Spatial Pattern, Inoculum Density-Disease Incidence Relationship, and Population Dynamics of *Sclerotium rolfsii* on Apple Rootstock

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ABSTRACT

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Nursery plots of apple at Tahlequah and Stillwater, OK, showing southern blight (*Sclerotium rolfsii*) symptoms were divided into 54 (2.75 × 2.75 m) and 30 (2.75 × 2.05 m) quadrats, respectively, and randomly sampled for sclerotia. Average preplant densities of sclerotia of *S. rolfsii* as determined by a wet-sieving/methanol assay technique ranged from 3.6 to 4.7 viable sclerotia per 1,000 g of dry soil at the apple nurseries. Numbers of sclerotia from individual quadrats ranged from 0 to 7. The spatial pattern of sclerotia extracted from bulked samples from a naturally and artificially infested field plot was random, according to Fisher's variance-to-mean ratio, frequency distribution analysis, and the k dispersion parameter of the negative binomial distribution. A mean density of 44 sclerotia per 525 g of dry soil was recovered from soil samples taken adjacent to dead trees in studies performed in microplots. These densities declined with increase in time between tree death and sampling. Densities of sclerotia adjacent to and at various distances from dead trees were best described by a polynomial regression equation. A positive linear relationship was observed between sclerotial densities in soil and disease incidence. Five sclerotia per 1,000 g of dry soil resulted in 19, 5, and 35% disease in container, microplot, and field studies, respectively. Placing sclerotia 3 cm from apple rootstock, 0.5 cm deep, grown in containers in the field, resulted in lower disease incidence than placing sclerotia in contact with the rootstock.

Sclerotium rolfsii Sacc. attacks apple rootstock (*Malus domestica* Borkh. cv. Delicious) used for the propagation of apple and crabapple cultivars and kills the tree by vascular girdling (20). Trees up to 3 yr old can become infected. Southern blight of apple has become an important disease in some eastern Oklahoma nurseries (4) as well as in Georgia (3), California (7), Arkansas, Texas, South Carolina, and Tennessee (K. Sears, Olin Chemical Co., Little Rock, AR, G. Philley, Texas A&M, Extension Plant Pathologist, Overton, and Charles Meister, University of Florida, IR-4 southern region coordinator, *personal communications*). Little is known about population dynamics of the sclerotial inoculum involved in this host-pathogen relationship. Previous work with *S. rolfsii* has involved high-density planting schemes, e.g., sugar beets (1,10), peanuts (17), and carrots (13). The apple-*S. rolfsii* system offers an opportunity to study the ecology and epidemiology of this pathogen in a low-density, evenly spaced, planting scheme involving a perennial woody host.

In this study, sampling and assay techniques were examined to ascertain sclerotial density and spatial patterns of *S. rolfsii* in apple nursery soils. The relationship of inoculum density and disease incidence between *S. rolfsii* and apple rootstock and the effect of inoculum placement on disease incidence were also investigated.

MATERIALS AND METHODS

Inoculum production. Two forms of inocula were used in this study, sclerotia and colonized oat seeds. An isolate of *S. rolfsii* from diseased apple rootstock was grown on autoclaved oat seeds in 2-L flasks (300 g of oats, 450 ml of water) for 4–5 wk at 27 C. Mature sclerotia were separated from oats by sieving and air-dried (exposed to natural air contaminants). One hundred sclerotia from each culture were placed on potato-dextrose agar (10 sclerotia per plate) to determine viability. Viability was near 100%. Oat seeds, fully colonized by *S. rolfsii* were also dried before first use.

