Epidemiology of Rhizoctonia solani Pre-emergence Damping-off of Radish: Influence of Pentachloronitrobenzene

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ABSTRACT

Curves for the inoculum density-disease incidence relationship of *Rhizoctonia solani* preemergence damping-off of radish (*Raphanus sativus* 'Early Scarlet Globe') were parallel for different concentrations of pentachloronitrobenzene (PCNB). Position of the curves was significantly different with an ID_{50} value (inoculum density required for 50% incidence of disease) of 5.2 propagules/gram of soil (p/g) for 0 μgPCNB/g soil, 11.0 p/g for 7.5 μgPCNB/g soil, 26.0 p/g for12.5 μgPCNB/g soil and 50.0 p/g for 20 μgPCNB/g soil on a log-log basis. Synergism between *R. solani* propagules was suggested when transformations proposed for the inoculum density-disease incidence curve were used to analyze the data.

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Additional key words: infection court, spermosphere.

Increases in inoculum density of soil-borne plant pathogens usually result in greater disease until a plateau is reached (2). Mathematical models have been suggested to describe this inoculum density-disease curvilinear relationship (3). For models which could apply to *Rhizoctonia solani* Kuehn, a slope of 1.0 on a log-log basis would be expected if there was a rhizosphere effect while a rhizoplane effect should be implied by a 0.67 slope. When slopes greater than 1.0 are found, synergism between fungal propagules is suggested (2, 12). Linear regression analysis (2) of the data of Martinson (8) and Sneh et al. (11) suggested a rhizosphere relationship for diseases incited by *R. solani* although the effects of synergism (which could not be quantified) had to be assumed. Experiments were designed to quantify the inoculum density-disease relationships of *Rhizoctonia solani* preemergence damping-off, and show how a fungitoxin used in control affects the various transformations of data (2).

MATERIALS AND METHODS.— A loam soil (pH 7.9) collected near Fort Collins, Colorado, was infested with *Rhizoctonia solani* (isolate R-3) by culturing the pathogen on autoclaved lettuce leaves for 7 days, homogenizing the leaves in a Waring Blendor for 30 sec and adding the homogenate to the soil. Successive plantings of radish (*Raphanus sativus* L. 'Early Scarlet Globe') at 5-day intervals were made for about 20 days until a large population of *R. solani* was established. To achieve inoculum densities above 30 propagules/g of soil (p/g), chopped potato-soil cultures were used (7). Anastomosis tests with isolate R-3 placed it in AG-4 (9), commonly called the "praticola-type."

The infested soil was mixed thoroughly and its inoculum density determined by a modification of a technique of Ko and Hora (7). Instead of plating out small bits of moistened soil, a 10-g soil sample (soil moisture, −18 bars, matric potential) was diluted with water to concentrations between 1:1 and 1:20 (v/v). While the mixture was stirred on a magnetic stirrer to keep soil particles in suspension, a 0.1-ml aliquot was removed and deposited on the surface of the selective medium. Five drops of soil-water suspension, each containing 100 mg to 5 mg of soil/drop depending on dilution were distributed on each plate. Two 10-g samples were used for each determination and four plates were used per sample. The plates were incubated for 48 hr at room temperature and the five spots per plate were examined for the presence of typical *R. solani* hyphae with the 10 × microscope objective. The percentage of drops containing *R. solani* multiplied by the dilution factor was adjusted to the p/g (dry weight basis). The soil was then diluted with calculated quantities of similar noninfested soil to achieve the desired inoculum densities.

Pentachloronitrobenzene (PCNB) at 7.5, 12.5, or 20 μg active/g soil was added to soil containing various inoculum densities and mixed for 5 min in a twin-shell blender. After the soil was moistened to −0.7 bars matric potential (6), it was distributed into four 11.4-cm (4.5-inch) diam plastic pots, leveled, and 15 radish seeds/pot were placed on the soil surface so that seed spacing was ≥2 cm. A 2-cm layer of soil with the same inoculum density was spread over the seeds and the pot was covered with mylar film to prevent evaporation during the seed germination period. The pots were maintained at 20 C and radish emergence counts were made daily over a period of 3-5 days. Experiments were repeated twice.

Growth of isolate R-3 through soil at various PCNB concentrations was measured by placing severely infected radish seed (obtained by exposing the seed to a PDA culture of *R. solani* for 3-4 days and then air-drying) on the smoothed surface of noninfested soil in a petri dish. The seed and exposed soil surface were covered with mylar film followed by another layer of soil and the petri dish lid. The soil was moistened to −0.7 bars matric potential and incubated for 2 days at 20 C. The top layer of soil and mylar film was then removed. A dissecting microscope

was used to determine the extent of growth of *R. solani* hyphae through soil from the infected seed.

