Influence of Soil on Inoculum Density-Disease Incidence Relationships of *Rhizoctonia solani*

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Accepted for publication 31 May 1987.

ABSTRACT

Kinsbursky, R. S., and Weinhold, A. R. 1988. Influence of soil on inoculum density-disease incidence relationships of *Rhizoctonia solani*. Phytopathology 78:127-130.

A method was developed to study inoculum density-disease incidence (ID-DI) relationships of *Rhizoctonia solani* in soil under controlled temperature and moisture conditions. ID-DI relationships were assessed by estimating disease in radish seedlings after 3 days in soil artificially infested with *R. solani*. There was a significant linear relationship ($P = 0.01$) between mean number of hypocotyl infections per plant and inoculum density for all soils tested except those in which disease incidence was very low. When 75 soils were compared, disease incidence in radish ranged in a continuum from 0 to 1.52 mean number of hypocotyl infections per plant ($\bar{x} = 0.51 \pm 0.32$). Analysis of variance indicated that sample mean differences were significantly different. Native inoculum densities of these soils ranged from 0 to 35.9 propagules per 100 g. There was no relationship between native inoculum density and DI values of artificially infested soils. A disease suppressive soil was identified, having a high native ID (30.0 propagules per 100 g) and a consistently low infection incidence. Four soils, when given heat treatments of 55 C for 1 hr, had DI values that increased in response to the treatment, the effect being greater in soils with low DI values.

Additional key word: suppressive soils.

Recently there has been considerable interest in soils suppressive to *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank) Donk). Henis et al (7) were able to induce suppression of *R. solani* by monocropping radish in the presence of the pathogen. Liu and Baker (10) attributed the mechanism of suppression to a buildup of the mycoparasite *Trichoderma harzianum* Rifai. Chet and Baker (3,4) established that suppression was more effective at a low pH and subsequently identified a naturally suppressive soil from Colombia with a high population level of *Trichoderma* spp. and low pH. Ko and Ho (8) identified several soils suppressive to *R. solani* in Hawaii by measuring mycelial growth rate in soil.

The objective of this study was to develop a procedure for measuring the extent that soil influences the inoculum potential of *R. solani*. This involved developing a system that could quantitatively evaluate inoculum density-disease incidence (ID-DI) relationships, yet be simple enough to be used in an extensive survey. When it was established that soil can suppress the capacity of *R. solani* to function as a pathogen, an investigation was begun to determine the mechanism of this effect.

MATERIALS AND METHODS

Soil collection. Soils used to develop the assay method were collected from five agricultural fields located in the San Joaquin Valley of California. Soil properties were analyzed by standard methods and are given in Table 1 (11). Approximately 20 kg of soil, taken with a shovel from the top 10-cm soil layer, were collected within a 9-m^2 area at each site. The samples were air dried and passed through a 2-mm-mesh sieve. For the soil survey, 15 soils each were collected from the Dixon (D), Salinas (S), Westley (W), Westside (WS), and Shafter (SH) areas of California. The inoculum density of *R. solani* was determined by a wet-screening procedure (12). Anastomosis group 4 was the only *Rhizoctonia* detected in these soils.

Description of the laboratory assay for ID-DI relationships. Two types of inoculum were used to investigate the relationship between inoculum density and disease incidence (ID-DI). Natural inoculum was obtained by wet-screening a field soil (W-5) heavily infested with *R. solani* (AG-4) and collecting the 0.50–2.00-mm plant debris fraction. Welch (13) determined that this fraction contained virtually all of the propagules of *R. solani* in assays from several California soils. The debris collected from soil W-5 contained 350 propagules of *R. solani* per gram dry weight. The inoculum was air dried and stored at room temperature. Laboratory inoculum was prepared by growing *R. solani* (AG-4, isolate R-24) on autoclaved millet seed for 2 wk. The colonized seed was air dried and ground in a Wiley mill using a 20-mesh screen. The millet inoculum was refrigerated because it was determined that inoculum efficiency decreased by 50% in 2 wk when stored at room temperature. The number of propagules per gram of inoculum was determined by plating out a weighed amount of millet inoculum on water agar.

