Evaluations of Biological and Chemical Controls for Southern Blight of Apple Rootstock in Oklahoma Nurseries

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Received: 1995 Jul 12; Revised: 1996 Jul 10

A survey of nurseries in eastern Oklahoma has identified southern blight, caused by *Sclerotium rolfsii,* as an economically important disease in the production of field-grown apple seedlings and rootstock. *S. rolfsii* can infect trees up to three years old. Chemical and biological control studies were conducted to evaluate rates and time of application of three fungicides and three formulations of the biological control-fungus, *Trichoderma harzianum.* On the basis of "dummy" regression models for analysis of disease progress, the fungicides benodanil and pentachloronitrobenzene (PCNB) were effective in delaying the onset of the disease and in slowing disease progress. Combinations of PCNB and *Trichoderma harzianum* were more effective in reducing disease rate than PCNB alone. *T. harzianum* formulated with a food-base (infested oat-seed) was more effective in reducing disease progress than either conidial or chlamydospore formulations. In addition to sclerotia, incorporation of residue from infested woody plant parts provided overwintering sites and initial inoculum for *S. rolfsii* in the spring. Weed control, rouging of diseased trees, and avoidance of highly infested fields and fields previously planted to soybean are important management practices to reduce losses in seedling production due to southern blight.

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. Southern blight was first reported on apple nursery stock *(Malus* × *domestica* Bork) in the United States in Maryland in 1935 (*1*) and subsequently in Indiana in 1953 (*2*) and in Georgia in 1980 (*3*); however, few reports have been documented. A preliminary report on the occurrence of southern blight of apple in Oklahoma nurseries has been published (*4*). Southern blight of apple has also been reported from New South Wales, the Republic of South Africa, Australia (*5*) and Israel (*6*). This paper reports on the characteristics, symptomatology, and incidence of southern blight of domestic apple rootstock in Oklahoma nurseries, and on evaluations of chemical and biological controls.

MATERIAL and METHODS

Nursery survey. Commercial nurseries near Tahlequah, OK were surveyed for the presence of southern blight on apple rootstock in the summers of 1981 and 1982. Plants exhibiting southern blight symptoms similar to those previously reported on apple were examined. If a symptomatic tree did not exhibit signs of the fungus, the plant was uprooted and the crown and upper root area was removed and placed into a moist chamber at 26 °C to confirm the presence of *S. rolfsii*. Isolations were made from diseased tissue samples, surface sterilized in 0.5% NaOCl, and plated onto potato dextrose agar amended with streptomycin sulfate (300 μg/ml) (PDSA). Isolates of *S. rolfsii* from diseased trees were used to confirm Koch's postulates on either apple seedlings grown from stratified seed or 2-yr-old seedling rootstock.

Inoculum production. Pathogenicity of isolates of *S. rolfsii* was tested using either sclerotia produced on PDSA or infested-oat seeds. Oat seeds were autoclaved twice and the moisture content adjusted to 67.0% w/v prior to inoculation with an actively growing culture of *S. rolfsii*. Infested seeds were air dried before use. Field soils were artificially augmented before each test with the infested-oat seed preparation.

Trichoderma harzianum, isolate OK-86,

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was used as the biological treatment. This fungus was prepared either as conidia, chlamydospores, or as infested-oat seeds. Conidia were produced on PDA, gently washed from the surface and incorporated into a 1.5% Laponite 508 gel (synthetic magnesium silicate, Laporte Industries Inc., Continental Plaza, 411 Hackensack, NJ 07601) at a density of 1×10^7 conidia/ml gel. Chlamydospores were prepared as a molasses-wheat bran fermentation product (*7*) and incorporated into the gel at 1×10^6 propagules/ml. Infested-oat seed was prepared in a manner similar to the *S. rolfsii* preparation and was applied to the soil either as whole seeds or as a powder. The powdered formulation was prepared by chopping the seeds in a blender, finely grinding the residue with a mortar and pestle and screening through a 500 μm sieve. The powder was mixed into the gel to provide a density of 1×10^6 propagules/ml.