Density and spatial pattern of sclerotia (field plots). Plots were established at nurseries in Tahlequah and Stillwater, OK. The plot at Tahlequah (sandy loam soil, pH 6.2, 1.5–2% organic matter) measured 24.7 × 16.5 m and was a naturally infested area that had a high incidence of southern blight of apple (10–50%) during the previous 2 yr. The plot at Stillwater (clay loam soil pH 7.6, 0.7% organic matter) measured 13.7 × 12.2 m and was artificially infested with *S. rolfsii* in 1982 and planted with apple rootstock for 2 yr before the beginning of this investigation in 1984. Disease

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incidence in this plot was also high (10–15%) during the 1982 and 1983 growing seasons. Field plots at both locations were disked, divided into quadrats, and sampled to determine sclerotial inoculum density and spatial pattern before planting. Domestic apple rootstocks (cultivar Delicious, Greenleaf Nursery, Tahlequah, OK) were used as host plants in all experiments unless stated otherwise. In April 1983, the Tahlequah plot was divided into 54 (2.75 × 2.75 m) quadrats with 22 apple rootstocks planted per quadrat (two rows, 11 trees per row). In April 1984 and 1985, the Stillwater plot was divided into 30 (2.75 × 2.05 m) quadrats with 16 apple rootstocks planted per quadrat (two rows, eight trees per row). Trees at both nursery sites were spaced 23 cm apart, and 1.4 m separated each row. The Stillwater plot was irrigated as necessary. The Tahlequah plot received no supplemental irrigation.

Preplant inoculum density. At Tahlequah in 1983, nine samples were removed at random from each quadrat with a 1.5-cm-diameter probe inserted 6 cm deep. Samples from each quadrat were bulked and analyzed as a single sample with an average dry weight of 400 g. Similar sampling techniques were followed at the Stillwater nursery during 1984 and 1985, except a 2-cm-diameter probe was used and 10 samples were removed to a depth of 8 cm in each quadrat with an average bulked dry weight of 550 g. Samples were stored at 7 C until extraction. All soil samples were taken in April after cultivation but before planting apple rootstock. Weights were converted to a 1,000-g basis for easier interpretation.

Microplots and container studies. Raised microplots (2.1 × 2.1 m) containing sandy loam soil 2 cm deep (pH 5.7–6.3, 1% organic matter) were used for several experiments. Microplots contained three rows of rootstock (nine per row), which were planted in April 1985. Rootstocks were spaced 23 cm apart, and 91 cm separated each row. Microplots were infested with dried oat seed inoculum (10 g/microplot) of *S. rolfsii* before planting. During October 1985, 10 diseased trees were chosen at random from microplots and the soil around the trees was sampled with a 1-cm-diameter probe to a depth of 5 cm to determine sclerotial production and distribution. Twenty soil samples per rootstock were taken at 1-cm increments along four perpendicular radii from trees to a distance of 5 cm. Samples (four per increment) from each distance were bulked (30 g dry weight total) and assayed by the wet-sieving/methanol method (described later) to determine the number of sclerotia. Regression analysis between sample distance and the corresponding sclerotial density was used to describe the data statistically.

Twelve-liter plastic containers (30 cm diameter) filled with sandy loam soil (same as in microplots) were used for more controlled studies. One rootstock was planted in each container and inoculated during May 1985 with 1–4 sclerotia of *S. rolfsii* per rootstock placed within 3 cm of each tree. In October 1985, entire volumes of soil (average 525 g dry weight) around and on the infected rootstock were removed and assayed for viable sclerotia; the sample area occupied a region 5 cm in radius and 8 cm in depth around the tree. Trees were grouped according to the number of days between death and sampling to determine the population dynamics of the sclerotia during this period. These groupings were: 45, 75, 105, and 135 days with eight, six, six, and two trees sampled from each group, respectively. Both microplot and container studies were located near Stillwater.

