Epidemiology of Rhizoctonia solani Preemergence Damping-Off of Radish: Inoculum Potential and Disease Potential Interaction

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ABSTRACT

Preemergence damping-off of radish incited by *Rhizoctonia solani* was most severe at 26 C, with only 0.76 propagules/g soil required for 50% disease incidence. As temp became less favorable for disease development, greater inoculum densities were required to incite the same level of disease. Comparison of the inoculum density-disease mcidence (ID-DI) curve at 15, 20, 22, 26, and 30 C by either the semilogarithmic, log-probit, or log-log transformations indicated that curves were parallel at 22, 26, and 30 C. At 15 and 20 C, slope values were significantly greater than those at the higher temp. As slope values were over 1.0 on a log-log basis, synergism between *R. solani* propagules was suggested. The change in slope of the ID-DI at 15 and 20 C was explained by the interaction of inoculum potential and disease potential. Inoculum potential was greatest at 26 C, and least at 15 C. Duration of radish susceptibility to preemergence damping-off was longest at 15 C, at which 100 h were required before a disease incidence of 10% or less occurred; and shortest at 26 C, where only 40 h were required. Disease potentials of radish at 22, 26, and 30 C were similar. The hypothesis that slopes of ID-DI curves give information on mechanisms of disease induction was confirmed.

Phytopathology 64:957-962.

Additional key words: infection court, seed germination.

The semilogarithmic (15), log-probit (10), and log-log (5) transformations have been used (2) for relating inoculum density to disease. At least two advantages are gained by transforming inoculum density-disease incidence (ID-DI) curves. First, for soil-borne plant pathogens, rhizosphere and rhizoplane effects can be elucidated by determining the slope of the ID-DI curve (3, 5). Second, for plant pathogens in general, the effect of the inoculum potential and disease potential interactions, as expressed in the ID-DI curve, can be determined. For example, if ID-DI curves are developed experimentally so that they are parallel when plotted in log-log or log-probit transformations, this may indicate that inoculum is more or less efficient in producing disease, depending on the position of the curves, but that relative interactions have not changed (3, 8).

Recent application of the transformations was hindered by a lack of suitable data so far reported in the literature for quantitative analysis (3). Data from the transitional, or even the plateau region, of the ID-DI curvulinear relationship often had to be used to obtain the minimum number of points necessary in regression analysis. Such data in some instances resulted in slope values of curves less than would be expected if only points on the logarithmic portion of the ID-DI curvulinear relationship were used.

To test theory (15) and value (1, 2) of the transformations in more detail, a system involving

957

preemergence damping-off of radish incited by *Rhizoctonia solani* Kuehn was used. The short time (3-7 days) required for disease expression makes this host-pathogen relationship ideal for accumulation of the large quantity of data needed for analysis. *R. solani* is considered a primitive parasite (17) surviving in soil as "resistant hyphae" and sclerotia (11), but capable of growth through soil when provided with a food base (9). Susceptibility to damping-off is limited in time, due to increased seedling resistance with age (6).

Previously, *R. solani* ID-DI experiments with pentachloronitrobenzene (PCNB) resulted in parallel slopes of curves, with position dependent on PCNB concn (8). Slope values of curves greater than 1.0 on a log-log and 2.0 on a log-probit basis were attributed to synergism (3). Our present study determined the influence of temp on the ID-DI curve. A preliminary report has been published (7).

MATERIALS AND METHODS.—Techniques used to detect and increase inoculum of *R. solani* (isolate R-3) in soil, and procedures for ID-DI experiments were described previously (8). Procedures for determining soil
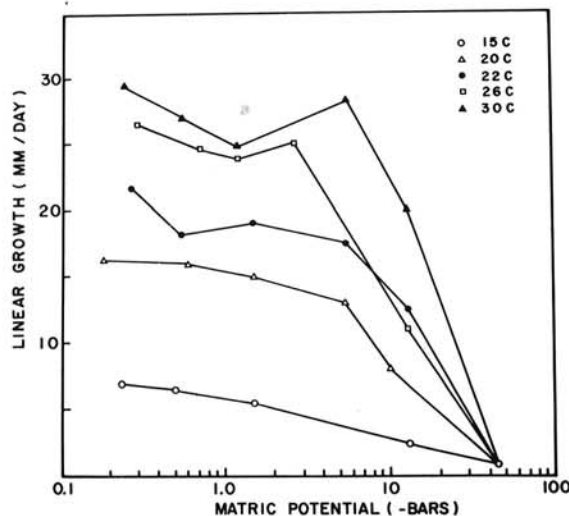