Control-Field Trials. A site where *S. rolfsii* did not occur was selected at Stillwater, OK in 1982 to establish an apple nursery. The Soil type was Norge loam. Seedling rootstocks (cv. Delicious) were planted 10 March. Fungicide treatments consisted of six split combinations of pentachloronitrobenzene (PCNB, Terraclor 10G, Uniroyal Chemical Co.), applied at these rates (kg of active ingredient [a.i.] per hectare): 11.2/0, 11.2/5.6, 11.2/11.2, 5.6/5.6. 5.6/11.2, and 0/11.2, for the first/second application. The first application was made 15 March and the second on 23 July. At planting, five oat seeds infested with *T. harzianum* were placed into each planting hole and an additional 2.0 g of infested seed was applied to the soil surface around each tree prior to hilling. Twenty grains of infested-oat seed culture of *S. rolfsii* was used as inoculum to infest soil along both sides of each row. Each row contained 20 trees, spaced 0.3 m within row, and rows were separated by 1.5 m. Treatments were arranged in a randomized block design, with each of the four blocks separated by 1.6-m alleys. Trifluralin was applied, at 1.75 l (a.i.)/ha, to soil in the entire plot area for weed control. Fertilizer was added to this area each year according to the recommendations of the Oklahoma State University Soil Testing Laboratory. Water was applied, as needed, by overhead sprinkler irrigation or, in later tests, by drip irrigation. Soil samples, 1.0 cm i.d. and 15 cm deep, were removed at random within rows from treatments with *T. harzianum* and controls at a distance of 3.0 cm from selected trees. Soil samples from each row were bulked and subsamples used for dilution assay onto *Trichoderma*-selective medium (*8*) to determine population densities of *T. harzianum.* Incidence of southern blight was recorded throughout the growing season. Apple trees were left in the field with no further treatment and disease incidence was also recorded during 1983.

During the winter of 1983, all trees in the Stillwater plot were flail-mowed and the woody residues were incorporated into the soil by discing. On 26 April 1984, apple seedlings were planted into the plot with 23 cm between trees in the row and 1.37 m between rows. Five treatments were evaluated for control of southern blight: a split application of PCNB (8.9 kg a.i./ha: 3 kg/ha at planting and 5.9 kg/ha on 11 July); a captafol (Difolatan 4F, Chevron Chemical Co.) drench (0.43 ml a.i./l/tree); a combination of PCNB (3 kg/ha) and *T. harzianum* (10 g infested-oat seed/tree) at planting; *T. harzianum* (10 g infested-oat seed/ tree) at planting; and a conidial suspension of *T. harzianum* (1×10^7 conidia/ml gel) applied to the entire length of the root at planting. Treatments were arranged in a randomized block design with 8 trees per plot and ten replications. Prior to planting, soil samples were removed from the field in a stratified random design with each quadrat measuring 3×3 m. Sclerotial densities of *S. rolfsii* were determined using a modified extraction technique (*9,10*). Disease incidence was recorded throughout the growing season based on visual symptoms and recovery of the pathogen through isolations on agar.

In 1987, the fungicide benodanil (Benefit MF-654 50WP, Monsanto Chemical Co) was evaluated at various rates and times of application for southern blight control. Apple rootstock was planted 2 April and in-line drip irrigation lines were installed which would deliver to each tree approximately 2 l/hr. Fungicide treatments consisted of two concentrations of benodanil (0.45 g/l and 0.9 g/l) applied either once, twice, or three times during the growing season. Treatments were replicated six times and each block contained 25 seedlings. Water was dripped around each tree 1 hr prior to and 1 hr after application of the

fungicide drench (52 ml/tree). Supplemental irrigation was applied throughout the season as needed. The first drench was applied to all treated plots on 6 April, the second was applied to two plots each/block on 20 May, and the last was applied to one plot on 15 July. Disease incidence was monitored weekly from 4 May to 2 November and the causal agent was confirmed either through the presence of signs or by isolation from crown tissue.

