

PATHOLOGY & NEMATOTOLOGY

John Olive
Section Editor and Moderator

Twenty-eight students competed in the Bryson L. James Student Research Competition and twenty-nine research projects were presented in poster form, which were displayed for review during the SNA Research Conference and Trade Show, this year. Their research is presented in the topical sections which follow and are designated as Student or Poster papers.

**Co-culture of *Microsphaera pulchra* and *Cornus florida*
(Flowering dogwood) Microshoots**

**L.A. Klein, M.T. Windham and R.N. Trigiano
Tennessee Agricultural Experiment Station
Knoxville, TN 37901-1071**

Nature of Work: Nursery and ornamental plantings as well as stands of native flowering dogwoods have been subjected to epidemics of powdery mildew since 1993. Epidemics have been ascribed to either *Phyllactinia guttata* (4) or *Microsphaera pulchra* (5,6). However, Klein et al. (2) demonstrated that *M. pulchra* is the only fungus that causes powdery mildew of flowering dogwood. Among the 100 or so *Cornus florida* cultivars grown, only 'Cherokee Brave' showed significant resistance, not immunity, to the disease (6), whereas most *C. kousa* and *C. kousa* x *C. florida* crosses exhibited resistance to the disease (4,6).

Powdery mildew fungi must have living host tissue to grow and complete their life cycle — we cannot grow it on nutrient agar in a petri dish. Presently, dogwood trees with foliage must be sustained year-round to support the fungus. The first objective of this preliminary study was to establish growing and sporulating colonies of *M. pulchra* on tissue cultures of dogwood in petri dishes. Our second objective was to compare the morphological and physiological relationship between the fungus and the host as it occurred on leaves from trees in the greenhouse and on leaves from microshoots in petri dishes.

Proliferating axillary bud cultures of *C. florida* were established according to method of Kaveriappa et al. (1). Depulped seeds from several trees were cold-stratified (40 F) in moist medium for four months. Cultures were initiated from seedlings after the first set of true leaves developed. Cotyledons and leaf blades were cut off and stems with a small piece of petiole were washed in soapy water. The stems were then briefly dipped in 95% ethanol and ignited by quickly passing them through a flame to remove hairs. Plant tissues were surface disinfested with 20% (1.05% NaOCl) Clorox® for 10 min and then rinsed with sterile, distilled water three times. Petioles and apical meristems were removed and single nodes, each with two axillary buds, were placed upright in petri dishes containing Woody Plant Medium (2) augmented with 6-benzyladenine (BA). Cultures were incubated at 75 F with fluorescent lighting for 15 h each day. Cultures were transferred to the same medium every four weeks.

Six trees infected with *M. pulchra* were maintained in a greenhouse with supplemental light for 15 h each day. Trees were watered directly into pots to avoid wetting foliage and spores. Plants were shaken vigorously 24 h before inoculation of the tissue cultures to remove old spores and to ensure inoculum of similar aged spores.

Twenty four-week-old microshoot cultures were inoculated with powdery mildew spores in the greenhouse by tapping infected leaves over the exposed tissue in petri dishes. Culture dishes were sealed with parafilm and incubated in the conditions described previously. Microshoots were examined with a dissecting scope every day for the development of powdery mildew signs and symptoms. Samples of leaf tissue were checked for fungal spores and germination, leaf penetration structures, and colonization using a combination of standard paraffin histology and scanning electron microscopy (SEM) techniques. Microshoot cultures were transferred to fresh, sterile, growth medium when contaminating fungi and bacteria were discovered.

Results and Discussion: Twenty-four h after inoculation, spores had germinated, but penetration of the leaf surface did not occur until 48 h. Spore germination and formation of penetration structures on microshoot leaves were similar to that observed on leaves in the greenhouse. Numerous secondary hyphae were formed by 144 h after inoculation and colonies of *M. pulchra* were considered established on microshoots when spore forming structures were observed after 168 h. SEM demonstrated that spore formation on microshoots were comparable to that observed on intact leaves. Paraffin histology revealed that haustoria (structure used by fungus to obtain nutrients from the plant cell) formed in epidermal cells of microshoots and leaves from the greenhouse had the same general morphology.

Freshly produced (less than 24 h) spores of *M. pulchra* were capable of infecting and establishing colonies on microshoots of flowering dogwood grown in petri dishes. The pathogen maintained a compatible physiological relationship with host tissue and produced asexual spores. However, viability and the ability of spores to infect new, healthy tissue from other microshoots and intact leaves needs was not examined.

The greatest impediment to establishing powdery mildew on tissue cultures of flowering dogwood was contamination with other microorganisms. Within 120 h, 14 of 20 cultures had fungal contamination of either the microshoots (*Botrytis* and/or *Alternaria*) or on the agar growth medium. All of the cultures became contaminated with either bacteria or fungi after 264 h. A possible solution to the contamination problem is to

carefully inoculate new cultures of microshoots with spores produced on leaves grown in vitro. Eventually, pure dual cultures would be established.

Significance to Industry: This study determined that *M. pulchra* infected and colonized flowering dogwood microshoots grown in petri dishes. It may be possible to screen promising flowering dogwood seedlings for resistance to powdery mildew using this method. Furthermore, the dual culture system would permit maintenance of powdery mildew collections from different geographical locations for studies of fungal genetics and host resistance.

Literature Cited:

1. Kaveriappa, K.M., L.M. Phillips and R. N. Trigiano. 1997. Micropropagation of flowering dogwood (*Cornus florida*) from seedlings. Plant Cell Rep. 16:485-489.
2. Klein, L.A., M.T. Windham and R.N. Trigiano. 1998. Natural occurrence of *Microsphaera pulchra* and *Phyllactinia guttata* on two *Cornus* species. Plant Dis. 82:383-385.
3. Lloyd, G. and B. McCown. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Intl. Plant Prop. Comb. Proc. 30:421-427.
4. McRitchie, J.J. 1994. Powdery mildew of flowering dogwood. Plant Pathol. Cir. Gainesville, No. 368.
5. Ranney, T.G., L. F. Grand, and J.L. Knighten. 1994. Resistance of *Cornus kousa* taxa to dogwood anthracnose and powdery mildew. Proc. Southern Nurseryman's Assoc. Res. Conf. 39:212-216.
6. Windham, M.T., W.T. Witte, R.N. Trigiano, S. Schlarbaum and A.S. Windham. 1997. Reactions of *Cornus* species to powdery mildew. Proc. Southern Nurseryman's Assoc. Res. Conf. 42:227-231.

'Appalachian Spring': A New Flowering Dogwood Cultivar Resistant to Dogwood Anthracnose

M. T. Windham, E. T. Graham, R. N. Trigiano, and W. T. Witte
Tennessee Agricultural Experiment Station,
Knoxville, TN 37901-1071

Nature of Work: One of the reasons for the rapid spread of dogwood anthracnose, *Discula destructiva* Redlin, has been the uniform susceptibility of flowering dogwood, *Cornus florida* L. Santamour et al. (1) tested seed provenances of flowering dogwood from twenty states and concluded there was little hope of selection and development of anthracnose-resistant plants. The objective of this project was to search for and select flowering dogwoods potentially resistant to dogwood anthracnose and if possible, to release them as new disease resistant cultivars.

Several dogwoods found surviving a severe epidemic of dogwood anthracnose on Catoctin Mountain, MD, have been previously reported as displaying resistance to this disease (2). The best of these trees, designated as tree #4, was bulked by budding at a commercial nursery and one year liners of that plant were returned to the Tennessee Agricultural Experiment Station for additional testing. Budded liners of tree #4 were placed in a double blind test at the U.S. Forest Service Bent Creek Disease Resistance Testing Facility under extremely high dogwood anthracnose disease pressure. Three other dogwood lines were tested as controls. Tree #4 once again passed the test by the Forest Service and will be released by the Tennessee Agricultural Experiment Station as 'Appalachian Spring'.

Results and Discussion: 'Appalachian Spring' is a white-bracted flowering dogwood with upright growth habit and prolific blooming. Two trees grown from cuttings in 1990 were transplanted to the field in 1994. They have grown to 2.3 m (8.5 ft) in height x 1.7 m (5.5 ft) in width and have a trunk caliper of 4 cm (1.6 in). Inflorescence number per tree averaged 194 and 299 in 1996 and 1997, respectively. Bract length averaged 7.1 cm (2.8 in) for the largest pair of bracts (typically the inner pair of bracts which immediately subtend the inflorescence receptacle) of 160 blooms in 1997, but bracts are not overlapping as in 'Cloud 9'. Bracts are not presented on a flat plane, but have a sculptural gently curving aspect.

Foliage of 'Appalachian Spring' is apple-green, turning to red in fall and the abundant berries are bright red. When observed in a field as one year budded liners, foliage size of 'Appalachian Spring' is unusually larger than most other flowering dogwood cultivars. For example, when

compared to foliage of 'Cherokee Brave', another cultivar known to have large leaves, the largest leaves of 'Appalachian Spring' liners averaged 9.6 cm wide x 17.4 cm long (3.8 x 6.9 in) whereas the largest leaves of 'Cherokee Brave' liners averaged 8.1 cm wide x 14.2 cm long (3.2 x 5.6 in).

'Appalachian Spring' has demonstrated unusual resistance to dogwood anthracnose in independent tests conducted by the Tennessee Agricultural Experiment Station in Ozone, TN and in western North Carolina by the U.S. Forest Service. Disease resistance tests were completed in 1992 and in 1996. In 1992 when the Forest Service screened thousands of dogwoods for resistance, 'Appalachian Spring' was the only tree to survive the trial.

A DNA amplification fingerprint (DAF) was developed for 'Appalachian Spring' and fingerprints of 'Appalachian Spring', 'Cloud 9', 'Springtime', 'Fragrant Cloud', 'Cherokee Princess', and 'Cherokee Daybreak' were compared. Five distinctive markers for 'Appalachian Spring' were identified. Using Principle Coordinate Analysis, DAF placed 'Appalachian Spring' outside the cluster of the other white-bract dogwoods.

Since 'Appalachian Spring' was found in a forested area, it cannot be patented. However, the name 'Appalachian Spring' for use as a dogwood cultivar and the use of 'Appalachian' for a series of dogwood cultivars can be trademarked. Trademarks for these names are being pursued.

Significance to Industry: In recent years, bad publicity from dogwood diseases such as dogwood anthracnose have hurt dogwood sales. A new dogwood anthracnose resistant cultivar will not only give nurseries a new product to sell, but will hopefully reverse some of the bad publicity that this industry has suffered over the last ten years.

Literature Cited:

1. Santamour, F.S., Jr., A. J. McArdle, and P. V. Strider. 1989. Susceptibility of flowering dogwood of various provenances to dogwood anthracnose. *Plant Dis.* 73:590-591.
2. Windham, M. T. and E. T. Graham. 1993. Resistance to dogwood anthracnose in *Cornus florida*. *Proc. of the Southern Nursery Research Conference* 38:216-217.

Influence of Application Timing with a Fungicide on the Control of Entomosporium Leaf Spot on Cultivars of Indian Hawthorn

A. K. Hagan, K. L. Bowen, J. R. Akridge, and J. W. Olive
Department of Plant Pathology, Auburn University, AL 36849;
Brewton Experiment Field, Brewton, AL 36427
Ornamental Horticulture Substation, Mobile, AL 36689

Nature of Work: Entomosporium leaf spot, which is caused by the fungus *Entomosporium mespili*, is a common and sometimes damaging disease of Indian hawthorn in both the nursery and landscape. Information, regarding the chemical control of this disease on Indian hawthorn, has largely been extrapolated from studies on red-tip photinia (1,2). Significant differences in the susceptibility of Indian hawthorn cultivars to Entomosporium leaf spot have, however, been noted (3). As a result, the relative value of protective fungicide treatments for controlling this disease on leaf spot-susceptible and resistant cultivars of Indian hawthorn has not been determined. The objective of this study was to assess whether fungicides or the selection of disease resistant cultivars would be the preferred method of managing Entomosporium leaf spot in a simulated landscape planting of Indian hawthorn.

In March 1994, cultivars of Indian hawthorn were established in a Benndale fine sandy loam at the Brewton Experiment Field (Zone 8a) on 5 ft. centers with 10 ft. between rows. Prior to planting, soil fertility and pH were adjusted according to the results of a soil assay. Plants were watered as needed with a drip irrigation system and were mulched annually with aged pine bark. Twice each spring, approximately one half cup of Osmocote 17-7-12 was uniformly distributed around each plant. A tank-mix of Gallery and Surflan was applied for pre-emergence weed control. Hand weeding and directed applications of Roundup were also used to control weeds. The experimental design was a randomized complete block with the 6 three-plant replications as whole plots and fungicide treatments as sub-plots. Fungicide sub-plot treatments were Daconil 2787 4.17F at a rate of 2 pt./100 gal. of spray volume applied at 2 and 4 week intervals as well as an unsprayed control. Each fungicide treatment was applied to one randomly selected plant in each three-plant whole plot at the above specified intervals from February 21 to June 4, 1997 for a total of 8 and 4 applications respectively, for the plants treated at 2 and 4-week intervals. Entomosporium leaf spot damage was assessed on March 6, April 20, May 19, June 29, and September 8, 1997 on a scale of 1 to 5 where 1 = not disease, 2 = 1 to 25%, 3 = 26 to 50%, 4 = 51-75% and 5 = 76-100% of leaves diseases. Disease ratings were averaged over the five assessment dates for each cultivar. Cultivars

were separated into four classes on the basis of their susceptibility to Entomosporium leaf spot. The most disease resistant cultivars were placed in class I and most susceptible in class IV. Correlation coefficients were calculated among average disease ratings. Analysis of variance was performed to determine effects due to susceptibility class and application interval. Regression analysis was performed for application interval.

