SCLEROTIUM ROLFSII, A PATHOGEN ON APPLE ROOTSTOCK IN OKLAHOMA

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SCLEROTIUM ROLFSII, A PATHOGEN ON APPLE ROOTSTOCK IN OKLAHOMA

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PREFACE

Southern blight of apple rootstock, incited by Sclerotium rolfsii, was investigated in this study. Extensive soil sampling and improved assay techniques were used to enumerate sclerotia of S. rolfsii in apple nursery soils and to determine the horizontal spatial pattern of sclerotia. Other epidemiologial aspects such as inoculum density/disease incidence relationship, and the effect of inoculum placement on disease were determined. Also, the histological aspects of infection using thin sectioning techniques were studied.

I wish to express my deepest appreciation to my dissertation adviser, Dr. Kenneth Conway, for his criticism, encouragement, and guidance throughout this research. His continued support and dedication to my academic development have enabled me to reach my academic goals. Appreciation is also extended to my committee members for their continued support and professional expertise: Dr. Robert Hunger, Dr. Hassan Melouk, Dr. John Sherwood, and Dr. Michael Smith.

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Finally, to my mother, Mrs. Beulah Tomasino, for allowing me freedom and responsibility in my early years which have helped me become an independent, and strong individual. My accomplishments are yours as well as mine.

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CHAPTER I

INTRODUCTION

Sclerotium rolfsii Sacc., a soilborne fungus, is a pathogen of a wide range of horticultural and agronomic crops in the southern United States that causes a disease referred to as southern blight or southern wilt. Since *S. rolfsii* was first described by Rolfs in 1892 as a pathogen on tomato, much research interest has been generated due to the economic losses attributed to the fungus. The production of resistant sclerotia, a wide host range, and the saprophytic ability of *S. rolfsii* have made disease control difficult. Most control efforts have focused on reducing sclerotial populations in soil or inhibiting their germination. Southern blight has become an important problem to apple rootstock in some eastern Oklahoma nurseries. The rootstock are used for graft propagation of apple and crabapple cultivars. Several aspects of this host-pathogen system were investigated in this study.

Many studies pertaining to basic physiological aspects of *S. rolfsii* have been performed. However, information in the areas of sclerotial ecology and epidemiological aspects of the disease is lacking. Sclerotial density and spatial patterns, epidemiological aspects, and the infection process of *S. rolfsii* on apple rootstock were major areas of study

of this research.

This dissertation is presented in an alternative format which deviates from the traditional organization outlined in the Thesis Writing Manual provided by the Graduate College of Oklahoma State University. The dissertation is composed of three manuscripts (Chapters) written according to the format style established by the American Phytopathological Society for manuscripts submitted to the professional journals of PHYTOPATHOLOGY or PLANT DISEASE. The final Chapter (Appendix) includes three experiments not represented in the manuscripts.

Chapter II entitled "Southern Blight of Apple Rootstock in Oklahoma" is a descriptive report of the disease characteristics, symptomatology, and incidence of southern blight of apple rootstock in Oklahoma. This manuscript will be submitted to PLANT DISEASE to the report section of New Diseases and Epidemics. Chapter III entitled "Sclerotial Population Characteristics, Inoculum Density/Disease Relationship, and the Effect of Inoculum Placement of Sclerotium rolfsii on Apple" describes a study involving the use of soil sampling and assay techniques to determine sclerotial population characteristics, relationships between sclerotial density in soil to disease incidence, and the effect of inoculum placement on disease. This manuscript will be submitted to PLANT DISEASE. Chapter IV entitled "Histology Aspects of Infection and Internal Mycelium as an Over-wintering Inoculum Source of Sclerotium rolfsii on Apple

Rootstock" reports on the infection process by which S. rolfsii penetrates and occupies host tissue, the location of S. rolfsii in woody tissue after tree death, and the ability of S. rolfsii to survive in the mycelial form through the winter season. This manuscript will be submitted to THE CANADIAN JOURNAL OF BOTANY. The Appendix describes experiments dealing with the evaluation of the wet-sieving/methanol assay, a study performed to determine total sclerotial populations in nursery soil, and the evaluation of several control measures.

CHAPTER II

Southern Blight of Apple Rootstock in Oklahoma

ABSTRACT

Southern blight of apple rootstock, incited by Sclerotium rolfsii, is described for the first time in Oklahoma. Symptoms of the disease, found in apple nurseries in eastern Oklahoma, include rapid wilting of the foliage and tree death resulting from a vascular girdling of the tree. Fungal mycelium and sclerotia of S. rolfsii were present on most infected trees. The pathogen becomes active in early summer as infections occur from germinating sclerotia or inoculum harbored in previously infected trees. Disease expression and incidence depend on time of infection and environmental stress, as well as factors which favor disease development, e.g., the presence of weedy plant material between trees.

INTRODUCTION

Southern blight, incited by Sclerotium rolfsii Sacc., is a destructive disease of many horticultural and agronomic crops in the southern United States and tropical regions of the world. However, few reports concerning southern blight of apple trees have been published. Southern blight was first reported on apple nursery stock in the United States in Maryland in 1935 (5) and subsequently in Indiana in 1953 (6) and in Georgia in 1980 (3). A preliminary report of southern blight of apple in Oklahoma nurseries has been published (4). Aycock (2) indicated that southern blight of apple occurred in New South Wales, the Republic of South Africa, and Australia. Widespread southern blight of apple has also been reported in Israel (1). This paper reports on the characteristics, symptomatology, and incidence of southern blight of apple rootstock ('Delicious') in Oklahoma.

Materials and Methods

Commercial nurseries near Tahlequah, Oklahoma in Cherokee County were surveyed for the presence of southern blight of apple rootstock in the summers of 1981 and 1982. Plants exhibiting southern blight symptoms similar to those

previously reported were examined. Diagnostic characteristics of S. rolfsii in association with diseased trees were noted. If a diseased tree did not exhibit signs of the fungus, the plant was uprooted and placed in a moist chamber at 26 C to allow fungal growth and to confirm the presence of S. rolfsii. Diseased tissue samples were removed and plated on potato-dextrose agar for isolations.

Isolates of S. rolfsii from diseased trees were tested for pathogenicity and used to confirm Koch's postulates on ten six-month old apple seedlings (Malus sylvestris Mill) from stratified seed (Lawyer Nursery Inc., Plains, Montana 59859) and 1-year old apple rootstocks ('Delicious', Malus domestica Bork, Greenleaf Nursery, Tahlequah, Oklahoma 74451). Infested oat-seed culture or sclerotia were used as inocula. Potted seedlings were grown and inoculated (5 sclerotia/ plant) in a growth chamber (12 hr. fluorescent light, approximately 100% RH, 27 C). Rootstock were grown in 12-liter containers and inoculated with 1 g dried S. rolfsii oatseed culture. Containers were placed in field-microplots located near Stillwater, Oklahoma. In both tests, inoculum was placed in contact with the plants to a 0.5 cm depth.

Results and Discussion

Field observations in 1981 and 1982 confirmed the presence of S. rolfsii on apple rootstock in Oklahoma. Southern blight of apple had not been previously reported in

the state. Diseased trees were scattered throughout the nurseries, though it was common to observe 3 to 4 adjacent trees killed by S. rolfsii. Although disease incidence of southern blight of apple is usually low (5 to 10%), the economic loss can be substantial because of the high cash value of the trees (average $1.75/ 1-year old tree) and because growers may allow young trees to remain in the nursery for 2 years before being lifted and sold. One nursery owner near Tahlequah estimated a loss of 10% of 100,000 grafted apple trees to S. rolfsii in 1981. In the fall of 1981, the infected trees remaining in the nursery were destroyed by mowing and disking. In 1982, 50% of the 'Floribunda' flowering crabapples in a 1.2 ha field at the same nursery were killed by the fungus.

Disease symptoms observed in the fields surveyed were similar to those described in earlier reports (3,5,6,7). Infections from germinating sclerotia or actively growing mycelium were observed in early summer as day temperatures approached 30 to 35 C. Symptoms become more apparent under higher summer temperatures (35 to 40 C) and moisture stress. Infection by S. rolfsii produces a vascular girdling of the tree near the soil line which results in the rapid wilting and death of foliage and ultimately the entire tree. Light microscopy has shown the presence of the fungus in the vascular system of dead host trees (8). The leaves of infected trees tend to remain attached to the stems after death. This disease may have been overlooked for several years in Oklahoma because foliar symptoms are similar to

those of fire blight (Erwinia amylovora). Infections occurring later in the growing season (September and October) result in less severe, atypical symptoms such as lack of vigor and reddening of the foliage. Tree death may not immediately follow. However, a delay in leaf emergence and rapid tree death from late-season infections may occur the following spring. Inoculum harbored within the tissues of these "latent" infected trees may also provide active foci for disease development.

The fungus was readily identified in the field by the presence of coarse, white mycelium along the tap root of infected trees and the tan to brown colored sclerotia associated with the mycelium (Fig. 2). Typical mycelial fans of S. rolfsii formed around infected trees under moist conditions (Fig. 3). S. rolfsii bridged the 23 cm space between trees by colonizing weedy plant debris on the soil surface which served as an organic food source for the fungus. Another food base, apple leaf and woody debris from previous growing seasons, provided an excellent inoculum source if infested. Debris with visible mycelium and sclerotia were observed frequently in the surveyed fields.

In both growth chamber and container-microplot studies, Koch's postulates were fulfilled as disease symptoms were reproduced on all plants and S. rolfsii was recovered from the diseased tissues. Seedlings were killed by the fungus within one week after inoculation and all rootstock were dead 3 weeks after inoculation.

Based on our field observations, disease incidence may be

reduced by proper weed control, avoiding infested fields, and roguing diseased trees including those exhibiting latent infections. The severity of this disease in Oklahoma and information from several other southern states indicate that S. rolfsii can cause a significant economic loss in nurseries of 1 to 3 year old apple trees. Research is needed to determine strategies to control this disease and to increase our understanding of the ecology of the pathogen and epidemiology of the disease.

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Fig. 1. Foliar symptoms associated with southern blight of apple rootstock caused by Sclerotium rolfsii. Leaves commonly remain attached to stems after death.

