issue. A number of lines of evidence indicate that NO_2^- was not being utilized during this period.

12. Direct evidence indicates that the St. Lawrence estuary [M. Sinclair, *J. Fish. Res. Board Can.* **35**, 1171 (1878)], Charleston Harbor [(T. A. Wastler and C. M. Walter, *J. Sanit. Eng. Div. Am. Soc. Cin. Eng.* **94**, SA6, 1175 (1968), paper 6311], and the Gironde estuary [G. P. Allen, *Bull. Inst. Geol. Bass-d'Aquitaine Mem. 5* (1973)] all undergo a similar cycle of stratification-destratification related to the neap-spring tidal cycle. Indirect evidence indicates that Puget Sound [D. F. Winter, K. Banse, G. C. Anderson, *Mar. Biol.* **29**, 139 (1975)] and Saanich Inlet [M. Takahashi, D. L. Seibert, W. H. Thomas, *Deep-Sea Res.* **24**, 775 (1977)] do also.

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Inhibition of Cellular Proliferation of Imaginal Epidermal Cells by Diflubenzuron in Pupae of the Stable Fly

Abstract. *A second mode of action has been found for the inhibition of chitin synthesis by diflubenzuron. This compound blocks synthesis of the imaginal cuticle by preventing formation of the adult epidermis in the pupal stage of the stable fly* (Stomoxys calcitrans *L.*).

One means of designing methods of insect control is to exploit the basic physiological and morphological differences between vertebrates and invertebrates. One of the basic differences is that vertebrates do not synthesize chitin, a compound found in some invertebrate phyla and a major component of insect cuticle. On the basis of this difference, a new class of insecticides that inhibits chitin synthesis appears promising. One of these insecticides, diflubenzuron [Dimilin; 1-(4-chlorophenyl)-3-(2,6-difluorbenzoyl)urea], prevents synthesis of chitin in both larval and adult insects (*1-3*). Since many of these studies were made on species of insects in which the larval epidermis persists throughout the life cycle, we undertook to determine the effect of diflubenzuron on chitin synthesis during the pupal stage of the stable fly (*Stomoxys calcitrans* L.). During this stage, the larval epidermis degenerates and is replaced by an imaginal (adult) epidermis, which then proceeds to synthesize the adult cuticle (*4, 5*). We found that diflubenzuron topically applied at the white prepupal stage prevented formation of the imaginal epidermis and thus the subsequent synthesis of adult cuticle.

To determine the effect of diflubenzuron on the chitin synthesis occurring in this type of transitional epidermis, we performed the following experiments. (i) Histological preparations were made of pupae topically treated with diflubenzuron to determine whether this compound had any effect on the morphology of the larval or adult epidermal cells and (ii) radioactively labeled *N*-acetyl-D-glucosamine (GlcNac) was used in a biochemical analysis to determine the extent of inhibition of chitin synthesis, if any, produced by diflubenzuron.

