Influence of Atrazine on the Severity of Fusarium Root Rot in Pea and Corn

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ABSTRACT

Populations of *Fusarium* in a loam soil amended in the laboratory of field with atrazine at 10, 30, and 100 μg/g were increased up to 4-fold over those in nonamended soil. A steamed greenhouse soil mixture artificially infested with conidia of *Fusarium solani* f. sp. *pisi* and amended with 2.5 to 25 μg/g atrazine had increased numbers of the pathogen at all concentrations. In soil artificially infested with either conidia or chlamydospores of *F. solani* f. sp. *pisi* or *F. roseum* f. sp. *cerealis* 'Culmorum', then amended with atrazine at 30 μg/g, incidence of pea root rot was three times, and of corn seedling blight twice, that in nonamended soil. Germination of macroconidia, growth of macroconidial germ tubes, and subsequent chlamydospore formation by both fungi, were enhanced on soil amended with 10 μg atrazine/g soil. Virulence of the pathogens was not enhanced by growing inoculum in media containing atrazine, nor were pea or corn plants predisposed to greater susceptibility by exposure to atrazine.

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Additional key words: *Fusarium solani* f. sp. *pisi*, *Fusarium roseum* f. sp. *cerealis* 'Culmorum'.

Increasing attention is being given to effects of herbicides on diseases caused by soil-inhabiting phytopathogens. This subject has been reviewed most recently by Katan and Eshel (12). Though there is little direct evidence that the widely used herbicide, atrazine, affects root diseases (8), evidence from pure culture work indicates that this herbicide may influence the growth or metabolic activity of several pathogens or saprophytes. Both stimulatory and inhibitory effects have been observed, depending on the fungus and atrazine concentrations used. At approximately 10 μg/ml, atrazine tended to stimulate growth (16,22) or glucose catabolism (23) of several fungi, or was without effect (16,17), whereas higher concentrations were often inhibitory (16,18,19). An exception was *Trichoderma viride*, whose growth was not inhibited in a liquid medium containing atrazine at concentrations up to 80 μg/ml (19). Numbers of sclerotia produced by *S. rolfsii* on potato-dextrose agar decreased with increasing concentration of atrazine, but this was partially offset by their increased size (1).

In sterile soil amended with carbon and/or nitrogen sources, and containing 12 μg/g atrazine, respiratory activity of *Sclerotium rolfsii* was reduced (3), and that of *T. viride* enhanced (3,20), compared to that in soil without atrazine. In sterile soil without carbon or nitrogen amendments, respiration of *S. rolfsii* was enhanced at 8 μg/g, but was reduced at 40 μg/g (20). Respiration of *Fusarium oxysporum* f. sp. *vasinfectum* in sterile soil was stimulated by atrazine above 8 μg/g (17). Numbers of *Fusarium* propagules were reduced in corn-cropped field soil treated with atrazine, whereas populations of certain fungi antagonistic to *Fusarium* were increased (10).

The present research deals with the effect of the herbicide atrazine in increasing incidence and severity of pea root rot and corn seedling blight caused by *Fusarium*, and with the mechanism of disease enhancement.

MATERIALS AND METHODS.—*Laboratory bioassays of effects of atrazine on fungi.*—Conover loam soil was collected from an area to which pesticides had not been recently applied. The soil was sieved (2-mm screen), and was either used immediately or stored in closed plastic containers at 15% moisture (35% WHC) at 24 C for 2-4 days. Unless otherwise indicated, atrazine, formulated as an 80% wettable powder (Aatrex 80-W) in aqueous suspensions was added to soil to give concentrations of actual atrazine ranging from 2.5 to 100 μg/g air-dried soil. A concentration of 2 μg/g is approximately equivalent to 1 kg/hectare or 1 lb/acre. Normal application rates in Michigan are 2-3 lb/acre. In some experiments, acetone solutions of technical grade atrazine (99.1%) or aqueous suspensions of substances other than atrazine used in the 80-WP formulation were used.