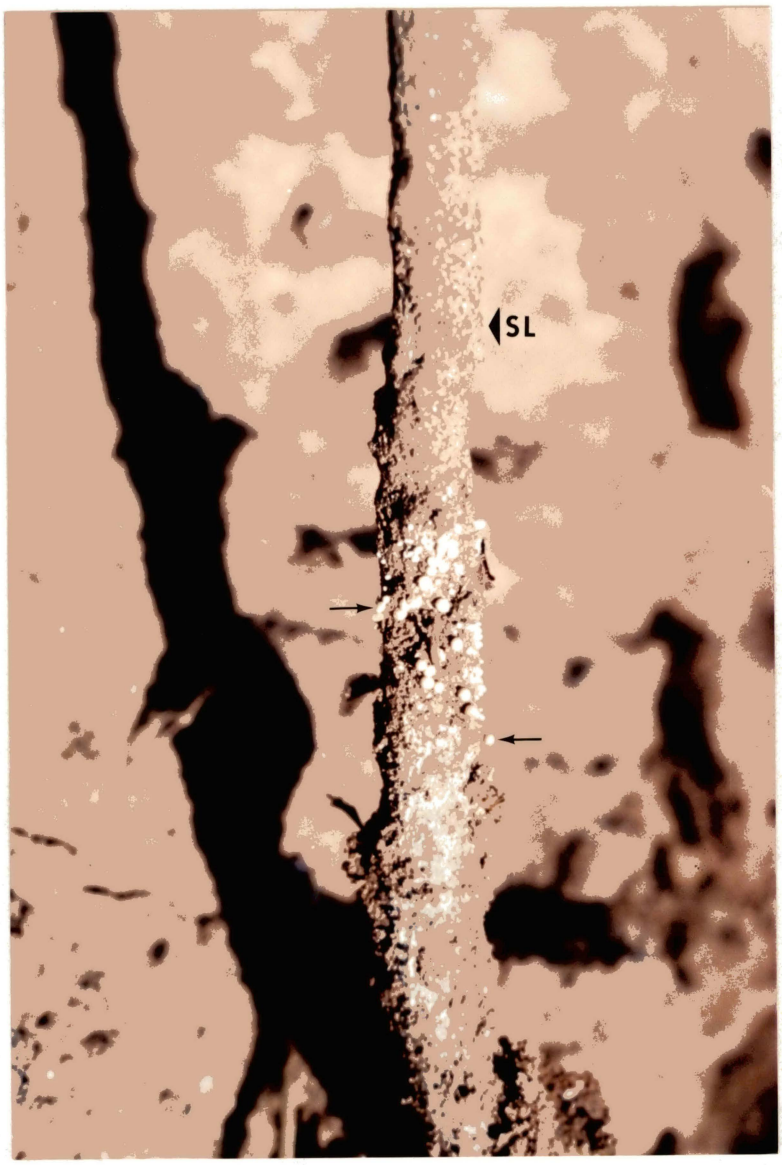

Fig. 2. Sclerotia of Sclerotium rolfsii produced on infected apple rootstock. Arrows indicate the characteristic tan colored sclerotia formed on the rootstock beneath the soil line (SL).

Fig. 3. Mycelial fan of Sclerotium rolfsii associated with a 1-year old infected apple rootstock. Note colonization of apple and weed debris near the rootstock by S. rolfsii.

CHAPTER III

Sclerotial Population Characteristics, Inoculum Density/ Disease Relationships and the Effect of Inoculum Placement of *Sclerotium rolfsii* on Apple Rootstock

ABSTRACT

Nursery plots exhibiting southern blight (*Sclerotium rolfsii*) of apple at Tahlequah and Stillwater, OK were divided into 54 (2.75 m X 2.75 m) and 30 (2.75 m X 2.05 m) quadrats, respectively, and randomly sampled for sclerotia. Pre-plant densities of sclerotia of *Sclerotium rolfsii* as determined by a wet-sieving/methanol assay technique ranged from 0.73 to 0.95 sclerotia/200 g soil at the two nurseries. The number of sclerotia from individual quadrats ranged from 0 to 7. The spatial pattern of sclerotia was determined to be random according to Fisher's variance/mean ratio, frequency distribution analysis, and the k dispersion parameter. A mean density of 44 sclerotia/525 g soil was recovered from soil samples taken adjacent to dead trees. These densities declined with increase in time between sampling and tree death. Densities of sclerotia adjacent to and at various distances away from dead trees were best described by a polynomial regression equation. A positive linear relationship existed between sclerotial densities in soil and disease incidence. One sclerotium/200 g soil produced 19, 5,

and 35% disease in container, microplot, and field studies, respectively. Placing sclerotia 3 cm from apple rootstock, grown in containers, resulted in lower disease incidence compared to sclerotia placed in contact.

INTRODUCTION

Sclerotium rolfsii Sacc. attacks seedling apple rootstocks ('Delicious', Malus domestica Bork) used for the propagation of apple and crabapple cultivars and kills the tree by vascular girdling (20). Trees up to 3 years 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), Arkansas and Texas (personal communication). Little is known about the population ecology of the sclerotial inoculum involved in this host-pathogen relationship. Most work with S. rolfsii has involved high density planting schemes with, e.g., sugar beets (1,10), peanuts (18), and carrots (13,). The apple-S. rolfsii system offers an opportunity to study the ecology and epidemiology of this pathogen in a low density 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

Density and spatial pattern of sclerotia. Plots were established at a nursery in Tahlequah, OK and at Stillwater, OK. The plot at Tahlequah (sandy-loam soil) was a naturally infested area that exhibited high incidence of southern blight of apple (10 to 50%) during the 2 previous years. It measured 24.7 m X 16.5 m. The plot at Stillwater (clay-loam soil) was artificially infested with S. rolfsii in 1982 and was planted with apple rootstock for 2 years prior to the begining of this investigation in 1984. It measured 13.7 m X 12.2 m. Disease incidence in this plot was also high (10 to 15%) during 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. Seedling apple rootstocks ('Delicious', Greenleaf Nursery, Tahlequah, OK 74451) were used as host plants in all experiments unless stated otherwise. In April, 1983, the Tahlequah plot was divided into 54 (2.75 m X 2.75 m) quadrats with 18 apple rootstock planted per quadrat (2 rows, 9 trees/row). In April, 1984 and 1985 the Stillwater plot was divided into 30 (2.75 m X 2.05 m) quadrats with 16 apple rootstock planted per quadrat (2 rows, 8 trees/row). The Stillwater plot was irrigated as

necessary. The Tahlequah plot received no supplemental irrigation. At Tahlequah in 1983, nine core samples were removed at random with a 1.5 cm diameter probe to a depth of 6 cm from each quadrat. Samples from each quadrat were bulked and analyzed as a single sample with an average weight of 400 g. Similar sampling techniques were followed at the Stillwater nursery during 1984 and 1985 except that a 2.0 cm diameter probe was used, and ten core samples were removed to a depth of 8 cm in each quadrat with an average bulked sample weight of 550 g. Samples were stored a 7 C until extraction. All soil samples were taken in April after cultivation but prior to the planting of apple rootstock.

Rootstock planted into microplot soil (sandy-loam) were utilized for several experiments described below. The microplots measured 2.1 m X 2.1 m. Twelve-liter plastic containers, filled with sandy-loam soil, were also used for more controlled studies. Microplot and container studies were located near Stillwater. The microplots contained three rows of rootstock which were planted in April, 1985. Microplots were infested with dried oat-seed culture (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 to determine sclerotial production and distribution. A total of 20 soil samples were taken along perpendicular radii from trees to a distance of 5 cm from each tree at 1-cm increments (4 samples/increment). Samples from each distance were bulked and assayed by the

wet-sieving/methanol method described below to determine the number of sclerotia. Regression analysis between sample distance and the corresponding sclerotial density was used to describe the data statistically.

To determine the total number of viable sclerotia produced on and adjacent to an infected apple root system, soil around 22 infected trees in containers was analyzed. Trees were inoculated during May, 1985 with 1 to 4 sclerotia of S. rolfsii per tree within a 3 cm distance from each 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 of time. These groupings were: 135, 105, 75, and 45 days with 2, 6, 6, and 8 trees sampled from each group, respectively. Entire volumes of soil 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.

Viable sclerotia were enumerated from soil samples using a modified extraction technique (10, 15). Each sample was mixed and air-dried. Soil was weighed and sieved sequentially through two nested sieves with openings of 2.4 mm and 0.5 mm. Contents retained in the final sieve were placed in 14-cm petri dishes and air-dried. Equal volumes of a dark soil base (Baccto potting soil, Michigan Peat Co., Houston, TX 77266) were added to each soil sample to provide color background 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 but ranged from 5 to 10 ml/plate. Saturation of the sample was avoided. Dishes were covered and incubated at 25 C. Samples were examined for germinating sclerotia 24 to 48 hours 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 distribution of sclerotia within plots was analysed by several procedures, and expected frequency numbers were generated and tested against observed values using the FORTRAN program developed by Gates and Ethridge (7). The Chi-Square function indicated the distribution model which best fit the data. The general models for Poisson and Negative Binomial were utilized in this study. Supplemental tests such as Fisher's Variance/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 comparing sclerotial counts from randomly sampled quadrats to the observed loss of trees in the corresponding quadrat. Ten quadrats with varying sclerotial densities (1 to 6 sclerotia/525 g soil) at the Stillwater nursery were used in

1984 for this determination. Trees (8/row) in each quadrat were observed throughout the growing season for disease.

Container Experiments. Non-sterile sandy-loam soil was placed in 108 plastic containers 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 non-sterile sclerotia of S. rolfsii produced from oat-seed cultures of an isolate obtained from diseased apple rootstock. Sclerotial viability was determined before inoculation and was found to be near 100%. Inoculum densities (sclerotia/1000 g 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 treatment had 18 trees and were arranged in a completely randomized design. Plants were monitored for infection and disease symptoms.

Microplot Experiments. Apple rootstocks were planted directly into microplots containing non-sterile sandy loam soil in April, 1984. Three rows with 9 trees /row were planted in 16 plots. The trees were spaced 22 cm apart, and 91 cm separated each row. All plots were sampled prior to planting and S. rolfsii was not recovered. Non-sterile sclerotia, at rates of 0, 2, 5, 10, 50 and 100/1000 g soil were randomly added to soil in narrow furrows (4 cm wide) on both sides of the rows to a depth of 2 cm. 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. The multiple infection transformation (Log_e (1/1-X) was utilized to estimate the number of infections per apple tree for all three experiments.

