Effects of Application of Fungicide During the Dormant Period on Phomopsis Cane and Leaf Spot of Grape Disease Intensity and Inoculum Production

M. Nita, M. A. Ellis, L. L. Wilson, and L. V. Madden, Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691

ABSTRACT

Nita, M., Ellis, M. A., Wilson, L. L., and Madden, L. V. 2006. Effects of application of fungicide during the dormant period on Phomopsis cane and leaf spot of grape disease intensity and inoculum production. Plant Dis. 90:1195-1200.

Efficacy of application of the fungicides calcium polysulfide or fixed copper during the dormant period on control of Phomopsis cane and leaf spot of grape (*Vitis* spp.), caused by *Phomopsis viticola*, was examined under field conditions during the 2003 and 2004 growing seasons in Ohio. Dormant-period fungicide applications were made either in the fall (after leaf drop and periderm tissue formation on the first-year canes, mid-November), or spring (at bud-swell, mid-April), or both. Disease incidence and severity on leaves and internodes were examined. In addition, effects of dormant-period application on sporulation of *P. viticola* were determined by examining the number of conidia in rain-splashed water in the spring and formation of mature pycnidia on cane sections in the winter. Fall-and-spring and spring applications of calcium polysulfide provided 12 to 88% reduction in disease intensity (incidence or severity), whereas calendar-based protectant mancozeb applications reduced overall disease intensity by 47 to 100%. Fixed-copper applications did not provide a consistent reduction of the disease. Fall applications of dormant-period fungicide provided little or no effect by itself. There was a significantly lower number of conidia observed in collected splashed rain water from vines treated with fall-and-spring applications of calcium polysulfide than in rain water from nonsprayed vines. Fall-and-spring and spring applications of calcium polysulfide provided a significant reduction in the number of mature pycnidia formed on incubated cane sections compared with the nonsprayed control (5 versus 10 pycnidia/cm²), whereas fixed copper did not provide a significant reduction.

Phomopsis cane and leaf spot is a disease of grape caused by the fungus *Phomopsis viticola* (Sacc.) Sacc. (16). Various tissues of the vine, such as leaf, cane, rachis, and fruit, can be infected by *P. viticola*. Infected leaves show small irregular or round pale-green to yellow spots with dark centers. Brown to black necrotic irregular-shaped lesions develop on the infected canes and rachises. Infected fruit appear as brown, shriveled berries near harvest (16,18). Infections on the canes and rachises weaken the plant and may cause premature fruit drop. Infections on fruit directly decrease yield and fruit quality. The disease is common in many parts of the United States and grape-growing regions around the world (9,12,15). Up to 30% loss of the crop has been reported in Southern Ohio grape vineyards (5).

The fungus survives winter in grape cane tissues that were infected in previous years. In spring, numerous pycnidia develop on infected canes, and a gelatinous mass of conidia (α - or β -conidia) called

cirrhi exudes out from the pycnidia. Conidia are splashed by rain onto new growth where they infect the plant tissues. The function of the β -conidia is not known (17,22).

Selective pruning and protective fungicide applications commonly are used to control the disease (16,18). Protective fungicides, such as mancozeb or captan applied on a 7- to 10-day calendar-based schedule have been used for disease control on green tissues and fruit (4,16). The fungus becomes active early in the growing season, when only about 2.5 cm of the new growth of the grape tissues is present (1,12,18). Close proximity between the source of inoculum (infected canes from the main trunk) and the susceptible tissues (new growth of canes) also may favor infection in the early growing season. It may not always be practical to apply fungicides at this time because the target plant tissue area is very small and rapidly growing. However, if fungicides are applied after this window of infection, disease intensity could be high no matter how many fungicide applications are made afterwards (M. A. Ellis, unpublished). Under favorable weather conditions, the fungus could become established in grape tissues regardless of the use of standard protectant fungicides.

Thus, the application of fungicide during the dormant period, which may aid in

controlling very early season infection, needs to be studied. Instead of applying the fungicide to newly produced tissue, a dormant-period application of fungicide targets the main trunk of the vine. Applications of fungicide during the dormant period are thought to reduce overall level of the inoculum before the beginning of a growing season (6). Examples of effective control provided with dormant-period application of fungicide include fire blight on apple and pear (caused by *Erwinia amylovora*; 8), powdery mildew on grape (caused by *Uncinula necator*; 6), anthracnose on grape (caused by *Elsinoe ampelina*; 4,16) and raspberry (caused by *Elsinoe veneta*; 2,4), and Phomopsis twig blight on blueberry (4). The potential effects of an application of fungicide during the dormant period against *P. viticola* have been reported in limited studies; however, the fungicide used may no longer be available (12) or experiments were not repeated (7,21). The objective of the current study was to determine the efficacy of applications of fungicide during the dormant period against *P. viticola* under field conditions in Ohio. Also, the effects of dormant-period applications of fungicide on sporulation were examined.