The histological study was performed

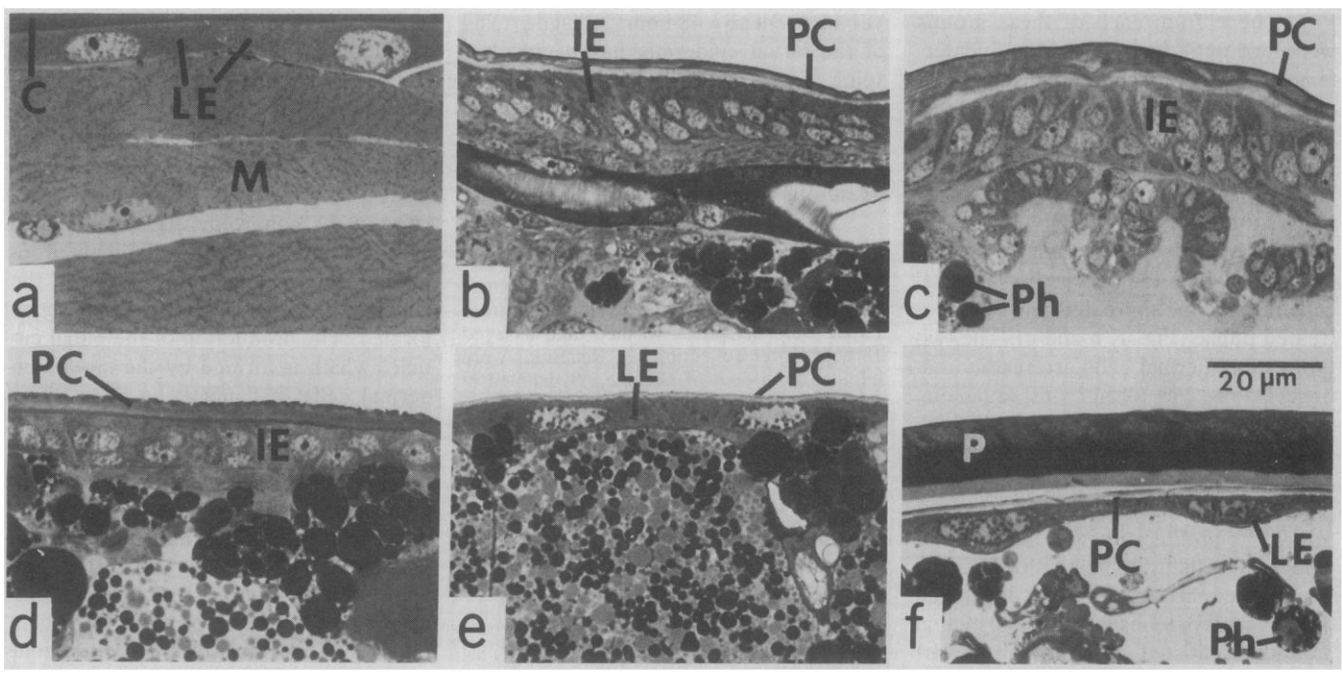

Fig. 1. (a) Transverse section through the integument of a white prepupal larva of *S. calcitrans*, the stage at which the various treatments were begun. The large squamous larval epidermal cells (*LE*) are still closely opposed to the thick larval cuticle that forms the puparium (*C*). Apolysis has not yet occurred nor has a pupal cuticle been secreted at this time (*M*, muscle). (b) Transverse section through epidermis of untreated pupa 24 hours after prepupal formation. The larval epidermis has been replaced by columnar, imaginal epidermal cells (*IE*) which is now overlain by the pupal cuticle (*PC*) formed by the larval epidermis. (c) Epidermis of a pupa 24 hours after treatment with acetone. The larval epidermis has been histolyzed and the imaginal epidermis has been formed (*Ph*, phagocyte). (d) Epidermis of a pupa 48 hours after treatment with acetone. The imaginal epidermal cells have lost their columnar appearance and have formed the cuboidal, monolayered adult epidermis. (e) Epidermis of a pupa 24 hours after treatment with diflubenzuron. The larval epidermis is still present. The pupal cuticle produced by these cells covers their apical surface. (f) Epidermis of a pupa 48 hours after treatment with diflubenzuron. No imaginal epidermis has formed. Histolysis of the larval epidermal cells has begun, resulting in loss of a large portion of the cytoplasm of these cells (*P*, puparium).

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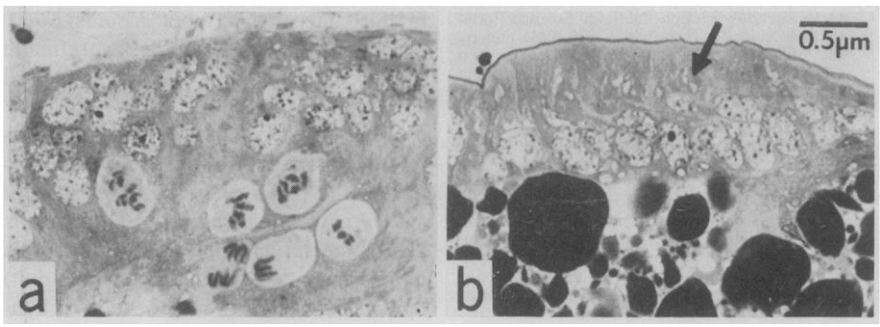