Effect of inoculum placement and density on disease incidence. Studies were initiated in growth chambers to determine the influence S. rolfsii sclerotia placement of on apple seedling disease. Apple seedlings (Malus sylvestris Mill) from stratified seeds (Lawyer Nursery Inc., Plains, Montana 59859) were used as host plants. The seedlings were grown in potting medium under greenhouse conditions. Three-month old seedlings were transferred to a growth chamber maintained at 30 C near 100% RH for the development of southern blight. Factorial experiments were designed to include combinations of placement of sclerotia 0.5 cm deep at contact, 1 cm, 2 cm, and 3 cm away from the host and varying numbers of sclerotia (1, 2, 3, 4) per plant. There were 8 trees per combination. Infection and death of seedlings were recorded for 2 weeks. In 1985, the same combinations were evaluated using container-grown rootstock. Again, containers were filled with sandy-loam soil and a rootstock was planted in each. Nine trees were used per combination. Infection and death due to S. rolfsii were recorded weekly throughout the growing season.

RESULTS

Density and spatial pattern of sclerotia. Average sclerotial densities in nursery soils ranged from 0.95/200 g soil in Tahlequah (1983) to 0.73/200 g soil in Stillwater (1985). Sclerotia (102 total) were recovered from 42 of 54 quadrats sampled in Tahlequah. In Stillwater (1984), all 30 quadrats exhibited sclerotia (89 total) while sclerotia (52 total) were recovered in 25 of 30 quadrats in 1985.

The number of sclerotia recovered in quadrats at both locations ranged 0 to 7 for all plots combined (Fig. 1). The variance to mean ratios (index of dispersion) for the three situations were low and close to 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 $k=mean^2/variance-mean$ was 6.25 for Tahlequah which further suggests a random pattern as values of "k" needed to indicate clustering should be "k"<2.0 (19). The variance to mean ratios for Stillwater 1984 and 1985 were less than 1 and 1.0 respectively which would reject a Negative Binomial

distribution for either data set since the variance is assummed to be greater than the mean for the negative binomial model.

Sclerotial population stability and arrangement of S. rolfsii surrounding dead trees were found to be dynamic characteristics. In the first study, of 150 sclerotia recovered in the total sample zones of 10 trees, 72% were found in the contact to 1 cm zone, 15% in the 1 to 2 cm zone, 5% in the 2 to 3 cm zone, 6.6% in the 3 to 4 cm zone, and 0.66% in the 4 to 5 cm zone. Two of the trees produced 0 sclerotia and were not included in the regression analysis. Other pathogens may have been responsible for tree death, explaining the absence of sclerotia. Mean numbers of sclerotia recovered were 13.5, 2.8, 1, 1.2, and 0.1 per 30 g soil for contact to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5 cm zones, respectively. Newly formed sclerotia occupied an area within a 3 cm distance of the host (Fig. 2). A polynomial regression equation ($r=0.79$, $P=0.01$) best described the relationship between sample distance and number of sclerotia. 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/30 g of soil and X=sample distance (cm).

The second study indicated that high densities of propagules could be produced as a result of infection, but densities are variable and decline over time. No correlation was observed between initial inoculum amounts (1 to 4

sclerotia) and numbers of sclerotia produced as a result of infection. A range of 0 to 68 sclerotia per 525 g soil existed in the soil sample area around 22 container grown trees. The mean number of viable sclerotia recovered from each tree in each group was: 135 days=3, 105 days=8, 75 days=24, and 45 days=44 (Fig. 3). Acccording to regression analysis, sclerotial populations decreased as the elapsed time between tree death and sampling increased ($r=-0.77$, $P=0.01$). The regression equation was $Y=-0.52(X)+65.6$, where Y=number of sclerotia and X= elapsed days between tree death and sampling.

Inoculum density/disease incidence relationships. Field Experiments. Disease incidence in infested field soils in 1983 (Tahlequah), 1984 (Stillwater), and 1985 were 6.2, 35.0, and 6.7% respectively. Only Stillwater (1984) disease incidence and sclerotial densities were high enough to allow analysis of the relationship between inoculum density and disease incidence. No correlation between inoculum density and percent disease could be detected in nursery plots during 1983 and 1985, because of low disease incidence. Disease incidence ranged from 0% to 75%. The number of sclerotia/ g soil was positively correlated to disease, ($r=0.86$, $P=0.01$) with a regression equation of $Y=0.18X + (-)0.04$, where Y=number of infections and X=sclerotia/525 g soil.

Container Experiments. Inoculations of container-grown trees during 1983 resulted in 44% disease in the 100 sclerotia/1000 g soil inoculation. The 2 sclerotia/1000 g

soil inoculation resulted in 0% disease. All controls remained healthy. A correlation coefficient of 0.79 (P=.01) was obtained through regression analysis of sclerotia/ 1000 g soil vs Log_e (1/1-X) (Fig. 4).

Microplot Experiments. Disease incidence of trees grown in microplots was less compared to the container-grown trees. Greatest disease incidence (22%) occurred in microplots in 100 sclerotia/1000 g soil inoculations and lowest disease incidence (2.7%) in 2 sclerotia/1000 g 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 containerized-rootstock studies, disease incidence decreased as sclerotia were placed at greater lateral distances from the host trees. Greatest disease occurred when sclerotia were placed in contact with the host and lowest at the 3 cm distance (Table 2). However, the presence of a viable sclerotium in contact with the host surface did not necessarily result in infection. The data from the growth chamber study indicated that to obtain 100% tree mortality (8/8), 4 sclerotia in contact with each seedling were required. However, a 100% mortality was never observed in any treatments using older, container-grown trees even with 4 sclerotia in contact with the root surface.

DISCUSSION

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

The effectiveness of random core-quadrat sampling for detection S. rolfsii sclerotia is an adequate approach to estimate sclerotial populations (20). Random core sampling, also referred to as stratified random sampling, exhibited lower percentage error of disease incidence estimates compared to diagonal and w-shaped sampling patterns in a simulated test (5).

The population of sclerotia in the two nursery soils ranged from 0.95 to 0.73 sclerotia/200 g soil when sampled in

April. 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 utilized. Shew (18) reported a density of 0.2 sclerotia/1000 g in peanut field soils and densities of 3.8 and 3.9 sclerotia/250 g soil were found by Rodriguez-Kabana et al (14) in Alabama peanut fields. Punja (13) reported densities of 0.3 to 53.7 sclerotia/300 cm soil in carrot fields while Leach and Davey (10) observed 3 to 91 sclerotia/200 g soil in sugar beet fields.

The analysis of horizontal pattern of sclerotia determined a randomness of sclerotia in the two apple nurseries. Data from both years at Stillwater resulted in a good fit of the Poisson model while the Negative Binomial was not suitable since the variance is greater than the mean. Neither null hypotheses could be rejected for either Poisson or Negative Binomial models for the Tahlequah data. The variance/mean ratio was low and the "k" parameter was high for the Tahlequah data which indicated randomness in the population. Data such as this are not common when compared to other studies dealing with soilborne pathogen population patterns. Other studies have dealt with smaller, more numerous propagules, or host systems where planting densities are much higher, thus supporting higher and more aggregated populations of propagules (9,11,13,17). Regardless of cropping system, comparing propagule population characteristics observed by other researchers is difficult

because standardized sampling and assay practices have not been utilized.

In one other nursery study, distribution analysis of microsclerotia of Cylindrocladium spp. in a walnut seedling nursery determined the propagules were clumped (16). The spatial pattern of S. rolfsii sclerotia has been studied in only one other host system. Punja (13) concluded the frequency distribution of sclerotia in carrot fields was best described by the Negative Binomial based on model fitting, the "k" parameter, and the variance/mean ratios from sample data gathered by using a large probe, single sampling of quadrats. However, he noted that variability and range was reduced and the Poisson distribution was best fit if multiple, smaller core samples were removed and bulked from quadrats. Removing smaller, more numerous samples requires more time and effort, however, it results in greater accuracy. In a study dealing with southern blight on peanuts (18), it was suggested that the frequency counts of sclerotia of S. rolfsii should follow a Poisson distribution based on low numbers of sclerotia observed in samples.

Randomness of sclerotia in apple nursery soils appears to be a result of cultivation as high densities of sclerotia are present around infected trees. Mean densities of 13.5 sclerotia/30 g soil (90/200 g soil) were found in the contact to 1 cm sampling zones and these values declined to 0.1 sclerotia/30 g soil at the 4 to 5 cm zones in undisturbed soil surrounding diseased rootstock. This observation corresponds

to the maximum lateral distance of 3 cm which sclerotia may be placed and still incite disease. Another factor influencing the density of sclerotia in soil at the end of the growing season is the time of year the infected trees were killed. Utilizing container-grown trees, fewer viable sclerotia were recovered from around dead trees which had died earlier in the growing season compared to ones 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 fields (2). Thus, late infections could be responsible for greater inoculum carry over 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 abiotic factors.

Incidence of disease using the apple rootstock system was easily determined as symptom expression is drastic and distinct. This desirable characteristic is critical in developing relationships of inoculum denisty and disease incidence. Densities of sclerotia recovered from field soil in this study were capable of producing high disease incidence. In the Talhequah nursery, 0.95 sclerotia/200 g soil incited 6.2% disease while in Stillwater (1984), 0.80 sclerotia/200 g soil incited 36% disease. Less disease occurred at Stillwater in 1985 as 0.73 sclerotia/200 g produced only 6.5% disease. This variation in disease incidence does not appear to be a result of a differnce in the pathogen population as all three density estimates were

similar. However, tests in this study indicate that disease incidence in the apple rootstock-*S. rolfsii* system responds to increased sclerotia densities. Therefore, this fluctuation of disease appears to involve other abiotic or biotic factors that influence disease development. The variation at Stillwater, especially during 1984 season, may have been a result of high inoculum quantities associated with debris from dead 2-year old trees still present in plots from the previous growing season. Thus, if infected woody trunk and root debris are not removed they may provide active sources of inoculum the following year. Inoculum in the form of mycelium was found to be present in late winter tissue samples from woody portions of infected trees (20). Inoculum in and on these tissues will most likely not be enumerated in soil samples thus making disease prediction less accurate. Even with low planting densities involved in nursery operations (29,500 trees/ha), sclerotial populations are maintained each year by the lack of sanitation practices and also by poor weed control.