Radish seeds (*Raphanus sativus* L. 'White Icicle') were planted in soils infested with various inoculum densities of *R. solani*. Brass rings (5.5 cm diameter × 3.0 cm height) placed on 0.5-bar ceramic pressure plate cells (Soil Moisture Equipment Corp., Santa Barbara, CA) were filled with soil to a height of 2.5 cm. Twelve radish seeds spaced 1 cm apart were placed 0.7 cm below the soil surface. The soils were saturated by placing the plates in water to a level just below the top of the rings. When water reached the soil surfaces, the plates and rings were placed in an extraction chamber. The plates were separated by corks (size #17) that were 0.7 cm taller than the rings. The extraction chamber had a capacity to hold five plates, each containing 12 rings. The extraction chamber was a modified pressure cooker (20.3 L liquid capacity) with an air inlet on the lid and five water outlets on the side wall each connected to one pressure plate cell inside. The chamber was placed in an incubator at 27 C. The applied air pressure was controlled by a nullmatic regulator (Moore Products Co., Spring House, PA) and measured using a mercury manometer. The system was considered to be in equilibrium when water ceased flowing from the outlets. Initial matric potential was adjusted to −0.15 bar during a 12–18-hr equilibration period.

After the equilibration, rings with soil were removed from the extraction chamber, and inverted Styrofoam cups with bottoms removed were fitted securely on the rings. The cups were covered with a sheet of Handi-Wrap plastic film (Dow Chemical Co., Indianapolis, IN) held in place with a rubber band. This was done to give seedlings additional space to grow and to reduce moisture loss. Rings were then placed in growth chambers at 27 C and a 12-hr photoperiod. Tests were terminated after 3 days to minimize secondary infection. The mean number of seedlings with a

hypocotyl lesion (DI) was determined and corrected for multiple infection (5).

Soil survey. A survey was done to determine the range and distribution of DI values in agricultural soils. Seventy-five soils (15 from each of five locations) were infested with 14 propagules of millet inoculum per 100 g of soil except for the Salinas soils, which were infested with 17 propagules per 100 g of soil. These inoculum densities were chosen at a level sufficient to show significant differences among DI values of artificially infested soils, but not so high that 100% infection would occur. Each group of 15 soils was tested simultaneously, and a reference soil was included as an internal standard for each test. The reference soil was arbitrarily given a DI value of 100 and adjusted DI values for soils were calculated based on a percentage of the reference DI value. Because of the logistics involved in doing a large number of samples, each soil was replicated only three times, except for the reference soil, which was replicated six times. Although precision of the method was reduced because of less replication, our primary concern was to be able to detect large differences in ID-DI values among soils.

Heat treatment of soils. Soils were moistened to −0.4 bar matric potential in the extraction chamber and allowed to equilibrate for 2 days. Samples of 250–300 g were placed in autoclavable bags and heated in a water bath for 1 hr at 55 C. Soil temperatures reached 55 C within a few minutes after immersion. After the treatments, soils were air dried and infested with enough millet inoculum to give 12 propagules per 100 g of soil, and disease incidence was determined after 3 days. Each treatment was replicated six times.

RESULTS

Laboratory assay for ID-DI relationships. ID-DI curves were constructed from data for four soils infested with the millet inoculum at densities of 0, 4, 7, and 14 propagules per 100 g (Fig. 1). The DI at each ID is the mean of six replications. ID-DI relationships were found to be linear and significant at $P = 0.01$. Additional ID-DI curves were constructed from 15 soils collected for the survey. ID-DI curves again were found to be linear at $P = 0.01$ (r values ranged between 0.51 and 0.86) for 10 of these soils, with slopes ranging between 0.22 and 0.88 mean number of hypocotyl infections per 10 propagules per 100 g of soil. Of the remaining five soils, all had slopes less than 0.18 mean number of hypocotyl infections per 10 propagules per 100 g of soil, and only one curve was linear at $P = 0.05$. Apparently at this level of replication, the mean differences of DI values at the four inoculum levels tested were too small to indicate a significant linear relationship. ID-DI curves intercepted the origin except for those soils that were naturally infested with *R. solani*. Deviations from the origin never exceeded 0.13 mean number of hypocotyl infections per 10 propagules per 100 g of soil of native inoculum and were often less. Preliminary tests had indicated that natural inoculum collected from different fields was considerably less aggressive than the millet seed inoculum.

