Effect of Atrazine on Growth of Fusarium oxysporum f. sp. vasinfectum

R. Rodriguez-Kabana and E. A. Curl

Assistant Professor and Professor of Plant Pathology, respectively, Auburn University Agricultural Experiment Station, Auburn, Alabama 36830.

Supported in part by Research Grant No. 12-14-100-8015 from the Crops Protection Research Branch, ARS, and Grant No. 916-15-23 from the Cooperative State Research Service, USDA.

Accepted for publication 28 July 1969.

ABSTRACT

Effect of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) on growth of *Fusarium oxysporum* f. sp. *vasinfectum* was studied in Czapek's liquid medium and in sterilized soil containing 0, 8, 20, 40, and 80 µg of active herbicide per ml of medium or per g of soil. In liquid culture, exposure of the fungus to 40 or 80 µg atrazine retarded mycelial growth during the first 6 days of incubation, but not thereafter. Conductivity values and concentrations of nutrients in the medium were inversely proportional to mycelial growth. The ratio values for amount of mycelium produced to nutrients utilized, calculated 6 days after inoculation, were lowest for the two highest herbicide treatments. Production of CO_2-carbon increased significantly in soil cultures of the fungus with atrazine concentrations of 20, 40, and 80 µg/g. This stimulation was first evident after 6 days' incubation, and remained so until the last sampling at 14 days. Also, the ratios of amount of carbon evolved to nitrate-nitrogen consumed were significantly higher for the three highest herbicide treatments at the last sampling. Phytopathology 60:65-69.

Reviews by Audus (1), Bollen (3), and Fletcher (8, 9) have indicated that most herbicides at recommended field rates generally do not significantly alter soil microbial populations, but Smith et al. (25) found that effects may be either toxic or stimulatory on specific groups of organisms. Kaufman (13) found that *s*-triazine and phenylurea herbicides had significant quantitative and qualitative effects on fungal populations in corn- and soybean-cropped soils; these effects varied with the herbicide, application rate, and crop.

Relatively little attention has been given to the selective action of herbicides on specific organisms. Inhibition of several plant pathogenic fungi by herbicides has been demonstrated by other workers (2, 4, 14). In our laboratory, certain herbicides either inhibited or stimulated growth of *Sclerotium rolfsii* (6, 7, 20, 21, 22, 23), *Rhizoctonia solani* (19), *Fusarium oxysporum* f. sp. *vasinfectum* ([Atk.] Snyd. & Hans.) (5), and *Trichoderma viride* (7, 20, 23, 24). Richardson (16, 17) found that some herbicides and insecticides increased disease severity in plants, while others suppressed disease development. Huber et al. (11) observed a reduction in severity of root rot of winter wheat, caused by *Cercosporella herpotrichoides* and *R. solani,* after fall applications of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea].

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is used primarily for control of weeds in corn, but the cotton-wilt fungus (*F. oxysporum* f. sp. *vasinfectum*) may survive long periods in soil organic matter and come in contact with a compound applied any time. The following study is part of a continuing investigation of interactions between herbicides and soil-borne plant pathogenic fungi.

MATERIALS AND METHODS.—Technical grade atrazine for this study was supplied by Geigy Chemical Corporation. *F. oxysporum* f. sp. *vasinfectum* was isolated from diseased cotton in Alabama and maintained on potato-dextrose agar (PDA).

Liquid culture.—The procedures were essentially as outlined in a previous publication (20). A stock solution of atrazine was prepared in 95% ethanol (v/v), and quantities of this solution were added to 50 ml of Czapek's solution in 250-ml flasks to provide concentrations of 0, 8, 20, 40, and 80 µg/ml, and adjusted to pH 3.00 with 10% lactic acid (w/v). All flasks, including controls, contained the same quantity of alcohol.

Each flask of solution was inoculated with a 5-mm mycelial disc from a 3-day-old culture of the fungus. Stationary cultures were incubated at 27 C, and sampling was performed from 8 flasks/treatment at 3, 6, and 10 days after inoculation. Contents of individual flasks were passed through fine dacron gauze (0.2-mm opening) over perforated bottoms of tared aluminum weighing-cups, and the retained mycelium was washed, dried, and weighed. Filtrates from pairs of flasks were pooled and brought to original volume with sterile water; thus, each treatment was represented at each sampling date by four filtrate replicates and eight mycelial samples.