A linear response was observed between increased disease incidence and increased inoculum density during 1984 at Stillwater. Disease incidence in 1983 and 1985 nursery assessments were reproduced artifically in microplots in 1984 in which 1 sclerotium/200 g soil produced 5% disease. In studies with container-grown trees, 1 sclerotium/200 g soil resulted in 19% disease. A linear relationship between inoculum and disease may not be as readily observed in host-

pathogen systems involving low propagule numbers and crops with low planting densities due to factors involving inoculum location and the ability of a single propagule to infect. The presence of 1 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. As sclerotia were placed closer to the trees and in greater numbers, disease incidence increased. In this study, 3 cm was determined to be the maximum lateral distance a sclerotium could be placed and possibly result in host infection. Gurkin and Jenkins (8) reported greater infection when sclerotia of S. rolfsii were placed in contact than at 1 cm from carrot roots.

Propagule distribution and density information of soilborne pathogens such as S. rolfsii are essential to development of control methods based on epidemiological principles. Distribution and density of propagules must be determined if one expects to develop efficient and accurate soil sampling techniques to better predict disease loss. Also, better estimates of inoculum production and survivabilty in the soil may be beneficial in evaluating disease control by methods which reduce densities of sclerotia within the field. Based on our results, a less extensive pre-plant sampling procedure may be used to estimate sclerotial populations in apple nurseries since sclerotia exist in a random pattern and in low densities. Fields exhibiting sclerotia populations at or above 1

sclerotia/200 g soil should be avoided, planted to a non-susceptible host or control measures should be planned in advanced since densities of 1 sclerotia/200 g soil were shown to produce 6 to 35% disease. 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. Each host system with it's own intrinsic cropping and cultural practices as well as the sampling method chosen may influence predictive models based on pathogen population characteristics.

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

		Poisson Distribution		Neg. Binomial Distribution		
Location[a]	S^2/X[b]	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/quadrat at Tahlequah and 10 random samples removed/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).

TABLE 2. Effect 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

	Growth Chamber[b]				Field-container[c]			
	Number of sclerotia							
Distance[a]	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 laterally away from the plants at a depth of 0.5 cm.

[b] Ratios represent number of dead seedlings out of 8.

[c] Ratios represent number of dead trees out of 9.

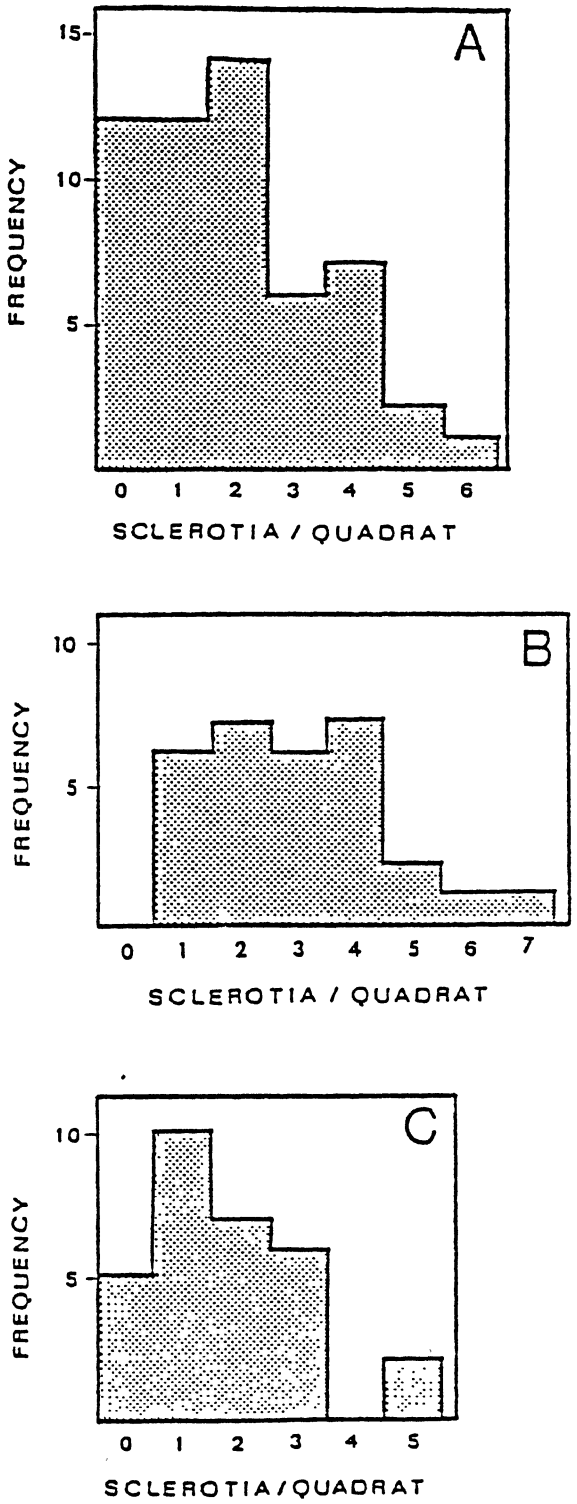

Fig. 1. Observed frequency distributions of sclerotia of Sclerotium rolfsii in two apple nursery soils. A)Tanlequan, 1983, quadrat size of 2.75 M X 2.75 M; B) Stillwater, 1984, quadrat size of 2.75 M X 2.05 M; C) Stillwater, 1985, same quadrats as 1984.

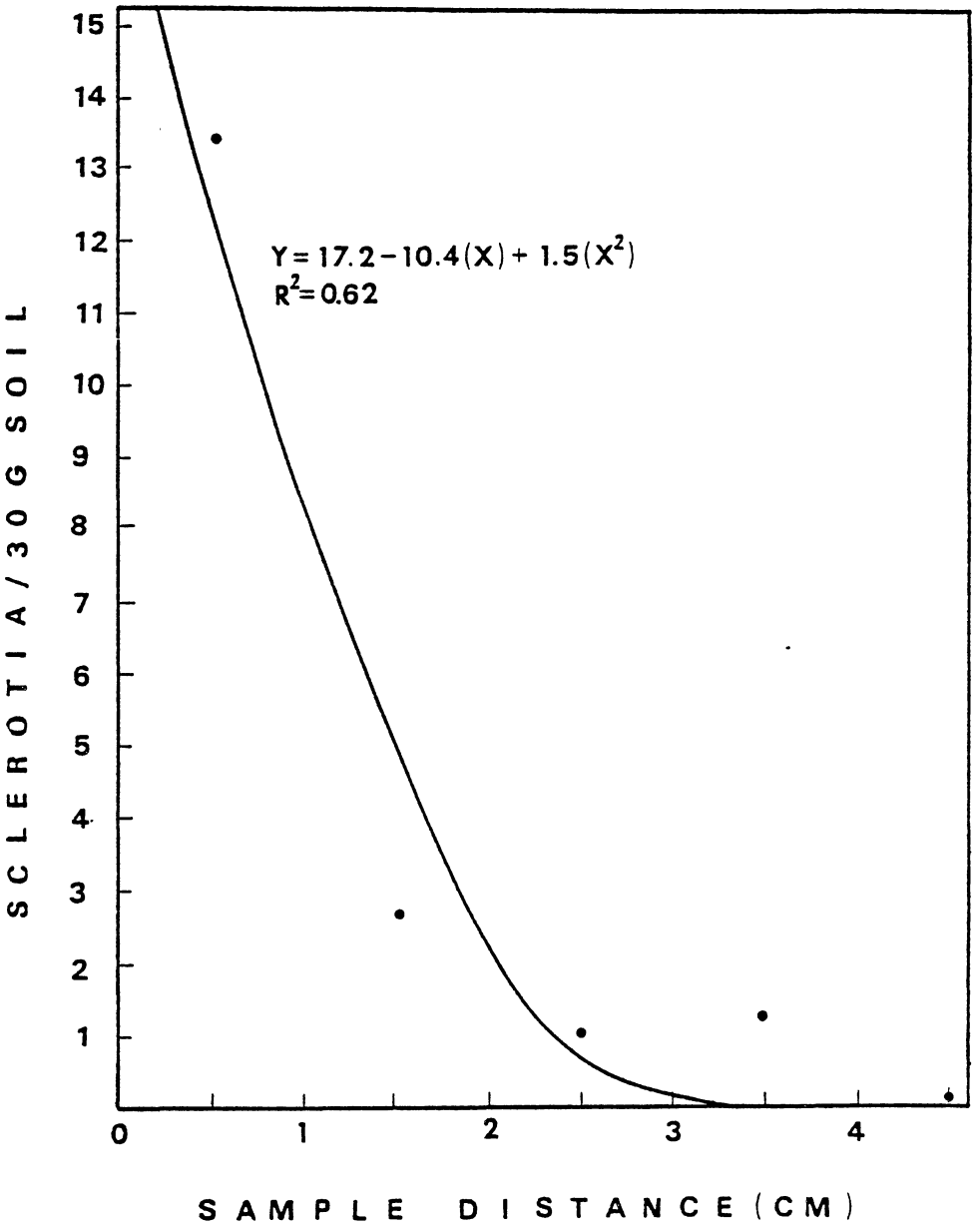

Fig. 2. Relationship between lateral soil sample distance and sclerotial density associated with apple rootstock killed by Sclerotium rolfsii. A second-degree regression equation is given where X=sample distance, and Y=number of sclerotia of S. rolfsii per 30 g soil. Points represent mean sclerotia densities determined at each sample distance from 8 diseased apple rootstock.