Results and Discussion: Indian hawthorn cultivars assigned to class IV, which were highly susceptible to Entomosporium leaf spot, were Springtime, Spring Rapture, White Enchantress, F6, Jack Evans, Heather, Janice, Enchantress, Harbinger of Spring, and Pinkie. Class III cultivars included Majestic Beauty, Rosalinda, Snow White, Clara, and Bay Breeze. Indian Princess, Dwarf Yedda, F1, F2, and F3 were assigned to Class II while Olivia and Eleanor Tabor, which have the best leaf spot resistance of available cultivars, were placed in Class 1.

Rankings of cultivars were similar for each month (data not shown); thus averages over the five assessment dates were considered representative of disease damage throughout the season. The interaction term for susceptibility class by application interval was significant ($P < 0.01$). Therefore, linear regression models listed below were developed for each susceptibility class.

Class I	$ADis = 1.99 - 0.017(n), P = 0.0908$
Class II	$ADis = 2.43 - 0.052(n), P = 0.0001$
Class III	$ADis = 3.02 - 0.074(n), P = 0.0001$
Class IV	$ADis = 3.88 - 0.103(n), P = 0.0001$

where ADis = average disease rating and n = number of fungicide applications.

Significant reductions ($P < 0.0001$) in disease were obtained with fungicide treatments in three of the four susceptibility classes (Figure 1). For classes II, III, and IV, the lowest Entomosporium leaf spot ratings were recorded at the two-week application interval while the highest were noted on the unsprayed controls (Table 1). For the above susceptibility classes, Daconil 2787 gave better disease control when applied on a 2 than on a 4-week intervals. For class I, fungicide application interval had little impact on leaf spot severity (Figure 1). Unlike the other three classes, the light spotting of the leaves noted on plants treated with Daconil 2787 at 2 and 4-week intervals was similar to that found on the unsprayed controls (Table 1).

Overall, disease resistance was a more effective tool for managing Entomosporium leaf spot on Indian hawthorn than an intensive fungicide treatment program. Leaf spot ratings for the disease-resistant class 1 cultivars, regardless of the treatment, were considerably lower than those for the leaf spot-susceptible class III and IV cultivars sprayed at 2-week intervals with Daconil 2787 (Table 1). For the class II cultivars sprayed at 2-week intervals, disease levels were comparable to those of both the unsprayed and treated class I cultivars and significantly below those for the class III and class IV entries.

Significance to Industry: Study results indicate that the adoption of resistant cultivars is the preferred method for controlling Entomosporium leaf spot on Indian hawthorn. Intensive fungicide treatment programs did significantly reduce the spotting of the leaves and premature defoliation on the leaf spot-susceptible cultivars. However, Entomosporium leaf spot intensity was much higher on the sprayed, susceptible cultivars as compared with the unsprayed, resistant cultivars. As a result, fewer costly fungicide and labor inputs would be required to produce or maintain a leaf spot resistant Indian hawthorn cultivar than a susceptible one of equal or lesser quality.

Literature Cited:

1. Bowen, K. L., A. K. Hagan, J. Olive, and W. Foster. 1994. Application rates and spray intervals of ergosterol-inhibiting fungicides for the control of Entomosporium leaf spot on photinia. *Plant Dis.* 78:578-581.
2. Cobb, G. S., A. K. Hagan, C. H. Gilliam, and J. M. Mullen. 1985. Fungicidal control of Entomosporium leaf spot on photinia. *Plant Dis.* 69:684-685.
3. Olive, J. W., A. K. Hagan, and J. R. Akridge. 1996. Evaluation of selected Indian hawthorn varieties for resistance to Entomosporium leaf spot. *Proc. SNA Res. Conf.* 41:184-187.

Table 1. Impact of application interval on the average disease rating of Indian hawthorn cultivars segregated according to their susceptibility to Entomosporium leaf spot.

Susceptibility Class	Application Interval		
	2 weeks	4 weeks	none
I	1.861	1.87	2.02
II	1.99	2.23	2.42
III	2.29	2.60	3.06
IV	3.09	3.62	4.02
LSD (P=0.05) ²	0.21	0.21	0.21

¹Entomosporium leaf spot was rated on a scale of 1 to 5 where 1 = no disease, 2 = 1 to 25%, 3 = 26 to 50%, 4 = 50 to 75%, and 5 = 76 to 100% of the leaves diseased. ²Mean separation was according to Fisher's protected least significance (LSD) test (P=0.05).

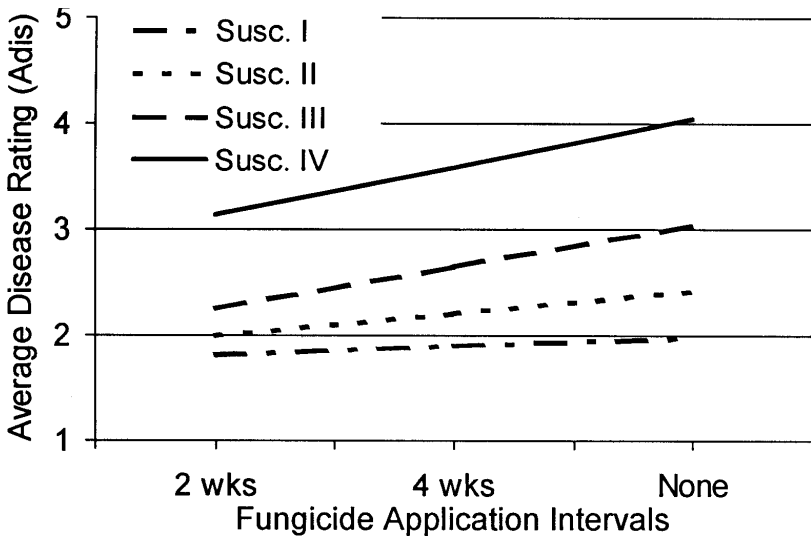


Figure 1. Effect of application interval between fungicide applications on the average disease rating (ADis) for the susceptibility classes of Indian hawthorn.

Suseptability of Cultivars of Indian Hawthorn to Fireblight, Anthracnose and Entomosporium Leaf Spot

A. K. Hagan, J. R. Akridge, J. W. Olive, and K. Tilt
Department of Plant Pathology, Auburn University, AL 36849;
Brewton Experiment Field, Brewton, AL 36427;
Ornamental Horticulture Substation, Mobile, AL 36689;
Department of Horticulture, Auburn University, AL 36849.

Nature of Work: Indian hawthorn, with its dark-green foliage, mounded canopy and often compact growth habit, has been a fixture for many years in residential and commercial landscapes across the South. Entomosporium leaf spot, which is caused by the fungus Entomosporium mespili, is widely recognized as common and often damaging disease of Indian hawthorn in both the nursery and landscape (3). Previously published results of this field trials (1,2) clearly illustrate that cultivars of Indian hawthorn differ significantly in their susceptibility to this disease. Although only light spotting of the leaves was previously noted on cultivars such as Olivia, Eleanor Tabor, and Indian Princess, others such as Springtime, Harbinger of Spring, Pinkie, Enchantress, and Spring Rapture consistently suffered each year from extensive leaf spotting and disease-related defoliation (1,2). Also, Tilt et al (4) reported that cultivars with good leaf spot resistance also had superior aesthetic ratings.

Entomosporium leaf spot is not the only foliar disease threat to Indian hawthorn. During the summer months, Rhizoctonia aerial blight often occurs on tightly jammed, container-grown dwarf Indian hawthorn cultivars. Outbreaks of fireblight, a bacterial disease caused by *Erwinia amylovora*, have occasionally been seen in Alabama nurseries and landscapes on Indian hawthorn. Other foliar diseases such as anthracnose (Colletotrichum gloeosporoides) may also threaten the quality and health of Indian hawthorn. The reaction of Indian hawthorn cultivars to these diseases is unknown. This report summarizes the reaction of selected cultivars of Indian hawthorn in a simulated landscape planting to the diseases Entomosporium leaf spot, fireblight, and anthracnose.

In March 1994, cultivars of dwarf and standard cultivars of Indian hawthorn were established in a Benndale fine sandy loam at the Brewton Experiment Field (Zone 8a) on 5 ft. centers with 10 ft. between the rows. Snow White and Rosalinda, which are dwarf and standard forms respectively, were added in March, 1995. In March 1996, the dwarf cultivar Bay Breeze was established. Prior to planting, soil fertility and pH were adjusted according to the results of a soil assay. A drip irrigation system was installed shortly after establishment and the plants were watered as needed. Plots have been mulched annually with aged pine bark. Twice

each spring, approximately one half cup of Osmocote 17-7- 12 was uniformly distributed around each plant. Pre-emergence weed control was obtained with two applications of a Gallery and Surflan tank mix per year. Hand weeding and directed applications of Roundup at recommended rates were also used to control weeds. In 1997, Entomosporium leaf spot (ELS) damage was assessed on May 19 while fireblight (FB) and anthracnose (ANTH) ratings were taken on June 29. Additional ratings for all above diseases were taken in 1997 at regular intervals through the growing season (data not shown). Disease rating scales are listed below in the table.

Results and Discussion: Weather patterns through much of the winter and spring, 1997 were wetter than normal. As early as mid-January, typical leaf spot symptoms were noted, particularly on those cultivars previously identified as susceptible to Entomosporium leaf spot (1,2). Disease intensification continued into early summer on all Indian hawthorn cultivars. In 1997, disease ratings for several leaf spot resistant cultivars were higher than those recorded in previous years (1,2)

Although no cultivar remained free of leaf spot, significant differences in the disease incidence were noted in 1997 among the 22 cultivars of Indian hawthorn screened (Table 1). Light to moderate spotting of the foliage along with a very low level of disease-related defoliation, as indicated by disease ratings of 2.0 to 2.6 were recorded for Olivia, F3, Indian Princess, Eleanor Tabor and Janice. Of these six cultivars, Olivia had the least spotting of the foliage and no disease-related defoliation. Nearly all the remaining cultivars suffered moderate to heavy spotting of the leaves along with extensive to near complete defoliation. As indicated by leaf spot ratings above 4.0, the worst damage was seen on Spring-time, Enchantress, Harbinger of Spring, Pinkie, F6 and White Enchantress.

The blossom blight and shoot dieback typically associated with fireblight was first noted on selected Indian hawthorn cultivars in mid-May (Table 1). Within two months of symptom onset, the cultivars Janice and Jack Evans had succumbed to this disease. Considerable fireblight-induced shoot dieback was also noted on the F3 and to a lesser extent on Olivia and Majestic Beauty. The remaining cultivars suffered little for no fireblight-related damage.

For the past two years, anthracnose has appeared on selected cultivars of Indian hawthorn between the mid-May and late June and was responsible for some damage to the leaves and premature leaf shed. Occurrence of this disease was restricted to the two larger forms, Majestic

Beauty and Rosalinda both of which are standard cultivars (Table 1). Of these two cultivars, incidence of anthracnose was significantly higher on Majestic Beauty than on Rosalinda.

Significance to Industry: In addition to Entomosporium leaf spot, fireblight and anthracnose are a potential threats to the health and quality to Indian hawthorn. Of these two additional diseases, fireblight is most likely to cause significant damage to Indian hawthorn in both the nursery and landscape. The dwarf cultivars Eleanor Tabor and Indian Princess suffered the least damage from all of the above diseases. Disease resistant cultivars of Indian hawthorn can be produced in a nursery or maintained in the landscape with little need for costly pesticide treatments.

Literature Cited:

1. Hagan, A. K., J. W. Olive, K. Tilt, and J. R. Akridge. 1995. Resistance of Indian hawthorn to Entomosporium leaf spot. Proc. SNA Res. Conf. 40:216-218.
2. Olive, J. W., A. K. Hagan, and J. R. Akridge. 1996. Evaluation of selected cultivars of Indian hawthorn varieties for resistance to Entomosporium leaf spot. Proc. SNA Res. Conf. 41: 184-187.
3. Schubert, T. S. and L. G. Brown. 1987. Entomosporium leaf spot of *Raphiolepis* sp. Fl. Dept. of Agric. Cons. Serv., Plant Pathol. Cir. 295.
4. Tilt, K., A. K. Hagan, J. D. Williams, and J. R. Akridge. 1997. Indian hawthorn: Cultivar selection is important. Proc. SNA Res. Conf. 42:519-521.

SNA RESEARCH CONFERENCE - VOL. 43 - 1998

Table 1. Susceptibility of cultivars of Indian hawthorn to Entomosporium leaf spot, fireblight, and anthracnose, 1997.

