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North American Grapevine Yellows Disease:
Current Knowledge and Management Recommendations for Wine Growers

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Abstract

North American grapevine yellows (NAGY) is a lethal, insect-transmitted disease of grapevines caused by phytoplasmas (cell wall-less bacteria). North American grapevine yellows occurs throughout the Mid-Atlantic region of the U.S., but it is particularly prevalent in the Blue Ridge and Piedmont regions of Virginia, where it causes significant vine loss in susceptible Vitis vinifera varieties. This publication reviews what is known about NAGY, provides management options based on our current knowledge, and describes ongoing research needed to better understand this complex disease.

Chardonnay vine in mid-summer showing North American grapevine yellows symptoms on left-hand cordon. Note the downward rolling of leaf margins, the yellowing and premature abscission of leaves, lack of periderm development on shoot stems, and loss of flower or fruit clusters. Note also the apparently unaffected shoots on the right-hand cordon of same vine.
Photo by Teresa Stoepler.

Introduction

North American grapevine yellows (NAGY) is a lethal, insect-transmitted disease of grapevines caused by cell wall-less bacteria called phytoplasmas. Regionally, the disease is routinely observed in Virginia, Maryland, southeast Pennsylvania, and, less commonly, in the Finger Lakes region of New York (Davis et al. 2012). It has not been observed in North Carolina to date; however, it has recently been observed and diagnostically confirmed in grapevines in Ohio and Missouri (R. E. Davis, personal communication).

In Virginia, NAGY is a statewide threat, but the incidence is particularly high in the Blue Ridge and Piedmont regions where the attrition rate in grapevines of susceptible varieties can exceed 25 percent of the original planting over a five- to 10-year period. For winegrowers in high-risk areas, the incidence and the potential for NAGY have become chief considerations when deciding which varieties to plant and where. In fact, the occurrence of NAGY in highly susceptible cultivars such as chardonnay, white riesling, and malbec has forced some growers to cease production of those varieties altogether.

Much of the ecology and epidemiology of the NAGY disease cycle remains poorly understood, impeding efforts to manage the disease. The purpose of this publication is:

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- To outline what is known about NAGY.
- To provide management recommendations based on our current knowledge.
- To describe research efforts aimed at better understanding this complex disease.
- To assist wine growers' efforts to minimize vine losses.

"Grapevine yellows" refers to any of a number of phytoplasma-caused diseases that affect grapes worldwide. Specific grapevine yellows diseases include flavescence dorée, Palatinate grapevine yellows and Bois Noir in Southern Europe and the Mediterranean region, Australian grapevine yellows, North American grapevine yellows in the U.S. and Canada, as well as other grapevine yellows diseases reported in South Africa and Chile (Weintraub and Jones 2010). A commonality of these diseases is the range of symptoms produced in susceptible grape varieties, described below. However, important distinctions among many of these diseases are the specific causal pathogens, the insect vectors, and the fate of infected vines.

North American grapevine yellows, which affects grapes principally in the Eastern United States, is caused by strains of phytoplasma that are distinct from the causal phytoplasmas of yellows diseases in other regions of the world (Davis et al. 1998; Olivier et al. 2009). North American grapevine yellows was first observed in the 1970s on riesling grapevines in the Finger Lakes region of New York, where symptoms closely resembled those of flavescence dorée in French vineyards (Pearson et al. 1985). The disease was subsequently confirmed in Virginia in 1993 (Prince et al.1993) where symptoms had been observed since 1987, principally on chardonnay and white riesling (Wolf, Prince, and Davis 1994). Identical symptoms have since been observed on chardonnay and other varieties in southeast Pennsylvania and Western Maryland.

In Virginia, two different phytoplasma strains — termed group I (aster yellows group, subgroup16SrI-A); and group III (X-disease group, subgroup16SrIII-I) — are implicated in NAGY disease (Davis et al. 1998; Davis et al. 2012) and are found to co-occur within the same vineyards. The two strains are categorized taxonomically based on the nucleotide sequence of the 16S ribosomal RNA gene, with groups I and III potentially containing multiple phytoplasma species. The two phytoplasma strains cause apparently identical disease symptoms; however, it is unknown whether differences in vector relationships or other ecological differences exist between them.

Disease Cycle

The causal pathogens of NAGY disease — phytoplasmas — are organisms in the class Mollicutes. These obligate, intracellular parasites lack cell walls and have small genomes. The term "obligate parasite" means that researchers have not been able to maintain cultures of the organism outside the host plant or insect, and this imposes certain limitations on our ability to study the organism and its disease expression. While phytoplasmas only cause disease in plants, other members of the Mollicutes cause disease in animals and humans.