Fig. 2. A comparison of the imaginal epidermal histoblast regions of untreated pupae and pupae treated with diflubenzuron. (a) The histoblast region of a pupa treated with acetone. There is a high degree of mitotic activity. (b) Histoblast region of pupa 24 hours after treatment with diflubenzuron. No mitotic division is discernible, and cells are becoming vacuolated (arrow).

in three groups of laboratory-reared stable fly pupae. One group was treated topically at the white prepupal stage with 0.1 μg of diflubenzuron dissolved in 0.5 μl of acetone. A second group was treated topically with 0.5 μl of acetone at the same time. A third group was left untreated. Then they were held at 27°C in divided petri dishes containing glass wool saturated with water. At 24 and at 48 hours after pupation, 12 pupae from each group were transected, fixed in glutaraldehyde solution, and prepared for resin sectioning; sections (1 μm) were made with a diamond knife and stained with azure B-methylene blue. Fifty pupae from each of these groups were held until time of eclosion to determine whether this compound prevented emergence of viable adult insects.

At the white prepupal stage, the epidermis of the stable fly is composed of larval epidermal cells (Fig. 1a). By 24 hours, these larval cells have begun to undergo histolysis and phagocytosis to be replaced by the imaginal epidermal cells in both the untreated and acetone-treated pupae (Fig. 1, b and c). The two types of epidermal cells are readily distinguishable, the former are squamous cells, much larger (85 by 15 μm) than the columnar (16 by 8 μm) adult epidermal cells. As the larval epidermis is being phagocytized, the imaginal epidermis is formed by proliferation and migration of epidermal cells from clusters of imaginal hypodermal histoblasts that developed during embryogenesis and persisted in an undifferentiated state through the larval instars (Fig. 2, a and b). During this period of cell proliferation, mitotic figures are readily discernible in the clusters of epidermal histoblasts in both the untreated and acetone-treated pupae (Fig. 2a). By 48 hours the imaginal epidermis has completely replaced the larval epidermis and the imaginal cells have become cuboidal and approximately 8 μm on a side (Fig. 1d). This newly formed epidermis is enclosed by a pupal cuticle (Fig. 1, b to f) produced by the larval epidermis just prior to its degeneration.

By 24 hours, the larval epidermis of the prepupae treated with diflubenzuron has secreted a pupal cuticle (Fig. 1e) as did the control groups. But these larval cells are still the only type of epidermal cells present in the integument. Although some of these cells have become vacuolated, many appear robust, with no sign of degeneration, and the plasma and nuclear membranes are still intact. No phagocytosis of the larval epidermis is yet apparent. By 48 hours, phagocytosis of the larval epidermis has begun, but some of these cells are still intact; however, this cytoplasm has become reduced in size and vacuolated (Fig. 1f). No imaginal epidermis has formed and no mitotic figures were found in the clusters of imaginal hypodermal histoblasts in the diflubenzuron-treated groups at 24 hours (Fig. 2b) or at 48 hours. Thus at 48 hours, the only form of integument enclosing the diflubenzuron-treated pupae is the fragile pupal cuticle and the larval epidermis (Fig. 1f).

Table 1. Inhibition of chitin synthesis in pupae of *Stomoxys calcitrans* by diflubenzuron. Pupae were collected, washed, and assayed 18 hours after collection (*2*). Reactions were initiated with 8 μl of GlcNac, ^{14}C-labeled in the C-1 position of the D-glucosamine moiety (specific activity, 58.18 mCi/mmole; New England Nuclear). The counting efficiency was 80 percent in Bray's solution.

Treatment	Incorporation* (count/min)	Inhibition (%)
None	2696 ± 328	0
Topical†		
Acetone	2754 ± 464	0
Diflubenzuron	703 ± 105	73.9
In vitro additions‡		
Acetone	3238 ± 518	0
Diflubenzuron	408 ± 81	87.4

*Mean ± standard deviation of four replicates. Each group contained six abdominal halves. †The pupae were treated topically with diflubenzuron (0.1 μg in 0.5 μl of acetone) or acetone alone (0.5 μl) after washing as white prepupae. ‡The diflubenzuron (0.2 μg in 1.0 μl of acetone) or acetone (1.0 μl) was added to the incubation reaction mixtures.