Glucose in the medium was determined by a modified Somogyi method as described by Nelson (15) for blood analysis. Inorganic phosphorus was determined by the vanadomolybdophosphoric yellow color method in a nitric acid system described by Jackson (12). Nitrate-nitrogen was determined by the nitrophenol-disulphonic acid procedure of Harper (10). Titratable acidity was determined by titrating 10 ml of the medium with 0.1 N NaOH to pH 9.00. Hydrogen ion concentration was measured with a Beckman H-2 meter, and conductivity determined by means of an Industrial Model RC 16 B2 Conductivity Bridge equipped with a conductivity cell (constant K=2).

Soil experiment.—A Norfolk sandy loam low in nutrients was used, the low nutrient status being desirable to avoid masking effects of added supplements. The soil was air-dried, screened through a 2-mm sieve, and lightly moistened. One hundred g of soil (oven-dry basis) were delivered to each of a series of 250-ml flasks fitted with two-hole No. 6 rubber stoppers; a short piece of glass tubing filled with loosely packed

cotton was inserted into each stopper hole to serve as a filter when the flasks were later attached to a CO_2-collecting assembly. The flasks of soil were autoclaved for 60 min at 121 C and again for 45 min 24 hr later. Eight 1-cm mycelial discs of the pathogen were chopped for 20 sec in a Monel semimicro blender with 50 ml of water, and 2 ml of the suspension were aseptically added to the soil surface of each flask. Twenty-four hr later each flask received 10 ml of a nutrient solution which provided (per g of oven-dry soil): 200 μg of P as K_2HPO_4; 800 μg of carbon as glucose; 120 μg of nitrogen as $NaNO_3$; and atrazine taken from the alcoholic stock solution. The solution was applied by pipetting uniformly over the soil surface. The herbicide concentrations were 0, 8, 20, 40, and 80 μg/g of soil, and the soil moisture after application was 60% of moisture-holding capacity. Each treatment was performed in duplicate, and the experiment was repeated.

Carbon dioxide evolved from the flasks was trapped in 1 N KOH solution and assayed 3, 6, and 14 days after inoculation; details of the procedure were described earlier (19). At termination of the CO_2 experiment, soil from the flasks was air-dried and ground in a mortar for further analyses.

Nitrate-nitrogen was determined by the phenyldisulphonic acid test described by Jackson (12). Glucose was determined by adding 10 g of soil to 50 ml of water, shaking the mixture for 20 min, and heating for 30 min at 75 C. The suspension was then filtered through Whatman No. 1 filter paper, and the filtrate was passed through an ion exchange column (10 mm diam and 10 ml bed volume) with a mixed-resin bed (Mallinckrodt, MB-3). The effluent was tested for reducing sugars according to Nelson (15).

All data were statistically analyzed by procedures for simple factorial experiments, and means were compared for significance by a sequential test outlined by Snedecor (26). All significant differences refer to the 0.01 level of probability unless otherwise stated.

RESULTS.—*Liquid culture.*—No significant differences between treatments were found for mycelial production (Fig. 1) 3 days after inoculation. Rate of growth (slope) increased between the first and second samplings, this interval initiating the logarithmic phase. The increase in growth rate was not identical in all treatments; rates for treatments of 40 and 80 μg were smaller than those for 0, 8, and 20 μg. The two highest herbicide treatments showed significantly reduced mycelial growth 6 days after inoculation. The final sampling showed increased growth rates for the 40- and 80-μg treatments that were higher than those for other treatments during the final 4 days of the experiment; consequently, no significant differences between the 80-μg and the other treatments were found. The 40-μg treatment still showed a significant ($P = .05$) but reduced difference from lower herbicide treatments.