Cultivar	ELS'	FB ²	ANTH'	Cultivar	ELS'	FB ²	ANTH'
Majestic Beauty	3.0	0.6	2.7	F6	4.3	0.0	1.0
Springtime	4.7	0.1	1.0	Rosalinda	3.0	0.1	1.3
Spring Rapture	4.0	0.0	1.0	Snow White	3.2	0.0	1.0
White Enchantress	4.2	0.0	1.0	Jack Evans	3.0	4.0	1.0
Indian Princess	2.3	0.2	1.0	Heather	4.0	0.2	1.0
Dwarf Yedda	2.8	0.0	1.0	Janice	2.6	3.7	1.0
F1	2.3	0.1	1.0	Enchantress	4.5	0.3	1.0
F2	2.6	2.0	1.0	Clara	3.0	0.1	1.0
F3	2.7	2.0	1.0	Harbinger of Spring	4.6	0.1	1.0
Olivia	2.0	0.7	1.0	Pinkie	4.2	0.1	1.0
Eleanor Tabor	2.5	0.2	1.0	Bay Breeze	3.6	0.0	1.0
LSD (P=0.05)	0.6	0.3	0.1		0.6	0.3	0.1

Entomosporium leaf spot (ELS) and anthracnose (ANTH) were rated on a scale of 1 to 5 where 1 = no disease, 2 = 1 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% of the leaves diseased. 2Fireblight (FB) severity was assessed on a scale of 0 to 5 where 0 = no disease, 1 = one or few diseased branch tips, 3 = numerous diseased branch tips with a few major branches killed 4 = major portion of tree killed, 5 = tree killed. 3Mean separation within columns was according to Fisher' K protected least significance (LSD) test (P=0.05).

Evaluation of Selected Fungicides for Control of Powdery Mildew of Dogwood

John W. Olive, Austin K. Hagan, and Leonard C. Parrott
AAES Ornamental Horticulture Substation, Mobile AL 36689

Nature of Work: Powdery mildew is a common pathogen on a number of ornamental plants. *Microsphaera pulchra* has been identified as the causal organism of this disease on *Cornus florida* (1) and epidemics on dogwood have occurred in various locations since the early 1990's. This disease can be devastating in the nursery where it has caused stunting of seedlings and lack of vigor in larger trees (2). It can cause serious problems in landscape plantings as well. A number of fungicides are labeled for control of powdery mildew and this study was initiated to determine the efficacy of selected fungicides for control of powdery mildew on dogwood.

Bare-root dogwoods, *C. florida* 'Cherokee Chief' were potted in 3 gallon containers in a pine bark:peat moss (3:1; vol:vol) medium amended with 14 lbs of Osmocote 17-7-12, 6 lbs dolomitic limestone, 2 lbs gypsum, and 1.5 lbs Micromax per cubic yard of mix. Trees were maintained under 47% shade with overhead impact sprinkler irrigation. The fungicides evaluated were thiophanate-methyl (Cleary's 3336 and Domain), propiconazole (Banner Maxx), myclobutanil (Eagle), triadimefon (Bayleton), fenarimol (Rubigan), and Triforine. The experimental design was a randomized complete block with eight single plant replicates. Treatments were applied to runoff with a CO₂ pressurized sprayer at 2 week intervals beginning on June 18 and ending on Sept. 17. The incidence of Powdery mildew on the foliage was visually assessed on Oct. 1 using a Horsfall/Barrett system for rating diseased foliage (Table 1).

Results and Discussion: All of the fungicides tested significantly reduced incidence of powdery mildew when compared to the unsprayed control (Table 1) and all of the fungicides tested are labeled for powdery mildew on dogwood. Treatments with the least colonization by powdery mildew (least disease) were Domain, Eagle, Rubigan, Banner Maxx, and Phyton 27.

Significance to Industry: Powdery mildew limits growth and quality of dogwood in the nursery. A number of fungicides were identified as effective in controlling this disease and when applied on a regular schedule, these fungicides can limit losses caused by this disease.

Literature Cited:

1. Klein, L.A., Windom, M.T. and Trigiano, R.N. 1998. Natural occurrence of *Microsphaera pulchra* and *Phyllactinia guttata* on two *Cornus* species. *Plant Dis.* 82:383-385.
2. Windom, M.T. 1996. Resistance to powdery mildew in flowering dogwood. *Proc. Southern Nurseryman's Assoc. Res. Conf.* 41:197-199.

Table 1. Efficacy of selected fungicides for control of powdery mildew on dogwood.

Treatment	Rate/100 gal	Disease Rating ¹
Bayleton T/O	4 oz	5.3
Triforine EC	12 fl oz	5.3
Cleary's 3336	20 fl oz	4.1
Banner Maxx	6 fl oz	2.5
Domain 50 W	1 lb	1.9
Rubigan A.S.	10 fl oz	1.9
Phyton 27	40 fl oz	1.9
Eagle 40 W	8 oz	1.6
Unsprayed Control	—	9.0
LSD (P=0.05)		1.0

¹Horsfall/Barratt rating scale: 1 = 0%, 2 = 0-3%, 3 = 3-6%. 4 = 6-12%, 5 = 12-25%, 6 = 25-50%, 7 = 50 - 75%, 8 = 75-87%, 9 = 87-94%, 10 = 94-97%, 11 = 97-100%, 12 = 100% of leaves colonized by the powdery mildew fungus.

Biorational Products for Powdery Mildew Disease Management in Dogwood (*Cornus* spp).

**Margaret T. Mmbaga,
Tennessee State University-Nursery Crop Research Station,
McMinnville, TN 37110.**

Nature of Work: Compounds for disease control that are relatively harmless to the environment and non-target organisms have been unofficially designated as "biorational" compounds (Stimmel 1996). Biorational compounds that have produced excellent control of powdery mildew in other crop systems include sodium bicarbonate, hydrophobic plant extract of neem oil, horticultural oils, antitranspirants, detergents, phosphate and nitrate salts, and some microbial fungicides (Horst *et al.* 1992, Ziv and Hagiladi 1993, Locke 1993, Reuveni *et al.* 1994, 1995, Clement *et al.* 1994). A relatively new hydrophobic product "M-96-018 Kaolin" (EngelHard Co. Iselin, NJ) has been derived from clay, and is reported to give excellent control of powdery mildew on apples (Mike Glenn, USDA Appalachian Fruit Crops Research Station, Kearneysville, WV). Some products that have been registered for use on powdery mildew of ornamental plants include a sodium bicarbonate formulation marketed as "Armcarb" (Church and Dwight Co. Inc. Princeton NJ), and as "Kaligreen" (TOAGOSEI Co. Ltd. Tokyo Japan). Neem seed oil extract marketed as "Triact" and as "Green Light Rose Defense" (Grace Biopesticides, Columbia, MD).

These reports show that a possibility exists to incorporate biorational compounds in powdery mildew disease management to reduce the use of synthetic fungicides that are realively less friendly to the environment (Clement *et al.* 1994, Stimmel 1996). The objective of this study was to identify biorational products that are effective in dogwood powdery mildew disease management. Two studies were conducted, (1) using 4-5ft plants of five cultivars, and (2) using small seedlings to test 13 products.

Based on preliminary studies, an insecticidal detergent "Safer Soap", and the new product "M96-018 Kaolin were selected for evaluation on five cultivars that represented moderate resistance (MR), moderate susceptibility (MS), and high susceptibility (S) to powdery mildew. Three replicates were used for each cultivar, 'Cherokee Brave' (MR), 'Cherokee Daybreak' (MS), 'Cherokee Sunset' (S), 'Pygmy' (S) and 'Ruth Ellen'(S). In the second study, susceptible seedlings derived from 'Cherokee Princess', were grown in two gallon containers for the evaluation of 13 biorational products. These products were evaluated individually and in a 3:1 rotation with a systemic fungicide Banner (Ciba Geigy Corporation

Greensboro, NC). Six common fungicides were included in the study to provide a comparison. A replication of ten plants per treatment was used and all plants were maintained in an open shade house with 50% shade and susceptible plants were placed at various locations to provide a continuous source of new inoculum. Plants were fertilized and irrigated by drip irrigation as recommended for *C. florida*.

Application of control products was started on June 2 and continued at two week intervals until the end of the season. All applications of control products followed the label recommendations and application rates reported in the literature. Disease evaluation was done in July, August and September using a scale of 0-5 with 0=no disease symptoms, 1=1-10%, 2=11-25%, 3=26-50% 4=51-75 and 5=75-100% of plants covered with powdery mildew disease.

Results and Discussion: Results are presented in Tables 1 & 2. Disease severity was highest in August for all treatments, thus severity rating performed in August gave the best evidence of product effectiveness. M98-018 Kaolin and Safer Soap, slightly reduced powdery mildew severity on the moderately resistant Cherokee Brave, but they were not effective on the susceptible or moderately susceptible cultivars (Table 1). When no control measures were used, Cherokee Brave developed almost as much disease as the more susceptible cultivars, but the infection started slightly later and developed more slowly. The fungicide Banner was the only product that gave effective control of powdery mildew in all cultivars (Table 1). Safer Soap was slightly more effective on seedlings, than on the larger plants, but M96-018Kaolin gave similar results on seedlings as on the older plants (Tables 2).

Most of the biorational products reduced disease severity on the seedlings, except foliar fertilizer (Blossom Plus) and M96-018Kaolin. Some products such as Total Grow, Transfilm and Armicarb reduced disease severity significantly, but the mean disease severity was still high and may be unacceptable in some commercial production systems. However, for the growers who abstain from using any traditional fungicides, these biorational products may provide an alternative that is better than not using any control treatment. The protection that these products provide may also be adequate for landscape use. The products that were most effective are Vapor Guard and Triact, but Vapor Guard tended to stunt plant growth.

One application of a systemic fungicide to every 3 applications of the biorational products, improved disease control except for phosphate salt, Vapor Guard, Triact and AQ10. The best rotation with fungicide was from Safer soap, Armicarb, Equate and Blossom Plus. These treatments were

almost as effective as the best fungicides. The dramatic change in the effect of the Blossom Plus when a fungicide rotation was added merits further investigation. Among the fungicides, Bordeaux mixture was the most effective, closely followed by Strike, Banner and Chlorothalonil. The Cleary's 3336 was not effective and gave results similar to the non-treated controls, Diathane and Kocide were also worse than some biorational products (Table 2).

These preliminary results have shown that biorational products have potential in powdery mildew disease management on dogwood either individually or in rotation with a standard fungicide. The identification of biorational products that are effective on powdery mildew of dogwood is an important component of the plant pathology program at the TSU Nursery Crop Research Station. The products that have shown good potential will be further evaluated using older plants and field situations. Additional products will also be evaluated.

Significance to the Industry: These preliminary results demonstrate that there is potential to attain effective management of powdery mildew and reduce the amount of traditional fungicides used in nursery production. Biorational products can play an important role in powdery mildew disease management. When used individually, some of the biorational products may not be effective enough for nursery production, but they may be adequate for landscape use. The biggest potential of biorational products in nursery production is as a component of integrated disease management systems that incorporate a judicious use of a highly effective traditional fungicide

Literature Cited:

1. Clement, D.L., S.A. Gill and W. Potts 1994. Alternative to Powdery mildew control in Lilac. *J. Arboriculture* 20(4): 227-230.
2. Horst, R.K., S.O. Kawamoto, and L.L. Porter 1992. Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of Roses. *Plant Dis.* 76:247-251.
3. Locke, J.C. 1993. Field evaluation of clarified neem seed oil, Sun Spray 6E Plus, horticultural oil, and funginex for control of powdery mildew on perennial garden phlox. *Phytopathology* 83:1337.
4. Reuveni, M., and R. Reuveni 1995. Efficacy of foliar sprays of phosphates in controlling powdery mildew in field grown nectarine, mango trees and grapevines. *Crop Protection* 14(4):311-314.

5. Reuveni, R., V. Agapov, and M. Reuveni 1994. Effect of powdery mildew (*Sphaerotheca pannosa*) of roses. J. Phytopathology 142:331-337.
6. Stimmel, James F. 1996. Biorational controls- Problem-free?. Regulatory Horticulture 22:13.
7. Ziv, O. and A. Hagiladi 1993. Controlling powdery mildew in Euonymus with polymer coatings and bicarbonate solutions. HortScience 28(2):124-126.

Table 1. Powdery mildew disease severity on 3-4 years-old trees of *Cornus florida* in 1997.

<i>Cornus florida</i> Cultivar	Mean disease severity and control treatments used				
	Banner*	M96-018 Kaolin	Safer Soap	Water Control	LSD
Cherokee Brave (MR)	0.0	2.2	2.0	3.7	1.29
Cherokee Sunset (MS)	0.7	3.0	3.3	4.3	1.85
Cherokee Daybreak (MS)	0.7	3.2	4.0	3.7	2.03
Pygmeae (Pygmy) (S)	0.7	3.7	3.7	4.0	0.93
Ruth Ellen (S)	0.7	3.7	4.3	4.3	0.92
LSD	1.19	1.10	1.61	1.97	

* A systemic fungicide used for comparison on cultivars moderately resistant (MR), moderately susceptible (MS), and susceptible (S) to powdery mildew.

Table 2. The effect of biorational agents and some synthetic chemical fungicides on powdery mildew disease severity on dogwood seedlings in 1997.