One isolate of each of the following fungi was used: *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (F.R. Jones) Snyd. & Hans., and *Fusarium roseum* (Lk. ex. Fr.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum'. *Fusarium* populations were determined by soil dilution using a selective medium (14). At given time intervals random 10 g (air-dried equivalents) samples from atrazine-treated and control soils were suspended in 95 ml aqueous 0.85% NaCl and shaken on a wrist action shaker for 10 minutes. One ml of a diluted (10^{-3}) soil suspension was mixed with 10-15 ml of the molten agar at 45 C in petri plates. Six plates were used for each treatment. Plates were incubated at 24 C and colony counts were made after 1 week.

Spore germination was determined on Conover loam and on water agar amended with atrazine. Macroconidia of *F. solani* and *F. roseum* from agar slants were washed three times by centrifugation at 2 C. The macroconidial pellet was resuspended in glass-distilled water, then adjusted to ca. 2.4×10^5 macroconidia/ml. Chlamydospores were produced by first germinating macroconidia on Nuclepore membranes on PDA for 24 hours, then floating the membranes bearing germlings on 0.03M Na_2SO_4 for 7 days (9). Samples (100 g) of soil, first air-dried to 8-10% moisture content, were amended with

10, 30, and 100 μg/g atrazine by thorough mixing in beakers before adding water to bring the moisture content to 25%. Each 100-g soil lot was then subdivided into three equal portions, placed in plastic petri plates (45 × 10 mm), and the surface smoothed. One-tenth ml of spore suspension was added to the surface of the soil plates and incubated at 24 C for 20 hours. The spores were stained with phenolic rose bengal, recovered with a plastic film, and observed microscopically (13); 300 spores were counted for each treatment. Some spores were allowed to remain in contact with the soil for 5 days to allow chlamydospore formation to occur.

Atrazine (technical) in acetone was incorporated into 250-ml lots of molten (50 C) 2.0% water agar at concentrations of 10, 30, and 100 μg/ml, and mixed with a magnetic stirrer for 2 hours to allow for acetone evaporation. The highest concentration of acetone in soil before evaporation was 2.0%. The agar was then poured into petri dishes and allowed to solidify. Macroconidia of *F. solani* and *F. roseum*, handled as previously described, were applied to the agar surface. The spores were stained with rose bengal after 20 hours incubation at 24 C and examined microscopically.

Greenhouse experiments.—The effects of atrazine on the incidence and severity of two *Fusarium* root rots were investigated. To infest soil, duplicate, 3-week-old cultures of *F. solani* and *F. roseum* in potato-dextrose agar (PDA) were homogenized with 50 ml distilled water. The inoculum suspension was mixed with 15 kg steamed greenhouse soil mixture of soil, coarse sand, and peat (1:1:1, v/v) using a rubber bulb fitted with a spray nozzle. The soil was either immediately amended with atrazine-80 WP, or first allowed to incubate at 24 C for 6 days to permit chlamydospore production. Fifty seeds of pea *(Pisum sativum* L. 'Little Marvel') or 25 of corn (*Zea mays* L. 'Michigan 555') were sown into each of four replicate flats of soil. In some experiments, seedlings were first germinated in sand or soil and then were transplanted into the amended soil.

Field experiment.—Conover loam at the Michigan State University farm was amended to contain 10, 30, and 100 μg/g atrazine. Atrazine was applied to the soil surface in late June, 1973, with a calibrated, hand-held, CO_2-powered sprayer. The herbicide was then mixed with the soil to a depth of 15.2 cm (6 inches) with a Rototiller. The soil had been previously plowed and harrowed, and thereafter was hand-weeded when necessary. The test plots were 1.8 × 6.1 m (6 × 20 ft). *Fusarium* populations were determined weekly for a period of three months. Random samples were taken from four locations in each plot at 2.5 cm below the soil surface, and were pooled for assay. Populations were estimated as described for the laboratory experiments.

