# Bioassays of TH6038 and Difluron Applied to Western Spruce Budworm[1] and Douglas-fir Tussock Moth[2,3]

NANCY L. GILLETTE, JACQUELINE L. ROBERTSON, and ROBERT L. LYON
Pacific Southwest Forest and Range Experiment Station, Forest Service, USDA, Berkeley, CA 94701

ABSTRACT

Two insect molt inhibitors, TH6038 (N-[[(4-chlorophenyl) amino]carbonyl]-2,6-dichlorobenzamide) and difluron (N-[[(4-chlorophenyl) amino]carbonyl]-2,6-difluorobenzamide), were tested for topical and feeding toxicity to the western spruce budworm, *Choristoneura occidentalis* Freeman, and the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough). Both compounds showed little topical activity, but both were quite toxic when fed to insects in artificial diet. Difluron was most active when fed to early instars of both species. Difluron sprayed on Douglas-fir seedlings showed substantial residual activity for 6 wk.

A new class of insecticides, the substituted phenylbenzyl ureas, has shown promise for the control of many forest defoliators. These chemicals work by inhibiting chitin deposition, which results in molting failure or rupture of the new cuticle (Mulder and Gijswijt 1973).

Difluron (Dimilin®, diflubenzuron, TH6040, PH60-40; N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide) has been tested on several economically important lymantriids (Granett and Dunbar 1974, Zabel and Ostojic 1973) and tortricids (Retnakaran and Smith 1975, Pree[4]); TH6038 (N-[[(4-chlorophenyl)amino]carbonyl]-2,6-dichlorobenzamide) has been tested on 2 tortricids (Anon. 1973 and Pree[4]); both have been tested extensively on many lepidopteran species (bibliography available from Thompson-Hayward, Box 3530, Visalia, CA 93277).

Reported here are results of laboratory tests of the 2 compounds applied to the western spruce budworm, *Choristoneura occidentalis* Freeman, and the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough)—2 major forest defoliators in western North America (Carolin and Honing 1972, Wickman et al. 1973). In a series of bioassays, we assessed the effects of mode of application (contact vs. feeding) and larval stage on susceptibility to these compounds. We also evaluated the residual effectiveness of difluron sprayed on the foliage of Douglas-fir, *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco, seedlings.

METHODS AND MATERIALS.—*Insects.*—Western spruce budworm larvae were selected from a laboratory colony reared on artificial diet (Lyon et al. 1972). Douglas-fir tussock moth larvae came from 2 sources: a laboratory colony reared from egg masses collected near LaGrande, OR. (Robertson and Lyon 1973), and the $F_1$ generation of a mixed field population from Oregon, Montana, and Idaho, reared by a modified procedure.[5] Because of the importance of exposure period before ecdysis (Anon. 1974), all insects used were newly-molted larvae. To determine instars, we used head capsule measurements of Lyon et al. (1972) for western spruce budworm and those of Robertson[5] for Douglas-fir tussock moth.

*Insecticide Formulations*[6].—Technical grade (>95% +) formulations of TH6038 and difluron and a 25% WP formulation of difluron were tested. The 25 WP formulation consists of particles airmilled to <5$\mu$ in an inert carrier (kaolin) with a small amount of dispersing agent.

For both feeding and topical toxicity studies, we prepared fresh solutions–suspensions daily in acetone: water (1:1) as wt/vol concentrations of the AI by dissolving the compound in acetone, then adding distilled water while stirring. Dilutions were made with a 1:1 solution of acetone:water. All formulations of the 25WP were agitated immediately before use to ensure suspension of kaolin particles. For formulations used in feeding studies, we added 0.1% methylene blue dye as a visual guide to ensure thorough mixing of the compounds with the artificial diet. The dye was nontoxic at that dose. Formulations sprayed on potted trees were suspensions of the 25 WP in water with 1.0% aniline blue dye added to allow colorimetric assessment of deposit.

*Treatment Procedures.*—Topical application.—Solutions of the compounds were applied at a rate of 1 $\mu$l/100 mg insect wt to the thoracic dorsa of $CO_2$-anesthetized larvae (Robertson et al. 1972). Control insects were treated with solvent only. Four concentrations were tested, and each test was replicated 3 times. Insects were treated in groups of 10 and were held until pupation in 100×20-mm sterile plastic petri dishes lined with filter paper. Artificial diet

[1] Lepidoptera: Tortricidae.
[2] Lepidoptera: Lymantriidae.
[3] Received for publication Sept. 1, 1977.
[4] Pree, D. J. 1973. Evaluation of PH60-40 and PH60-38 against spruce budworm and forest tent caterpillar. Unpublished report available from Thomson-Hayward Chemical Company, 5200 Speaker Road, Kansas City, KS 66110.
[5] Robertson, J. L. Contact, feeding, and residual activity of selected insecticides on *Orgyia pseudotsugata* (McDunnough). Manuscript in preparation.
[6] This paper describes research involving insecticides. It does not make recommendations for their use, nor does it imply that any uses described have been registered. All use of insecticides must be registered by the appropriate State and/or Federal Agencies before they can be recommended.

was provided until larval–pupal ecdysis. Since early tests had shown nearly complete correlation between pupal abnormalities and failure of the pupal–adult molt, pupal abnormalities were included in the mortality data.

