

Effect of Water Management and Soil Application of Nitrogen Fertilizers, Petroleum Oils, and Lime on Inoculum Production by *Mycosphaerella citri*, the Cause of Citrus Greasy Spot

SACHINDRA N. MONDAL¹, KELLY T. MORGAN², AND L.W. (PETE) TIMMER^{1*}

¹University of Florida, IFAS, Plant Pathology Department, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

²University of Florida, IFAS, Soil and Water Science Department, Southwest Florida Research and Education Center, 2686 SR 29 N, Immokalee, FL 34142-9515

ADDITIONAL INDEX WORDS. *Mycosphaerella citri*, grapefruit, irrigation, dolomite

Greasy spot, caused by *Mycosphaerella citri*, produces leaf spots and defoliation of citrus trees, which reduces tree vigor and yield. The fungus produces airborne ascospores from pseudothecia in decomposing leaf litter on the grove floor. Factors affecting production of inoculum on decomposing leaves were evaluated. Pseudothecial formation and ascospore production increased as greasy spot severity on the leaves increased. Applications of urea, dolomite, or increased irrigation frequency on leaf litter reduced inoculum production by 70% to 90%. Of the N fertilizer materials evaluated, urea and ammonium sulfate were very effective in reducing inoculum, whereas nitrate fertilizers were ineffective. The effects of ammonium fertilizer are thought to be due to the toxicity of ammonia gas to the fungus. Soil surface applications of petroleum oils also reduced inoculum, but had to be applied in large volumes of water for maximum effectiveness. These measures may provide practical methods of lowering inoculum levels of *M. citri* and may reduce fungicide applications to foliage. However, inoculum levels must be reduced greatly to delay symptom development and reduce disease severity.

Greasy spot, caused by *Mycosphaerella citri* Whiteside, produces lesions on mature citrus leaves that result in premature defoliation of the tree (Timmer and Gottwald, 2000). Excessive leaf loss debilitates trees and reduces vigor and fruit yield (Mondal and Timmer, 2006a; Timmer and Gottwald 2000; Whiteside, 1977). In addition to foliar symptoms, *M. citri* infects stomates on fruit producing necrotic lesions that reduce the acceptability of fruit for the fresh market (Mondal and Timmer, 2005; Timmer and Gottwald, 2000). Greasy spot is a significant problem on citrus in Florida and the Caribbean Basin (Mondal and Timmer, 2006a) requiring from one to three applications of fungicide for adequate control (Timmer and Chung, 2007) on all commercial citrus acreage.

Ascospores of *M. citri* are produced in pseudothecia in decomposing leaf litter on the grove floor (Mondal and Timmer, 2006a; Whiteside, 1972a, 1974, 1976). Ascospores are airborne and are deposited on the abaxial side of the leaf, where they germinate and the fungus grows epiphytically (Mondal and Timmer, 2003; Mondal et al., 2003). After several weeks, *M. citri* penetrates the stomates and invades the mesophyll. Symptoms do not usually develop until 3 to 4 months after infection. In Florida, most of the infection occurs during the summer rainy season, the symptoms appear from November to January, and most of the defoliation follows in February to March.

Changes in the Florida citrus industry have affected the seriousness of the greasy spot problem. Peak ascospore release now occurs at a drier time of the year in April and May (Mondal

et al., 2003; Timmer and Gottwald, 2000; Timmer et al., 2000) rather than during the rainy season in June and July as observed in earlier years (Whiteside, 1972a, 1976), which tended to reduce disease severity. However, after the freezes of the 1980s, the industry moved southward where temperatures are warmer and more favorable for greasy spot. Currently, herbicides, rather than tillage, are used for weed control. Thus, in the past, cultivation buried a portion of the inoculum and weed growth interfered with ascospore dispersal. Groves are now irrigated largely by microsprinklers, rather than by overhead irrigation. When rainfall is low and evapotranspirational demands are high, groves are irrigated three times weekly with microsprinklers, whereas previously they were watered only every 2 weeks by overhead sprinkler irrigation. Frequent wetting of the leaf litter accelerates the production of pseudothecia and ascospores (Mondal and Timmer, 2002). As a result, under present grove practices, leaves fall on a uniform flat surface under the tree that is not cultivated, leaves are moistened frequently, favoring maximal production of pseudothecia, and there are no weeds to interfere with ascospore dispersal to the tree canopy.

