Epidemiology of Rhizoctonia solani Preemergence Damping-Off of Radish: Survival

D. M. Benson and Ralph Baker

Former Research Assistant and Professor of Botany and Plant Pathology, respectively, Colorado State University, Fort Collins 80521. Present address of senior author: San Joaquin Valley Agricultural Research and Extension Center, University of California, Parlier 93648.

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ABSTRACT

When survival of *Rhizoctonia solani* was followed after infesting natural soil, a brief increase in density occurred occasionally during the first 1-2 days but, in most instances, this increase was followed by a rapid decrease in detectable propagules. Twelve days after infestation population counts were consistently low. Rates of inoculum survival following repeated planting of diseased radish seeds and seedlings in soil, or the addition of cultures of either cornmeal-sand mixture or chopped potato tubers to soil were not significantly different. Rates of population decline were similar at initial high (127 propagules/g), medium (85 propagules/g), or low (37 propagules/g) inoculum densities; the actual number of propagules surviving for 8-80 days being proportional to the amount of original inoculum added to soil. Survival of *R. solani* was greatest in cool dry soil. Soil pH had no significant effect on survival. Extrapolations for 50% survival (TS_{50}) and rate of decline were complementary when log-probit and semilogarithmic transformations were used. Quantitative analyses of the major epidemiological factors determining disease incidence for Rhizoctonia damping-off of radish are now possible.

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Additional key words: half-life equation, log-probit transformation, semilogarithmic transformation, *Raphanus sativus*, disease prediction.

Rhizoctonia solani Kühn apparently survives as sclerotia or thick-walled hyphae associated with plant debris (7). Detailed studies on population dynamics of survival amenable to quantitative analysis have not been done, however. Indeed, methods for concrete quantitative determination of populations of *R. solani* in soil have only recently been described (4, 13). Literature, reviewed by Baker and Martinson (3), also indicates variation in survival ability very often correlated with soil temperature and moisture content (8, 11, 12, 16, 17): cool dry soils favoring survival.

Mathematical equations and transformations have been proposed to describe survival of plant pathogens (2 9, 18). The half-life equation predicts the time required for death of 50% inoculum (18); however, since an initial lag phase may cause erroneous results when this equation is used, the graphical solution of survival data is recommended by Dimond and Horsfall (9). The lag phase results when multinucleate or multicellular survival structures are encountered.

Dimond and Horsfall (9) use the semilogarithmic transformation for survival data because the lag phase is accounted for and inoculum tends to die in soil at a logarithmic rate. The log-probit transformation (2, 6) has also been proposed for characterizing survival data. This transformation assumes that susceptibility of propagules to death follows a normal distribution in time. At least three advantages result when transformations are used for survival data (10). First, sigmoid-shaped survival curves are transformed to straight lines. Second, the time required for death of 50% inoculum can be interpolated. Third, the slopes of survival curves can be determined and compared among treatments.

The influence of pentachloronitrobenzene and temperature on the inoculum density-disease curve for preemergence damping-off of radish caused by *R. solani* has been reported (4, 5). The survival characteristics of *R. solani* in soil would establish the inoculum density available for inciting disease in radish populations over time. In this paper we use the semilogarithmic and log-probit transformations for analyzing effects of environmental factors on survival of *R. solani* in field soil.

MATERIALS AND METHODS.—Inoculum of *R. solani,* isolate R-3, was increased by successive planting of radish (*Raphanus sativus* var. 'Early Scarlet Globe') in artificially infested soil (SPS) (4), by the chopped potato-soil culture (CPS) method (13), or by the cornmeal-sand culture (CMS) method (14). The mixtures containing inoculum were thoroughly mixed in a twin shell blender with a loam field soil collected near Fort Collins, Colorado. Characteristics of this soil have been described (5). The infested soil (total 400 g) was then moistened and placed in quart jars fitted with a 1-mil thick mylar covering. Moisture potential was determined as previously described (4, 5). Jars were kept in the dark at 15, 20, 22, or 26 C (± 0.6 C). Two 10 g soil samples were withdrawn randomly at 2-day intervals for the first 20 days, followed thereafter by weekly sampling. Samples were assayed for *R. solani* as previously reported (4). Experiments were repeated twice and regression analysis was used to determine slope values of all curves in probit units/log unit in probit analysis and log I/I_0 units/unit of time (days) in the semilogarithmic analysis (10).