Soil analysis. Viable sclerotia were enumerated from soil samples by a modified extraction technique (10,15). Each sample was mixed and air-dried. Soil was weighed and wet-sieved through two nested sieves with openings of 2.4 and 0.5 mm. Contents retained in the bottom sieve were placed in 14-cm-diameter petri dishes and air-dried. Equal volumes of a dark soil base (Baccto potting soil, Michigan Peat Co., Houston, TX, pH 4.5, 95% organic matter) were added and mixed with each soil sample to provide a dark color and an additional organic substrate to stimulate sclerotial germination. A solution of 1% aqueous methanol was pipetted across this mixture. The volume of methanol added was adjusted according to soil type and sample amount and ranged from 5 to 10 ml per plate. Saturation of the sample was avoided. Dishes were covered and incubated at 25 C. Samples were examined for germinating sclerotia 24–48 hr after incubation. If sclerotia were present and viable, mycelia of the germinating sclerotia were easily visible against the dark soil mix.

Data analysis. Estimates of densities and the horizontal spatial pattern of sclerotia of *S. rolfsii* were determined from the sample data. The spatial pattern of sclerotia within plots was analyzed by several procedures, and expected frequency numbers were generated and tested against observed values with the FORTRAN program developed by Gates and Ethridge (5). The chi-square function indicated the distribution model that best fit the data. The general models for Poisson and negative binomial were used in this study. Supplemental tests such as Fisher's variance-to-mean ratio and the k parameter were used to further specify the spatial pattern of inoculum.

Inoculum density-disease incidence relationships. *Field experiments.* Inoculum density-disease incidence relationships in the field were determined by correlating preplant sclerotial counts from randomly sampled quadrats to the observed loss of trees at the end of the growing season in the corresponding quadrat. Ten quadrats with varying sclerotial densities (1.9–11.4 sclerotia per 1,000 g of dry soil) at the Stillwater nursery were used in 1984 for this determination. Trees in each quadrat were observed throughout the growing season for disease.

Container experiments. Nonsterile sandy loam soil was placed in 108 plastic containers (described earlier) in April 1983, and an apple rootstock was planted in the center of each container. Trees were inoculated in May by infesting soil with varying numbers of sclerotia of *S. rolfsii*. Inoculum densities (sclerotia per 1,000 g of dry soil) of 0, 2, 5, 10, 50, and 100 were used. Sclerotia were thoroughly mixed into the top 5 cm of soil in a circular area (10 cm diameter) around the tree. The soil was firmly pressed to reduce drying, and each container was then watered. Each inoculum level had 18 trees, and the containers were arranged in a completely randomized design in empty microplots. Plants were monitored for infection and disease symptoms.

Microplot experiments. In April 1984, apple rootstocks were planted directly in microplots (described earlier). Three rows with nine trees per row were planted in each of 16 plots. The trees were spaced 22 cm apart, and 91 cm separated each row. All plots were sampled before planting, and *S. rolfsii* was not recovered. Sclerotia at rates of 0, 2, 5, 10, 50 and 100 per 1,000 g of dry soil were randomly added to soil in narrow furrows (4 cm wide, 2 cm deep) on each side of the row. Treatments (replicated eight times) were arranged in a completely randomized design among 16 microplots. Disease incidence was recorded throughout the growing season. Regression analysis was used to examine the relationships between inoculum density and disease incidence for all three experiments. The multiple-infection transformation, $\log_e(1/1 - X)$, was used to account for multiple infections (6).

Effects of inoculum placement and density on disease incidence. Studies were initiated in growth chambers to determine the influence of inoculum placement on disease. Apple seedlings (*M. sylvestris* Mill.) from stratified seeds (Lawyer Nursery Inc., Plains, MT) were used as host plants. In 1984, seedlings were grown in nonsterile potting medium (pH 4.5, 95% organic matter) under greenhouse conditions. Three-month-old seedlings were transferred to a growth chamber set at conditions optimal for the development of southern blight (30 C, near 100% relative humidity). Factorial experiments were designed to include two variables, distance and density. Sclerotia were placed at 0 (contact), 1, 2, and 3 cm from the trees at a depth of 0.5

cm, with varying numbers of sclerotia (1–4) in each pot. There were eight trees per treatment. Infection and death of seedlings were recorded for 2 wk after inoculation. In 1985, the same treatment combinations were evaluated using container-grown rootstock in the field. Containers were filled with nonsterile sandy loam soil, and a rootstock was planted in each. Nine trees were used per treatment. Infection and death caused by *S. rolfsii* were recorded weekly throughout the growing season.