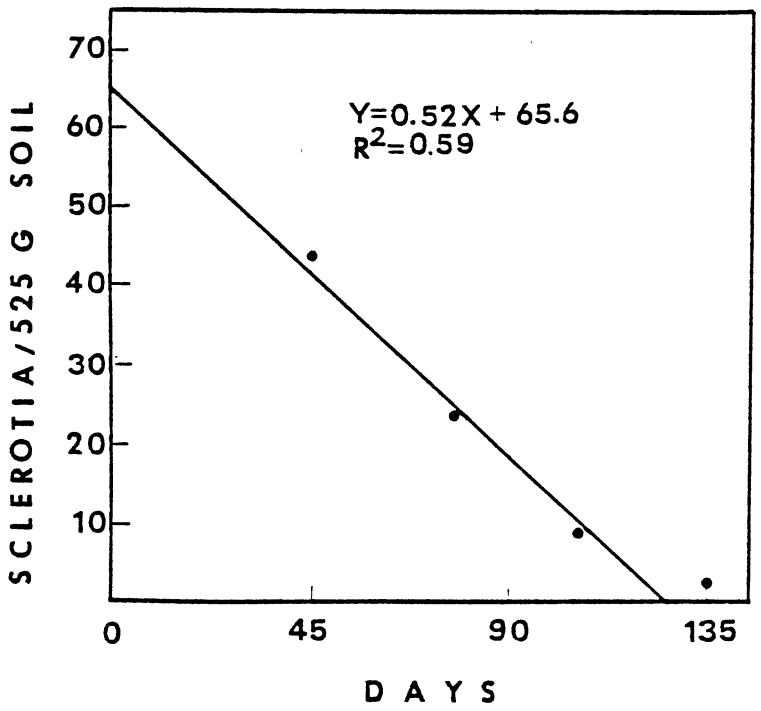

Fig. 3. Relationship between days elapsed from apple rootstock death to sample date and the sclerotial density of Sclerotium rolfsii. Data points are mean sclerotial densities associated with diseased apple rootstock determined for each time period.

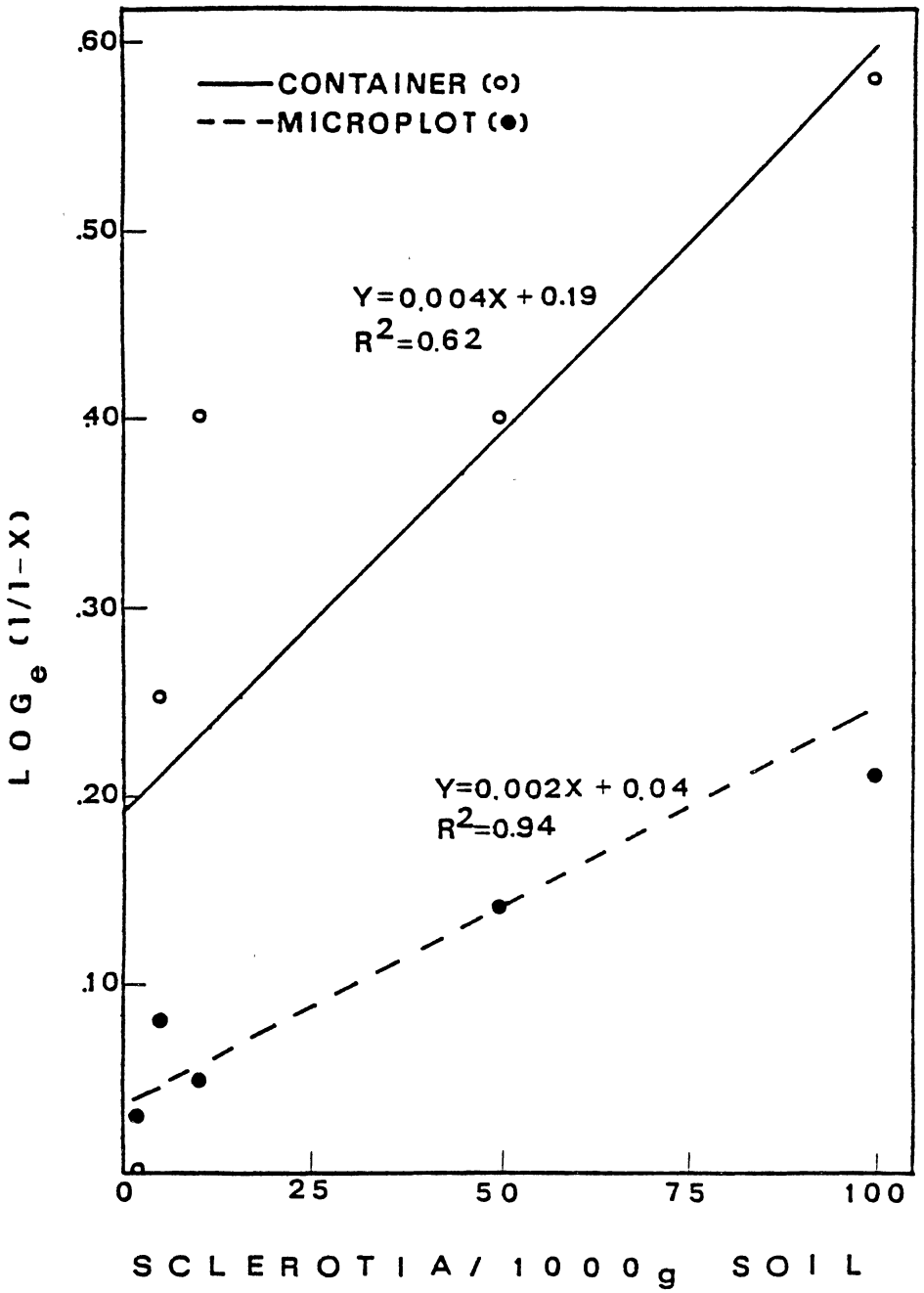

Fig. 4. Relationship between inoculum density (sclerotia) of Sclerotium rolfsii and number of infections on apple rootstock expressed as multiple infection transformation values of disease incidence (LOG_e (1/1-X). Data points are transformed values of disease incidence (X in equation) of 18 and 72 trees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

CHAPTER IV

Histology of Infection and Internal Mycelium as an Overwintering Inoculum of *Sclerotium rolfsii* on Apple Rootstock.

ABSTRACT

A histological study of the infection process of *Sclerotium rolfsii* on 6-month old apple seedlings and 1-year old rootstock determined that aggregates of mycelium (infection cushions) were formed in crevices of bark and serve as sources for hyphal penetration into host tissue. Death of apple seedlings and rootstock occurred 7 and 9 days after inoculation, respectively. Hyphae were observed in sections of phloem and xylem of inoculated trees and mycelium occupied a 3.5 cm zone encompassing the infection site after tree death. Mycelium within dead rootstock was present in tissue samples taken in the early spring from field material, demonstrating the overwintering capability of *S. rolfsii* mycelium. Trees killed later in the growing season have greater potential to harbor mycelium through winter months than those killed from early-season infections.

INTRODUCTION

Many aspects of the physiology and biology of Sclerotium rolfsii Sacc. have been studied in depth. However, there have been few reports describing the parasitic process by which this pathogen colonizes and penetrates host tissue. Two previous histological reports of the infection process by S. rolfsii describe appressorial (holdfasts) formation (3) and the presence of infection cushions (7). No reports have addressed the presence or potential of S. rolfsii mycelium as an overwintering inoculum source. Apple rootstocks, used for graft propagation of apple and crabapple cultivars, are important host plants of S. rolfsii (8). This host-pathogen system provides an opportunity to investigate the infection process of this fungus on a woody host and to examine the role of mycelium as an overwintering stage of the fungus. In this study the infection process of S. rolfsii on apple seedlings and older apple rootstock, the location of S. rolfsii in the tissues of apple rootstock after death, and the ability of S. rolfsii to overwinter in woody tissues of apple trees are examined.

MATERIALS AND METHODS

An isolate of S. rolfsii recovered from infected apple rootstock was used throughout this study. Sclerotia produced from infested oat-seed cultures were used as inoculum. Two plant sources were used; 6-month old apple seedlings (Malus sylverstris Mill) grown from seed (Lawyer Nursery Inc., Plains, Montana 59859) and one-year old domestic apple rootstock ('Delicious', Malus domestica Bork, Greenleaf Nursery, Tahlequah, Oklahoma 74451). Seedlings were grown initially in potting medium in the greenhouse and later transplanted into 15-cm pots containing 50% non-sterile sandy-loam soil and 50% potting medium. Rootstock were grown in the 50/50 combination throughout. Plants were placed in a growth chamber at 27 C, 12 hours fluorescent lighting (2000 lux) and approximately 100% RH.

Six seedlings and rootstock were inoculated in the growth chamber by placing four sclerotia in contact with the trees 0.5 cm below the soil surface. Plastic bags were placed over the seedlings to retain moisture. Two plants of each age were sacrificed 2, 4, and 7 days after inoculation. If typical disease symptoms (rapid wilting and browning of leaves) did not appear after 7 days, the infection was allowed to proceed until tree death occurred. Stem sections (4 cm) were removed from the base of the plants around the

infection sites and fixed in formalin-acetic acid-alcohol (FAA) (4) for 24 hours under vacuum. The fixed material was then dehydrated in a tertiary butyl alcohol series (5). Stem pieces were embedded in paraffin and cross and longitudinal sections (12 to 15 um) were cut with a rotary microtome. After removal of the paraffin with xylene, the material was hydrated in an ethanol series of 95, 80, 70, 50, 30, and 10% for 5 minutes and finally 2 minutes in distilled water. Material was stained with acid lacto-fuschin, and Pianeze IIIb (a double stain containing malachite green and lacto-fuschin). The lacto-fuschin was dropped onto the hydrated sections while double staining was performed according to Dhingra (2).

Two one-year old apple rootstock were inoculated with 5 mm potato-dextrose agar (PDA) plugs supporting active mycelial growth of S. rolfsii. Plugs were placed in contact with the trees 0.5 cm below the soil surface. Inoculations were performed in the growth chamber under conditions described earlier. After tree death, an 8 cm portion of the plant was removed with the primary infection site approximately in the middle. The stem/root section was surface sterilized 5 minutes in 10% sodium hypochlorite and rinsed in sterile water. Cross sections 0.5 cm in thickness were made sequentially 3.5 cm above and 3 cm below the infection site. Each of the 0.5 cm sections were placed on PDSA (potato-dextrose streptomycin agar; PDA containing 300 mg streptomycin sulfate/L) and incubated 24 hours at room

temperature. Sections were observed for the presence of S. rolfsii mycelium and which tissue layer (bark, phloem, xylem) supported its growth.

In March, 1986, 15 trees were chosen in microplots at Stillwater to represent a range of trees which had been killed by S. rolfsii at various dates the previous year. One latently infected tree was also chosen for sampling. Symptoms of latent infections (lack of vigor and reddening of leaves) occur as a result of infection by the fungus late in the growing season (September, October). Microplots, containing sandy-loam soil, were artificially infested with S. rolfsii in the form of colonized oat-seed culture in April, 1985. One-year old domestic apple rootstock ('Delicious') were planted into the microplots after infestation and observed for disease symptoms and death throughout the growing season. Tree deaths resulting from S. rolfsii infection were recorded from 6/13/85 to 10/7/85. A 4 cm portion of each tree was removed just below the soil surface, washed vigorously in tap water, and surface sterilized for 5 minutes in a 10% sodium hypochlorite. Sections were rinsed in sterile water and split longitudinally before being placed on PDSA and were incubated at room temperature for 24 to 48 hours. Observations were made for the presence of S. rolfsii mycelium from internal tissues of each sample.