At eclosion, which occurred 8 days after treatment of the prepupae, 98 percent of the untreated and 92 percent of the acetone-treated pupae eclosed. Only 2 percent of the diflubenzuron-treated emerged as adults. Dissection of these uneclosed pupae revealed that they had degenerated within the puparium.

During the pupal instar, incorporation of radioactive GlcNac is most pronounced on days 1 and 4 after the prepupal stage (*2*). These peaks are apparently associated with the production of the pupal and imaginal cuticles that form within 24 hours after prepupal formation, and with the production of the remaining imaginal cuticle, including formation of the setae by the fourth day after pupation (*2*). Since our studies showed that the effects of diflubenzuron were manifested on day 1 and that pupal death had probably occurred by day 4, we investigated the effects of diflubenzuron on incorporation of the chitin precursor, GlcNac, during the first burst of chitin synthesis. Eighteen hours after the treatment of white prepupae, these pupae were assayed in vitro (*2*) for the incorporation of GlcNac into chitin. Acetone alone did not inhibit GlcNac incorporation (Table 1). However, acetone plus diflubenzuron inhibited 73.9 percent of the chitin synthesis. This inhibition is presumably related to the nonproliferation of imaginal epidermal cells in the diflubenzuron-treated pupae, since they produced the imaginal cuticle which had not been formed. The production of the pupal cuticle, which is formed by the larval epidermal cells, had not been affected by this treatment. In addition, diflubenzuron was added directly to the in vitro chitin synthesis assay system. Untreated white prepupae from the above group were held until 18 hours after pupation, collected, and washed as before; they were then assayed for their ability to incorporate GlcNac into chitin (*2*). Then, either diflubenzuron (0.2 μg) or acetone was added just before the initiation of the reaction with labeled GlcNac. Diflubenzuron inhibited the incorporation of GlcNac by 87.4 percent, whereas acetone alone had little effect (Table 1).

Diflubenzuron, therefore, acts in at least two ways to inhibit chitin synthesis in insects. It prevents proliferation of the undifferentiated primordial cells that

form the imaginal epidermis, thereby blocking the subsequent cycle of cuticular deposition, and it inhibits chitin synthesis by established epidermal cells. Whether this cytostatic or antimitotic activity is a direct or indirect effect of diflubenzuron treatment remains to be determined. The effect of diflubenzuron on the formation of the imaginal epidermis does not appear to be the result of a moribund condition. The pupae appear to be healthy for at least the first 48 hours after treatment with diflubenzuron. During this time, the larval tissues not programmed for degeneration have intact plasma and nuclear membranes and an absence of autolytic vacuoles and pyknotic nuclei. That physiological processes are proceeding in the treated pupae in synchrony with those of the control groups is evident in the secretion of the pupal cuticle by the larval epidermis, continued selective phagocytosis of specific larval muscles, and cellular proliferation of other organ systems through the first 48 hours after diflubenzuron treatment. An interesting effect of diflubenzuron was that this compound retarded the programmed cell death of the larval epidermis. Although phagocytosis of other larval tissues proceeded at the same rate as in the control groups, degeneration of the larval epidermis was still occurring at 48 hours whereas phagocytosis of this tissue had been completed by 48 hours in the control groups.

The effect of diflubenzuron on the proliferation of imaginal epidermis has not been previously reported, probably because few studies on the mode of action have depended on histological observations for corroboration and because most studies have been made on insects in which the larval epidermis does not undergo metamorphosis (*3*). These studies have included hemimetabolous organisms such as the Orthoptera and Homoptera and also two orders of holometabolous insects, Coleoptera and Lepidoptera (*5*).

Although the effect of diflubenzuron on the stable fly may be limited to cyclorrhaphous Diptera, it should be noted that Hymenoptera, which include several economically important species, also form an imaginal epidermis. Therefore studies concerning the effect of diflubenzuron on this order should be made.