RESULTS

Density and spatial pattern of sclerotia. Average sclerotial densities from all samples of nursery soils ranged from 4.7/1,000 g of dry soil in Tahlequah (1983) to 3.6/1,000 g of soil in Stillwater (1985). Sclerotia were recovered from 42 of 54 quadrats sampled in Tahlequah. In Stillwater (1984), all 30 quadrats contained sclerotia (89 total), whereas sclerotia (52 total) were recovered in 25 of 30 quadrats in 1985.

The number of sclerotia recovered in quadrats at both locations ranged from 0 to 7 for all plots combined (Fig. 1). The variance-to-mean ratios for the three sampling times were low and near 1.0, indicating a randomness in horizontal sclerotial pattern (19). Chi-square analysis was performed on observed sclerotial frequencies to determine if a random or clustered sclerotial population existed. Observed sclerotial frequencies consistently fit the Poisson distribution, although the negative binomial distribution could not be rejected for the Tahlequah data (Table 1). The dispersion parameter k of the negative binomial estimated by $k = \text{mean}^2/\text{variance-mean}$ was 6.25 for Tahlequah, which further suggests a random pattern because values of k needed to indicate clustering should be <2.0 (19). The variance-to-mean ratios for Stillwater in 1984 and 1985 were <1 and 1.0, respectively, which would reject a negative binomial distribution for either data set because the variance is assumed to be greater than the mean for the negative binomial model.

Sclerotial population stability and arrangement of *S. rolfsii* around dead trees were dynamic. Of 150 sclerotia recovered in the total sample zones of 10 trees, 72% were found at 0–1 cm, 15% at 1–2 cm, 5% at 2–3 cm, 6.6% at 3–4 cm, and 0.66% at 4–5 cm. Two of the trees had no sclerotia and were not included in the regression analysis. Other pathogens may have been responsible for tree death, explaining the absence of sclerotia. Sclerotia were found within 3 cm of the host (Fig. 2). A polynomial regression equation best described the relationship between sample distance and number of sclerotia ($r = 0.79$, $P = 0.01$). The regression equation, $Y = 17.2 - 10.4(X) + 1.5(X^2)$ can be used as a predictive tool in estimating the number of sclerotia at a given distance, where Y = sclerotial density per 30 g of soil and X = sample distance (cm).

The second study indicated that a high density of propagules could be produced as a result of infection, but the recovery of viable sclerotia declined as the interval between seedling death and sampling increased. No correlation was observed between initial inoculum levels (1–4 sclerotia) and numbers of sclerotia resulting from infection. A range of 0–68 sclerotia per 525 g of soil was found in the soil sample area around 22 container-grown trees. The mean number of viable sclerotia recovered from each tree at each time period was: 45 days = 44, 75 days = 24, 105 days = 8, and 135 days = 3 (Fig. 3). The regression equation was $Y = -0.52(X) + 65.6$, where Y = number of sclerotia and X = elapsed days between tree death and sampling ($r = 0.77$, $P = 0.01$).

