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DIFLUBENZURON INHIBITS CHITIN SYNTHESIS IN *CULEX PIPIENS* L. LARVAE

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ABSTRACT. Diflubenzuron (DFB) inhibits growth and development of *Culex pipiens* L. larvae at 4 ppb. With 42 hr exposure, a period allowing two molts, it causes a dose-dependent reduction in body weight and chitin content, such that at 9 ppb these are only 66 and 27% of control respectively. There is also a dose-dependent increase in the instar duration and mortality. Following [^{14}C]glucose feeding, DFB has no effect on the transport of [^{14}C] glucose and its [^{14}C] metabolites into the integument of fourth instar larvae, but causes a dose-dependent decrease in their incorporation into the newly-formed chitin.

INTRODUCTION

Diflubenzuron (DFB), 1-(2, 6-difluorobenzoyl)-3-(4-chlorophenyl) urea, is a potent insecticide that acts on a wide variety of insects species (Verloop and Ferrell,1977). It is, however, particularly effective against a number of medically important mosquito species at very low doses under field (0.025–0.05 lb/acre) and laboratory (0.4 ppb) conditions (Arias and Mulla 1975, Mulla and Darwazeh 1976). Recently we reported on the *in vitro* inhibition of chitin synthesis by DFB in *Oncopeltus fasciatus* abdomen systems and demonstrated that inhibition proceeds very rapidly without hormonal involvement. DFB blocks the terminal polymerization step in chitin formation (Hajjar and Casida 1978, 1979). The present paper investigates the *in vivo* inhibition of chitin synthesis by DFB in *Culex pipiens* L. larvae.

MATERIALS AND METHODS

Studies were conducted on larvae from an autogenous strain reared in this lab-

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oratory (Dadd 1970). Late 2nd instar larvae were collected from rearing pans and distributed into 100 ml polyethylene beakers. Each beaker contained 13–17 larvae, 50 ml of the test solutions and about 50 mg diet consisting of liver extract concentrate, yeast and rat diet (7:3:2). Larvae were maintained at 28± 1°C and test solutions were changed daily until pupation. At the concentrations tested DFB is water soluble. Mortality, pupation, and adult emergence were recorded daily. Chitin content was determined from a second set of larvae 42 hr following treatment, a period allowing larvae to molt twice and develop to the early 4th instar. Larvae were harvested, their dry weight determined after lyophilization and from the resulting powders chitin was extracted, purified (Ishaaya and Casida 1974) and weighed.

Radiolabelled investigations utilized 2nd instar larvae treated with DFB for 24 hr, during which period they normally molt and develop to late 3rd instar. Larvae were then transferred into staining dishes containing 4 ml of 0.06% agar solution, to promote larval feeding (Dadd, personal communication) and 5 μCi [^{14}C(U)] D-glucose (Specific activity 125 mCi/mmole, New England Nuclear Corp., Boston, Mass) and allowed to feed for 2.3 hr. Fed larvae were collected, washed and transferred into freshly prepared test solutions where they were kept for 10-12 hr to allow them to molt into the 4th instar. From each treatment larvae were then collected, weighed and separated into 2 groups to determine [^{14}C] incorporation into the newly formed integument and chitin. [^{14}C]Chitin was determined from 20–50 larvae as follows: larvae were homogenized and the total [^{14}C] intake determined in an aliquot by liquid scintillation counting for the whole homogenate, which was then centrifuged for 10 min at 12,000 g and 2°C. To the precipitate, 50 mg of chitin (practical grade, prewashed several times with water and dried) were added as carrier and [^{14}C]chitin extracted. The purified chitin was weighed (to correct for loss during the purification procedure of 30%), combusted and the [^{14}C] content determined by liquid scintillation counting. Incorporation of [^{14}C] into the integument was determined from 10 larvae. Integuments were obtained by cutting off the heads and siphons and removing their alimentary canals and residual body fluids. The cuticles were weighed, combusted and the [^{14}C] content determined. The data are reported as the means with their standard errors. Results were analyzed by linear regression of the dependent variables studied, (e.g. % pupation, etc.) on ppb of DFB tested.

RESULTS AND DISCUSSION

DFB has a profound effect at very low concentration on the growth and development of *Cx. pipiens* larvae. It produces a dose-dependent reduction in normal pupation, adult emergence, larval dry weight and chitin content (Table 1). At 9 ppb there is a large decrease in chitin content so that only 27% of the control value is obtained for the exposure period which allowed 2 molts into the 4th instar. The physiological significance of this observation is more evident when the results are expressed as percent of larval dry weight, a value decreasing from 3 in control larvae to 1.2 at 9 ppb DFB is obtained. These results indicate that although larval weight decreased as a result of treatment, chitin content decreased at an even higher rate. Thus inhibition of chitin synthesis may be limiting larval development and causing eventual death. Similar results are reported for house fly (Ishaaya and Casida 1974) and *Oncopeltus fasciatus* (Hajjar and Casida 1979) immatures. There is also a dose-dependent increase in the time required for pupation and adult emergence (Table 1). Treated larvae do not grow to their normal size and die during the molt. In separate experiments DFB appears to exhibit a cumulative effect on continuous exposure, thus the extent of mortality increases with the number of molts.

