# Proceedings of the Symposium on Advances in Vineyard Pest Management

February 6–8, 2010
Midwest Grape and Wine Conference
Osage Beach, Missouri

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**Editors:**
R. Keith Striegler
Andy Allen
Satisha Jogaiah
Jackie Harris

Institute for Continental Climate Viticulture and Enology
College of Agriculture, Food and Natural Resources, University of Missouri
Missouri Wine and Grape Board

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## On the cover:

**Top row (left to right)**

(1) *Phomospsis viticola* damages basal internodes and leaves of vines early in the season and infects both berries and cluster rachises.

(2) Canker disesases caused by *Botryosphaeria* spp. and *Eutypa lata* cause slow death of cordons and trunks, with decreasing yields as fruiting wood dies.

**Middle row (left to right)**

(3) Foliar feeding by the aerial form of grapevine phylloxera, *Daktulosphaira vitifoliae*, causes severe deformation of leaves on susceptible cultivars.

(4) Sour rot, a disease complex caused by a combination of fungi, yeasts and bacteria, causes severe losses of several wine-grape cultivars when environmental conditions favor disease development.

**Bottom row**

(5) Japanese beetles, voracious feeders on grapevine foliage, are an increasing threat to regional vineyards.

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# Symposium Agenda

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*Dr. Douglas Gubler, University of California, Davis*

**9:00–10:00 a.m.** **Target Your Sprays and Save Money: Methods of Improving Deposition and Reducing Drift**
*Dr. Andrew Landers, NYSAES, Geneva, N.Y.*

**10:15–11:15 a.m.** **Studies on the Biology and Control of Phomopsis Cane and Leaf Spot**
*Dr. Michael Ellis, The Ohio State University, Wooster, Ohio*

**2:00–3:00 p.m.** **Developing an Effective Fungicide Spray Program for Wine Grapes in the Midwest**
*Dr. Michael Ellis, The Ohio State University, Wooster, Ohio*

**3:15–4:15 p.m.** **Canker Diseases: Their Causes and Control in Calfornia and the Midwest**
*Dr. Douglas Gubler, University of California, Davis*

**4:15–5:15 p.m.** **Evaluation of Cultural Practices to Reduce Bunch Rot in Vignoles Grapevines**
*Dr. Keith Striegler, University of Missouri, Columbia, Mo.*

***Sunday, Feb. 7, 2010***
***Eli Bergmeier, Moderator***

**8:30–9:30 a.m.** **Weeds in Vineyards: What's Out There, and Why?**
*Dr. Reid Smeda, University of Missouri, Columbia, Mo.*

**9:30–10:30 a.m.** **Management of Grape Phylloxera, Grape Berry Moths and Japanese Beetles**
*Dr. Donn Johnson, University of Arkansas, Fayetteville, Ark.*

**10:45–11:45 a.m.** **Emerging Viruslike Diseases in Chardonel, Vidal Blanc and Cabernet Sauvignon in Missouri**
*Dr. Wenping Qiu, Missouri State University, Mountain Grove, Mo.*

# Grapevine Trunk Diseases: Etiology, Epidemiology and Control

**Dr. W. Douglas Gubler, Dr. J.R. Urbes-Torres, F.P. Trouillas and R. Herche**
Department of Plant Pathology
University of California, Davis

**Dr. R. Keith Striegler**
Institute for Continental Climate Viticulture and Enology
University of Missouri

**Dr. Richard D. Cartright, J. Kreiddy and Dr. John C. Rupe**
Department of Plant Pathology
University of Arkansas

## Introduction

Grapevine trunk diseases are responsible for significant economic losses to the wine industry worldwide. Symptoms include dead spurs and cordon and trunk dieback due to canker formation in the vascular tissue, and in some cases, deformed leaves and shoots caused by fungal toxins. As cankers develop, yield reductions occur due to the loss of productive wood. The impact of grapevine trunk diseases can be significant in older vineyards and usually becomes more severe as vineyards age. Petri disease and esca (black measles) are caused by the vascular pathogens *Phaeomoniella chlamydospora* and numerous species of *Phaeoacremonium aleophilum* (*Togninia minima*), respectively. Eutypa dieback, caused by the fungus *Eutypa lata*, was originally thought to be responsible for most grapevine canker disease in California vineyards. However, recent findings have highlighted the importance of other fungi involved in the death and decline of grapevines in California. *Botryosphaeriaceae* species have been recovered from cankers, and were determined to be the main cause of canker diseases in some California vineyard production areas. Recent research has also indicated the occurrence of several new fungal pathogens causing trunk diseases. These fungi belong to the family Diatrypaceae. These species include *Eutypa leptoplaca*, *Cryptovalsa ampelina*, *Eutypella* spp., *Diatrypella* sp. and *Diatrype* species. We will present current information on the epidemiology and control strategies of fungal organisms responsible for grapevine spur, cordon and trunk dieback in California.

## Esca and young esca (vine declines)

Esca (black measles) and young esca (Petri disease, vine decline) have been documented to occur in all of the major grape production regions of California and the world. *Phaeomoniella chlamydospora* is the primary pathogen responsible for Petri disease. Esca can be caused by *Pa. chlamydospora* but *Phaeoacremonium aleophilum* (*Togninia minima*) is the primary pathogen. Other species of Phaeoacremonium have also been shown to be pathogens and probably are responsible for some mature vine esca. These include *Pm. mortoniae* (*Togninia fraxinopennsylvanica*), *Pm. parasiticum*, *Pm. rubrigenum*, *Pm. angustius* and two new species of *Togninia*, *T. californica* and *T. davisiana*. These fungi are also responsible for poor vineyard establishment in many newly planted vineyards, in which case, the young vines may have been infected prior to planting. We know that all of these fungi are endophytes and as such they may infect nursery stock. When this occurs the vines are generally but not always of reduced quality and may not survive well after planting. This is particularly the case when vines are planted poorly, fruited early or not irrigated properly. The infection court for these fungi are wounds, generally the xylem parenchyma and vessels of mature grapevine xylem, and we suspect that nursery infection occurs through these structures. It is suspected that the pathogens may be passed from mother vines to progeny vines via spores or mycelium, carried either in the sap flow or by external contamination of bark by the release of ascospores from perithecia. However, these pathogens are also soil-borne and have the capacity to infect young roots directly through the bark after planting.

Young vine decline and Petri disease have become common diseases of 1- to 9-year-old grapevines, mostly in California's North Coast production area and in other production areas around the world. The occurrence of the disease coincided with massive replanting of grapevines as a result of Phylloxera infestation of AXR1 rootstock. AXR1 was replaced with rootstocks that were resistant to Phylloxera; however, these rootstocks were more susceptible to the Petri disease and other vine-decline pathogens. Although widespread in occurrence, vines showing decline due to Petri disease usually constitute a minor portion (1 to 5 percent) of a newly planted vineyard. Likewise, the chronic type of esca has significantly increased in California over the past 12 years. Chronic symptoms of esca, which were not common on grapevines unless the vine was older than 10 years

of age, are now commonly seen on vines of 1 to 6 years old also as a result of the use of Phylloxera-resistant rootstock.

Esca is characterized by the presence of bright tiger-striped patterns on the leaves of affected shoots, which can vary in occurrence and severity from one year to the next. Fruit symptoms range from superficial brown to purple spots on the berry skins to complete collapse of the rachis causing withering of fruit. Entire clusters can become affected, making fruit of table grape varieties unmarketable and fruits of both wine and table grapes have an acrid taste. Despite past research efforts, no information was available regarding etiology, disease epidemiology and management.

It is now known that the young esca and esca pathogens are endophytes in vines in all production areas of California and the world. The pathogens produce ascospores or pycnidiospores, depending on species, which are released with rainfall; new infections occur through pruning wounds. It was demonstrated that pruning wounds are susceptible to infection by conidia of both *P. aleophilum* and *Pa. chlamydospora* and ascospores of *T. minima*. A detection method using nested-PCR was developed to provide a rapid and sensitive test to determine the presence of these fungi in soils and plants.

Spores of several Phaeoacremonium species and *Pa. chlamydospora* were trapped in infected vineyards during periods of rainfall. Also, propagules of both fungi were found on the surface of clusters, leaves and trunks of grapevine in infected vineyards in California. Perithecia of *T. minima* have been identified on rotted vascular tissue in infected grapevine wood in California and Australia.

Two other species which have been reported only once before on grapevines in California were also found to be somewhat common in California: *Pm. angustius* and *Pm. mortoniae*. After confirming the presence of *Togninia* spp. and *Pa. chlamydospora* in nursery propagation wood and as overwintering structures in California vineyards, we have begun to examine different applications for disease management. Our results indicate that pruning wounds can be protected by application of fungicides immediately after pruning. These wounds commonly were susceptible to several other fungal pathogens responsible for shoot dieback and canker formation on grapevine, and we also have recovered several of these fungi in consent from wood cankers.

## Grapevine canker diseases

### *Eutypa dieback*

Eutypa dieback and Bot canker are estimated to cost California winegrape growers over $260 million per year in lost fruit production, pruning of infected vines, retraining and replanting. Many growers consider canker diseases to be the most significant diseases of grapevines.

Typical symptoms of *Eutypa lata* include formation of a wedge-shaped canker in the water-conducting tissue, and stunted shoots with cupped, tattered, chlorotic and necrotic leaves that are best seen at springtime. Foliar symptoms are due to toxins produced by *E. lata*. Differences in susceptibility of grapevine cultivars to infection have been reported, although no cultivars are immune. Cankers are perennial and develop both basipetally and acropetally and increase in diameter over time. Extended infection of grapevines by *E. lata* leads to death of the vine.

*E. lata* spreads to new pruning wounds by wind-driven and water-splashed ascospores released after rainfall. Ascospores are the primary inoculum and develop inside fungal fruiting bodies called perithecia (sexual stage). Perithecia form in a black stroma that is embedded in the cortical tissue of the grapevine or other hosts. The sexual stage develops in regions that receive more than 16 inches of rain per annum. It is common to find stroma and perithecia on old grapevines and other types of wood in the North Coast and Delta production areas. Ascospores infect grapevines through fresh pruning wounds during the dormant season. Ascospores germinate, invade xylem vessels and weaken the plant by producing an array of toxins and decaying the wood over years by excreting cell wall–degrading enzymes. Pruning wounds become less susceptible with age. After six weeks, wounds are generally not susceptible. *E. lata* also produces asexual spores called conidia. These are formed inside pycnidia (asexual fruiting bodies) that develop on wood, but these spores do not play a role in the epidemiology of the disease. In California, ascospore discharge of *E. lata* occurs from the first rain of the early fall until the last rains in the spring. Ascospore discharge drops off noticeably in late February and remains low to nil by early March. However, important ascospore releases may occur during the occasional rains of March and April if no winter rains have occurred. Such releases may occur because perithecia are able to recover in productivity during the dry period, or because spores that would have been released in the winter months are released in the spring simply because

they were not released in the winter. This scenario is more likely to occur in years when there is little rainfall during the winter months. Ascospore release may occur continuously for approximately 24 hours during periods of rainfall, starting a few hours after the onset of a rain. Low temperatures in California seem to stop ascospore release. Spore-trapping studies in the North Coast show very few to no spores released at or below 40 degrees Fahrenheit (5 degrees Celsius).

More than 80 plant species around the world have been reported to be potential hosts for *E. lata*. In the 1970s, grapevine, apricot and Ceanothus were found to be natural hosts of *E. lata*. In California, 22 tree or shrub species were found to be hosts of *E. lata*, including big leaf maple, kiwifruit, blueberry, cherry, apple, pear, crabapple, almond, oak, California buckeye, willows and oleander. All of these species bore perithecia and occurred in the vicinity of vineyards. We now know that these species serve as natural reservoirs of *E. lata* inoculum.

Perithecia of *E. lata* were found to be particularly well established on dead branches of various willow species occurring along natural creeks and irrigation waterways. It appears likely that the flora surrounding vineyards is a key factor in disease epidemiology and surely acts as an inoculum reservoir for some canker organisms. Sanitation of the dead wood of the potential hosts of *E. lata* in areas surrounding vineyards is advised in order to decrease the inoculums level. Surveys inside vineyards and apricot and cherry orchards have revealed an abundance of pathogen inoculums in plantings of about 20 years and older. Only a few perithecia have been found in almond orchards.

Perithecia of *E. lata* were found to be prevalent in the counties of Napa, Sonoma, Yolo, Sacramento, Contra Costa, San Benito, El Dorado, Mendocino, San Joaquin, Stanislaus and Merced. Perithecia of *E. lata* were not found in Madera, Fresno, Kings, Tulare and Kern counties. Large amounts of viable inoculum were found in several old vineyards near Healdsburg in Sonoma County. Perithecia were particularly well developed on vines that had been previously grafted for variety change. Stroma on those vines had developed below the grafting wound down to the callus union with the rootstock.

It is our opinion that even though *E. lata* ascospores can travel considerable distances and cause disease, Eutypa dieback is primarily a disease of local origin, developing in the vicinity of where the ascospores are released.

## Botryosphaeria "bot" canker

Botryosphaeria canker disease of grapevine is a wood disease caused by various species in the fungal family Botryosphaeriaceae in California and grape-production regions around the world. Although it is well accepted that some Botryosphaeriaceae species have been the causal agent of canker diseases of various woody plants in California, the importance of these fungi as pathogens of grapevines has been largely ignored in the most important grapevine areas throughout the world. To date, 14 Botryosphaeriaceae species placed in the anamorphic genera Fusicoccum, Neofusicoccum, Diplodia, Lasiodiplodia, Spencermartinsia and Dothiorella have been reported as pathogens on grapevines in the U.S., South Africa, Australia, New Zealand, France, Italy, Spain, Portugal, Egypt, India, Mexico, Chile and Brazil.

Since 1990, *Lasiodiplodia theobromae* has been known to cause wedge-shaped cankers and dieback symptoms in vineyards of southern California. This species was previously known as Botryodiplodia theobromae, and the disease it causes was referred to as bot canker. It is now considered an endemic species in many vineyards in warm and hot climate areas in California.

Typical symptoms caused by Botryosphaeriaceae on grapevines in California are the wedge-shaped canker in the trunk and cordons and dead spur positions. It is important to emphasize that no foliar symptoms associated with Botryosphaeriaceae-induced canker disease have been observed in California grapevines. This is in contrast to other areas in the world where foliar symptoms were observed on grapevines infected by different species of Botryosphaeriaceae such as *Diplodia mutila*, *Diplodia seriata*, and *Botryosphaeria dothidea*. The wedge-shape cankers caused by Botryosphaeriaceae are visually indistinguishable from those formed by both *Eutypa lata* and *E. leptoplaca*. All three fungi can cause cankers and can be detected in the vine at the same time. The best distinguishing characteristic is the total absence of the stunted or chlorotic spring growth, which is typical of infections by *E. lata*.

Botryosphaeriaceae spp. are pruning-wound pathogens entering the vine through fresh pruning wounds and wounds up to 12 weeks old. Large numbers of Botryosphaeriaceae conidia are exuded from fruiting bodies (pycnidia) found on old diseased vine parts under the bark of cordons, trunks and spurs. Another important pycnidial reservoir may be the residual infected pruning wood left in

the vineyards. We also have found that many of these species cause disease on different hosts around the vineyards. The formation of numerous fruiting bodies provides an excellent source of spores for further infections in the vineyard. Conidia may be easily distributed over the vineyard due to wind, or they may be waterborne in splashed drops from rain or sprinkler irrigation.

Like *E. lata*, the canker formed by Botryosphaeriaceae grows more rapidly basipetally. The canker develops for several years in the trunks and cordons, depending on where the infection was located. Death of the infected vine part occurs when the last live tissue is killed by the growth of the fungus. In California, wedge-shaped cankers caused by Botryosphaeriaceae can be found on vines 8 years old and older, especially where large pruning wounds have been made in retraining vines.

Eutypa dieback and bot canker disease decrease the life of the vineyard, reduce the yield, and increase production costs due to the application of control treatments including cultural practices to prevent infections and pruning of diseased tissues.

In a recent field survey (2003 to 2006), more than 1,900 samples showing the typical wedge-shaped cankers were collected from 172 vineyards in 23 counties (Mendocino, Napa, Sonoma, Marin, Yolo, Solano, Sacramento, El Dorado, Amador, San Joaquin, Madera, Stanislaus, Fresno, Kern, Tulare, Riverside, Santa Clara, San Benito, Santa Cruz, Monterey, San Luis Obispo and Santa Barbara). Botrysophaeriaceae spp. were found in every grape-growing area surveyed and were the main fungi recovered from cankers in 17 out of 23 counties. *E. lata* was the only fungus isolated in a higher percentage than Botryosphaeriaceae in Napa, Sacramento, Yolo, Solano, Stanislaus and San Joaquin counties. In addition, a small percentage of both *E. lata* and Botryosphaeriaceae spp. were isolated from the same canker in California vineyards.

It is important to note that Phomopsis viticola was the principal fungus found in cankers from Fresno and Tulare counties. We have yet to initiate a project there to evaluate the significance of this finding. The fungi associated with esca (black measles), *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, were also isolated in a low percentage in this study. These pathogens produce different types of internal symptoms and were not a target of this work.

To date, 10 Botryosphaeriaceae species have been associated with the wedge-shape canker symptoms in California: *Botryosphaeria dothidea*, *Diplodia seriata*, *Diplodia mutila*, *Diplodia*

*corticola*, *Lasiodiplodia theobromae*, *Neofusicoccum parvum*, *Neofusicoccum luteum*, *Neofusicoccum australe*, *Dothiorella iberica* and *Spencermartinsia viticola*.

Recent studies conducted in our laboratory showed all 10 Botryosphaeriaceae spp. were able to infect both young and mature tissues, as well as green shoots of the new vegetative growth, causing cankers, vascular discoloration and/or otherwise dark streaking of the wood. However, virulence varied among species. *Lasiodiplodia theobromae* was the most virulent species followed by *Neofusicoccum luteum*, *N. parvum* and *N. australe*, all categorized as highly virulent. Botryosphaeria dothidea was considered intermediately virulent and *Diplodia mutila*, *D. corticola*, *D. seriata*, *Dothiorella iberica* and *S. viticola* were shown to be less virulent.

Botryosphaeriaceae species infect grapevines through pruning wounds. The seasonal abundance of Botryosphaeriaceae spores was studied in nine different locations throughout California. Spore traps placed on grapevine cordons and Burkard volumetric spore traps placed within the vineyards were used to determine when and under what environmental conditions Botryosphaeriaceae spores were released in California. During the period of study, spore discharge of Botryosphaeriaceae occurred from the first fall rain through the last spring rains in California vineyards. However, the highest numbers of spores were trapped following rain events during the winter months of December, January and February, which correlates with the grapevine pruning season in California. Botryosphaeriaceae spore release was much lower in fall and early spring, and very few or no spores were trapped in late spring and summer. In addition to rainfall, overhead irrigation triggered spore release, beginning less than one hour after event onset and ending within two hours after event end.

## *Other wood decay fungi*

Besides *Eutypa lata* and Botryosphaeriaceae spp., several other fungi were recovered from cankers of grapevines. These include *Phomopsis viticola*, *Phaeomoniella chlamydospor* and *Phaeoacremonium aleophilum*. Others, such as *Diatrypella* sp., were also found in the margins of cankers. Both Phaeomoniella and Phaeoacremonium are known to cause esca and vine decline disease in grapevines. These fungi are ubiquitous in California vineyards, and the pycnidia of *Phaeomoniella chlamydospora* and the perithecia of *Togninia minima* and two other species of Togninia have been found on grapevine pruning wounds.