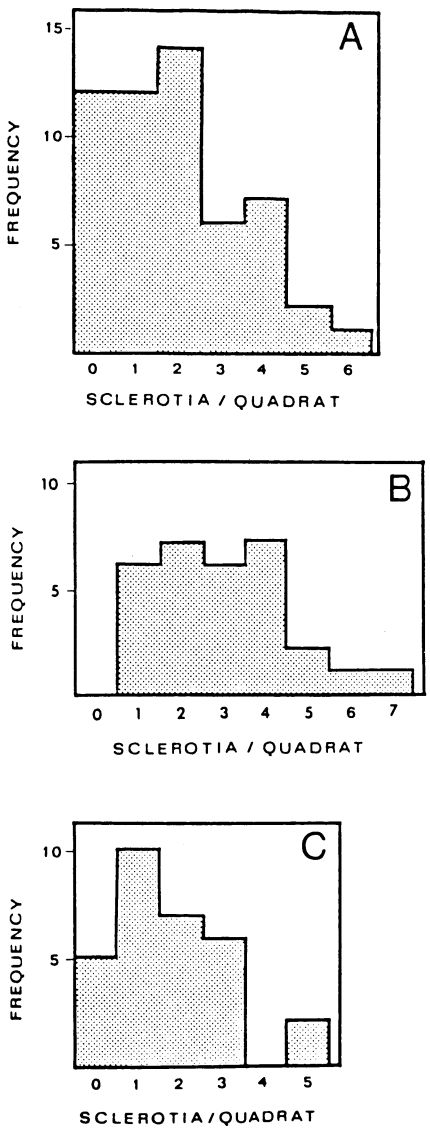

Fig. 1. Observed frequency distributions of sclerotia of *Sclerotium rolfsii* in two apple nursery soils: **(A)** Tahlequah, 1983, quadrat size 2.75 × 2.75 m; **(B)** Stillwater, 1984, quadrat size 2.75 × 2.05 m; and **(C)** Stillwater, 1985, same quadrats as 1984.

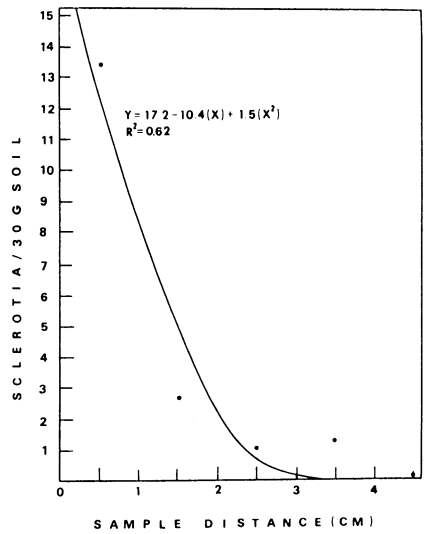

Fig. 2. Relationship between lateral soil sample distance and sclerotial density associated with apple rootstock killed by *Sclerotium rolfsii*. A second-degree regression equation is given, where X = sample distance and Y = number of sclerotia per 30 g of soil. Points represent mean sclerotial densities determined at each sample distance from eight diseased apple rootstocks.

Table 1. Indices of dispersion and distribution model testing of sclerotial populations of *Sclerotium rolfsii* in two Oklahoma apple nursery soils

Location[a] (year)	S^2/X[b]	Poisson distribution		Negative binomial distribution		
		Chi-square	P[c]	Chi-square	P[c]	k[d]
Tahlequah (1983)	1.27	5.50	0.24	3.34	0.30	6.25
Stillwater (1984)	0.82	3.26	0.66	...	...	...
Stillwater (1985)	1.02	0.93	0.82	...	...	...

[a] Nine random samples removed per quadrat at Tahlequah and 10 random samples removed per quadrat at Stillwater.
[b] Variance-to-mean ratio (values of 1.0 indicate randomness).
[c] Probability of exceeding the chi-square value.
[d] Negative binomial distribution parameter (values less than 2.0 indicate clustering).

Inoculum density-disease incidence relationships. *Field experiments.* Disease incidence in infested field soils in 1983 (Tahlequah), 1984 (Stillwater), and 1985 was 6.2, 35.0, and 6.7%, respectively. Only Stillwater (1984) disease incidence and inoculum densities were high enough to allow analysis of the relationship between inoculum density and disease incidence. No correlation between inoculum density and disease incidence could be detected in nursery plots during 1983 and 1985 because of low disease incidence. In 1984, the number of sclerotia per 1,000 g of soil was positively correlated to the multiple-infection transformation values of percent disease ($r = 0.86$, $P = 0.01$).