Control - Microplot Studies. Various formulations of *Trichoderma harzianum* were evaluated during 1985 in raised microplots (2.1 × 2.1 m) filled to a depth of 20 cm, with sandy loam soil. Soil in each microplot was infested 9 da prior to planting with *Sclerotium rolfsii*-infested oat seeds (10 g dried oat seed/microplot). Formulations of *T. harzianum* consisted of either conidia, chlamydospores or infested-oat seed. Each formulation was added to Polysurf-C gel (1.5%) (hydrophobically modified hydroxyethylcellulose, Hercules, Inc., Wilmington, DE) to provide 1×10^6 propagules/ml gel. Gel mixtures were applied as a slurry to the entire length of the apple rootstock. Each microplot contained a row (eight trees/row) for each treatment and a control (non-amended gel treatment). Treatments were randomly arranged within each microplot and all treatments were replicated eight times. Trees were planted on 25 April. Incidence of southern blight was recorded throughout the growing season as described before.

Data analyses. Analysis of variance was performed using the CoStat program (CoHort Software, Berkeley, CA) and, when appropriate, means were separated with a Student-Newmans-Keuls multiple range test. Percentages of mortality were transformed to adjust for multiple infections (*11*) and plotted against time. Linear regression lines were determined by using the SAS procedure (SAS Institute Inc., Cary, NC) and differences in slopes and intercept values were compared to the control by using a "dummy" variable multiple regression model (*12*). Data was also subjected to polynomial regression in order to determine the best-fit equations for disease progress. Fungicides used in these studies have been replaced by newer, more effective compounds and further investigations (*13*) have identified fungicides such as flutolanil 50WP (Moncut 50W) (AgrEvo) and the insecticide Lorsban 15 G (chlorpyrifos) (Dow Elanco) to be more effective than fungicides used in these studies.

RESULTS

Nursery Survey. 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 three to four adjacent trees killed by *S. rolfsii*. Although disease incidence of southern blight of apple is usually low (5-10%), the economic loss can be substantial because the cash value of the trees is high (average = $1.75/ 1-yr-old tree) and because growers may allow young trees to remain in the nursery for two years before being lifted and sold. One nursery owner estimated a loss of 10% of 10^5 planted, 1-yr-old, grafted trees to *S. rolfsii* in 1981. In the fall of 1981, the infected trees remaining in the nursery were destroyed by mowing and discing. In 1982, 50% of 1-yr-old flowering crabapples in a 1.2-ha field at the same nursery were killed by this fungus.

Disease symptoms observed on trees in the fields surveyed were similar to those described previously (*2-4,10*). Infections from germinating sclerotia or actively growing mycelium were observed in early summer as maximum daily temperatures approached 30-35 °C. Symptoms became more apparent under high summer temperatures (35-40 °C) and moisture stress. Infection by *S. rolfsii* produced a girdling of the tree near the soil line, which resulted in the rapid wilting and death of foliage, and ultimately death of the entire tree. 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 caused by *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