Fig. 1. Linear growth of *Rhizoctonia solani*, isolate R-3, through raw soil at five temp and various matric potentials.

Fig. 2. Effect of five temp on germination of radish.

moisture matric potential from a standardized curve (16), and methods for measuring the linear growth of *R. solani* through soil were reported (8). A loam of pH 8.0 (determined colorimetrically in 1:2 soil: $0.01 M$ $CaCl_2$ suspensions) with the following properties was used: organic matter 1.4%; $NO_3^- - N$, 15.5 $\mu g/g$; lime, >1%; P_2O_5, 33 $\mu g/g$; K_2O, 492 $\mu g/g$; Fe, 9.4 $\mu g/g$; Zn, 3.85 $\mu g/g$; and conductivity 2.6 mmhos/cm.

For germination tests, radish seeds (*Raphanus sativus* L. var. 'Early Scarlet Globe') were germinated on soil in a petri dish whose matric potential was −0.7 bars. The time required for the appearance of radicles or cotyledons at 15, 20, 22, 26, and 30 C was determined within 2-h intervals, beginning 16 h after placing the radish seed on the soil. Twenty-five seeds were used in each of four replications at each temp. Data of this type were compiled, synthesized, and recorded as the time required for 50% seed germination or TG_{50}. The coefficients of velocity of emergence (CVE) were also determined at various temp (21).

To measure the time during which radishes were susceptible to damping-off, five radish seeds were placed on 2 cm radii on a smoothed soil surface in 4.0×9.5 cm plastic dishes. The seeds were covered with a 2 cm layer of soil, and a glass tube, 3 cm long × 0.3 cm diam, was inserted in the soil equidistant from and in the center of the five seeds. The plastic dishes were incubated at 15, 20, 22, 26, or 30 C with soil moisture at −0.7 bars. Soil containing the germinating radish seeds was infested at different times by inserting a radish seed infected with *R. solani* 2 cm below the soil surface through the glass tube. Disease incidence (DI) was calculated after a correction factor was applied as the percentage of plants that failed to emerge for the 99% germinability of the seed lot in noninoculated controls.

Soil pH (measured in 1:2 suspensions of 0.01 M $CaCl_2$) was adjusted with aluminum sulfate [$Al_2(SO_4)_3 \cdot 18 H_2O$] for linear growth experiments. Temperature variation during all experiments was ±0.6 C of the stated values. All experiments were repeated at least twice with four replications/trial.

RESULTS.—*Effect of soil moisture, temperature, and pH on growth of R. solani through soil.*— At a given temp, growth rate was not significantly different at matric potentials less than −5.4 bars (Fig. 1). However, over the entire matric potential range studied (−.02 to −50 bars), the growth rate at 15 C was significantly lower than the growth rate at the higher temp. The growth rates at 20 and 22 C were significantly lower than the growth rates at 26 and 30 C according to Tukey's Q. Greatest growth rate was 29.6 mm per day at −0.27 bars and 30 C.

Growth rate of *R. solani* through soil was 14.0 mm per day at pH 5.3, 14.4 mm per day at pH 6.1, 13.8 mm per day at pH 6.9, and 15.6 mm per day at pH 7.9, when growth rate values at 15, 20, and 26 C were averaged. Significant differences in means were not found at any pH, regardless of temp. The interaction between temp and pH was significant.

