Effect of Trifluralin on Inoculum Density and Spore Germination of Fusarium oxysporum f. sp. vasinfectum in Soil

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ABSTRACT

The herbicide trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,N-dipropyl-p-toluidine) enhanced production of chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum* in sandy loam and clay soils when applied at concn in the range of 0.6-40 μg/g. Most spore production occurred in the range of 0.6-5.0 μg/g herbicide and was more pronounced in clay soil. Numbers generally declined with increasing concn of the compound in both soils. The effect of trifluralin on germination of chlamydospores in nonsterilized clay soil was similar to that for spore production. Percentage germination was highest at the lowest herbicide concn (2 μg/g), then decreased at higher levels. Populations of fungi, bacteria, and actinomycetes also were higher in trifluralin treatments of 2-10 μg/g than in 20- and 40-μg treatments or in herbicide-free soil. These results suggest the possibility of a relationship of trifluralin effect to inoculum density and disease potential. Phytopathology 60:1082-1086.

Intensified use of herbicides on agricultural soils has not been accompanied by adequate evaluation of their effects on soil-borne phytopathogenic fungi. Literature reviews (2, 3, 8) indicate that most herbicides used at recommended field rates generally do not greatly alter soil microbial populations, but studies of whole populations or groups of organisms may not reveal the selective action of a herbicide on individuals. Previous work in our laboratory has revealed inhibitory or stimulatory effects of organic herbicides on growth activity of *Sclerotium rolfsii* (7, 14, 15, 16) and *Fusarium oxysporum* f. sp. *vasinfectum* (6, 17) in soil. Other evidence indicates that certain soil-applied herbicides may influence disease incidence and severity (1, 5, 9, 13).

Trifluralin (Treflan) is usually recommended for preemergence application rates of 0.5-1.0 lb/acre (0.56-1.12 kg/ha) for control of annual grasses and many broadleaf weeds in cotton and several other crops. The cotton-wilt pathogen, *F. oxysporum* f. *vasinfectum,* is a common inhabitant of organic matter in soil and may frequently come in contact with this herbicide. The following investigation was made to determine the interactions between trifluralin and the pathogen, with emphasis on spore production and germination.

MATERIALS AND METHODS.—The isolate of *F. oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. used throughout this study was obtained from the American Type Culture Collection, ATCC No. 7808, and maintained on potato-dextrose agar (PDA). Technical grade trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,N-dipropyl-p-toluidine) was supplied by Eli Lilly Co.

Spore production experiment.—Two soil types were used to determine the influence of trifluralin on production of chlamydospores. A Norfolk sandy loam was collected to a depth of 4-6 inches from a fertile experimental rotation plot of the Auburn University Agronomy farm. For 10 years the plot had received the necessary fertilization for maximal plant growth. The rotation system included cotton, corn, oats, soybeans, and a winter legume. The soil contained approximately 60% sand, 25% silt, and 15% clay, and had a pH of 5.5 at time of sampling. The other soil was a Sumpter clay from a grass-covered plot at the Black Belt Substation, Marion Junction, Alabama. This soil contained about 75% clay and 25% silt and sand, with a pH of 7.8. In each case the soils were air-dried, screened (1.5 mm screen), and stored in plastic bags at room temp (25-28 C) until used; this storage period did not exceed 48 hr.

Fifty g (oven-dry wt) of lightly moistened soil were placed in 125-ml flasks to provide three replications for seven treatments, and these were sterilized by autoclaving. Inoculum of *F. vasinfectum* was prepared by chopping young liquid cultures of the fungus in a Monel semimicro blender for 30 sec and further diluting the suspension with sterile water to make a total volume of 250 ml. Three ml were pipetted aseptically in a straight line across the soil surface to each soil flask, and the cultures were incubated at 28 C for 48 hr before herbicide treatments. Each flask then received 5 ml of a nutrient solution containing 30 g dextrose, 2.2 g KNO_3, and 1 g K_2HPO_4/liter of demineralized water. The adjusted pH of the solution was 5.5. Also at this time, stock solutions of trifluralin in 95% ethyl alcohol were applied to provide concentrations of 0.6, 1.25, 5, 10, and 40 μg/g of soil. Special care was taken to distribute the solutions uniformly over the soil surface. Appropriate alcohol and water controls without herbicide were included. The final alcohol content of all flasks (except water controls) was the same. Final soil moisture was approximately 17%. The cultures were further incubated at 28 C, and sporulation was determined after 10 and 20 days.