MATERIALS AND METHODS

A vineyard of cv. Concord (*Vitis labrusca*), planted in 1970, was used for this experiment. In addition, a portion of a vineyard of cv. Catawba (*V. labrusca*), planted in 1995, was used for an additional experiment. In both vineyards, vines were trained to an umbrella Kniffin system, and distance between vines was 2.1 m, with 3 m between rows. The two vineyards were at the Ohio Agricultural Research and Development Center, Wooster, and about 100 m apart from each other. Both the Concord and Catawba vineyards were known to have relatively high incidence of Phomopsis cane and leaf spot in previous years (M. A. Ellis, unpublished).

In the Concord vineyard, treatments consisted of different fungicide types and application timings. Calcium polysulfide (Suregard [liquid lime sulfur], 29% a.i.; Value Garden Supply, LLC, St. Joseph, MO) or copper oxychloride sulfate (COCS WGD [fixed copper], 50% metallic copper equivalent; Clean Crop, Platte Chemical Co. Greeley, CO) were applied to dormant vines in the fall (after leaf drop and after formation of periderm of canes, early No-

Corresponding author: L. V. Madden
E-mail: madden.1@osu.edu

Accepted for publication 5 May 2006.

vember), the spring (at bud swell, mid-April), or both fall and spring. A control consisted of nonsprayed vines. Calcium polysulfide was applied at 95 liters/ha, and fixed copper was applied at 3.4 kg/ha in 948 liters of water. Treatments were applied using a handgun sprayer at a pressure of 689 kPa. Vines were sprayed to the point of runoff. Fall and spring applications for the 2003 season were applied on 7 November 2002 and 17 April 2003, respectively; and fall and spring applications for the 2004 season were applied on 6 November 2003 and 20 April 2004, respectively. No other fungicides were applied in the vineyard.

In the Catawba vineyard, a dormant-period application of calcium polysulfide in the spring was compared with a calendar-based protectant program (7-day interval) of mancozeb (Dithane M-45; Dow Agrosciences LLC, Indianapolis, IN) and a nonsprayed control. The experiment was conducted in 2003 and 2004. Dates of dormant-period application were the same as in the Concord vineyard. The calendar-based applications of mancozeb started on 29 April and ended on 26 June in 2003 (consisting of nine sprays), and started on 6 May and ended on 17 June in 2004 (seven sprays).

Experimental design was a randomized complete block, replicated four times in both experiments in each year. In the Concord vineyard, each experimental unit consisted of three adjacent vines, and the

center vine was assessed for disease in order to minimize influence of chemical drift on disease development. Also, the same treatments were applied to the same vines for the two consecutive years. In the Catawba vineyard, each replication consisted of a single vine and treatments were randomized each year.

Levels of disease intensity (incidence and severity) were assessed visually on 27 June 2003 and 28 June 2004 in both vineyards. The five basal internodes and leaves on 10 randomly chosen shoots per vine were evaluated (50 internodes and leaves per vine). Direct estimation of percentage of diseased area was used for internode disease severity assessments. Disease severity of leaves was assessed by estimating the number of lesions on each leaf using a scale with seven levels, where 0 = no lesions, 1 = 0 to 10, 2 = 10 to 25, 3 = 25 to 50, 4 = 50 to 75, 5 = 75 to 100, and 6 = more than 100 lesions. The midpoint of each level was used as the lesion count for a leaf. Disease incidence was based on percentage of infected internodes or leaves per shoot using the same internodes or leaves used in severity estimate.

Data were analyzed using a linear mixed model with PROC MIXED of SAS (SAS Institute, Inc., Cary, NC) in order to determine effects of treatments on disease intensity. Because the treatment locations were not randomized between years, a mixed-model spatial analysis was performed to detect and correct for any corre-

lation of the disease values in neighboring vines. Using the methods described in Littell et al. (10) and Schabenberger and Pierce (20), a linear mixed model was fitted using a spatial first-order autoregressive model for the residuals. A comparison of the log likelihood statistics (10), and also the AIC statistics, for models with and without a spatial correlation component revealed that results were not affected by treatment location in the vineyard (*data not shown*). Thus, results for the simpler linear mixed model are reported.

In the model, treatments were considered as fixed effects and blocks as random effects. Values of internode and leaf disease incidences and of internode disease severities were transformed using an angular transformation ($\arcsin \sqrt{\text{proportion}}$), and a square-root transformation was used for lesion counts on leaves. Statistical analyses and treatment comparisons were based on least square means of transformed values, which then were back transformed after the analysis to obtain the presented means.