Recently, Diatrypaceae fungi have been isolated from cankered wood of grapevines (Vitis vinifera L.) in California. Overall, we isolated 12 species of Diatrypaceae from the wood of diseased grapevines: *Cryptosphaeria pullmanensis*, *Cryptovalsa ampelina*, *Diatrype oregonensis*, *D. stigma*, *D. whitmanensis*, *Diatrype* sp, *Diatrypella verrucaeformis*, *Eutypa leptoplaca* and four putative species of *Eutypella*. *Eutypa leptoplaca*, *D. stigma* and *C. ampelina* were found to be pathogenic on grapevines. Species of *Cryptosphaeria*, *Diatrype*, *Diatrypella*, *Eutypella*, and *Cryptovalsa* belong to the same fungal family as *E. lata* and closely resemble *E. lata* by their spore shape and size, and their appearance in culture. These fungi also have been found commonly on various host plants in the vicinity of vineyards.

With these results, it is evident that *E. lata* is not the only cause of grapevine canker diseases in California vineyards. More studies should be conducted to elucidate the impact of the different species of Botryosphaeriaceae, as well as the other fungi detected in cankers. The virulence of many of these species remains to be determined.

These recent discoveries have led to the conclusion that grapevine trunk diseases are more complicated than initially thought and that a complex of fungi is obviously involved. Development of information regarding the biology, epidemiology and control of each of these fungi and diseases is underway. Nevertheless, vineyard sanitation through the removal of infected parts of vines is highly advised, as is sanitation of surrounding areas where other potential hosts of these pathogenic fungi reside.

## Occurrence of canker pathogens in Missouri and Arkansas

Grapevine cankers and consequent dieback have been recently observed in Missouri and Arkansas grape-growing regions. However, identity of the grapevine canker-causing agents occurring in both states has not been reported. Between 2007 and 2009, diseased grapevine samples showing perennial cankers from eight vineyards in Arkansas and 20 in Missouri were collected and inspected for fungal identification. A total of 70 samples from Arkansas and 190 samples from Missouri showing typical wedged-shape cankers and vascular streaking were collected. Samples were collected from the most predominant grapevine cultivars in both states, including Concord,

Chambourcin, Norton, Vidal Blanc, Niagara, Vignoles, Catawba, Rougeon, Chardonel, Cabernet Franc and Cabernet Sauvignon.

Morphological identification along with phylogenetic analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA), a partial sequence of the beta-tubulin gene (BT2), and a partial sequence of the elongation-factor gene (EF1) showed that at least 15 different fungi occur on grapevines in both Arkansas and Missouri. These fungi comprised six Botryosphaeriaceae spp. (*Botryosphaeria dothidea*, *Diplodia seriata*, *Lasiodiplodia theobromae*, *Neofusicoccum parvum*, *Neofusicoccum vitifusiforme* and *Dothiorella sarmentorum*), three Diatrypaceae spp. (*Eutypa lata*, *Diatrypella* sp., and *Eutypella vitis*), two Basidiomycete spp. (*Schizophyllum commune* and *Trametes versicolor*), *Phaeomoniella chlamydospora*, *Togninia minima*, *Phomopsis viticola* and *Pestalotiopsis* sp. All 15 fungi were isolated from vineyards in Missouri, and all but *D. seriata*, *L. theobromae*, *N. vitifusiforme*, *D. sarmentorum* and *E. lata* were isolated from vineyards in Arkansas.

Botryosphaeriaceae spp. were the most prevalent fungi isolated from wedge-shaped cankers in both Arkansas and Missouri vineyards, followed by *Pestalotiopsis* sp., *Diatrypaceae* spp. and *Phomopsis viticola*. *Phaeomoniella chlamydospora* and *Togninia minima* were the most prevalent species isolated from vascular-streaking symptoms of the wood. *Pestalotiopsis* sp., *Botryosphaeria dothidea* and *Neofusicoccum parvum* were isolated from streaking of the wood as well. Pathogenicity studies to determine the virulence of all fungal species isolated from cankered wood in Arkansas and Missouri is currently underway on Chambourcin, Norton, Vignoles and Traminette dormant rooted cuttings.

## Control

### *Pentra-Bark*

Pentra-Bark (Agrichem Manufacturing Industries) is a superior nonionic wetting agent designed for fast-spreading, uniform distribution and absorption of spray on leaf and stem surfaces and has been used in combination with systemic fungicides. Preliminary results obtained in our laboratory have shown that Pentra-Bark can transport fungicide residues into the vascular cambium at concentrations that can inhibit the growth of *E. lata*. Accordingly, we continued testing

Pentra-bark in mixtures with various fungicides for efficiency in spray application and to evaluate the control of pruning wound invading canker disease pathogens. Pristine (BASF Corporation Agricultural Products) has been shown to prevent infections caused by *Erysiphe necator* and *Botrytis cinerea* during the growing season and has been shown to increase plant health through currently unknown means. Therefore, Pristine was tested to determine its ability to mitigate the severity of esca by use in early spring and summer applications.

## *Dormant fungicide*

This was the second year of dormant fungicide trials conducted in a Chardonnay and Cabernet Sauvignon vineyard in Napa County. Grapevines were prepruned to the second trellis wire and sprayed within 12 hours after pruning to the point of drip with single applications of Enable 2F, Rally 40W, Topsin M, as well as a combination of all three. All treatments were amended with Pentra-Bark at a high label rate of 16 mL/L to ensure maximum penetration of the cork cambium. An untreated control was reserved for statistical comparison and treatments were arranged in a split-plot design. Pruning wounds were separately inoculated two days after treatment with the pathogens *Lasiodiplodia theobromae*, *Eutypa lata*, *Phaeoacremonium aleophilum* (*Pal*) and *Phaeomoniella chlamydospora* (*Pch*) by placing 25 µl spore suspension (200 spores/µl) for each fungi on the pruning wound. Inoculated canes were allowed to remain on the vine, then collected before bud-break and brought to the laboratory for isolation of pathogens and determination of extent of vascular invasion. Fungi were identified morphologically, and the incidence of infection was determined for each treatment/pathogen combination.

**In-season fungicide:** Three nondormant foliar applications of Pristine at a high label rate of 172 g/100 L (23 oz/acre) were tested. Each vine was injected with 10 ml of $10^5$–$10^6$ spore suspension, and symptoms were visually rated within the same growing season. Experimental design was completely randomized, and the data was analyzed using Analysis of Variance (ANOVA).

## *Evaluation of cultural practices to reduce infection by wood fungal pathogens (double pruning)*

Botryosphaeriaceae spp. are wood pathogens infecting grapevines mainly through conidial deposit on pruning wounds. Therefore, infections of fresh pruning wounds appear to lead to new infections and, subsequently, the development of perennial cankers that rapidly kill portions of the

vine. Double pruning has recently been shown to be an effective cultural practice to reduce pruning wound infection by *E. lata*. However, pathogenicity studies conducted by our laboratory have shown *Botryosphaeria* spp. to grow much more rapidly, being able to colonize wood tissue at least three times faster than *E. lata*. For this reason, we continued to study the efficacy of double pruning to reduce infection by Botryosphaeriaceae. Trials were established in a mature Chardonnay vineyard and a mature Cabernet Sauvignon vineyard in Napa Valley. Grapevines were prepruned to the top trellis wire and separately inoculated with a 105 spores/mL spore suspension of *Lasiodiplodia theobromae* and *Neofusicoccum parvum* during both dormant seasons 2007–08 and 2008–09. These fungi were selected in this experiment because they were shown to have the fastest growth rate among all Botryosphaeriaceae spp. found in California. Prepruning and inoculations occurred at mid-month in October, November, December, January, February and March. In both seasons, canes were collected the second week of March and brought to the laboratory. Extent of the vascular discoloration was measured for each pruning/inoculation time in order to evaluate the progression of the pathogen and risk of cordon infection using double pruning.

**Fungicide treatments:** Fungicides reduced *L. theobromae* and *Pal* incidence significantly on Chardonnay grapevines. Rally 40WSP was the most effective treatment on *L. theobromae* and *Pal*. The untreated control vines were uninfected on Cabernet Sauvignon grapevines, so there was no statistical separation. Fungicides significantly reduced the incidence of all canker pathogens in Trials 2 and 3 on Chardonnay grapevines. Similar results were found on Cabernet Sauvignon grapevines. Results from 2008 and 2009 were comparable. Enable + Rally + Topsin (ERT) was the most effective treatment for all pathogens, although Rally alone was as effective against *E. lata* as ERT. Topsin performed better than the FRAC G1 fungicides against L. theobromae.

Fungicides reduced *L. theobromae* and *E. lata* incidence significantly on Chardonnay grapevines. Incidence was zero for *Pal* and *Pch* in Trial 4. Results from 2008 and 2009 were comparable: There was a low level of canker pathogen incidence on Cabernet Sauvignon grapevines. There was zero canker pathogen incidence on canes treated with Rally + Topsin.

Among standalone fungicides, Topsin was most effective against *L. theobromae*, and Rally was most effective against *E. lata*. Incidence of all canker pathogens was higher in canes inoculated in January compared with canes inoculated in February. Fungicides significantly reduced canker

pathogen severity. Rally + Topsin was significantly more effective than Rally alone against *L. theobromae*.

**Pruning time:** For all canker pathogens, incidence increased significantly from November to December, then decreased significantly each month through March. Fungicides reduced canker pathogen incidence to zero in each month for which isolations were made.

**Pruning wound age:** For all canker pathogens, incidence decreased significantly from inoculations one day to 21 days after pruning. Fungicide reduced canker pathogen incidence to zero except for *L. theobromae* and *E. lata* inoculated seven days after pruning.

**Phytotoxicity:** Incidence of bud failure was similar for all treatments in Trials 1 to 7. There was overlap among all 95-percent confidence intervals for the mean bud failure incidence for each treatment within each trial. This indicates that the fungicides did not cause injury to the grapevines.

## Conclusion

Since *E. lata* was first identified in California in 1975, canker formation and subsequent dieback of grapevines have been attributed mainly to Eutypa dieback in the state. However, in the past few years, our research has shown that dieback of grapevines in California is a much more complex situation than originally thought, and grapevine cankers can be caused by at least 20 different fungi in the Diatrypaceae, Botryosphaeriaceae and Valsaceae families. Furthermore, our studies have indicated Botryosphaeriaceae fungi to constitute the main pathogens isolated from grapevine cankers statewide. *In vitro* pathogenicity studies in the laboratory as well as *in vivo* studies in commercial vineyards have shown all Botryosphaeriaceae spp. to be pathogenic. Moreover, four out of the 10 Botryosphaeriaceae spp. found in California appeared to be much more pathogenic than *E. lata*. In addition, species of *Eutypella* and *Phomopsis viticola* were commonly isolated from diseased vines from the table and raisin grape-growing regions of Southern San Joaquin Valley and Coachella Valley. Preliminary pathogenicity tests have suggested that these fungi constitute new pathogens in table grape areas capable of colonizing wood and producing cankers. Identification work suggested that these fungi may constitute new species, and more work is being conducted to characterize these fungi.

Spore trapping studies conducted for the family Botryosphaeriaceae have allowed us a better understanding of the epidemiology of this new group of pathogens in California. Spore trap studies have shown Botryosphaeriaceae spores to be mainly trapped following rainfall events and overhead and/or drip irrigation. Botryosphaeriaceae spores were trapped frequently after the first rainfall in September-October to March-April. These studies have allowed us to characterize low infection risk periods throughout the growing season, thereby improving appropriate timing periods for pruning. Results from the spore-trapping study conducted in Coachella Valley showed a high incidence of *Eutypella* spp. In this case it was documented that spore release occurred during sprinkler and drip irrigation. Surveys for the perithecia of Botryosphaeriaceae in California have shown various grapevine cultivars with perithecia of *B. dothidea*, suggesting that the sexual stage could also play an important role in the epidemiology of the disease. More work is being done to understand the role of native and ornamental trees adjacent to vineyards in the epidemiology and disease cycle of canker diseases.

Our laboratory has developed chemical, cultural and organically acceptable control methods to reduce infections caused by these fungi. Double pruning was shown to be an effective cultural practice that completely eliminates canker formation by *Diatrypaceae* spp. We have also shown that double pruning is effective against the Botryosphaeriaceae fungi and the esca fungi as well. Dormant application of Rally alone or in combination with Enable (another DMI fungicide) and Topsin M with or without a bark penetrant reduced infection by *E. lata* and *Botryosphaeria* spp. Also, Rally treatments significantly reduced *Phaeoacremonium* infection. More work is being done to evaluate single and combined applications of different active materials to control canker diseases. Finally, dormant application of fungicides with a penetrating surfactant was not a significant phytotoxicity hazard to grapevines.

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# Target Your Sprays and Save Money: Methods of Improving Deposition and Reducing Drift

**Dr. Andrew Landers**
New York State Agricultural Experiment Station
Cornell University

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## Introduction

Application of pesticides, particularly methods of reducing drift and improving deposition, has been of concern for many years. There are many interrelated factors that affect spray application depending upon such factors as the target, the efficacy of the spray, the attitude of the operator, the standard of management and the weather (Figure 1).

The operation of the sprayer often leaves much to be desired. Most growers know the three factors that affect application rate — forward speed, nozzle size and system pressure — but often overlook the factors that get the spray onto the target — airflow, liquid flow, forward speed and canopy structure. Progress lies in a better understanding of the factors involved in getting the spray from the tank to the vines. Adjusting both airflow and liquid flow are the key factors to match the growing canopy as the season progresses.


**Figure 1. Interrelated factors affecting spray application.**

## Airflow

Airflow is an extremely important part of the application process, and excessive air speed and volume are responsible for spray drift. The purpose of air is to carry the droplets from the nozzles to the target as well as create a small amount of turbulence within the canopy to aid penetration. Too much air blows the spray through the canopy onto the ground or into the air (drift) or dislodges

the droplets previously deposited into the canopy when the other side of the row was sprayed. Many vineyard sprayers use some form of air assistance from fans, which are frequently too large for modern, well-pruned training systems, as the large-diameter fans create too much air for the target canopy. The ideal air volume should match the canopy volume. Canopies vary along the row; sometimes vines are missing, presenting no resistance to air movement, resulting in air traveling through the target row and away. Air speed and volume need to be adjustable according to the growth stage of the canopy (Balsari et al. 2001, 2005; Perger 1995, 2005). There are a number of simple methods a grower can adopt to do this, such as changing PTO speed, fitting an air-limiting system to the air intake or outlet, or using a variable speed hydraulic motor drive to the fan.

**Figure 2. Cornell doughnut outlet.**

**Figure 3. Adjustable louver on the air outlet.**

Trials with various types of vineyard sprayers have been conducted at Cornell University to study how changes in fan speed affect air speed, volume and direction (Landers 2000, 2002, 2005, 2008; Farooq and Landers 2004; Landers and Gil 2006). For some years I have shown growers that reducing airflow via reduced air intake design will improve deposition in the canopy and reduce drift. A simple device, christened the Cornell doughnut, is made of plywood or metal (Figure 2). It is the same size as the fan, with a hole at the center to cut about one-third, one-half or two-thirds of the air intake. The doughnut reduces air intake, and the grower can select the larger holes as the canopy develops.

We have also recently developed an adjustable air outlet for both air blast and tower sprayers (Figure 3). An electric actuator moves an adjustable louver, allowing the operator to change air volume to match the changing canopy and reduce drift by as much as 71 percent in vineyards in early-season application. Where the air blows, the droplets will surely follow. Therefore, if drift is

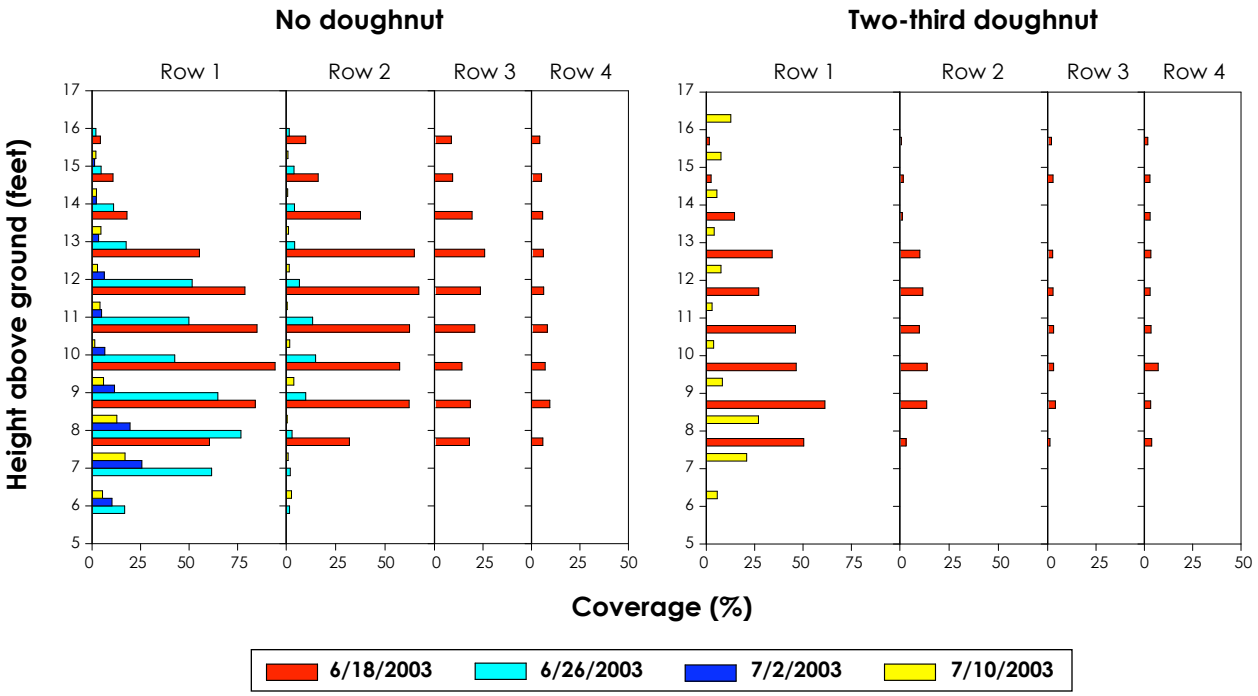



**Figure 4. Drift reduction using a "doughnut" air restrictor.**

reduced, deposition within the canopy must be improved. Modifying air flow at the air intake or outlet has resulted in up to 30 percent improvement in canopy deposition (Landers and Farooq, 2004) (Figure 4).

Traditional air-blast sprayers using a fan rotating in a counterclockwise direction move air downwards on the left side of the sprayer and vice-versa on the right side. This often results in a large plume of spray going upwards and outwards on the right side of the sprayer and uneven application within the canopy. This also does nothing to help public perception of the application of pesticides to fruit crops!

Air-blast sprayers fitted with towers, adjustable air outlets or multihead fans provide better airflow characteristics and, therefore, better deposition into the canopy than do traditional designs. In trials, Landers (2002, 2008b) has shown up to 30 percent better deposition throughout the canopy by using tower sprayers. Adjustment of top and base deflector plates on traditional air-blast sprayers should also be carried out to direct the air toward and confine it to the target canopy. Sets of air deflectors were developed for both traditional air-blast sprayers and Kinkelder style sprayers (Figures 5 and 6) (Tables 1 and 2).

Adjusting the airspeed can considerably improve deposition of pesticides. Field trials were conducted using a sprayer fitted with air shear nozzles operating at two fan speeds, 2076 rpm (540 rpm PTO) and a 25 percent reduced speed of 1557 rpm (405 rpm PTO). Drift was detected using water-sensitive cards and then analyzed using image analysis software. At the higher fan speed of 2076 rpm, drift was detected up to 80 feet from the target row where 10 percent card coverage occurred. Reducing fan speed by 25 percent with a slower PTO speed resulted in considerably less drift, with card coverage at 24 meters (78.74 feet) being 0.20 percent.

