Relationships Among Soil Depth, Soil Texture, and Inoculum Placement in Infection of Carrot Roots by Eruptively Germinating Sclerotia of *Sclerotium rolfsii*

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ABSTRACT

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The maximum lateral distance (competence distance) at the surface of pasteurized or nonsterile soil from which mycelium from an eruptively germinating (competent) sclerotium of *Sclerotium rolfsii* grew to infect a carrot root was 3 cm. With increasing depth in either soil, the competence distance was reduced. At depths of 7–8 cm, sclerotia had to be in contact with the root surface to infect. The volume of soil (competence volume) surrounding the root within which a competent sclerotium had a probability ($P \geqslant 0.1$) of initiating an infection was shaped like the inverted frustum of a cone. Within this volume, the probability of infection was reduced from a value of 1.0 with each increment in distance from the root surface and with increasing depth in soil. The average infection efficiency (obtained by averaging all probability values) for a sclerotium located within the competence volume was 0.45 in a pasteurized sandy loam soil and 0.34 in nonsterile field soil; the values were slightly higher when the proportion of sand was increased. Infection efficiencies were always greater at the soil surface and close to the root surface. An average inoculum density of 2.5 sclerotia per 100 cm^3 of soil resulted in 100% infection of carrot root slices under greenhouse conditions.

Additional key words: inoculum density, rhizosphere, soilborne disease.

Sclerotia of the soilborne plant pathogen *Sclerotium rolfsii* Sacc. serve as the overwintering structures and primary source of inoculum. Two forms of sclerotial germination have been described, eruptive and hyphal (8). Eruptive germination is characterized by protrusion of mycelial plugs through the sclerotial rind. Subsequent mycelial growth can be extensive (9), and energy for growth is derived from stored reserves within the sclerotium (8). Mycelia from eruptively germinating sclerotia can initiate infection of host tissue without an exogenous nutrient source or food base (9). In contrast, hyphal germination is characterized by production of individual hyphal strands from the sclerotium. Growth of mycelia is not extensive unless a nutrient source is provided (8). A food base usually is required for infection by hyphally germinating sclerotia to occur (9). Eruptive germination can be induced in sclerotia by drying them for short periods of time at low relative humidity (8) or exposing them to volatile compounds from dried and remoistened plant tissues (8,9,11).

The distance over which mycelium from eruptively germinating (competent) sclerotia of *S. rolfsii* can grow to infect host tissue in the absence of an exogenous nutrient source (competence distance; other terms are rhizosphere width and pathozone [4]) has been measured at the surface of soil (9). The influence of soil depth or soil texture (proportion of sand:silt:clay) on the competence distance has not been determined. If the distances at various depths were known, the competence volume, i.e., the volume surrounding a growing root within which a sclerotium has a probability of initiating an infection (or volume of the pathozone, sensu Gilligan [4]), could be calculated. In addition, the relationship of inoculum placement to the probability of infection of roots by sclerotia of *S. rolfsii* has not been investigated. Factors influencing infection efficiency (proportion of functional or competent sclerotia that cause a successful infection) also are unknown. This information is essential for a proper understanding of the ecology and infection behavior of *S. rolfsii* and could provide empirical data to test the validity of some recently proposed probability models for soilborne fungi (4).

In the southeastern United States, root rot of processing carrots caused by *S. rolfsii* is a major factor limiting commercial production. The uniform taproot of the carrot plant provides an ideal system for studying the host-pathogen interaction for a soilborne pathogen such as *S. rolfsii*.

The objectives of this study were to determine: 1) the influence of inoculum placement, soil depth, and soil texture on the probability of infection of carrot roots by sclerotia of *S. rolfsii*, 2) the competence distance and average infection efficiency for an eruptively germinating sclerotium, 3) the competence volume of carrot roots of increasing radii in which infection by *S. rolfsii* can occur, and 4) the relationship of inoculum density to infection of carrot roots in the greenhouse.