Phytoplasmas are responsible for disease in hundreds of plant species worldwide, including many important crops (Lee, Davis, and Gundersen-Rindal 2000). Phytoplasmas reside in the plant's food-conducting tissues (phloem), where they are thought to impair the function of sieve tubes (elongated food-conducting cells in the phloem). Phytoplasmas are only known to be transmitted in the field by phloem-feeding, hemipteran insects with sucking mouthparts, including leafhoppers, planthoppers, and psyllids (Weintraub and Beanland 2006). From a disease management standpoint, this essentially eliminates the potential for transmission by dormant pruning or other vine management practices. On the other hand, there is some potential for distributing infected plant material through propagation, grafting, and other nursery operations.

Phytoplasma cells are ingested by the insect while feeding, multiply in the insect's gut, and eventually reach the insect's salivary glands where they are incorporated into the insect's saliva. When the insect later feeds on a healthy plant, it injects saliva containing phytoplasma cells into the phloem, infecting the plant where the phytoplasma cells multiply once again.

Disease Symptoms, Cultivar Susceptibility, and Patterns of Infection

Diagnostic symptoms of North American grapevine yellows in cultivated *Vitis vinifera* include leaf discoloration and downward rolling of leaves, dieback of shoot tips, abortion of developing fruit, uneven periderm (bark) development on shoots, and eventually vine death (fig. 1A-D). Leaves become brittle and leathery, easily breaking off from the shoot. In white-fruited varieties, leaves may yellow and exhibit veinal chlorosis (yellowing of leaf veins; fig. 1A). Shoots appear rubbery due to a failure to develop periderm and lack of or incomplete lignin production in vascular tissues (fig. 1B). Similar symptoms have also been observed in vidal blanc, and other interspecific hybrids, but at much lower frequency than in *V. vinifera* cultivars. In red-fruited varieties, leaves turn dark red (fig. 1C).

Often, a single shoot exhibits symptoms on an otherwise apparently healthy vine in the first year, followed by expansion of symptoms to other parts of the vine in the second and subsequent years. However, we lack an understanding of the distribution of phytoplasmas within the plant. Presumably, the localized expression of disease symptoms on a few shoots of a vine, while other shoots of the vine remain asymptomatic, reflects localized differences in phytoplasma concentration or titer.

Symptoms may be observed any time after bloom. Infected vines usually die within two to three years. While the disease progresses relatively rapidly in highly susceptible varieties such as chardonnay and more slowly in less susceptible varieties such as cabernet sauvignon, vines become progressively weaker and death of the vine occurs in all cases (Wolf, Prince, and Davis 1994). The inevitable death of vines infected with NAGY differs somewhat from reports of the European grapevine yellows disease, flavescence dorée, wherein vines may undergo an apparent remission of symptoms but remain infected. Reasons for the discontinuous symptom expression of flavescence dorée are unknown.

Although it is not clear whether vines that express NAGY symptoms are an efficient inoculum source of phytoplasmas for insect vectors, we recommend their removal from the vineyard to eliminate this potential inoculum source and because there is no precedent to suggest that they will recover.

The symptoms of NAGY can be confused with other diseases or nutritional disorders, and careful diagnosis of NAGY should be made before making disease management decisions such as roguing of infected vines. The combination of (1) characteristic discolored, rolled, and brittle leaves; (2) uneven bark development; and (3) cluster abortion is diagnostic for NAGY (fig. 1A-D).

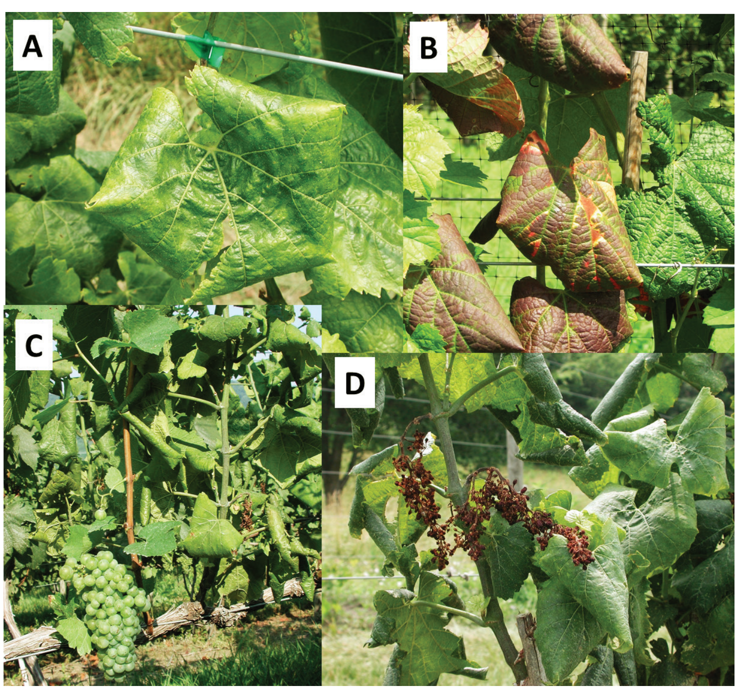

Figure 1. Symptoms of North American grapevine yellows (NAGY). Discolored, downward-curled leaves in (A) chardonnay, and (B) malbec; (C) a comparison of periderm development on an unaffected (left) and NAGY-affected (right) shoot showing the characteristic "rubbery shoot" appearance caused by lack of periderm development; and (D) an aborted fruit cluster in chardonnay. When observed together on the same vine, these three symptoms — leaf discoloration and rolling, lack of periderm development on shoots, and fruit cluster abortion — are diagnostic for North American grapevine yellows. The lack of one of these symptoms may suggest another disease or nutritional deficiency. Photos by Tony Wolf.