Effect of temperature on radish seed germination.—The rate of germination of radish seed decreased with a decrease in temp (Fig. 2). The time required for emergence of root hairs and cotyledons was greatest at 15 C and least at 26 C. To obtain an estimate of
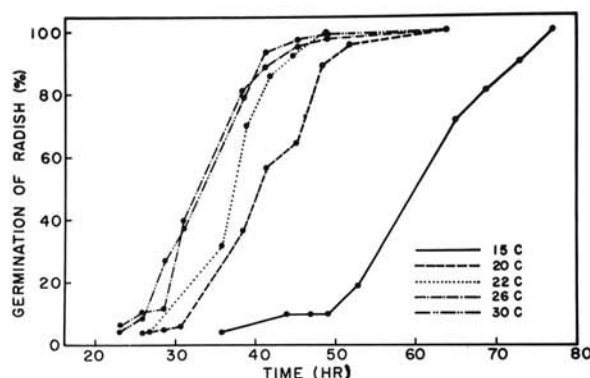
TG_{50} (time required for 50% seed germination), the germination data were transformed by the log-probit transformation (10) and analyzed by linear regression. The TG_{50} values were all significantly different: 32 h at 30 C, 34 h at 22 and 26 C, 40 h at 20 C and 60 h at 15 C. The slope values at each temp were not significantly different when tested by analysis of covariance.

Effect of host age on susceptibility to preemergence damping-off.—By combining the information obtained on growth of *R. solani* through soil at various temp (Fig. 1) with that obtained by infesting soil containing radish seeds at various times (previously described under Materials and Methods), the time of fungal contact with the germinating seed was determined and plotted against DI (Fig. 3). Hyphae of *R. solani* which encountered the infection court of radish before 24 h after planting induced 90-100% DI at all temp tested (Fig. 3-A). At 20, 22, and 26 C the change from highly susceptible (90-100% DI) to highly resistant (0-10% DI) to preemergence damping-off required about 20 h (actual time: 24 to 44 h). At 30 C, this interval was about 30 h, whereas at 15 C the interval was about 60 h (Fig. 3-A). Slope value and position of the susceptibility curve at a given temp in relation to the time required for 10% DI (TD_{10}) was estimated using linear regression analysis of the log-probit transformed data. Slope values for the change from susceptible to resistant varied from −3.36 at 15 C to −18.38 at 26 C (Fig. 3-B). By interpolation, the TD_{10} value was 53 h at 30 C, 42 h at 26 C, 47 h at 22 C, 46 h at 20 C and 100 h at 15 C.

Effect of temperature on inoculum density-disease incidence curves.—Over the range of inoculum densities used (0.5 to 10.0 propagules/g soil), a sigmoid-shaped curve characteristic of the ID-DI curvilinear relationship (3), resulted at each temp (Fig. 4-A). As temp decreased from 26 to 15 C the position of the ID-DI curve was displaced to the right with respect to the x-axis. The range of ID required for an increase of 20 to 80% DI was 0.5 to 1.5 propagules/g (p/g) soil at 26 C, whereas 6.0 to 10.0 p/g soil were required for the same disease increase at 15 C. The most favorable temp for preemergence damping-off of radish was 26 C.

The ID-DI curve at each temp was plotted using semilogarithmic, log-log, and log-probit transformations (Fig. 4-B, C, D). Extrapolation of slopes from the higher values in the ID-DI curves at 22, 20, and 15 C in the semilogarithmic plot of points (Fig. 4-B) resulted in intercepts passing to the right of the origin. Since this indicates that propagules were more efficient in producing infections at higher ID, synergism was probable (25).

The ID-DI data from three experiments at each temp (minimum 900 observations at each temp) were pooled and transformed to a log-log plot of points. A common slope was estimated using linear regression analysis for the ID-DI curve at each temp. At the various temp, slope values varied from 1.31 to 2.57 at 22 and 15 C, respectively. Analysis of covariance indicated significant differences in slope values ($P = 0.05$). When only the slope values of curves at 22, 26, and 30 C were compared, the curves were parallel. Positions of curves, however, were significantly different.