MATERIALS AND METHODS

Influence of inoculum placement on root infection. Two isolates of *S. rolfsii*, 159 from sorghum and 2672 from annual bluegrass-bentgrass golf greens (North Carolina State University Culture Collection, Raleigh 27695), were used. Sclerotia from 3-mo-old oat cultures were dried for 24 hr in a laminar flow hood to induce eruptive germination (8). Dried sclerotia with an average diameter of 1.0–1.5 mm were used as inoculum. The viability of these sclerotia was 98–100%. Harvested carrot roots (with the tops removed) about 15 cm long and with a crown diameter of approximately 2.5 cm were inserted 4 cm deep into one of the following soil mixtures contained in 10-cm-diameter pots:

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pasteurized sandy loam (sand:silt:clay ratio of 72:20:8, organic matter content about 1%); a 2:1 mixture of pasteurized sandy loam and sand; nonsterile fine sandy loam from a field in Maxton, NC (sand:silt:clay ratio of 65:19:16, organic matter content about 2%); and a 2:1 mixture of nonsterile fine sandy loam and sand. Each root was inoculated with a single sclerotium placed at lateral distances ranging from 0 to 4 cm from the root surface (at 0.5-cm increments) and at depths ranging from 0 to 9 cm (at 0.5-cm increments); all possible distance-depth combinations were used, for a total of 171. To achieve the various depths, 0–9 cm of soil was added to the 4-cm layer in the pot before inoculation (no soil was required for the 9-cm depth), a sclerotium was placed on the soil, and soil was layered over the inoculum. The depths represented measurements made from the position of the sclerotium to the soil surface, i.e., height of overlying soil. The soil was moistened to approximately field capacity and allowed to drain, and the pots were sealed in polyethylene bags and arranged in a randomized complete block design on greenhouse benches. Presence or absence of root infection was determined after 7 days by pulling up the carrot root and scoring for lesions or soft-rotting and for characteristic white mycelium. Each distance-depth combination in each soil mix was replicated four times. All experiments were conducted five times.

Estimation of competence distance, competence volume, and infection efficiency. Competence distance at each depth in a given soil mix was determined as the maximum lateral distance (R, cm) from the root surface at which an eruptively germinating sclerotium could initiate an infection with a 10% probability. Competence volume, denoting the volume of soil (cm^3) surrounding the root within which a sclerotium has a probability ($P \geqslant 0.1$) of infecting, was calculated from maximum distance and depth values in each soil mix. The probability of infection at a specific distance or depth in a given soil mix was determined by averaging the results from the five trials (with four replicates in each). Infection efficiency, representing the average probability for a sclerotium located within the competence volume to initiate an infection, was calculated by dividing the number of roots infected by the number inoculated; all the distance-depth combinations confined to the competence volume, i.e., that could potentially result in infection, were used. Thus, the infection efficiency value represented the average of the probabilities for every sclerotium located within the competence volume; each probability was the mean of 20 experimental trials of each distance-depth combination.

Increase in root growth and competence volume over time. Carrot crown diameters in a field located at Maxton, NC, were measured at monthly intervals from April to August 1984. About 40–50 plants were selected arbitrarily, the tops were removed, and the root crown diameters were measured and averaged. These data were used in calculating the increments in competence volume over time as a function of root growth.

Relationship of inoculum density to root infection. Dried sclerotia from oat cultures were used as inoculum. Batches of precounted sclerotia were mixed with 1.8 L of a 2:1 mixture of pasteurized sandy loam:sand in a twin-shell blender for 1 min. The soil containing inoculum was placed in perforated metal trays (19.5 × 19 × 5 cm deep, total volume = 1,850 cm^3), moistened to saturation, and allowed to drain for 10 min. Uniform rectangular carrot segments (1 cm^2 × 5 cm long) were cut from mature harvested roots, surface-disinfested in 0.5% NaClO, and inserted into the soil. Each flat contained 18 root segments distributed in a regular pattern to ensure that each segment was surrounded by about 100 cm^3 of soil. A range of inoculum density from 0 to 3 sclerotia per 100 cm^3 of soil, at increments of 0.5, was tested. Each inoculum density was replicated five times. The flats were sealed in polyethylene bags and arranged in a randomized complete block design on a greenhouse bench (temperature range 24–28 C). The number of root segments infected by *S. rolfsii* and showing characteristic mycelial growth at each inoculum density was determined after 5 days. The experiment was repeated six times over a 3-mo period. Reported values of percentage of infection are the means of the five replications of each experiment.