Container experiments. In 1983, inoculations of container-grown trees with 100 sclerotia per 1,000 g resulted in 44% disease; inoculations with 2 sclerotia per 1,000 g of soil resulted in no disease. All controls remained healthy. A correlation coefficient of 0.79 ($P = 0.01$) was obtained from regression analysis of sclerotia per 1,000 g of soil vs. $\log_e (1/1 - X)$ (Fig. 4).

Microplot experiments. Disease on trees grown in microplots was less than in those grown in containers. In microplots, highest disease incidence (22%) occurred with 100 sclerotia per 1,000 g of soil and lowest disease incidence (2.7%) occurred with 2 sclerotia per 1,000 g of soil. Regression analysis of inoculum density-disease incidence determined the line of best fit and the correlation coefficient of $r = 0.97$ ($P = 0.01$) (Fig. 4).

Effect of inoculum placement and density on disease incidence. Based on data obtained in both growth chamber and container studies, disease incidence was highest when sclerotia were placed in contact with the host and lowest at the 3-cm distance (Table 2). The presence of a viable sclerotium in contact with the host surface, however, did not always result in infection. Data from the growth chamber study indicated that 2–4 sclerotia in contact with the seedling caused 100% mortality (8/8); however, 100% mortality was never observed in any treatment using older, container-grown trees even with 4 sclerotia in contact with the root surface.

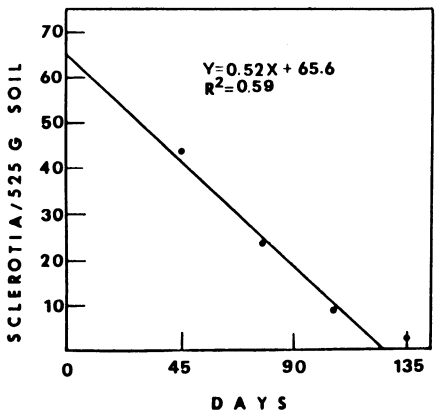

Fig. 3. Relationship between days elapsed from apple rootstock death to sample date and the sclerotial density of *Sclerotium rolfsii*. Data points are mean sclerotial densities associated with diseased rootstock determined for each time period.

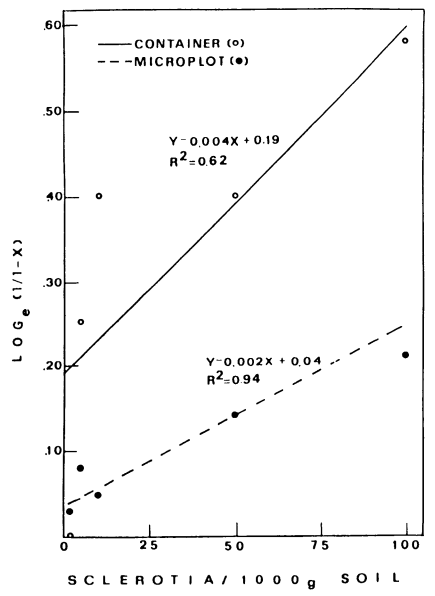

Fig. 4. Relationship between inoculum density (sclerotia) of *Sclerotium rolfsii* and number of infections on apple rootstock expressed as multiple-infection transformation values of disease incidence [$\log_e (1/1 - X)$]. Data points are transformed values of disease incidence of 18 and 72 trees per treatment in container and microplot studies, respectively.

DISCUSSION

A wet-sieving/methanol assay, modified from techniques developed by Rodriguez-Kabana et al (15) and Leach and Davey (10), was used to detect viable sclerotia of *S. rolfsii* in soil samples. Punja et al (13) observed that the methanol assay (15) had a lower recovery and a higher coefficient of variability when tested with soil-produced sclerotia and stated that this may be a less effective technique for enumerating viable field sclerotia. A comparison with wet-sieving procedure based on visual enumeration of sclerotia was found more time consuming than the methanol and flotation techniques, but recovery was the highest (13). The technique used in this study combines attributes of the methanol technique and the wet-sieving assay of Leach and Davey (10), which included placing sclerotia recovered from sieving onto peat soil.