Reduction of ascospore inoculum by treatment of leaf litter has been used successfully in the control of apple scab caused by *Venturia inaequalis* (Cke.) Winter. Treatment of leaf litter with urea and other nitrogenous fertilizers, application of lime, or shredding of the litter have substantially reduced inoculum production and disease severity the following season (Burchill, 1968; MacHardy and Lord, 2000; Miller and Rich, 1968; Spotts et al., 1997). Treatment of leaf litter with chemicals such as phenylmercuric chloride (Burchell and Hutton, 1965) and ap-

*Corresponding author; email: lwtimmer@ufl.edu; phone: (863) 956-1151.

plication of fungal antagonists (Carisse et al., 2000) have also been successful in reducing ascospore inoculum.

Benomyl treatment of decomposing leaves reduced ascospore production, but copper fungicides, captafol, or chlorothalonil did not (Whiteside, 1973). In small plot studies, we found that urea or lime applied to leaf litter reduced pseudothecia production and ascospore release from decomposing leaf litter (Mondal and Timmer, 2003).

The purpose of this study was to evaluate the effect of greasy spot severity on the production of inoculum of *M. citri* and to examine more closely the effects of urea, dolomite, and extra irrigations on pseudothecial production in the field and their mode of action. We also evaluated the effects of other nitrogen fertilizers and petroleum oil applications on the production of pseudothecia.

Materials and Methods

GENERAL METHODS. For all microplot experiments, field-collected mature grapefruit leaves affected by greasy spot were used in the experiments. Prior to initiation of the experiments, leaves were placed on the soil surface and allowed to air dry in the microplots consisting of 50-cm × 50-cm square frames constructed from PVC pipe and fitted with plastic mesh with 2-cm × 2-cm openings to contain the leaves within the frame. About 200 dried leaves were placed in each microplot and treatments were usually initiated 2 to 3 weeks after placement of the leaves. All microplots were located on the floor of a grapefruit grove near Lake Alfred, FL, and were subjected to normal rainfall and grove irrigation. Pseudothecial density was rated on a scale of 0 to 5 where 0 = none; 1 = 1% to 20%; 2 = 21% to 40%; 3 = 41% to 60%; 4 = 61% to 80%; and 5 = >80% of the leaf area was covered with pseudothecia.

Treatments in each experiment were arranged in a completely randomized design. As appropriate, data were subjected to analysis of variance or linear regression analysis using SAS Version 9.0 (SAS Institute, Cary, NC). Means were separated using the Waller-Duncan *k* ratio *t* test, $P \leq 0.05$.

EFFECT OF GREASY SPOT SEVERITY ON PSEUDOTHECIAL PRODUCTION. Greasy spot-affected leaves were collected in February in 2001 and 2002. The leaves were grouped into five severity classes based on area covered by the disease and placed in the microplots. The severity classes were: 0 = no symptoms; 1 = 1% to 5% of the leaf area covered with greasy spot; 2 = 6% to 10%; 3 = 11% to 15%; 4 = 16% to 20%; and 5 = >20%. Each treatment was replicated three times in each year. The leaves were examined periodically and rated for pseudothecial density when more than 50% of the pseudothecia were mature. To measure ascospore production, pseudothecia were hydrated by soaking the leaves for about 30 min and then placed in a wind tunnel apparatus designed by Whiteside (1973) and modified by Mondal and Timmer (2002). Ascospore production was expressed as the number of ascospores per leaf. Data from both years were combined since variances between years were homogeneous as determined by the two-tailed *F*-test.