Treatments	Type of product	Mean disease severity(1-5 scale) when the products are used			
		Individually		In rotation with a fungicide ³	
		July 30	August 28	July 30	August 28
1. Total Grow	Foliar fertilizer	2.1 cd	2.5 def	0.7 ghij	1.5 ijklmno
2. Safer Soap	Insecticidal soap	0.4 hij	1.7 ghijklm	0.3 hij	0.7 pqr
3. M-96-018Kaolin	Clay	1.7 cde	4.1 a	0.5 ghij	2.7 cde
4. Armicarb	Bicarbonate	1.6 def	2.1 defghi	0.3 hi	0.7 pqr
5. Transfilm	Antitranspirant	1.7 cde	2.3 defgh	1.3 defg	1.1 lmnopq
6. M-Pede	Insecticidal soap	0.6 ghij	1.8 fghijkl	1.7 cde	1.3 jklmnop
7. K2HPO4	Phosphate salt	1.3 defg	1.2 klmnop	0.1 j	1.2 klmnop
8. Triact	Plant extract ¹	0.4 hij	1.1 mnopq	0.5 ghij	2.0 efg hij
9. Vapor Grad	Antitranspirant	0.0 j	1.0 mnopqr	0.0 j	1.2 klmnop
10. Equate	Antibacterial soap	1.0 efghi	1.5 ijklmno	0.4 hij	0.7 pqr
11. AQ10	Biocide ²	0.0 j	1.3 jklmnop	0.6 ghij	1.9 fghijk
12. Blossom Plus	Foliar fertilizer	3.1 ab	4.0 ab	2.1 cd	0.3 r
13. Ultra Fine	Horticultural oil	0.0 j	1.6 hijklmn	0.0 j	0.8 opqr
14. Water	Control	3.5 a	3.8 ab		
15. Banner	Fungicide	0.2 ij	0.9 nopqr		
16. Chlorothalonil	Fungicide	1.0 efghi	0.9 nopqr		
17. Dithane	Fungicide	0.5 ghij	2.8 cd		
18. Kocide	Fungicide	1.1 efgh	2.4 defg		
19. Cleary's 3336	Fungicide	2.5 bc	3.3 bc		
20. Bordeaux	Fungicide	0.0 j	0.4 qr		
21. Strike	Fungicide	0.8 fghij	0.8 opqr		

1=1-10%, 2=11-25%, 3=26-50% 4=51-75 and 5=75-100% plant infection. Disease severity readings followed by same letters in the same date of sampling are statistically similar. ¹Plant extract from neem oil, ²Microbial pesticide from *Ampelomyces quisqualis*; ³A systemic fungicide Banner was used in a rotation of a 3:1 biorational to fungicide applications

Controlling Rust on Zonal Geraniums with Fungicides

Steven N. Jeffers and Lynn A. Luszcz

Department of Plant Pathology & Physiology, Clemson University,
Clemson, SC 29634-0377

Nature of Work: In the spring of 1997, an epidemic of rust on zonal geraniums (*Pelargonium x hortorum*) occurred in plant beds on the coast of South Carolina (3). This was one of the most serious epidemics of geranium rust that has occurred in the United States in recent years. In addition, diseased geraniums were found at nurseries and greenhouses and were distributed to retail outlets in South Carolina and other parts of the Southeast. This situation occurred despite applications of fungicides (primarily triadimefon products) for rust control. Geranium rust (caused by *Puccinia pelargonii-zonalis*) is not endemic to the United States and occurs only sporadically and usually insignificantly each year when the pathogen inadvertently is brought into the United States on infected or contaminated geranium cuttings or plants coming from locations where the disease is endemic (1,2). Some people in the industry consider rust to be one of the most potentially threatening diseases to the production and cultivation of zonal geraniums in the United States because there are not adequate methods for detecting the pathogen on symptomless but contaminated or infected propagation stock coming into this country from foreign locations. Research on the efficacy of fungicides for controlling geranium rust has been very limited (2,4,5). Consequently, a project was initiated to evaluate the effectiveness of currently available fungicides for managing rust on zonal geraniums. This is a preliminary report.

Fungicides were evaluated on geranium plants (cv. Veronica) that were grown from rooted cuttings in a greenhouse for approximately two months. Plants were moved into a growth chamber (1721°C [62-70°F], 14-h photoperiod) for inoculation and symptom development after treatments were applied because geranium rust does not develop well at temperatures above 25°C [77°F] (2). In all, seven fungicides were evaluated (Table 1) at rates recommended on product labels or by the manufacturer. Strike, Systhane, Daconil, and Dithane are registered for control of geranium rust in the United States, but Strike is used most frequently. Baycor is registered for geranium rust control outside the United States—where it is becoming popular. Rubigan and Phyton are registered for use on ornamental crops in the United States but are not registered specifically for geranium rust. Strike, Systhane, Baycor and Rubigan are newer chemistry, demethylation-inhibiting (DMI) fungicides—which provide protective as well as some eradicated activity—whereas Daconil, Dithane, and Phyton are traditional broad-spectrum, protestant fungicides. Phyton was evaluated at two rates because of the broad range of rates recommended on the product label. Combinations

of Strike or Systhane mixed with Daconil or Dithane were evaluated to take advantage of two different types of chemistry in a single treatment; full rates of both ingredients were used in all mixtures. Treatments were prepared with tap water in 500-ml volumes (Table 1) and applied with a CO₂ powered sprayer equipped with a single-nozzle spray boom. Treatments were reapplied one week later.

Plants were inoculated approximately 24 hours after treatments were applied. *P. pelargonii-zonalis* was collected on infected plants in 1997 and was maintained on geranium plants in a growth chamber. An aqueous suspension of urediniospores (3.5 x 10⁵ spores/ml) was prepared as inoculum by washing leaves with sporulating uredinia in 0.1% Tween 80. The suspension was applied to plants with a hand-pump spray bottle, and plants were maintained at 100% relative humidity for 24 hours. Plants were evaluated five weeks later by counting the number of lesions per leaf and the number of leaves with lesions and measuring diameters of up to five randomly selected lesions on each plant. Data were analyzed by one-way analysis of variance (ANOVA), and means were separated by Fisher's Protected Least Significant Difference (LSD; $P=0.05$). The experiment was repeated.

Results and Discussion: When treatments were applied before inoculation under controlled conditions, all fungicides significantly reduced the incidence of geranium rust compared to the untreated control (Table 2). Although the number of lesions per plant ranged from zero (0) to 9.4 for the twelve fungicide treatments, there was no significant difference among treatments—which was due in part to the high variability associated with the control treatment. However, when the numbers of diseased leaves per plant were analyzed, significant differences among treatments were observed (Table 2). Baycor, Daconil, Dithane, and the four combination treatments—which contained either Daconil or Dithane—protected plants completely; disease incidences with Systhane and Rubigan were not significantly different from these treatments. Strike and the low rate of Phyton were not as effective as any of these products. On plants where lesions developed, lesions were smallest after treatment with Systhane whereas treatment with Phyton, at either rate, had no effect on lesion size compared to the untreated control. Similar results were observed when this experiment was repeated and in other experiments. Phytotoxicity was observed with the high rate of Phyton; some leaves developed marginal necrosis 24 hours after application. In addition, some plants treated with Dithane developed a mild chlorosis and a mottled appearance.

These results suggest that excellent control of rust on zonal geraniums can be achieved with timely applications of broad-spectrum protectant fungicides containing chlorothalonil (as found in Daconil Ultrex and

related products) or mancozeb (as found in Dithane T/O and related products). Of the newer chemistry DMI fungicides evaluated, Baycor provided excellent control but is not registered in the United States; it should be considered for rust management programs in geranium production operations outside the United States. Of the DMI products available in the United States—Sythane, which is registered for geranium rust, provided excellent control and Rubigan, which is not registered for this disease, looked promising. In theory, the best management strategy should be to use a mixture of a protectant fungicide and a DMI fungicide because applications can not always be made before inoculum is present. However, we were unable to prove this theory in these experiments because the protectants alone provided complete protection. Efficacies of these products may be different in a production greenhouse or the field where inoculum is present much of the time and infection periods occur daily. In conclusion, producers should be able to manage rust on zonal geraniums with the products currently available; however, they should not rely solely on one product—which has been done in the past. Additional research on managing geranium rust with fungicides is in progress.

Significance to Industry: Identifying the most effective fungicides for managing geranium rust should aid in limiting the introduction of this pathogen on plant material coming into this country, should prevent the disease from becoming established in our nursery and greenhouse industries, and should avoid disastrous epidemics like the one that occurred in South Carolina in 1997.

Literature Cited:

1. Daughtrey, M. L., R. L. Wick, and J. L. Petersen. 1995. *Compendium of Flowering Potted Plant Diseases*. APS Press, The American Phytopathological Society, St. Paul, MN. 90 pp.
2. Harwood, C. A., and R. D. Raabe. 1979. The disease cycle and control of geranium rust. *Phytopathology* 69:923-927.
3. Jeffers, S. N. 1998. Geranium rust: A potentially serious threat to the zonal geranium industry. *Greenhouse Product News* 8(2):20-23.
4. Kauffman, P. 1980. Control of geranium rust with foliar sprays, 1979. *Fungicide and Nematicide Tests* 35:136-137.
5. Raabe, R. D. 1991. Control of rust on geranium, 1990. *Fungicide and Nematicide Tests* 46:345.

Table 1. Fungicides and application rates used to control rust on zonal geraniums.

Trade Name	Formulation	Common Name	Recommended Rate ^x	Application Rate ^y
Strike	25 WP	triadimefon	2.0 oz/50 gal	150 mg
Systhane	40 WP	myclobutanil	2.0 oz/50 gal	150 mg
Rubigan	1 EC	fenarimol	5.0 fl oz/100 gal	0.2 ml
Baycor	300 EC (metric)	bitertanol	38.0 fl oz/100 gal	1.5 ml
Daconil Ultrex	82.5 WDG	chlorothalonil	1.4 lb/100 gal	840 mg
Dithane T/O	75 WDG	mancozeb	1.5 lb/100 gal	900 mg
Phyton 27 ^z	21.36 S	copper sulfate pentahydrate	2.5 fl oz/10 gal	1.0 ml
			5.0 fl oz/10 gal	2.0 ml

^x The amount of formulated product added to a designated volume of water recommended on the product label or by the manufacturer.

^y The amount of formulated product added to 500 ml of water and applied to geranium plants.

^z Phyton 27 was evaluated at two rates: 1x and 2x.

Table 2. Efficacy of fungicides for geranium rust control when applied before inoculation.

Treatment	No. Lesions per Plant ^x	No. Diseased Leaves per Plant ^x	Lesion Diameter (mm) ^x
Strike	9.4 a	2.4 c	2.5 c
Sythane	0.8 a	0.8 ab	1.3 b
Rubigan	4.6 a	1.0 ab	2.5 c
Baycor	0.0 a	0.0 a	0.0 a
Daconil	0.0 a	0.0 a	0.0 a
Dithane	0.0 a	0.0 a	0.0 a
Phyton/1x	9.0 a	2.8 c	5.4 d
Phyton/2x	2.8 a	1.8 bc	5.2 d
Strike + Daconil	0.0 a	0.0 a	0.0 a
Strike + Dithane	0.0 a	0.0 a	0.0 a
Sythane + Daconil	0.0 a	0.0 a	0.0 a
Sythane + Dithane	0.0 a	0.0 a	0.0 a
Control	76.2 b	6.8 d	5.2 d
P value ^y	<0.001	<0.001	<0.001
LSD ^z	17.80	1.10	0.80

^x Values are means of five replicates; means followed by the same letter are not significantly different.

^y P values for F statistics from one-way analyses of variance (ANOVAs).

^z Fisher's Least Significant Difference with P=0.05 for separating means within a column.

Extraction and Analysis of Common Nursery Pesticides From Runoff Water

Melissa Riley, Rosemary Cancro, and Ted Whitwell
Clemson University, Dept. of Plant Pathology,
Clemson, SC 29631

Nature of Work: Containerized plant nurseries utilize many different chemicals for the control of plant diseases, insects, and weeds. These chemicals may have very different chemical properties which result in problems in analyzing runoff and surface water at nurseries for their presence. A simple analysis procedure is needed for determining residues of the most common pesticides in water in order to compare management protocols for reducing pesticide runoff. Previous studies have shown that many of the herbicides commonly used in nurseries can be extracted from water utilizing C18 solid phase extraction cartridges (2). This study was begun by making modifications to this initial extraction procedure and to the analytical procedures used for quantitation of pesticide residues. An analytical method for detecting and quantifying pesticides in water samples using gas chromatography-mass spectrometry (GC-MS) and/or high pressure liquid chromatography (HPLC) was developed.

Fifteen common nursery pesticides with different uses and chemical properties (Table 1) were analyzed using HPLC and GC-MS to determine the best detection method for each pesticide and to determine conditions needed for separation and quantification. Once a detection method was established, sample solutions containing all of the pesticides (0.2 ug/ml, total sample volume of 200 ml) were extracted and analyzed to determine percent recovery.

Water samples containing pesticides were run through a C₁₈ solid phase extraction cartridge to concentrate the pesticides. In the first two procedures, the pH of the water samples was adjusted to either pH 2.5 or 10 prior to running through C₁₈ cartridges. In a third procedure water pH was adjusted to pH 2.5, run through the C₁₈ cartridge with water collected, pH adjusted to 10 and run back through the cartridge. In the fourth procedure water pH was adjusted to pH 10, run through the C₁₈ cartridge and collected, pH adjusted to 2.5 and run back through the cartridge. Pesticides were eluted from the cartridges with 2 ml of acetone, yielding a final concentration of 20 ug/ml (assuming 100% recovery). Samples were then run on both HPLC and GC-MS for quantitation. Three replicates of each procedure were conducted for each pesticide.

GC-MS method was as follows: The column was a DB-5MS (30m X 0.25mm I.D., 0.25 μ m film) run from 150°C to 310°C at 5°C/min, followed by a 3 min hold at 310°C. The injector was 250°C, transfer line at 245°C, and 2 μ l were injected. Mass spectrometer was set to monitor from 50-318 mass/charge with 0.810 sec/scan.