Reports of inoculum density of *S. rolfsii* have been varied and appear to be influenced by many factors including cropping and cultural practices, type of sampling technique, time of sampling, and the assay used. Random-core quadrat sampling for detecting *S. rolfsii* sclerotia in this study may be an adequate method to estimate sclerotial populations (20). In this study, randomly sampled quadrats of the two apple nurseries contained 3.6–4.7 viable sclerotia per 1,000 g of soil. These values are within the range of sclerotial densities reported from other cropping systems. Shew et al (17) reported a density of 0.2 sclerotia per 1,000 g in peanut field soils, and Rodriguez-Kabana et al (14) found densities of 3.8 and 3.9 sclerotia per 250 g of soil in Alabama peanut fields. Punja et al (13) reported densities of 0.3–53.7 sclerotia per 300 cm^3 of soil in carrot fields, and Leach and Davey (10) observed three to 91 sclerotia per 200 g of soil in sugar beet fields.

The horizontal pattern of sclerotia was close to random in both apple nurseries. Data from both years at Stillwater gave a good fit to the Poisson model, whereas the negative binomial was not suitable because the variance is greater than the mean. Neither of the models could be rejected for the Tahlequah data; however, although the variance-to-mean ratio was low, the k parameter was high for the Tahlequah data, indicating randomness in the population. Other studies on spatial pattern of propagules have dealt with smaller, more numerous propagules or host systems where planting densities are much higher, resulting in higher and more aggregated populations of propagules (9,11,13,18). Regardless of cropping system, comparing propagule population characteristics reported by other researchers is difficult because

Table 2. Effects of number and placement of sclerotia of *Sclerotium rolfsii* on disease incidence of apple seedlings in the growth chamber and apple rootstock in a field-container study

Distance[a] (cm)	Number of sclerotia							
	Growth chamber[b]				Field-container[c]			
	1	2	3	4	1	2	3	4
0	4/8	8/8	8/8	8/8	3/9	5/9	5/9	7/9
1	4/8	6/8	4/8	6/8	0/9	2/9	2/9	3/9
2	2/8	2/8	4/8	6/8	0/9	3/9	1/9	2/9
3	0/8	2/8	0/8	2/8	1/9	0/9	1/9	1/9

[a] Values represent distance (cm) sclerotia were placed from the plants at a depth of 0.5 cm.
[b] Ratios represent number of dead seedlings of eight.
[c] Ratios represent number of dead trees of nine.

standardized sampling and assay practices have not been used.

In one other nursery study, distribution analysis of microsclerotia of *Cylindrocladium* spp. on walnut showed that the propagules were clumped (16). The spatial pattern of *S. rolfsii* sclerotia has been determined in only one other host system. Punja et al (13) concluded that the frequency distribution of sclerotia in six carrot fields was best described by the negative binomial based on model fitting, the k parameter, and the variance-to-mean ratios from sample data gathered by using a large probe and single sampling of quadrats. They noted, however, that variability among samples and range of inoculum density were reduced and the data were best fit by the Poisson distribution if multiple, smaller core samples were removed and bulked from quadrats, as we did in our study. Removing smaller, more numerous samples required more time and effort; however, it resulted in similar means. In a study dealing with southern blight on peanuts (17), it was suggested that the frequency counts of sclerotia of *S. rolfsii* should follow a Poisson distribution only because low numbers of sclerotia were observed in samples.