A number of manufacturers now offer adjustable airflow. For example, some adjust the airflow by changing fan blade pitch or by altering hydraulic or electricity flow to multihead fan sprayers.

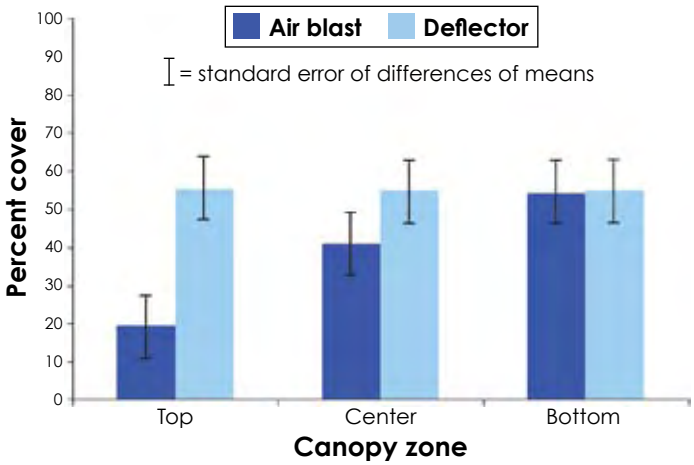
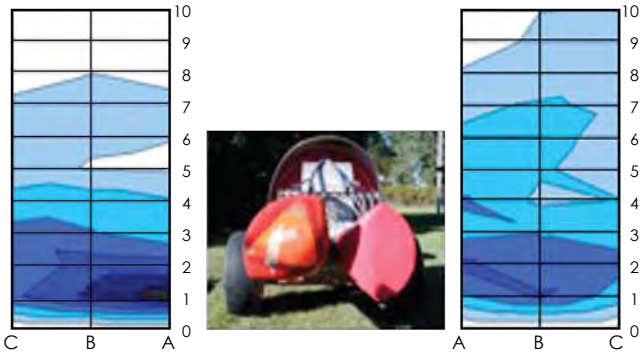


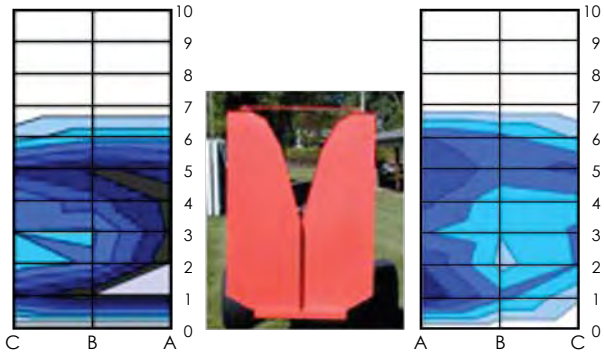

**Figure 5. Improved deposition throughout the canopy using a deflector or tower.**

**Kinkelder Conventional**




| | Left | Right |
|---|---|---|
| **Average** | 9.00 | 9.54 |
| **Std dev** | 8.08 | 5.38 |
| **CV (%)** | 89.76 | 56.39 |
| **Max** | 32.93 | 21.17 |
| **Min** | 0.39 | 0.68 |

**Kinkelder Deflectors**




| | Left | Right |
|---|---|---|
| **Average** | 17.93 | 12.56 |
| **Std dev** | 13.42 | 8.75 |
| **CV (%)** | 74.87 | 69.72 |
| **Max** | 43.01 | 29.90 |
| **Min** | 0.64 | 0.47 |

**Figure 6. Airflow and deposition with a Kinkelder sprayer with a deflector.**

## Liquid flow and canopy structure

The two main aspects to consider when applying liquids are the volume of product and the volume of water. Many growers typically apply X gallons per acre prebloom and then Y gallons per acre postbloom with the intention of getting good leaf coverage. Unfortunately, poor spray coverage is a major factor contributing to poor insect and disease control. Better coverage leads to better control, and a thorough application of an effective material is required. Uneven coverage increases the amount of pesticides that must be applied in order to provide adequate control on poorly covered areas and can increase the number of sprays required if it allows insects or diseases to become established. Applying the correct amount of spray at the correct time to the correct target is good practice.

Canopy size and shape will affect application volume, and there are as many dangers in not applying enough spray as there are in applying too much. There is an optimum quantity required for thorough coverage of the target. The old adage that you should spray until the leaves drip is misplaced. Likewise, lowering spray rates to below the minimum that offers control is also misguided advice. Increasing spray application volumes leads to higher losses to the ground and lower deposition on foliage.

The tunnel or recycling sprayer provides the ultimate in both drift control and canopy sensing. As only the vine and foliage intercepts the spray it requires, excess is returned to the tank, providing savings of up to 75 percent in spray use in early season and an average of about 30 percent over the whole season.

A number of new techniques to assess canopy volume or area are being investigated or practiced. The Unit Canopy Row (UCR) method from Australia, which uses canopy volume, and the Leaf Wall Area (LWA) method from Europe (Koch 2007) are both recently devised methods being used to assess the volume of liquid required to give satisfactory coverage without applying and transporting vast quantities of liquid around the vineyard. In New York State, we have conducted field trials for three seasons using the computer-based planning method Dosavina, which was developed originally in Spain (Gil 2006) and modified for New York conditions (Landers and Gil 2007). Dosavina is based on multiple data obtained over several years in real working conditions

using different types of sprayers in vineyards, and by adding a complete database about crop characteristics, such as structure, crop stage, leaf area and Leaf Area Index. The objective of this work has been to develop an easy and useful tool, based on a Microsoft Excel spreadsheet, that takes into consideration all the parameters involved during the application process. By selecting and choosing the different options in the different files (crop, chemical, working conditions, weather conditions, sprayer and droplet characteristics) the program calculates the theoretical volume rate (gpa) based on two different methods (optimal coverage and Vine Row Volume), showing the final result of real volume to apply by adding the R value. In order to make a complete and useful tool, the program includes the possibility of calculating the working parameters (pressure, nozzle type and size) according to the recommendations on volume rate (gpa) obtained.

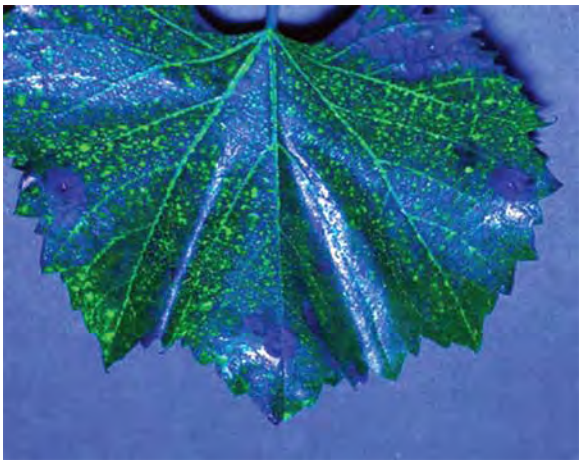


**Figure 7. Coverage – 35 gpa at 3 mph.**

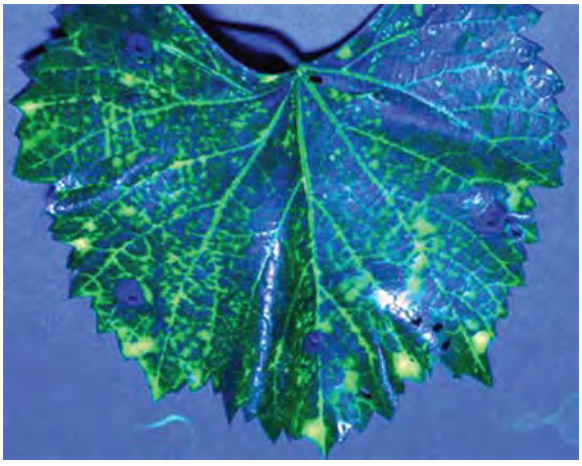


**Figure 8. Coverage – 75 gpa at 3 mph.**

Trials were conducted on three varieties at vineyards belonging to three cooperating growers, two in the Finger Lakes region and one in the Lake Erie region of New York State. The savings in pesticide use, particularly in early season, were quite substantial. Average seasonal savings in pesticide use were 40 percent in 2006 and 32 percent in 2007, representing a total saving of $125 per hectare (Landers and Gil 2007, 2008).

What is the optimum volume to be applied per acre? The aim of good pesticide application is to provide more small-to-medium droplets that will stick effectively to the leaf surface, considering each vine canopy is different due to variation in growth stages, varieties, trellis system, canopy microclimate and other factors that can be determined by close observation of the canopy. Growers

can use a variety of safe methods to determine the optimum volume to be applied using clean water with:

- Water sensitive cards and strips attached to the leaves with paperclips or staples, as long as the canopy is dry and the user wears rubber gloves. They are quite expensive but show exactly where water droplets have hit either on upper or lower leaf surfaces and how close the water deposits are to the grape cluster.
- High-quality photographic paper cut into 2-inch by 1-inch strips attached to the leaves and used in conjunction with a readily available kitchen food dye. Quality photo paper made for printing digital photos can be purchased at office supply shops. Alternatives include plain glossy business cards or file cards.
- Surround as a tracer**.** Surround, an organic insecticide based upon Kaolin clay, is highly visible on all green leaves and grapes. It should be premixed in a bucket before putting into the spray tank, otherwise it will cause blockage in the filters. Keep tank contents agitated. The spray will dry rapidly on a summer day, and in about 10 minutes you will see all the droplets over the leaves and grapes.
- Fluorescent tracers and an ultraviolet **(**black-light) lamp provide an excellent means for visualizing the deposition of spray droplets (Figures 7 and 8). Figure 8 also shows excessive application leading to run-off, resulting in fewer products being retained on the leaf. Growers have to wait until dark to see the droplets in the canopy or remove leaves to view in a darkened area. Information and vendors of inexpensive black lights are available on the Internet.

## Nozzles

When applying pesticides, growers know that small or fine/medium droplets give the best coverage, as large droplets, in excess of 300 μm, will bounce off the leaves onto the ground. Good coverage is critical for all contact pesticides. Unfortunately, small or fine droplets (less than 150 microns) are drift-prone if they do not become attached to the target leaf, insect or clusters. Directed deposition is needed if the pesticide is to be applied to the target zone. Drift results in damage to susceptible off-target crops, environmental contamination to water resources, and an unintentionally

reduced rate of application to the target crop, thus reducing the effectiveness of the pesticide. Pesticide drift also affects neighboring properties, often leading to public outcry. Air-induction nozzles can be used in the canopy sprayer to reduce drift considerably. They can reduce drift occurring from as far as seven rows away down to one or two rows and are ideal when spraying next to sensitive areas.

The orientation of nozzles can also be adjusted on many sprayers to reduce drift. For example, nozzles set in the "typical growers" pattern, that is, pointing radially outwards, resulted in a large quantity of liquid being blown above the target row. The quantity overshooting the target varies according to canopy height, density and size and speed of the fan. There is a great imbalance of distribution between the left and right sides of the sprayer due to the airflow characteristics and nozzle orientation. When nozzle orientation is adjusted for differences in fan rotation, a 20 percent improvement in spray deposition in grapevines can be achieved (Farooq and Landers 2004).

To assist in adjusting the nozzles, a vertical patternator can be used. The Cornell University patternator comprises nine 14-inch by 48-inch wide fly screens connected via hooks to two 14-foot-high, 4-inch by 2-inch wooden boards. A small gutter is attached, at an angle, to the bottom edge of each screen. The gutter slopes to one end, where a plastic hose connects it to a box containing graduated measuring cylinders. The sprayer tank is filled with clean water, the patternator is placed at the end of a row, and the sprayer is operated but remains stationary. As the spray cloud hits the fly screen, air passes through and liquid runs down the front of the screen, into the gutter and then, via the plastic hose, into the collecting cylinders. Plans for the construction of the Cornell patternator are available on the Internet at http://www.nysaes.cornell.edu/ent/faculty/landers/pestapp/PATTERNATOR.htm.

The results show the importance of correct nozzle orientation for effectively applying pesticides onto the target. It should be noted that each sprayer design will vary, due to fan size and air volume, so no blanket recommendation can be made.

The patternator is a very useful tool in both research and extension. In research, it allows us to make changes to the sprayer and see repeatable results compared to the original settings. In extension, it demonstrates to growers the quantity of spray plume going up and over the canopy; it shows the symmetry, or lack of it, between the left and right sides of the sprayer; and it also teaches

the importance of adjusting the sprayer correctly to improve deposition and reduce drift. More than 75 sprayers have been evaluated in the past six years, in both orchards and vineyards. One apple grower, for example, has adjusted nozzle orientation and reduced his pesticide use by 20 percent while maintaining the same coverage. Other growers choose to apply the full 100 percent of spray into the target area.

## The operator

The operator should follow standard practices such as correct filling routine, personal protective clothing and calibration. Videos of calibration of vineyard sprayers showing both measuring liquid flow and nozzle selection can be seen on the Internet at YouTube. The person who operates the sprayer needs to remain alert, checking changing weather conditions. The use of a hand-held anemometer is recommended. It is also advisable to participate in a one-day operator training class, which provides in-depth instruction on sprayer operation, a subject often neglected.

## Forward speed

The sprayer should be operated at a speed consistent with spray penetration into the canopy. Driving too slowly in a sparse, early-season canopy will result in spray blowing through the row; conversely, driving too fast in a full canopy results in poor penetration. Watching what is happening, along with checking on deposition as mentioned earlier, will result in the optimum speed.

## Automatic spraying

The ultimate variable-rate, fully automatic canopy sprayer may comprise many of the aspects described in this paper. The sprayer travels along the vine rows, monitoring either presence or absence of the canopy, plus canopy size and volume. Ultrasonic or infrared sensors monitor the dimensions of the canopy and thus alter both airflow output from the fan and liquid flow (application rate/acre) according to the variable canopy. Patches of diseases or insect activity may have been located previously by scouting the crop, and their exact location recorded on a handheld GPS device. Research has shown how the application rate of pesticides varies considerably with canopy volume and growth stage (Barber and Landers 2002; Koch 2007). In Riesling and Cabernet Franc varieties on a VSP trellis, for example, Landers and Farooq (2004) found the application rate

requirement of the canopy varies from 16 gpa in early season to 50 gpa in full canopy. To monitor the variation in the canopy, we have recently developed infrared sensors mounted on the sprayer to detect the missing vines and the height of the canopy, which in turn switch on and off nozzles corresponding to the canopy height. Infrared sensors provided a reduction in pesticide use of up to 40 percent in the first three sprays of the season (Table 3).

## Conclusions

Paying attention to every minor detail allows the operator to make adjustments to the sprayer. Changing airflow direction and volume not only improves deposition but also reduces drift. Novel techniques such as adjustable louvers allow air adjustment on the move and match airflow to the changing canopy. Measuring canopy volume and adjusting spray volume accordingly reduces spray use when applied with a correctly adjusted sprayer. Sensors can also adjust liquid flow to match canopy size and reduce spray use, particularly in early season when minimum foliage exists to intercept the spray. An air-induction nozzle reduces the spray drift. As with all farm operations, spraying requires thorough preparation, attention to detail and constant vigilance if mistakes are to be avoided and an efficient application is to be made.

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**Table 1. Differences in foliar powdery mildew infection on Concord grapes in Pennsylvania, 2006.**

| | Leaf infection* |
|---|---|
| **Treatment** | **Percent leaf area** |
| New deflector head | 26.6 a |
| Old design | 32.2 b |

* Values represent the means from four replicate plots per treatment, 25 leaves per plot. Means not followed by a common letter are significantly different according to Fisher's Protected LSD test (P≤0.05) performed on Barratt-Horsfall ratings (% area). Converted values are presented.

**Table 2. Differences in cluster powdery mildew infection on Concord grapes in Pennsylvania, 2006.**

| | Cluster infection* | |
|---|---|---|
| **Treatment** | **Percent clusters** | **Percent area** |
| New deflector head | 68.8 a | 2.3 a |
| Old design | 75.0 a | 1.6 a |

* Values represent the means from four replicate plots per treatment, 20 clusters per plot. Means not followed by a common letter are significantly different according to Fisher's Protected LSD test (P≤0.05) performed on arcsine square-root transformed data (% clusters) or Barratt-Horsfall ratings (% area). Nontransformed and converted values, respectively, are presented. (Landers, A.J. and W. Wilcox, *Viticulture Consortium Report 2006*)

**Table 3. Reduced spray use with infrared sensors.**

| Infrared sensor trial 2009 | Reduction in spray use |
|---|---|
| Early season, June 3 | 40.0% |
| Mid-season, June 17 | 18.0% |
| Full canopy, July 6 | 0.3% |

# Studies on the Biology and Control of Phomopsis Cane and Leaf Spot

**Dr. Michael A. Ellis**
Ohio Agriculture Research and Development Center
Department of Plant Pathology
The Ohio State University

## Acknowledgements

The author thanks Dr. Wayne Wilcox of Cornell University, Geneva, N.Y., for providing much of the information presented in this review and the New York Agricultural Experiment Station for permission to use the *Disease cycle of Phomopsis cane and leaf spot* figure.

## Introduction

For many years, the grape industry in the Eastern U.S. recognized a disease called dead-arm, which was thought to be caused by the fungus *Phomopsis viticola.* In 1976, researchers demonstrated that the dead-arm disease was actually two different diseases that often occur simultaneously. Phomopsis cane and leaf spot, caused by the fungus *Phomopsis viticola*, is the name for the cane- and leaf-spotting phase of what was once known as dead-arm. Eutypa dieback, caused by the fungus *Eutypa lata*, is the new name for the canker- and shoot-dieback phase of what was once known as dead-arm. At present, the name dead-arm is no longer used. Growers should be aware that Phomopsis cane and leaf spot and Eutypa dieback are distinctly different diseases, and their control recommendations vary greatly.

Disease incidence of Phomopsis cane and leaf spot appears to be increasing in many vineyards throughout the Midwest (Erincik et al. 2005). Crop losses up to 30 percent have been reported in some of the vineyards in Ohio during growing seasons with weather conditions favorable for disease development. Phomopsis cane and leaf spot can affect most parts of the grapevine, including canes, leaves, rachises (cluster stems), flowers, tendrils and berries, and can cause significant loss in vineyards by:

- Weakening canes, making them more susceptible to winter injury

- Damaging leaves, which reduces photosynthesis
- Infecting cluster stems (rachises), which can result in poor fruit development and premature fruit drop
- Infecting berries, resulting in fruit rot at harvest

## Symptoms

Spots or lesions on shoots and leaves are common symptoms of Phomopsis cane and leaf spot. Small black spots on the internodes at the base of developing shoots are probably the most common disease symptom. These spots are usually found on the first three to four basal internodes. The spots may develop into elliptical lesions that may grow together to form irregular black crusty areas. Under severe conditions, shoots may split and form longitudinal cracks. Although cane lesions often appear to result in little damage to the vines, these lesions are the primary source of overwintering inoculums for infections during the next growing season.

Leaf infections first appear as small light-green spots with irregular, occasionally star-shaped, margins. Usually the basal four leaves on a shoot are affected. In time, the spots become larger, turn black and have a yellow margin. Leaves become distorted and die if large numbers of lesions develop. Infections of leaf petioles may cause leaves to turn yellow and fall off.

All parts of the grape cluster (berries and rachises or cluster stems) are susceptible to infection throughout the growing season; however, most infections appear to occur early in the growing season, from prebloom through shortly after bloom. Lesions developing on the first one or two cluster stems on a shoot may result in premature withering of the cluster stem. Infected clusters that survive until harvest often produce fruits of inferior quality.

