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## Chemical Inducers of Oviposition for the Corn Earworm, Heliothis zea (Boddie)

Abstract. *The corn earworm moth lays its eggs in the vicinity of triacetin, an ingredient of felt-tipped marking pens. Related compounds also induce this behavior. A bioassay was devised to measure the activity of chemicals as oviposition inducers.*

Gravid insects, usually of the order Diptera (flies), have been induced to lay their eggs in the vicinity of certain chemicals. Some compounds eliciting this response are associated with decaying proteinaceous matter such as ammonia, its salts, or amines (*1*), and some sulfur-containing materials such as mercaptans and sulfides (*2*). In one instance aliphatic monoesters (found in food flavors) were reported to be oviposition attractants for *Aedes aegypti* mosquitoes (*3*). In another, chemicals inducing phytophagous insects to oviposit were ones present in the preferred host plants (*4*). Thus, these chemicals may be correlated with a source of sustenance for the young larvae that emerge from the eggs oviposited by the adult (*5*). Chemicals that actively induce oviposition might be useful as a means of controlling insect pests because larvae emerging from eggs laid near an appropriately placed chemical would not find the food normally present and would perish. An attractive feature of this approach is that no toxic chemicals need be used.

In 1968, as part of a program for large-scale rearing of the corn earworm, *Heliothis zea* (Boddie), we began to use felt-tipped marking pens to number cloths on which the insects oviposit. Shortly thereafter we noted that the moths laid more eggs near the ink markings than elsewhere on the cloth. The active ingredient in the ink was isolated and identified as triacetin.

The first felt-tipped pens to induce the oviposition response were manufactured by the Zip Mark Corp., Bordentown, N.J. (*6*). Subsequent tests showed that Aqua Mark (Chemolene Industries, Bordentown, N.J.) and Marks-A-Lot (Carter's Ink Co., Cambridge, Mass.) pens also produced active markings.

The pure active principle in the ink wick was isolated after a series of exploratory experiments in which fractions found active by bioassay were progressively purified. The following procedure worked well. Two ink wicks were sliced into disks about 5 mm thick and allowed to dry overnight (*7*). The wick disks were then extracted in a Soxhlet apparatus with 100 ml of ethyl ether for 3 hours. The ether extract was washed twice with 50-ml portions of distilled water, dried over anhydrous sodium sulfate, decolorized with activated charcoal, and finally concentrated to 1 to 2 ml. Chromatography of this ether extract on a column of silica gel (*8*) with a mixture of ether and hexane (1 : 1) as eluant gave active material between 40 and 140 ml. Gas chromatography of the active material on an OV-17 column (*9*) disclosed the presence of two compounds. Only one was active, and it comprised 27 percent of the material based on peak area. The active fraction from the silica gel column was concentrated to a few milliliters and chromatographed again on silica gel as already described except that the column was developed serially with 100 ml each of 10, 20, 30, 40, and 50 percent ether in hexane by volume. The inactive component was found in the 10 percent eluate and the active one in the 30 percent fraction. Yield of active compound was 20 mg per wick.

The infrared spectrum of the compound showed prominent characteristic acetate absorption (1745 and 1220 $cm^{-1}$). The nuclear magnetic resonance (NMR) spectrum of the compound in $CCl_4$ was obtained on an HA-100 Varian spectrometer. It showed an intense singlet at 2.01 parts per million (ppm) and two multiplets centered at 4.13 and 5.11 ppm (from tetramethylsilane) with respective proton absorptions of 81 : 36 : 8. The singlet at 2.01 ppm was undoubtedly due to acetate protons, and the multiplets were tentatively assigned to -C*H*-O- protons. These data suggested the compound was a triacetate; that is, if the multiplet at 5.11 ppm were one proton, the acetate absorption would be equivalent to roughly nine protons, or three acetate groups. Triacetin, with a proton ratio of 9 : 4 : 1 (acetate, primary and secondary protons), fit the 81 : 36 : 8 proton ratio very well. Comparison of the infrared and NMR spectrums of authentic triacetin with the corresponding spectrums of the active compound from the wick showed that they were virtually the same and that the active substance was indeed tri-

Table 1. Oviposition ratio (eggs in treated area divided by eggs in untreated area) for chemicals related to triacetin and for triacetin determined concurrently at two concentrations.

| Chemical | Oviposition ratio | | | |
|---|---|---|---|---|
| | Chemical at | | Triacetin at | |
| | 0.1 mg/ml | 1.0 mg/ml | 0.1 mg/ml | 1.0 mg/ml |
| Diacetin | 1.00 | 3.84 | 3.82 | 3.24 |
| 1-Monoacetin | 0.99 | 1.84 | 2.29 | 5.13 |
| Tripropionin | 2.40 | 3.44 | 3.82 | 3.24 |
| Dipropionin | 2.64 | 4.70 | 3.38 | 5.92 |
| Monopropionin | 1.26 | 1.42 | 3.38 | 5.92 |
| Tributyrin | 1.28 | 3.88 | 3.82 | 3.24 |
| Ethylene glycol, diacetate | 1.01 | 1.50 | 2.29 | 5.13 |
| 1,3-Propanediol, diacetate | 0.85 | 1.34 | 3.82 | 3.24 |
| 1,3-Butanediol, diformate | 1.18 | 1.81 | 3.38 | 5.92 |

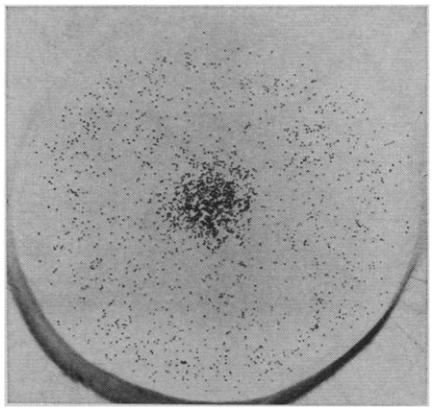

Fig. 1. Oviposition cloth showing the concentration of eggs at the site of the chemical triacetin. (Eggs were blacked with India ink for contrast.)