Collection of splashed rain water. To assess the effects of applications of fungicide during the dormant period on sporulation in the field during the spring, spore traps, consisting of a 20-cm-diameter funnel attached to a 2-liter plastic bottle, were placed under the center vine of the three-vine plot, starting at bud break until the end of bloom. A trap was placed in each replication of treatments receiving both fall

Table 1. Efficacy of dormant applications of calcium polysulfide and fixed copper, and their application timing, on disease incidence and severity of Phomopsis cane and leaf blight in a 'Concord' vineyard in 2003 and 2004 in Ohio

Treatment ^u	Timing ^v	Incidence ^t				Severity ^t			
		Leaf		Node		Leaf		Node	
		Percent ^w	PC ^x	Percent ^w	PC ^x	Number ^y	PC ^x	Percent ^w	PC ^x
2003									
Calcium polysulfide	Fall	65.3 a	−41.3	65.3 ac	17.5	4.9 a	−78.3	1.5 ab	35.3
Calcium polysulfide	Spring	41.5 bc	10.2	41.4 bc	47.7	1.7 bc	37.7	0.4 c	82.0
Calcium polysulfide	Both	33.9 c	26.6	32.7 b	58.6	1.2 b	55.3	0.3 c	88.4
Fixed copper	Fall	50.7 ab	−9.8	78.6 a	0.7	3.1 ab	−12.3	1.9 ab	13.7
Fixed copper	Spring	56.0 ac	−21.2	47.0 bc	40.5	1.6 bc	40.1	0.8 bc	66.1
Fixed copper	Both	33.7 b	26.9	78.7 a	0.5	4.2 ac	−53.2	2.1 a	8.6
Control	NS ^z	46.2 ab	...	79.1 a	...	2.7 ab	...	2.2 a	...
2004									
Calcium polysulfide	Fall	76.7 ab	8.4	81.3 ab	11.5	6.5 ab	32.6	3.9 ab	29.5
Calcium polysulfide	Spring	59.8 b	28.6	63.5 bc	30.9	2.9 b	70.2	1.3 bc	75.6
Calcium polysulfide	Both	60.6 b	27.7	56.5 c	38.5	3.1 b	67.8	0.8 c	85.5
Fixed copper	Fall	68.7 ab	18.0	85.2 ab	7.3	5.6 ab	42.1	3.7 ab	32.8
Fixed copper	Spring	64.6 b	22.8	79.2 ac	13.8	3.6 b	62.6	2.6 ac	52.4
Fixed copper	Both	77.1 ab	7.8	86.4 a	6.0	8.3 a	14.3	3.8 ab	30.0
Control	NS ^z	83.7 a	...	91.9 a	...	9.6 a	...	5.5 a	...

^c Means within a column followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares treatment means determined with a linear mixed model fitted to the data.

^a Calcium polysulfide (lime sulfur) was applied at a rate of 94.7 liters/ha and fixed copper (COCS) was applied at 3.4 kg/ha.

^b Fall and spring applications for the 2003 season were applied on 7 November 2002 and 17 April 2003, respectively, and fall and spring applications for the 2004 season were applied on 6 November 2003 and 20 April 2004, respectively.

^e Percent = back-transformed value of mean disease incidence or severity. Disease severity was estimated by visual assessment of proportion of internode covered by lesions. Disease incidence is a proportion of leaves or internodes showing visible symptoms of Phomopsis cane and leaf spot. Disease incidence or severity were angular transformed [$\arcsin \sqrt{\text{proportion}}$] before analysis with Proc MIXED of SAS.

^x PC = percent control = $100[(C - T)/C]$, where C is disease intensity (incidence or severity) for the nonsprayed control and T is disease intensity for the treatment.

^y Number = back-transformed value of the number of lesions per leaf. Disease severity was estimated by visual assessment of lesion numbers based on a 7-class scale (0 = no lesion and 6 = more than 100 lesions) per leaf, then square-root transformed before analysis with Proc MIXED of SAS.

^z Nonsprayed control.

and spring applications of lime sulfur and in the nonsprayed control. Traps were placed under the main trunk where the canes emerged from the main trunk. After each rain event, rainwater in the container was collected and the number of conidia was counted. Collected water was mixed constantly with a magnetic stir plate, six 20- μ l samples per replication were collected, and α -conidia were counted using a hemacytometer. Some samples were plated on potato dextrose agar (PDA) to confirm *P. viticola* with growth morphology. Mean number of conidia collected per month was analyzed using the linear mixed model, with treatment and month as fixed factors.