RESULTS

Influence of inoculum placement on root infection. The maximum distance at the surface of a 2:1 mixture of pasteurized sandy loam:sand from which an eruptively germinating sclerotium infected a carrot root with a 10% probability was 3 cm. With each increment in depth, the competence distance was reduced almost proportionately. At depths of 7–8 cm, sclerotia had to be in contact with the root surface to initiate infection (Fig. 1A). No infection was initiated by sclerotia placed deeper than 8 cm. The shape of the competence volume was approximated to an inverted frustum of a cone, with the root represented as a cylinder (Fig. 1A). The soil mixtures tested did not affect the maximum distance or depth at which infection from germinating sclerotia was observed; thus, the size and shape of the competence volume were not altered. To calculate competence volume, the volume represented by the root, given by:

$$V = \pi r^2 h, \qquad (1)$$

was subtracted from the total volume represented by the frustum of a cone:

$$V = \pi h/3\,(R^2 + Rr + r^2), \qquad (2)$$

where h = 8 cm (maximum depth in soil at which infection was observed), R = (3 cm [maximum distance at the soil surface from

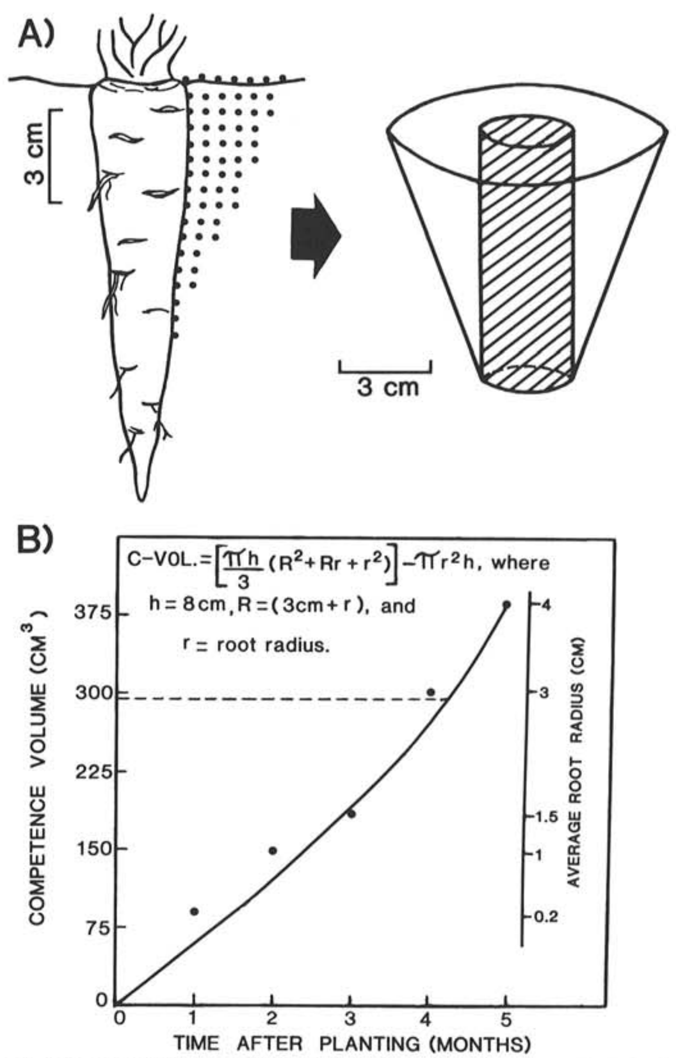

Fig. 1. Competence volume (C-VOL.) of soil for infection of carrot roots at $P \leqslant 0.10$ by eruptively germinating sclerotia of *Sclerotium rolfsii*. **A,** Successful infections from sclerotia placed at various distances and depths away from a carrot root were mapped (•) and converted to an infectious volume of soil whose shape was approximated to the inverted frustum of a cone, with the root represented as a cylinder. **B,** Increase in competence volume of soil over time in relation to the increase in radii of carrot roots and time after planting. The average competence volume was calculated to be about 300 cm^3.

which infection occurred] + r), and r = root radius (Fig. 1B). This expression is analogous to that of a shell model (4). The radius of the inoculum (r_i) was disproportionately small compared with the R value and therefore was excluded.

The average infection efficiency for a sclerotium of *S. rolfsii* was highest in the 2:1 mixture of pasteurized sandy loam:sand and lowest in the nonsterile field soil (Table 1). In the 2:1 mixture of pasteurized sandy loam:sand, the probability of infection by a sclerotium was reduced with increasing distance from the root surface and also with increasing depth in soil (from 1 to 4 cm) at a given distance (Fig. 2A). Thus, infection efficiency within the competence volume was always higher at the soil surface and close to the root. The probability of infection by a sclerotium was lower in nonsterile soil (Fig. 2B); addition of sand increased the overall probabilities only slightly.