RESULTS.—*Effect of inoculum type on survival.*—Although equal amounts of either CPS inoculum or CMS inoculum were added to soil (−0.7 bars matric potential), different inoculum densities were noted at day 0 (Fig. 1-A). The use of CPS-15 inoculum (i.e., chopped potato-soil inoculum added at the rate of 15% by weight to 400 g soil) resulted in an initial inoculum density of 84 propagules/g soil which was about three times that found when CMS-15 inoculum (i.e., cornmeal-sand inoculum added at the rate of 15% to 400 g of soil) was used. Inoculum density 4 days after infestation and subsequent population levels in successive planting soil (designated SPS-100 in Fig. 1) were slightly higher than in soil infested with CMS-15 inoculum.

Slope values of the survival curves transformed to the log-probit transformation ranged from −2.30 for CPS-15 to −6.10 for SPS-100 (Fig. 1-B). Position of the curves was determined by interpolating a TS_{50} value (time required for 50% of the propagules to die in soil) at probit value 5.0. This value for CPS-15 inoculum was 11 days, while for CMS-15 inoculum and SPS-100 it was 2.6 and 9.4 days respectively. Positions of the survival curves but not slopes were significantly different ($P = 0.05$) by analysis of covariance.

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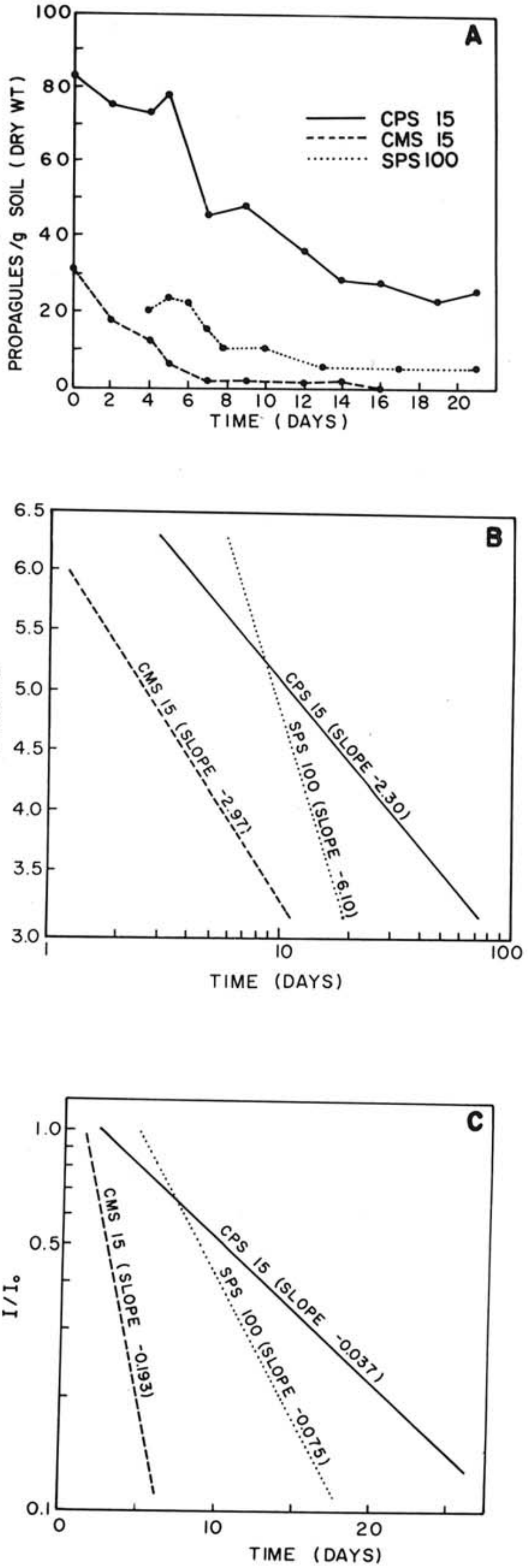

Fig. 1-(A-C). Effect of inoculum type on survival of *Rhizoctonia solani* in soil at 22 C and −0.7 bars matric potential: **A)** arithmetic plot; **B)** log-probit transformation; **C)** semilogarithmic transformation. Correlation coefficients are significantly positive ($P = 0.05$) in the transformations except CMS-15 in the log-probit transformation. CPS 15, chopped potato soil medium added equivalent to 15% (w/w) of raw soil; CMS 15, cornmeal-sand medium added equivalent to 15% (w/w) of raw soil; SPS 100, successive crops of radish planted and returned to soil to increase inoculum density.

