The Nature of Control of Rhizoctonia solani by Pentachloronitrobenzene in Soil

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ABSTRACT

Beet seeds did not accumulate sufficient pentachloronitrobenzene (PCNB) from soil in 12 hr to protect them from infection by *Rhizoctonia solani*. The pathogenicity and population of *R. solani* in soil also were not affected by PCNB treatment. Direct microscopic observation of *R. solani* in soil indicates that control of *R. solani* by PCNB in soil is a consequence of strong suppression of growth of this fungus rather than destruction of it.

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Additional key words: selective medium, vertical-illumination microscope.

Pentachloronitrobenzene (PCNB) is a common soil fungicide used in the control of *Rhizoctonia solani* Kuehn, which causes root diseases of many economic crops throughout the world. However, because of the lack of adequate techniques, very little was known about the mode of action of this compound in soil. Recently, a selective medium for the quantitative determination of *R. solani* in soil (5) and a vertical-illumination microscope technique for direct observation of fungal activity on soil (4) were developed in our laboratory. With the help of these techniques, we studied the mechanism of control of *R. solani* by PCNB in soil.

MATERIALS AND METHODS.—*Rhizoctonia solani* was isolated from a diseased bean seedling and maintained on potato-dextrose agar (PDA). Soil was infested with *R. solani* which had been grown for 7 days at 26 C in sterilized soil (500 ml fine sandy loam soil plus 50 g chopped potato tubers). Natural soil was infested by mixing it with varying amounts of this inoculum preparation (hereafter referred to as "inoculum").

To determine the time required for infection of seeds by *R. solani*, 20 beet seeds (*Beta vulgaris* L. 'Detroit Dark Red') were planted in soil containing 1% (w/w) *R. solani* inoculum in a plastic container (125 X 125 X 35 mm). After 12- or 24-hr incubation, seeds in two containers were recovered, washed in running tap water for 5 min, surface-sterilized for 3 min in 0.8% sodium hypochlorite solution, and placed on a selective medium (five seeds/plate) (5). The number of beet seeds containing *R. solani* was determined microscopically after 48 hr. Sixty-six and 100% of the beet seeds were infected by *R. solani* within 12 and 24 hr, respectively. Consequently, a 12-hr period was used for all later experiments.

RESULTS AND DISCUSSION.—Bristow & Katan (2) reported that bean seedlings accumulated sufficient PCNB to partially protect them from infection by *R. solani* in 1 week. To determine whether beet seeds can accumulate PCNB to a sufficient level to protect them against *R. solani*, 20 seeds were preincubated in soil containing 100 μg/g PCNB (Terraclor, 75% wettable powder, Olin Chemical Co.). This concentration of PCNB was used in all experiments. Beet seeds preincubated in soil without PCNB were used as controls. After 12-hr incubation, seeds were recovered from soil, washed with running tap water for 5 min, and dried by blotting them with paper towels. Each set of 20 seeds was then planted in soil containing 1% *R. solani* inoculum. After 48-hr further incubation, the percentage of seeds infected by *R. solani* was determined as previously described. All beet seeds were infected regardless of PCNB treatment. Appparently, beet seeds were unable to accumulate a sufficient amount of PCNB in 12 hr to protect them from infection by *R. solani*.

Decreasing *R. solani* population by PCNB as a possible mechanism of controlling this pathogen was studied by the planting of 20 beet seeds in PCNB-treated soil which contained 1 or 5% *R. solani* inoculum. Germination of beet seeds was recorded after 10 days, and the population of *R. solani* was

TABLE 1. Effects of PCNB treatment of soil on pre-emergence damping-off of beets and on population of *Rhizoctonia solani*

Treatment of soil	Damping-off (%)	Population (No./g dry soil)
1% *R. solani*[a]	100	20
1% *R. solani* + PCNB[b]	4	18
5% *R. solani* + PCNB[b]	2	41

[a] Natural soil was infested by mixing it with 1 or 5% (w/w) *R. solani* inoculum prepared by growing *R. solani* for 7 days at 26 C in a sterilized mixture of 500 ml fine sandy loam and 50 g chopped potato tubers.

[b] Natural soil was amended to contain 100 μg/g pentachloronitrobenzene.

determined by the selective-medium method (5), using 0.1 g soil distributed in 10 clumps/plate, 15 plates for each treatment. In soil containing 5% *R. solani* inoculum, but without PCNB treatment, pre-emergence damping-off was 100% (Table 1). Percentage damping-off decreased to 4% when soil was treated with PCNB. Even in soil containing 5% *R. solani* inoculum, only 2% damping-off occurred in PCNB-treated soil. The population of *R. solani* at the end of the experiment for soils containing (i) 1% inoculum, (ii) 1% inoculum and PCNB, or (iii) 5% inoculum and PCNB was 20, 18, or 41 propagules/g dry soil, respectively. Since the population of *R. solani* in PCNB-treated soil was approximately the same or higher than that in nontreated soil, it is unlikely that PCNB controlled *R. solani* by decreasing its population in soil.

It has been reported that *R. solani* mycelium is able to accumulate PCNB from soil (3, 6). Therefore, the possibility that control of *R. solani* by PCNB in soil is due to a decrease in pathogenicity as a result of accumulation of PCNB was investigated. Ten sclerotia of *R. solani* were placed about 1 mm below the smoothed surface of untreated soil or soil treated with PCNB. After 12-hr incubation, sclerotia were recovered from soil with a needle, washed with distilled water, and placed in soil containing 20 beet seeds. After 48 hr, seeds were recovered, washed, surface-sterilized, and plated on the selective medium. The number of seeds containing *R. solani* was determined microscopically after 48 hr. Ninety-seven per cent of the beet seeds were infected by *R. solani* pretreated with PCNB, whereas 92% were infected by *R. solani* without PCNB treatment. Thus, control of *R. solani* by PCNB in soil is not due to a decrease in its pathogenicity.