RESULTS.—*Effect of atrazine on Fusarium populations.*—Populations of *Fusarium* were significantly increased in Conover loam soil amended with atrazine 80-WP in the laboratory (Fig. 1-A). The population levels at 30 and 100 μg/g reached a maximum at about day 9 then decreased slightly, but were stabilized by day 15. After 15 days, numbers in soil amended with 10, 30, and 100 μg/g were 2-, 4-, and 10-fold greater, respectively, than those in non-treated soil, and remained at these high levels through the 60th day ($P<0.05$).

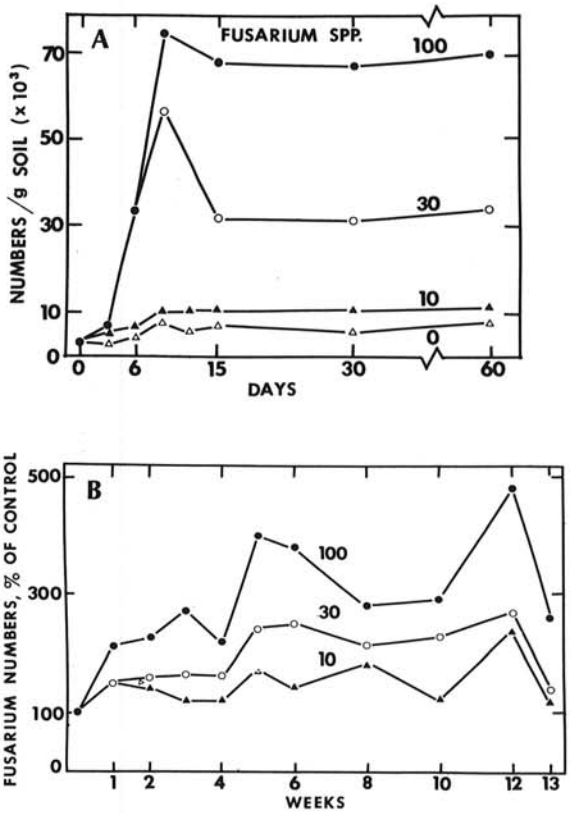

Fig. 1-(A, B). Effect of atrazine at 0, 10, 30, and 100 μg/g on numbers of *Fusarium* spp. in Conover loam soil as determined in dilution plates with a selective medium. **A)** Laboratory experiment. **B)** Field experiment.

The initial temporary stimulation of *Fusarium* at 30 and 100 μg/g (Fig. 1-A) was probably due to the formulants in the commercial 80-WP preparation. In other work (15), when these substances were applied in amounts equivalent to those found in 100 μg 80-WP/g, increases in total fungi, bacteria, and actinomycetes occurred. However, this stimulation was transitory, peaking at day 9. By day 15, populations had returned to the level of the untreated soil. In contrast, the stimulating effect of atrazine itself (technical) was first observed between days 9 and 15, and did not decrease during the 60 days of the experiments.

Fusarium populations in the field were also enhanced by atrazine. Mean increases over nonamended soil from day 7 through day 91 were 1.5-, 2-, and 3-fold with 10, 30, and 100 μg/g atrazine ($P<0.05$) (Fig. 1-B). Fluctuations in population levels were in part related to the moisture conditions of the soil at the time of sample collection. When soil moisture was high due to a recent rainfall, populations within a particular treatment period tended to increase, whereas under drought conditions they tended to decrease. However, some variability was not accounted for by soil moisture.

Soil previously infested with *F. solani* in the laboratory was treated with atrazine to give concentrations of 2.5, 5, 10, or 25 μg/g. After 6 days incubation at 24 C, populations of *F. solani* were significantly increased at all

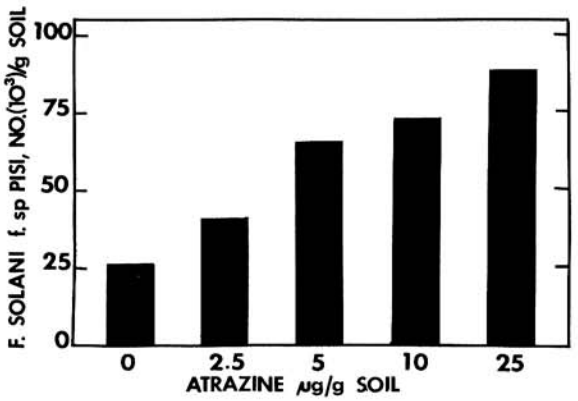

Fig. 2. Numbers of *Fusarium solani* f. sp. *pisi* propagules in artificially infested soil six days after amendment with atrazine.

concentrations, and in proportion to the concentration of herbicide (Fig. 2).