Similar patterns were obtained using the semilogarithmic transformation (Fig. 1-C). Slope values ranged from −0.037 for CPS-15 inoculum to −0.193 for CMS-15 inoculum; TS_{50} values were similar to those for the log-probit transformation (Fig. 1-B). Since initial inoculum levels in soil were greatest and thus more easily followed using CPS inoculum of *R. solani,* it was used in the following experiments.

Effect of the amount of CPS inoculum on survival.—When CPS inoculum of *R. solani* was mixed with field soil (−0.7 bars matric potential) at the rate of 50, 15, and 4.5% by weight, the initial inoculum density was 127, 85, and 37 propagules/g soil, respectively (Fig. 2-A). Regression analysis of the three survival curves for the first 14 days transformed to the log-probit transformation (Fig. 2-B) gave slope values ranging from −2.30 for CPS-15 to −4.48 for CPS-50. Survival curves were parallel with either the log-probit or semilogarithmic transformation (Fig. 2-B, C) when compared by analysis of covariance ($P > 0.05$). Positions of the survival curves also were not significantly different; TS_{50} values for *R. solani* were 8.5 days CPS-50 inoculum, 11.0 days for CPS-15 inoculum, and 7.6 days for CPS-4.5 inoculum. After 14-16 days the inoculum density at each inoculum level remained fairly uniform for the next 66 days. These persistent survival values over time had significantly different means ($P < 0.01$) of 49 propagules/g soil, 31 p/g soil, and 9.5 p/g soil, for CPS-50, CPS-15, and CPS-4.5 inoculum, respectively.

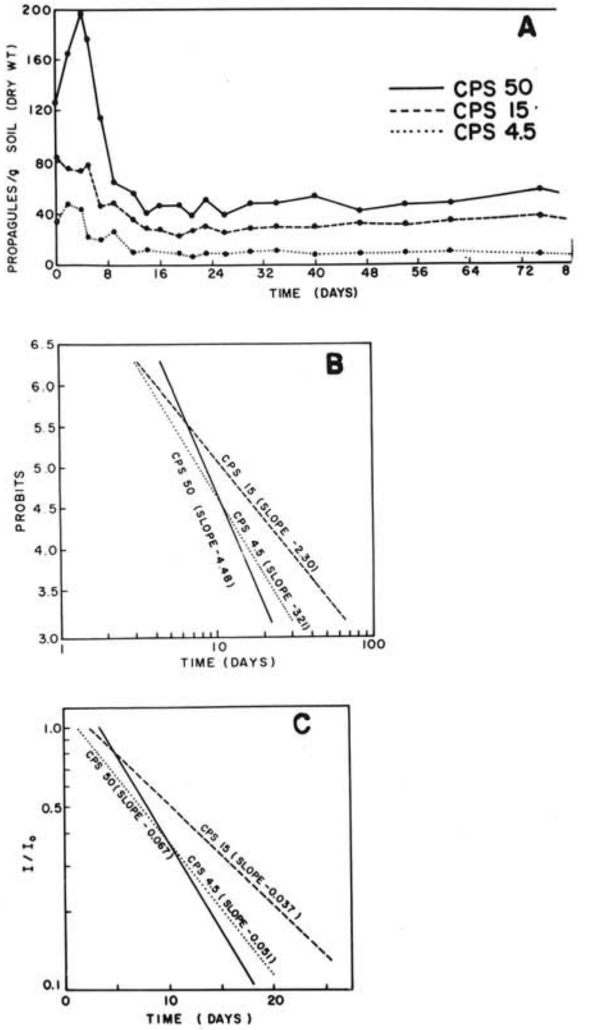

Fig. 2-(A-C). Effect of the initial amount of CPS inoculum on survival of *Rhizoctonia solani* in soil at 22 C and −0.7 bars matric potential. Chopped potato soil medium added equivalent to 50% (CPS 50), 15% (CPS 15), and 4.5% (CPS 4.5) (w/w) of soil. **A)** arithmetic plot; **B)** log-probit transformation; **C)** semilogarithmic transformation. Correlation coefficients are significantly positive ($P = 0.05$) in the transformations.