RESULTS AND DISCUSSION

Mycelium from germinating sclerotia became visible around the base of plants 24 hours after inoculation. Typical disease symptoms occurred on day-7 for apple seedlings; however, older rootstock remained symptomless until day-9.

Both stains were beneficial in detecting superficial and internal mycelium. Day-2 sections from both seedlings and rootstock clearly showed the superficial growth of mycelium on the bark of the trees. There was no indication of appressorial development or association of mycelia with lenticels or lateral buds. Day-4 sections of both seedlings and rootstock exhibited aggregations of fungal mycelium of S. rolfsii in crevices and under sheaths of bark tissue (Fig. 1). Mycelium composing the aggregates were highly branched, with numerous cross walls, and measured 5 to 10 µm in diameter. These aggregations were termed infection cushions (IC). Day-4 IC ranged from 90 to 125 µm in diameter. Extensive IC measuring 400 to 500 µm in diameter were observed at day-7 on seedlings. Sections of infected apple seedlings revealed hyphal penetration originating from the IC (Fig. 2). The mycelium ramified within the phloem and xylem tissues both inter- and intracellulary and resulted in rapid vascular girdling. Day-9 sections from the older rootstock also exhibited organized

IC, and penetration appeared similar (Fig. 3).

Longitudinal sections of day-7 (seedlings) and day-9 (rootstock) stem pieces clearly showed the mycelium of S. rolfsii in the xylem vessels (Fig. 4). The mycelium was shown to invade adjacent cell types such as parenchyma by constriction of hyphae through the vessel pits (Fig. 5).

The location of mycelium of S. rolfsii in infected apple rootstock tissues was confined to a limited area above and below the infection point (Table 1). Mycelium was observed to 2 cm above and 1.5 cm below the infection point. The isolations corresponded to the extent of the water soaked, lesioned portion of the tree base.

Mycelium of S. rolfsii grew from 5 of the 15 infected rootstock samples (Table 2). The sample taken from a latent infected tree was also positive for S. rolfsii. Based on these isolations, trees killed in September and October have a greater potential to harbor mycelium in internal tissues through winter months than those killed earlier in the season.

The observation of infection cushions in apple seedling and rootstock infection by S. rolfsii agree with findings of an earlier study of S. rolfsii infection of bean hypocotyls (7). However, Higgins (3) reported the presence of holdfasts, flattened hyphae with thickened ends, on the surface of infected tomato and soybean seedlings. He also observed that the underlying parenchyma cells were killed before there was any evidence of hyphal penetration. The active production of

oxalic acid and polygalacturonases by S. rolfsii is well documented and appear to cause host cell death before penetration (1,6). The compound effects of infection cushion formation and chemical production by S. rolfsii aid in the breakdown of host barriers such as bark. In this study, the mycelium was shown to grow through the stem tissues both inter- and intracellulary. The presence of the fungus in xylem vessels may be a secondary characteristic of the infection because many vessels of dead trees did not contain mycelium and physical blockage of water could not occur. Thus, the rapid dysfunction of phloem caused by the physical presence of mycelium and metabolite and enzyme production appears to be responsible for disease symptoms. Knowledge of the infection process of fungi on host plants will enable researchers to better understand mechanisms involved in host resistance, both structural and chemical. That is, varieties partially or completely resistant to S. rolfsii may be a product of reduced infection through mechanical barriers or chemical properties within the host.

Mycelium of S. rolfsii can successfully overwinter in dead apple tree tissues. Trees infected and killed earlier in the growing season are less likely to harbor viable mycelium through the winter months. This may be a result of food depletion, or competition with other microorganisms. This additional source of inoculum presents problems in control as well as disease prediction based only on sclerotia populations from soil samples. Although the amount of

inoculum harbored in wood debris varies with time of tree death and probably with winter severity, the reduction of this inoculum source by roguing and adequate sanitary practices appears necessary for disease control. More research directed towards an understanding of the occurrence and importance of overwintering mycelium of S. rolfsii in initiation of disease epidemics is needed in the apple rootstock system and in other host-pathogen systems.

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TABLE 1. Recovery and location of mycelium of Sclerotium rolfsii in tissues of two infected apple trees

Section (cm)[a]	Tree #1[b]		Tree #2[b]	
	Bark/Phloem	Xylem	Bark/Phloem	Xylem
H (3-3.5).....	-	-	-	-
G (2.5-3).....	-	-	-	-
F (2-2.5).....	-	-	-	-
E (1.5-2).....	+	-	+	+
D (1-1.5).....	+	-	+	+
C (0.5-1).....	+	+	+	+
B (0-0.5).....	+	+	+	+
A Infection site.	+	+	+	+
B' (0-0.5).....	+	+	+	+
C' (0.5-1).....	+	+	+	+
D' (1-1.5).....	+	-	+	+
E' (1.5-2).....	-	-	-	-
F' (2-2.5).....	-	-	-	-

[a]Sections B-H indicate tissue samples above the infection site and B'-F' indicate samples below the infection site.

[b](+)=positive isolation on Potato-Dextrose-Streptomycin Agar (PDSA) and (-)=pathogen not isolated on PDSA.

TABLE 2. Occurrence of overwintering mycelium of *Sclerotium rolfsii* in woody tissues of dead apple rootstocks

Rootstock death (1985)[a]	*S. rolfsii* isolations[b]
6/13	-
7/11	-
7/29 (2)	-,-
8/3	-
8/22	-
9/9 (3)	+,-,-
9/13	+
9/17 (4)	+,+,-,-
10/7	+
Latent infected tree	+

[a]Tissue samples removed on 3/10/86.

[b](+)=positive isolation on Potato-Dextrose-Streptomycin Agar (PDSA) and (-)=pathogen not isolated on PDSA.

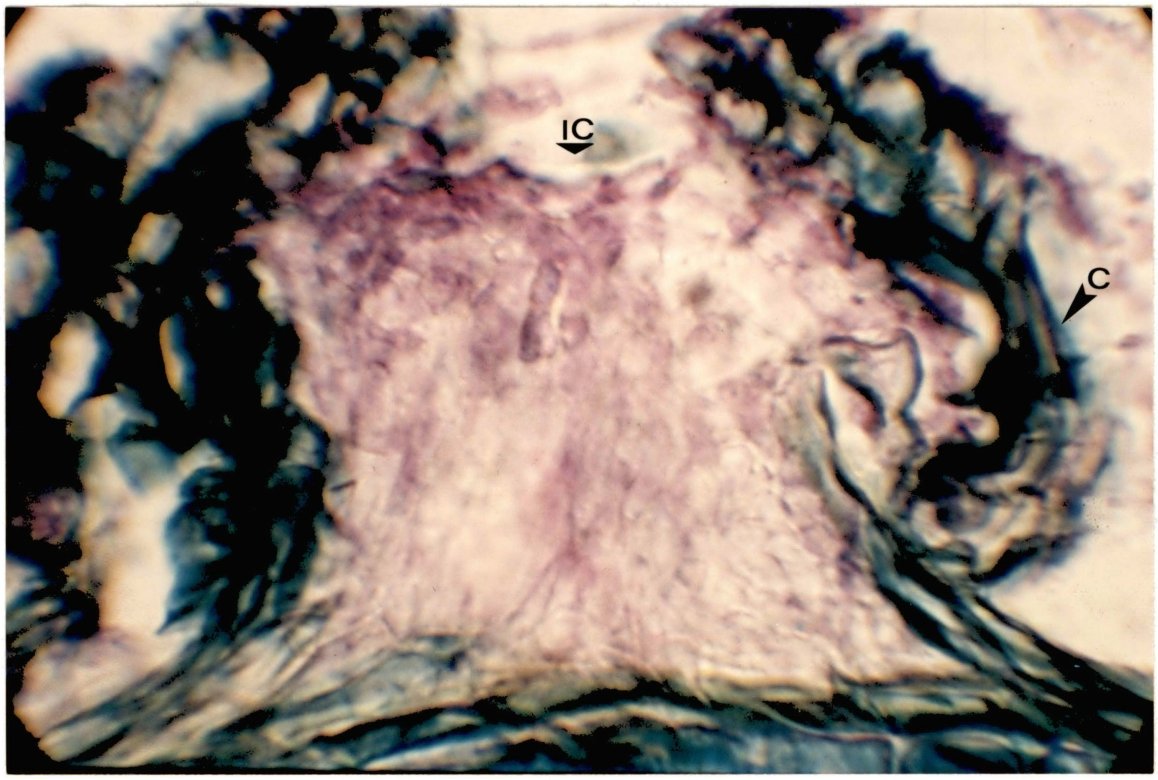

Fig. 1. Transverse section of a day-4 infection cushion (IC) of Sclerotium rolfsii on an apple seedling. Colonization has occurred beneath sheaths of cork (C), X400.