At the end of each incubation period, soil suspensions were prepared for microscopic spore counts. Fifty ml of demineralized water were added to each of three flasks/treatment and shaken for 30 min on a mechanical shaker. Each flask was then shaken by hand and, while the suspension was in motion, 10 ml were pipetted immediately into another 125-ml flask containing 40 ml of water. This was shaken again for 30 sec, and the heavier particles were allowed to settle for exactly

1 min. Ten ml were quickly withdrawn, transferred to a small vial, and further diluted with 10 ml of water. Two vials were prepared for each of the three flasks, or six vials/treatment. Each vial was further shaken briefly, drops were applied to a hemacytometer counting chamber, and microscopic counts were made at ×430; two counts were made from each vial. The large chlamydospores were readily distinguishable from soil particles without staining. The number of spores per 0.1 mm^3 volume of the chamber was determined. Thus, the final figure for spore production per treatment was the average of 12 suspension drops or hemacytometer counts.

Spore germination study.—The influence of trifluralin on fungistasis or germination of chlamydospores was determined with Sumpter clay soil. An initial experiment was conducted with soil which had been stored in loosely covered plastic containers for 5 weeks and, later, freshly collected soil of the same type and source was used. Preliminary tests revealed that chlamydospores did not germinate on nonsterilized undiluted soil. Therefore, the natural soil was diluted with sterilized soil of the same type in the ratios 6:1 (sterile: natural) for the previously stored soil and 4:1 for fresh soil. The soil was screened and 20 g (oven-dry wt) were placed in small petri dishes (50 × 15 mm). Some of these were sterilized by autoclaving for use as controls.

Trifluralin from an alcoholic stock solution was applied aseptically by pipette to each dish of soil in sufficient water to provide concn of 2, 5, and 20 μg/g of soil and a final soil moisture of about 25%. Control treatments consisted of water only and alcohol only. The soil surface was packed and smoothed with a sterile spatula, and 5-6 drops of thrice-washed chlamydospores in water were applied to each of three dishes/treatment. These were incubated in humidity chambers at 28 C for 6 hr, the period predetermined as the time for maximum spore germination on water agar.

Following incubation, spores were stained with aqueous phenolic rose-bengal solution and recovered with 2% polystyrene essentially as described by Lingappa & Lockwood (11). Chlamydospore germination was determined for twelve microscope fields per treatment.

Effect on microbial populations.—A study of fluctuations in populations of microorganisms in herbicide-treated soil was made primarily to determine if changes may relate to results of the preceding experiments. Fresh sandy loam and clay soils were collected from the same sources as before. Twenty-five g of screened soil were placed in 250-ml flasks, and 10 ml of an alcoholic stock solution of trifluralin were applied uniformly over the soil surface to provide concentrations of 2, 5, 10, 20, and 40 μg/g. Duplicate flasks were prepared for each treatment, and herbicide-free water and ethanol controls were included. After 4 days of incubation at 28 C, water was added to each flask to bring the volume to 250-ml; this was transferred to a 500-ml flask and processed by the standard soil-dilution and plate-count method (10). A soil:water ratio of 1:5,000 was used for plating fungi in OAES agar (18), and 1:500,000 for bacteria and actinomycetes in soil extract agar (4).