Evaluation of laboratory assay. To evaluate the reliability of the laboratory disease assay, two soils, WS-7 and W-5, were assayed three times with six replications per assay. The soils were infested with enough millet inoculum to give an inoculum density of 14 propagules per 100 g. The mean number of hypocotyl infections per plant (at confidence limits, $P = 0.95$) and the coefficients of variation were 0.24 ± 0.14, 54%; 0.19 ± 0.12, 58%; and 0.13 ± 0.12, 84% for W-5 and 0.88 ± 0.40, 43%; 0.90 ± 0.45, 48%; and 0.96 ± 0.42, 42%, for WS-7. Thus, six replications were sufficient to indicate significant mean differences of DI values between these two soils.

Comparison of radish hypocotyl infection in five soils infested with native inoculum or millet seed inoculum. Soil W-5 was of particular interest because, not only was it quite suppressive to *R. solani* (Fig. 1), but in addition it had a native ID of 30 propagules per 100 g of soil. This raised the question whether the population of *R. solani* from this soil was weakly virulent or whether the soil was suppressing the parasitic activity of the fungus. To test this, four soils were infested with native inoculum screened from soil W-5 to give an ID of 30 propagules per 100 g of soil. For comparison, these soils were also infested with 14 propagules per 100 g of soil of millet inoculum. With both types of inoculum, the ranking of soils by the level of host infection was the same, and in both cases seedlings in WS-7 had disease levels that were significantly higher than W-5 or SH-13 (Table 2). When soil WS-7 was infested with inoculum that

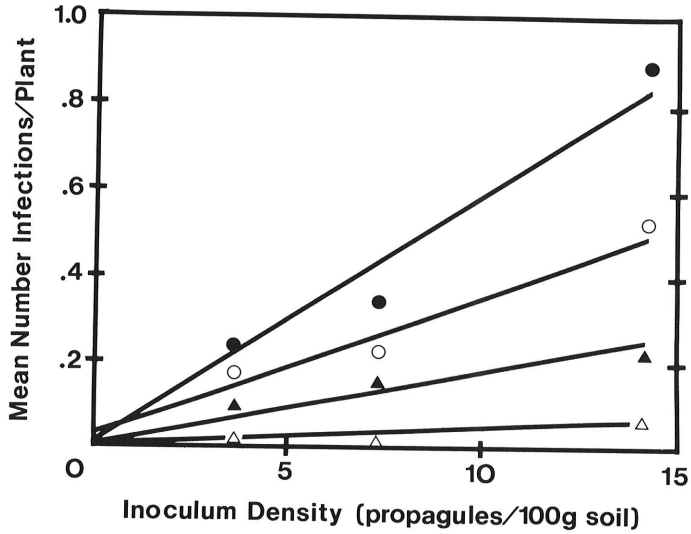

Fig. 1. Relationship between inoculum density of *Rhizoctonia solani* and mean number of hypocotyl infections per plant. Soils were infested with 0, 4, 7, and 14 propagules of millet seed inoculum per 100 g of soil. Infection incidence was determined 3 days after planting, and the data were transformed ($\ln 1/1-x$) to account for multiple infection. Each value is the mean of six replications. WS-7 (dark circle), W-14 (open circle), W-5 (dark triangle), and SH-13 (open triangle). Slopes of ID-DI curves and correlation coefficients were 0.60, 0.75; 0.33, 0.62; 0.15, 0.51; and 0.03, 0.49; respectively.