Effect of atrazine on severity of pea root rot and corn seedling blight.—*F. solani* macroconidia were mixed with steamed greenhouse soil and atrazine at 30 µg/g was immediately added. After 6 days incubation at 24 C, one volume of the treated soil was diluted with 9 volumes of nontreated steamed greenhouse soil to reduce the concentration of atrazine to 3 µg/g, a level nontoxic to pea (21). Soil amended with 30 µg/g atrazine, but without *F. solani*, soil amended with *F. solani* only, and nontreated steamed greenhouse soil also were diluted similarly.

Mortality of seedlings germinated in soil with only *F. solani* was 30% after 10 days, whereas it was 100% in soil amended with atrazine and *F. solani*. Three-day-old seedlings transplanted into soil containing only the pathogen had a disease loss of 10%. Mortality increased to 60% of seedlings transplanted into soil containing both the herbicide and pathogen. Severity of disease symptoms was also enhanced (Fig. 3). Seeds sown or seedlings transplanted into soil containing only atrazine were healthy. Another experiment gave similar results.

In similar experiments with macroconidia of *F. roseum* as inoculum, the incidence of corn seedling blight was increased in the presence of atrazine. Disease incidence was 73, 83, 95, and 100% in soil amended, respectively, with 5, 10, 30, and 100 µg/g, whereas soil infested only with the pathogen had 50% diseased plants. Corn plants grown in soil containing the fungus and herbicide were severely stunted and had more severely diseased roots than plants grown in soil containing only the fungus. Plants in soil containing only atrazine at the four concentrations, or nontreated steamed soil, showed no disease nor herbicide injury. The experiment was repeated twice with similar results.