HPLC method was as follows: Rexchrom C₁₈ column (3 μ , 10cm X 4.6mm I.D.) with a 0.5 ml/min flow rate and initial mobile phase of 70:30 (water with 2% acetonitrile and 0.1% phosphoric acid:acetonitrile with 0.1% phosphoric acid) changing to 25:75 over the first 30 min, holding for 5 min, then to 0:100 over 5 min and held 5 min. The column was re-equilibrated for 4 min with 70:30 initial solvent. Injections were 50 μ l and the diode array detector was set to monitor the wavelengths of 206, 310, 254, 265 and 215 nm.

Results and Discussion: Ten pesticides were detected utilizing GC-MS (Table 2). However, acephate recoveries were low, perhaps due to degradation. Fluralinate and metalaxyl yielded high recoveries possibly due to split peaks which introduce error into quantitation. Fourteen pesticides were detectable utilizing HPLC (Table 3); however, coelution of chlorpyrifos and dicofol occurred, resulting in high recoveries for chlorpyrifos and no recovery of dicofol. Chlorpyrifos was detectable utilizing GC-MS which eliminates this problem. Imidicloprid recoveries were high, possibly due to interference with the solvent peak.

Standard deviations of replicate samples indicate that in procedures where the pH was adjusted twice (particularly in pH 2.5 to 10 procedure) results were less reproducible. Adjusted samples that began with a pH of 10 and were later adjusted to 2.5 have lower standard deviations, probably due to a majority of the compounds interacting optimally with the C₁₈ cartridge at pH 2.5. When the pH of water was adjusted to pH 10, then to pH 2.5, chlorothalonil, endosulfan, thiophanate-methyl, and pendimethalin were lost. The overall best procedure for extraction of pesticides from water samples is the pH 2.5 procedure.

Significance to Industry: These data indicate that it is possible to monitor pesticide loss and runoff in nursery water using a relatively simple extraction method. The techniques developed will be used in future integrated pest management projects aimed at lowering environmental impact of runoff water by adjusting time, frequency and quantity of pesticide application.

Literature Cited:

1. Meister R. T., 1998. Farm Chemicals Handbook '98. Vol 84. Meister Publishing Company, Willoughby, OH.
2. Riley M. B. and R. J. Keese. 1996. Comparison of Solid Phase Extraction Techniques for Herbicides. Weed Science 44:689-693.

Table 1. Common pesticides used in ornamental nurseries and their chemical class and usage (1)

Compound	Trade Name	Chemical Class	Useage
Acephate	Orthene	Organophosphate	Insecticide
Bifenthrin	Talstar	Pyrethroid	Insecticide, Miticide
Chlorothalonil	Daconil	Benzonitrile	Fungicide
Chlorpyrifos	Dursban	Organophosphate	Insecticide
Dicofol	Kelthane	Chlorinated benzhydrol	Acaricide
Diquat	Reward	Bipyridilium	Herbicide, Dessicant
Endosulfan	Thiodan	Cyclodiene	Insecticide, Acaricide
Fluvalinate	Mavrik	Pyrethroid	Insecticide
Imidacloprid	Marathon	Chloropyridinyl	Insecticide
Isoxaben	Gallery, Snapshot ¹	Benzamide	Herbicide
Metalaxyl	Subdue	Acylanine	Fungicide
Oryzalin	Surflan, Rout ²	Dinitroaniline	Herbicide
Oxyfluorfen	Goal, Rout ² , OH-II ³	Diphenyl ether	Herbicide
Pendimethalin	Pendulum, Pre-M, OH-II ³	Dinitroaniline	Herbicide
Thiophanate-methyl	3336, Domain, Fungo	Benzimidazole	Fungicide
Trifluralin	Treflan, Snapshot ¹	Dintroaniline	Herbicide

¹ combination of isoxaben and trifluralin

² combination of oryzalin and oxyfluorfen

³ combination of oxyfluorfen and pendimethalin

TABLE 2: GC-MS DATA Retention time, major ions of mass spectrum and percent recovery of pesticides from various methods of solid phase extraction.

Compound	Retention Time (min)	Major Ion	Percent Recovery		
			pH 2.5	pH 10	pH 10-2.5
Acephate	4.2	136	4.0±6.9	nd	10.9±18.9
Bifenthrin	20.9	181	77.7±2.5	nd	60.0±33.2
Chlorpyrifos	12.3	207	132.6±41.7	121.4±18.2	111.8±44.4
Chlorothalonil	9.3	266	106.4±13.7	16.5±9.1	3.9±3.39
α Endosulfan	15.2	159, 207	116.0±10.3	nd	19.4±17.0
β Endosulfan	17.4	207	110.9±16.6	2.4±2.7	26.8±23.5
Fluvalinate	28.5	251	188.4±36.5	nd	114.9±111.7
Metalaxyl	11.3	161, 206	227.4±23.7	181.6±18.0	110.3±36.4
Oxyfluorfen	16.3	253	166.6±17.0	179.5±12.5	120.1±36.8
Pendimethalin	13.6	252	79.0±6.0	83.8±16.3	85.6±35.6
Trifluralin	6.9	264	126.8±32.1	103.2±8.1	85.0±10.0
					193.0±16.9
					10.1±1.7
					130.7±25.3
					69.7±17.6
					75.3±9.4

Table 3: HPLC DATA Retention time, wavelength used for quantitation and percent recovery of pesticides from various methods on solid phase extraction.

Compound	Retention Time (min)	λ Observed	Percent Recovery		
			pH 2.5	pH 10	pH 10-2.5
Bifenthrin	41.8	206	144.7 \pm 32.3	66.9 \pm 20.3	107.8 \pm 103.1
Chlorpyrifos	31.8	206	143.1 \pm 46.9	40.7 \pm 28.4	33.6 \pm 26.2
Chlorothalonil	20.2	215	124.3 \pm 30.6	nd	nd
Dicofol	32.0	206	nd	nd	nd
α Endosulfan	31.1	215	83.4 \pm 10.4	nd	nd
β Endosulfan	29.2	215	55.1 \pm 11.7	nd	nd
Fluvalinate	39.9, 40.2	215	120.2 \pm 6.4	nd	nd
Imidicloprid	3.2, 4.2	265	188.4 \pm 63.8	254.2 \pm 8.6	101.3 \pm 95.4
Isoxaben	19.5	206	161.6 \pm 32.9	75.4 \pm 32.7	112.5 \pm 65.6
Metalaxyl	11.6	206	85.4 \pm 27.3	56.8 \pm 5.2	73.6 \pm 23.8
Oryzalin	20.6	206	141.6 \pm 10.9	nd	37.5 \pm 33.1
Oxyfluorfen	30.6	206	143.7 \pm 28.1	89.5 \pm 65.8	39.1 \pm 34.8
Pendimethalin	31.6	206	81.7 \pm 16.0	73.4 \pm 17.7	37.7 \pm 15.3
Thiophanate-methyl	8.2	206	54.7 \pm 13.3	nd	nd
Trifluralin	32.6	206	83.2 \pm 30.9	18.2 \pm 18.2	10.1 \pm 8.8
					123.2 \pm 8.3
					128.3 \pm 69.5
					84.8 \pm 7.3
					32.4 \pm 5.7
					69.3 \pm 2.3
					102.8 \pm 5.3
					nd
					nd
					56.8 \pm 8.1

Black Spot Management on Hybrid Tea Rose 'Peace'

A. S. Windham and M. T. Windham
University of Tennessee, Entomology and Plant Pathology,
Nashville, 37211

Nature of Work: Blackspot of rose is a fungal disease caused by *Diplocarpon rosae*. Symptoms of this disease include black spots on foliage, purple to black spots on canes, defoliation, decline in vigor and death of canes or entire plants. This disease is often severe in the Southeastern United States on hybrid tea and floribunda roses. Normal disease management requires spraying roses with a fungicide on a 7 - 14 day schedule (1).

Bareroot hybrid tea rose 'Peace' were planted into ground beds at the Plant and Pest Diagnostic Center in full sun. *Diplocarpon rosae* the causal agent of black spot was introduced to the planting on June 10 by scattering infected leaves at the base of each plant. Five fungicide treatments were evaluated for black spot management. Fungicide treatments and rates per 100gal of water were: Systhane 40WSP (myclobutanil) @ 4 oz; Sentinel 40WG (cyproconazole) @ 3.5 oz; Daconil Ultrex (chlorothalonil) @ 16 oz; Heritage 50WG (azoxystrobin) @ 4 oz and Heritage 50WG (azoxystrobin) @ 38.4 oz. Fungicides were applied with a two gallon, compressed air, hand sprayer using a hollow cone nozzle. Spray solutions were applied to the point of runoff. Fungicide treatments and an unsprayed control were arranged in a randomized complete block design with 3 replications. Fungicide applications were initiated on April 29 and follow up sprays applied on May 15, May 30, June 20, July 17 and August 6. Disease severity of black spot and percent defoliation were estimated using the following scale: 0 = healthy foliage; 1 = <2% of foliage symptomatic; 2 = <5% of foliage symptomatic; 3 = <10% of foliage symptomatic; 4 = <25% of foliage symptomatic; 5 <50% of foliage symptomatic; 6 =>50% of foliage symptomatic. Black spot and defoliation data were collected on July 18 and 28 and August 22.

Results and Discussion: Sentinel and Daconil Ultrex provided good control of black spot with little defoliation (Table 1). Systhane and both rates of Heritage provided less satisfactory control of black spot and defoliation approached 50% for all three treatments. All fungicides may have been more efficacious if applied on a consistent 14 day schedule throughout the summer. Sentinel is labeled for production roses, but not landscape plantings. Heritage is presently labeled for commercial turf diseases only.

Significance to Industry: Sentinel was extremely effective in controlling black spot but may only be used in commercial field production of roses. Daconil Ultrex continues to be an acceptable, preventative treatment for landscape roses in the Upper South (2).

Literature Cited:

1. Roark, S., B. Behe, K. Bowen and R. Kessler, Jr. 1997. Evaluation of weather-based scheduling of antitranspirant treatments for control of rose blackspot disease. Proceedings of the Southern Nursery Research Conference. Vol 42. pp. 266-269.
2. Windham, M. T. and A. S. Windham. 1997. Evaluation of oils and fungicides for control of rose diseases. Proceedings of the Southern Nursery Research Conference. Vol 42. pp.474-475.

Table 1. Efficacy of fungicide treatments for black spot of hybrid tea rose 'Peace'.

Treatment	Black Spot Severity			Defoliation		
	18 July	28 July	22 Aug	18 July	28 July	22 Aug
Untreated Control	3.3	5.3	4.3	3.7	4.3	5.7
Sythane	3.0	3.3	2.7	2.3	3.7	4.7
Sentinel	0.3	0.0	1.3	0.3	0.7	1.3
Daconil Ultrex	1.3	1.7	2.3	1.3	2.0	3.0
Heritage (low rate)	3.0	5.0	3.0	1.3	3.3	5.0
Heritage (high rate)	2.7	4.7	3.0	2.3	4.3	4.7

Transport of Entomogenous Nematodes in Porous Media

Sam O. Dennis¹, Teferi Tsegaye² and Robert E. Harrison¹

¹Co-operative Agricultural Research Program, Tennessee State
University, Nashville TN 37209

²Department of Plant & Soil Science, Alabama A&M University,
Normal, AL. 35762.

Nature of Work: With the impacts of pesticides on both surface and groundwater quality being under increased scrutiny, the search for alternative means of controlling farm pests, especially soil-inhabiting insects, in nursery fields is necessary. Entomogenous nematodes have been tested as biocontrol agents against soil inhabiting insects like the white grubs and Japanese beetle grubs (Forschler and Gardner, 1991, Simoes et al., 1993, Mannion, 1997). With the passing of chlorinated hydrocarbon pesticides, white grubs have become an increasing problem in the nursery industry. Additionally, Japanese beetles have been trapped in some major nursery crop producing areas of Tennessee. Thus, nursery stock shipped in ball and burlap might contain soils infested with the Japanese beetle grubs. Shipment of Japanese beetle grubs into non-infested areas could threaten the nursery industry's ball and burlap market. It is postulated that entomogenous nematodes, especially those belonging to the families of Steinernematidae and Heterorhabditidae, could be ideal biocontrol agents of the Japanese beetle grubs. However, studies of their transport behavior and the influence of water application rates on their movement in soils (porous media) is minimal. With this in mind, the primary objective of the study was to examine the transport (movement) of *Steinernema carpocapsae* (Weiser) (Tn25 strain) in disturbed and undisturbed soil columns while subjected to relatively low water application rates delivered from a compact rain simulator. The soil columns were made of PVC pipes 6 inches (id) x 16 inches long. The base was fitted with a plastic sieve that has greater pore size than the nematodes. Two elbow tensiometers were also fitted on the sides of the columns to monitor the water status of the soil. The rain simulator devices (Ogden et al., 1997) were made of clear acrylic plastics with coiled capillary tubing at the bottom. The tubing serves as drippers. The rain simulator device was calibrated to deliver a darcian flux of 0.4 cm/hr which amounts to a water delivery rate of approx. 120 ml/hr. The columns were saturated with water dripping from the rain simulator devices. The devices allowed the columns to become saturated evenly without the occurrence of surface ponding. Figure 1 shows the schematic setup of the soil column and the rain simulator.