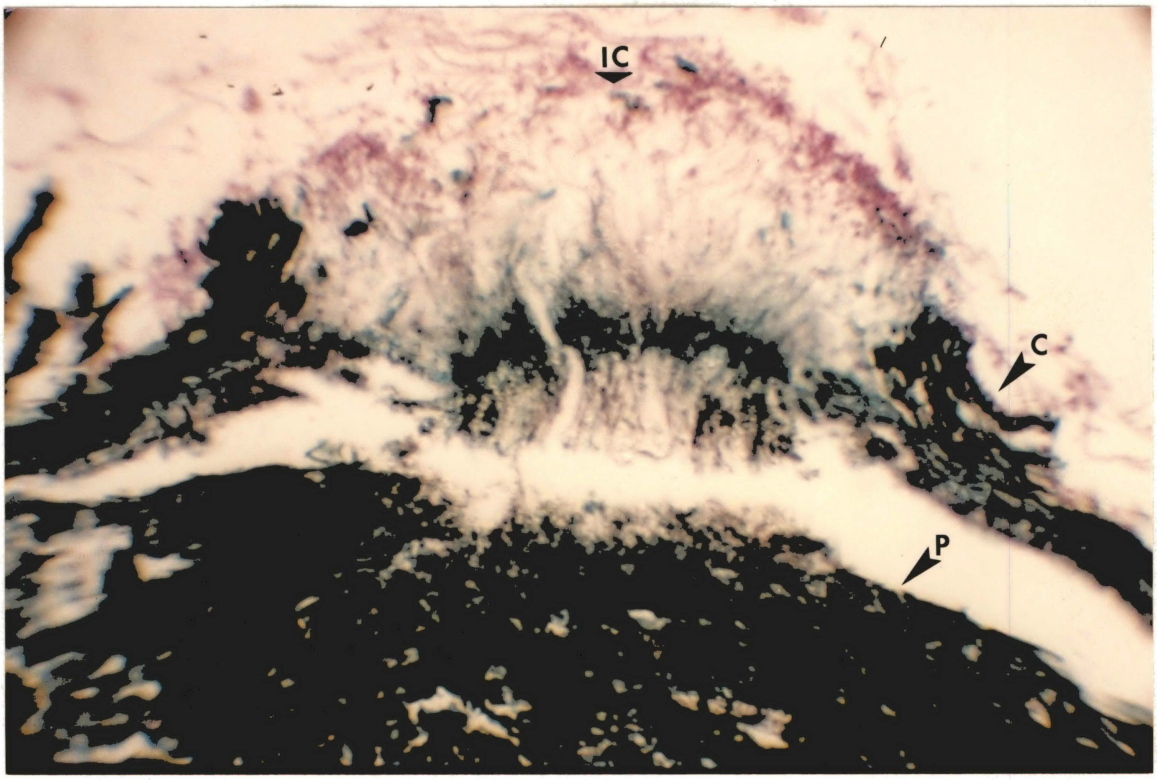

Fig. 2. Day-7 transverse section of apple seedling infected by Sclerotium rolfsii exhibiting hyphal penetration through cork tissue (C) and into the phloem (P). Actively growing hyphae originate from an infection cushion (IC), X100.

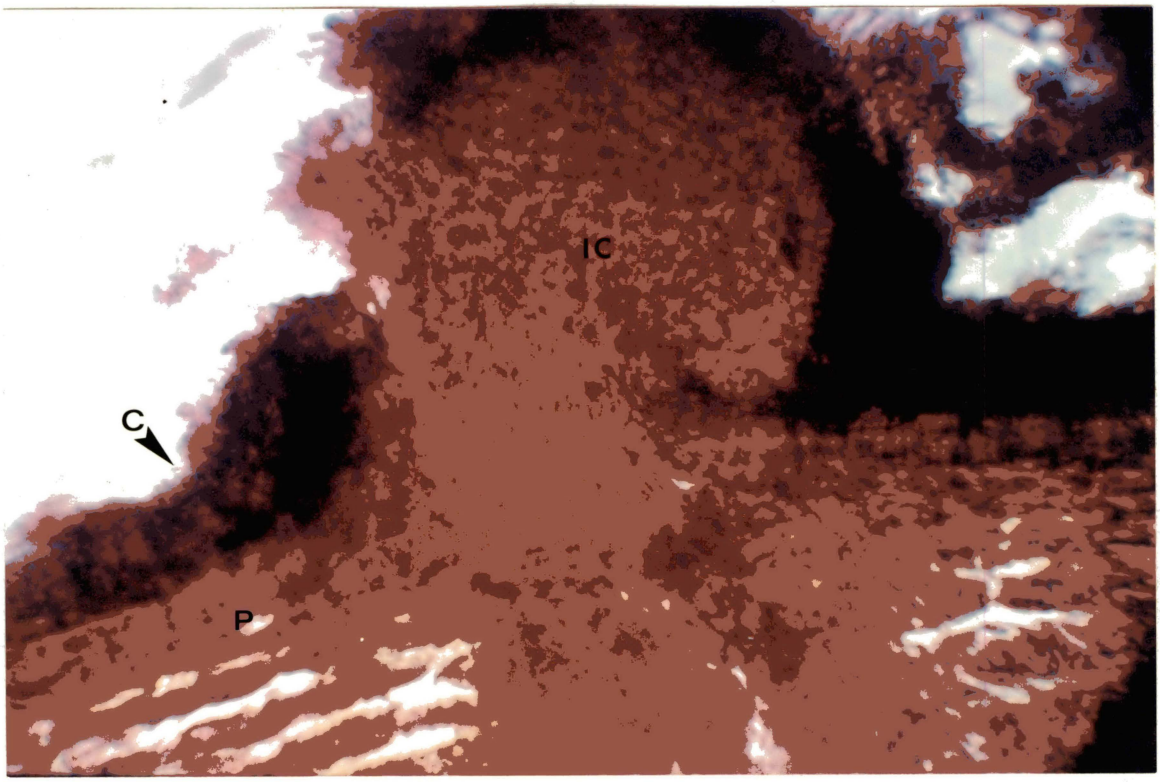

Fig. 3. Day-9 transverse section of apple rootstock infected by Sclerotium rolfsii exhibiting hyphal penetration through cork tissue (C) and into the phloem (P). Actively growing hyphae originate from a well developed, sclerotial-like infection cushion (IC), X200.

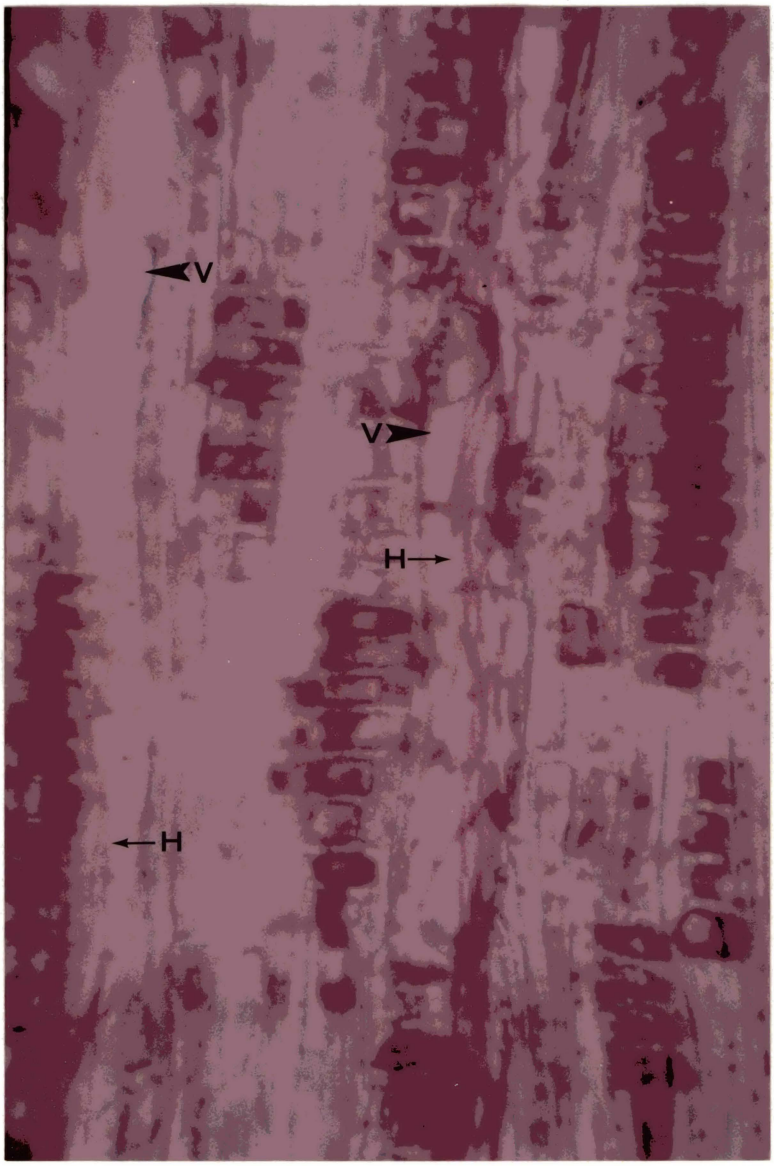

Fig. 4. Longitudinal section of xylem of apple seedling infected by Sclerotium rolfsii after 7 days. Hyphae (H) of S. rolfsii have invaded and blocked both xylem vessels (V) shown, X400.

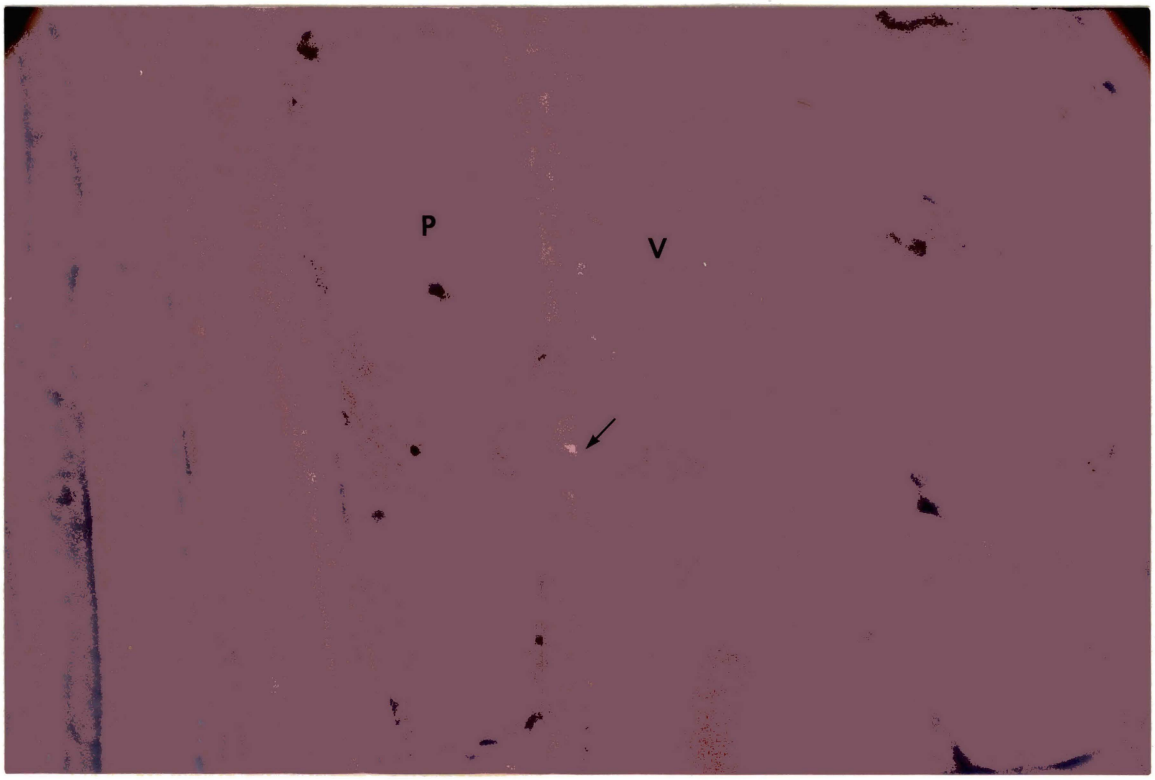

Fig. 5. Londitudinal section of infected apple rootstock xylem demonstrating constriction and penetration of Sclerotium rolfsii hypha within a vessel (V) into an adjacent parenchyma cell (P). Arrow indicates constriction of the hypha occurring in the vessel pit, X1000.