Similar enhancement of both diseases occurred in

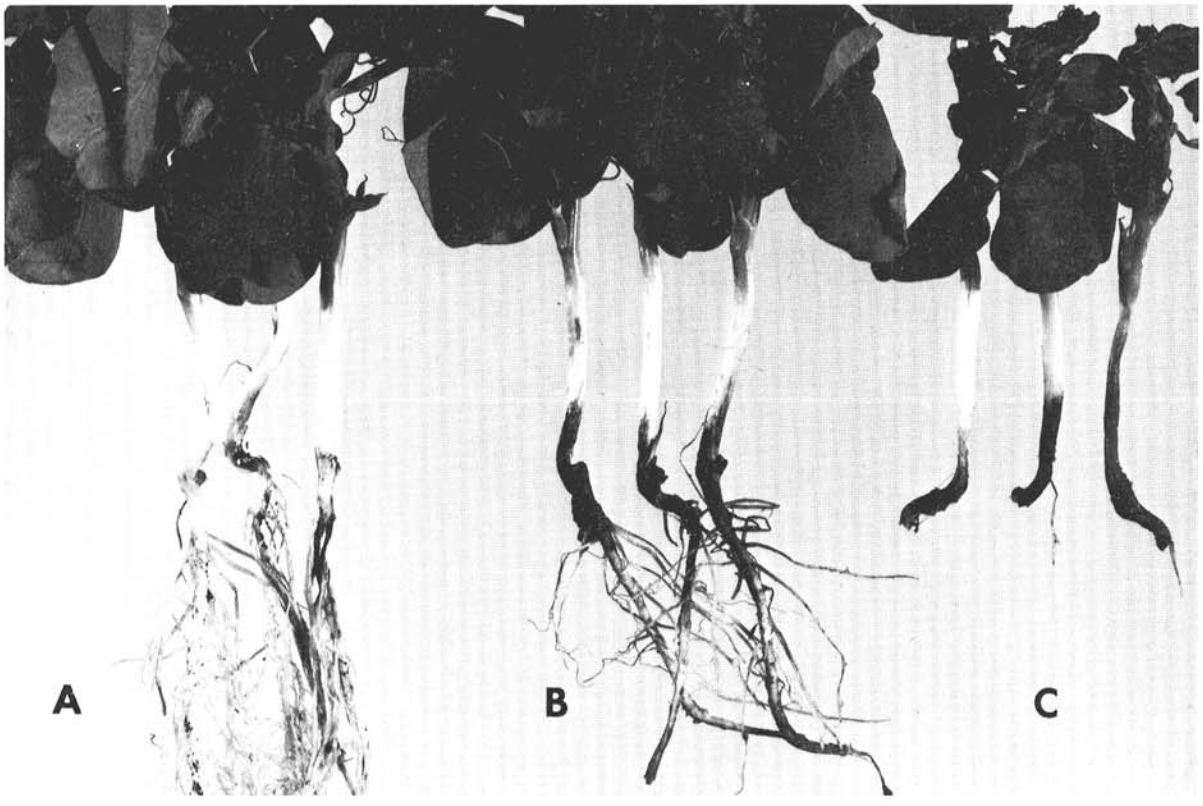

Fig. 3. Severity of root rot of pea in soil amended with **A)** 30 µg/g atrazine, **B)** *Fusarium solani* f. sp. *pisi*, and **C)** atrazine and *F. solani*.

three experiments when chlamydospore inoculum was used (Fig. 4).

Predisposition of pea and corn by atrazine.—Seeds of pea and corn were sown in flats of soil containing 3 and 30 μg/g atrazine, respectively. After 16 days, seedlings were transplanted into herbicide-free soil previously infested with either *F. solani* or *F. roseum*. In two such experiments, neither incidence nor severity of disease was increased as compared with plants transplanted from untreated soil into pathogen-infested soil.

Effect of atrazine on virulence of F. solani and F. roseum.—Cultures of *F. solani* and *F. roseum* were grown in potato-dextrose broth (PDB) containing 30 μg/ml technical atrazine. After 7 days, the cultures were washed on a sieve with running distilled water to remove the macroconidia, which were collected and used as the inoculum. Soil was infested by atomizing 2.5×10^7 conidia, with mixing, into each of four 15-kg lots of soil per treatment. Seeds of pea and corn were then sown into the infested soil. In three such experiments, neither disease incidence nor severity was enhanced as compared with inoculum prepared from cultures grown in PDB without atrazine.

Germination of macroconidia and subsequent chlamydospore formation.—Atrazine (80-WP) amendment at 0, 10, 30, and 100 μg/g soil resulted in markedly increased germination of macroconidia of *F. solani* and *F. roseum* on soil in three experiments. Germination was directly correlated with concentration of atrazine (Fig. 5-A, B). Soil amended with increasing concentrations of technical atrazine also gave correspondingly increased germination. Respective germ tube lengths for the four treatments after 20 hours were