TABLE 1. Properties[a] of soils used in laboratory study on the inoculum density-disease incidence relationships of *Rhizoctonia solani* and radish

Soil	Type	pH	Cation exchange capacity (meq/100g)	Conductivity (mmhos/cm)	%C[b]	%N[c]	DTPA extractable micronutrient[d] (ppm)		
							Fe	Mn	Zn
W-5	Vernalis loam	7.7	20	0.75	0.52	0.07	3.2	10.3	1.6
W-14	Salado fine sandy loam	7.8	16	0.86	0.72	0.10	7.4	6.8	0.8
WS-7	Panoche clay loam	7.6	16	0.78	0.52	0.07	4.2	7.8	5.2
WS-14	Panoche clay loam	7.7	16	1.29	0.52	0.07	3.3	8.2	3.6
SH-13	Hanford sandy loam	7.7	8	2.74	0.28	0.04	2.2	5.2	0.9

[a] Soil properties were determined by standard procedures (11).
[b] Walkley-Black procedure for organic carbon.
[c] Kjeldahl method for total nitrogen.
[d] DTPA: diethylenetriamine penta acetic acid.

had been collected in a similar manner from other fields heavily infested with *R. solani*, disease levels were 5–10% higher, which was not significantly greater.

Soil survey. The results of the survey are summarized in Table 3 and Figure 2A. The survey revealed that within each region there was a wide range in the incidence of disease among soils resulting from a given level of added inoculum. Analysis of variance of the data indicated that there was a significant ($P < 0.01$) variance component between soils; its estimate was $\sigma^2_s = 0.05$, and $\sigma^2_e = 0.16$. This suggests that soil can have an appreciable influence on ID-DI relationships. Confidence limits around the overall mean were $CL_{95} = 0.51 \pm 0.46$. Eight of the 75 soils had DI values beyond (four each above and below) these limits. Estimated confidence limits, calculated on the basis of six replications per sample were $CL_{95} = 0.51 \pm 0.32$. On this basis, 18 soils would have DI values beyond (nine each above and below) these limits. Although mean DI values for the reference soil were not significantly different, among runs there was the possibility that these mean differences were in fact real. However, when DI value were calculated as a percentage of the reference DI value the same trend was apparent that DI values lie on a continuum over a wide range. There did not appear to be any regional influences, however, as the mean DI values for each region were not significantly different.

The differences in native inoculum densities of *R. solani* among regions were more dramatic (Fig. 2B). The mean inoculum densities of soil by regions (at confidence limits, $P = 0.95$) were Salinas 0.4 ± 0.4, Westside 1.5 ± 1.2, Shafter 4.4 ± 3.7, Dixon 9.6 ± 5.4, and Westley 12.1 ± 6.9 propagules per 100 g of soil. Of the 15 fields sampled in the Salinas area, *R. solani* was detected in only five fields, and the highest ID was 2.0 propagules per 100 g. By comparison, of the 15 fields sampled in the Westley area, only one field did not have a detectable level of the pathogen, and the highest ID was 34.7 propagules per 100 g.

When native ID was compared with DI values for the 75 soils in the laboratory assay, the correlation coefficient ($r = 0.12$) was not significant even at $P = 0.10$. Thus, it appears that native inoculum density was not related to the DI value of a soil.

The effect of heat treatment on soil suppression. The DI values of four soils were increased in response to a heat treatment of 55 C for 1 hr (Table 4). The increase in disease was significant for the three soils that had DI values (untreated) in the suppressive range. This suggests that a biological component may be involved in the suppression of *R. solani* in soil. Soil W-5 was given additional heat treatments at 40, 60, and 80 C. The mean numbers of infections/plant (at confidence limits, $P = 0.95$) were 0.32 ± 0.28, 1.46 ± 0.62, 0.88 ± 0.84, respectively, compared with 0.19 ± 0.13 for the untreated control.