Increase in root growth and competence volume over time. The average root radius of carrots at various times after planting is shown in Figure 1B. At each sampling date, the competence volume was calculated by subtracting equation 1 from equation 2 and plotted as a function of root radius and time after planting. The average competence volume in the 90- to 150-day period after planting in Maxton, NC (the period during which disease caused by *S. rolfsii* on carrots generally progresses) was approximately 300 cm^3 (for an average root radius of 3 cm).

TABLE 1. Average infection efficiency for carrot root infection by an eruptively germinating sclerotium of *Sclerotium rolfsii* in four soil mixes[a]

Soil mix	Infection efficiency[b]
Pasteurized sandy loam	0.45
Pasteurized sandy loam:sand (2:1, v/v)	0.52
Nonsterile field soil	0.34
Nonsterile field soil:sand (2:1, v/v)	0.40

[a] Carrot roots were inoculated with a single sclerotium placed at various distance-depth combinations from the root surface.

[b] Values represent average probability for a sclerotium located within the competence volume of soil to initiate an infection and were calculated from the mean percentage of roots infected in five trials comprising 61 distance-depth combinations, with four replications in each trial.

Relationship of inoculum density to root infection. The relationship of percentage of infection of root slices to number of sclerotia per 100 cm^3 of soil was almost linear throughout the range of inoculum density (Fig. 3); the coefficient of determination (R^2) for linear and quadratic regressions was 0.85 and 0.99, respectively. Under greenhouse conditions, 100% infection was attained at an average inoculum density of 2.5, i.e., 45 randomly distributed sclerotia per 1.8 L of soil.

DISCUSSION

The competence distance at the soil surface over which mycelium from an eruptively germinating sclerotium of *S. rolfsii* could grow to infect a carrot root in this study, i.e., 3 cm (at $P \leqslant 0.01$) was not affected by soil texture and was similar in pasteurized and nonsterile soil. Punja and Grogan (9) reported that infection of sugar beet leaf petioles by germinating sclerotia occurred from distances of up to 3.5 cm at the soil surface and that the chance of infection lessened as distance from the tissue increased. The probability of infection in this study was also reduced with increasing distance from the root surface and with increasing depth in soil. Infection efficiency was lower in nonsterile soil and in soil mixtures containing a lower proportion of sand. Other investigators have shown that the probability of infection by *Pythium aphanidermatum* (12) and *Gaeumannomyces graminis* var. *tritici* (14) was reduced with increasing distance from the root surface. Incidence of disease caused by *S. rolfsii* on rice and infection efficiency were reported to be higher in sandy than in clay soil (13).

Recent theoretical models (4) and previous attempts to model infection by soilborne fungi have assumed the competence distance (rhizosphere width or pathozone) (4,5) to be the same at all soil depths; thus, the volume of soil surrounding the root that influenced pathogen growth was represented by a cylinder (4,5). To establish the shape of the competence volume from empirical data in this study, the shape of the root was approximated to that of a