Sporulation on cane pieces. To assess the effects of treatments on subsequent sporulation, three canes per vine were collected at the end of the growing season, after formation of periderm tissues on the canes (4 November 2003 and 10 February 2005). These 1-year-old canes, with or without symptoms, were chosen randomly from the main trunk of each treatment replication. Each cane was cut into three 10-cm sections, then surface disinfested for 5 s with 70% EtOH, followed by 1 min of 0.5% sodium hypochlorite (10% Clorox) and 2 min of rinse with deionized H₂O. Canes then were placed in a moist chamber at approximately 20°C for 3 weeks to induce fungal sporulation. The moist chamber consisted of a 15-cm plastic petri dish with a moistened paper towel at the bottom, covered with a 0.5-cm mesh steel screen to prevent the canes from touching the paper towels directly. The lids were sealed with Parafilm.

Sporulation of *P. viticola* was assessed by counting the number of pycnidia with cirrhi per square centimeter of cane. Nine 1-cm² sections per replication (36 per

treatment) were assessed. A linear mixed model was used to analyze the efficacy of the treatments on sporulation with treatment as a fixed effect factor.

RESULTS

Disease incidence and severity—Concord. In 2003, spring application of calcium polysulfide (lime sulfur) or fixed copper (COCS) both provided a significant ($P \leq 0.05$) level of control of Phomopsis cane and leaf spot (Table 1). Leaf disease incidence was decreased by 10% and cane internode incidence was decreased by 48% by a spring application of calcium polysulfide compared with the nonsprayed control. Leaf severity was decreased by 38 and 40% with spring application of calcium polysulfide and fixed copper, respectively, and internode severity was decreased by 82 and 66% with spring application of calcium polysulfide and spring application of fixed copper, respectively (Table 1).

Generally, there were no significant differences in disease levels between the spring-only application and the fall-and-spring application of calcium polysulfide; however, the fall-and-spring application of fixed copper resulted in significantly higher disease intensity compared with the spring-only application of the same fungicide in many cases (Table 1). Overall, a fall-only application of fungicide resulted in disease incidence and severity not different from the nonsprayed control.

In 2004, there was higher disease incidence and severity; however, percentage of control provided by the treatments, especially for disease severity, was similar to that found in 2003 (Table 1). Spring application of calcium polysulfide provided better control than did the fall application, resulting in 29 and 31% reduction in dis-

ease incidence of leaves and internodes, respectively, and 70 and 76% reduction in disease severity of leaves and internodes, respectively, compared with that with the nonsprayed control. Spring application of fixed copper provided 23 and 14% reduction in disease incidence of leaves and internodes, respectively, and 63 and 52% reduction in disease severity of leaves and internodes. However, among fixed copper treatments, only leaf disease incidence and severity for the spring-only application were significantly lower than that in the nonsprayed control (Table 1). The fall-and-spring dormant application of fixed copper provided poorer control than just the spring application of the same fungicide, as in the 2003 season. Again, fall-only application was not effective for either fungicide.

Disease incidence and severity—Catawba. For the experiment in the Catawba vineyard, the results were consistent in 2003 and 2004 (Table 2). The dormant-period application of calcium polysulfide in spring provided moderate level of control, with disease incidence and severity of leaves and internodes significantly lower ($P \leq 0.05$) than that found in the nonsprayed control. The percent control of disease severity ranged from 75 to 98% (Table 2). However, the calendar-based protectant applications of mancozeb provided significantly ($P \leq 0.05$) lower disease incidence and severity than did the dormant-period fungicide treatment (Table 3). Percent control based on severity ranged from 87 to 100%.

Collection of conidia in splashed rain water. In 2003, the peak of spore discharge was relatively early (mid-April) in the field, with a secondary peak around late May (Fig. 1). In 2004, the peak was

Table 2. Efficacy of a dormant (spring) application of calcium polysulfide and of a calendar-based (7-day) mancozeb application on disease incidence and severity of Phomopsis cane and leaf spot in the 'Catawba' vineyard in 2003 and 2004 in Ohio

Treatment ^u	Timing ^v	Incidence ^t				Severity ^t			
		Leaf		Node		Leaf		Node	
		Percent ^w	PC ^x	Percent ^w	PC ^x	Number ^y	PC ^x	Percent ^w	PC ^x
2003									
Mancozeb	Calendar	13.1 c	81.0	10.5 c	87.8	0.3 c	96.8	0.0 b	100.0
Calcium polysulfide	Dormant	33.8 b	51.0	28.2 b	67.3	1.1 b	88.4	0.1 b	97.7
Control	NS ^z	69.0 a	...	86.2 a	...	9.5 a	...	4.3 a	...
2004									
Mancozeb	Calendar	52.2 c	46.8	33.3 c	66.7	1.7 c	86.7	0.7 c	94.0
Calcium polysulfide	Dormant	68.8 b	29.9	81.0 b	18.9	3.2 b	75.0	2.2 b	81.2
Control	NS ^z	98.1 a	...	100.0 a	...	12.8 a	...	11.7 a	...