EFFECT OF UREA AND LIME APPLICATIONS, AND EXTRA IRRIGATION ON LEAF DECOMPOSITION AND PSEUDOTHECIAL DENSITY. In this experiment, all treatments were applied to leaves that had fallen naturally in a mature grapefruit grove near Lake Alfred, FL, with severe greasy spot. Each plot consisted of three trees and treatments were replicated three times. Samples were collected from predesignated 3-m² areas under the center tree in each plot.

Treatments were 1) urea application, 2) dolomite application, 3) extra irrigation, 4) all of the treatments combined on the same plot, and 5) an untreated control receiving only rainfall and normal irrigations. Leaves were sampled to determine the state of decomposition and pseudothecial density before application of treatments on 22 Mar. 2002. Leaf litter in urea-treated plots was sprayed with a 5% solution of urea applied at the rate of 15 g·m⁻² three times at 30-d intervals beginning on 16 Mar. 2002. Dolomitic limestone was spread over the leaf litter in the corresponding plots at 250 g·m⁻² once in mid-April. Plots with extra irrigations were sprayed with water to wet the leaf litter using a handgun sprayer at 150 KPa of pressure on days when no irrigation was applied and there was no measurable rainfall. Thus, the leaf litter was saturated at least five times per week. On sampling dates 16 Apr., 3 May, 17 May, 30 May, and 25 June, 40 decomposing leaves were collected from each plot and leaf decomposition and pseudothecial density were assessed. Decomposition was rated using a scale of 0 = dead, not decomposed, leaf firm; 1 = not decomposed, flexible, still intact; 2 = leaf slightly decomposed, no loss of lamina; 3 = moderately decomposed, some loss of lamina; 4 = moderately decomposed, considerable loss of lamina; and 5 = highly decomposed, skeletonized leaves.

EFFECT OF NITROGEN FERTILIZERS ON PSEUDOTHECIAL DENSITY. Different types of nitrogen fertilizers were evaluated in a microplot experiment. Leaves were sprayed with solutions of calcium nitrate, potassium nitrate, ammonium nitrate, ammonium sulfate, or urea and those treatments compared to a water-sprayed control on 22 Apr. 2003. Each 0.25-m² plot was sprayed with 350 mL of the solutions or water. The amounts of each fertilizer were adjusted to give 10 g·m⁻² of actual N, i.e., 227 kg of N·ha⁻¹. Leaf samples were collected and rated for pseudothecial density in late May to early June.

EFFECT OF SPRAY VOLUME AND UREA RATE ON THE INHIBITION OF PSEUDOTHECIAL DEVELOPMENT. A factorial experiment was established in microplots using three rates of urea: 0, 10, and 20 g of actual N·m⁻² applied in three different volumes of water: 0, 0.5, or 1 L per 0.25 m². Where no water was applied, urea was spread dry on the surface of the plots. Control plots received no urea and no water. The experiment was replicated three times. Treatments were applied on 22 Mar. 2004 and the soil 2.5 cm below the leaf litter was collected for analysis of ammonium nitrogen (NH₄-N) and nitrate N (NO₃-N) at 1, 24, and 48 h after the applications. NH₄-N and NO₃-N were extracted using 2 N KCl. Extracts were sent to the Waters Agricultural Laboratories, Inc. in Camilla, GA, for determinations of ammonium and nitrate nitrogen. The leaf litter was moistened with a hand-pressurized sprayer twice a week in addition to normal irrigation and rainfall to speed pseudothecial development. Pseudothecial density was determined at the end of May.

EFFECT OF PETROLEUM OIL APPLICATION ON PSEUDOTHECIAL DENSITY. A factorial experiment was established in microplots using petroleum oil concentrations of 0%, 5%, 10%, 30%, and 50% in spray volumes of 25, 100, or 250 mL·m⁻². Control plots received no oil, but were sprayed with 250 mL of water per square meter. Petroleum oil sprays were applied with a hand-pump sprayer on 12 Sept. 2005 and each treatment was replicated twice. The petroleum oil was provided by Petro-Canada Lubricants, Mississauga, ON, Canada, and was a proprietary formulation designed for this use. Leaves were wetted with water using a hand-pressurized sprayer every other day throughout the course of the experiment. Pseudothecial density was evaluated 3 months after the initiation of the experiment.