Further evidence for chitin synthesis

Table 1. Development, mortality and chitin content of 2nd instar *Cx. pipiens* larvae exposed to DFB for 42 hr.

Measurement	DFB in media, ppb				Rate of change/ ppb inccrease of DFB
	0	4	6	9	
Pupation and emergence[a]					
Percent pupation	74±6	51±4	24±6	3±2	8.12±0.73
Days to pupation[b]	5.1±0.1	6.8±0.5	8.0±0.9	8.2±0.8	0.36±0.10
Percent emergence	67±7	32±5	11±4	1.1±0.8	7.58±0.73
Days to emergence[b]	6.8±0.2	8.6±0.5	8.9±1.2	9.5±1.5	0.30±0.15
Larval dry weight, μg[c]					
Initial, when added to media	35±1	35±1	35±1	35±1	—
After 42 hr in media	213±3	171±2	161±6	140±5	8.04±0.66
Chitin content[c]					
μg/larva	6.3±0.2	4.2±0.3	2.8±0.1	1.7±0.2	0.52±0.03
% of dry weight	3.0	2.5	1.7	1.2	—

[a] Ten replicates of 15 larvae each. Values reported are from the same experiments.
[b] Measured from the first instar.
[c] Four to six replicates of 100–150 larvae each.

inhibition by DFB is obtained from the radiolabelled studies. Incorporation of [^{14}C] from glucose into the chitin during molting from 3rd to 4th instar larvae is decreased in a dose-dependent manner by DFB (Table 2). When incorporation into chitin is expressed as pmoles glucose (and glucose equivalents), intake per larval fresh weight, a value decreasing progressively from 0.74 for the control to 0.54 at 9 ppb DFB, is obtained. However, total [^{14}C] from glucose intake into the integument is unaffected by either decrease in body weight or chitin content. Reduced [^{14}C]chitin biosynthesis following [^{14}C]glucose administration has been reported for *O. fasciatus* (Hajjar and Casida 1979) and *Pieris brassicae* L. (Deul et al. 1978) larvae.

It is therefore evident that the mode of action of DFB is similar in mosquito larvae and in other species, however, DFB is extremely more toxic to mosquito larvae.

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Table 2. Incorporation of [^{14}C]glucose and its metabolites into the integument and chitin of 3rd instar *Cx. pipiens* larvae exposed to DFB.

Measurement	DFB in media, ppb				Rate of change/ ppb increase of DFB
	0	4	6	9	
[^{14}C]*Glucose intake*, %					
In integument[a]	76.2±6.7	82.9±3.5	64.6±3.0	73.5±5.9	0.64±0.77
In chitin[b]	9.5±0.9	8.7±0.5	6.8±0.5	5.7±0.4	0.44±0.09
pMoles [^{14}C]*Glucose*					
Per larva[b]	7.2±0.6	6.8±0.6	7.8±0.6	7.3±0.8	—
In integument/mg integument	27.0	30.9	30.6	35.7	—
In chitin/mg larva	0.74	0.65	0.60	0.54	—

[a] Eight replicates of 10 larvae each.
[b] Eight replicates of 20–50 larvae each.

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OBITUARY

WILLIAM N. SULLIVAN

Bill Sullivan, whose work with Dr. Lyle Goodhue produced the aerosol bomb and thus changed the course of history not only in mosquito control but in world economics, died on 2 March 1979 at the Veterans Administration Hospital in Washington, D.C., of the effects of a brain tumor. He had retired last June from active work at the Beltsville Agricultural Research Center in Maryland, but had continued to serve as a consultant in the ongoing program of the U.S. Department of Agriculture to rid aircraft of hitch-hiking insects.

The invention of the aerosol method of insecticide dispersal, now in some disputed disfavor, was without doubt a major factor in the successful stemming of the insect vectors of disease in World War II and led to much of the methodology still used in insecticide dispersal today. In addition to honors from the World Health Organization, Dr. Sullivan had won international recognition for his work on chemical and biophysical control of insects and insect biorhythms.

Dr. Sullivan was born in Lawrence, Massachusetts and graduated from what is now the University of Massachusetts in Amherst, a school which was a pioneer in the teaching of Entomology and has remained one of the leading institutions in this subject. His doctorate was from the University of Tokyo.

Bill was a quiet and unassuming man but a friendly and warm associate and a good companion remembered with affection by those of us who worked with him in Beltsville or knew him in the Army during the War years and the early post-War years of the Occupation.

—AUSTIN W. MORRILL, JR.