) \rangle} = 0.456 \]
\[ \xi (wy) = 13.682 \]
\[ \bar{y} = \frac{\langle (\text{wy}) \rangle}{\langle (w) \rangle} = 5.421 \]
\[ \xi (wx^2) = 0.571 \]
\[ A = (\langle wx^2 \rangle) - \bar{x} \langle \text{wx} \rangle = 0.047 \]
\[ \xi (wxy) = 6.357 \]
\[ b = \frac{\langle (\text{wxy}) \rangle - \bar{x} \langle \text{wy} \rangle}{A} = 2.511 \]
\[ Y = \bar{y} + b(X-\bar{x}) = 4.276 + 2.511x \]
\[ @ x = 0.2, \quad y = 4.778 \]
\[ @ x = 0.3, \quad y = 5.078 \]
\[ @ x = 0.8, \quad y = 6.285 \]
\[ \xi (wy^2) = 74.196 \]
\[ \chi^2 = (\langle wy^2 \rangle) - \bar{y} \langle \text{wy} \rangle (\langle wxy \rangle) - \bar{x} \langle \text{wy} \rangle \]
\[ \chi^2 = 0.00306 \text{ (experimental)} \]
\[ \chi^2 = 7.81 \text{ (theoretical), } p = 0.05, \text{ df } = 3 = n \]
\[ V(a) = \frac{\chi^2}{n \langle (w) \rangle} = 0.000404 \]
\[ V(b) = \frac{\chi^2}{nA} = 0.0217 \]
\[ t = 3.18 \quad \text{df } = 3 = n \]
Figure III. *T. confusum*: dosage-mortality plot

Experimental

Calculated
<table>
<thead>
<tr>
<th>Dosage (ppm)</th>
<th>Observed Kill</th>
<th>% Net Kill</th>
<th>Log of Empirical Probits</th>
<th>Probits Coefficients</th>
<th>Weighting Sample Factor</th>
<th>Sample Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Medium</td>
<td>20/91</td>
<td>22</td>
<td>52.7</td>
<td>5.068</td>
<td>5.07</td>
<td>1.000</td>
</tr>
<tr>
<td>Acetone (0.000)</td>
<td>24/93</td>
<td>26</td>
<td>52.7</td>
<td>5.418</td>
<td>5.50</td>
<td>1.000</td>
</tr>
<tr>
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<td>52/80</td>
<td>65</td>
<td>52.7</td>
<td>5.068</td>
<td>5.07</td>
<td>0.635</td>
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<td>74/99</td>
<td>75</td>
<td>66.2</td>
<td>5.418</td>
<td>5.50</td>
<td>0.581</td>
</tr>
<tr>
<td>0.050</td>
<td>81/94</td>
<td>86</td>
<td>81.1</td>
<td>5.822</td>
<td>5.83</td>
<td>0.493</td>
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<td>89/98</td>
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<td>87.8</td>
<td>6.165</td>
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<td>0.405</td>
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<td>0.080</td>
<td>81/85</td>
<td>95</td>
<td>93.2</td>
<td>6.491</td>
<td>6.53</td>
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<tr>
<td>0.100</td>
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<td>100</td>
<td>100.0</td>
<td>6.491</td>
<td>6.53</td>
<td>0.259</td>
</tr>
<tr>
<td>0.150</td>
<td>87/87</td>
<td>100</td>
<td>100.0</td>
<td>6.491</td>
<td>6.53</td>
<td>0.259</td>
</tr>
<tr>
<td>0.200</td>
<td>79/81</td>
<td>98</td>
<td>97.3</td>
<td>1.301</td>
<td>1.301</td>
<td>0.248</td>
</tr>
<tr>
<td>0.400</td>
<td>97/97</td>
<td>100</td>
<td>100.0</td>
<td>1.602</td>
<td>1.602</td>
<td>0.248</td>
</tr>
<tr>
<td>0.800</td>
<td>88/91</td>
<td>97</td>
<td>95.9</td>
<td>1.904</td>
<td>1.904</td>
<td>0.248</td>
</tr>
<tr>
<td>1.500</td>
<td>90/92</td>
<td>98</td>
<td>97.3</td>
<td>2.176</td>
<td>2.176</td>
<td>0.248</td>
</tr>
</tbody>
</table>
Table V. continued

\[ \bar{x} = \frac{\zeta(wx)}{(w)} = 0.656 \]

\[ \bar{y} = \frac{\zeta(wy)}{(w)} = 5.682 \]

\[ \zeta(wx^2) = 1.213 \]

\[ A = (wx^2) - \bar{x} (wx) = 0.049 \]

\[ b = \frac{(wxy) - \bar{x} (wy)}{A} = 3.569 \]

\[ Y = \bar{y} + b(X-\bar{x}) = 3.341 + 3.569x \]