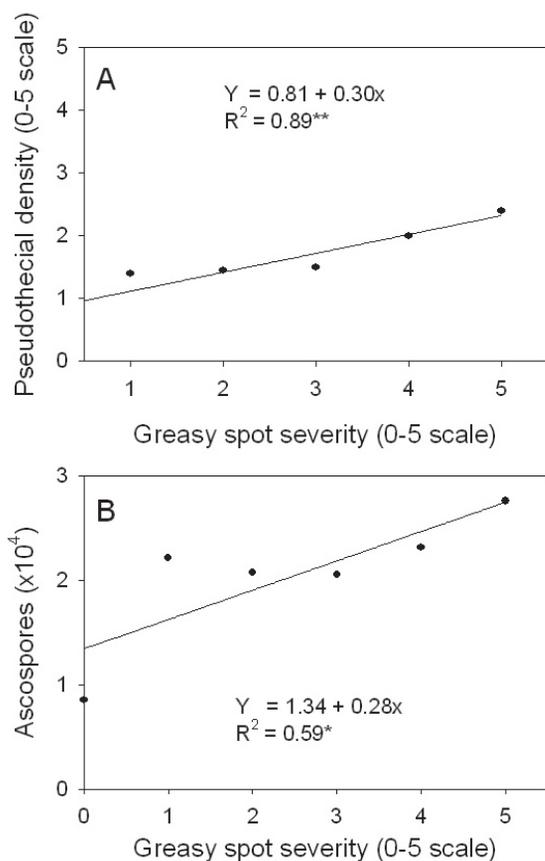


Fig. 1. Effect of greasy spot severity on the density of pseudothecia of *Mycosphaerella citri* on decomposing leaves and the production of ascospores from those leaves. R² values significant at P ≤ 0.05 (*) or P ≤ 0.01 (**).

Results

EFFECT OF GREASY SPOT SEVERITY. There was a significant linear relationship between greasy spot severity on the leaves at the time of collection and the pseudothecial density on decaying leaves 3 months later (Fig. 1A). The R² value for the combined data from both years was 0.89. There was also a linear relationship between severity and ascospore release for the combined data from both years (Fig. 1B). However, some pseudothecia and ascospores were produced on leaves that had no symptoms.

EFFECT OF UREA AND LIME APPLICATIONS, AND EXTRA IRRIGATION ON LEAF DECOMPOSITION AND PSEUDOTHECIAL DENSITY. In these field studies, application of extra irrigation accelerated the decomposition of the leaves compared to the non-treated control on the first two assay dates (Table 1). In contrast, application of urea significantly delayed decay compared to the control and to most other treatments on the first two dates of sampling. Dolomite did not affect decomposition of the leaves compared to the non-treated control on any assay date. The combination treatment showed results similar to the water treatment only possibly due to washing of urea and dolomite from the leaves. Subsequently, there were no differences among any of the treatments with regard to leaf decomposition. With regard to pseudothecial density, the water and the combination treatments reduced density on the first assay date when compared to the non-treated control, but the other treatments were not significantly different from the control. On subsequent assay dates, all treatments reduced density compared to the control. There were few differences among the urea, dolomite, water, and combination treatments with respect to pseudothecial density. The combination treatment was no more effective than the individual ones applied separately, indicating no synergistic or additive effects of the treatments.

EFFECT OF NITROGEN FERTILIZERS ON PSEUDOTHECIAL DENSITY. The urea and ammonium sulfate treatments significantly reduced pseudothecial density compared to the control, but neither the calcium nor the potassium nitrate applications had any effect on pseudothecial density (Table 2). Pseudothecial density in the ammonium nitrate treatment was not significantly different from that in the nitrate treatments or from that in the urea or ammonium sulfate treatments.