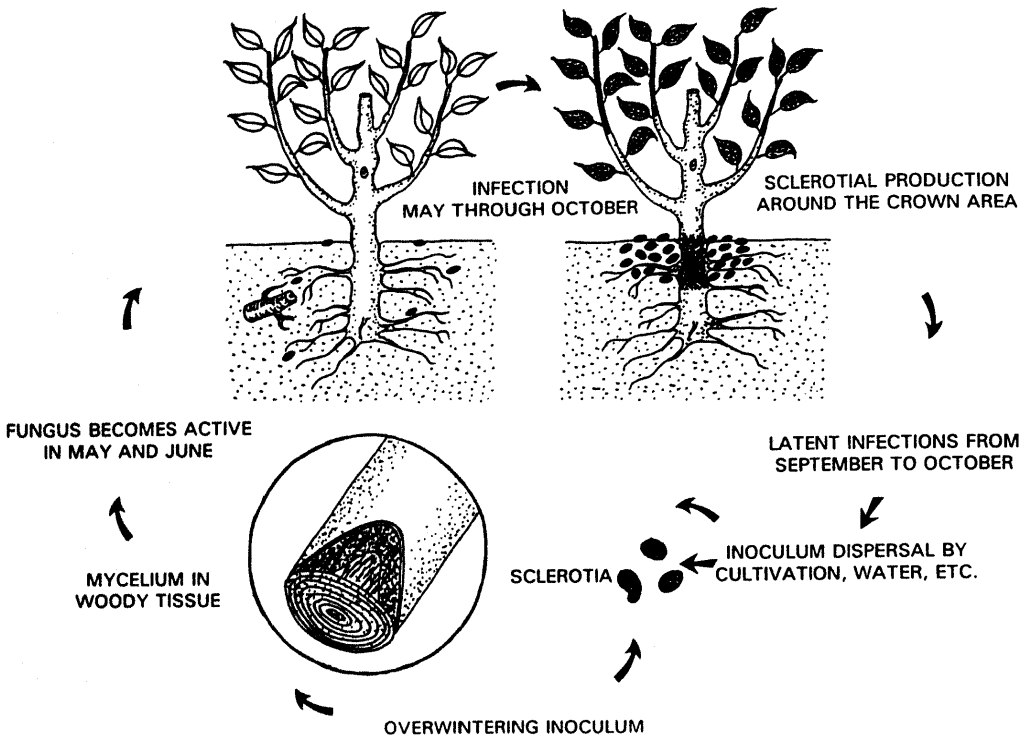

Figure 1. The disease cycle of southern blight caused by *Sclerotium rolfsii* on apple seedling rootstock. Note that both infested debris (woody tissue) and sclerotia may act as initial inoculum in the spring.

TABLE 1. Effect of application of *Trichoderma harzianum* (*T. harz.*) and various rates and times of application of pentachloronitrobenzene (PCNB) on death of apple seedlings due to southern blight during a 2-year period, 1982-1983, at Stillwater, OK.

Treatment[a]	Rate (kg/ha)	Average Percent of dead trees[b] 1982[c]	1983[d]	Total[e]
T. harz.		2.50xz	2.50	5.00
PCNB	11.2–0	0.00z	13.75	13.75
PCNB	11.2–5.6	1.25z	3.75	5.00
PCNB	11.2–11.2	0.00z	11.25	11.25
PCNB	5.6–5.6	2.50xz	5.00	7.50
PCNB	5.6–11.2	0.00z	11.25	11.25
PCNB	0.0–11.2	2.50xz	11.25	13.75
Control		6.25x	6.25	12.50
			N.S.	N.S.

a PCNB first treatment applied 15 March; second treatment applied 23 July 1982. *T. harzianum* applied as infested oat seed (approximately 2.0 g/tree) at planting.

b Data are expressed as percent of trees killed by *Sclerotium rolfsii*. Total number of trees per treatment = 20, replicated four times. Data were transformed using the arcsine transformation prior to ANOVA and have been retransformed to percentages for inclusion into this Table.

c Numbers followed by different letters are significantly different (P=0.05) by Student-Newman-Keuls test. N.S.=non-significant.

d Numbers represent new infections developing in 1983, no biological or chemical treatments applied during 1983.

e Numbers represent average disease loss for both years.

latently infected trees may also provide active foci for future disease development (Figure 1).

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 sclerotia associated with the mycelium. Typical mycelial fans of *S. rolfsii* formed around infected trees under moist conditions. *S. rolfsii* bridged the space between trees by colonizing weedy plant debris on the soil surface which served as a food source for the fungus. Another food base, apple leaf and woody residue from previous growing seasons, provided an excellent inoculum source if infested. Debris with visible mycelium and sclerotia were observed frequently in the surveyed fields.

Control-Field Trials. Incidence of southern blight on apples during 1982 ranged from 0 to 6.25% (Table 1). Although percent disease was low in all treatments, there were significant differences among treatments. All rates and combinations of PCNB and the *T. harzianum* treatment provided sufficient control of southern blight, with some of the chemical application rates

TABLE 2. Linear regression statistics for disease progress of southern blight, induced by *Sclerotium rolfsii*, on apple trees in relation to time for various fungicides and formulations of *Trichoderma harzianum* (*T. harz.*).