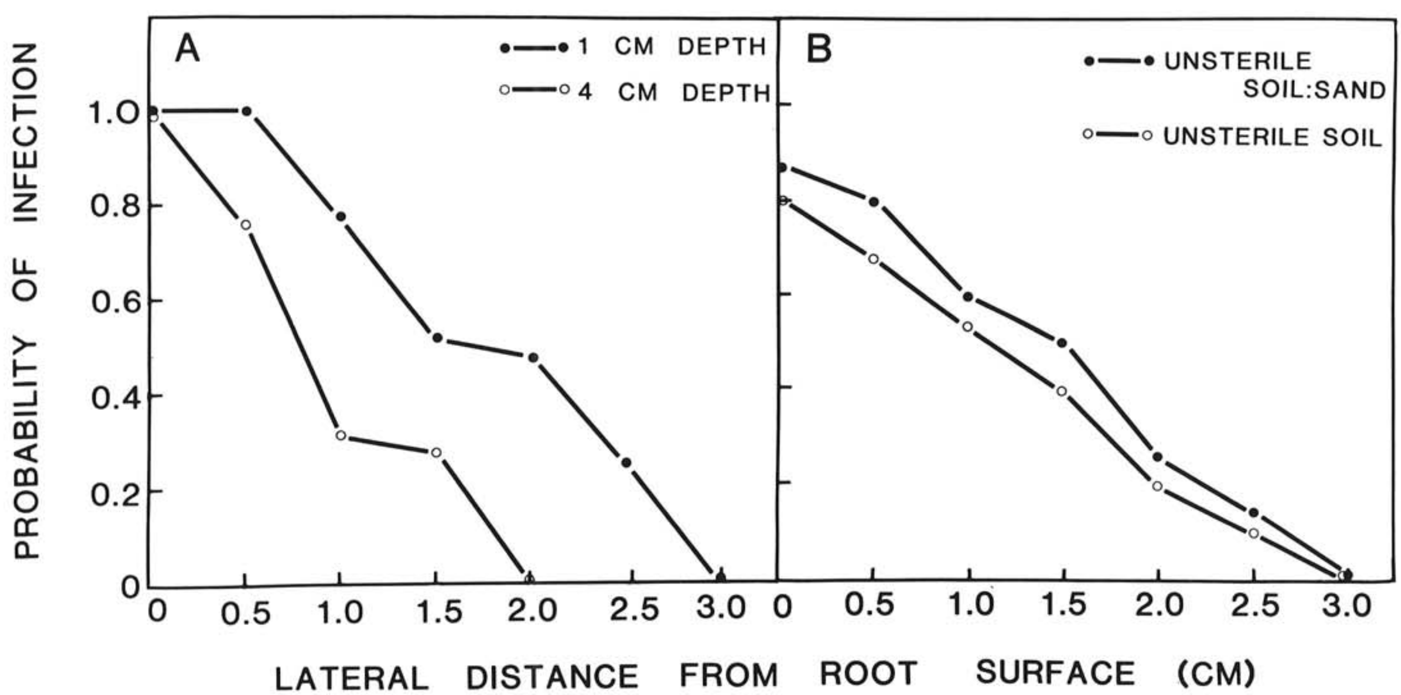

Fig. 2. Average probabilities for infection of carrot roots by eruptively germinating sclerotia of *Sclerotium rolfsii* under greenhouse conditions. Sclerotia were placed at various distances from the root surface and presence or absence of infection was rated after 7 days of incubation. Each point represents the mean of four replications; the experiment was repeated five times. **A,** Average probabilities at two depths in a 2:1 mix of pasteurized sandy loam soil:sand. The overall infection efficiency at the 1- and 4-cm depth was calculated to be 0.58 and 0.47, respectively. **B,** Average probabilities at a 1-cm depth in nonsterile soil and a 2:1 mix of nonsterile soil:sand. The overall infection efficiency was calculated to be 0.38 and 0.45, respectively.

cylinder. The assumptions were that infections occurring anywhere on the upper 8 cm of root would result in symptoms of disease and that infection sites would not be limiting. The shape of the competence volume for *S. rolfsii* on carrot was found to resemble that of the inverted frustum of a cone (Fig. 1A).

The decrease in competence distance and infection efficiency of *S. rolfsii* with increasing soil depth may result in part from inhibition of linear mycelial growth by lower oxygen and/or higher carbon dioxide (10) deeper in soil, or to inhibition of sclerotial germination by pressure imposed by weight from overlying soil (10). Thus, soil mixtures containing a higher proportion of sand and with lower bulk density may be better aerated and exert less pressure over sclerotia. Both of these factors could have contributed to the higher infection efficiency observed.

The term "competence volume" (5) is similar conceptually to a recent description by Gilligan (4), in which he proposed the term "volume of the pathozone." Arguments against use of the term competence (4) are semantic, since ability, i.e., competence, particularly of large propagules such as the sclerotia of *S. rolfsii*, to germinate and infect can be readily determined. The extent to which infection actually occurs, i.e., infection efficiency, can also be determined. With fungal propagules of smaller size, such as the oospores of *P. aphanidermatum* (12), microsclerotia of *Cylindrocladium crotalariae* (2), or hyphae of *G. graminis* (14), competence distances or pathozones may be considerably smaller and more difficult to estimate directly. Infection efficiencies were easily determined in this study because the sclerotia used were large and uniform in size and germinated consistently. Sclerotia produced in nonsterile soil that are surface-contaminated with microorganisms and germinate poorly (11) predictably have a lower infection efficiency because their competence distance is reduced (6). In natural soil, therefore, more sclerotia per volume of soil are required to cause 100% root infection by *S. rolfsii* under optimal environmental conditions. Dillard and Grogan (3) similarly reported that seven sclerotia of *Sclerotinia minor* per competence volume of nonsterile soil (100 cm^3) would result in the highest probability of infection of lettuce plants with the greatest inoculum efficiency. The concept of competence volume has been extended further and used as the basis to develop a strategy for sampling fields to determine the relationship of disease incidence to inoculum density (3,5). The infection efficiency of pathogen propagules may be dependent on propagule or inoculum size (2,7,15), the availability of nutrients (12,14), and the form of the colonized nutrient substrate (15). In *S. rolfsii*, the presence of a food base (6,9), volatile compounds (9,11), and host exudates (11) may increase the probability of infection by increasing the distances over which mycelia can grow (6,10). Under these conditions, infection efficiencies may be higher than those reported in this study.