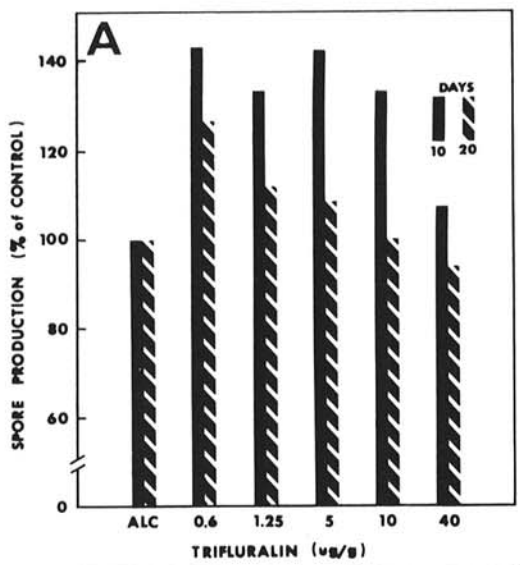
Data from all experiments in this investigation were analyzed statistically, and means compared according to procedures described by Snedecor (19).

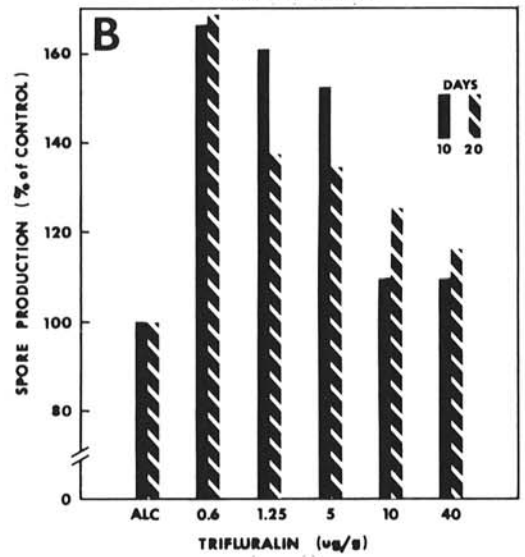
RESULTS.—*Spore production.*—In sandy loam (Fig. 1-A) during 10 days' incubation, all concn of trifluralin induced higher chlamydospore production than in the alcohol control (expressed as percentage of control). After 20 days, spore production was higher in herbicide treatments of 0.6-5 μg than in the control. Numbers tended to decline with increasing concn of herbicide. Although statistical analysis revealed no significant differences between treatments, the trend persisted with repeated tests. In clay soil (Fig. 1-B), the trend was similar to that for the 20-day period in sandy loam, except that differences between treatments were significant for both 10 and 20 days. Highest spore production was in the lowest herbicide treatment (0.6 μg/g), and decreased with increasing herbicide concentration. Little difference was evident between the 10- and 20-day incubation periods. Comparison of the water and alcohol controls showed that alcohol alone had a slight inhibitory effect on spore production.

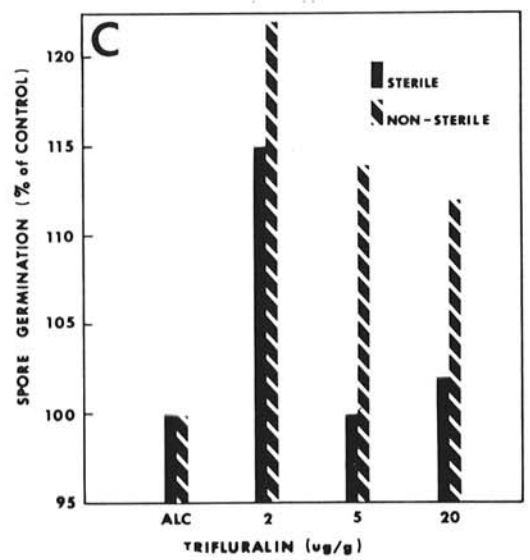
Spore germination.—The effect of trifluralin on chlamydospore germination in clay soil somewhat resembled the effect observed for spore production. Also, the results were similar for previously stored soil and freshly collected soil. Figure 1-C shows results for the fresh soil only, with germination data expressed as percentage of the alcohol control. The percentage germination was highest at the lowest herbicide level (2 μg/g); difference from both the control and the 20-μg treatment was highly significant. In nonsterilized soil (diluted with sterile soil), the difference between 5 or 20 μg and the control also was highly significant. Contrary to expectation, higher germination occurred in nonsterilized than in sterilized soil.