If this disease is not controlled during the early part of the growing season, berry infection under favorable environmental conditions can result in serious yield loss. Symptoms of berry infections do not appear until close to harvest, at which time infected berries develop a light-brown color. Black spore-producing structures of the fungus (pycnidia) then break through the berry skin, and the berry soon shrivels. At this advanced stage, Phomopsis cane and leaf spot can be easily mistaken for black rot. Growers should remember that the black rot fungus only infects green berries and will not infect berries after they start to mature. Berries become resistant to black rot infection

by three to four weeks after bloom. Fruit rot symptoms caused by *Phomopsis* generally do not appear until close to harvest (veraison and later) on mature fruit. Severe fruit rot has been observed in several Ohio vineyards.

Research in Ohio has shown that berry and rachis infection can occur throughout the growing season (Erincik et al. 2001 and 2002). However, most fruit rot infections probably occur early in the season, from prebloom to shortly after bloom. Established fungal infections on fruits can be seen as soon as clusters appear after bud break very early in the spring. This fact helps to emphasize the importance of early season fungicide applications for effective control of this disease. Once the fungus establishes inside green tissues of the berry, it becomes inactive, or latent, and the disease does not continue to develop (Erincik et al. 2001). Infected berries do not exhibit any symptoms until fruit matures late in the season, at veraison or later. Thus, fruit rot that develops at harvest is probably due to infections that occurred prior to or during bloom.

## *Causal organism and disease cycle*

The fungus overwinters in lesions or spots on old canes or rachises infected during the previous growing season, and requires cool, wet weather (free water) for spore release and infection during early spring (Figure 1). The fungus produces flask-shaped fruiting bodies called pycnidia in the old diseased wood (canes or rachises). These pycnidia release spores in early spring and are spread by splashing rain droplets to developing shoots, leaves and clusters. In the presence of free water, the spores germinate and cause infection. Shoot infection is most likely during the period from bud break until shoots are 6 to 8 inches long. Research in Ohio has determined that the optimum temperature for leaf and cane infections is between 60 and 68 degrees Fahrenheit, and wetness duration (free water) of at least six hours is required at these temperatures. The chance of infection further increases with increase in wetness duration. A disease predictive system based on temperature and wetness duration in the vineyard has been developed and validated for the disease in Ohio (Erincik et al. 2003; Nita et al. 2006a).

Lesions on leaves appear at seven to 10 days after infection. Fully expanded leaves become resistant to infection. Lesions on canes require three to four weeks to develop. As cane tissues become mature, they also become highly resistant to infection. Recent research in New York, Ohio and Michigan indicates that the majority of spores are produced and released very early in the

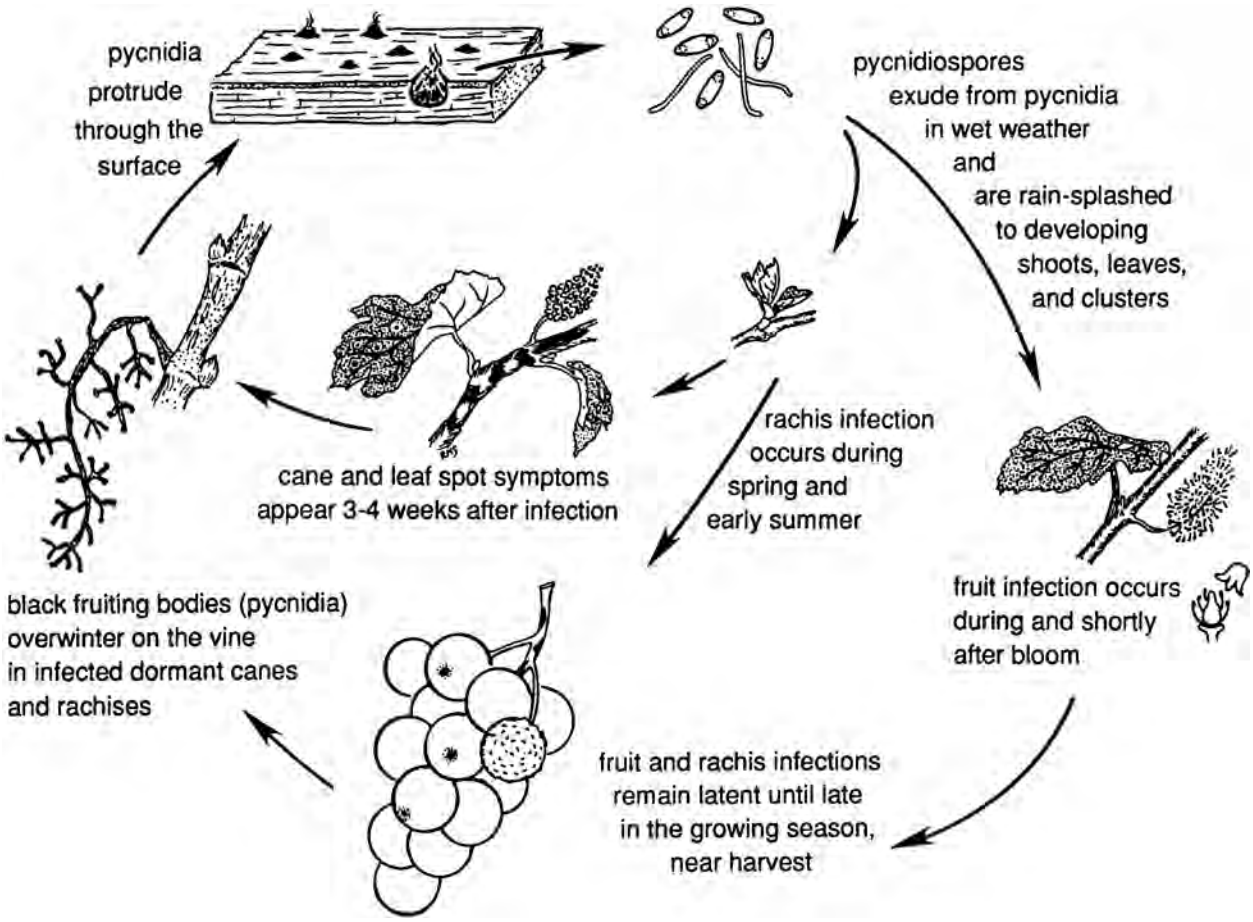


**Figure 1. Phomopsis cane and leaf spot.**

growing season. It appears that the vast majority of spores are released from bud break through bloom and that few, if any, spores are left by early to mid-July. This is probably the reason that almost all infections appear only on the first three to four internodes of the cane and the leaves on those internodes. Although young leaves and cane tissues are susceptible to infection throughout the growing season, no spores are left to infect them later in the growing season. If there are not enough spores in the vineyard (inoculum), then there is no chance of infection. This is another reason early season fungicide sprays are critical for controlling the disease.

One very important aspect of this disease that most growers do not realize is that Phomopsis cane and leaf spot is what we call a monocyclic disease. Monocyclic is a pathological term that means the fungus does not produce spores, or secondary inoculum, on current-season infected tissues. The fungus within cane and rachis lesions and leaf spots that develop on tissue infected

during the current growing season will not produce pycnidia and spores. Thus, secondary infections do not occur later in the growing season. This is unlike most of the other important grape diseases, such as black rot, downy mildew and powdery mildew. When these fungi infect grape tissues and form lesions, the fungus sporulates (produces spores) in the infected tissues, which can cause additional infections throughout the growing season. We call these types of diseases polycyclic. Polycyclic diseases can have many infection cycles throughout the current growing season, which is why they often appear to explode later in the growing season, especially in wet years. The fact that Phomopsis cane and leaf spot is a monocyclic disease makes it somewhat easier to control and further emphasizes the importance of early season fungicide application for effective disease control.

For unknown reasons, the fungus that causes Phomopsis cane and leaf spot is not capable of producing pycnidia and spores until after cane tissues harden off in the fall. The fungus overwinters inside infected canes and produces pycnidia and spores to cause new infections the following spring. This completes the disease cycle.

Although the fungus does not appear to be capable of producing spores on living tissues of current season infections, research in New York has shown that the fungus can sporulate throughout the growing season on old dead wood and pruning stubs. For this reason, sanitation is a very important part of the overall disease management program. Selective dormant pruning to remove infected canes and dead wood, including old pruning stubs, from the trellis aids greatly in disease control.

## Disease management

### *Site selection*

Select planting sites that receive adequate sunlight throughout the day and provide good soil drainage and air circulation. Avoid sites with excessive shade. Orient rows to take full advantage of sunlight and wind movement. Cultural practices that increase air circulation and light penetration in the vineyard will reduce wetting periods and should be beneficial for control of this disease.

## *Sanitation*

During dormant pruning, remove and destroy all infected canes. Select only strong, healthy canes that are uniform in color to produce next season's crop. Cut out old dead wood and pruning stubs. Although the fungus does not produce spores on infected canes during the first year of infection, it can produce spores on older dead wood for years and may produce spores on older dead wood late in the growing season. Removal of older dead wood and pruning stubs is very important for successful control.

## *Dormant applications of fungicide*

Over the past several years, many Ohio growers have asked questions regarding dormant applications of fungicide for disease control in grapes. From 2003 through 2005, we conducted several evaluation studies on dormant applications of liquid lime sulfur and fixed copper (copper hydroxide, COCS) for control of Phomopsis cane and leaf spot on grapes. We applied lime sulfur at 10 gallons per acre and copper at 3 pounds per acre in 100 gallons of water per acre. We made applications in the fall after leaf drop, in the spring at bud swell, and at both times.

Our results indicate that both lime sulfur and copper applied in the spring result in a significant reduction of Phomopsis leaf and internode infection in the growing season (Nita et al. 2006 and 2007b). Lime sulfur was more effective than copper. There were no differences in disease control between the spring-only application and the fungicide application done during both spring and fall. Applications in the fall only were not effective. Although we observed a significant level of disease control — about 28 percent and 70 percent reduction in disease incidence and severity, respectively — we never achieved 100 percent control of Phomopsis with the dormant application. Therefore, the dormant application did not reduce the need for fungicide applications for Phomopsis control during the season.

The following comments are based on over two years of research:

1. Dormant applications of lime sulfur or copper will provide some degree of Phomopsis control but will not reduce the need for the standard recommended fungicide sprays for Phomopsis control during the growing season. We have no evidence to indicate that the dormant applications are effective against any of the other grape diseases.

In short, they could help, but if you have a good fungicide spray program during the growing season, they probably will not result in much of an increase in disease control at the end of the season. The bottom line is that if you have a good spray program and your vineyards are reasonably clean, you probably do not need a dormant application of fungicide in the spring. I do not recommend a dormant application of fungicide in the fall for disease control.

2. I do recommend the use of dormant applications of lime sulfur in the following situations:
   a. In organic vineyards, this should be an important spray.
   b. In vineyards where control of Phomopsis is getting out of hand, this spray should be considered. In some Concord vineyards that are mechanically pruned, Phomopsis incidence is increasing. A dormant spray of lime sulfur would probably be beneficial here, but the economics on Concord need to be considered.
   c. For wine grape vineyards where the level of Phomopsis infection is severe, the dormant spray should be considered. A dormant application of lime sulfur in the spring will aid in disease control when combined with effective sprays during the early part of the growing season. The economics of these dormant applications are questionable. In other words, the level of control you get may not be worth the cost of its application. Over the past several years, we detected some level of Phomposis in almost every vineyard we inspected. And in our studies, the dormant application of lime sulfur plus a good full-season spray program has never resulted in 100 percent control of Phomopsis. Therefore, in my opinion it is probably not realistic to expect 100 percent control of Phomopsis on internodes even with a good full-season spray program.
   d. If anthracnose is present in the vineyard, a dormant application of lime sulfur at the rate of 10 gallons per acre is very important. This spray is the major means for controlling anthracnose. We have seen serious anthracnose in several Ohio vineyards over the past few years, mainly on Vidal and Reliance grapes.

In summary, a dormant application of lime sulfur — lime sulfur appears to be more effective than copper — in the spring is beneficial for control of Phomopsis and even necessary in some situations, as mentioned above; however, it is not a silver bullet that is going to reduce the need for a full-season fungicide spray program on wine grapes.

### *Properly timed early-season fungicide sprays*

Application of fungicides very early in the growing season is critical for successful control of Phomopsis cane and leaf spot, as fungicide timing trials in New York, Michigan and Ohio over the past several years have clearly demonstrated. The laboratory of Dr. Wayne Wilcox, professor of Plant Pathology at Cornell University in Geneva, N.Y., has conducted numerous fungicide timing trials for control of Phomopsis. Dr. Wilcox reported that early sprays are most important for control of rachis infections and that they also provide significant control against berry infections. He found that applications during early shoot growth period — as clusters first become visible, at about 3 inches of shoot growth — are the most important for control of this disease, especially on the rachises, and they also significantly controlled fruit rot and cane infections. Dr. Wilcox recommends that a minimal fungicide spray program for Phomopsis should include at least one application during the period soon after cluster emergence. His research has shown that waiting until the immediate prebloom spray is far too late if there is any significant disease pressure in the vineyard.

Fungicides such as mancozeb, captan and ziram provide good to excellent control of Phomopsis. The strobilurin fungicides Abound and Pristine also have some activity against Phomopsis; however, they certainly do not appear to have any advantage over mancozeb, captan or ziram, and they are much more expensive.

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# Developing an Effective Fungicide Spray Program for Wine Grapes in the Midwest (2010)

**Dr. Michael A. Ellis**
Ohio Agriculture Research and Development Center
Department of Plant Pathology
The Ohio State University

## Acknowledgment

The author thanks Dr. Anne DeMarsay of the University of Maryland for permission to use the *Resistance-prone fungicides and risk of resistance by chemical class* table.

## Introduction

The following information should be considered when developing a fungicide spray program for wine grapes in Ohio. This spray schedule presents various fungicide options. It is important to note that the schedule is intended to provide simultaneous control of black rot, powdery mildew, downy mildew and Phomopsis cane and leaf spot. The schedule is also intended to provide some level of fungicide resistance management, primarily against the powdery mildew and downy mildew pathogens. **Please pay close attention to the notes and comments.**

## Important note on powdery and downy mildew fungicide resistance

### *Powdery mildew*

In some locations, the powdery mildew fungus has developed resistance to the sterol-inhibiting fungicides (Rally, Rubigan and Elite) and the strobilurin fungicides (Abound, Sovran and Flint). All of these materials were highly effective for control of powdery mildew when they were first introduced. However, in vineyards where these materials have been used for several years, reduced sensitivity or resistance may be present. In some vineyards, all of these materials may still be effective, but at present there is no way to know the level of resistance in your vineyard. Having a control failure and crop loss due to fungicide resistance is a hard way to discover you have resistance. Reports from Virginia suggest that resistance may develop after as few as 10 applications of the

material over the life of vineyard. If these materials have been used in a vineyard on a regular basis for several years, growers should consider not using these materials alone for powdery mildew control. If resistance is of concern, these materials should be replaced or mixed with sulfur fungicide, JMS Stylet Oil, Quintec, Endura or potassium salts (Table 1). Pristine is a combination of a strobilurin fungicide and Endura; therefore, it should be safe to use alone for control of powdery mildew. Sulfur fungicides are very effective for control of powdery mildew, are relatively inexpensive and are not at risk for resistance development. On sulfur-tolerant varieties, the use of sulfur should be considered.

### *Downy mildew*

The strobilurin fungicides (Abound, Sovran and Pristine) provided good to excellent control of downy mildew when they were first introduced. Several reports from various areas in Europe and, most recently, from Virginia indicate that the downy mildew pathogen has developed resistance or is at least less sensitive to the strobilurin fungicides. Growers should consider not using strobilurin fungicides for downy mildew control. If these products are used to control other diseases and downy mildew control is also required, they should be tank mixed with another downy mildew–controlling fungicide. Alternative downy mildew fungicides include Mancozeb, Captan, Ridomil Gold MZ, Ridomil Gold Copper, Revus, Presidio, a copper fungicide or a phosphorous acid (phosphite) fungicide. Pristine still provides good control of powdery mildew when used alone and was the only material that would control almost all of our major diseases when used alone. Unfortunately, it should now be combined with a downy mildew fungicide when downy mildew control is required.

### *To aid in resistance management*

Do not apply more than two sequential sprays of any material that is at risk for resistance development before alternating to a fungicide with a different mode of action (Table 2). In addition, the less specific a fungicide or class of fungicide used in a vineyard, the less likely a fungus is to develop resistance against it. Most of the fungicides that are at risk for resistance development have a limited number of applications that can be made per season (Table 2). **Always read the label.**

Note that at any specific time of application, there are usually several fungicide options that can be selected. This schedule does not contain all of the fungicides currently registered for use on

grapes. Remember, these are only **suggested guidelines** for use in developing a fungicide program. The final program that you develop will depend upon the disease incidence in your vineyard, as well as economic considerations.

## Suggested guidelines for developing a fungicide spray program for wine grapes in Ohio

**This program is intended to provide simultaneous control of black rot, powdery mildew, downy mildew and Phomopsis cane and leaf spot, as well as fungicide resistance management.**

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| 1-inch shoot | Mancozeb (3 lb/acre). Mancozeb alone is only for control of Phomopsis. If powdery mildew is of concern during this stage, use one of the following chemicals along with Mancozeb:<br>• A sterol-inhibiting fungicide [Elite (4 oz/acre) or<br>• Rubigan (3 fl oz/acre) or<br>• Rally (4 oz/acre)] or<br>• Endura 70WG (4.5 oz) or<br>• Quintec 2.08F (4 fl oz) or<br>• Flowable sulfur 6F (3–4 qt/acre) or<br>• Wettable sulfur (6–10 lb/acre) or<br>• JMS Stylet Oil (1% concentration) or<br>• Potassium salts (see notes below) |

**Note: These early sprays are most critical for control of Phomopsis.**
**Notes on potassium salts:**

- Several potassium salts are currently registered as fungicides for control of powdery mildew on grapes. These include Nutrol (monopotassium phosphate), Kaligreen and Amicarb 100 (potassium bicarbonate). They provide moderate to good control of powdery mildew when applied to developing powdery mildew colonies. They do not provide protectant activity, and they are not effective against the other grape diseases caused by fungi. See label of each material for usage rates and other recommendations.
- Do not combine JMS Stylet Oil with sulfur fungicides or Captan, as it causes serious vine injury. The products should not be sprayed on vines within 14 days of each other.
- Do not apply sulfur to sulfur-sensitive varieties.

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| 3- to 5-inch shoot or 7 to 10 days after first spray | Mancozeb (3 lb/acre) plus:<br>• A sterol-inhibiting fungicide [Elite (4 oz/acre) or Rubigan (3 fl oz/acre) or<br>Rally (4 oz/acre)] or<br>• Quintec 2.08F (4 fl oz) or<br>• Flowable sulfur 6F (3–4 qt/acre) or<br>• Wettable sulfur (6–10 lb/acre) or<br>• JMS Stylet Oil (1% concentration) or<br>• Potassium salts |

**Note:** If powdery mildew is of concern, an effective fungicide for powdery mildew control should be used at this time. If fungicide resistance is not a problem, the sterol-inhibiting fungicides (Rally, Rubigan and Elite) are excellent for powdery mildew control. In some vineyards, reduced sensitivity or resistance to the sterol-inhibiting fungicides has been reported in the powdery mildew fungus. If there is resistance buildup for these materials, alternative materials must be used. Alternatives for powdery mildew control include sulfur fungicides, Endura, Quintec, potassium salts and JMS Stylet Oil. Sulfur fungicides are very effective for powdery mildew control, relatively inexpensive and not at risk for resistance development. The use of sulfur for powdery mildew control should be considered on sulfur-tolerant varieties.