Effect of soil moisture on survival.—Air-dry soil (−540 bars matric potential) was mixed with an equal portion of CPS inoculum and moistened to either −18.0 or −0.7 bars matric potential. Samples were maintained at 22 C. At −18 or −0.7 bars, there was an initial increase in colony counts followed by a rapid decrease 2-8 days after the addition of inoculum to soil (Fig. 3-A). After 9-10 days, however, inoculum densities remained relatively constant. At −540 bars the initial fluctuations were not apparent and inoculum persisted at a relatively constant level.

Survival curves were not parallel when either semilogarithmic or log-probit transformations were used (Fig. 3-B,C). The slope values (−0.008 and −1.80 respectively) for survival at −540 bars were the lowest observed in any experiment.

TS_{50} values were 39.0 days (derived by extrapolation) at −540 bars, 8.5 days at −18 bars, and 8.0 days at −0.7 bars, using the semilogarithmic transformation. These values were 40.0 (extrapolated), 8.5, and 8.5 days respectively, using the log-probit transformation.

Effect of soil temperature on survival.—CPS inoculum was mixed with an equal portion of soil, adjusted to −0.7 bars matric potential, and maintained at either 15, 22, or 26 C. Typical curves, in which survival values initially decreased rapidly followed by a relatively stable population count, were found at the three temperatures (Fig. 4-A).

Slope values for the survival curves at 15, 22, and 26 C were significantly different ($P < 0.05$) when either transformation was used (Fig. 4-B,C). The lowest slope value was −0.025 at 15 C, while the greatest was −0.067 at 22 C (Fig. 4-C). The TS_{50} values for *R. solani* survival at 15, 22, and 26 C were 12.5, 8.0, and 4.6 days, respectively.

Effect of soil pH on survival of R. solani.—Soil pH was altered from 7.9 to either 6.3 or 6.8 with aluminum sulfate. Soil was infested with equal quantities of CPS inoculum and incubated at 22 C. The initial inoculum density at the beginning of the experiment varied from 69-83 propagules/g soil and gradually declined to 25-35 propagules/g soil at termination of the experiment (Fig. 5-A). No change in pH was detected at the end of the experiment.

Slope values ranged from −1.90 at pH 6.3 to −3.26 at pH 7.9 using the log-probit transformation (Fig. 5-B). These were not significantly different ($P > 0.05$). Similar TS_{50} values of 15.0, 11.0, and 10.5 days at pH 6.3, 6.8, and 7.9, respectively, were found with either transformation (Fig. 5-B,C).

DISCUSSION.—Slope values of survival curves were not significantly different between propagules increased in a chopped potato-soil medium and propagules produced by the more "natural" method of repeatedly growing and returning the parasitized host to soil (Fig. 1). Thus, survival characteristics of propagules of *R. solani* added to raw soil using the CPS method should simulate natural conditions.