Position of the ID-DI curve at each temp was

determined from the ID_{50} value (ID required for 50% DI). This value was interpolated at $y = 0.693$ (Table 1 and Fig. 4-C). Hence, the lowest ID_{50} was at 26 C and the value increased as temp became less favorable for preemergence damping-off of radish.

Analyses and relative extrapolation values for the log-probit transformation were similar to those obtained using the log-log transformation (Fig. 4-D). Slope values ranged from 2.57 at 22 C, to 4.99 at 15 C. An analysis of covariance indicated that there was a significant difference in slope values when all five temp were compared; although, curves were parallel at 22, 26, and 30 C.

The ID_{50} also was used to determine the position of the ID-DI curves in the log-probit transformation. Slightly lower ID_{50} values compared to the log-log transformation were found for the ID-DI curve at each temp. The values were 0.72 propagules/g soil at 26 C, 1.5 p/g at 30 C, 2.6 p/g at 22 C, 4.8 p/g at 20 C, and 5.5 p/g at 15 C (Fig. 4-D).

Relationship of coefficient of velocity of emergence (CVD) to pathogen growth rate.—Emergence data from noninoculated controls in the ID-DI experiments were used to calculate the CVE value (21). CVE values ranged from 17.8 at 15 C to 31.0 at 26 C (Table 1). CVE values for
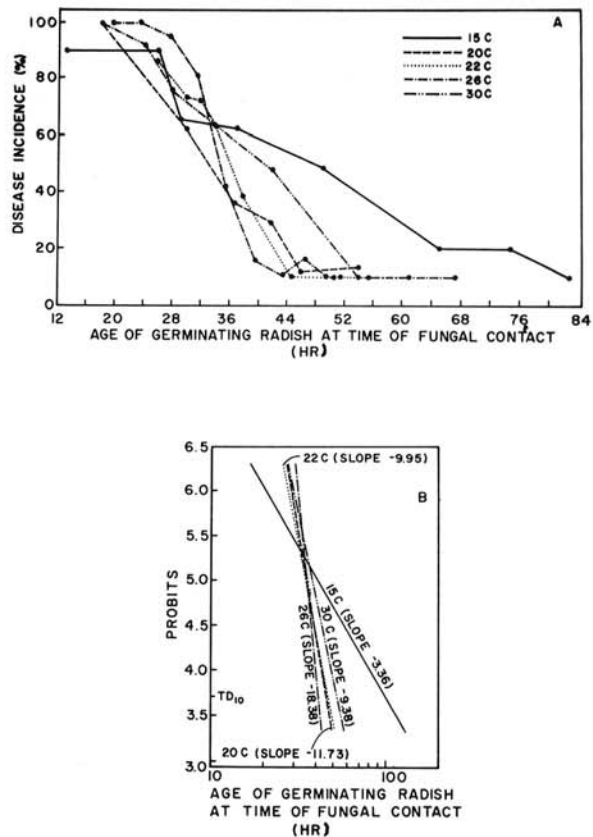