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References and Notes

1. L. C. Post, B. J. de Jong, W. R. Vincent, *Pestic. Biochem. Physiol.* **4**, 473 (1974); G. W. Ivie and J. E. Wright, *J. Agric. Food Chem.* **26**, 90 (1978); J. N. Kaplanis, J. Elliott, M. Taher, *J. Econ. Entomol.* **71**, 620 (1978); D. H. Deul, B. J. de Jong, J. A. M. Kortenback, *Pestic. Biochem. Physiol.* **8**, 98 (1978); N. P. Hajjar and J. E. Casida, *Science* **200**, 1499 (1978); B. A. Sowa and E. P. Marks, *Insect Biochem.* **5**, 855 (1975); R. F. Ker, *J. Insect Physiol.* **23**, 39 (1972).
2. R. T. Mayer, S. M. Meola, D. L. Coppage, J. R. DeLoach, *J. Insect Physiol.* **25**, 677 (1977); ———, *J. Econ. Entomol.*, in press. Tests including exposure to chitinase and hot KOH were positive for chitin or chitin-like material synthesized at 18 hours and 4 days after pupation.
3. A. Verloop and C. D. Ferrell, *Am. Chem. Soc. Symp. Ser.* **37**, 236 (1977).
4. M. Demerec, *Biology of Drosophila* (Hafner, New York, 1965); G. E. Cantwell, A. J. Nappi, J. G. Stoffolano, *U.S. Dep. Agric. Tech. Bull. No. 1519* (1976).
5. M. Luscher, in *Insects and Physiology*, J. W. L. Beament and J. E. Treherne, Eds. (Elsevier, New York, 1967), pp. 39–51.

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Phosphorus Sources for Aquatic Weeds: Water or Sediments?

Abstract. *Nine common species of aquatic macrophytes took all their phosphorus from the sediments when grown in situ in both a mesotrophic and a mildly eutrophic bay. Even under hypertrophic conditions, the sediments contributed an average of 72 percent of all the phosphorus taken up during growth. These experiments unambiguously demonstrate for the first time that submergent macrophytes in nature overwhelmingly depend on the sediments for their phosphorus supply and characterize them as potential nutrient pumps to the open water.*

Whether aquatic macrophytes (*1*) take their nutrients from the sediments or from the open water has been a long discussed but unresolved question. A quantification of the relative contribution of water and sediments in nutrient uptake would identify macrophytes as nutrient pumps or sinks and would contribute to weed control strategies in areas where excessive growth is a problem.

Several investigators have attempted to establish the relative importance of roots and shoots in the phosphorus (P) nutrition of macrophytes under artificial conditions (*2*). These studies have qualitatively shown the ability of macrophytes to take up and translocate some P by way of the root system. However, they were not designed to resolve the question of whether macrophytes in nature obtain their nutrients from the water or the sediments, or both sources.

Our approach to the question was to grow in situ various species rooted in ^{32}P-labeled sediments of known available P-specific activity (*3*), with the shoot in free contact with the unlabeled open water P. If during growth the plants obtain P exclusively from the water, they should not show any ^{32}P activity; conversely, if the sediment is the only source of P, the plants should show a specific activity equal to the specific activity of the available sediment P. If P is assimilated both from the sediments and the water, the ratio of plant P to available sediment P-specific activities should provide a direct measure of the relative importance of each source. As the relative contribution of water and sediments in P uptake is probably related to the relative P availability of sediments and water, the study was carried out in three locations representing a broad range of relative water P and sediment P availabilities (Table 1).

At each site, the sediments were collected with a Petersen dredge and pooled as large single batches (100 kg). They were then labeled with ^{32}P as $H_3{}^{32}PO_4$ (*4*), carefully homogenized, and allowed to equilibrate for 4 weeks, after which growth experiments were initiated. In early June 1977 and 1978, small sprouting plants were collected, weighed, and potted in closed 1.5-liter polyethylene

Table 1. Water P and sediment P characteristics for the three southern Quebec sites investigated. The interstitial soluble reactive P (SRP) was sampled by dialysis; TP designates total P.

Site	Water		Sediment		
	Mean TP (μg/liter)	Mean SRP (μg/liter)	Mean TP (μg/g)	Mean available P (μg/g)	Mean interstitial SRP (μg/liter)
Central Lake Memphremagog	9.7	0.5	799	66.7	169
Southern Lake Memphremagog	29.8	1.9	785	195	1200
Rivière-du-Sud	290	167	1199	228	1490

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