@ \( x = 0.5 \), \( y = 5.126 \)

@ \( x = 1.0 \), \( y = 6.910 \)

\[ \zeta(wy^2) = 87.890 \]

\[ X^2 = (wy^2) - \bar{y} (wy) (wxy) - \bar{x} (wy) \]

\[ X^2 = 0.104 \text{ (experimental)} \]

\[ X^2 = 7.81 \text{ (theoretical)}, \ p = 0.05, \ df = 3 = n \]

\[ V(a) = \frac{X^2}{n(w)} = 0.0128 \]

\[ V(b) = \frac{X^2}{nA} = 0.707 \]

\[ t = 3.28 \quad df = 3 \]
Figure IV. *T. audax*: dosage-mortality plot

Experimental __________

Calculated ___ ___ ___
<table>
<thead>
<tr>
<th>Dosage (ppm)</th>
<th>Observed Kill Total Sample</th>
<th>% Kill</th>
<th>% Net Log of Empirical Probits</th>
<th>Provisionsal Coefficients</th>
<th>Weighting Factor</th>
<th>Sample Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(x)</td>
<td>(y)</td>
<td></td>
<td>(w)</td>
<td>(wx)</td>
</tr>
<tr>
<td>Pure Medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone (0.000)</td>
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<td>9/96</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.030</td>
<td>26/92</td>
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<td>20.9</td>
<td>0.477</td>
<td>4.190</td>
<td>4.25</td>
</tr>
<tr>
<td>0.040</td>
<td>49/94</td>
<td>52</td>
<td>47.3</td>
<td>0.602</td>
<td>4.932</td>
<td>4.88</td>
</tr>
<tr>
<td>0.050</td>
<td>60/92</td>
<td>65</td>
<td>61.5</td>
<td>0.699</td>
<td>5.292</td>
<td>5.38</td>
</tr>
<tr>
<td>0.060</td>
<td>75/95</td>
<td>79</td>
<td>76.9</td>
<td>0.778</td>
<td>5.736</td>
<td>5.79</td>
</tr>
<tr>
<td>0.080</td>
<td>86/94</td>
<td>91</td>
<td>90.1</td>
<td>0.903</td>
<td>6.287</td>
<td>6.43</td>
</tr>
<tr>
<td>0.100</td>
<td>92/95</td>
<td>97</td>
<td>97.7</td>
<td>1.000</td>
<td>6.995</td>
<td>6.94</td>
</tr>
<tr>
<td>0.150</td>
<td>94/94</td>
<td>100</td>
<td>100.0</td>
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</tr>
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</tr>
<tr>
<td>0.400</td>
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<td>0.600</td>
<td>97/97</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1.500</td>
<td>97/97</td>
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<td>100.0</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**TABLE VI.**

_T. madana_

Dosage-Mortality Calculation Worksheet
Table VI. continued

\[ \xi (w) = 2.739 \]
\[ \xi (wx) = 1.884 \]
\[ \ddot{x} = \frac{(wx)}{(w)} = 0.688 \]
\[ \xi (wy) = 14.476 \]
\[ \ddot{y} = \frac{(wy)}{(w)} = 5.285 \]
\[ \xi (wx^2) = 1.357 \]
\[ A = (wx^2) - \ddot{x} (wx) = 0.061 \]
\[ \xi (wxy) = 10.262 \]
\[ b = \frac{(wxy) - \ddot{x} (wy)}{A} = 4.959 \]

\[ Y = \ddot{y} + b(X-\ddot{x}) = 1.873 + 4.959x \]

@ \( x = 0.5, \quad y = 4.353 \)