RESULTS AND DISCUSSION.— Sigmoid curves resulted from plotting inoculum densities and disease incidence (Fig. 1-A). As PCNB concentration increased, greater inoculum densities were required to achieve the same amount of preemergence damping-off. The intervals between the lowest inoculum density which would incite disease and the highest density required for maximum damping-off increased as the concentration of PCNB increased. For example, disease incidence increased from 15% to 98% with an inoculum increase of only 18 p/g at 7.5 μg/g PCNB. In contrast, an increase of 55 p/g was necessary for the same increase in disease incidence at 20 μg/g PCNB (Fig. 1-A).

The semilogarithmic (5), log-log (2, 3), and log-probit transformations (4) of the data suggested synergism between *R. solani* propagules in all treatments and the control. First, in the semilogarithmic transformation (Fig. 1-B) the plot of points of the higher levels of successful infections (computed from $\log_e \frac{1}{1-x}$) for 0 μg/g PCNB, 7.5, and 20 μg/g PCNB had their intercepts with the x axis displaced to the right of the origin (12). Secondly, slopes derived from linear regression analysis in the log-log transformation (2) were from 2.05–2.32 (Fig. 1-C) which were greater than the predicted value of 1.0 for this type of host pathogen relationship (3). Finally, slopes for the log-probit transformation (3.09–3.99, Fig. 1-D) exceeded the predicted value of 2 (10), indicating synergism.

As PCNB concentration increased, position of the inoculum-density vs. disease-incidence curve was shifted to the right in the log-log and log-probit analyses (Fig. 1-C, D). Interpolated ID_{50} value or inoculum density required to produce 50% disease incidence (0.693 on the y axis which is $\log_e \frac{1}{1-0.5}$) in the former transformation was 5.2 propagules/g for the control, 11.5 propagules/g for 7.5 μg/g PCNB, 26.0 propagules/g for 12.5 μg/g PCNB and 50.0 propagules/g for 20 μg/g PCNB. The ID_{50} values were similar for the log-probit transformation, 5.2 propagules/g for 12.5 μg/g PCNB and 46.0 propagules for 20 μg/g PCNB. According to analysis of covariance, with either transformation, position of the