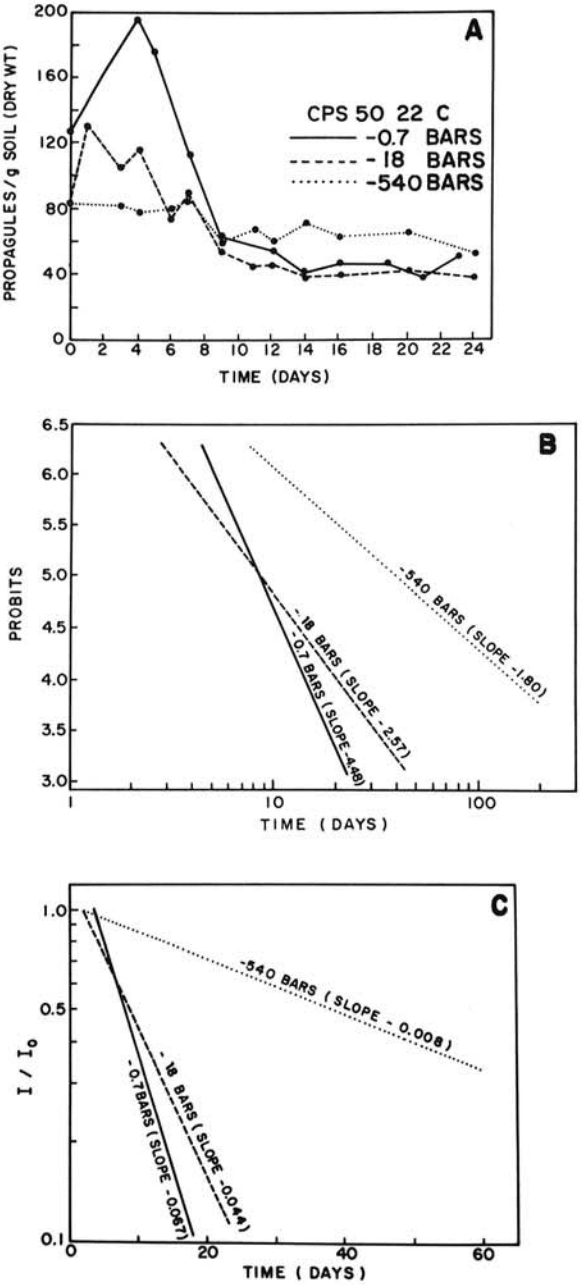

Fig. 3-(A-C). Effect of moisture on survival of *Rhizoctonia solani* in soil at 22 C. **A**) arithmetic plot; **B**) log-probit transformation; **C**) semilogarithmic transformation. The correlation coefficients are significantly positive ($P = 0.05$) in the transformations.