@ \( x = 1.0, \quad y = 6.832 \)

\[ \xi (wy^2) = 78.054 \]

\[ \chi^2 = (wy^2) - \ddot{y} (wy) (wxy) - \ddot{x} (wy) \]

\[ \chi^2 = 0.469 \text{ (experimental)} \]

\[ \chi^2 = 9.49 \text{ (theoretical), } p = 0.05, \text{ df = 4} \]

\[ V(a) = \frac{\chi^2}{n (w)} = 0.0428 \]

\[ V(b) = \frac{\chi^2}{nA} = 1.922 \]

\[ t = 2.78 \quad \text{df = 4} \]
Figure V. *T. madens*: dosage-mortality plot

Experimental  

Calculated  


DISCUSSION

As the data show, *T. castaneum*, *T. confusum*, *T. audax*, and *T. madens* are all strongly affected by very small concentrations of the juvenile hormone analogue, Altosid. It was interesting to note that most of the larvae of all four species successfully pupated. The highest mortality occurred either before or during the pupal-adult molt. The pupal-adult intermediates ranged from partially emerged adults to successfully emerged but grossly misshapen adults which quickly died.

Successful development of the most resistant of the four species to the compound, *T. confusum*, is effectively limited at Altosid concentrations well below one part per million, and the other three species are effectively limited at Altosid concentrations of less than one-tenth of a part per million. These figures are well below the standard screening dosages used by Strong and Diekman (1973), who stated that *T. castaneum* and *T. confusum* are strongly affected by Altosid at five parts per million.

The effectiveness of Altosid as a control agent for the four species of flour beetles tested is seen when the data presented here are compared to those presented by Dyte (1972), who used a similar juvenile hormone analogue, methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate in tests on *T. castaneum*. This compound was shown to exert little or no morphogenetic effects at
0.3 parts per million. Unfortunately, due to the nature of his study, Dyte did not determine the lowest effective dosage for the compound. But in a study of many insect growth regulating substances, Henrick et al. (1973) determined that the compound used by Dyte was ten times less morphogenetically active than Altosid.

Another study which used Altosid on T. castaneum shows results quite different from those given here. Williams and Amos (1974) state that Altosid at five parts per million does not affect development of the beetles from the egg to the adult stage. Due to differences in experimental technique, however, their work cannot be directly compared with the data presented in this paper. Williams and Amos (1974) treated whole grain wheat kernels with Altosid, and then ground them into flour. Because of this, they must have lost a lot of the juvenile hormone activity of the compound by the time the medium was ready for the introduction of the beetles.

Table I shows that T. confusum is considerably more resistant to the effects of Altosid than the other three species tested. A possible explanation for this phenomenon is elucidated when one looks into the taxonomy of the genus Tribolium. T. castaneum, T. audax, and T. madens are very closely related species, whereas T. confusum is only a distant relation (Sokoloff, 1972). T. audax and T. madens are so closely related that
hybridization between these two can produce viable, but sterile offspring (Halstead, 1969).

These genetic relationships are substantiated by many of the physiological differences which have been found to exist between T. castaneum and T. confusum. In regards to this paper, one of the most significant of these differences lies in the beetles' gut pH. The foregut pH and midgut pH to T. castaneum are 5.2 and 7.2-7.6 respectively. For T. confusum, these figures are 4.6-5.2, and 5.2-6.8 respectively (Sokoloff, 1974). The more acetic gut pH of T. confusum would not only foster a higher rate of Altosid hydrolysis, but probably reflects differences in the digestive enzymes present. Although this work on gut pH was done on adult beetles, it has been shown that the larvae of both species employ almost identical enzymes in both their foregut and midgut (Sokoloff, 1974). Therefore, the pH relationships between the larvae of the two species should be maintained.

CONCLUSIONS

Altosid has been shown to be an effective control agent when mixed in the nutrient growth medium of T. castaneum, T. confusum, T. audax, and T. madens. The very low dosages required for control of these stored grain products pests is even more appealing in light of the observation that juvenile hormones and many of their
analogues, including Altosid, have not been shown to have any deleterious effects on other animal groups, and are rather easily degraded in the environment. Vertebrate enzyme systems quickly degrade these compounds to harmless by-products (Williams, 1967; Staal, 1972; Schneiderman, 1972).
BIBLIOGRAPHY