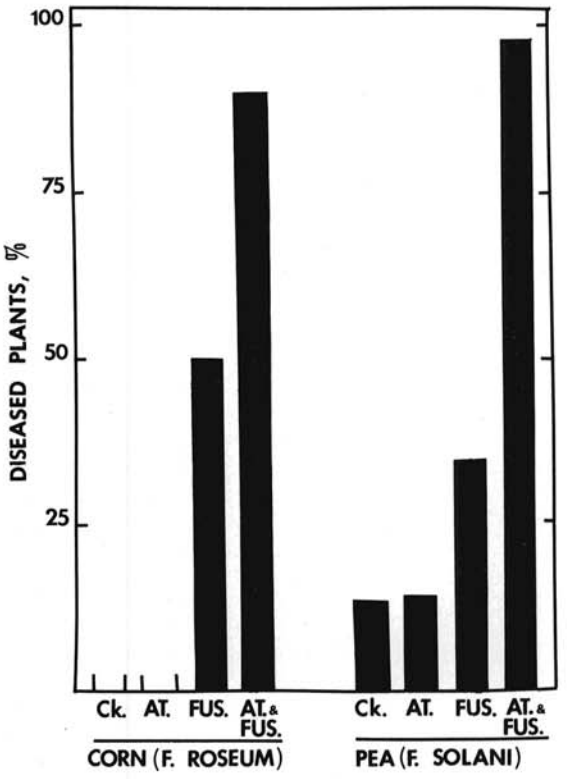

Fig. 4. Incidence of pea root rot and corn seedling blight in soil amended with 30 μg/g atrazine, and infested with chlamydospores of *Fusarium solani* f. sp. *pisi*, or *F. roseum* f. sp. *cerealis* 'Culmorum'. Ck. = Untreated control, AT. = Atrazine, FUS. = *Fusarium*.

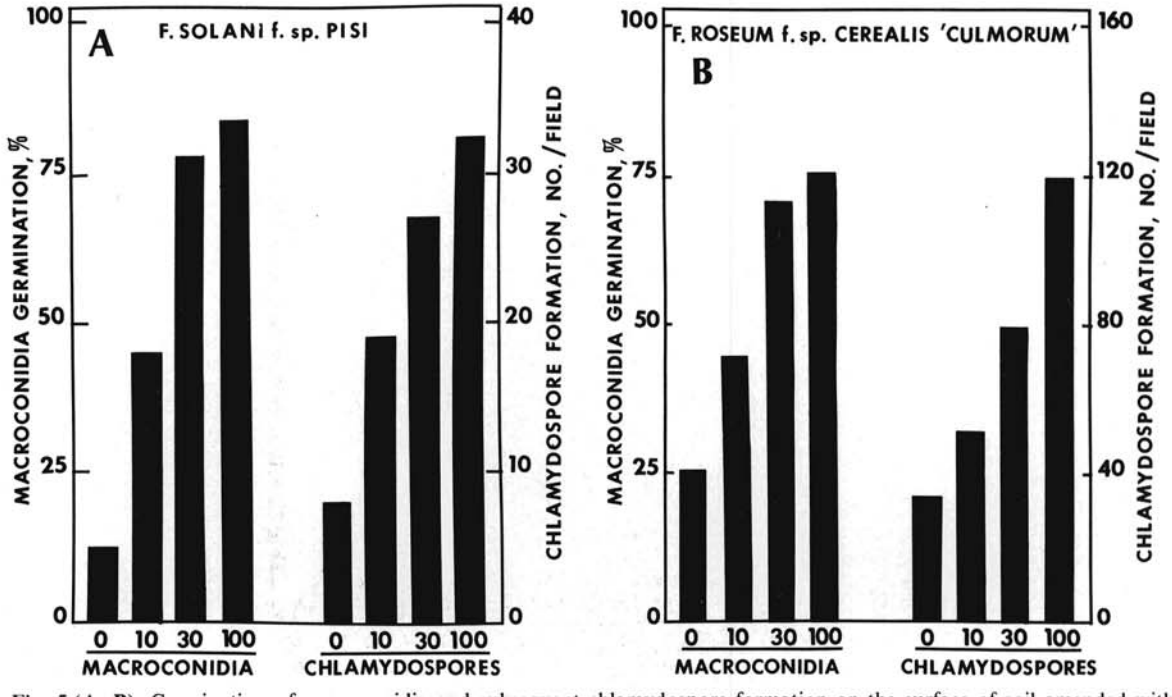

Fig. 5-(A, B). Germination of macroconidia and subsequent chlamydospore formation on the surface of soil amended with atrazine. **A)** *Fusarium solani* f. sp. *pisi*. **B)** *F. roseum* f. sp. *cerealis* 'Culmorum'.

15, 45, 65, and 172 μ for *F. solani*, and 60, 80, 120, and 160 μ for *F. roseum*.

Chlamydospore formation by both fungi was also more abundant on soil amended with atrazine (Fig. 5-A, B). The enhancement was also in proportion to the level of atrazine amendment.