The soil used for both the disturbed and undisturbed columns was collected from the Tennessee State University Nursery Crop Research Station in McMinnville Tennessee. The predominant soil type at the site is Waynesboro sandy loam. Soil samples were collected from this site at the depth increments of 0-4 inches, 4-8 inches and 8-15 inches. The soil samples were crushed and passed through a 2 mm sieve. Soil samples were analyzed for particle size (texture), organic matter and pH. A sub-sample of the soils collected was sterilized in an autoclave with temperature set at 254°F for 60 minutes (to kill existing nematodes). This sample was used to pack the disturbed (crushed) soil into the PVC columns. The soil in the columns was packed to a bulk density of 1.20 gm/cm³. The undisturbed soil columns were collected in-situ in 15-cm diameter PVC pipe to a depth of 35 cm. The encased soil columns were put in an oven for 72 hours (to kill existing nematodes) with temperature set at 105°C. Undisturbed soil cores were also collected with an Uhland core sampler (Blake and Hartge, 1986) for the determination of saturated hydraulic conductivity (K_{sat}). Porosity for each soil core was determined by assuming a soil particle density of 2.65gm/cm³. The entomogenous nematodes (*Steinernema carpocapsae*, Tn25) used in the study were isolated from soils collected from commercial nursery fields in Middle Tennessee. The nematodes were baited with greater wax moth larvae to verify their pathogenicity. The pathogenic isolates were used for the transport study. The nematodes were uniformly applied to the surface of the soil columns. Each column received approximately 11,000 nematodes which amount to the standard field application rate of about 1 billion nematodes per acre. The nematodes were left to equilibrate with the soil for 24 hrs. After this time, a bromide tracer, 0.8M (0.095kg/L) from (KBr), non-toxic to the nematodes, was added to the soil's surface. Bromide (Br-) does not adsorb to the soil and thus will indicate a breakthrough of the water that was applied to the surface of the soil. After bromide application, the rain simulator devices were placed on top of the soil columns to initiate leaching. Leachate samples were collected and assayed for the presence of nematodes that have been transported through the soil columns. The number of nematodes recovered from the undisturbed soil columns versus the disturbed soil columns was noted.

Results and Discussion: The results of some soil physical and chemical properties of the study site are shown in Table 1. Entomogenous nematodes were detected in the leachate samples, suggesting transport of the nematodes in porous media. Preliminary results show that the number of nematodes recovered from the undisturbed columns tended to be more than those recovered from the disturbed columns. This is probably due to macropores that might be present in the undisturbed soil and as such have allowed for the preferential transport of more nematodes through the undisturbed soil columns than the disturbed columns.

Breakthrough curves for bromide and the nematodes, although not shown, indicate that nematodes in the leachate samples reached a steady state but tended to fluctuate over the course of the breakthrough.

Significance to Industry: It appears that entomogenous nematodes, a potential bio-control agent of some soil inhabiting pests, are capable of movement in the direction of water flow. The goal of most growers will be to maximize the effectiveness of these nematodes around the root zone of the crop, while minimizing their movement below it. This is the area where recalcitrant pests like the Japanese beetle grubs do most damage.

Literature Cited:

1. Blake, G.R., and K. H. Harge. 1986. Bulk Density. In A. Klute et al. (Ed.) *Methods of Soil Analysis*. Part I 2nd ed. Agronomy 9:363-375.
2. Forschler, B.T. and W.A. Gardner. 1991. Field efficacy and persistence of entomogenous nematodes in management of white grubs in turf and pasture. *J. Econ. Entomol.* 84 (5) 1454-1459.
3. Mannion, C. 1997. Management of Japanese beetle grubs in field-grown nursery. Poster Presentation. Tennessee Nursery Association, 1997 Trade Show. Nashville, TN.
4. Ogden, C.B., H.M. Van Es, and R.R. Schindelbeck. 1997. Miniature rain simulator for field measurement of soil infiltration. *Soil Sci. Soc. Am. J.* 61:1041-1043.
5. Simoes, N., C. Laumond, and E. Bonifassi. 1993. Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popilla japonica* in the Azores. *J. Nematol.* 480-485.

Table 1. Average values of some soil physical and chemical properties of the study site.

Soil Properties	Average Values
pH	5.90
Organic Matter	1.80
Bulk Density	1.25 gm/cm ³
Porosity	0.51
Saturated Hydraulic Conductivity	3.65 cm/hr

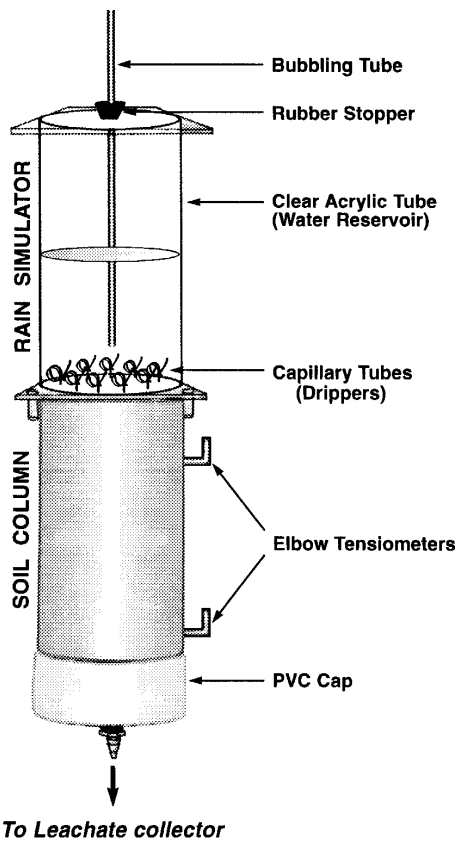


Figure 1. Rain Simulator and Soil Column Setup.

Occurrence of *Phytophthora* spp. on Woody Ornamentals in South Carolina Nurseries

D. T. Ducharme and S. N. Jeffers

Dept. of Plant Pathology & Physiology, Clemson University,
Clemson, SC 29634

Nature of Work: *Phytophthora* species are plant pathogens that attack a diverse and ever-increasing number of host plants causing serious diseases like root rots, trunk and stem cankers, and foliar blights. Diseases caused by *Phytophthora* spp. have become an important concern in the production of many economically important crops in ornamental horticulture, as well as in agriculture and forestry (Jones, 1982). Most infections occur during propagation or juvenile stages of growth with symptoms of disease appearing in the later stages of production or in the landscape (Ribeiro & Linderman, 1991). These extraordinary plant-pathogenic fungi are characterized by an ability to increase rapidly in soil and on foliage and the production of motile zoospores that can be spread by moving water to attack the root systems of other plants (Erwin & Ribeiro, 1996).

In managing *Phytophthora* diseases in commercial nurseries, an integrated approach, including use of cultural practices, fungicides, biocontrols, and host resistance, is recommended. Applications of fungicides usually are necessary for consistent control in the nursery. Treatment with metalaxyl (SUBDUE) or fosetyl-AI (ALIETTE) have proven effective on most woody ornamentals in both nursery and landscape plantings if applied prior to root infection and with repeat applications (Benson, 1987; Ribeiro & Linderman, 1991). However, these fungicides are only suppressive so repeat applications are needed, which increase the likelihood of resistance developing (Erwin & Ribeiro, 1996). Little knowledge currently is available on the incidence of and nursery losses due to *Phytophthora* spp. in South Carolina nurseries. Consequently, an assessment is needed to better understand the epidemiology of the diseases and to apply effective control measures before epidemics develop and losses are incurred. Recently, our laboratory devised a baiting bioassay for detecting *Phytophthora* spp. in nursery container mixes prior to symptom development (Ferguson & Jeffers, 1997). The objective of this study is to use this bioassay to determine the prevalence of *Phytophthora* spp. and to identify potential sources of inoculum in South Carolina nurseries producing container-grown woody ornamentals.

A list of 22 nurseries was established with the assistance of the Plant Problem Clinic at Clemson University, South Carolina Cooperative Extension Agents, the Department of Plant Industry Area Nursery Specialists, and nursery trade magazines. Nurseries from a wide geographical distribution around South Carolina were selected for sampling. Nurseries were commercial wholesale/retail operations that grew containerized woody ornamentals, which were propagated onsite from rooted cuttings or from liners from an outside source. Woody ornamental plants targeted for sampling include some of the most common landscape plants that are susceptible to *Phytophthora* spp., e.g., azalea, boxwood, juniper, holly, and camellia.

At each nursery, samples were taken from (1) container mix prior to use, (2) field soil around native woody plants, and (3) container mix with roots from three to five woody ornamental species. Both fresh and air-dried subsamples were baited with three replications used per sample (Ferguson & Jeffers, 1997). Three different bait types were used for all samples—camellia leaf disks and whole needles of shore juniper and eastern hemlock. To bait fresh subsamples, 100 ml of mix were flooded with 200 ml of distilled water and six baits of each type were floated on the water. Soil and mix subsamples also were air-dried and then held at 25 C for 3 additional days prior to flooding and baiting as described above. For all subsamples, three baits were removed at 24 and 72 hr, placed onto PARPH-V8 agar, a selective medium (Ferguson & Jeffers, unpublished), and incubated at 20 C for up to one week. *Phytophthora* species were recovered from plates, and isolates were stored on CMA slants at 15 C. Currently isolates are being identified and characterized.

Results and Discussion: *Phytophthora* spp. have been detected at 19 of the 22 nurseries sampled (86%). These pathogens have been isolated from 10% of the bulk container mix samples, 24% of the field soil samples, and 52% of the samples from container-grown plants. Plant species with the highest incidence of *Phytophthora* spp. are: *Pieris japonica* (67%), azalea spp. (61%), *Ilex* spp. (58%), *Buxus* spp. (33%), and *Juniperus* spp. (30%). Based on tentative identification, *P. cinnamomi* and *P. nicotianae* have been the species most frequently encountered.

These data indicate that container mix, field soil, and container-grown plants all are potential sources of inoculum. The highest incidence of *Phytophthora* spp. was on the nursery stock itself. Therefore, many nurseries have the possibility of an outbreak of a *Phytophthora* disease if conducive environmental conditions occur and appropriate management practices are not initiated. It is likely that infected plants are coming into nurseries and also are being transported to landscapes where they can serve as a source of inoculum for future plantings.

Significance to Industry: The distribution of *Phytophthora* spp. and the relative importance of diseases caused by these fungi in South Carolina nurseries have not been determined previously. Detection from containerized plants prior to onset of disease symptoms can be beneficial for control and reduction of economic loss. Our data suggest that a relatively high percentage of ornamental crops at commercial nurseries are infested with *Phytophthora* spp. even though symptoms are absent. By identifying the source of inoculum, nurseries will be able to apply control measures at the most effective place in the disease cycle—the source. Knowledge from this study should assist in reducing disease occurrence, avoiding epidemics, and preventing spread to landscapes where a nursery's reputation is at stake and further economic losses can result. Most importantly, identification and characterization of *Phytophthora* spp. occurring in South Carolina nurseries will aid in developing an integrated disease management strategy that will be effective and reliable in years to come.

Literature Cited:

1. Benson, D. M. 1987. Occurrence of *Phytophthora cinnamomi* on roots of azalea treated with preinoculation and postinoculation applications of metalaxyl. *Plant Disease* 71(9):818-820.
2. Erwin, D. C., and O. K. Ribeiro. 1996. *Phytophthora Diseases Worldwide*. APS Press, The American Phytopathological Society. St. Paul, MN. 562 pp.
3. Ferguson, A. J., and S. N. Jeffers. 1997. Detection of *Phytophthora* species in container mixes from ornamental crop nurseries. (Abst.) *Phytopathology* 87(Supplement): S29.
4. Jones, R. K., and R. C. Lambe, eds. 1982. *Diseases of Woody Ornamental Plants and their Control in Nurseries*. NC Agricultural Extension Service, Raleigh, NC 130pp.
5. Ribeiro, O. K., and R. G. Linderman. 1991. Chemical and biological control of *Phytophthora* species in woody plants. Pages 399-410, in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge, UK.

**Survival of Conidia of the Dogwood Anthracnose
Fungus Through the Digestive System of an Insect
(Student)**

**Bryan Hed, Mark T. Windham, and Jerome F. Grant
The University of Tennessee, Knoxville, TN 37901**

Nature of Work: Dogwood anthracnose, caused by the fungus *Discula destructiva* Redlin, is a lethal disease of flowering dogwood, *Cornus florida* L. (3, 5). The sticky conidial matrix of *D. destructiva* makes possible the transport of viable conidia on the external surfaces of insects, making insects potential vectors of this pathogen. Viable conidia of *D. destructiva* also can be carried internally by convergent lady beetles (CLBs), *Hippodamia convergens* Guerin-Meneville (1, 2). Using CLBs as a model insect, the objectives of this study were to determine if conidia of *D. destructiva* carried internally by the beetles can be discharged in viable condition in frass, and if so, for how long.

Two groups of 30 CLBs were exposed to sporulating cultures of *D. destructiva* for 1 h. Beetles were surface-disinfested to ensure that their frass would not be contaminated by conidia carried externally. After disinfestation, 17 survivors of one group were provided tobacco aphids, *Myzus nicotianae* Blackman, as food for the duration of the experiment. Twenty-four survivors of the other group were provided pieces of 'Red Delicious' apple, as food (0.125 cm³/beetle). Beetles were transferred to clean petri dishes after 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 96, and 120 h, and frass pellets were collected in each interval. Pellets were dissolved in 1 ml of sterile water and spread on petri plates of potato dextrose agar amended with chlortetracycline hydrochloride and streptomycin sulfate (PDA+). *Discula*-like colonies from the frass pellets were transferred to petri dishes of PDA+ amended with autoclaved leaves of *C. florida*. Colony, conidial, and fruiting body characteristics were used to confirm the identity of *D. destructiva* isolates. The percentage of beetles carrying viable conidia at each time interval was calculated and described by a three parameter, single exponential decay equation (SAS Institute Inc., Cary, NC).