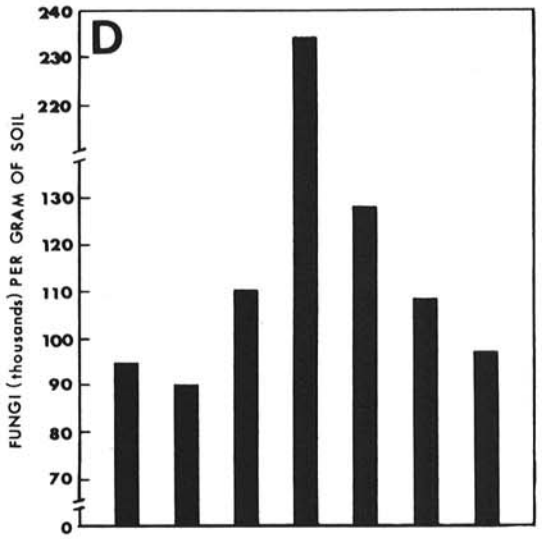
Microbial populations.—The influence of trifluralin on microbial populations in the two soils was quite similar; therefore, only data for clay soil (Fig. 1-D, E, F) are presented. Numbers of all three groups of organisms were higher in herbicide treatments of 2-10 μg/g than in either the alcohol control or the 20- or 40-μg treatments. Fungal populations (Fig. 1-D) were significantly higher at 5 μg trifluralin, and actinomycetes (Fig. 1-F) higher at 2 μg than for any other treatment. Bacterial numbers (Fig. 1-E) were equally high in the treatment range of 2-10 μg. Thus, the pattern for effect of the herbicide on the general soil microflora resembled that observed for production and germination of chlamydospores of *F. oxysporum* f. *vasinfectum*.

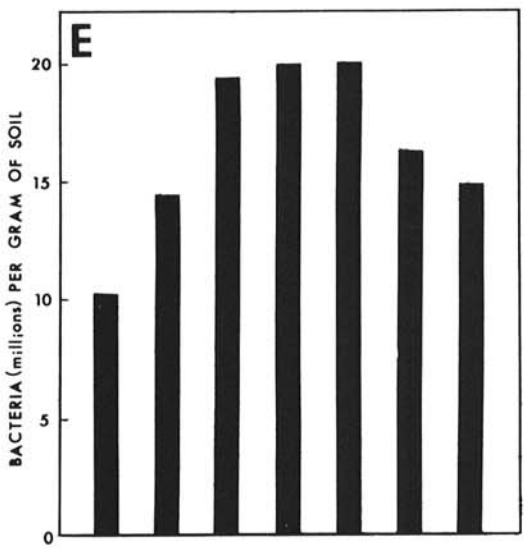
DISCUSSION.—The lower trifluralin concn (0.6-5 μg/g) used in this investigation are well within the range of recommended field rates when one considers that the compound is usually incorporated at 0.5-1.0 lb/acre in the upper 3 inches of soil. Considering further that the water solubility of trifluralin is less


A
SPORE PRODUCTION (% of CONTROL)
DAYS
10 20
ALC 0.6 1.25 5 10 40
TRIFLURALIN (ug/g)


B
SPORE PRODUCTION (% of CONTROL)
DAYS
10 20
ALC 0.6 1.25 5 10 40
TRIFLURALIN (ug/g)


C
SPORE GERMINATION (% of CONTROL)
STERILE
NON-STERILE
ALC 2 5 20
TRIFLURALIN (ug/g)