CHAPTER V

APPENDIX

A. Evaluation of the Wet-Sieving/Methanol Assay For Estimates of Viable Sclerotia of *Sclerotium rolfsii* From Soil

The wet-sieving/methanol technique (described in Chapter III) was evaluated for estimating populations of sclerotia of *S. rolfsii*. Soil samples were collected from areas in microplots with higher levels of sclerotia of *Sclerotium rolfsii* (i.e., near diseased trees). The samples were wet-sieved and the sclerotia were removed with forceps and counted with the aid of a stereomicroscope. A known number of sclerotia (air-dried) were placed on a layer of 50% potting medium/50% sandy-loam soil in several petri dishes. Methanol (1%) was applied and the plates were incubated at room temperature for 48 hr to allow sclerotial germination.

Ninty-six percent of the 371 sclerotia recovered from the soil samples eruptively germinated when exposed to the assay conditions. The wet-sieving/methanol assay appears to be an adequate technique for emumeration of viable sclerotia from field soil samples.

B. Total Sclerotia Population of *Sclerotium rolfsii* at Six Randomly Chosen Sites in an Apple Nursery

Six locations were chosen at random in the Stillwater

apple nursery (described in Chapter III) in 9/1985 to compare actual sclerotial populations to those estimated by random core sampling. Entire volumes of soil were removed from six areas measuring 0.5 m X 0.5 m to a depth of 10 cm. Prior to the removal of the soil, four core samples (400 g total) were taken to provide sample data. An average of 30 kg soil was removed from each site, air-dried, weighed, and processed through the wet-sieving/methanol technique (described in Chapter III) to detect viable propagules. Sclerotial populations recovered from each sample site were compared to the estimate provided by sampling.

Total sclerotial densities ranged from 0.25 to 0.44 sclerotia/200 g soil. The mean density for all six total soil volumes was lower (0.37/200 g soil) than what was estimated earlier in the year (0.73/200 g soil) by sampling quadrats in the same area in April. Estimates (provided by sampling) of sclerotial densities from the six sites ranged from 0 to 0.86/200 g soil. The composite ratio for all six samples was 0.40/200 g soil, also lower than previous counts made ealier in the year. No sclerotia were recovered by sampling in the two sites exhibiting fewest sclerotia from total soil volumes. Based on an LSD value of 0.31, there was not a significant difference between mean values of the total and sample data. The standard deviation was lower for the total volumes (0.075) to that of the samples (0.33). The narrow range of ratio values found for the total counts would suggest uniformity in sclerotial populations between the

sample sites, thus supporting the random distribution described earlier in this report.

C. Evaluation of two fungicides and Trichoderma harzianum for control of southern blight of apple rootstock incited by Sclerotium rolfsii

Pentachloronitrobenzene (PCNB) (Terraclor 10-G, Uniroyal Chemical Co.) and Difolatan (Chevron Chemical Co.) were evaluated for control of southern blight of apple. Also, the efficacy of Trichoderma harzianum (#86-Abbott Laboratories) as a biological control agent of S. rolfsii in two delivery systems, oatseed culture and gel (Laponite) was determined.

Two field plots were established in Oklahoma to conduct this study; in Tahlequah and Stillwater (both described in Chapter III). Domestic apple rootstock (Red Delicious) were planted at both locations in April, 1983 and 1984 for Tahlequah and Stillwater, respectively. Treatments were PCNB (8.9 kg/ha; 1/2 X at plant and 1 X in July), Difolatan (52.8 ml/121.6 L water/9 m; applied as a drench in July), T. harzianum (colonized oatseed culture; 10 g/tree at plant), and T. harzianum (10^7 conidial suspension in laponite gel applied on rootstock at plant). Stand counts were made on a biweekly basis and disease progression (apparent infection rate) was calculated using regression analysis between time and the multiple infection transformation values of disease incidence (Log_e (1/1-X).

Disease incidence at Tahlequah was low for all treatments (Table 1). The apparent infection rate was 0.0005

for the controls. Thirty-five percent disease occurred in the controls at Stillwater with an apparent infection rate of 0.0026 (Fig. 1). All treatments reduced the incidence of southern blight at Stillwater, however, based on a SNK test (P=0.05), the reductions were not significantly different from disease incidence in controls. The lowest apparent infection rate at Stillwater was 0.0013 (T. harzianum oatseed treatment) (Fig. 1).

As shown in this study, control of Sclerotium rolfsii by chemical or biological control measures is often inconsistent. Research focusing on the population dynamics of S. rolfsii, either as sclerotia or mycelium, will further enable us to better understand the success or failure of control measures. Also, further investigations exploring the abiotic and biotic factors which influence southern blight epidemics are needed.

Table 1. Effectiveness of two fungicides and *Trichoderma harzianum* for control of southern blight (*Sclerotium rolfsii*) of apple rootstock in two Oklahoma nurseries.

	Tahlequah (1983)	Stillwater (1984)	
Treatment	% Disease	% Disease	A. I. R.[a]
Terraclor	6.2	22.5	0.0020
Difolatan	6.2	28.7	0.0027
Trichoderma-Oats	7.4	15.0	0.0013
Trichoderma-Gel	5.5	28.7	0.0031
Control	6.2	35.0	0.0026

[a]Apparent Infection Rate; Values based on linear regression between time (days) and multiple infection transformation values (LOG_e (1/-X)) of percent disease.

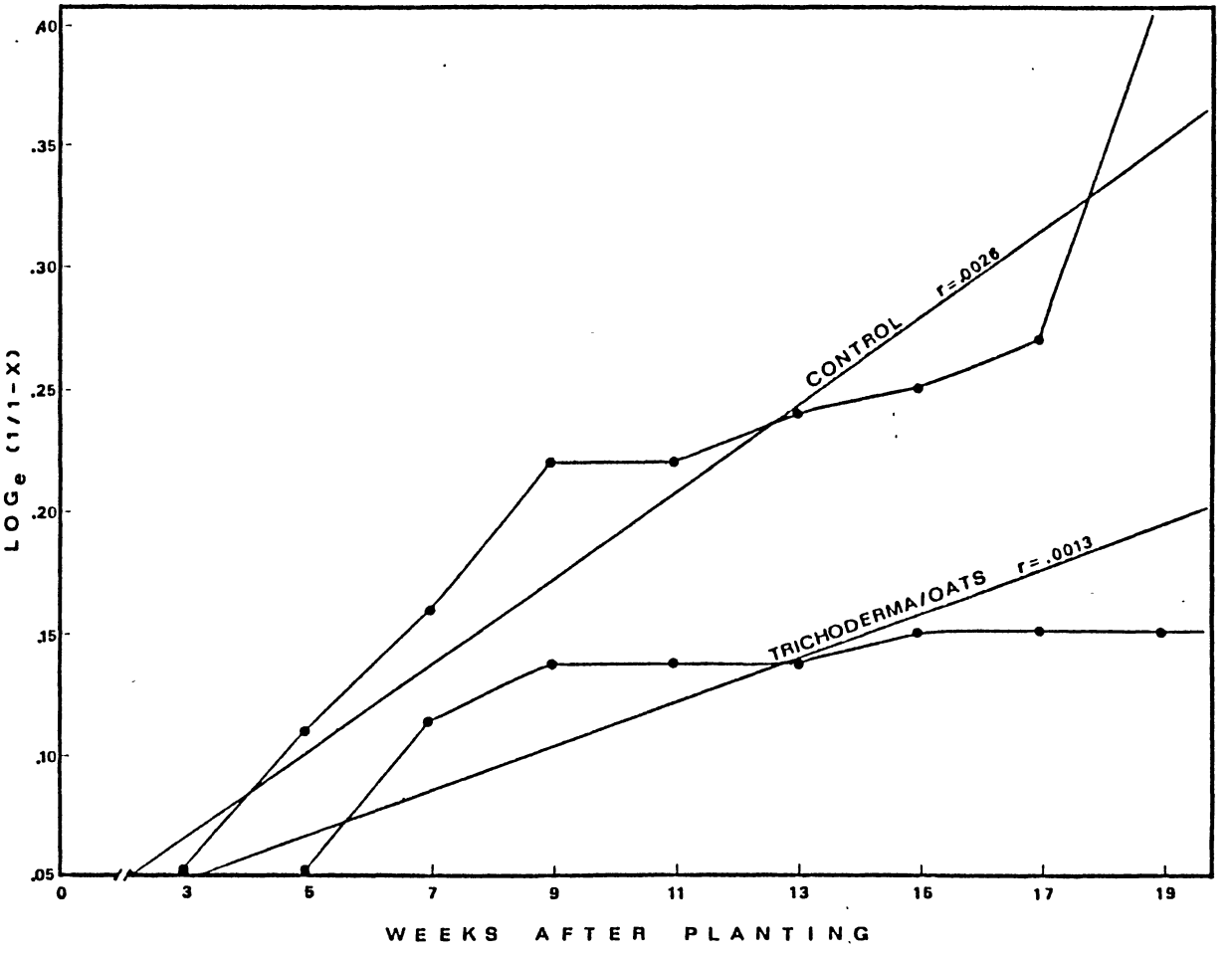

Fig. 1. Disease progression of Sclerotium rolfsii on apple rootstock in unamended control and Trichoderma harzianum/oat culture treatment.

VITA

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