Rhizoctonia solani is among the few fungi which

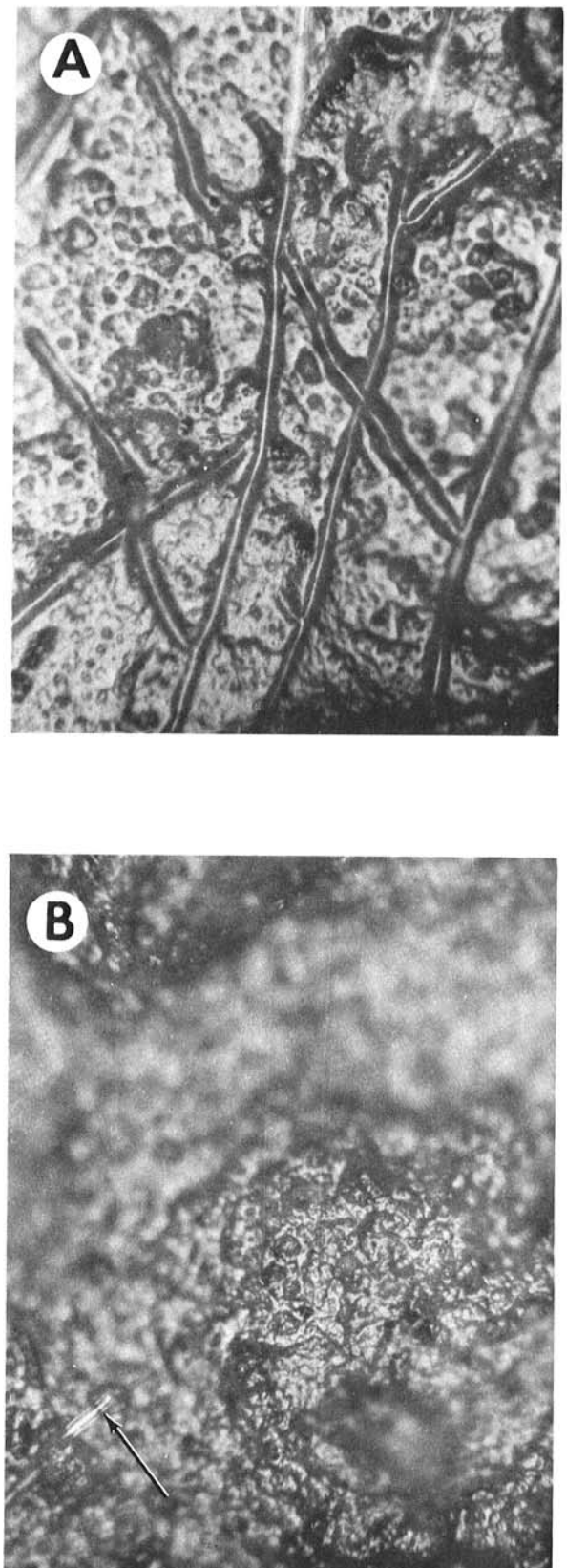

Fig. 1. Effect of pentachloronitrobenzene (PCNB) on germination of sclerotia and growth of germ tubes of *Rhizoctonia solani* on soil. **A)** Soil without PCNB treatment. **B)** Soil treated with 100 μg/g PCNB. Pictures were taken after 24-hr incubation from the edge of places (bottom) where sclerotia were embedded 1 mm below soil surface.

can grow in natural soil (1). We, therefore, studied the effect of PCNB on growth of germ tubes from sclerotia of *R. solani* in soil, and the possible relationship of this effect to the mechanism of control. The vertical-illumination microscope technique (4) was used for direct observation of growth of *R. solani* on soil. The microscope consisted of a Zeiss Universal microscope equipped with a Model II C vertical illuminator and 16X Epiplan objective. A heat reflection filter was used to reduce heat generated. Approximately 4 g of soil with or without PCNB treatment was placed on a glass slide and compressed, and the surface smoothed with a spatula to give a final soil mass of about 35 X 20 X 4 mm. Two sclerotia were embedded about 18 mm apart and 1 mm below the soil surface. Each slide was elevated on a neoprene matting in a petri dish containing 1 ml of distilled water. The mycelial growth on the soil surface from sclerotia beneath the surface was observed directly with the vertical-illumination microscope after incubation for 24 hr at 26 C. Three slides were used for each treatment, and the experiment was repeated twice. After incubation for 24 hr, the average number of germ tubes per sclerotium and the length of germ tubes were 3 and 318 μ, respectively, in PCNB-treated soil, and 80 and 3,436 μ in nontreated soil (Fig. 1). Twenty beet seeds were planted in soil containing 1, 0.1, 0.01, 0.001, or 0% *R. solani* inoculum to determine the relationship between amount of inoculum present in soil and the severity of the pre-emergence damping-off. Percentage germination of beet seeds was recorded after 10 days. The severity of pre-emergence damping-off of beets was directly correlated with the density of the inoculum. The percentage damping-off of beets in soil that contained 1, 0.1, 0.01, 0.001, and 0% *R. solani* inoculum was 100, 64, 24, 10, and 0, respectively. Therefore, control of *R. solani* by PCNB in soil is apparently due to the strong suppression of the growth of this fungus in soil.

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