Year – site	Regression coefficients[a]						
Treatment	a	s(a)	b	s(b)	R^2	MSE	Model[b]
1984 – Field[c]							
PCNB	3.69*	3.49	0.17*	3.5	0.80	1.33	Q
PCNB & *T. harz.*	2.21	4.30	0.14*	4.3	0.65	2.02	Q
T. harz. – oat seed	-4.65	3.05	0.16*	3.1	0.82	1.02	L
T. harz. – gel	-12.38	4.23	0.33	4.3	0.91	1.95	L
Captafol	0.88	3.74	0.23	3.8	0.87	1.53	Q
Control	-6.79	2.36	0.31	2.4	0.97	0.61	L
1985 – Microplot[d]							
T. harz. – oat seed	3.1*	0.77	0.12*	0.7	0.94	0.22	L
T. harz. – chlamydospore	5.3	2.21	0.17	1.9	0.80	1.84	C
T. harz. – conidia	5.8	1.79	0.20	1.6	0.89	1.21	Q
Control	7.4	1.38	0.18	1.2	0.91	0.72	C
1987 – Field[e]							
Benodanil 1-1	-4.6*	0.66	0.17**	0.5	0.98	0.19	L
Benodanil 1-2	-6.2**	0.73	0.17**	0.5	0.98	0.23	C
Benodanil 1-3	-12.2**	1.85	0.20*	1.4	.89	1.51	Q
Benodanil 2-1	-5.1**	0.55	0.10**	0.4	0.96	0.14	L
Benodanil 2-2	-8.9**	0.94	0.16**	0.7	0.95	0.39	Q
Benodanil 2-3	-9.3**	1.45	0.16**	1.1	0.90	0.93	Q
Control	1.6	2.17	0.24	1.6	0.90	2.09	4th

a a, b are the intercept and the slope, respectively; s(a), s(b), their standard errors; R^2, the coefficient of determination; MSE, the mean square error. Data points in each analysis: for 1984–Field, 8; for 1985–Microplot, 23; for 1987–Field, 27. Numbers followed by * or ** are significantly different from the control, (P=0.5) or (P=0.001) respectively.

b Model is the best–fit equation for the data; L=Linear, Q=Quadratic, C=Cubic, and 4th=fourth-order response curves.

c PCNB (Pentachloronitrobenzene) applied (8.9 kg/ha) at planting; *T. harzianum* – gel – conidia suspended in Laponite 508 gel at planting, 1×10^7 propagules/(ml of gel) applied to the entire root. Combination treatment: *T. harzianum* (10 g infested oatseed/tree) applied at planting with PCNB(3 kg a.i./ha). Captafol (Difolatan 4F) applied as a drench (0.43 ml a.i./l/tree).

d *T. harzianum* formulated as either ground-infested-oatseed, conidia, or chlamydospores added to polysurf-C gel at 1×10^6 propagules/ml.

e Benodanil (Benefit MF-654) formulated at two concentration, 0.45 g/l or 0.9 g/l and each applied either once, twice, or three times during the growing season.

providing complete suppression of the disease. No further treatments were applied to the surviving trees during 1983 to determine if 2-yr-old trees were more resistant to infection. Incidence of new southern blight during 1983 ranged from 2.5 to 13.75%. Percent increase in trees killed during this second year was greater in all treatments except the control and in the treatment with *T. harzianum,* where the percent dead trees remained the same as in 1982, 6.25 and 2.5%, respectively. Although there were no differences (P=0.05) among treatments for either new infections during 1983 or for the total amount of disease for the two-year period, use of high rates of PCNB (11.2 kg/ha) or the lack of treatment in July tended to increase disease during the second year. Population densities of *T. harzianum,* approximately 1 mo after planting in 1982, were 0.1 to 6.0×10^4 propagules/g dry soil at 3 cm distance from apple trees in treated plots compared to less than 1×10^2 propagules/g soil in controls. During 1984, the incidence of southern blight in the field at Stillwater ranged from 15.0 to 35.0%, with the greatest disease incidence occurring in the control treatments. Preplant sclerotial densities of *S. rolfsii* were approximately four sclerotia/1000 g dry soil (*2,14*). Comparison of linear regression statistics (Table 2) for all treatments showed lower slope values for the combination treatment with PCNB and *T. harzianum* (P = 0.05), the oat seed formulation of *T. harzianum* (P = 0.05), and the PCNB treatment (P = 0.05) compared to the control. Only the PCNB treatment had lower intercept values (P = 0.05) than the control. Linear regression lines best fit the data from the *T. harzianum* oat seed and conidia/gel formulations and the control, while disease progress curves for the other treatments best fit quadratic equations (Table 2). Lower intercept values for a treatment compared to the control would indicate a delay in disease development,