EFFECT OF SPRAY VOLUME AND UREA RATE ON THE INHIBITION OF PSEUDOTHECIAL DEVELOPMENT. The concentration of NH₄-N in the soil beneath the leaf litter was affected significantly by the application of urea to the soil surface (Table 3). However, *t* tests indicated that there was no difference in the concentration of NH₄-N between the low and high rates of urea applied. The time of collection of the soil samples, 1, 24, or 48 h, for measurement of NH₄-N did not significantly affect the concentration of NH₄-N nor did the volume of water in which it was applied. Application of urea did not affect the concentration of NO₃-N in any treatment and the concentration in all treatments was less than 10 μg·kg⁻¹ (data not shown). Pseudothecial density was greatly reduced by application of urea at both rates. However, there was no significant affect of the water volume in which the urea was applied (Table 3).

Table 1. Effect of applications of urea, dolomitic lime, extra irrigation, and a combination treatment on the decay of leaf litter and the production of pseudothecia of *Mycosphaerella citri* under field conditions.

Treatment	16 Apr.		3 May		17 May		30 May		25 June	
	Decay ^z (0-5)	Pseudo ^y (0-5)	Decay (0-5)	Pseudo (0-5)	Decay (0-5)	Pseudo (0-5)	Decay (0-5)	Pseudo (0-5)	Decay (0-5)	Pseudo (0-5)
Control	0.7 b	0.9 a	1.4 c	1.6 a	2.6	1.6 a	3.8	1.7 a	4.7	2.4 a
Water	1.2 a	0.5 bc	2.4 b	0.7 b	2.8	0.5 c	3.8	0.4 cd	4.7	0.6 c
Urea	0.6 b	0.7 abc	0.9 d	0.7 b	2.2	0.5 c	3.5	0.7 bc	4.5	0.4 d
Dolomite	0.6 b	0.8 ab	1.1 cd	0.8 b	2.5	0.8 b	3.2	0.9 b	4.7	0.9 b
Combination ^x	1.2 a	0.4 c	2.9 a	0.6 b	2.9	0.5 c	3.9	0.3 d	4.7	0.4 d
					NS		NS		NS	

^zExtent of decay from 0 = none to 5 = completely disintegrated.

^yPseudo = pseudothecial density rated on a scale of 0 = none to 5 = 75% to 100% of the leaf surface covered.

^xAll of the above treatments applied to the same blocks.

^{ns}Nonsignificant.

Table 2. Effect of different nitrogenous fertilizers on the pseudothecial density of *Mycosphaerella citri* on decaying grapefruit leaves.

Fertilizer	Rate (g·m ⁻²) ^z	Pseudothecial density (0–5) ^y
Ca(NO ₃) ₂ ·4 H ₂ O	167	3.6 ab
KNO ₃	48	3.1 ab
NH ₄ NO ₃	29	2.3 bc
(NH ₄) ₂ SO ₄	63	1.0 c
Urea	22	1.0 c
Control	0	3.9 a

^zApproximately 10 g of actual N·m⁻² in all cases.

^yDensity of pseudothecia from 0 = none to 5 = 75% to 100% coverage.

Table 3. Effect of different amounts of urea applied in different volumes of water on the density of pseudothecia of *Mycosphaerella citri* and levels of ammonium N in the soil 1, 24, and 48 h after application.

Treatments N (g·m ⁻²)	Water (L·m ⁻²)	Ammonium N (µg·kg ⁻¹ soil)			Pseudothecial density (0–5) ^z
		1 h	24 h	48 h	
0	0	1.9	2.6	2.0	2.3
0	1	3.7	3.5	2.6	2.8
0	2	3.7	4.8	3.5	2.9
10	1	30.5	73.6	27.8	1.5
10	2	28.7	177.0	49.1	0.9
20	1	62.0	198.0	140.0	0.9
20	2	48.5	48.5	73.5	1.4

^zPseudothecial density from 0 = none to 5 = 75% to 100% coverage.