Chlamydospores of *F. solani* were produced from germinated macroconidia by incubation in 0.03 M Na_2SO_4 (9). Triplicate membranes bearing the chlamydospores were transferred to petri dishes of soil containing 0, 10, 30, and 100 μg atrazine (technical)/g. Germination, based on counting 100 chlamydospores per membrane, was 8, 30, 72, and 80%, respectively. Germination of macroconidia of both fungi also was increased on water agar amended with technical atrazine at the same concentrations. Mean germination of *F. solani* was 43, 64, 71, and 80%, whereas corresponding germination by *F. roseum* was 23, 29, 58, and 71%, respectively.

DISCUSSION.—Propagules of *Fusarium* in a loam soil were increased in proportion to the amount of atrazine amendment. A concentration as low as 10 μg/g resulted in significant population increases which were sustained in both the laboratory and field for at least two or three months. This is a sufficient time to cover a normal growing season for many field and vegetable crops. Since chlamydospores are the chief survival spores of most *Fusarium* spp. in soil, any stimulation in their production by atrazine would be reflected in increased propagule levels for some period of time due to their longevity in soil. The possibility exists, therefore, that the use of atrazine coupled with continuous cropping of corn, or in corn rotation with other *Fusarium*-susceptible crops such as soybeans and vegetables, may result in increased inoculum potential. The concentrations of atrazine used in the present work were higher than most field rates, if one assumes that atrazine is uniformly distributed through the top several inches. However, in normal field operations atrazine is sprayed on the soil surface without incorporation, which would result in concentrations near the soil surface being in excess of some of those employed in these studies. Moreover, the levels of atrazine accumulating in organic matter in soil can exceed the application rate many-fold (5,7). The presence of such high concentrations of atrazine on colonizable organic micro-sites may have important ecological and biological significance for many pathogens.

Incidence and severity of pea root rot and corn seedling blight were increased in soil amended with atrazine. Katan and Eshel in a recent review (12), gave four possible mechanisms for disease increase due to herbicides: (i) direct stimulatory effects of herbicide on the pathogen, (ii) enhanced virulence of the pathogen, (iii) increased host susceptibility and (iv) effect on relationships between pathogens and other organisms favorable to the pathogen.

In the present work, atrazine stimulated macroconidial germination, germ tube growth, and subsequent chlamydospore formation in *F. solani* and *F. roseum* on natural soil and on agar. The degree of stimulation was directly correlated with concentration of atrazine. The increased numbers of chlamydospores formed may have resulted from the longer germ tubes, which would contribute more biological material for subsequent chlamydospore formation. Whether the *Fusarium* stimulation in this work was due to utilization of atrazine as an energy source was not studied. However, this possibility is suggested by the growth of several fungi, including *Fusarium roseum*, occurring on water agar containing atrazine at 10 μg/ml, whereas no growth occurred in the absence of atrazine (6). *F. roseum* was also shown to degrade ^{14}C-labelled atrazine to hydroxyatrazine and other unidentified products (2). Fungal utilization of the related triazine herbicide, simazine, as a nutrient is well-documented (11).

Evidence for increased virulence of pathogens resulting from exposure to herbicides is sparse (4). Our present work indicates that virulence of *F. solani* and *F. roseum* were not increased by growing the fungi in the presence of atrazine.

No predisposition of corn or pea plants by atrazine to *Fusarium* attack was found when corn or pea seedlings grown in atrazine-treated soil were later transplanted into herbicide-free soil infested with either pathogen. Since the transplanted plants were removed from association with atrazine, the possibility that atrazine has short-lived effects on susceptibility of the hosts was not completely eliminated.

Soil dilution plates indicated that *Fusarium* numbers increased with increasing concentrations of atrazine. In related, unpublished work, total fungi, actinomycetes, and bacteria also increased in atrazine-amended soil (15). Therefore, since atrazine was generally stimulatory to all three groups of soil microorganisms, it seems unlikely that disease enhancement was due to suppression of antagonists of *Fusarium*.

The most probable mechanism for the disease enhancement by atrazine occurring in the present research appears to be by direct stimulation of the pathogens.

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