Fig. 3. Effect of age of germinating radish at time of *Rhizoctonia solani* contact on disease incidence at five temp. **A)** arithmetic plot; **B)** log-probit transformation. The correlation coefficients are significantly positive, $P = 0.05$, in the log-probit transformation.
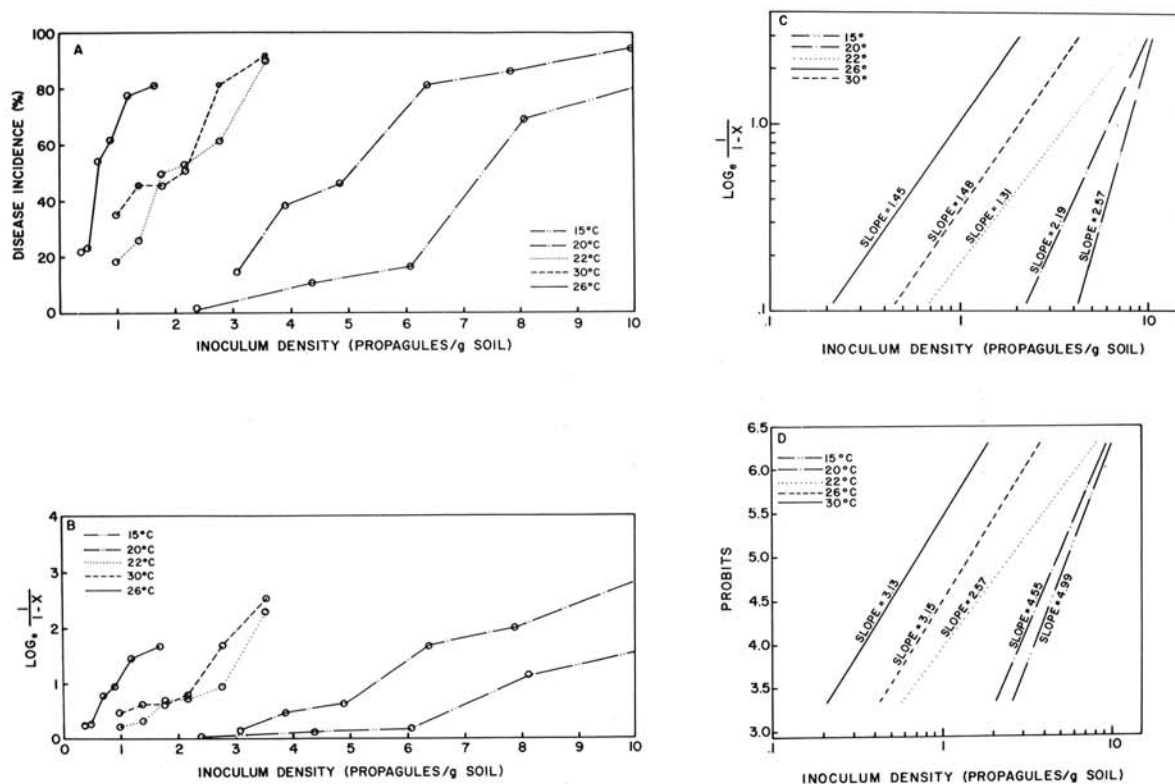
Fig. 4. Effect of temp on inoculum density-disease incidence relationship for *Rhizoctonia solani* preemergence damping-off of radish. Analysis A) arithmetic plot; B) semilogarithmic transformation; C) log-log transformation; D) log-probit transformation. The correlation coefficients are significantly positive, $P = 0.01$, in the last two transformations.

radish between 20 to 30 C were not significantly different $(P < 0.05)$. The CVE value for radish at a given temp divided by the pathogen growth rate at that temp yields the host/pathogen ratio (22). At all temp this ratio was above 1.0.

DISCUSSION.—Slope values above 2.0 in the log-probit and above 1.0 in the log-log transformations suggest synergism (3, 15). Transformed data in all the ID-DI experiments attempted, gave slopes above these

TABLE 1. Coefficient of velocity of emergence (CVE) of radish seedlings, *Rhizoctonia solani* growth rates, host/pathogen ratios, and ID_{50} for five temp regimes

Temp (C)	CVE[w,x]	Pathogen growth (mm/day)[y]	Host/Pathogen ratio	ID_{50}[z]
15	17.8 a	6.2	2.9	7.0
20	25.9 b	15.7	1.7	5.2
22	25.9 b	18.5	1.4	2.8
26	31.0 b	24.5	1.3	0.76
30	29.8 b	26.5	1.1	1.6

[w] Determined by averaging at least seven experiments at each temp.

[x] Means with common letter not significantly different, $P = 0.05$.

[y] Linear growth at −0.7 bars matric potential and pH 7.9.

[z] ID_{50} is inoculum (propagules/gram) required for 50% disease incidence (from log-log transformations).

values. Evidence of synergism in other experiments and explanations based on visual observations have been presented elsewhere (6).