Results and Discussion: Of the CLBs fed aphids, *D. destructiva* was isolated from frass of all intervals into the 36-48 h interval and from 59% (10/17) of the CLBs. *Discula destructiva* was isolated from 13% (37/280) of the pellets collected. More than 70% (26/37) of the pellets from which *D. destructiva* was isolated were discharged in the first 12 h. Ninety-two percent and more than 97% of the pellets from which *D. destructiva* was isolated had been discharged after 24 and 36 h, respectively. An expo-

nential decay equation was generated where Y = percent CLBs carrying viable conidia internally and X = the time interval. For the aphid diet, $Y = -0.0452 + 0.716e^{-0.0466X}$, $R^2 = 0.96$ (Figure 1).

Of the CLBs fed apple, *D. destructiva* was isolated from frass of all intervals and from 87.5% (21/24) of the CLBs. *Discula destructiva* was isolated from 39% (106/275) of the pellets collected. Only 36% (38/106) of the pellets from which *D. destructiva* was isolated were discharged in the first 12 h. Sixty-four percent, 81%, 90%, and 97% of the pellets from which *D. destructiva* was isolated had been discharged after 24, 48, 72, and 96 h, respectively. For the apple diet, $Y = 0.0897 + 0.850e^{-0.0331X}$, $R^2 = 0.95$ (Figure 1).

The majority of lady beetles exposed to *D. destructiva* ingested and discharged viable conidia in their frass. A small percentage of beetles carried viable conidia for as many as 4 days. The sticky and fluid conidial matrix of *D. destructiva* may enhance the ingestion of conidia in the event of casual contact with insects and protect conidia (4) from the rigors of digestion.

Significance to Industry: Insects may play an important role in the spread of dogwood anthracnose. Future recommendations for controlling the spread of this disease will have to take management of insect populations into consideration.

Literature Cited:

1. Colby, D. M., Windham, M. T., and Grant, J. F. 1995. *Hippodamia convergens* (Coleoptera: Coccinellidae) dissemination of dogwood anthracnose fungus (Melanconiales: Melanconeaceae). *Environ. Entomol.* 24:1075-1079.
2. Colby, D. M., Windham, M. T., and Grant, J. F. 1996. Transportation and viability of conidia of *Discula destructiva* on *Hippodamia convergens*. *Plant Dis.* 80:804-805.
3. Hibben, C. R., and Daughtrey, M. L. 1988. Dogwood anthracnose in northeastern United States. *Plant Dis.* 72:199-203.
4. Nicholson, R. L., and Moraes, W. B. C. 1980. Survival of *Colletotrichum graminicola*: importance of the spore matrix. *Phytopathology* 70:255-261.
5. Redlin, S. C. 1991. *Discula destructiva* sp. nov. cause of dogwood anthracnose. *Mycologia* 83:633-642.

**A Viral Disease of *Chionanthus Virginicus*
Associated with Tomato Ringspot Virus
(Poster)**

**A. S. Windham, M. T. Windham, W. T. Witte, P. Flanagan
and H. P. Conlon**

**University of Tennessee, Entomology and Plant Pathology,
Ornamental Horticulture and Landscape Design,
Knoxville, TN 37901**

Nature of Work: Fringetree, *Chionanthus virginicus*, is one of the best native shrubs for landscape plantings. It has relatively few pest problems. The objective of this work was to determine the causal agent of virus-like symptoms on fringe tree seedlings.

Two hundred and seventy *C. virginicus* seedlings in their second year of growth were brought into a greenhouse for forcing on 25 February, 12 March and 27 March 1998. One hundred and six seedlings were left in an overwintering house and moved outside approximately the first of May. Plants in the greenhouse were fertilized weekly with Peter's 20-20-20 at about 200 ppm N. Those moved outside from the overwintering house were not fertilized. Plants forced in the greenhouse were grown at a day/night cycle of 75°/60-65° F with ambient photoperiod. Symptoms associated with virus diseases were observed on seedlings in May and leaf samples from affected plants were collected and sent to Agdia, Elkhart, Indiana for an ornamental virus screen.

Results and Discussion: Seedlings that appeared to have a viral infection had one or more of the following symptoms: stunting, mosaic, and strap-shaped leaves. Plants that were forced in the greenhouse expressed virus symptoms at a greater frequency than plants that were simply moved out of the overwintering house in early-May. Frequency of plants showing virus-like symptoms for plants forced on 25 February, 12 March, 27 March was 21.1, 25.6, and 17.8 %, respectively. The number of plants outside showing symptoms was 7.5%. It is possible that environmental and cultural conditions of the plants forced in the greenhouse were more favorable for symptom expression than for plants that were simply moved from the overwintering house in early May. Agdia screened symptomatic leaf tissue for the following viruses: alfalfa mosaic virus, cucumber mosaic virus, impatiens necrotic spot virus, potato virus X, prunus necrotic ringspot virus, tobacco mosaic virus - c, tobacco ringspot virus, tobacco streak virus, tomato aspermy virus, tomato spotted wilt virus, arabis mosaic virus, tomato ringspot virus and potato virus Y. Tomato ringspot virus (ToRSV) was the only plant virus detected in symptomatic leaf tissue. As ToRSV is seed transmitted, leaves were

collected from the two, female trees that were the seed source for this experiment. ToRSV was not detected in these samples. It's possible that high air temperature (>90° F) for two weeks prior to testing may have caused a drop in virus titer in the source trees to undetectable levels (Brad Reddick, personal communication). ToRSV has been associated with viral diseases of other woody plants such as florist's hydrangea (1) and euonymus (2).

Significance to Industry: Although seed-transmitted virus diseases in woody ornamentals are uncommon, they may be quite damaging. Viral symptoms may be mistaken for nutritional deficiencies. Plants with virus-like symptoms may be screened for common ornamental plant viruses through plant diagnostic laboratories located at land grant universities.

Literature Cited:

1. Brierley, P. 1954. Symptoms in the florist's hydrangea caused by tomato ringspot virus and an unidentified sap-transmissible virus. *Phytopathology* 44:696-699.
2. Puffinberger, C. W. and Corbett, M. K. 1985. *Euonymus chlorotic ringspot disease caused by tomato ringspot virus*. *Phytopathology* 75:423-428.

**Chemical Control of Powdery Mildew in
Flowering Dogwood**
(Poster)

M.T. Windham, A.S. Windham, and M.A. Halcomb
University of Tennessee, Dept. of Entomology and Plant Pathology,
Knoxville, TN 37901

Nature of Work: An evaluation of fungicides for control of powdery mildew, *Microsphaera pulchra* Cook and Peck, on flowering dogwood (*Cornus florida* L.) was conducted on two year old white dogwoods trees located at a nursery in Warren County, TN. Five fungicides, Heritage, Rubigan, Cleary's 3336, Systhane 40 WP, and Immunex, were applied at labeled rates with the exception of Heritage (24 lb/100 gal - 10X rate) to the foliage of trees assigned to each treatment. Fungicide treatments and an unsprayed control were arranged in a randomized complete block design with six replications. Each replication consisted of eight trees. Chemicals were applied to the foliage at approximately two week intervals from 4 June through 18 August, 1997.

Data were collected for disease severity of powdery mildew, amount of new growth during the growing season, and the amount of leaf scorch apparent on the trees in late summer. Powdery mildew disease severity was estimated on 4 and 19 June and on 24 July using the following scale: 0 = healthy foliage, 1 = <2%, 2 = <10%, 3 = <25%, 4 = <50%, 5 = >50% diseased foliage. New growth was estimated for each tree using the following scale: 0 = no new growth, 1 = <5%, 2 = <10%, 3 = < 25%, 4 = <50%, 5 = > 50% of branches having new growth. To evaluate the amount of leaf scorch the following scale was used: 0 = no scorched leaves, 1 = <2%, 2 = < 10%, 3 = <25%, 4 = <50%, 5 = >50% of the foliage with scorched leaves. Data for each variable were analyzed using Proc ANOVA and means were separated using Duncan's New Multiple Range Test ($p < 0.05$).

Results and Discussion: Low levels of powdery mildew occurred in all plots when the initial fungicide applications were made (Table 1). Although all fungicide treatments significantly reduced the level of powdery mildew disease severity, some fungicides such as Cleary's 3336, Systhane 40 WP and Immunex were significantly more effective in reducing disease severity than Rubigan or Heritage.

All fungicide treatments improved the level of new growth when compared to the controls (Table 1). However, the fungicide treatments that yielded the highest levels of new growth were not necessarily the treatments that had most significantly reduced disease severity.

Trees that were used as controls displayed symptoms of leaf scorch that was similar in appearance to that expected when trees are under extreme drought conditions (Table 1). Scorch symptoms were so severe, that powdery mildew ratings could not be conducted by 19 August. Trees sprayed with fungicides, regardless of compound, had significantly less scorch symptoms than did trees used as controls.

Significance to Industry: Fungicide treatments for control of powdery mildew are highly effective in reducing the impact of this disease on flowering dogwood production. The use of fungicides at labeled rates on a two week spray interval also enhanced the number of new shoots on the trees and reduced scorch symptoms found on trees that served as controls.

Table 1. Effects of fungicide treatments on disease severity of powdery mildew, amount of new growth and the level of scorch symptoms on flowering dogwood.

<u>Treatment</u>	<u>Powdery Mildew Ratings</u>			<u>New</u>	<u>Scorch</u>
	4 June ¹	19 June	24 July	<u>Growth</u> 24 July	19 Aug.
Control	1.7 a ²	3.6 a	5.0 a	1.6 a	4.5 a
Cleary's 3336	1.7 a	1.8 cd	2.3 d	3.8 c	1.6 c
Heritage	1.9 a	2.5 bc	3.8 c	3.2 bc	2.0 bc
Immunex	1.9 a	1.4 d	2.1 d	3.8 c	1.6 c
Rubigan	1.9 a	2.3 c	3.6 c	3.6 c	3.2 b
Systhane 40 WP	1.7 a	1.5 d	1.8 d	2.1 ab	1.7 bc

¹ Disease ratings on 4 June indicate the level of disease at the first chemical application.

² For each column, means followed by the same letter do not differ according to Duncan's New Multiple Range Test ($p \leq 0.05$).

**Winter Survival and Source of Primary Inoculum for
Powdery Mildew of Dogwood**
(Poster)

Margaret T. Mmbaga and Hongyan Sheng
Tennessee State University
Nursery Crop Research Station, McMinnville, TN 37110

Nature of Work: The natural occurrence of cleistothecia of *Microsphaera pulchra* in powdery mildew infected dogwoods (Mmbaga, 1997, Leigh *et al.* 1998) suggest that they may play an important role in the perpetuation of this disease. However, previous reports show that powdery mildew fungi often survive in the form of mycelia in dormant buds (Jones 1982), and that cleistothecia of some powdery mildew fungi become degenerate during winter and are of minor or no importance in the disease cycle. There are no previous studies to determine either the mode of survival of the dogwood powdery mildew pathogen, or the primary source of infection in spring. A study to assess the role of cleistothecia and dormant buds in the winter survival of powdery mildew in dogwood was initiated in 1996. Initial results showed that cleistothecia, and not mycelia, is the major mechanism of winter survival and source of primary inoculum for dogwood powdery mildew in Tennessee (Mmbaga 1997). This present report is a confirmation of the initial results.

(1) Cleistothecia formation and winter survival.

The development of cleistothecia initials on dogwood was monitored and at the end of season, leaves that carried cleistothecia were cut into small pieces and placed in polyethylene mesh-bags. These bags were then placed at different over-wintering locations: (a) outdoors on the ground, exposed to all conditions that leaf debris normally experience, (b) outdoors hanging on a shrub, (c) outdoors in a zip-lock on the ground, protected from moisture and microbial activities, and an indoor location in a non-heated area protected from extreme temperatures, direct moisture and microbial activity.

At 7 days interval starting mid March to mid June 1998, cleistothecia were harvested. Their state of development was evaluated and their viability was assessed using the method described by Pearson and Gadoury (1987). The ability of the overwintered cleistothecia to cause infection was tested on disease-free seedlings grown under growth chamber conditions of 20/28°C and 85% relative humidity.

(2) Assessment of ascospores in the air.

Sticky slides used as spore traps were placed underneath dogwood trees in the landscape, in the forest undergrowth, and in a dogwood container yard. These spore traps were used to sample the air for periods of 7 days, starting mid March to mid June, the same periods used to study cleistothecia on leaf debris. The spores trapped on sticky slides were compared with those harvested from leaf debris.

(3) Assessment of initial infection from dormant buds.