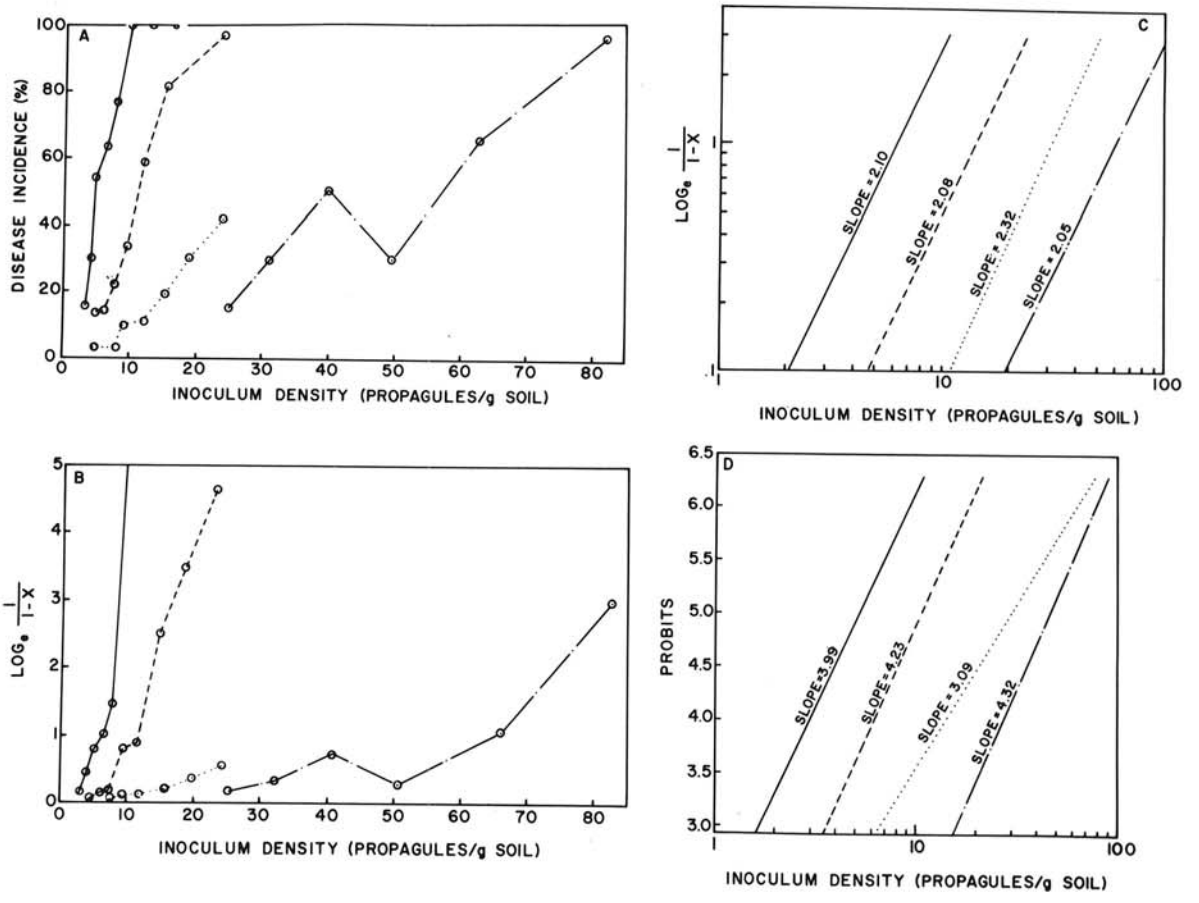

Fig. 1-A, B, C, D. Effect of pentachloronitrobenzene (PCNB) on inoculum density-disease incidence relationships for *Rhizoctonia solani* preemergence damping-off of radish. **A)** arithmetic plot; **B)** semilogarithmic transformation; **C)** log-log transformation; **D)** log-probit transformation. The correlation coefficients are significantly positive at the 5% level of significance in the latter two transformations. Legend: ———— = control; ---- = 7.5 μg PCNB/g soil; ····· 12.5 μg PCNB/g soil; ——— . ——— . 20 μg PCNB/g soil.

PCNB treatments, but not slope, was significantly different depending upon concentration of PCNB.

A direct correlation between increasing PCNB concentration and reduced growth of *R. solani* through soil was observed. For instance, growth of *R. solani* hyphae from an infected radish seed was 17.8 mm per day at 20 C, while values of 9.3 and 3.8 mm per day were measured for 7.5 and 20 μg/g PCNB, respectively. This fungistatic effect would result in fewer hyphae from an inoculum source reaching the infection court as more PCNB was added to soil. Even so, at the PCNB concentrations tested, the effects interpreted as synergism (slopes greater than 1.0 on a log-log basis) were still apparent. Visual observations were made of radish seeds in infested soil which might explain this phenomenon. When only a few hyphae of *R. solani* came under the influence of the host spermosphere, a massive proliferation of these hyphae over the seed coat was observed. The rate of hyphal proliferation was rapid enough to engulf the entire seed coat and emerging radicle in a mycelial sheath. The process could be interpreted as: (i) initial contacts between host and pathogen directly related to inoculum density and thus to the number of hyphae ultimately capable of touching the seed, and (ii) subsequent proliferation in which inoculum potential increases at an accelerated rate at the infection court. Thus the processes responsible for synergism operated in the spermosphere where exudates could at least partially overcome the influence of the applied fungistat and where there was opportunity for the pathogen to pool energy.

Because reduction in germination or viability of soil-borne propagules occurs when increasing concentrations of fungicide are applied, reduction in values of slopes of inoculum density-disease arithmetic plots of data has been predicted (1). Transformations (log-probit or log-log) of such data should result in a series of parallel lines: the relative positions of such lines being determined by the concentration of the fungicide. The hypothesis was also advanced that fungistatic substances like PCNB might reduce the effective range from the root, seed, or below-ground stem within which affected propagules could germinate, penetrate, and infect. In this situation, values of inoculum density-disease slopes for log-probit or log-log transformations should be reduced as the effective rhizosphere influence "shrinks" to a rhizoplane (3). Indeed, analyses of the data reported by Martinson (8), who exposed radishes to various inoculum densities of *R. solani* at different concentrations of PCNB, indicated a reduction in slope from 1.34 in the nontreated control to 0.92 when soil contained 10 ppm PCNB (2). However, at the concentrations used, our results indicate that, position but not slope of transformed inoculum density-disease curves was changed as a result of adding a fungistatic compound like PCNB to soil.

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