TABLE 2. Effectiveness of natural inoculum from soil W-5 and millet inoculum on hypocotyl infection in radish in soils exhibiting a range of suppressiveness to *Rhizoctonia solani*

Soil[a]	Densities of native inoculum (propagules/100 g)	Mean infections/plant[b]	
		Millet inoculum	W-5 inoculum
W-5	30.7	0.16 ± 0.17	0.08 ± 0.07
SH-13	0	0.25 ± 0.16	0.09 ± 0.06
WS-14	4.7	0.42 ± 0.20	0.14 ± 0.15
W-14	3.3	0.67 ± 0.36	0.20 ± 0.11
WS-7	0.7	1.07 ± 0.64*[c]	0.29 ± 0.09*

[a] Soils were infested with 14 propagules per 100 g of millet inoculum or 30 propagules per 100 g of W-5 inoculum. Assays were done at 27 C and −0.15 bar matric potential.

[b] Values are the mean of six replications. Confidence limits at $P = 0.95$ are indicated. Data have been transformed by the equation $\ln 1/(1 - x)$ to account for multiple infection.

[c] * = Values are significantly different from W-5 and SH-13.

TABLE 3. Native inoculum densities of *Rhizoctonia solani* and radish hypocotyl infection values of representative soils from a survey of 75 California soils[a]

Soil	Native inoculum density (propagules/100 g)	Mean infections[b] per plant	Percent of reference soil[c]
WS-3	0	0.29 ± 0.20	35
S-13	0	1.83 ± 0.46	159
S-12	0.7	0.34 ± 0.16	30
D-11	1.3	1.24 ± 0.78	155
W-9	3.3	0.70 ± 0.12	61
D-3	6.7	0.03 ± 0.04	3
WS-15	7.0	0.76 ± 0.59	92
SH-1	12.7	0.19 ± 0.12	28
SH-15	13.3	0.96 ± 0.36	136
W-5	30.7	0.14 ± 0.07	12

[a] Soils were infested with 14 propagules of millet inoculum per 100 g of soil, except for Salinas (S) soils, which were infested with 17 propagules per 100 g. Assays were done at 27 C and −0.15 bar matric potential.

[b] Values are the mean of three replications with standard error. Data have been transformed by the equation $\ln 1/(1 - x)$ to account for multiple infection.

[c] A reference soil (WS-7) was used for comparison in each test of fifteen soils. The reference soil was arbitrarily given an infection rating of 100.