D
FUNGI (thousands) PER GRAM OF SOIL


E
BACTERIA (millions) PER GRAM OF SOIL

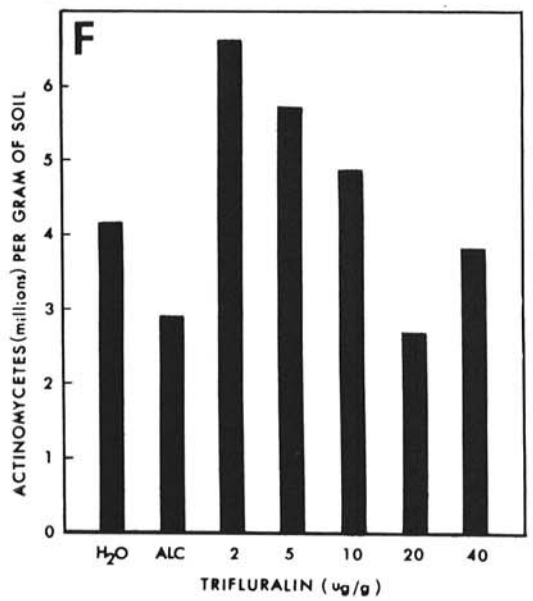

F
ACTINOMYCETES (millions) PER GRAM OF SOIL
H2O ALC 2 5 10 20 40
TRIFLURALIN (ug/g)

Fig. 1. Effect of trifluralin on *Fusarium oxysporum* f. *vasinfectum* and microbial populations in soil. **A)** Production of chlamydospores of *Fusarium* in sandy loam after 10 and 20 days of incubation. **B)** Production of chlamydospores in clay soil. **C)** Germination of chlamydospores in sterilized and nonsterilized clay soil. **D-F)** Populations of fungi, bacteria, and actinomycetes in clay soil.

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than 1 ppm at 27 C and volatility is relatively high, the observed effects on the fungus may have resulted from actual concn even lower than those indicated here.

It seems apparent that chlamydospore production was enhanced, particularly in a clay soil at herbicide concn of 0.6-5 μg/g. Since no information is available at this time on the effect of trifluralin on growth or respiration of *F. oxysporum* f. *vasinfectum* in soil, the spore data cannot be discussed in relation to growth. A previous study (12) in liquid culture, however, showed little effect on mycelial production of the pathogen. The increased sporulation in our present study probably was not a response to restricted growth of the fungus, since mycelium in the flasks was visibly more abundant at the lower herbicide concn. In work with *Sclerotium rolfsii* (15), respiratory activity was stimulated by trifluralin at 6.25 and 12.5 μg/g of soil, with inhibition at higher concn. Chopra (6) found that CO_2 production of *F. oxysporum* f. *vasinfectum* was increased by the herbicide prometryne [2,4-bis(isopropylamino)-6-methylmercapto-*s*-triazine] in soil only at a high concn (20 ppm). Also, sporulation increased at 20 and 80 ppm and was not significantly affected at lower concn.

Chlamydospore germination was greatly enhanced at a relatively low herbicide concn (2 μg/g) in both sterilized and nonsterilized soil. This further suggests that vegetative growth of the fungus was probably stimulated at low concn. The higher percentage germination obtained in diluted nonsterile soil as compared to sterile soil was contrary to expectation, since lower germination usually occurs in soil with living organisms. Chopra (6) found a direct inhibitory effect of prometryne on spore germination with increasing rates of the herbicide in sterilized soil; this could be altered to reflect a stimulatory effect by introducing individual antagonistic organisms into the soil prior to fungistasis tests. Such an effect might also have occurred with trifluralin.

The data for trifluralin on microbial populations cannot be directly related to the spore germination results, since the soil, though from the same source, was collected at a different time. The apparent stimulatory effect of low concn of the herbicide observed on spore production and germination was also evident for whole populations. In the case of fungi, this may suggest an increase in sporulation of the general fungal flora, since colonies obtained by the dilution-plate procedure develop principally from spores rather than from mycelia.