In 1997, one-year old seedlings from *C. florida* 'Cherokee Princess' were severely infected with powdery mildew. Plants were observed for the presence of cleistothecia, and were over-wintered outdoors covered with a commercial winter fabric. On March 1st 1998, plants were placed at: (i) outside exposed to air-borne inoculum, (ii) in growth chambers sheltered from air-borne spores, and (iii) in green house conditions protected from air-borne spores. At each location plants were separated into two groups, one treated with fungicide, the other was untreated. A systemic Banner (Ciba Geigy Corporation Greensboro, NC) was used according to label recommended rate of 3fl oz per 100gal water, sprayed to run-off. Plants in growth chambers were maintained at 28/20° C day/night temperatures and 85% relative humidity; and in the greenhouses they were maintained at 28/20° C and 70% relative humidity. Development of disease symptoms was monitored daily. The experiment was terminated in mid June during which disease outbreaks were widespread in the local area.

Results and Discussion: Cleistothecia development and winter survival. In 1997, cleistothecia initials were first observed in late October/ early November, about a month later than the previous year. Young and older leaves developed ascocarps and the density varied in different locations and cultivars. Cleistothecia was more abundant in the severely infected cultivars like Pygmy, Ruth Ellen and Cherokee Princess; the moderately resistant cultivar Cherokee Brave had relatively few cleistothecia. Cleistothecia was abundant in some locations and absent at some landscape locations. Since the presence of two mating types is required for the formation of cleistothecia, the presence or absence of cleistothecia may indicate the relative abundance of the two mating types. All cleistothecia observed were of *Microsphaera pulchra* and confirms our previous report that *M. pulchra* is the main pathogen that causes powdery mildew in dogwood (Mmbaga, 1997). Most of the cleistothecia were mature and had well developed ascospores before leaf abscission at the end of season and no cleistothecia were observed early in the season.

Leaf debris placed at different winter conditions showed similar trends of cleistothecia development and numbers after winter (Figs. 1-4). The number of immature ascocarps decreased steadily over time, while the number of mature cleistothecia that had not opened remained steady through early May and then started to decline. Cleistothecia that had opened were empty and suggest that they had liberated their ascospores, their number increased over time and the highest number was in May and early June. The first disease symptoms were observed in early May, about four weeks after full bloom (Fig. 5).

Evidence that cleistothecia on leaf debris survived winter and remained viable the following spring is corroborated by their ability to reproduce powdery mildew infection on disease-free plants (Table 1). There was no infection from leaf debris that overwintered indoors in a non-heated area, and the viability of the ascocarps at this treatment was also the lowest. These observations suggest that we need to better understand the environmental conditions that favor the release of inoculum from leaf debris. Such information will allow the prediction of inoculum release for initial infection using meteorological data and thus better time the application of fungicides.

Table 1. The winter survival of cleistothecia as measured by their viability and ability to incite powdery mildew infection on disease-free plants.

Location	Mean percentage of cleistothecia open and empty	viable+	non viable	Infection reproduced ¹
Outdoors on the ground	25.3 b	38.6 ab	36.1	2/4
Outdoors protected*	16.7 b	43.5 a	39.8 a	2/4
Outdoors hanging	24.9 b	36.6 b	38.4 a	0
Indoors unheated	62.7 a	11.2 c	26.1 b	1/4

+Viable cleistothecia had ascospores, *protected with a zip-lock bag, ¹ Number of plants that got infected from leaf debris over total number inoculated. Numbers in a column followed by same letters are statistically similar at 5% level.

Ascospores in the air.

Highest numbers of ascospores trapped from the air was in April and the spores were similar to those of *M. Pulchra* harvested from leaf debris. The appearance of initial disease symptoms in early May suggest that infection may have originated from the ascospores (Fig. 6). The numbers of air-borne ascospores and mature asci trapped from the different

locations reflect the relative abundance of cleistothecia and primary inoculum for initial infection (Fig. 7). These results clearly show that cleistothecia on leaf debris release ascospores in spring and constitute an important source of primary inoculum. While infection from ascospores continue through May, the production of conidiospores that cause subsequent infections start early in the season and may help in the rapid spread of infection. Early intervention of powdery mildew infection is therefore critical to a successful and economic control strategy.

Dormant buds as a source of powdery mildew initial infection.

All plants that were maintained under growth chamber and greenhouse conditions protected from outside source of infection other than the dormant bud, did not develop powdery mildew under conditions that normally favor powdery mildew infections. All the plants maintained outside, developed disease symptoms (Table 2). If the dormant buds were an important source infection, the non treated plants in growth chamber and greenhouse would have developed infection, but this did not happen. These observations confirm our previous results that the dormant buds do not constitute an important source of initial infection for powdery mildew of dogwood.

Table 2. The dormant bud as the source of initial infection for dogwood powdery mildew.

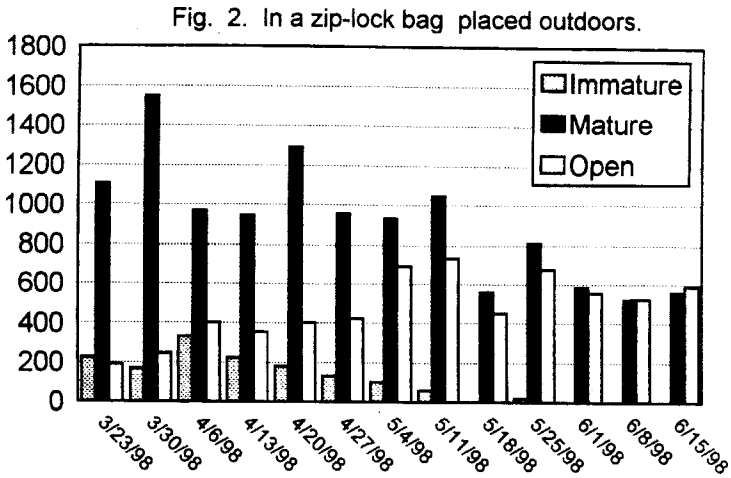
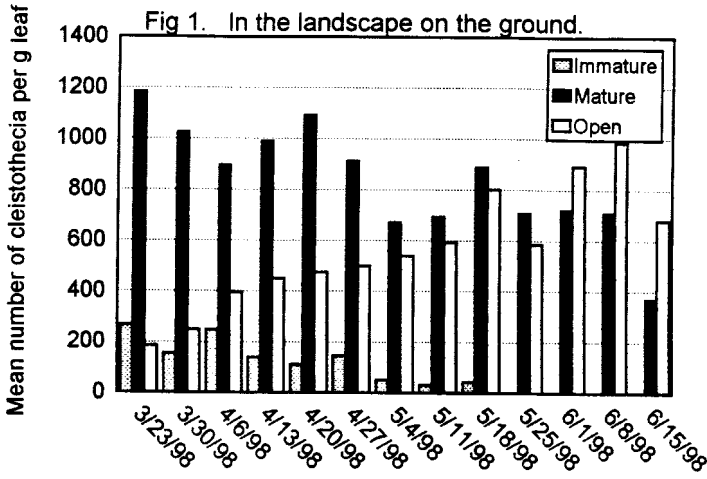
Location	Number of plants per location	Number of plants that developed powdery mildew symptoms	
		Fungicide treated*	Non treated
Growth chamber	16	0	0
Greenhouse	40	0	0
Outside	16	8	8

*A systemic fungicide 'Banner' was applied to eliminate mycelia in dormant bud

Significance to the Industry: These studies show that leaf debris from infected plants rather than the dormant buds constitute the main source of initial infection for powdery mildew of dogwood. Measures that eliminate or reduce cleistothecia density or the overwintered leaf debris, will reduce primary inoculum and thus reduce initial infection. Since infection starts very early in the season, fungicide applications should start early in spring and should aim to eliminate any established infections and protect the foliage from new infection. Such strategy would reduce the amount of initial infection, reduce the amount of secondary inoculum, and thus reduce the rate of disease progress. This would improve powdery mildew control and possibly reduce the total amount of fungicide used.

Literature Cited:

1. Jones R.K. 1982. Powdery mildew. *In* R.K. Jones, (ed). Diseases of woody ornamental plants and their control in nurseries pg 24-25. North Carolina Agric. Ext. Serv., Raleigh, NC. 130p.
2. Leigh, Ann Klein, Mark T. Widham, and Robert N. Trigiano. 1998. Natural occurrence of *Microsphaera pulchra* and *Phyllactinia gutata* on two *Cornus* species. *Plant Dis.* 82(4): 383-385.
3. Mmbaga, Margaret T. 1997. Over-winter survival of powdery mildew pathogen of dogwood (*Cornus* spp.). *Proceed. Southern Nurserymen's Association Research Conference.* 42:505-511.



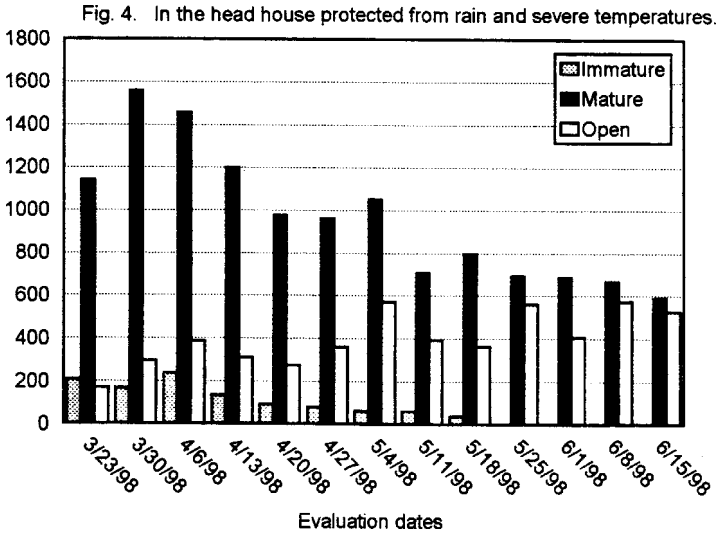
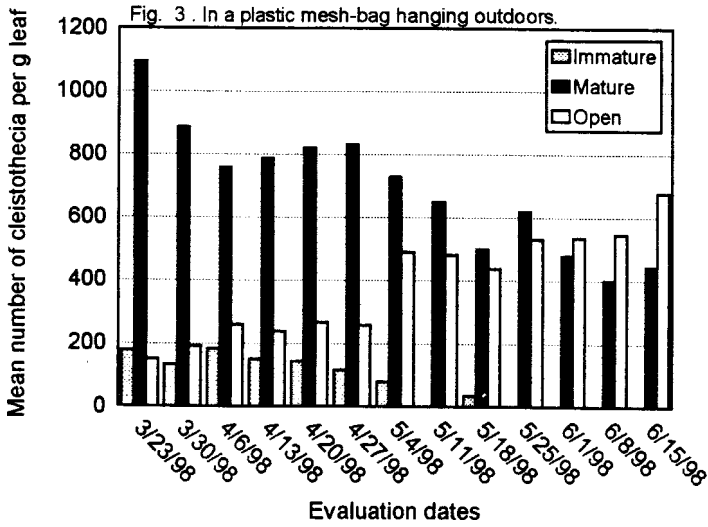
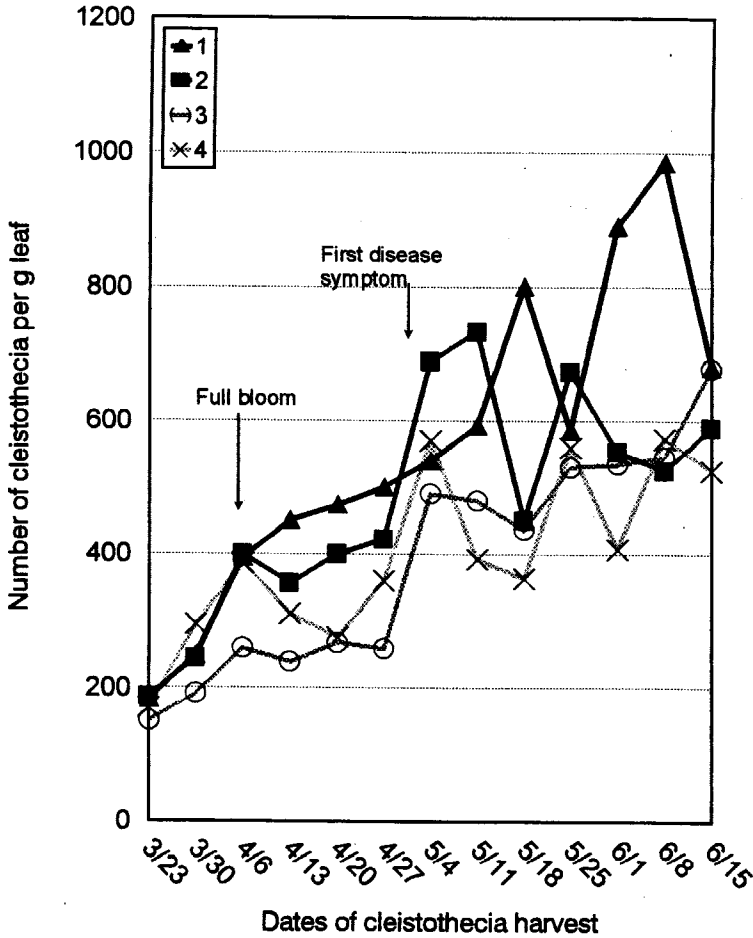


Fig.5. Number of cleistothecia that opened and released ascospore in spring 1998 after winter survival on leaf debris at different conditions.



Leaf debris in mesh bag placed (1) Outdoors on the ground; (2) Outdoors in zip-lock bag on the ground;; (3) Outdoors hanging on a plant; (4) Indoors non-heated.

Figs.6-7. Mean number and type of spores trapped from the air around dogwood foliage shown by dates and by location .