whereas lower slope values indicate lower disease progress during the growing season. Polynomial regression models indicate several periods of pathogen activity during the growing season. The rate of PCNB used in the combination treatment with *T. harzianum* was approximately the rate used in the PCNB treatment (3 kg a.i./ha vs. 8.9 kg a.i./ha, respectively). All rates and times of applications of benodanil lowered slope and regression values for disease progress ($P = 0.01$) compared to the control. Although all linear regression lines had high coefficient of determination (R^2) values, only the disease progress in plots receiving one application of the fungicide were best fit by linear equations. Disease progress in the control was best fit by a fourth-degree equation (Table 2).

Control - microplot studies 1985. Total disease developing in the microplot experiments ranged from 21.9% to 32.8% in the *T. harzianum* oat seed formulation and the control, respectively. There were no significant differences for total disease development. However, regression analysis of disease progress indicated that the oat seed formulation of *T. harzianum* had both lower slope and intercept values ($P = 0.05$) representing a lower infection rate and a delay in disease development than the control and was best fit by a linear regression equation (Table 2). The R^2 value for the regression line of disease progress in the treatment with the chlamydospore formulation was the lowest and best fit a cubic model

DISCUSSION

Southern blight of apple seedlings or ornamental trees with common apple rootstock can cause great economic losses to the nursery industry if control measures are not instituted in a timely and effective manner. Apple trees or root stocks are usually grown for 2 to 3 yrs in the nurseries to attain the desired salable size. *Sclerotium rolfsii* can infect and kill trees up to 3 yrs old, which compounds disease control problems. Application of fungicides to the base of the trees is necessary each year that the trees are in the field. Increases in the incidence of southern blight during 1983 when no fungicides were applied indicate that PCNB was fungistatic and did not reduce the sclerotial density in the soil. Furthermore, incidence of this disease can dramatically increase if dead trees are flail-mowed during the winter season and the residue left on the soil. This was illustrated in the increase in the growers field during 1982 and at our field site during 1984 following the incorporation of infected woody tissue into the soil. Light microscopy has shown the presence of the fungus in the vascular system of dead host trees (*7,15*). On the basis of our field observations and research, disease incidence may be reduced by proper weed control, avoiding infested fields, and roguing diseased trees including those exhibiting latent infections in the fall and spring.

Fungicides used in this research did provide control of *S. rolfsii* by delaying the onset of the disease and slowing disease progress during the growing season. All rates and times of application of benodanil were effective in reducing losses due to southern blight compared to the control. Although multiple applications of benodanil did not reduce disease ($P = 0.05$) compared to one application, they did affect the equations for the best fit model equation from a linear response to quadratic. PCNB was also an effective fungicide and combinations with *T. harzianum* were even more effective in controlling southern blight. However, there are currently no fungicides labeled for the control of southern blight on apple.

The use of biological control agents to colonize around the crown area of seedling trees and protect against infection by *S. rolfsii* has great potential. The manner in which these agents are formulated can have a great impact on their efficacy. Formulations of *T. harzianum* with a food base (oat seed) were very effective in controlling southern blight, while those using either conidia or chlamydospores were not as effective. The addition of a food base in formulations of biological control agents has been reported to be essential to achieve control of several soilborne pathogens (*16*). The chlamydospore formulation tended to be more effective than the conidial formulation, which supports the findings of others (*7,8,11,17*). Research should be continued to evaluate other biocontrol agents either alone, in combinations or with combinations of fungicides to control southern blight of apple seedlings. Increasing the density of

propagules in the gel, the use of other gel formulations, and the addition of different food bases may enhance the control potential of *T. harzianum* or other biocontrol agents and reduce the variability of this agents between locations and years.

ACKNOWLEDGMENTS

We thank Mr. Ben Davis, Hill Country Nursery and the management of Greenleaf Nursery, Tahlequah, OK for their kind donation of apple rootstock used in these studies. We deeply appreciate the technical assistance of Carol Foor, Christine Fisher and Bryan Brown.

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