Inoculum densities required to produce 50% disease incidence (ID_{50}) were low compared to reported ID in the field for other soil-borne plant pathogens (12, 19, 24). However, the observed ID_{50} values (0.76 to 7.0 p/g) were similar to the ID of *R. solani* reported for Hawaiian soils (20).

Growth of isolate R3 through soil was little effected by soil pH. This is consistent with the generalization that over the hydrogen ion concns for good plant growth, there is little effect of pH on severity of diseases incited by *R. solani* (4). Soil matric potential and temp, however, had a profound influence on growth through soil (Fig. 1). Since soil temp may be the more variable environmental factor within and between seedbeds in cultivated and irrigated soils, its influence on inoculum and host resistance was studied more intensively.

Slope values for ID-DI curves were parallel at temp of 22, 26, and 30 C, but were significantly different at 15 and 20 C in both log-log and log-probit transformations (Fig. 4-C, D). Dimond and Horsfall (15) have suggested that the slopes of such curves give information on the mechanism of disease induction. Analyses of data from appropriate experiments reported in the literature, however, have indicated that there are no good examples of changes in slope values due to environment,

fungitoxins, or different host susceptibilities (3, 13, 14, 23). Thus, it would be of some interest to determine the reason for slope value differences in ID-DI curves at different temp in our experiments.

The effects of temp on the various responses of host, pathogen and their interaction are presented graphically in Fig. 5. Disease incidence at various temp is compared using ID_{50} values; i.e., the ID (or dosage) required to incite 50% disease incidence. It has been suggested (1, 3, 4) that DI or severity is a function of inoculum potential [energy available for colonization of the host at the surface of the host to be colonized (17)] and disease potential [ability of the host to contract disease (18)].

Interaction of capacity (environmental) factors (15) with ID determines inoculum potential (1). Since moisture and pH were constant in the ID-DI experiments, temp was the environmental variable changing inoculum potential. Martinson (23) has demonstrated a close correlation between saprophytic invasion of substrate by *R. solani* and preemergence damping-off of radishes confirming Garrett's (17) suggestion of direct relationships between primitive parasitism and competitive saprophytic ability where both rely on high mycelial growth rate for success. Thus, the capacity variable for inoculum in these experiments should be correlated with fungal growth rate in Fig. 5 (extracted from Fig. 1) at the matric potential (−0.7 bars) of soil used in the ID-DI experiments.

In Fig. 5 fungal growth rate is inversely proportional to ID_{50}—the higher the growth rate, the less inoculum required for 50% DI. Since there are no inconstant points on the fungal growth curve in Fig. 5 which violated this generalization, we conclude that inoculum potential was not responsible for slope value differences between ID-DI curves at the three higher temp and 15 and 20 C.

Can slope value differences be explained by corresponding differences in disease potential at various temp? Martinson (23) used the coefficient of the velocity of emergence and the host/pathogen ratio suggested by Leach (22) to explain his results with preemergence damping-off of radish incited by *R. solani*. The CVE (21) is interpreted by both of these workers as being a quantitative measurement of host resistance to damping-off, and thus as a measure of disease potential. Host/pathogen ratios in our experiments were always above 1.0 at all temp (Table 1) which would predict severe damping-off (22). This occurred, but absolute values of ratios were not well correlated with ID_{50}; however, in Fig. 5-C, CVE and ID_{50} are inversely related. Further, CVE values at various temp approximately paralleled fungal growth rate. Synthesis of these two factors to predict disease incidence would generate ID-DI curves at various temp with approximately the same slope values. Thus CVE, in this case, does not explain slope value differences in Fig. 4-C, D.