*Feeding Tests.*—Small molds of artificial diet containing TH6038 and difluron were fed to larvae; the molds were prepared as follows: diced artificial diet was liquefied in a double-boiler, then transferred to 1-oz plastic jelly cups (10 ml/cup) using disposable syringes; a 200-$\mu$l aliquot of the insecticide solution was added to each 10 ml of diet; the mixture was stirred immediately with a sterile toothpick until the dye was evenly distributed; then it was allowed to gel for 1 h. Solidified molds were removed from the jelly cups and placed in petri dishes lined with filter paper; each dish contained 10 larvae. Control insects were fed diet molds prepared with solvent and dye only. Five to 8 concentrations were tested, and each concentration was replicated 4 times. Insects were held through pupation; data on larval and pupal mortalities were combined.

*Spray Chamber Tests.*—Two-yr-old potted Douglas-fir seedlings were sprayed for 5 sec with 25WP in a stainless steel spray chamber (E. E. Moellman, patent pending) equipped with a fine Potter spray nozzle. Atomization pressure was 703 g/cm$^2$; exposure time totaled 60 sec. Five trees were sprayed at each of 6 concentrations for each postspray weathering interval (0, 1, 2, 4, and 6 wk). Trees were then weathered outdoors where sunlight and moisture were not regulated. Rainfall was negligible during these periods, but the trees were watered daily. After the prescribed interval, each tree was covered with a tubular cage made of 22×22 mesh nylon screening. Cages were tightly cinched around the trunk below the foliage, and 20 larvae were inserted in each cage. Cages were then sealed across the top. After the larvae had consumed all the foliage, they were transferred to petri dishes with artificial diet until the larval–pupal molt.

Dosage of the compound deposited in the spray chamber was determined by a modification of the method of Rayner (1956). Aniline blue dye deposited on a 9-cm filter paper was eluted with distilled water to a volume of 10 ml. The amount of dye was then determined by colorimetric analysis; percent transmittance was converted to mg dye using a standard curve for aniline blue ($\lambda$=600) in water; weight of dye was then translated into volume of insecticide/ha by using the formulae of Robertson et al.[7] Calibration experiments showed that a 0.25-ml volume delivered from the nozzle was roughly equivalent to a deposit of 9.36 liters/ha; deposits of dye and solvent alone averaged 9.36±0.94 liters/ha.

Because of the physical properties of the WP formulation, it was necessary to make separate deposits to determine the dosage in g/ha. For weight determinations, deposits were made with both insecticide and dye in the solvent. The eluted samples were centrifuged before colorimetric analysis to remove suspended kaolin particles which otherwise would occlude transmitted light. The avg of these deposits was used to determine the dosage deposited.

*Data Analysis.*—Log-dose probit lines were constructed for topical application and feeding tests (Daum 1970).

RESULTS AND DISCUSSION.—Technical TH6038 and difluron showed no toxicity when applied topically to 6th-instar spruce budworm and to 4th-instar tussock moth. This finding is in agreement with earlier studies showing difluron to be active only by ingestion (Mulder and Gijswijt 1973, Wellinga et al. 1973). However, the 25 WP formulation of difluron showed topical activity against 4th-instar tussock moth ($LD_{90}$ for difluron = 70 $\mu$g/g, compared with 0.45 for resmethrin, 5.5 for DDT-pp′, 40 for trichlorfon, and 70 for methamidophos (Robertson and Lyon 1973)). Cerf and Georghiou (1974) also found difluron to be active topically against prepupae of *Musca domestica* L. However, since our feeding studies with 2nd-instar tussock moth larvae show no significant difference in the feeding toxicities of the 2 difluron formulations (Table 1), the topical activity of the 25 WP formulation in our tests may be an artifact resulting from the physical characteristics of the WP formulation. Perhaps the kaolin particles adsorbed difluron as the solvent evaporated after the insects were treated, and these particles contaminated the diet and were subsequently ingested. The extremely high order of toxicity of difluron when ingested would lend support to this explanation. Retnakaran and Smith (1975) reported similar findings, i.e., slight contact and high feeding toxicity, in tests with *C. fumiferana* (Clemens); Tamaki and Turner (1974) reported similar results in tests with the zebra caterpillar, *Ceramica picta* (Harris). Ascher and Nemny (1976), however, found that technical difluron showed substantial topical activity ($ED_{50}$ = 0.074

[7] Robertson, J. L., M. Page, N. L. Gillette, R. L. Lyon, E. Moellman and T. L. Andrews. A unique spray chamber for laboratory application of insecticides. Manuscript in preparation.