Low inoculum densities of *S. rolfsii* were required to infect 1 $cm^2 \times 5$ cm root slices (with a zone of influence calculated to be about 100 cm^3, based on competence distances of 3 cm at the soil surface and 1 cm at a 5-cm depth) in the greenhouse because temperature and moisture conditions were maintained near the optimum for sclerotial germination. Also, the inoculum was randomly distributed in soil to ensure contact with all root segments, especially at the higher inoculum densities. Under these conditions, 100% infection was attained at 2.5 sclerotia per 100 cm^3 of soil (infection efficiency = 0.4). The short duration of the experiment (5 days) prevented secondary spread of mycelium from root to root. Previous investigators (1) reported that an inoculum density of 130 sclerotia per 100 cm^3 of soil (converted from grams using a soil bulk density value of 1.3 g/cm^3) was required to attain 75% infection of beans in the greenhouse (infection efficiency = 0.07). The proportion of sclerotia capable of eruptive germination, their distribution in soil, and their proximity to the root or stem surface in that study (1) are unknown. Inoculum of low competency or essentially wasted (superfluous) by virtue of being located outside the competence volume could in part account for the excessively high inoculum density required and extremely low infection efficiency reported by these investigators (1). Implicit in the definition of competence volume is the need to avoid or exclude nonfunctional or superfluous inoculum.

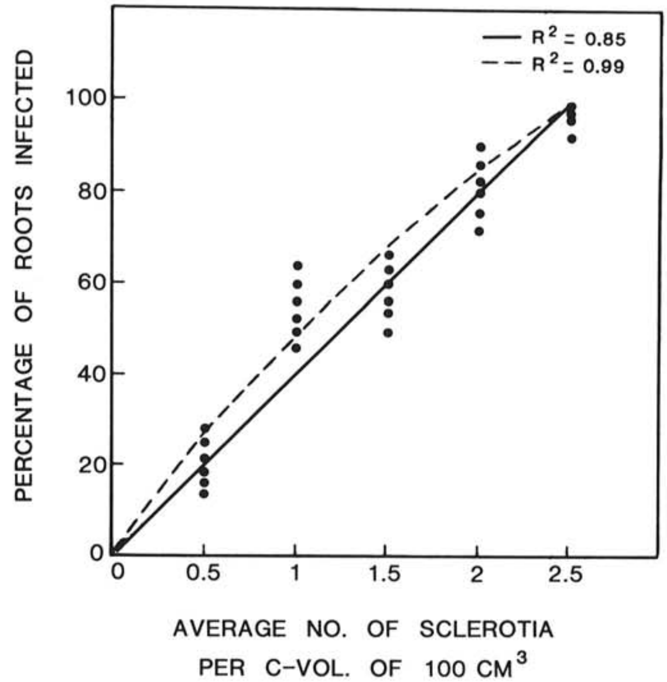

Fig. 3. Relationship of percentage of infection of carrot root slices to number of sclerotia of *Sclerotium rolfsii* in 100 cm^3 of a 2:1 mix of pasteurized sandy loam soil:sand. Infection was rated after 5 days of incubation in the greenhouse. Points represent the mean of five replications of each inoculum density; the experiment was repeated six times. Regression lines were fit using the mean value from the six experiments. Significant fit was obtained to a linear and quadratic function.

The infection efficiency of 0.4 in the greenhouse study was lower than the expected value of 0.52 calculated from the distance-depth study. This could have been due to downward movement of inoculum when the flats were watered. Also, exudation of nutrients from the cut root surfaces may have reduced the proportion of sclerotia that germinated eruptively (11).

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