Inhibition of F-2 (Zearalenone) Biosynthesis and Perithecium Production in Fusarium roseum 'Graminearum'

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ABSTRACT

Tracer incorporation studies and quantitative analysis showed that dichlorvos is a potent inhibitor of Zearalenone (F-2) biosynthesis by *Fusarium roseum* 'Graminearum'. Dichlorvos also strongly inhibits production of perithecia by the fungus, and its inhibitory action can be reversed by F-2. This evidence suggests that F-2 may be a true fungal hormone which governs development of the sexual stage.

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In 1968, Eugenio (1) suggested that Zearalenone (F-2), a metabolite of *Fusarium roseum* (Lk.) Snyd. & Hans. 'Graminearum', could enhance production of perithecia by the fungus. This was finally demonstrated by Wolf in 1971 (3), suggesting that F-2 might be a true fungal hormone, provided that production of perithecia could be shown to require F-2. We therefore sought to find a chemical that would inhibit the biosynthesis of F-2, and to determine whether the same chemical would also inhibit production of perithecia by *F. roseum* 'Graminearum'. A suitable compound proved to be dichlorvos (Shell Chemical Co., San Ramon, Calif.). Because F-2 was shown to be an acetogenin (2), incorporation of acetate-1-^{14}C was a convenient measure of F-2 biosynthesis. The fungus was cultured on autoclaved, moist rice, and 5-μc quantities of the

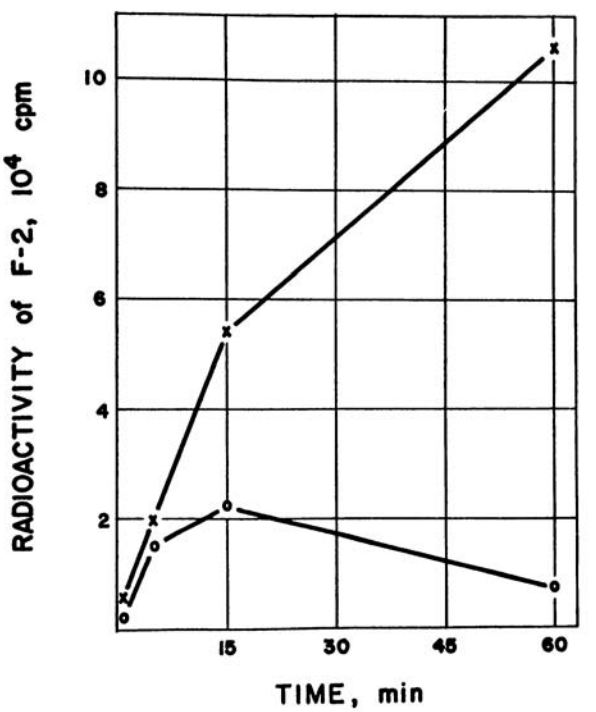

Fig. 1. Incorporation of acetate-1-^{14}C into Zearalenone (F-2) by slices of a culture of *Fusarium roseum* 'Graminearum' grown with 4 mg dichlorvos in 310 g of medium (wet weight before autoclaving) (0's), and by control slices (x's).