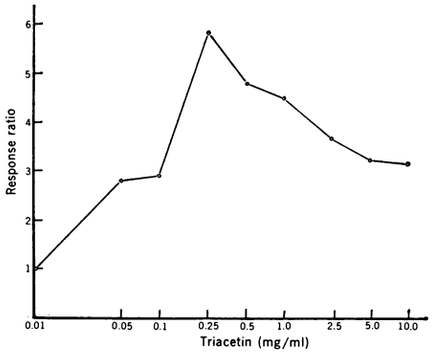


Fig. 2. Relationship between response ratio (eggs in treated area divided by eggs in untreated area) and concentration of triacetin applied to the oviposition cloth.

acetin. Identity and purity of the compound were further substantiated by bioassay and by the identical retention times and peak shapes obtained in the gas chromatography of the isolated and authentic compound on the OV-17 column already described and on a second column of different polarity (*10*).

We devised a bioassay to evaluate the potency of the isolated fractions and to compare the activity of triacetin with that of related compounds. Insects were reared as described by Burton (*11*). As part of the rearing procedure, moths of both sexes are placed in a 1-gallon cylindrical ice cream carton (17 cm high, 16 cm in diameter) that has its top circular cardboard end replaced with white cotton sheeting on which the females oviposit. The moths are allowed to feed freely on beer contained in a cotton wad inside the container. For our bioassay, we applied the appropriate concentration of the fraction or chemical in 0.25 ml of redistilled acetone on a spot (1 cm in diameter) in the center of the oviposition cloth while an air stream was directed on the cloth to remove the acetone solvent. The oviposition cloth was fastened over the open end of 3- to 4-day-old cartons containing 10 male and 10 female corn earworm moths with the chemical at the center of the opening. Oviposition was allowed to take place overnight (16 hours) under a lighting regime of 14 hours of daylight and 10 hours of darkness. The next morning, the number of eggs laid in the treated area was compared with the number laid in an untreated area. To facilitate the counting of the eggs, we made a white paper template on which concentric circles with radii of 2.54, 3.64, 4.39, and 5.08 centimeters were drawn. The area of the inner circle and the areas between adjacent drawn circles were each 20.23 $cm^2$. These areas were designated circles 1, 2, 3, 4 from the inner circle outward.

To count the eggs, we positioned the template under the cloth so that the site of application was in the center of circle 1. Also a light was placed below the template so that it could be readily seen. The ratio of the number of eggs in circle 1 to the average number of eggs in the two outermost circles 3 and 4 proved to be an adequate measure of activity. The number of eggs in circle 2 was disregarded because the concentration of eggs about the chemical in circle 1 sometimes extended into this area. Controls (no chemical) were treated in a like manner.

Egg deposition on triacetin at the center of the oviposition cloth was typically abundant (Fig. 1). Results of bioassay (mostly in triplicate) for triacetin over a 1000-fold range of concentration are graphed (Fig. 2). The oviposition ratio varied between 3 and 6 for concentrations of triacetin from 0.05 to 10 mg/ml. Since 0.25 ml was applied, good activity was obtained with as little as 10 $\mu$g of triacetin. Results with controls (12 tests) averaged 1.00 with a standard deviation of 0.14.

Tests were made with 33 compounds related to triacetin at concentrations of 0.01, 0.1, and 1.0 mg/ml. Results with compounds most closely related to triacetin and those that showed good activity are given in Table 1. Data are included on the bioassay of triacetin conducted concurrently for comparison. (Results with 0.01 mg/ml are not listed because no chemical was active at this concentration.) Some of the compounds are quite active, and further exploration of related compounds is warranted.

The emphasis on safe methods of insect control favors investigation of oviposition-inducing chemicals such as triacetin. In integrated control programs (a combination of several control procedures) inducers of oviposition might provide an extra margin of safety by reducing the concentration of insecticide needed to effect adequate control.

RICHARD L. JONES
ROBERT L. BURTON
MALCOLM C. BOWMAN
*Entomology Research Division, Agricultural Research Service, Tifton, Georgia 31794*
MORTON BEROZA
*Entomology Research Division, Agricultural Research Service, Beltsville, Maryland 20705*

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6. Mention of proprietary products is for identification only and does not constitute a recommendation by the U.S. Department of Agriculture.
7. Failure to remove the volatiles by air drying results in excessive uptake of dyes in the subsequent ether extraction.
8. J. T. Baker Chemical Co. No. 3405. Column was 1.5 cm in diameter and 18 cm long; the silica gel contained 3.5 percent water.
9. An F and M Model 700 instrument equipped with flame ionization detector and a glass column (4 mm internal diameter by 180 cm) containing 5 percent OV-17 on 80-100 mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) was used. The temperature was programmed from 100° to 250°C at 10°C/min and then held at 250°C for 10 minutes. The $N_2$ carrier gas flow was 100 ml/min. In isothermal analyses, one compound eluted at 160°C in 1.70 minutes and was active; the other eluted at 270°C in 2.05 minutes and was inactive.
10. A 5 percent OV-225 liquid phase was used. Other parameters were the same as in the OV-17 analysis except that the column length was 240 cm and column temperature was 170°C; retention time was 1.80 minutes.
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