Nutrient concentrations closely reflected mycelial growth. No significant differences were evidenced at the first sampling for glucose (Fig. 2), NO_3-N (Fig. 3), and P (Fig. 4). Significantly higher amounts of residual nutrients in the medium were recorded for the 40- and

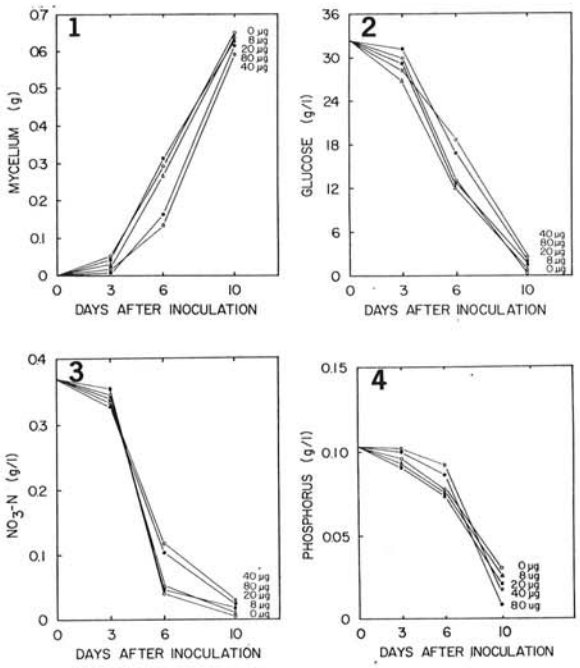

Fig. 1-4. Effect of different concentrations of atrazine on growth of *Fusarium oxysporum* f. sp. *vasinfectum* in liquid culture (○—○, 0 μg/g; △—△, 8 μg/g; ■—■, 20 μg/g; □—□, 40 μg/g; ●—●, 80 μg/g). **1)** Mycelial dry wt produced. **2)** Residual glucose in the medium, indicating quantity consumed. **3)** Residual NO_3-N in the medium. **4)** Residual inorganic P in the medium.

80-μg concentrations of herbicide 6 days after inoculation. Only the 40-μg treatment showed somewhat higher nutrient concentrations at the last sampling 10 days after inoculation. Economic coefficients, calculated at the final sampling, indicated a decrease in efficiency of utilization of glucose (Fig. 5), NO_3-N (Fig. 6), and P (Fig. 7) by the fungus, with increasing concentrations of the herbicide. This effect was particularly striking at the two highest concentrations used.

Conductivity of the medium (Fig. 8) decreased with growth of the fungus. Significant differences occurred after 6 days' incubation between the two highest atrazine levels and the other treatments. Differences at other sampling dates were not significant. Acidity of the culture medium (Fig. 9) decreased (pH increased) with time after inoculation. Although the same pattern of differences could be observed for pH as for other variables, these differences were less striking. Titratable acidity in treatments of 40 and 80 μg of atrazine was significantly different from other treatments 6 days after inoculation.

Soil experiment.—Little difference in CO_2 production (Fig. 10) was observed between treatments during the first 3 days. At the second sampling, 6 days after inoculation, significantly higher CO_2 evolution was associated with herbicide treatments than with the check. This divergency increased to the last sampling date for treatments of 20, 40, and 80 μg which produced significantly more CO_2 than the check. The value for