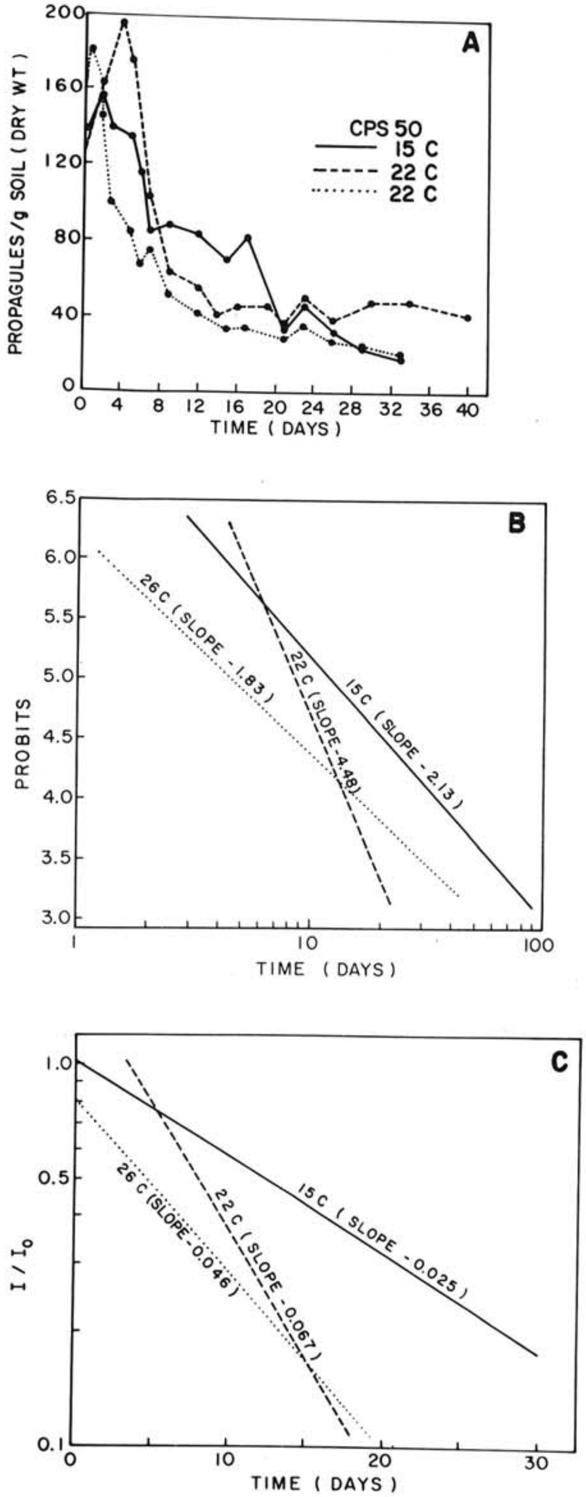

Fig. 4-(A-C). Effect of temp on survival of *Rhizoctonia solani* in soil at −0.7 bars matric potential. **A**) arithmetic plot; **B**) log-probit transformation; **C**) semilogarithmic transformation. Correlation coefficients are significantly positive ($P = 0.05$) in the transformations.

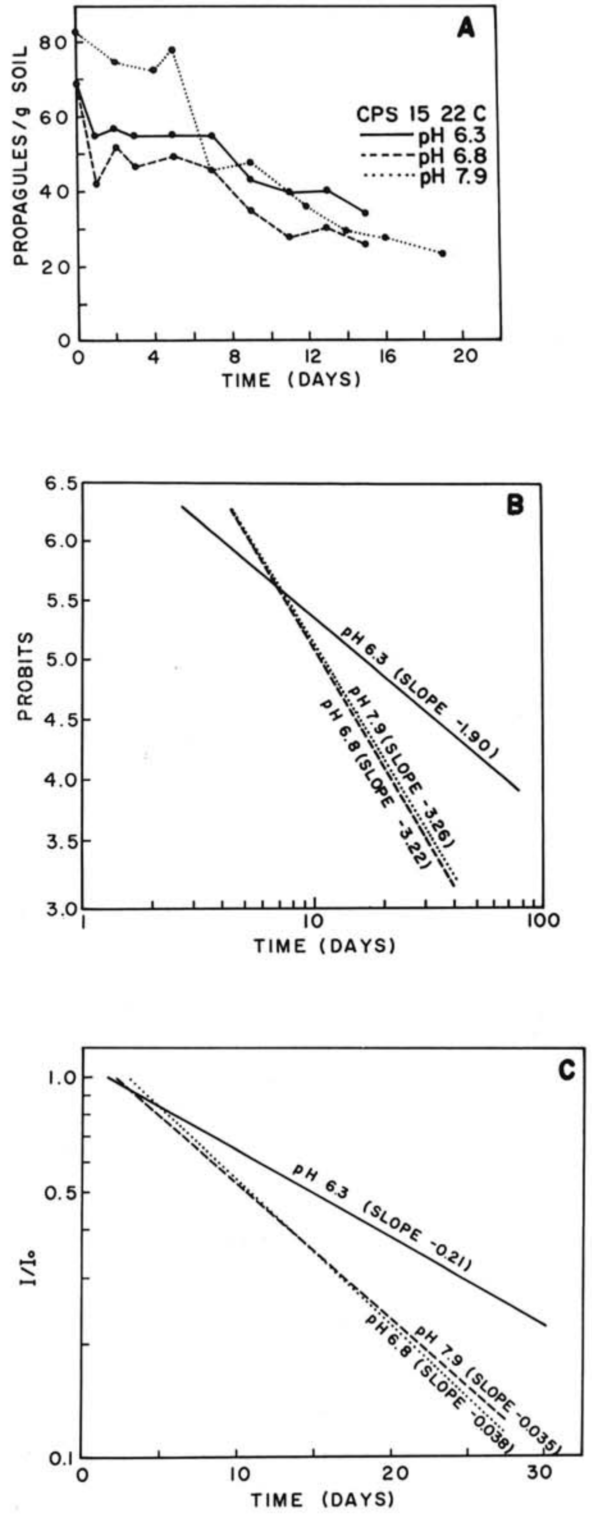

Fig. 5-(A-C). Effect of pH on survival of *Rhizoctonia solani* in soil at 22 C and −0.7 bars matric potential. **A)** arithmetic plot; **B)** log-probit transformation; **C)** semilogarithmic transformation. Correlation coefficients are significantly positive ($P = 0.05$) in the transformations.