ANOVA—Ammonia N

Factor	df	Mean		
		square	F value	P < F
Time (T)	2	0.010	0.1	0.95
N rate (N)	2	17.091	78.1	<0.0001
Water volume (W)	1	0.036	0.2	0.69
N × W	1	1.312	6.0	0.006
T × N	2	0.011	0.1	0.99
T × W	4	0.008	0.04	0.96
T × N × W	2	0.003	0.01	0.99

ANOVA Pseudothecia				
N rate (N)	2	5.69	24.9	<0.0001
Water volume (W)	1	0.001	0.01	0.93
N × W	2	0.449	1.97	0.18

EFFECT OF PETROLEUM OIL APPLICATION ON PSEUDOTHECIAL DENSITY. The concentration of petroleum oil as well as the volume of water in which it was applied significantly affected the pseudothecial density on decomposing leaves (Table 4). The interaction of the two factors was nonsignificant at $P < 0.05$, but the results were significantly different in the two replications. Pseudothecial density was only reduced slightly when oil was applied at 25 mL·m⁻² compared to the non-treated control. The greatest reduction in pseudothecial density was observed at the highest concentration of oil applied in the greatest volume of water.

Discussion

In a previous study (Mondal and Timmer, 2003), we found that application of urea, CaCO₃, or dolomitic limestone to leaf litter reduced production of pseudothecia and ascospores of

Table 4. Effect of application of petroleum oil at different rates and spray volumes on the density of pseudothecia of *Mycosphaerella citri* on decaying leaf litter.

Water volume (mL·m ⁻²)	Pseudothecial density (0–5)				
	Petroleum oil rate (% v/v)				
	0	5	10	30	50
25	---	1.25	1.24	1.27	1.49
100	---	1.33	1.32	1.25	0.096
250	1.96	1.18	1.16	1.02	0.76

ANOVA				
Factor	df	Mean		
		square	F value	P < F
Volume (V)	2	0.335	12.25	0.0016
Oil rate (R)	3	0.131	4.78	0.0228
V × R	6	0.0596	2.18	0.1248
Block	1	0.5046	18.45	0.0013

--- Treatment not done.

Mycosphaerella citri. In the current study, we confirmed and extended those findings.

First, we demonstrated that leaves with the most severe greasy spot produced the most pseudothecia and ascospores. This may seem self-evident. However, Whiteside (1972b) found that pseudothecia often form in areas of the leaves that have no symptoms of greasy spot. Our observations confirm those of Whiteside, and we often observe clusters of pseudothecia outside of lesion areas. Thus, *M. citri* appears to colonize the decaying leaves and potentially could produce large numbers of pseudothecia on lightly affected leaves. In fact, in this study, many pseudothecia were produced on the old leaves that had no visible symptoms. However, detached leaves from the current season's growth did not form pseudothecia when exposed to the appropriate regime of wetting and drying (Mondal and Timmer, 2002; Mondal, unpublished). Thus, reduction of pseudothecial density can be important even on old leaves without symptoms that fall to the grove floor.

The ability of urea and lime applications to reduce pseudothecial density was confirmed in this study using relatively low rates of N. As in our previous study (Mondal and Timmer, 2003), urea application decreased pseudothecial density and reduced leaf decay. Previous studies on apple scab (Burchill and Hutton, 1965; Crosse et al., 1968; Miller and Rich, 1968) suggested that urea acted by promoting leaf decomposition. However, our results suggest that urea was directly toxic to the fungus. Urea readily breaks down to NH₄-N, which can then volatilize as ammonia (NH₃). Such NH₃ formed from ammonium fertilizers can even be toxic to plants (Schumann and Mills, 1996). Thus, we hypothesize that urea is toxic to pseudothecia due to formation of NH₃ gas. It is also probably toxic to other microorganisms involved in leaf decay. Our finding in this study, which indicates that nitrate fertilizers do not inhibit pseudothecial development, supports that conclusion. Doubling the volume of water in which the urea was applied did not seem to impair the effectiveness of the urea treatment. However, application of similar amounts of urea through an irrigation system might not be effective since the urea might be leached beyond the area where it would be active.