**Note:** If powdery mildew is not a problem, Mancozeb alone can be used. It is important to use Mancozeb in all sprays where it is recommended. Mancozeb will provide excellent control of Phomopsis cane and leaf spot, black rot and downy mildew. It will not control powdery mildew. For this reason it is recommended for use in a tank mix with a powdery mildew fungicide.

**I consider Mancozeb to be the backbone of the fungicide program for wine grapes in Ohio.**

**Note:**

- Do not combine JMS Stylet Oil with sulfur fungicides or Captan, as it causes serious vine injury. The products should not be sprayed on vines within 14 days of each other.
- Do not apply sulfur to sulfur-sensitive varieties
- Always check the price (cost per acre per application) of each fungicide. At the rates recommended, fungicides vary considerably in cost.

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| 10- to 12-inch shoot or 7 to 10 days after last spray | Same fungicides as in 3- to 5-inch shoot stage. |

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| Immediate prebloom or early bloom or 7 to 10 days after last spray | Mancozeb (3 lb/acre) plus:<br>• A sterol-inhibiting fungicide [Elite (4 oz/acre) or Rubigan (3 fl oz/acre) or Rally (4 oz/acre)] or<br>• Endura 70WG (4.5 oz) or<br>• Quintec 2.08F ( 3–4 fl oz) or<br>• Flowable sulfur 6F (3 qt/acre) or<br>• Wettable sulfur (8-10 lb/acre) or<br>• JMS Stylet Oil (1% concentration) or<br>• Potassium salts or<br>• **Pristine** 38WG (6–10.5 oz/acre) (see note below) |

**Note:** Due to possible resistance to powdery and downy mildew, the strobilurin fungicides are no longer recommended. They will still provide excellent control of black rot; however, during this period, all of the major diseases need to be controlled.

**Note on Pristine:** Pristine is combination of a strobilurin fungicide (pyraclostrobin) and the fungicide Endura (boscalid); therefore, it should be effective for controlling all of the major grape diseases except downy mildew. If downy mildew is of concern, probably Pristine should not be used alone. Do not make more than two sequential applications of Pristine without switching to another fungicide in a different class of chemistry, and do not make more than six applications per season.

- If conditions are highly conducive for downy mildew development, Ridomil Gold MZ or Ridomil Gold Copper should be considered.
- Rally and Elite provide excellent control of black rot and have excellent curative activity (3 to 4 days) against black rot. If powdery mildew is resistant to the sterol-inhibiting fungicides in your vineyard, an alternative material for powdery mildew control should be used. Also, if two sequential sprays of a sterol-inhibitor have been made, switch to a powdery mildew fungicide with a different mode of action.
- Do not combine JMS Stylet Oil with sulfur fungicides or Captan, or serious vine injury can occur. The products should not be sprayed on vines within 14 days of each other.

**VERY IMPORTANT NOTE:** The period from immediate prebloom through 3 to 4 weeks after bloom is the **MOST CRITICAL PERIOD** for controlling fruit infection by black rot, powdery mildew and downy mildew. During this period, fruits are highly susceptible to infection by all of these diseases. Around 4 weeks after bloom, the fruit become resistant to infection.

**Note on downy mildew:** If conditions are highly conducive for downy mildew development during this critical period, Ridomil Gold MZ or Ridomil Gold Copper should be considered at this time. I do not think any material is more effective than Ridomil for downy mildew control. However, a good protectant program with Mancozeb should provide effective downy mildew control during most growing seasons. The PHI for Ridomil Gold MZ is 66 days and for Ridomil Gold Copper is 42 days. Revus and Presidio are two new fungicides that are reported to be highly effective against downy mildew. It is very important to remember that these materials will need to be tank mixed with other fungicides because they will not provide adequate control of powdery mildew or black rot (Table 1).

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| First postbloom spray no longer than 10 days after last spray | Same fungicides as immediate prebloom or early-bloom stage. |

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| Second postbloom spray no longer than 10 days after last spray | Mancozeb (4 lb/acre) or Captan 50W (3–4 lb/acre) or phosphorus acid (see note below), plus:<br>• A sterol-inhibiting fungicide [Elite (4 oz/acre) or Rubigan (6 fl oz/acre) or<br>Nova (4 oz/acre)] or<br>• Endura 70WG (4.5 oz) or<br>• Quintec 2.08F ( 3–4 fl oz) or<br>• Flowable sulfur 6F (3 qt/acre) or<br>• Wettable sulfur (8-10 lb/acre) or<br>• Potassium salts or<br>• **Pristine** 38WG (6–10.5 oz/acre) |

**Note on phosphorous acids:**
Several products containing phosphorous acid (phosphonates, phosphites) are sold as nutritional supplements and "plant conditioners," but a few products (ProPhyt, Phostrol, Agri-Fos, Topaz) are registered for use as fungicides for downy mildew control on grape. Phosphorous acid has been used successfully for over 30 years in Australia for downy mildew control on grape. Phosphorous acid is a good fungicide for control of downy mildew. Usage rate recommendations vary among different products. The products mentioned here have a 4-hour reentry interval and a 0-day preharvest interval. Obtain and read the label of each product prior to use.

**Note:** The second postbloom spray should be near the end of the **CRITICAL PERIOD** for controlling fruit infection by black rot, powdery mildew and downy mildew (immediate prebloom through 3 to 4 weeks after bloom). By this time, the fruit of most varieties should be resistant to infection.

It is very important to maintain excellent fungicide coverage (protection) during this period until the fruit become resistant. Failure to provide adequate fungicide protection can result in the development of **diffuse infections** of powdery mildew on fruit. It is difficult to see these infections with the naked eye, and they can result in increased problems with various fruit rots later in the season. The importance of protecting the fruit during this critical period cannot be overemphasized.

Remember that cluster stems (rachis) and leaves will remain susceptible to powdery and downy mildew throughout the growing season; therefore, a good fungicide program needs to be maintained throughout the season.

**Note on downy mildew:** If conditions are highly conducive for downy mildew development during this period, Ridomil Gold MZ or Ridomil Gold Copper should be considered at this time. I do not think any material is more effective than Ridomil for downy mildew control. The PHI for Ridomil Gold MZ is 66 days and for Ridomil Gold Copper is 42 days. Revus and Presidio are two new fungicides that are reported to be highly effective against downy mildew. Both of these materials have a 12-day PHI. It is very important to remember that these materials will need to be tank mixed with other fungicides because they will not provide adequate control of powdery mildew or black rot (Table 1).

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| Third postbloom spray 10 to 14 days after last spray<br><br>**Late-season summer sprays should not exceed a 14-day interval**<br><br>Under heavy disease pressure, spray at shorter intervals | Mancozeb (3–4 lb/acre) or Captan 50W (3–4 lb/acre) or phosphorus acid, plus:<br>• Endura 70WG (4.5 oz) or<br>• Quintec 2.08F ( 3–4 fl oz) or<br>• Flowable sulfur 6F (3 qt/acre) or<br>• Wettable sulfur (8–10 lb/acre) or<br>• Potassium salts |

**Note: Watch the 66 days PHI on Mancozeb.** On late-maturing varieties, Mancozeb can be used later in the season as long as it is not applied within 66 days of harvest. I recommend keeping it in the spray program as long as it is legal to use.

If you get within 66 days of harvest, Captan, a phosphite fungicide, Ridomil Gold Copper, Revus, Presidio or a copper fungicide can be used in place of Mancozeb for downy mildew control. If you have more than 66 days to harvest, Mancozeb would be the fungicide of choice. If weather is dry and downy mildew is not a problem, these downy mildew fungicides are not required. However, you will need to maintain a good program for powdery mildew control, even if weather is dry. The danger of black rot infection should be over by this time. Berries should be resistant to black rot.

| Stage of application | Rate of fungicide application (per acre) |
|---|---|
| Fourth postbloom spray 10 to 14 days after last spray<br><br>**Maintain a 10- to 14-day spray schedule through harvest. The suggested fungicides can be used through harvest.** | Captan 50W (3–4 lb/acre) or phosphorous acid, plus:<br>• Endura 70 WG (4.5 oz) or<br>• Quintec 2.08F (4 fl oz) or<br>• Wettable sulfur (8–10 lb/acre) or<br>• Flowable sulfur 6F (3 qt) or<br>• Potassium salts<br>OR<br>Fixed copper fungicides, used alone<br>OR<br>Pristine 38WG (6-10.5 oz), used alone |

**Note:**

- If dry weather persists and the risk of downy mildew is low, a downy mildew fungicide may not be required and sulfur can be used alone for powdery mildew control. If weather is wet and downy mildew is a problem, a downy mildew material should be included. A fixed copper fungicide will give good control of both downy and powdery mildew. Especially on susceptible varieties, powdery mildew will need to be controlled throughout the growing season.
- Do not apply Captan, sulfur or copper fungicides within 30 days of harvest, or fermentation may be affected; and **DO NOT** combine Captan or Sulfur with any form of oil.
- Under heavy disease pressure, use a shorter spray interval.

**For controlling Botrytis bunch rot, the following fungicides can be used:**

- Rovral (1.5 lb/acre) plus Latron B1956 (6 fl oz/100 gal) or
- Vangard (10 oz/acre) used alone or
- Elevate (1 lb / A) used alone or
- Scala (18 fl oz/acre) used alone or
- Endura (8 oz /acre) used alone or
- Pristine (18.5 to 23 oz/acre) used alone

These fungicides should be used as special (additional) sprays for control of Bortytis bunch rot only on tight-clusterd, bunch rot–susceptible cultivars. The first spray should be made when disease is first observed or at veraison, or shortly thereafter. Then wait until threatening weather (wet conditions) and/or disease develops and make a second spray (at least two weeks after the first spray). On late-maturing varieties, a third spray may be required.

**Importance of bloom sprays for Botrytis bunch rot control:** Botrytis can enter fruit on dead flower parts or other plant debris on the cluster during bloom. Therefore, bloom applications of fungicide may be beneficial for its control. In some years, bloom sprays seem to be very effective and in others, they appear to have no or little effect. Some growers make a Botrytis spray during bloom every year and many do not. On bunch rot–susceptible and high-value wine grapes, a bloom application may be a good form of insurance against Botrytis bunch rot. One practical approach for providing protection against bunch rot infections during bloom is to use a fungicide such as Pristine during bloom, which would be a standard application within the critical period for fruit infection by black rot, powdery mildew and downy mildew. Pristine at the higher concentration listed above should provide excellent control of Botrytis, in addition to the other diseases that need to be controlled at this time.

**Note:** Some tests in New York have indicated that Rovral at 1 lb/acre plus Vangard at 5 oz/acre may have an additive effect and provides good bunch rot control.

**Pristine applied at normal harvest for ice wine:** Grapes for ice wine production must hang for long periods past normal harvest prior to picking. An application of Pristine at normal harvest time may aid in controlling some fruit rots of ripe grapes, especially during fall and early winter when temperatures remain high.

**Table 1. Effectiveness of fungicides for the conrol of grape diseases.**

| Fungicide | Phomopsis cane and leaf spot | Black rot | Downy mildew | Powdery mildew | Botrytis rot | Bitter rot |
|---|---|---|---|---|---|---|
| Abound | + | +++ | +++ (FRP) | +++ (FRP) | ++ | ? |
| Bayleton | 0 | +++ | 0 | +++ (FRP) | 0 | 0 |
| Captan | +++ | + | +++ | 0 | + | ++ |
| Elevate | 0 | 0 | 0 | 0 | +++ | 0 |
| Elite | 0 | +++ | 0 | +++ (FRP) | 0 | 0 |
| Endura | 0 | 0 | 0 | +++ | ++ | 0 |
| Ferbam | + | +++ | + | 0 | 0 | ++ |
| Fixed copper & lime | + | + | +++ | ++ | + | + |
| Flint | + | +++ | + (FRP) | +++ (FRP) | ++ | 0 |
| JMS Stylet Oil | 0 | 0 | 0 | +++ | 0 | 0 |
| Mancozeb | +++ | +++ | +++ | 0 | 0 | ++ |
| Nova | 0 | +++ | 0 | +++ (FRP) | 0 | 0 |
| Potassium salts | 0 | 0 | 0 | ++ | 0 | 0 |
| Phosphorous acid | 0 | 0 | +++ | 0 | 0 | 0 |
| TablePresidio | 0 | 0 | +++ | 0 | 0 | 0 |
| Pristine | ++ | +++ | +++ (FRP) | +++ | ++ | ? |
| Procure | 0 | ++ | 0 | +++ (FRP) | 0 | 0 |
| Quintec | 0 | 0 | 0 | +++ | 0 | 0 |
| Revus | 0 | 0 | 0 | +++ | 0 | 0 |
| Ridomil Gold MZ | + | ++ | +++ | 0 | 0 | ++ |
| Ridomil Gold Copper | + | + | +++ | ++ | + | + |
| Rovral | 0 | 0 | 0 | 0 | +++ | 0 |
| Rubigan | 0 | ++ | 0 | +++ (FRP) | 0 | 0 |
| Scala | 0 | 0 | 0 | 0 | +++ | 0 |
| Sovran | + | +++ | ++ (FRP) | +++ (FRP) | ++ | 0 |
| Sulfur | + | 0 | 0 | +++ | 0 | 0 |
| Topsin M[1] | ++ | + | 0 | +++ | ++ | ++ |
| Vangard | 0 | 0 | 0 | 0 | +++ | 0 |
| Ziram | ++ | +++ | ++ | 0 | 0 | 0 |

Key to ratings:

+++ = highly effective
++ = moderately effective
+ = slightly effective
0 = not effective
? = effectiveness unknown or not established

FRP = Fungicide resistance possible, especially if the material has been used in the vineyard for several years. Generally, if they have not been used extensively, resistance may not be a problem.

[1] Where Topsin M–resistant strains of the powdery mildew and Botrytis fungi have been detected, Topsin M will be ineffective and should not be used.

**Table 2. Resistance-prone fungicides and risk of resistance by chemical class.**

| Fungicide class | Rating | Common (chemical) name(s) | Trade name(s) |
|---|---|---|---|
| Benzimidazole (Group 1) | High | Thiophanate-methyl | Topsin-M |
| Phenylamide (Group 4) | High | Mefenoxam<br>Mefenoxam (+ copper)<br>Mefenoxam (+ mancozeb) | Ridomil Gold<br>Ridomil G old/Copper<br>Ridomil Gold MZ |
| Strobilurin (QoI) (Group 11) | High | Azoxystrobin<br>Kresoxim-methyl<br>Pyraclostrobin (+ boscalid)<br>Trifloxystrobin | Abound<br>Sovran<br>Pristine<br>Flint |
| Dicarboximide (Group 2) | Medium to High | Iprodione | Rovral |
| Sterol Inhibitors (Group 3) | Medium | Fenarimol<br>Myclobutanil<br>Tebuconazole<br>Triflumizole | Rubigan<br>Nova<br>Elite<br>Procure |
| Carboximide (anilide) (Group 7) | Medium | Boscalid<br>Boscalid (+ pyraclostrobin) | Endura<br>Pristine |
| Anilinopyrimidine (Group 9) | Medium | Cyprodinil<br>Pyrimethanil | Vangard<br>Scala |
| Quinolines (Group 13) | Medium | Quinoxyfen | Quintec |
| Hydroxyanilid (Group 17) | Medium | Fenhexamid<br>Fenhexamid + captan | Elevate<br>CaptEvate |
| (Group 40) | Medium | Mandipropamid | Revus |
| (Group 43) | Medium | Fluopicolide | Presidio |

Resistance ratings apply to all members of a class of fungicides. All fungicide classes with a medium or high risk of resistance development must be used in accordance with resistance management guidelines listed on the label. Tactics for avoiding or slowing resistance development include:

1) Rotating among fungicides from different classes. Make no more than two consecutive applications of a resistance-prone fungicide (or fungicides from the same class) before switching to a fungicide from a different class (has a different mode of action).

2) Use high-risk fungicides as little as possible. The fewer times a fungicide is applied in a vineyard, the less likely that resistance will develop. Always use fungicides only when needed and at the proper time to obtain the disease control that is required. Always use fungicides as one integral part of an integrated disease management program.

# Evaluation of Cultural Practices to Reduce Bunch Rot in Vignoles Grapevines

**Dr. R. Keith Striegler, R. Andrew Allen, Eli A. Bergmeier and Jackie L. Harris**
Institute for Continental Climate Viticulture and Enology
University of Missouri

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## Introduction

Vignoles is an important interspecific hybrid grapevine cultivar that is grown extensively in the Eastern and Midwestern U.S. This cultivar has moderate to low vigor and produces a medium-sized vine when grown on its own roots. Vignoles is very cold hardy and has a late bud burst, which reduces the risk of spring freeze injury. Fruit from Vignoles can develop relatively high sugar content with a distinctive, pleasing flavor profile while maintaining high acidity. Because it possesses these fruity characteristics, Vignoles is commonly used to produce off-dry or dessert wines. Well-made Vignoles wine is popular with consumers and has been referred to by Missouri winemakers as "the only wine that sells itself."

Problems associated with growing Vignoles include that this cultivar has small, compact clusters that are susceptible to *Botrytis* bunch rot and the summer bunch rot complex (Figure 1), as well as having low to moderate yield primarily due to low bud fruitfulness. Of these production issues, susceptibility to bunch rot is by far the most pervasive and costly problem for grape growers.

Unfortunately, in Missouri and many other areas of the East and Midwest, reliable control of bunch rot in Vignoles has not been achieved by even the best spray programs. For Missouri grape growers, the reasons for this are complex but certainly include environmental conditions during the

growing season (heat and high humidity), as well as differences in pathogen populations responsible for bunch rot when compared to cooler grape-production reasons. Recent research has demonstrated that *Botrytis cinerea* is only a minor bunch rot pathogen under Missouri conditions (T. Sutton, R.A. Allen, and R.K. Striegler, unpublished data, 2009). All these factors point to the need for use of integrated approaches to bunch rot control rather than reliance on fungicides alone. Manipulation of cultural practices may be an important component of increased use of integrated approaches for bunch rot control in Missouri.

**Figure 1. The tight, compact architecture of Vignoles' clusters make them highly prone to bunch rots.**

Several experiments have examined the relationship between cluster compactness and the incidence and severity of bunch rot (Hed et al. 2009; Percival et al. 1993; Vail and Marois 1991; Vail et al. 1998). A consistent result from these research projects was that increased cluster compactness led to a greater incidence and severity of bunch rot. Thus, cultural practices that reduce cluster compactness would likely reduce bunch rot losses for Missouri growers.

## Leaf removal

The cultural practice of leaf removal is generally used during the berry set to veraison period on vines with excessive canopy density. This practice improves light penetration into the canopy and modifies other environmental factors, such as humidity and evaporative potential, within the canopy. Benefits from leaf removal can include increased yield, improved fruit composition and reduced levels of bunch rot (English et al. 1993; Gubler et al.1991 and 1987; Percival et al. 1994; Zoecklein et al. 1992). Grower experiences have indicated improvements in bunch rot control with leaf removal, but for tight-clustered cultivars such as Vignoles, acceptable control is often not attained. This is especially true for seasons with high rainfall postveraison.