The CVE measures rate of seedling emergence. Rate of germination of seeds measured by emergence of root hairs and cotyledons when host tissue is more susceptible may give more appropriate indications of susceptibility to preemergence damping-off. Data from such measurements are recorded in Fig. 5-A as TG_{50} (time required for 50% germination of radish seeds) at various temp. Points for TG_{50} at 15 and 20 C are inversely related

to fungal growth rate and directly related to ID_{50}; however, values for TG_{50} at 22, 25, and 30 C are approximately the same. If TG_{50} is correlated with disease potential (i.e., the faster the germination, the shorter the
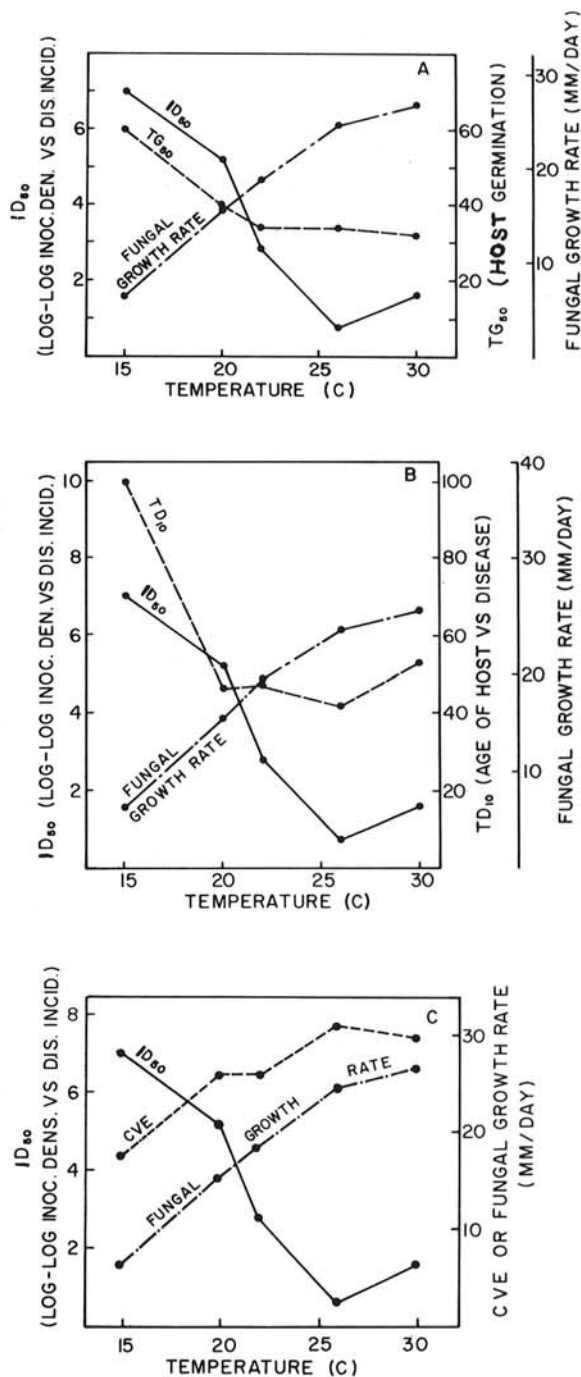

Fig. 5. Interaction of *Rhizoctonia solani* growth rate and inoculum density required for 50% disease incidence (ID_{50}), with **A**) time required for 50% seed germination (TG_{50}); **B**) time required for only 10% disease incidence (TD_{10}); **C**) coefficient of velocity of emergence (CVE), as influenced by temp.

period in which preemergence damping-off is possible), the host was relatively more susceptible at 15 and 20 C than at higher temp. This explains why slope values of ID-DI curves were different at 15 and 20 C; disease potential was not constant at all temp while inoculum capacity (measured by fungal growth rate) was inversely but consistently related to ID_{50} or DI.

An analogous situation is apparent when the time required for the host to change from highly susceptible (90-100% DI) to highly resistant (0-10% DI, Fig. 3) or TG_{10} was plotted in Fig. 5-B. Values of TG_{10} in this case were relatively constant, however, from 20 to 25 C.

These relationships indicate that nonlinear values of disease potential are responsible for changes in slope values of ID-DI curves at various temp. Dimond and Horsfall (15) were correct; slopes of the ID-DI curves give information on the mechanisms of disease induction.

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