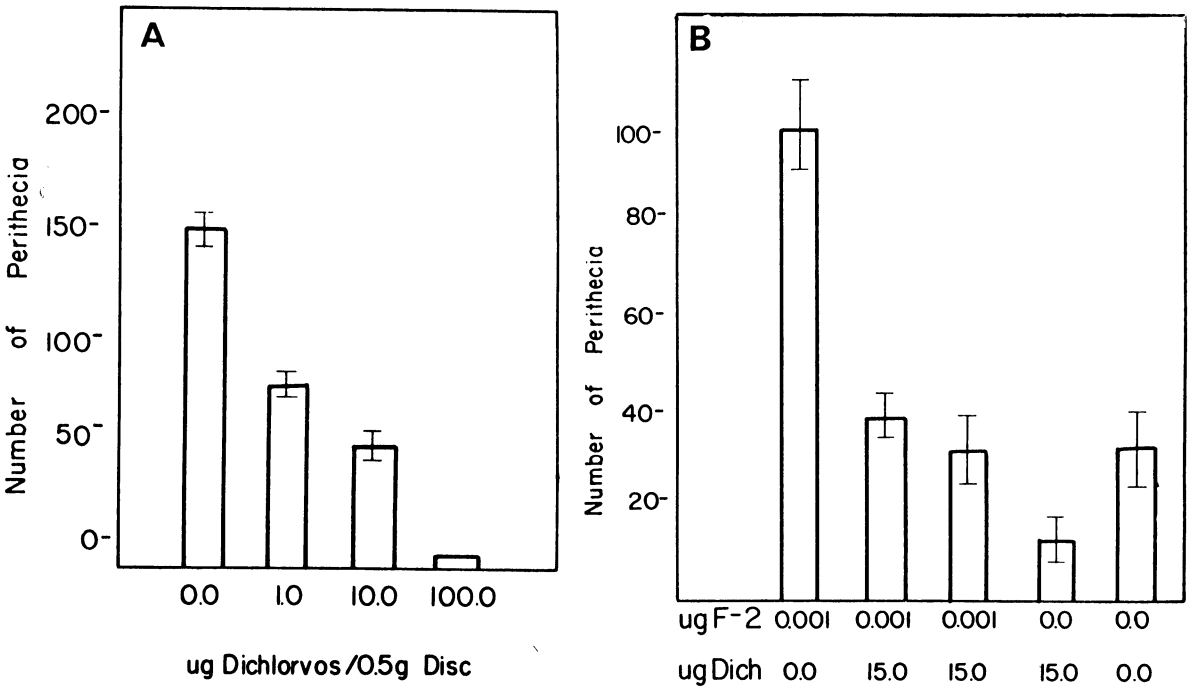

Fig. 2. A) Effect of dichlorvos on the perithecia produced by *Fusarium roseum* 'Graminearum'. **B)** Prevention or reversal of the inhibition of perithecial production as caused by dichlorvos in *F. roseum* 'Graminearum'. Zearalenone (F-2), (0.001 μg) was applied either simultaneously (second column) with dichlorvos or 3 hr later (third column). Culture age was 4 days at the time of application. Number of perithecia are those produced per 0.5 g disc.

radioactive precursor were applied to slices of such cultures at an appropriate age.

Treatment of such slices with dichlorvos invariably caused some inhibition of acetate incorporation, even when a 1.4-mg droplet of dichlorvos was placed in a petri dish with the slice and allowed to contact the slice only by spontaneous vaporization at 25 C.

Best results were obtained when dichlorvos was added to the autoclaved, moist rice prior to inoculation; i.e., 4 mg dichlorvos in 2 ml diethyl ether was added to a bottle which had previously been filled with 200 g Uncle Ben's Converted (parboiled) Rice and 110 ml distilled water and autoclaved. The control bottle was treated only with 2 ml diethyl ether. As shown in Fig. 1, acetate incorporation by the culture treated with dichlorvos was greatly inhibited. Moreover, actual F-2 concentration in the dichlorvos-treated culture was 50-60% less than in the control. The latter was determined by gas chromatography or by measuring its absorbance at 274 nm after extraction with ethyl acetate and purification by thin-layer chromatography.

The effect of dichlorvos on production of perithecia was examined by applying solutions of dichlorvos in diethyl ether-dimethyl sulfoxide (DMSO), 97:3 (v/v) to 0.5 g discs of Coon's agar which had been seeded with a suspension of *F. roseum* 'Graminearum' macrospores. Ten μliters of solution were applied in each test. Controls were treated with diethyl ether-DMSO. As little as 1 μg of dichlorvos inhibited production of perithecia by 50%, and 100 μg caused complete inhibition (Fig. 2-A). In none of the experiments with dichlorvos was there any visible inhibition of vegetative growth by the toxicant.

Alternatively, to determine if dichlorvos was acting in a manner similar to or independent of that of F-2 in influencing perithecial formation, it was necessary to determine whether F-2 could reverse the inhibition caused by dichlorvos. To do this, 0.001 μg of F-2 and 15.0 μg of dichlorvos were applied to the cultures in the following combinations: F-2 only; F-2 and dichlorvos simultaneously; dichlorvos followed by F-2, 3 hr later; dichlorvos only, and the nontreated control. When 0.001 μg of F-2 were applied to 4-day-old cultures, enhancement of perithecial production occurred. Fifteen μg of dichlorvos inhibited perithecial production, but when applied simultaneously with 0.001 μg of F-2, the inhibition was prevented. Furthermore, 0.001 μg of F-2 either prevented or reversed the dichlorvos-caused inhibition even when it was applied 3 hr after the dichlorvos was applied (Fig. 2-B). However, in no case did enhancement of perithecial formation occur when the F-2 and dichlorvos were applied simultaneously. It appears, therefore, either that dichlorvos inhibits

the production of perithecia through a mechanism either related to F-2 biosynthesis, or its site of action is similar to that of F-2, or both.

We have thus added credence to the hypothesis that F-2 may indeed be a true fungal hormone which controls sexual stage development. Our evidence for this lies in the fact that dichlorvos, which inhibits F-2 biosynthesis by *F. roseum* 'Graminearum', also inhibits the production of perithecia by the same fungus. Conversely, F-2 reverses or prevents the inhibition caused by dichlorvos.

LITERATURE CITED

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