A random pattern of sclerotia in apple nursery soils could result from cultivation, because high and clumped densities were present around infected trees. A mean density of 13.5 sclerotia per 30 g of soil was found in the sampling zone at 0–1 cm, but these values declined to 0.1 sclerotia per 30 g of soil at 4–5 cm in undisturbed soil surrounding rootstock. This observation corresponds to the maximum lateral distance of 3 cm at which sclerotia may be placed and still incite disease. With container-grown trees, fewer viable sclerotia were recovered around trees that had died earlier in the growing season than around trees killed later in the growing season. Reduction in numbers of viable sclerotia of *S. rolfsii* over a period of time was also observed in peanut (2) and carrot (13) fields. Thus, late infections could be responsible for greater inoculum carryover for the next growing season. Lower numbers of viable sclerotia associated with trees infected earlier in the season may be a result of microbial degradation or unfavorable abiotic factors.

Densities of sclerotia recovered from field soil in this study were associated with high disease incidence. In the Tahlequah nursery, 4.7 sclerotia per 1,000 g of soil incited 6.2% disease, whereas in Stillwater (1984), 4 sclerotia per 1,000 g of soil incited 36% disease. Less disease occurred at Stillwater in 1985; 3.6 sclerotia per 1,000 g produced only 6.5% disease. This variation in disease incidence does not appear to be a result of a difference in the pathogen population, because all three density estimates were similar. Furthermore, tests in this study indicate that disease incidence in the apple rootstock-*S. rolfsii* system responds to increased inoculum density. Therefore, variable disease incidence appears to involve other abiotic or biotic factors that influence disease development. The high disease incidence at Stillwater during 1984 may have resulted from inoculum associated with debris from dead 2-yr-old trees still present in plots from the previous growing season. Thus, infected woody trunk and root debris may provide active sources of inoculum the following year. Inoculum in the form of mycelium was found in late-winter tissue samples from woody portions of infected trees (20). Inoculum in and on these tissues will probably not be enumerated in soil samples, thus making disease prediction less accurate.

A linear relationship was observed between disease incidence [transformed to $\log_e (1/1 - X)$] and inoculum density during 1984 at Stillwater. Disease incidence in nurseries in 1983 and 1985 was similar to that observed in microplots in 1984, where 5 sclerotia per 1,000 g of soil produced 5% disease. In studies with container-grown trees, 5 sclerotia per 1,000 g of soil resulted in 19% disease. A linear relationship between inoculum density and disease incidence may not be readily observed in host-pathogen systems involving low propagule numbers and in crops with low planting densities because of factors involving inoculum location, i.e., the ability of a single propagule to infect. The presence of one sclerotium in contact with a host such as apple may not result in infection. Host morphology must be considered as one factor responsible in reducing infection; however, disease incidence increased as sclerotia were placed closer to trees and in greater numbers, because the probability of infection was higher. In this study, 3 cm was determined to be the maximum lateral distance a sclerotium could be placed and possibly result in host infection. Similarly, Punja and Grogan (12), using sugar beet leaf petioles, showed that sclerotia of *S. rolfsii* in field soil can infect from 3.5 cm. Gurkin and Jenkins (8) reported greater infection when sclerotia of *S. rolfsii* were placed in contact than when placed 1 cm from carrot roots.

Estimates of inoculum production and survivability in the soil may be beneficial in evaluating disease control methods that reduce densities of sclerotia within the field. Even with low planting densities involved in nursery operations (29,500 trees per hectare), sclerotial populations are maintained each year by poor weed control and the lack of sanitation practices. Careful removal of diseased trees from the nursery, especially those killed in the later part of the growing season, may result in an immediate reduction of sclerotia in soil. Based on our results, a less extensive preplant sampling procedure may be used to estimate sclerotial populations in apple nurseries, because sclerotia exist in a random pattern and in low densities. Fields with sclerotial populations at or above 5 sclerotia per 1,000 g of soil should be avoided or planted to a nonsusceptible host, or control measures should be planned in advance, because densities of five sclerotia per 1,000 g of soil were shown to produce 6–35% disease. Similar recommendations were made by Punja et al (13) for carrots.

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