^c Means within a column followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares treatment means determined with a linear mixed model fitted to the data.

^a Calcium polysulfide (lime sulfur) was applied at a rate of 94.7 liters/ha and mancozeb was applied at 4.5 kg/ha in 948 liters of water.

^b Spring application of calcium polysulfide were applied on 17 April 2003, and 20 April 2004. Applications of mancozeb were made from 29 April 2003 to 26 June 2003 (nine applications) and from 6 May 2004 to 17 June 2004 (seven applications).

^e Percent = back-transformed value of mean disease incidence or severity. Disease severity was estimated by visual assessment of proportion of internode covered by lesions. Disease incidence is a proportion of leaves or internodes showing visible symptoms of Phomopsis cane and leaf spot. Disease incidence or severity were angular transformed [$\arcsin \sqrt{\text{proportion}}$] before analysis with Proc MIXED of SAS.

^x PC = percent control = $100[(C - T)/C]$, where C is disease intensity (incidence or severity) for the nonsprayed control and T is disease intensity for the treatment.

^y Number = back-transformed value of the number of lesions per leaf. Disease severity was estimated by visual assessment of lesion numbers based on a 7-class scale (0 = no lesion and 6 = more than 100 lesions) per leaf, then square-root transformed before analysis with Proc MIXED of SAS.

^z Nonsprayed control.

around mid-May (Fig. 1). In 2003, there was a significant interaction of treatment and sampling month on spore density ($P \leq 0.05$). The untreated control had a significantly higher mean than treated vines for the first month after spring application, but not for the second month (Table 3), suggesting a short time effect of the fungicide.

In 2004, month and treatment both had significant effects ($P \leq 0.05$), but the interaction was not significant ($P \leq 0.05$). Thus, comparisons are limited to the main-effect means. Mean spore density trapped under treated vines was significantly less than that found for nonsprayed vines (Table 3), and the mean was higher in the first month compared with the second. Because of the lack of interaction, effectiveness of the fungicide did not depend on sampling month in 2004.

Sporulation on cane pieces. In 2003, there were significant reductions ($P \leq 0.05$) in density of mature pycnidia on the canes collected at the end of the season that were treated with a calcium polysulfide dormant application in the Concord vineyard (Table 4). Reductions in number of mature pycnidia were about 55% compared with the control, regardless of timing of application. Means for fixed copper treatments were not significantly different from that in the nonsprayed control (Table 4).

Results from the 2004 season were similar to those in 2003. Dormant-period application of calcium polysulfide in both fall and spring, and also the spring application of fixed copper, resulted in mean density of mature pycnidia significantly less than that in the nonsprayed control (Table

4). Although there was about a 57% reduction in the mean density of mature pycnidia, the mean for the spring application of calcium polysulfide was not significantly different from that in the nonsprayed control (Table 4). As in 2003, means for fixed copper treatments were not significantly different from that in the nonsprayed control (Table 4).

DISCUSSION

Over 2 years, the efficacy of applications of two different fungicides (calcium polysulfide and fixed copper) during the dormant period of grape vine was examined under field conditions for control of *Phomopsis* cane and leaf spot. The results from the Concord vineyard indicate that calcium polysulfide application generally suppressed the disease on leaves and internodes better than did fixed copper application when applied in the spring (Table 1). With this application timing of calcium polysulfide, mean disease incidence or severity on leaves and internodes were reduced relative to that obtained with the nonsprayed vines.

In the Catawba vineyard, the calendar-based mancozeb application had consistently better control than did the dormant-period spring application of calcium polysulfide, although the latter also significantly reduced disease incidence and severity (Table 2). This showed the importance of protection of the canes and internodes during the spring in order to control *Phomopsis* cane and leaf spot.

Fall applications of either chemical did not provide significant effects on disease

control (Table 1), and would not be recommended for disease management. With calcium polysulfide, the fall-and-spring application tended to have lower disease intensity on leaves and canes compared with the spring-only application, but the differences were not significant or consistent (Table 1), which suggests that the contribution of the fall application of the fungicide to the degree of disease control was low. Applications of fixed copper in the fall and spring resulted in significantly higher disease incidence and severity than with application just in the spring for some years and some measurements of disease (Table 1). Because the treatment was applied 2 years in a row to the same vines, it is possible that one particular vine with higher inoculum doses affected the results (*data not shown*). Because of poorer and inconsistent control, an application of fixed copper during the dormant period would not be recommended for management of *P. viticola*.