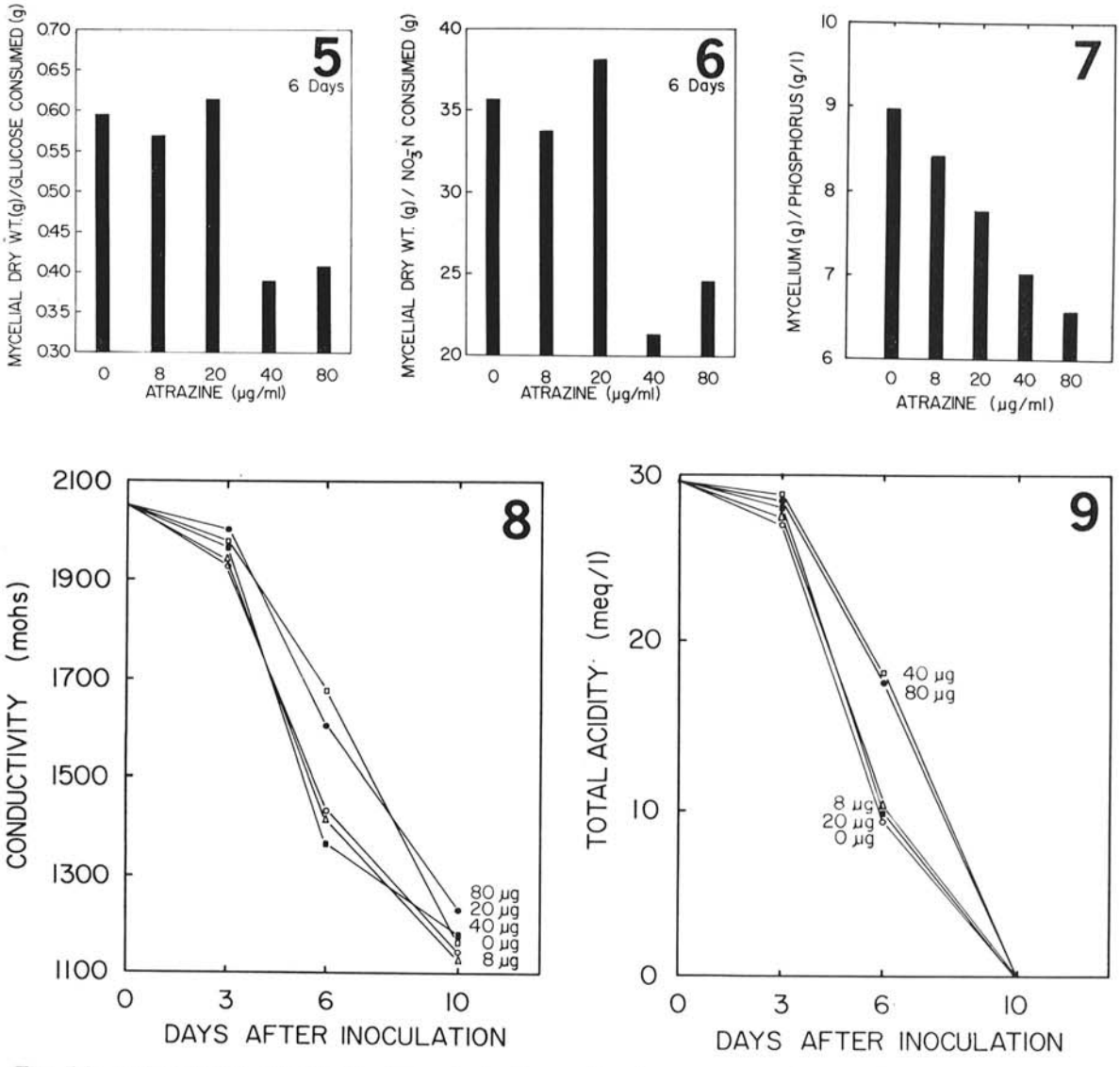

Fig. 5-9. Effect of different concentrations of atrazine on *Fusarium oxysporum* f. sp. *vasinfectum* in liquid culture (○—○, 0 µg/g; △—△, 8 µg/g; ■—■, 20 µg/g; □—□, 40 µg/g; ●—●, 80 µg/g). **5)** Ratio of mycelium produced to glucose consumed (economic coefficient). **6)** Ratio of mycelium to NO_3-N. **7)** Ratio of mycelium to P. **8)** Conductivity of the medium during growth. **9)** Titratable acidity produced.

the 8-µg treatment was similar to the check at the final sampling. The highest rates of CO_2 production occurred during the initial 6 days, then continued at slower rates during the remainder of the experiment.

Little difference among treatments was found in removal of glucose and NO_3-N by the fungus. Economic coefficients, relating CO_2-C evolved to each of these two nutritional variables (Fig. 11, 12), showed an increase with increased concentrations of the herbicide. The soil pH did not significantly vary among treatments.