Colony counts often increased 2-4 days following infestation especially when inoculum was added to soil which had just been moistened (Fig. 3) or when high proportions of inoculum including culture substrate were incorporated in soil (Fig. 2). Apparently this increase in population of *R. solani* was related to its well-known capacity for saprophytic growth through soil (1, 3, 15).

Upon further incubation of infested soil, colony counts declined rapidly except at very negative moisture potential (Fig. 3). After 7-14 days, the propagule population remained relatively uniform indicating persistent and long term survival. This phenomenon can be explained by the assumptions of the log-probit transformation that the ability of propagules to survive follows a normal distribution. Evidence for this can be seen in Fig. 2. The number of persistent propagules was proportional to the original amount of inoculum added: ratios of initial inoculum densities detected to average number of propagules surviving from 14 to over 80 days being 0.37 for heavily infested soil (CPS 50), 0.36 for moderately infested soil (CPS 50), and 0.28 for the lowest amount of inoculum added (CPS 4.5). Thus, the inoculum level added initially did not affect the rate at which propagules of *R. solani* became inactive in soil (i.e., slope values were not significantly different) but it did affect the ultimate inoculum density maintained by the fungus; a higher number of resistant propagules was added proportionately with higher initial densities of added inoculum.

Lowest slope values for survival were observed in dry soil with very negative matric potential (Fig. 3). The TS_{50} value was relatively high and thus the matric potential of −540 bars (ca. 2.5% soil moisture) was most favorable for survival of the moisture levels tested. Slope values were significantly more negative and TS_{50} values lower at −0.7 and −0.18 bars. Thus, moist soil had an adverse effect on survival of *R. solani*.

A soil temperature of 15 C favored survival of *R. solani*. At temperatures of 22 and 26 C, slope values of survival curves were more negative. At 15 C, the time required for 50% survival was almost three times that for 26 C. Soil pH, however, had little influence on *R. solani* survival.

Use of the semilogarithmic and the log-probit transformations changing sigmoid curves to straight lines permitted, (i) the time required for 50% death of inoculum to be estimated, and (ii) the slope values of survival curves to be determined thus making comparisons among treatments possible. Good agreement was found between TS_{50} values determined from data analysis using the two transformations and statistical comparison of slope values among treatments was similar. Hence, the semilogarithmic and log-probit transformation complement each other as tools for evaluating survival data of soil-borne plant pathogens. Considerable biological information carried by the arithmetic plots (e.g., initial saprophytic growth) is lost, however, when data are transformed.

The value of accumulating these detailed data on persistence of, and environmental influences on, survival of a soil-borne pathogen like *R. solani* is that quantitative analyses using appropriate transformations are now possible. The host-pathogen interaction involved with

Rhizoctonia damping-off of radish was chosen as a model system because data related to its epidemiology could be rapidly accumulated and applied to theory–not because of its economic importance. Such data may be typical of epidemiology of other diseases incited by *R. solani,* however. For instance, when we applied the log-probit transformation to data of Papavizas and Davey (15) for the saprophytic colonization of buckwheat stem segments by *R. solani* in soil of 50% moisture holding capacity (MHC) and 22-24 C, the resulting survival slope values were −2.59 for the Greenhouse loamy soil and −2.86 for the Elsinboro sandy loam. Interpolation of TS_{50} values for these data were 10 and 13 days, respectively. At −18 bars (approx. 50% MHC) and 22 C in our experiments we found a slope value of −2.57 with a TS_{50} of 8.5 days for CPS 50 inoculum. Therefore, rate of decolonization of buckwheat stems and the rate of decline of inoculum density in soil infested with CPS inoculum appear to be similar.

Combining analyses of survival data reported in this paper with the other major factors involved in epidemiology of Rhizoctonia damping-off of radish for inoculum potential, disease potential (5), and influence of fungitoxins (4) should permit a systems analysis. Thus, given any combination of host susceptibility, environmental parameters, and inoculum density, prediction of resultant disease should be possible.

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