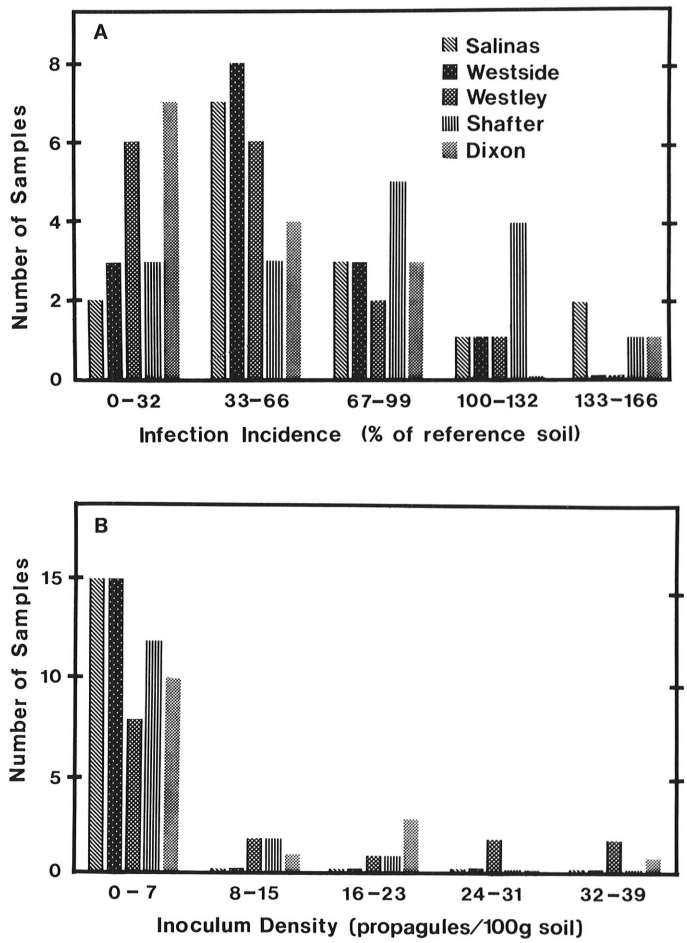

Fig. 2. A, Infection incidence and **B,** native inoculum densities of *Rhizoctonia solani* in agricultural soils from California. Seventy-five soil samples were collected in 15 fields from five different agricultural areas. Infection incidence data for radish seedlings were based on three replications with 12 seeds per replication. Inoculum levels were 14 propagules of millet inoculum per 100 g of soil except for Salinas soils, which were 17 propagules per 100 g of soil. Infection incidence values of Salinas soils were multiplied by a correction factor of 0.82 to account for the higher inoculum levels. Infection incidence was determined by comparing infection data with a reference soil (WS-7), which was arbitrarily given a value of 100. The reference soil was retested for each group of 15 soils tested.

TABLE 4. Influence of heat treatment of soil on suppression of radish hypocotyl infection by *Rhizoctonia solani*[a]

Soil	Mean infections/plant[b]	
	Control	55 C Treatment
SH-13	0.08 ± 0.05	0.49 ± 0.20
W-5	0.24 ± 0.12	0.76 ± 0.32
WS-14	0.23 ± 0.12	0.53 ± 0.12
W-14	0.58 ± 0.20	0.81 ± 0.37
WS-7	0.63 ± 0.24	...

[a] Soils were heated in a water bath for 1 hr at 55 C. After treatment soils were air dried, infested with millet inoculum at 12 propagules per 100 g. Assays were done at 27 C and −0.15 bar matric potential.

[b] Values are the mean of six replications. Confidence limits at $P = 0.95$ are indicated. Data have been transformed by the equation $\ln 1/(1 - x)$ to account for multiple infections.

DISCUSSION

This study has demonstrated that agricultural soils in California can have a dramatic effect on the pathogenic activity of *R. solani*. Whether measured as the slope of an ID-DI curve or as the DI at a given ID, disease incidence was found to lie on a continuum, with the extremes being significantly different.

The question may be asked whether soils with low DI values may be considered suppressive soils. We would advise caution in concluding this because nonsoil factors such as cropping history, agricultural chemicals, or seasonal fluctuations may have dramatic transitory effects on the behavior of the pathogen. For example, soil W-5 was consistently suppressive to the pathogen, whereas other soils, when sampled at different times, had host infection values that fluctuated along the disease incidence continuum.

Soil W-5 could clearly be characterized as a disease suppressive soil. It was found to have low DI values over several sampling periods, even though it had a high (30.7 propagules per 100 g of soil) native ID of *R. solani*. When a conducive soil was infested with inoculum from this soil, a significantly greater level of infection occurred (Table 2). Although population virulence may vary from field to field, this suggests that inoculum from soil W-5 is pathogenic, but factors in the soil are suppressing the efficiency of the pathogen. Heat treatments reduced the suppressive effect of soil W-5, suggesting that a biological factor may be involved in suppression.

The results of this study also indicate that there is not a close relationship between native inoculum densities and the laboratory ID-DI values. This may be quite significant from a disease management perspective, because it suggests that ID-DI relationships may be a more useful tool for disease prediction than inoculum density of the pathogen. Bouhot (2) has also suggested that inoculum density is not a good measure for disease prediction. The laboratory findings, however, have not been correlated with disease studies in the field. Therefore, conclusions regarding the use of the laboratory disease assay as a predictive tool is still premature. Nonsoil factors such as host plant-temperature interactions would have to be considered in any predictive assay for seedling diseases (1,6,9).

The assay system described above is easy to perform, requiring only small amounts of soil (~500 g/assay of six replications) and is done under conditions of controlled temperature and moisture. With further studies correlating infection levels of seedlings in the laboratory and field it could become a useful tool, providing growers with predictive information before planting that may improve the management of seedling disease.

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