Recently, leaf removal earlier in the season, near bloom, has been shown to control crop level and improve fruit composition (Intrieri et al. 2008; Poni et al. 2006, 2008 and 2009). The mechanism employed is induction of a resource limitation at bloom, which reduces fruit set. Cluster weight and compactness were reduced by leaf removal executed near the time of bloom. The implications of this research for control of bunch rot on cultivars with compact, tight clusters are apparent. Initial research in this area was conducted in Michigan (G.S. Howell, 2004–2005 Viticulture Consortium-East Final Report) with favorable results for Vignoles and other cultivars. Hed et al. (2009) recently reported the results of an experiment where leaf removal was applied at trace bloom on Vignoles vines during the 2004 and 2005 seasons. Leaf removal (four most basal leaves removed) at trace bloom reduced cluster weight and cluster compactness in both seasons of the study. In addition, consistent reductions in bunch rot were observed, although the differences were not always statistically significant. More information on the impact of cultural practices such as early leaf removal would be beneficial for Missouri grape producers. This article reports on a project that was designed to manipulate cluster compactness and reduce bunch rot of Vignoles grapevines.

## Materials and methods

A study was conducted during the 2008 and 2009 seasons to evaluate treatments that alter cluster architecture and reduce compactness of Vignoles grape clusters. The experiments were conducted in an own-rooted Vignoles vineyard located near Hermann, Mo. Vines were trained to a high-wire, single-curtain trellis system and balance-pruned each season using a 20+20 pruning severity (20 nodes retained per pound of cane prunings). Treatments were selected to reduce compactness either by resource limitation–induced fruit set reduction or physical removal of flowers or immature berries. The treatments used were control (no treatment), removal of eight basal leaves on shoots with clusters, brushing (passing a brush with semirigid teeth through the cluster twice), and spraying vines to the point of run-off with a 2-percent solution of Stylet Oil, which inhibits photosynthesis. All treatments were applied at trace bloom. Data collected included yield, components of yield, fruit composition, cluster compactness, percentage bunch rot and dormant pruning weight.

## Results and discussion

Results from this study are presented in Table 1. Leaf removal at trace bloom significantly reduced yield in 2008. During the 2009 season, vines receiving leaf removal and brushing displayed reduced yield as compared to control vines. Cluster weight was significantly reduced when early leaf removal was used in both seasons. The reduction in yield observed for the leaf-removal treatment was likely the result of the lower cluster weights produced by this treatment. Cluster compactness and percent bunch rot were not altered by treatment in 2008; however, in 2009, vines that received leaf removal had lower cluster compactness and percent bunch rot. Hed et al. (2009) reported that Vignoles clusters with less than nine berries per centimeter of rachis had reduced bunch rot. Our results are consistent with this observation.

The results of this study indicate that early leaf removal has potential to reduce cluster compactness and bunch rot of Vignoles grapevines in Missouri. However, enthusiasm for this must be tempered by the realization that these results were obtained at the cost of a yield loss between 1.4 and 2.0 tons per acre. This yield reduction occurred in both seasons even though a reduction in bunch rot was observed in only one of the two. Hence, the economics of this practice on a multiyear basis need to be closely examined before being adopted commercially, both from the standpoint of reduced yields and the cost required to execute early leaf removal. As with any hand-executed cultural practice, cost and availability of hand labor to perform early leaf removal must be carefully considered. Some success has been achieved using mechanical leaf removal to obtain results similar to early leaf removal performed by hand (Intrieri et al. 2008). The yield reduction caused by early leaf removal could potentially be partially offset by increasing retained node numbers at the time of pruning. However, training system selection places practical constraints on this by limiting the number of shoots that can be retained while maintaining adequate canopy light microclimate and, hence, bud fruitfulness.

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**Table 1. Effect of treatment on yield, cluster weight, cluster architecture and percentage bunch rot of Vignoles grapevines (Hermann, Mo.).**

| | Yield (tons/acre) | | Cluster weight (g) | | Berries/cm of rachis | | Bunch Rot (%) | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **2008** | **2009** | **2008** | **2009** | **2008** | **2009** | **2008** | **2009** |
| Control | 4.1 a* | 3.2 a | 81.7 a | 90.8 a | 5.1 | 10.8 a | 2.7 | 23.3 a |
| Leaf removal | 2.1 b | 1.8 b | 45.4 b | 49.9 c | 4.6 | 8.2 b | 2.0 | 5.9 b |
| Brushing | 4.0 a | 2.0 b | 77.2 a | 72.6 b | 4.8 | 9.9 a | 1.6 | 18.0 a |
| Stylet oil | 3.6 a | 2.5 ab | 81.8 a | 81.7 ab | 5.3 ns | 10.7 a | 4.0 ns | 21.4 a |

* Means followed by the same letter do not differ significantly at the 0.05 level; ns=not significant. Mean separation by Tukey's Studentized Range (HSD) Test.

# Management of Grape Phylloxera, Grape Berry Moth and Japanese Beetles

**Dr. Donn T. Johnson and Barbara Lewis**
Department of Entomology
University of Arkansas

**Jackie Harris, Andy Allen and Dr. R. Keith Striegler**
Institute for Continental Climate Viticulture and Enology
University of Missouri

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## Abstract

A brief description of the biology and damage caused by foliar grape phylloxera (GP) is provided, as well as a list of grape cultivars economically damaged by foliar grape phylloxera. On susceptible vines, it is best to apply recommended insecticide sprays against exposed, young second-generation GP crawlers, which emerge from early May to early June. During this period, growers should inspect weekly for a rash-like appearance of expanding terminal leaves indicating the leaf is beginning to form galls around the crawlers. Field efficacy studies confirmed that Danitol, Movento, Assail, Surround kaolin clay protective film and Admire adequately protected susceptible cultivars against galling caused by GP crawlers. To further improve timing of insecticide applications, a degree-day (DD) model is being developed to pinpoint when to begin and end scouting of terminal leaves for GP crawler activity.

Grape berry moth (GBM) larvae tunnel in berries creating entry points for bunch rot fungi. The first-generation GBM larvae cause fruit damage on perimeter vines from late May to early June. The later generations move to the vineyard interior causing berry damage until harvest. In the vineyards where we are demonstrating pest management practices, we integrated the biofix date (first consistent trap catch) with a DD model (400 to 700 DD and > 1,300 DD) to aid timing of insecticide sprays against GBM larvae. This program resulted in a significant drop in the season total number of GBM trapped and less than 5 percent cluster damage.

Since mid 1990s, Japanese beetles (JB) have spread from infested areas east of the Mississippi River to Arkansas, Kansas, Missouri and Oklahoma. The adult Japanese beetle defoliates grapes and 300 other plant species, whereas immature Japanese beetle grubs eat grass roots in and around vineyards. We determined that vines had minimal JB-damaged foliage after being treated from late June and July with one spray of Danitol followed by two weekly sprays of Mustang Max or four sprays of Surround protective film.

Separate tables list insecticides and biopesticides labeled against GP, GBM and JB, with respective mode of action for rotating formulations to prevent resistance in pest populations.

## Introduction

**Grape phylloxera** (GP), *Daktulosphaira vitifoliae* (Fitch): GP is becoming a more important pest of grapes as growers in the central and eastern United States plant more hybrid cultivars derived from French *V. vinifera* and American *Vitis* species crosses. Leaves of susceptible cultivars that expand after early June may sometimes have more than 100 grape phylloxera galls. These galls reduce the effective leaf surface area for photosynthesis leading to less sugar accumulation, thereby resulting in reduced grape yield. Many cultivars grown in this region reported to suffer economically damaging levels of leaf galling by GP: Aurora, Cascade, Catawba, Cayuga White, Chambourcin, Chancellor, Chelois, DeChaunac, Delaware, Himrod, Lakemont, Norton/Cynthiana, Rayon D'Or, Reliance, Rougeon, Seibel, Seyval, Vidal Blanc, and Vignoles (Johnson et al. 2009).

The seasonal biology of the GP begins soon after first grape leaves begin to expand. In April, yellow six-legged crawlers hatch from eggs that overwintered on the vine. These crawlers walk to the end of new terminals and suck on expanding leaves. Usually, these stem mother galls appear on the first to third expanded leaves of the season near the base of new shoots. In response to sucking, the leaf forms a gall around each crawler. These crawlers mature into stem mothers that produce second-generation eggs and crawlers from late April to early June. From mid-May to late August, you can expect three or more additional generations of galls to form on expanding terminal leaves.

We are developing the following degree-days (DD) model for GP crawlers. On each GP-susceptible cultivar with a history of foliar galling, record the date when grapevines begin to expand their first leaves in late March to early April (biofix). After this biofix date, begin accumulating daily DD with a developmental base temperature of 43 degrees Fahrenheit (Belcari and Antonelli 1989) and upper threshold of 96.8 degrees Fahrenheit (Fisher and Albrecht 2003) by using the following


**Figure 1. Grape phylloxera (A) mature stem mother galls (oval) on first to third mature leaves, (B) stem mother with eggs and two crawlers (circle and inset) inside gall, and (C) mature gall (circle) and small, rash-like galls on the underside of expanding terminal leaf before they enclose crawler (inset) on June 15 in Hillsboro, Mo. (2009).**

equation: DD = average daily temperature – 43. After the biofix date, crawler emergence periods for the second and third generations occur from 500 to 800 DD (early to late May = insecticide spray period) and after 1,200 DD (mid-June).

Insecticides labeled against GP crawlers (Table 1) should not be applied to susceptible cultivars until you detect crawlers in stem mother galls or on expanding terminal leaves.

Scouting for crawlers should be restricted to blocks of grape cultivars historically susceptible to foliar GP (listed above). Locate vines with mature stem mother galls on the three oldest leaves at the base of new shoots (Figure 1A). Beginning at 400 DD since biofix, weekly cut open 10 stem mother galls and use a 10X hand lens to check for presence of crawlers (Figure 1B). You can also inspect expanding terminal leaves for immature, pin-sized galls that appear rashlike on the lower leaf surface and pitted on the upper leaf surface (Figure 1C). Inspect pits of immature galls on upper leaf surface with a hand lens to see if a yellow crawler is present.

We have conducted efficacy studies to demonstrate efficacy of insecticides labeled against grape phylloxera (Table 1). In a Norton block in St. James, Mo., a foliar application of Danitol or Assail via a Solo backpack sprayer and a soil drench of Admire each applied on May 11, 2006, (crawlers active) had significantly fewer GP-galled shoots (1.8-fold less) than the untreated check (Johnson et al. 2008). In a Vignoles block in Altus, Ark., foliar applications of Danitol, a low rate and high rate of Movento and Surround kaolin clay particle film were applied via a Solo backpack sprayer on June 16, 2009. A week later, after a significant rain, Surround-treated plots received a second foliar application to maintain whitewashed foliage. At the time of treatment, all vines had similar numbers

of shoots with GP galls on old leaves (Table 2). By Aug. 14, the untreated check vines had developed significantly more GP-galled leaves than had the single foliar application of Danitol, either rate of Movento and two applications of Surround to maintain whitewash appearance.

**Grape Phylloxera Fact Sheet:** An extension fact sheet titled Biology and Management of Grape Phylloxera is available online at http://comp.uark.edu/~dtjohnso/GP Fact Sheet 09.pdf (Johnson et al. 2009).

**Grape Berry Moth** (GBM), *Endopiza viteana* Clemens: Risk of damage to grape clusters by GBM corresponds directly to past history of damage in a block and the percentage of vineyard perimeter adjacent to woods where GBM pupae overwinter. The categories are low risk — less than 25 percent wooded edge; moderate risk — 25 to 50 percent wooded edge; and high risk — more than 50 percent of vineyard perimeter adjacent to woods. In our previous survey, the percentage of growers that reported their vineyard risk percentage for grape berry moth damage as low, moderate and high risk were 36 percent, 50 percent and 7 percent, respectively, in Arkansas; compared to 69 percent, 16 percent and 2 percent, respectively, in Missouri.

The Food Quality Protection Act (http://www.epa.gov/) was enacted in 1996 mandating the cancellation or mitigation of more than 50 percent of the registered uses of insecticide classes like the organophosphate and carbamate insecticides that pose high risk to human health or cause other environmental problems. These compounds are being replaced by "softer," more narrowly targeted, newer chemistries (ovicides like Entrepid and Esteem) and biopesticides such as the bacteria *Bacillus thuringiensis* (*Bt*) (Table 3). These *Bt* products represent about 80 percent of all biopesticides sold, with *Bt* use in grapes increasing from 5 percent of total U.S. grape acreage in 1993 to 30 percent in 2000 (Whalon and Wingerd 2003).

Currently, management of GBM in moderate- to high-risk vineyards involves spraying vines shortly after berries reach pea size with either insecticides targeted against eggs (ovicides like Intrepid or Esteem) or larvicides or biopesticides against larvae (Table 3).

Since 2006, we have been training grape growers about best management practices (BMP), including pest management and spray timing. In addition, we have six BMP demonstration vineyards in Missouri and one each in Arkansas and Kansas. Local GBM populations were

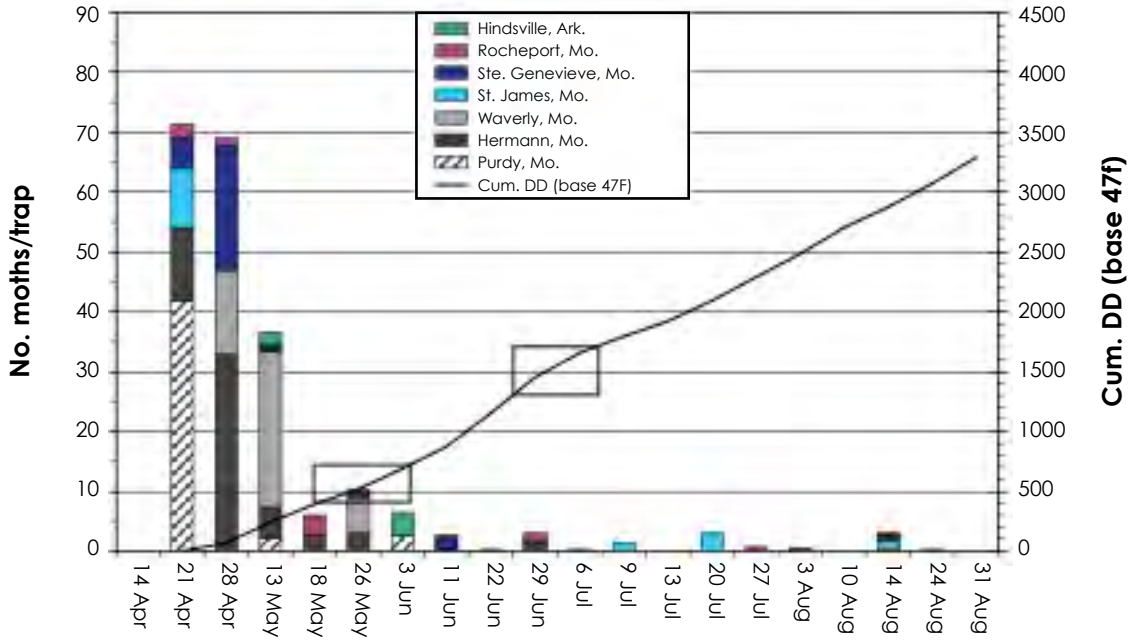


**Figure 2. Weekly numbers of grape berry moths (GBM) captured in pheromone traps where each bar is stacked of values from a vineyard in Hindsville, Ark., and six vineyards in Missouri. Rectangles note hatch periods for first (500 to 700 DD) and second (1,300 to 1,700 DD) generation GBM larvae (DD = degree-days accumulated since first trap catch on April 23, 2009, given lower and upper developmental temperatures of 47 and 93 degrees Fahrenheit) (Tobin et al. 2001).**

estimated weekly or biweekly with three pheromone sticky traps set out on April 1 in the edge of woods adjacent to the vineyard perimeter and moved 100 feet into the vineyard in mid-May. During each larval generation we assessed the percentage of 100 clusters damaged in perimeter vines in Hermann, Mo. (Table 4). We recorded the date (biofix date) when we began to consistently catch GBM in traps, and we continued sampling to monitor moth flights throughout the season. After biofix, we begin accumulating daily degree-day (DD) (base 47 degrees Fahrenheit to upper limit 93 degrees Fahrenheit) (Tobin et al. 2001; Teixeira et al. 2009). The larvae hatch period occurs from 500 to 700 DD from late May to early June (Figure 2). During that period, we recommend weekly inspections of 100 clusters on perimeter vines for damage caused by GBM berry tunneling and application of insecticide sprays to perimeter vines if damage is detected. In late July from 1,300 to 1,800 DD (Figure 2), growers reported increasing numbers of GBM larvae in fruit and percentages of GBM-damaged clusters in the perimeter vines (Figure 3).

The recorded season total GBM trap catches have dropped significantly since 2006 (Table 4), and the corresponding percentage cluster damage in the perimeter vines remained at or below 5

percent in all BMP vineyards except the new BMP vineyard in Waverly, Mo., (9 percent damage) added in 2008 and in Paolo, Kan., (27 percent damage) (Table 5). In 2009, trap catch often exceeded 10 moths per trap for the emerging overwintered generation of GBM but dropped to below five and one moth per trap, respectively, for second (May and mid-June), third and fourth generations (July and August) of GBM (Figure 2).

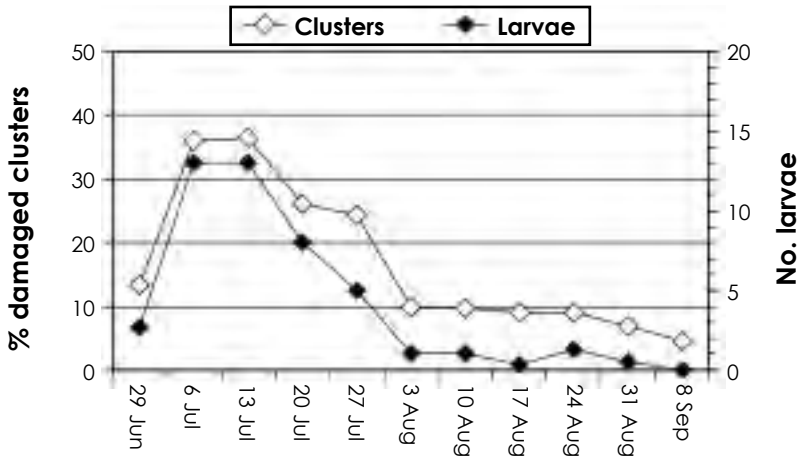


**Figure 3. Weekly changes in percentage cluster damage by grape berry moth larvae and number of live grape berry moth larvae per 100 clusters in the edge of the vineyard in Hermann, Mo. (2009).**

Spray timing is being improved by pest monitoring and DD models to pinpoint oviposition or larval periods (Teixeira et al. 2009). In 2010, we plan to demonstrate the efficacy of various GBM tactics during three periods in a season as follows:

1. Standard larvicide insecticides (Sevin, Danitol), ovicides (Intrepid and Esteem) or biopesticides (Entrust and *Bt* formulations) applied to vines from 500 to 800 DD as needed against first generation GBM attacking perimeter vines by woods.
2. Setting out 200 or 400 Isomate GBM PLUS sex pheromone dispensers per acre, respectively, for low- and high-GBM-risk vineyards for GBM mating disruption, which will minimize number of fertile eggs laid by GBM from late May (900 DD) to August.
3. Weekly applications of *Bt* in September to reduce the number of GBM pupae entering overwintering.

**Japanese beetle** (JB), *Popillia japonica* Newman: JB populations have been causing defoliation of grapes and other crops from Fayetteville and Hindsville, Ark., to Purdy, Mo., and several locations along the Mississippi River valley in eastern Missouri. In 2009, the first adult JB were observed on June 15 and caused significant foliar damage from June 24 to early August. Several insecticides are labeled against JB for grapes (Danitol, Mustang Max, Sevin, Brigade, Assail,

Avaunt, Clutch and Imidan), but Admire Pro is not. Each formulation appears to provide about a week of partial protection against JB foliar feeding due to the fact that new, untreated terminal leaves continually develop.