Discussion.—Results from the liquid culture study revealed that *F. oxysporum* f. sp. *vasinfectum* was not as greatly affected by atrazine as two previously tested plant pathogenic fungi. Mycelial production by *R. solani* (21) was significantly reduced by atrazine concentrations of 10-70 µg/ml, and the effect persisted throughout an experimental period of 20 days. For *S. rolfsii* we found (20) that atrazine caused reduction in efficiency of nutrient utilization, particularly at high concentrations (40 and 80 µg/ml); this effect lasted throughout a 13-day experimental period. Thus the initial action of atrazine on *F. oxysporum* f. sp. *vasinfectum* and *S. rolfsii* is similar, in that concentrations of 40 and 80 µg are necessary to significantly affect mycelial production. However, action of the compound on the *Fusarium* species was not as persistent, since its retardation of mycelial growth at high concentrations (Fig. 1) had essentially disappeared by the time of final sampling. The effect of atrazine on *F. oxysporum*

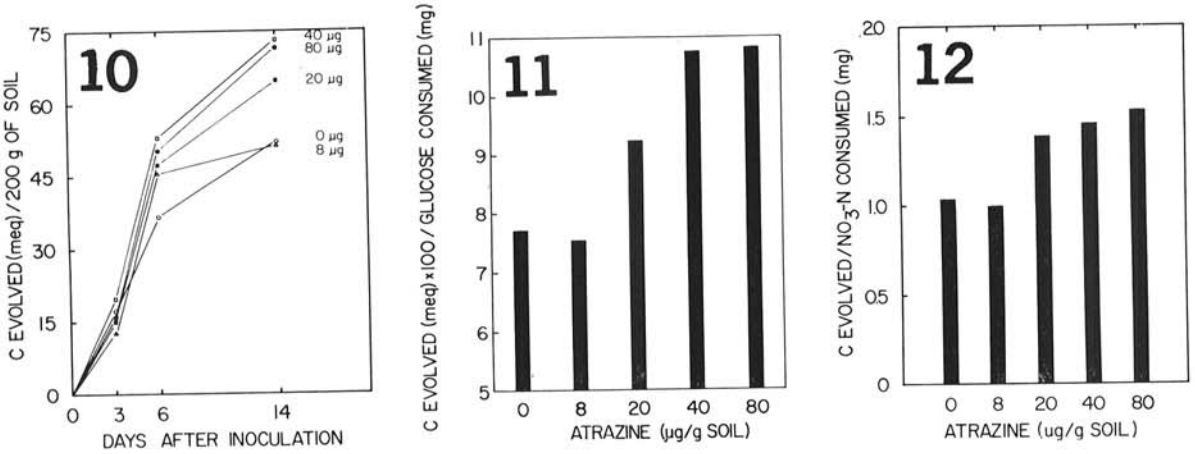

Fig. 10-12. Effect of different concentrations of atrazine on growth response of *F. oxysporum* f. sp. *vasinfectum* in soil culture (○—○, 0 µg/g; △—△, 8 µg/g; ■—■, 20 µg/g; □—□, 40 µg/g; ●—●, 80 µg/g). **10)** Evolution of CO_2-C with time. **11)** Ratio of C evolved to glucose consumed at 10 days. **12)** Ratio of C evolved to NO_3-N consumed.

f. sp. *vasinfectum* contrasts with that on *T. viride* (20), where herbicide treatments of 8, 20, 40, and 80 µg/ml resulted in increased mycelial production by the saprophyte with correspondingly higher economic coefficients.

The data on conductivity reflect ion removal from the medium and therefore parallel data for removal of inorganic P and NO_3-N.

Data from the soil experiment clearly indicate a stimulatory effect of atrazine concentrations above 8 µg/g on respiratory processes of *F. oxysporum* f. sp. *vasinfectum* (Fig. 10). This is particularly evident when the amount of CO_2-C evolved is related to the amount of nutrients consumed (Fig. 11, 12). This type of effect has been observed with this herbicide in soil cultures of other fungi (23). Atrazine induced a stimulatory action on CO_2 production by *S. rolfsii* at 8 µg/g of soil, but production declined with higher concentrations of herbicide. The herbicide also stimulated CO_2 production in soil by *T. viride* at concentrations above 8 µg/g.