**Table 1.—Toxicity of TH6038 and difluron fed to 2nd instar *O. pseudotsugata*.**

| Compound | Formulation | No. treated | Control mortality (%) | Slope ±SE | $LC_{50}$ (ppm in diet) | Range | $LC_{90}$ (ppm in diet) | Range |
|---|---|---|---|---|---|---|---|---|
| TH6038 | Tech | 200 | 11 | 2.91±0.15 | 0.020 | 0.018–0.022 | 0.054 | 0.048–0.060 |
| Difluron | Tech | 350 | 6 | 4.08±0.79 | 0.028 | 0.014–0.056 | 0.056 | 0.036–2.200 |
| Difluron | 25WP | 1000 | 6 | 2.94±0.51 | 0.028 | 0.018–0.040 | 0.076 | 0.052–0.170 |

**Table 2.—Toxicity of difluron fed to selected instars of *C. occidentalis* and *O. pseudotsugata*.[a]**

| Insect and instar | No. treated | Slope±SE | $LC_{50}$ (ppm in diet) | Range (ppm in diet) | $LC_{90}$ (ppm in diet) | Range (ppm in diet) |
|---|---|---|---|---|---|---|
| *C. occidentalis:* | | | | | | |
| 3,4 | 450 | 1.16±0.09 | 2.27 | 1.71 –2.90 | 29 | 21–43 |
| 6 | 330 | 0.96±0.15 | 5.32 | 2.92 –8.12 | 114 | 60–350 |
| *O. pseudotsugata:* | | | | | | |
| 1 | 810 | 1.85±0.40 | 0.024 | 0.012–0.042 | 0.11 | 0.056–1.30 |
| 2 | 1000 | 2.94±0.51 | 0.028 | 0.018–0.040 | 0.076 | 0.052–0.17 |
| 3 | 795 | 3.25±0.38 | 0.046 | 0.036–0.058 | 0.11 | 0.088–0.18 |
| 4 | 360 | 3.32±0.67 | 0.060 | 0.036–0.092 | 0.15 | 0.096–0.67 |
| 5 | 659 | 1.72±0.25 | 0.048 | 0.028–0.076 | 0.27 | 0.15 –0.85 |

[a] Mortality of control insects in *C. occidentalis* tests average 11% and in *O. pseudotsugata* tests, 9%.

μg/100g larva) against *Spodoptera littoralis* (Boisduval). In their study, cross-contamination and potential ingestion of difluron particles were partially controlled by isolating larvae.

There appeared to be no significant difference between the feeding toxicities of TH6038 and difluron (Table 1). Although both compounds showed promise, all subsequent work was done with difluron because its shorter half-life enhances its potential for registration.

Both western spruce budworm and Douglas-fir tussock moth were most susceptible to difluron in the earlier instars (Table 2), and the tussock moth was considerably more susceptible than the spruce budworm. For example, at the $LC_{50}$, the last instar tussock moth was ca. 100 times more susceptible than the last instar spruce budworm.

Difluron demonstrated lasting residual activity when applied as a spray to potted trees which were subjected to weathering. When applied at 33.6 g/ha, its activity against the tussock moth decreased from 100% mortality at 2 wk to 51% at 6 wk after spraying (J. L. Robertson and L. M. Boelter, unpublished data).

The same bioassay, when using western spruce budworm as a test organism (Table 3), also showed lasting residual activity, but the dosage required for nearly complete kill was ca. 25 times that required for tussock moth. At that dose, the 6-wk postspray mortality was 87%.

**Table 3.—Residual activity of difluron applied to potted Douglas-fir seedlings and assayed with 6th-instar *C. occidentalis*.**

| Weathering interval (wk) | % insect mortality[a] at dosage of (g/ha) 25 | 51 | 105 | 203 | 406 | 840 |
|---|---|---|---|---|---|---|
| 0 | 40 | 47 | 47 | 57 | 95 | 99 |
| 1 | 35 | 46 | 41 | 56 | 91 | 87 |
| 2 | 31 | 23 | 42 | 38 | 87 | 89 |
| 4 | 16 | 17 | 27 | 38 | 84 | 89 |
| 6 | 9.0 | 5.9 | 21 | 28 | 84 | 87 |

[a] Abbott's formula used to correct for control mortality; total of 100 larvae, 20/tree caged on 5 trees/dosage. Control mortality averaged 3%.

These laboratory tests suggest that the best strategy for field testing would be to spray just before hatch or in the earliest larval stages of both species. Larval exposure may not be an important consideration in spray timing because of the good residual life and mode of action of difluron. Such early application would also give maximum protection to the new foliage.

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