In 2009, we compared the efficacy of three treatments against JB on grape foliage (Table 6). On June 26, 2009, Surround was applied at the rate of 25 pounds per acre to the first four rows (each 600 feet long) of a Norton block in 100 gallons of water in an air-blast sprayer. This was followed by 0.86 inches of rain on June 27–28, which necessitated reapplication of Surround on June 28. A total of 0.69 inches of rain was received from June 29 to July 12, so the last application of Surround was done on July 13, mostly to cover new green foliage being attacked by JB. The next two rows of vines were untreated from June 25 to July 9. Vines in rows 7 to 40 were treated with 10.5 fluid ounces of Danitol 2.4EC on June 26 and 4 fluid ounces of Mustang Max EC on June 30 and July 9.

By July 9, untreated vines on row ends recorded about 50 percent defoliation, so the grower sprayed Mustang Max insecticide to all untreated vines to maintain good vine health. On July 6, digital photographs were taken from the upper side of the canopy of half of each of 10 or 21 randomly selected vines in each treatment row and the number of JB per vine side was counted. On July 15, we also recorded the number of JB adults per vine side for each of 10 or 21 vines in each of two untreated rows or three treated rows.

On July 6, there were significantly fewer JB (Table 6) and skeletonized foliage per vine on vines treated with Surround or the insecticide (one Danitol followed by two Mustang Max sprays) than on untreated check vines. By July 15, only the Surround-treated vines had significantly fewer numbers of JB adults per vine and less foliar damage (Table 6) than the insecticide-treated or the untreated check vines, and hence both of them needed another foliar treatment against Japanese beetle attack. The participating grower said that this Surround treatment would drive off the JB for a short time until there was new growth or even the least bit of rain (greater than 0.3 inches): "I was hoping the persistence would have been better since it was clay and wouldn't break down like regular pesticides. I would say that Surround would only have application in a vineyard that is wanting to go organic and doesn't want to use other materials since it is the most expensive JB treatment, difficult to apply (couple of passes per row to get whitewash coverage) and very sensitive to rainfall."

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**Table 1. Insecticide formulations labeled for use against foliar grape phylloxera (GP), noting active ingredient, insecticide class and mode of action.***

| Formulations | Active Ingredient | Class | Mode of action |
|---|---|---|---|
| Movento | Spirotetramat | Tetramic acid | Inhibit acetyl CoA carboxylase, lipid synthesis, growth regulation |
| Danitol 2.4EC | Fenpropathrin | Pyrethroid | Na channel modulators |
| Surround WP | Kaolin clay | Earth-derived | Repellent |
| Admire Pro, Admire 2F, Advise 2FL, Couraze 2F, Nuprid 2F | Imidacloprid | Neonicitinoid | Nicotinic acetylcholine receptor agonists |
| Assail WSP, Assail 30SG, Assail 70WP | Acetamiprid | | |
| Platinum | Thiamethoxam | | |

* Growers can rotate among these insecticides with different modes of action to delay development of insecticide resistance in GP populations.

**Table 2. Numbers of shoots with grape phylloxera (GP) galls on old leaves present at the time treatments were applied (June 16) and number of shoots and terminal leaves with mature GP galls (Aug. 14) on Vignoles grapevines, Altus, Ark. (2009).**

| Treatment | Rate per acre | No. galled shoots[a] | No. shoots with mature galls on leaves[a] | No. leaves with mature galls[a] |
|---|---|---|---|---|
| Check (untreated) | — | 10.5 ± 1.12a | 8.5 ± 1.16a | 26.3 ± 4.36a |
| Danitol | 10 fl oz | 9.0 ± 0.72a | 2.4 ± 0.42b | 4.1 ± 0.68b |
| Movento | 8 fl oz | 12.4 ± 1.59a | 4.7 ± 0.73b | 9.0 ± 2.02b |
| Movento | 5 fl oz | 11.8 ± 1.40a | 3.8 ± 0.76b | 6.2 ± 1.20b |
| Surround | 33 lbs | 9.4 ± 0.88a | 3.5 ± 0.94b | 6.8 ± 1.54b |
| *F* (4, 56) | | 1.53 | 7.64 | 15.15 |

MSO = Methylated seed oil used with Movento treatments at rate of 0.9 fl oz MSO / 3 gal water

[a] Means followed by a different letter are significantly different, Waller-Duncan k-ratio t-test ($P < 0.001$)

**Table 3. Insecticides labeled against grape berry moth (GBM) as ovicides (shaded area) and larvicides, noting active ingredient, insecticide class and mode of action.***

| Formulations | Active Ingredient | Class | Mode of action |
|---|---|---|---|
| Altacor | Chlorantraniliprole | Diamides | Ryanodine receptor modulators |
| Baythroid XL | B-cyfluthrin | Pyrethroids | Na channel modulators |
| Brigade WSB | Bifenthrin | | |
| Danitol 2.4EC | Fenpropathrin | | |
| Intrepid | Methoxyfenozide | Diacylhydrazine | Ecdysone agonists, molting disruptors |
| Esteem 0.86EC | Pyriproxyfen | None | Juvenile hormone mimics |
| Avaunt | Indoxacarb | None | Voltage-dependent sodium channel blockers |
| Clutch 50WDG | Clothianidin | Neonicitinoid | Nicotinic acetylcholine receptor agonists |
| Sevin 80WSP | Carbaryl | Carbamate | Acetylcholine esterase inhibitor |
| Diazinon AG500 | Diazinon | Organophosphate | Acetylcholine esterase inhibitor |
| Imidan 70W | Phosmet | | |
| Delegate WG | Spinetoram | Spinosyns | Nicotinic acetylcholine receptor allosteric activators |
| SpinTor 2SC or Entrust (***organic***) | Spinosad | Spinosyn | |
| Biobit HP, Dipel DF (***organic***) | *Bt* subsp. *kurstaki* | Bacteria (microbial) | Toxin destroys gut |

* Growers can rotate among these insecticides with different modes of action to delay development of insecticide resistance in GBM populations.

**Table 4. Mean grape berry moth (GBM) catch per trap for three seasons at monitoring sites in Missouri and Arkansas.**

| | | Mean No. GBM/trap | | | |
|---|---|---|---|---|---|
| **Vineyard** | **Risk of GBM** | **2006** | **2007** | **2008** | **2009** |
| Ste. Genevieve, Mo. | Low to moderate | 26 | 0 | 27.0 | 34.8[a] |
| St. James, Mo. | Moderate to high | 180 | 121 | 13.0 | 17.5 |
| Hermann, Mo. | High | 112 | 27 | 22.0 | 54.2 |
| Rocheport, Mo. | Low | 23 | 55 | 28.0 | 12.9 |
| Purdy, Mo. | Low | — | — | 20.6 | 15.5 |
| Waverly, Mo. | Moderate to high | — | — | 32.9 | 47.3[b] |
| Hindsville, Ark. | Low to moderate | 76 | 27 | 13.0 | 4.5 |
| Paolo, Kan. | Moderate | — | — | — | 55.5[c] |

[a] Moved GBM traps to new location, a block by the winery with woods across the road.

[b] New location in 2008.

[c] New location added in 2009.

**Table 5. Harvest mean grape berry moth (GBM)–damaged clusters in perimeter row in vineyards in 2009.**

| **Vineyard** | **Risk of GBM** | **% GBM damaged clusters** |
|---|---|---|
| Farmington, Mo. | Low to moderate | 1 |
| St. James, Mo. | Moderate to high | 0 |
| Hermann, Mo. | High | 5 |
| Rocheport, Mo. | Low | < 1 |
| Purdy, Mo. | Low | 0 |
| Waverly, Mo. | Moderate to high | 9 |
| Hindsville, Ark. | Low to moderate | 0 |
| Paolo, Kan. | Moderate | 27 |

**Table 6. Mean number of Japanese beetles (± SE) per vine for each management treatment applied to two or more rows of Norton vines in Purdy, Mo. (2009).**

| | | Mean No. Japanese beetles per vine | |
|---|---|---|---|
| **Treatments** | **No. vines** | **July 6** | **July 15** |
| Surround WP (kaolin clay)* | 21 | 0.4 ± 0.16 b | 0.5 ± 0.11 b |
| Insecticide sprays* | 10 | 4.8 ± 1.02 b | 7.4 ± 0.83 a |
| Unsprayed | 10 | 48.0 ± 3.37 a | 5.0 ± 0.96 a |

* Spray dates: Surround on June 26 and 28 and July 5 and 13; Danitol on June 22, followed by Mustang Max on June 30 and July 9.

Means followed by a different letter are significantly different in Tukey's Studentized Range (HSD) test ($P < 0.05$)

# Emerging Viruslike Diseases on Chardonel, Vidal Blanc and Cabernet Sauvignon in Missouri

**Dr. Wenping Qiu and Dr. Shaista Lunden**
Center for Grapevine Biotechnology
Missouri State University

**Liying Wang and Yali Wang**
The State Key Laboratory of Seedling Bioengineering
Ningxia Forestry Institute

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## Abstract

Grapevine vein-clearing complex (GVCC) was first observed on Chardonnay grapevines in Missouri in 2004. GVCC symptoms include vein-clearing, short internodes and vine decline. Similar symptoms were observed on Chardonel, Vidal Blanc and Cabernet Sauvignon vines in the past four years in Missouri. We conducted a survey of two common viruses, *Tomato ringspot virus* (ToRSV) and *Grapevine fanleaf virus* (GFLV), in Chardonel vines from four commercial vineyards by the reverse-transcription polymerase chain reaction (RT-PCR). ToRSV and GFLV were detected in the sampled vines. There was no significant correlation, however, between the appearance of symptoms and the presence of the two viruses. It is probable that viruses other than ToRSV and GFLV that are indigenous to Missouri soil are associated with the emergence of viruslike diseases on *Vitis vinifera*-derived varieties and are associated with vine decline syndrome.

## Introduction

Virus and viruslike diseases emerged on Vitis vinifera–derived grape varieties such as Chardonnay (Qiu and Avery 2007; Qiu et al. 2007; Lunden et al. 2009) and hybrid grape varieties

(French–American hybrids), severely damaging vines in commercial vineyards in the Midwest region (Qiu and Avery 2007). The diseases slowly spread in these vineyards. Grapevine vein-clearing complex (GVCC) was found in a Chardonnay vineyard in Missouri in 2004 and affected Chardonnay vines so severely that the entire vineyard was removed in 2007 (Qiu et al. 2007; Lunden et al. 2009). Decline syndrome in grapevines with similar symptoms was also described in commercial vineyards in neighboring states (B. Taylor and S.A. Walters, personal communication). From the surveys of commercial vineyards in 2008 and 2009, it is clear that viruslike diseases are becoming epidemic on grape varieties such as Chardonel, Vidal Blanc and Cabernet Sauvignon.

Typical symptoms of viruslike diseases include mosaic and yellowing leaves with vein clearing, short internodes and decline of vine vigor that resemble those caused by Tomato ringspot virus (ToRSV), Grapevine fanleaf virus (GFLV) and other nematode-transmitted viruses. In a survey of GFLV, Grapevine fleck virus (GFkV), Grapevine leaf roll-associated virus 1 (GLRaV 1), GLRaV 3 and Grapevine virus A (GVA) by the enzyme-linked immune-sorbent assay (ELISA) in Missouri vineyards, GFLV was not detected in collected samples (Milkus and Goodman 1999). A survey of the four nepoviruses ToRSV, Tobacco ringspot virus (TRSV), Peach rosette mosaic virus (PRMV) and Arabis mosaic virus (ArMV) was conducted on five hybrid grape varieties, Vidal Blanc, Seyval Blanc, St. Vincent, Norton and Catawba, by the ELISA in commercial vineyards of Missouri, and found that ToRSV and ArMV were detected in the five hybrid grape cultivars (Milkus 2001). In this study, we described the characteristic symptoms that were observed on Chardonel, Vidal Blanc and Cabernet Sauvignon vines in geographically separated vineyards. We sampled 151 Chardonel vines from four vineyards and investigated the incidences of ToRSV and GFLV. The results showed that both ToRSV and GFLV were detected in Chardonel vines but were not closely correlated with the exhibition of symptoms, which is consistent with the previous findings in Chardonnay vines (Lunden et al. 2009).

## Materials and Methods

### *Samples and sample collection*

The grape variety Chardonel was selected, and four Chardonel vineyards designated as A, B, C and D were chosen for this study in 2008. The third fully expanded leaves of young shoots were

sampled. Six to eight leaves were collected randomly from both sides of each vine to compose one sample. Nine rows of Chardonel vines were divided into nine blocks, three samples were collected randomly from each block, and a total of 27 samples were collected from vineyard A on July 30, 2008, and from vineyards C and D on August 1, 2008. Sixteen rows of Chardonel vines were divided into 16 blocks, four samples were randomly collected from each block, and a total of 64 samples were collected from vineyard B on July 31, 2008. Samples were kept on ice in a cooler until they were frozen in liquid nitrogen in the laboratory. From Vineyard A, 27 samples were collected for repeated testing on June 2, 2009, from the same vines as in 2008.

### *RNA extraction*

Two grams of frozen leaf tissues were ground to a fine powder with 0.6 g polyvinyl polypyrrolidone (PVPP) in liquid nitrogen in a cold mortar. A 20 mL RNA extraction solution containing 17 mL of extraction buffer (2% CTAB, 2.5M NaCl, 1M Tris, pH 8.0, 0.5M EDTA, pH 8.0), 2 mL 10% SDS, and 1 mL 5% ß-marcaptoethanol was added and mixed well with ground leaf tissue powder. The mixed leaf tissues were distributed in 2-mL centrifuge tubes and frozen in a –80°C freezer overnight. Two tubes containing a total of 4 mL mixed leaf tissues were incubated at 42°C for 10 minutes and then mixed vigorously before centrifugation at 13,000 *g*, 4°C, for 20 minutes. Approximately 800 µl supernatant was transferred to a new tube and centrifuged at 13,000 *g*, 4°C, for 10 minutes. Then supernatant was transferred to a new tube and an equal volume of chloroform was added and mixed by vortex. After centrifugation at 13,000 *g*, 4°C, for 20 minutes, supernatant was combined and transferred into a new tube, and nucleic acids were precipitated with LiCl with a final concentration of 0.2M by incubation on ice for 2 hours (or overnight at 4°C). After incubation, nucleic acids were collected by centrifugation for 30 minutes at 13,000 *g*, 4°C. The pellet was washed twice with cold 80% ethanol. The pellet was dried under vacuum for 10 to 20 minutes and dissolved in 30 µl double DEPC-treated sterile water. The quality of RNA was analyzed by electrophoresis, and the concentration was measured in a spectrophotometer. Afterwards, RNA concentration was adjusted to 1 µg/µl water, and stored at –80°C until use. Unless mentioned otherwise, all steps were performed on ice in a RNA laboratory.

Total RNA from samples of vineyard D and 2009 samples of vineyard A were treated with DNase I in TURBO DNA-free reagents following the supplier's recommendation (Ambion Inc., Austin, Texas), and then further purified using RNeasy MinElute Cleanup Kit (Qiagen, Valencia, Calif.). Total RNA from vineyards A, B and C were used directly in the subsequent steps.

### *Reverse-transcription polymerase chain reaction*

Total RNA were transcribed to cDNAs by the MultiScribe reverse transcriptase Taqman Kit (Applied Biosystems) or by the SuperScript-III reverse transcriptase (Invitrogen), with addition of 50 µM random hexamer primer. Reverse-transcription conditions were: 25°C for 10 minutes, 48°C for 30 minutes, and 95°C for 5 minutes. A nested PCR procedure was adopted for detecting ToRSV. For the primary PCR, a pair of ToRSV CP-specific primer, ToRSV-620C (5'-GGCAACGGATTGGCACTTAACTCA-3') and ToRSV-71V (5'-GAGGAACGCTCTTGCACACTCT-3'), was used. Each 20-µl PCR reaction contained 12.8 µl $H_2O$, 4 µl 5× buffer, 2 µl dNTP (2.5 mM each), 1 µl 10 mM ToRSV-620C primer, 1 µl 10 mM ToRSV-71V, 1 µl cDNA, and 0.2 µl DNA Polymerase. Thermal cycle conditions were: 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 56°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 7 minutes. For the nested PCR, 1 µl primary PCR product was used as template with 20 µl of PCR reactions using the following primer pair: ToRSV-6697 (5'-ATCAGAGAGACTGATAACATCAGTT-3') and ToRSV-6698 (5'-GTAAGAGTATGAGTCTCCTAAGGTACAAG-3'). Nested-PCR conditions were: 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 7 minutes, and stored at 4°C. For the detection of GFLV, RT-PCR reaction and thermal cycle conditions the same as above except that GFLV-specific primer GFLV-CP433v (5'-GAACTGGCAAGCTGTCGTAGAAC-3') and GFLV-CP912c (5'-GCTCATGTCTCTCTGACTTTGACC-3'), and annealing at 55°C for 45 seconds were used. Tested PCR using a pair of internal primers GFLV-CPnf (5'-GCBGAAYTGGAAGARGCCDC-3') and GFLV-CPnr (5'-CCATAGTGGTCCCGTTCCACTC-3') with anneal temperature of 58°C for 45 seconds was performed to confirm the GFLV identity of PCR-amplified DNA fragments. This two-step RT-PCR was applied to detect ToRSV and GFLP in the samples of vineyard D, and for

2009 samples of vineyard A for which a combination of 50 μM random hexamer and oligo(dT) in a ratio of 3:1 was used in the cDNA synthesis.

For 2008 samples from vineyards A, B and C, ToRSV and GFLV were detected by the SuperScript III One-Step RT-PCR kit following the protocol by the manufacturer (Invitrogen), except for the fact that a 20-μl reaction was used for each RNA sample containing 1 μg total RNA. The reaction conditions were: cDNA synthesis, 1 cycle of 50°C for 30 minutes and 94°C for 2 minutes, and PCR amplification 35 cycles of 94°C for 15 seconds, 55°C for 30 seconds, 68°C for 1 minute, then final extension at 68°C for 5 minutes. Similarly, nested PCR was performed for detecting ToRSV using the primer pair of ToRSV-6697 and ToRSV-6698 under the same condition.

### *Analysis of PCR-amplified product*

Aliquots (10 μl) of the PCR products were analyzed on a 1.0% agarose gel by electrophoresis. The DNA fragments were visualized on a UV transilluminator following staining of the gel with ethidium bromide, and photographed by an imaging system.

## Results and Discussion

### *Vein-clearing and short internodes are typical symptoms*

New leaves on the young shoots that grew out of overwintering buds started to show vein-clearing symptoms in late May and early June under Missouri conditions. Mosaic and mottle symptoms were occasionally observed on some vines. Vein clearing is distinguishable and translucent when the symptomatic leaves were held up to light (Figures 1 and 2). Mosaic and mottle leaves were more frequently observed and short internodes were more pronounced on diseased vines in

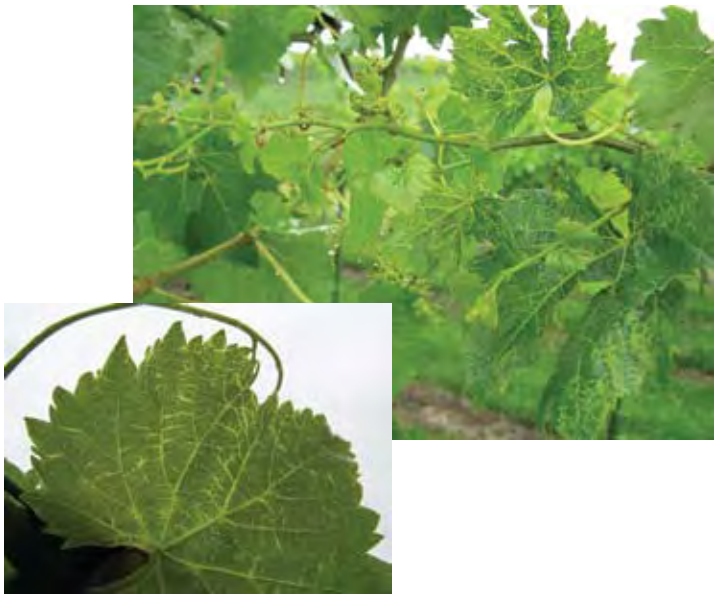

**Figure 1. Vein-clearing symptoms on Charonel vines (June 2, 2009).**

July and August than in late May and early June (Figures 3 and 4). A few symptomatic vines began to decline and die (Figure 5). These typical symptoms appeared on Chardonel in four geographically separate vineyards (Figure 6). Similar symptoms were also observed on Vidal Blanc (Figure 7) and on Cabernet Sauvignon (Figure 8).