Interpretation of the effect of atrazine in soil on this species of *Fusarium* hinges on the relationship between CO_2 production and growth. The liquid culture data indicate reduced mycelial formation at the two highest concentrations. If increased CO_2 production resulted from increased mycelial production, the effect of the herbicide in soil would be in direct opposition to its effect in liquid culture. It is more likely that the increased production of CO_2 is not accompanied by increased mycelial growth in soil, but results from some metabolic disturbance. Clarification of this question is currently being sought by more direct means (18) of determining actual mycelial growth in soil.

LITERATURE CITED

1. Audus, L. J. 1964. 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, New York.
2. Bain, D. C. 1961. Effect of various herbicides on some soil fungi in culture. Plant Dis. Reptr. 45:814-817.
3. Bollen, W. B. 1961. Interactions between pesticides and soil microorganisms. Annu. Rev. Microbiol. 15: 69-92.
4. Chappell, W. E., & L. I. Miller. 1956. The effects of certain herbicides on plant pathogens. Plant Dis. Reptr. 40:52-56.
5. Chopra, B. K. 1968. Effects of prometryne and biotic soil factors on growth and survival of *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. Ph.D. Thesis. 137 p. Auburn University, Auburn, Alabama.
6. Curl, E. A., R. Rodriguez-Kabana, & H. H. Funderburk, Jr. 1966. Effect of dipyridyl and toluidine herbicides on growth responses of *Sclerotium rolfsii* in soil. Phytopathology 57:7 (Abstr.)
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. 1960. The effects of herbicides on soil microorganisms, p. 20-62. *In* E. K. Woodford [ed.] Herbicides and the soil. Blackwell Sci. Pub., Oxford, England.
9. Fletcher, W. M. 1966. Herbicides and the bioactivity of the soil. Landbouwkundig Tijoschrift 78:274-281.
10. Harper, H. J. 1924. The accurate determination of nitrates in soil. Ind. Eng. Chem. 16:180-183.
11. Huber, D. M., C. I. Seeley, & R. D. Watson. 1966. Effects of the herbicide diuron on foot rot of winter wheat. Plant Dis. Reptr. 50:852-854.
12. Jackson, M. L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
13. Kaufman, D. D. 1964. Effects of *s*-triazine and phenylurea herbicides on soil fungi in corn- and soybean-cropped soil. Phytopathology 54:897 (Abstr.)
14. Millikan, D. F., & M. L. Fields. 1964. Influence of some representative herbicidal chemicals upon the growth of some soil fungi. Phytopathology 54:901 (Abstr.)
15. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375-380.
16. Richardson, L. T. 1957. Effect of insecticides and herbicides applied to the soil on the development of plant diseases. I. The seedling disease of barley

caused by *Helminthosporium sativum*. P. K. and B. Can. J. Plant Sci. 37:196-204.
17. 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.
18. RODRIGUEZ-KABANA, R., & E. A. CURL. 1968. Saccharase activity of *Sclerotium rolfsii* and the mechanism of antagonistic action by *Trichoderma viride*. Phytopathology 58:985-992.
19. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1966. Effect of four herbicides on growth of *Rhizoctonia solani*. Phytopathology 56:1332-1333.
20. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1967. Effect of atrazine on growth response of *Sclerotium rolfsii* and *Trichoderma viride*. Can. J. Microbiol. 13:1343-1348.
21. 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.
22. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1968. Effect of trifluralin on growth of *Sclerotium rolfsii* in liquid culture and soil. Phytopathology 59:228-232.
23. RODRIGUEZ-KABANA, R., E. A. CURL, & H. H. FUNDERBURK, JR. 1968. Effect of atrazine on growth of *Sclerotium rolfsii* and *Trichoderma viride* in soil. Can. J. Microbiol. 14:1283-1288.
24. SIKKA, H. C., R. W. COUCH, D. E. DAVIS, & H. H. FUNDERBURK, JR. 1965. Effect of atrazine on the growth and reproduction of soil fungi. Proc. SWC 18:616-622.
25. SMITH, N. R., V. T. DAWISON, & M. E. WENZEL. 1945. The effect of certain herbicides on soil microorganisms. Proc. Soil Sci. Soc. Amer. 10:197-201.
26. SNEDECOR, G. W. 1956. Statistical methods. Iowa State College Press, Ames.