Throughout the literature one can find many statements to the effect that most herbicides do not persist more than a few weeks in soil and, therefore, do not cause lasting changes in the microflora. For fungal reproduction and spore germination, however, only a few hours of contact with a compound would be necessary to induce a significant response of an organism. Whereas this study indicates that trifluralin may enhance a pathogen in a controlled laboratory environment, it is recognized that many other ecological soil factors not yet studied may be expected to influence these interactions. The study does emphasize the possibility of a relationship between herbicide rate of application and inoculum density, and hence the potential for plant disease occurrence.

LITERATURE CITED

1. ALTMAN, J., & M. ROSS. 1967. Plant pathogens as a possible factor in unexpected preplant herbicide damage in sugarbeets. Plant Dis. Reptr. 51:86-88.
2. AUDUS, L. J. 1965. Herbicide behavior in soil. II. Interactions with soil microorganisms, p. 163-206. *In* L. J. Audus [ed.] The physiology and biochemistry of herbicides. Academic Press, N.Y.
3. BOLLEN, W. B. 1961. Interactions between pesticides and soil microorganisms. Annu. Rev. Microbiol. 15:69-92.
4. BUNT, J. S., & A. D. ROVIRA. 1955. Microbiological studies of some subantarctic soils. J. Soil Sci. 6:119-128.
5. CHANDLER, J. M., & P. W. SANTELMANN. 1968. Interactions of four herbicides with *Rhizoctonia solani* on seedling cotton. Weed Sci. 16:453-456.
6. CHOPRA, B. K. 1968. Effects of prometryne and biotic soil factors on growth and survival of *Fusarium oxysporum* f. sp. *vasinfectum*. Ph.D. Thesis, Auburn Univ., Ala. 124 p.
7. CURL, E. A., R. RODRIGUEZ-KABANA, & H. H. FUNDERBURK, JR. 1968. Influence of atrazine and varied carbon and nitrogen amendments on growth of *Sclerotium rolfsii* and *Trichoderma viride* in soil. Phytopathology 58:323-328.
8. FLETCHER, W. M. 1966. Herbicides and the bio-activity of the soil. Landbouwkundig Tijdschrift 78:274-281.
9. HUBER, D. M., C. I. SEELY, & R. D. WATSON. 1966. Effects of the herbicide diuron on foot rot of winter wheat. Plant Dis. Reptr. 50:852-854.
10. JOHNSON, L. F., E. A. CURL, J. H. BOND, & H. A. FRIBOURG. 1959. Methods for studying soil microflora-plant disease relationships. Burgess Pub. Co., Minneapolis, Minn. 178 p.
11. LINGAPPA, B. T., & J. L. LOCKWOOD. 1963. Direct assay of soil for fungistasis. Phytopathology 53:529-531.
12. PEEPLES, J. L., & E. A. CURL. 1969. Effect of paraquat, EPTC, and trifluralin on growth of *Fusarium oxysporum* f. sp. *vasinfectum* in liquid culture. Phytopathology 59:117 (Abstr.).
13. RICHARDSON, L. T. 1959. Effect of insecticides and herbicides applied to soil on the development of plant diseases. II. Early blight and *Fusarium* wilt of tomato. Can. J. Plant Sci. 39:30-38.
14. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1967. Effect of paraquat on growth of *Sclerotium rolfsii* in liquid culture and soil. Phytopathology 57:911-915.
15. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1969. Effect of trifluralin on growth of *Sclerotium rolfsii* in liquid culture and soil. Phytopathology 59:228-232.
16. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1968. Effect of atrazine on growth activity

of *Sclerotium rolfsii* and *Trichoderma viride* in soil. Can. J. Microbiol. 14:1283-1288.

17. Rodriguez-Kabana, R., E. A. Curl, & H. H. Funderburk, Jr. 1970. Effect of atrazine on growth of *Fusarium oxysporum* f. *vasinfectum*. Phytopathology 60:65-69.

18. Schmitthenner, A. F., & L. E. Williams. 1958. Methods for analysis of soil-borne pathogens and associated soil fungi. Ohio State Univ. Bot. Plant Pathol. Mimeo. Series No. 29.

19. Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa. 534 p.