Often, disease severities of treated vines were reduced significantly by fungicide, whereas incidences were not, or percent reduction in incidence was much less than percent reduction in severity (Tables 1 and 2). The large reductions in internode disease severity were especially noteworthy, because infected canes are the source of the inoculum in subsequent years (16,18). However, observations from incubation of canes showed that mature pycnidia can be formed on nonsymptomatic canes (M. Nita, *unpublished*).

A dormant application of a protectant fungicide does not protect susceptible plant tissues produced after application from infection; thus, other mechanisms must underlay the measured control. One explanation for the positive effect of an application of calcium polysulfide during the dormant period is in reduction of spore production that can potentially infect new tissues. In our study, reduction of conidial density in splashed rain water was observed during spore sampling in the spring, where significantly fewer conidia were collected under treated vines compared with the control vines (Table 3). However, there was still a substantial amount of spore release with dormant-period fungicide applications during both years (Fig. 1; Table 3).

Because conidia were collected with rain water, absence of significant rain events from 27 April to 7 May 2004, and relatively less rainfall in 2004 than 2003, may have affected the monthly spore total results (Fig. 1; Table 3). Also, the variability of rain water collection should be noted. Although spore traps were placed in a relatively small field (approximately 25 by 15 m), there was large variation in the amount of rain water collected in each trap. Sometimes, one spore trap collected more than 1 liter of rain water while another trap located approximately 2 m away

Table 3. Mean number of conidia per milliliter of splashed rain per rain event in the first and second month after applications of dormant fungicides in spring

Treatment ^a	Conidia ^a		
	Month 1 ^w	Month 2 ^w	Mean
2003			
Calcium polysulfide	3,056 b ^x	3,426 b	3,194
Control (nonsprayed)	9,744 a	1,542 b	6,178
Mean	6,161	2,434	4,654
2004			
Calcium polysulfide	4,130	1,637	3,071 a ^y
Control (nonsprayed)	9,029	2,800	6,750 b
Mean	6,580 a ^z	2,183 b	4,776

^a Conidia were collected with traps that were located next to the main trunk. Rain water was collected after each rain event, and then six samples were taken from the collected water to count number of conidia per milliliter using a hemacytometer.

^w Calcium polysulfide (lime sulfur) was applied at 95 liters/ha in 348 liters of water. Fall and spring applications for the 2003 season were applied on 7 November 2002 and 17 April 2003, respectively, and fall and spring applications for the 2004 season were applied on 6 November 2003 and 20 April 2004, respectively.

^w Month 1 in 2003 and 2004 represents the periods between 17 April and 17 May 2003 and 20 April to 20 May 2004, respectively, and month 2 in 2003 and 2004 represents the periods between 17 May and 17 June 2003 and 20 May and 20 June 2004, respectively.

^x Means within a column or row for 2003 followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares treatment means determined with a linear mixed model fitted to the data.

^y Means within a column for 2004 followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares treatment means determined with a linear mixed model fitted to the data.

^z Means within a row for 2004 followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares month means determined with a linear mixed model fitted to the data.

collected no water (*data not shown*). This may be due to a slight difference of angle of the funnel of the traps, raindrop trajectory, or rainfall heterogeneity. To provide stronger evidence of the effects of the dormant fungicide application on the sporulation of *P. viticola*, more consistent sampling methods or a larger number of sampling sites are needed.

A study conducted in California by Cucuza et al. (1) showed that the peak of inoculum production of *P. viticola* is in early-April, followed by a gradual decline over the course of the season. Also, grape tissues seem to be more susceptible to *P. viticola* infection during the early stages of growth (19). Relative proximity of the source of the inoculum (infected old canes) and the susceptible young tissues in early growth stages in the spring increases the chance of infection because the conidia are splashed by rain and, in many cases, the distance traveled by dispersal of conidia is short (i.e., <1 m; 11,13,14). The limited range of spore dispersal also was indicated by the lack of spatial autocorrelation of disease in neighboring vines within vineyards. Thus, the reduction of spore release early in the season by the dormant fungicide application may have played an important role in the reduction in disease incidence and severity we observed. This tendency of reduction of sporulation activity by the dormant application of fungicide also was discussed previously (1,12).