As demonstrated previously (Mondal and Timmer, 2003), dolomite also reduced pseudothecial density, but it was less effective than urea. Wetting of the leaf litter five times a week also proved effective in promoting decay and decreasing pseudothecial density. The combination treatment was probably no more effective

tive because urea tends to reduce decay whereas dolomite and extra irrigations tend to promote decomposition.

Ground application of petroleum spray oils also reduced pseudothecial density. However, it appears that the amounts of oil needed and the high volume in which they need to be applied would make use impractical. Apparently, the leaf litter needs to be thoroughly wetted with the oil suspension to be effective.

Thus, there appear to be multiple ways that cultural operations and applications of products or extra irrigations to leaf litter can be used to reduce greasy spot inoculum. Of the possibilities, petroleum oils, as pointed out above may prove impractical. Dolomite may also not be practical since multiple applications would be required and such frequency would impact soil pH and not be consistent with normal grove operations. However, it may be feasible to apply urea as soil surface applications with a herbicide sprayer. Since most of the leaf drop occurs in the spring, two appropriately timed sprays might be sufficient to greatly reduce inoculum. Previously, we showed that two applications of 40 g·m⁻² each to leaf litter reduced ascospore production by 97% compared to the untreated control. However, that amount would total about 400 kg·ha⁻¹, considering that only about 50% of the grove area was treated in these studies and this rate is nearly double the normal application rate of 225 kg·ha⁻¹. In the current study, we reduced rates to more practical levels and still achieved considerable reduction of inoculum. However, such sprays would require extra trips through the field, but it would supply most of the N requirement for fertilization.

Any treatment would have to greatly reduce ascospore production to be effective. In our studies, reducing ascospore concentration by 90% delayed symptom development and reduced severity (Mondal et al., 2006b). However, we think a reduction of 99% must be achieved to be highly effective. There are also complications in the method of application. Use of low volumes of water may result in volatilization of most of the N, which may not affect the ability of urea to reduce inoculum, but may reduce its value for fertilizer. On the other hand, application with more water may decrease its value for inoculum reduction, but increase its availability to the plant. Thus, more research will be required to optimize a functional system.

Another approach would be to increase irrigation frequency to promote leaf litter decomposition. This could be combined with shredding of the leaf litter. Shredding has not been evaluated for greasy spot control on citrus, but has proven effective for apple scab (MacHardy and Lord, 2000). Increasing irrigation frequency may not require greater volumes of water and could be highly effective, especially in combination with leaf shredding.

Although inoculum reduction may not be highly effective in the first season, with repeated uses, it may gradually reduce inoculum and disease pressure. Such an approach may not eliminate all fungicide sprays but could improve disease control.