**Figure 2. Vein-clearing symptoms on Charonel vines (June 2, 2009).**

Vein-clearing symptoms were observed first on 8-year-old Chardonnay vines in 2004, and different vines exhibited slight variations of the typical vein-clearing symptom most prominent on newly emerged young leaves. The vein-clearing symptom also appeared on Cabernet Franc, Baco Blanc and LN-33 that were grafted with the buds of originally diseased Chardonnay vines. Thus the disease was referred to as the grapevine vein-clearing complex (GVCC) (Lunden et al. 2009). The vigor and fruit set of GVCC-affected Chardonnay vines declined significantly so that the entire vineyard was unproductive and unprofitable and, hence, was uprooted in 2007. GVCC-like symptoms were noticed also on Chardonel vines in commercial vineyards in recent years. Since Chardonel is a hybrid variety of Seyval and Chardonnay, it is not surprising that the same symptoms appeared on both affected Chardonel and Chardonnay vines.

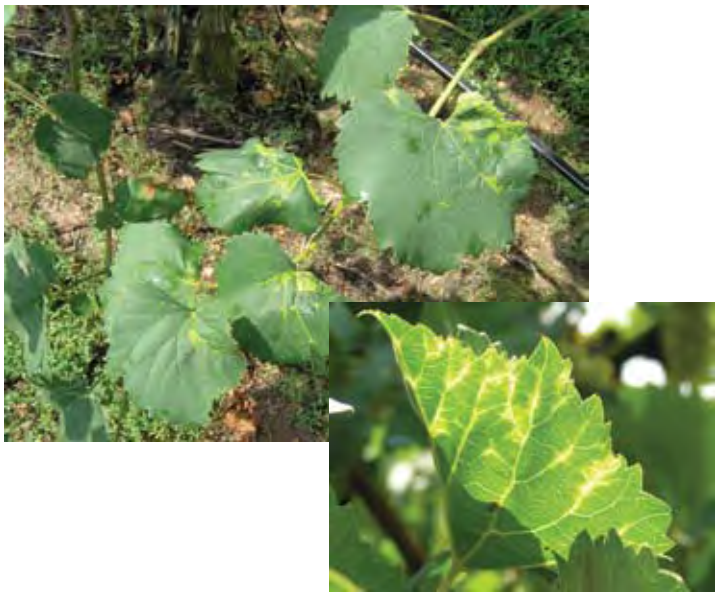

**Figure 3. Vein-clearing symptoms on Charonel vines (Aug, 11, 2009).**

Interestingly, Vidal Blanc and Cabernet Sauvignon vines also exhibited GVCC-like symptoms (Figures 7 and 8). One speculation is that the causal pathogens were present in the original vines but did not cause conspicuous symptoms in the first few years of establishment until the titers of virus complex reached such a high level that the complex of pathogens started debilitating vines under ambient climate and soil conditions in later years. It is also likely that soil-borne pathogens indigenous to Missouri soils and cover-crop plants were transmitted to newly planted grapevines and brought severe damage to most *V. vinifera*-derived grape varieties or hybrids with the genetic background largely from *V. vinifera*.

**Figure 4. Vein-clearing symptoms on Charonel vines (Aug. 27, 2009).**

**Figure 5. Vein-clearing syndrome causes decline and dying of Chardonel vines (June 2, 2009).**

## *No close correlation between symptoms and the presence of GFLV and ToRSV in Chardonel vines*

The results from the 2008 survey of four Chardonel vineyards for ToRSV and GFLV are summarized in Table 1. An example of detecting virus by the reverse-transcription polymerase chain reaction (RT-PCR) is provided in Figure 9. From the 27 Chardonel samples of vineyard A, three vines were infected with ToRSV and one was infected with GFLV. From the 64 Chardonel vines of vineyard B, ToRSV was detected in 16 samples and GFLV in 13 samples. Both viruses were present in one Chardonel vine. A total of 45 percent of Chardonel vines were infected by either ToRSV or GFLV in vineyard B. From 27

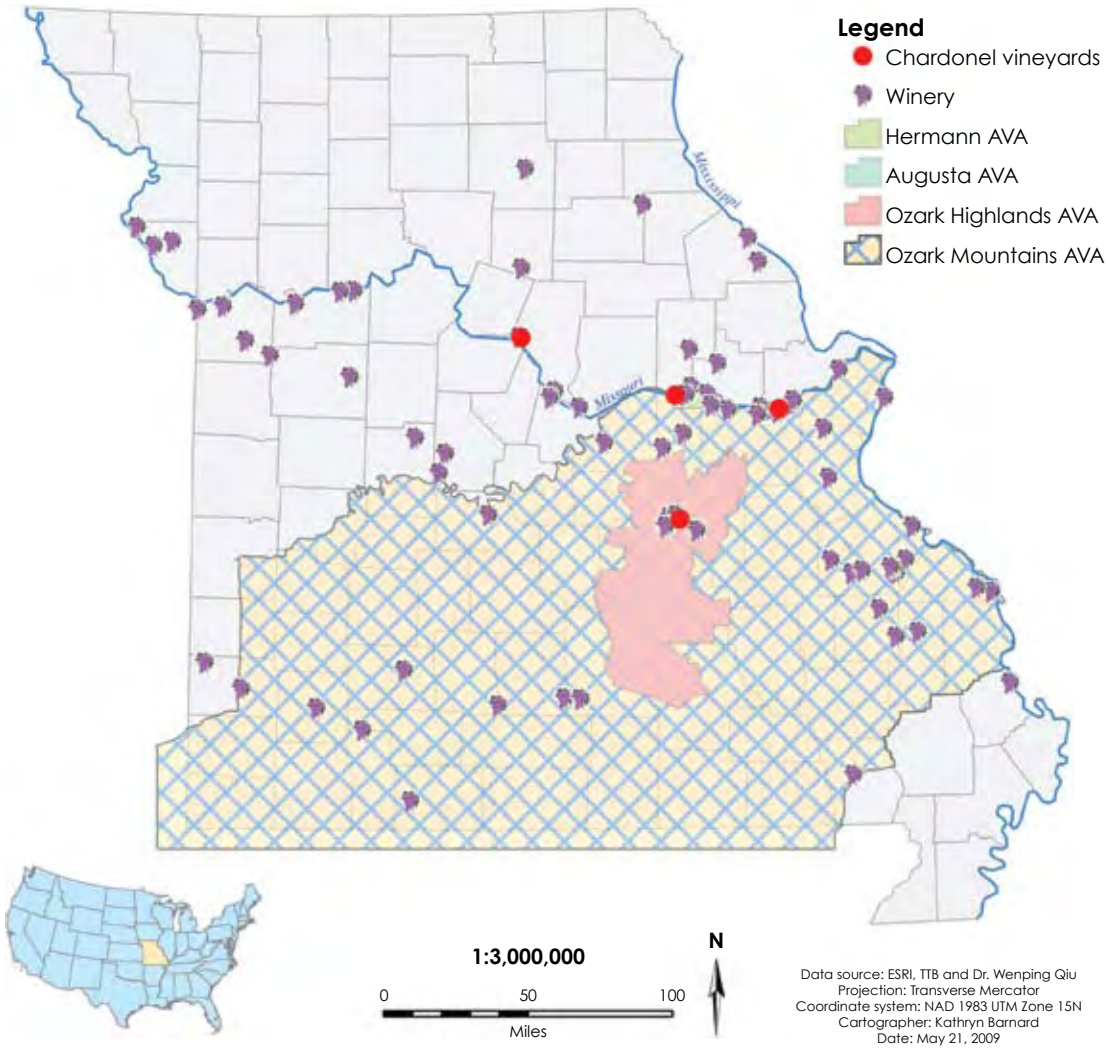


**Figure 6. Locations of four Chardonel vineyards that were sampled for detection of Tomato ringspot virus (ToRSV) and Grapevine fanleaf virus (GFLV) in 2008.**

Chardonel vines in vineyard C, four samples were positive for ToRSV and one was positive for both ToRSV and GFLV. Among 27 Chardonel vines from vineyard D, only one vine was infected by ToRSV and nine vines were positive for GFLV. The infection rate of ToRSV is highest in vineyard B while vineyard D has the highest incidences of GFLV.

The results from the 2009 survey of the same 27 vines in vineyard A showed that GFLV was detected in more vines in the 2009 samples than in the 2008 samples (Table 2). On the other hand, ToRSV was not detected in the 2009 samples of vineyard A (Table 2). A total of 16 vines (60 percent) tested positive for GFLV. The 2008 samples were collected on July 31; the 2009 samples were collected on June 2. Leaves of the 2009 samples were collected from the young shoots that

**Figure 7. Vein-clearing symptoms on Vidal Blanc vines (June 2, 2009).**

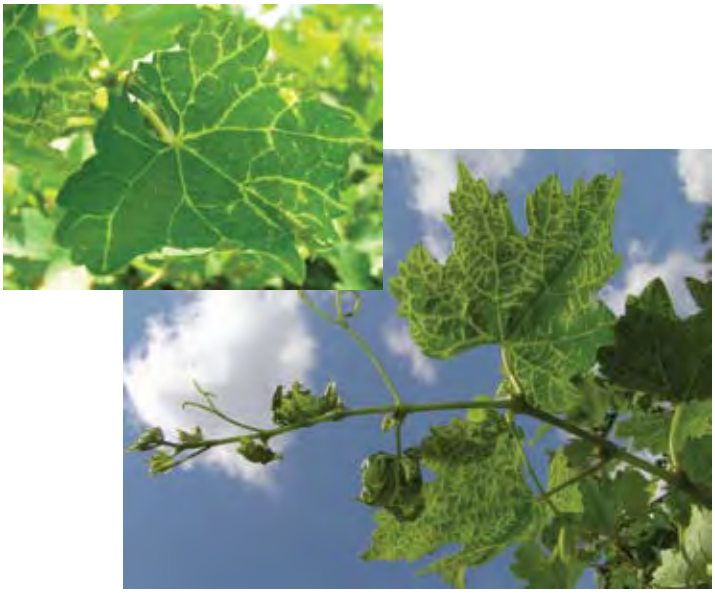

**Figure 8. Vein-clearing symptoms on Cabernet Sauvignon vines (Aug. 11, 2009).**

sprouted from the primary buds. It is likely that the sampling time and tissue type might influence the sensitivity of GFLV detection so that GFLV is detected more frequently in young leaf tissues of the shoots from primary buds. This observation is in agreement with previous findings of Rowhani et al. (2005).

Appearance of viruslike symptoms did not ensure the presence of two viruses in the sampled vines. Most Chardonel vines did not show symptoms but contained either one of the two viruses. It is speculated that viruslike pathogens other than ToRSV and GFLV may also be associated with the induction of vein clearing, short internodes and vine-declining symptoms.

This is the first report on the incidences of ToRSV and GFLV in hybrid Chardonel vines in commercial vineyards of Missouri. This survey confirmed that GFLV is epidemic in Chardonel vines in four major grape-growing regions in Missouri. The origin of GFLV cannot be verified at this time, nor can the speed of GFLV-spread in vineyards. It is not surprising that ToRSV was detected in Chardonel vines because it has a broad-spectrum host range. Existence of *Xiphinema* species of nematodes in vineyards and surrounding soils may provide means for the spread of ToRSV and GFLV among commercial vineyards.

## Conclusions

GVCC was first discovered on Chardonnay vines in Missouri in 2004. Causal agents of GVCC were graft-transmissible and caused vein clearing on bud-grafted Chardonnay, Cabernet Franc, Baco Blanc and LN-33. At present, GVCC-like symptoms have been observed on Chardonel in four viticultural areas of Missouri, and recently on Vidal Blanc and Cabernet Sauvignon. ToRSV and GFLV were detected in some GVCC-affected vines but were not closely correlated with the appearance of symptoms. It is hypothesized that other viruslike agents are also associated with GVCC, and these agents are indigenous to Missouri soils and cover-crop plants, and spread to newly planted grapevines and cause substantial damage to most *V. vinifera*-derived varieties or hybrids with a major genetic portion from *V. vinifera*.

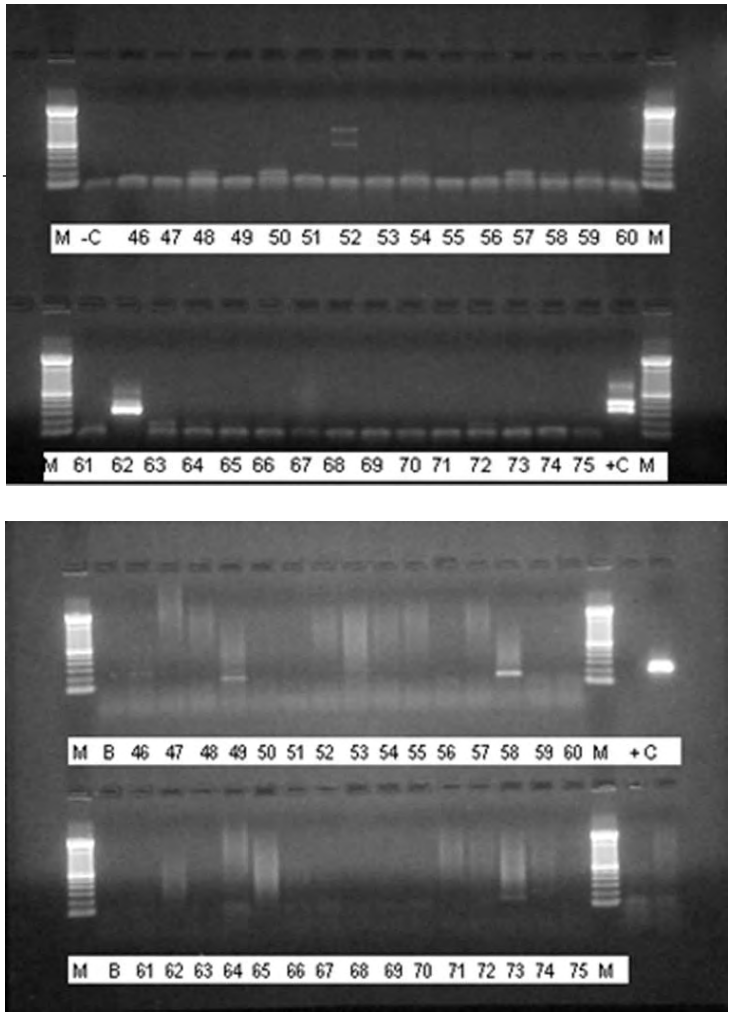


**Figure 9. A representative agarose gel image of detecting Tomato ringspot virus (ToRSV) and Grapevise fanleaf virus (GFLV) in Chardonel vines from Vineyard A. Virus-specific DNA was amplified in the reverse-transcription polymerase chain reaction with GFLV- or ToRSV-specific primers.**

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**Table 1. Incidences of *Tomato ringspot virus* (ToRSV) and *Grapevine fanleaf virus* (GFLV) in four Missouri vineyards and correlation with symptoms in 2008.**

| Vineyard | Vines Tested | ToRSV (% infected)* | GFLV (% infected)* | ToRSV+GFLV | Symptomatic Samples | Correlation |
|---|---|---|---|---|---|---|
| A | 27 | 3 (11%) | 1 (4%) | 0 | 3 | 0 |
| B | 64 | 16 (25%) | 13 (20%) | 1 | 8 | 2 |
| C | 27 | 4 (15%) | 1 (4%) | 1 | 4 | 2 |
| D | 27 | 1 (4%) | 9 (33%) | 0 | 2 | 2 |

* Percentage of virus-infected vines among the vines sampled and tested.

**Table 2. Incidences of *Tomato ringspot virus* and *Grapevine fanleaf virus* in the same Chardonel vines of Vineyard A (N: 38.02.598; W: 91.30.923) that were sampled on July 31, 2008, and June 2, 2009.**

| | | ToRSV | | GFLV | |
|---|---|---|---|---|---|
| **Sample ID** | **Symptoms** | **2008** | **2009** | **2008** | **2009** |
| MOV-47 | | | | | n/t |
| MOV-48 | | | | | + |
| MOV-49 | | + | | | + |
| MOV-50 | | | | | + |
| MOV-51 | | | | | + |
| MOV-52 | | | | | + |
| MOV-53 | | | | | + |
| MOV-54 | | | | | + |
| MOV-55 | | | | | + |
| MOV-56 | | | | | + |
| MOV-57 | | | | | + |
| MOV-58 | | + | | | n/t |
| MOV-59 | symptomatic | | | | |
| MOV-60 | | | | | |
| MOV-61 | | | | | + |
| MOV-62 | | | | + | + |
| MOV-63 | | | | | |
| MOV-64 | symptomatic | | | | + |
| MOV-65 | | | | | |
| MOV-66 | | | | | |
| MOV-67 | | | | | + |
| MOV-68 | symptomatic | | | | n/t |
| MOV-69 | | | | | |
| MOV-70 | | | | | + |
| MOV-71 | | | | | |
| MOV-72 | | | | | |
| MOV-73 | | + | | | + |

n/t: not tested.

# Speaker Contact Information

**Dr. Douglas Gubler**
Professor and Cooperative Extension Specialist
Department of Plant Pathology
University of California
460 Hutchison Hall
Davis, CA 95616
E-mail: wdgubler@ucdavis.edu

**Dr. Mike Ellis**
Professor
Ohio Agriculture Research and
Development Center
Department of Plant Pathology
The Ohio State University
224 Selby Hall
1680 Madison Avenue
Wooster, OH 44691
E-mail: ellis.7@osu.edu

**Dr. Donn Johnson**
Professor
Department of Entomology
AGRI 320
University of Arkansas
Fayetteville, AR 72701
E-mail: dtjohnso@uark.edu

**Dr. Andrew Landers**
Pesticide Application Technology Specialist
Department of Entomology
New York State Agricultural Experiment Station
Cornell University
312 Barton Lab
Geneva, NY 14456
E-mail: ajl31@cornell.edu

**Dr. Wenping Qiu**
Associate Research Professor
Missouri State Fruit Experiment Station
Department of Agriculture
Missouri State University
9740 Red Spring Road
Mountain Grove, MO 65711
E-mail: WenpingQiu@MissouriState.edu

**Dr. Reid Smeda**
Associate Professor
Division of Plant Sciences
University of Missouri
204 Waters Hall
Columbia, MO 65211
E-mail: SmedaR@missouri.edu

**Dr. R. Keith Striegler**
Director and Viticulture Program Leader
Institute for Continental Climate Viticulture
and Enology
University of Missouri
108 Eckles Hall
Columbia, MO 65211
E-mail: StrieglerK@missouri.edu

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