A potential longer-term effect of an application of fungicides during the dormant period could be seen in lowered inoculum density on canes in the spring following fungicide application prior to the growing season. Calcium polysulfide treatments significantly reduced the density of mature pycnidia with cirrhi, compared with the control (Table 4). Fixed copper fungicide clearly was less effective as a dormant-period application compared with lime sulfur in this regard. However, even though there were significant reductions in density of mature pycnidia with both the spring and fall-and-spring dormant-period application of calcium polysulfide, there were still 5 to 6 pycnidia/cm², on average, which could produce numerous conidia (Table 4). Overall disease intensity on leaves and internodes increased from 2003 to 2004 when the same treatments were applied to the same vines; therefore, the application of fungicide during the dormant period alone clearly does not provide satisfactory long-term control of *P. viticola*. This lack of long-term effects by the dormant-period fungicide application was discussed previously (1,12).

Overall, there is apparent benefit in applying lime sulfur early in the spring in order to control *Phomopsis* cane and leaf spot. In general, an application of lime sulfur around bud-swell provided moderate disease control, ranging from 10 to 67% and 38 to 98% in disease incidence and

Fig. 1. Number of *Phomopsis viticola* conidia per milliliter of splashed rain water in the 'Concord' vineyard, and amount of rainfall recorded in 2003 and 2004 (vertical bars). Arrows indicate the date of the spring-dormant fungicide application of calcium polysulfide (CP). Solid lines represent mean number of conidia observed per rain event for the fall-and-spring application of calcium polysulfide, and dashed lines represent the mean for the nonsprayed control.

Table 4. Mean number of mature pycnidia with cirrhi of *Phomopsis viticola* per square centimeter of grape cane from the 'Concord' vineyard collected during the winter following dormant application of fungicides

Treatment ^v	Timing ^w	2003 ^u		2004 ^u	
		Number ^x	PC ^y	Number ^x	PC ^y
Calcium polysulfide	Fall	5.3 bcd	54.3	8.0 ab	19.2
Calcium polysulfide	Spring	4.9 cd	57.8	4.2 ac	57.6
Calcium polysulfide	Both	4.6 d	60.3	2.1 c	78.8
Fixed copper	Fall	8.7 ab	25.0	5.4 ac	45.5
Fixed copper	Spring	7.8 acd	32.8	3.2 bc	67.7
Fixed copper	Both	8.4 ab	27.6	5.3 ac	46.5
Control	NS ^z	11.6 a	...	9.9 a	...

^u Canes were sampled on 2 November 2003 and 10 February 2005. Means within a column followed by same letter are not significantly different ($P \leq 0.05$) based on multiple comparisons of least-squares treatment means determined with a linear mixed model fitted to the data.

^v Calcium polysulfide (lime sulfur) was applied at a rate of 94.7 liters/ha and fixed copper (COCS) was applied at 3.4 kg/ha in 948 liters of water.

^w Fall and spring applications for the 2003 season were applied on 7 November 2002 and 17 April 2003, respectively, and fall and spring applications for the 2004 season were applied on 6 November 2003 and 20 April 2004, respectively.

^x Number = back-transformed value of mean number of mature pycnidia with cirrhi per square centimeter. Number of mature pycnidia per square centimeter was angular transformed [$\arcsin \sqrt{\text{proportion}}$] before analysis with Proc MIXED of SAS.

^y PC = percent control = $100[(C - T)/C]$, where C is disease incidence for the nonsprayed control and T is disease incidence for the treatment

^z Nonsprayed control.

severity, respectively (Tables 1 and 2). Other limited studies (7,21) showed very similar results. There was, however, variation among years and experiments, and the magnitude of control is not sufficient to recommend this treatment alone for season-long control of *Phomopsis* cane and leaf spot. However, in this study, even a calendar-based 7-day-interval application of protective fungicide provides only 47 to 88% control of disease incidence (Table 2). Because frequent rains in the spring can prevent the timely application of protective fungicides, an application of a fungicide during the dormant period can provide some level of control during this critical period when plant tissues would be otherwise unprotected. A dormant-period fungicide application in combination with a standard protective fungicide program may lead to better control of *Phomopsis* cane and leaf spot of grape than either program alone. The economics of using applications of fungicides during the dormant period in relation to the amount of disease control needs to be studied.

Applications of fungicide during the dormant period may have the greatest potential in organic grape production systems where the choices of fungicides currently are very limited. Calcium polysulfide, as well as fixed copper, are registered for use with organic production of grape (3). A study showed that the dormant-period application of calcium polysulfide (at 284 liters/ha) also can reduce powdery mildew intensity on grape by reducing viable cleistothecial numbers (6). Although it was not recorded, no major outbreak of powdery mildew of grape or other common diseases in Ohio, such as black rot (caused by *Guidnardia bidwellii*) or downy mildew

(caused by *Plasmopara viticola*), was observed in our experimental fields during these two seasons, even when no other fungicides were applied (M. Nita, *unpublished*). In addition, protective foliar applications of lime sulfur (4.5 kg/ha) applied weekly can be used as an effective control for *P. viticola* (M. Nita, *unpublished*), and *Uncinula necator* in grape production (3,16).