Literature Cited

- Burchill, R.T. 1968. Field and laboratory studies on the effect of urea on ascospore production of *Venturia inaequalis* (Cke.) Wint. Ann. Appl. Biol. 62:297–307.
- Burchill, R.T. and K.E. Hutton. 1965. The suppression of ascospore production to facilitate the control of apple scab (*Venturia inaequalis* (Cke.) Wint). Ann. Appl. Biol. 56:285–292.
- Carisse, O., V. Pillion, D. Rolland, and J. Bernier. 2000. Effect of fall application of fungal antagonists on spring ascospore production of the apple scab pathogen, *Venturia inaequalis*. Phytopathology 90:31–37.
- Crosse, J.E., M.E. Constance, and R.T. Burchill. 1968. Changes in the microbial population of apple leaves associated with the inhibition of the perfect stage of *Venturia inaequalis* after urea treatment. Ann. Appl. Biol. 61:203–216.
- MacHardy, W.E. and W.G. Lord. 2000. Effects of shredding or treating apple leaf litter with urea on ascospore dose of *Venturia inaequalis* and disease buildup. Plant Dis. 84:1319–1326.
- Miller, P.M. and S. Rich. 1968. Reducing spring discharge of *Venturia inaequalis* ascospores by composting overwintering leaves. Plant Dis. Rep. 52:728–730.
- Mondal, S.N. and L.W. Timmer. 2002. Environmental factors affecting pseudothecial development and ascospore production of *Mycosphaerella citri*, the cause of citrus greasy spot. Phytopathology 92:1267–1275.
- Mondal, S.N. and L.W. Timmer. 2003a. The relationship of epiphytic growth of *Mycosphaerella citri* to greasy spot development on citrus and to disease control with fenbuconazole. Plant Dis. 87:186–192.
- Mondal, S.N. and L.W. Timmer. 2003b. The effect of urea, CaCO₃, and dolomite on pseudothecial development and ascospore production of *Mycosphaerella citri*, the cause of citrus greasy spot. Plant Dis. 87:478–483.
- Mondal, S.N., T.R. Gottwald, and L.W. Timmer. 2003. Environmental factors affecting the release and dispersal of ascospores of *Mycosphaerella citri*. Phytopathology 93:1031–1036.
- Mondal, S.N. and L.W. Timmer. 2005. Ascospore deposition and epiphytic growth in relation to fungicide timing for control of greasy spot rind blotch caused by *Mycosphaerella citri*. Plant Dis. 89:739–741.
- Mondal, S.N. and L.W. Timmer. 2006a. Greasy spot, a serious endemic problem for citrus production in the Caribbean Basin. Plant Dis. 90:532–538.
- Mondal, S.N. and L.W. Timmer. 2006b. Relationship of the severity of citrus greasy spot, caused by *Mycosphaerella citri*, to ascospore dose, epiphytic growth, leaf age, and fungicide timing. Plant Dis. 90:220–224.
- Schumann, A.W. and H.A. Mills. 1996. Injury of leatherleaf fern and tomato from volatilized ammonia after fertilization. J. Plant Nutr. 19:573–593.
- Spotts, R.A., L.A. Cervantes, and F.J.A. Niederholzer. 1997. Effect of dolomitic lime on production of asci and pseudothecia of *Venturia inaequalis* and *V. pirina*. Plant Dis. 81:96–98.
- Timmer, L.W. and T.R. Gottwald. 2000. Greasy spot and similar diseases, p. 25–28. In: L.W. Timmer, S.M. Garnsey, and J.H. Graham (eds.). Compendium of citrus diseases. APS Press, St. Paul, Minn.
- Timmer, L.W. and K.-R. Chung. 2007. Greasy spot, p. 81–83. In: M.E. Rogers and L.W. Timmer (eds.). 2007 Florida citrus pest management guide. Publ. No. SP-43. Univ. Fla., IFAS, Gainesville.
- Timmer, L.W., P.E. Roberts, H.M. Darhower, P.M. Bushong, E.W. Stover, T.L. Peever, and A.M. Ibáñez. 2000. Epidemiology and control of citrus greasy spot in different citrus-growing areas in Florida. Plant Dis. 84:1294–1298.
- Whiteside, J.O. 1972a. Etiology and epidemiology of citrus greasy spot. Phytopathology 60:1409–1414.
- Whiteside, J.O. 1972b. Histopathology of citrus greasy spot and identification of the causal fungus. Phytopathology 62:260–263.
- Whiteside, J.O. 1973. The possibilities of using ground sprays to control citrus greasy spot. Proc. Fla. State Hort. Soc. 86:19–23.
- Whiteside, J.O. 1974. Environmental factors affecting infection of citrus leaves by *Mycosphaerella citri*. Phytopathology 64:115–120.
- Whiteside, J.O. 1976. Epidemiology and control of greasy spot, melanose, and scab in Florida citrus groves. PANS 22:243–249.
- Whiteside, J.O. 1977. Behavior and control of greasy spot in Florida citrus groves. Proc. Intl. Soc. Citricult. 3:981–986.