LITERATURE CITED

1. Cucuzza, J. D., and Sall, M. A. 1982. *Phomopsis* cane and leaf spot disease of grape vine: Effect of chemical treatments on inoculum level, disease severity, and yield. *Plant Dis.* 66:794-797.
2. Ellis, M. A., Converse, R. H., Williams, and R. N., Williamson, B., eds. 1991. *Compendium of Raspberry and Blackberry Diseases and Insects*. American Phytopathological Society Press, Inc., St. Paul, MN.
3. Ellis, M. A., and Nita, M. 2004. *Disease Management Guidelines for Organic Grape Production in the Midwest*. The Ohio State University OARDC/OSUE, Wooster, OH.
4. Ellis, M. A., Welty, C., Funt, R. C., Doohan, D., and Williams, R. N., eds. 2004. *Midwest Small Fruit Pest Management Handbook*. Ohio State University Extension, Columbus, OH.
5. Erincik, O., Madden, L. V., Ferree, D. C., and Ellis, M. A. 2001. Effect of growth stage on susceptibility of grape berry and rachis tissues to infection by *Phomopsis viticola*. *Plant Dis.* 85:517-520.
6. Gadoury, D. M., Pearson, R. C., Riegel, D. G., Seem, R. C., Becker, C. M., and Pscheidt, J. W. 1994. Reduction of powdery mildew and other diseases by over-the-trellis applications of lime sulfur to dormant grapevines. *Plant Dis.* 78:83-87.
7. Gubler, W. D., and Baldwin, L. G. 2000. Evaluation of fungicides for the control of *Phomopsis* cane and leaf spot of grapes, 1999. *Fungic. Nematicide Tests* 55:97.
8. Jones, A. L., and Aldwinckle, H. S., eds. 1990. *Compendium of Apple and Pear Diseases*. American Phytopathological Society Press, Inc., St. Paul, MN.
9. Lal, B., and Arya, A. 1982. A soft rot of grapes caused by *Phomopsis viticola*. *Indian Phytopathol.* 35:261-264.
10. Littell, R. C., Milliken, G. A., Stroup, W. W., and Wolfinger, R. D. 1996. *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC.
11. Madden, L. V. 1992. Rainfall and the dispersal of fungal spores. *Adv. Plant Pathol.* 8:39-79.
12. Malathrakias, N. E., and Baltzakis, N. G. 1976. Control of dead-arm of grapes. *Poljopr. Znan. Smotra* 39:261-269.
13. McCartney, H. A., and Fitt, B. D. L. 1998. Dispersal of foliar fungal plant pathogens: Mechanisms, gradients and spatial patterns. Pages 138-160 in: *The Epidemiology of Plant Diseases*. Kluwer, Dordrecht, The Netherlands.
14. McDonald, O. C., and McCartney, H. A. 1987. Calculation of splash droplet trajectories. *Agric. For. Meteorol.* 39:95-110.
15. Mostert, L., Crous, P. W., and Petrini, O. 2000. Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. *Sydowia* 52:46-58.
16. Pearson, R. C., and Goheen, A. C., eds. 1988. *Compendium of Grape Diseases*. American Phytopathological Society Press, St. Paul, MN.
17. Pezet, R., Pont, V., and Girardet, F. 1983. Microcycle conidiation in pycnidial cirrhii of *Phomopsis viticola* Sacc. induced by elemental sulfur. *Can. J. Microbiol.* 29:179-184.
18. Pscheidt, J. W., and Pearson, R. C. 1989. Effect of grapevine training systems and pruning practices on occurrence of *Phomopsis* cane and leaf spot. *Plant Dis.* 73:825-828.
19. Pscheidt, J. W., and Pearson, R. C. 1989. Time of infection and control of *Phomopsis* fruit rot of grape. *Plant Dis.* 73:829-833.
20. Schabenberger, O., and Pierce, F. J. 2002. *Contemporary Statistical Models for the Plant and Soil Sciences*. CRC Press, Boca Raton, FL.
21. Schilder, A. M. C., Gillett, J. M., and Sysak, R. W. 2000. Evaluation of fungicides for control of *Phomopsis* cane and leaf spot of grape, 1999. *Fungic. Nematicide Tests* 55:105.
22. Sergeeva, V., Nair, N. G., Barchia, I., Priest, M., and Spooner-Hart, R. 2003. Germination of β conidia of *Phomopsis viticola*. *Australas. Plant Pathol.* 32:105-107.