The lack of one or more of these symptoms could point to a disease or disorder other than NAGY. For example, a common but unrelated disease of Mid-Atlantic grapevines — grape leafroll disease, which is caused by several RNA viruses — causes similar discoloration and downward leaf rolling but does not cause cluster abortion, failure of periderm maturation, or shoot tip dieback (fig. 2).

Similarly, deficiencies of nutrients such as nitrogen or phosphorus (fig. 3) may also cause leaf discoloration, but nutrient deficiencies do not cause uneven bark development or cluster abortion. Further, unlike NAGY, which affects individual vines or vine parts, nutrient deficiencies normally affect a broad area of the vineyard. Potato leafhoppers (*Empoasca fabae*), which can feed on grapevines, also cause leaf cupping, but their feeding does not cause cluster abortion or failure of periderm development, and it typically affects a large, contiguous area of vines.

Vitis vinifera varieties differ significantly in susceptibility to NAGY, with chardonnay, riesling, and malbec among the most susceptible varieties commonly grown in the Mid-Atlantic United States (fig. 4). The reasons for this variation in cultivar susceptibility are unknown; however, this variation is common to grapevine yellows diseases in other grape-growing regions of the world.

Previous work has documented an "edge effect" on vine infection by NAGY, with a higher incidence of infected vines on the edge of vineyards closest to deciduous forests, treelines, or scrub, suggesting that the insect vectors are moving into the vineyard from alternative host plants in these bordering habitats. Because NAGY symptoms are not normally observed in the first year after vineyard planting and because the observed pattern of vine infection suggests an insect vector, there is no compelling reason to believe that NAGY is introduced into the vineyard from nursery stock produced within the U.S. (Beanland, Noble, and Wolf 2006).

Figure 2. A chardonnay vine displaying symptoms of grape leafroll disease caused by several different leafroll viruses. Grape leafroll disease causes leaf discoloration and downward leaf rolling similar to North American grapevine yellows (NAGY), but unlike NAGY, grape leafroll does not cause cluster abortion, failure of periderm maturation, or shoot-tip dieback. Photo by Mizuho Nita.

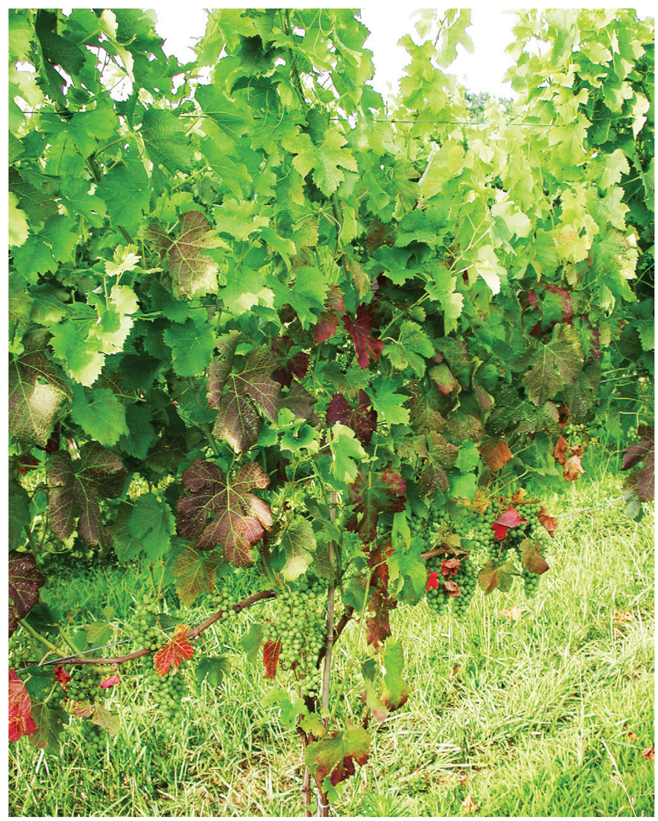

Figure 3. A merlot vine showing symptoms of nutritional deficiency (leaf discoloration). Unlike North American grapevine yellows (NAGY), nutritional deficiencies do not cause uneven bark development or fruit abortion. Further, nutrient deficiencies generally affect a broad area of the vineyard, unlike NAGY. Photo by Tony Wolf.

LOW				HIGH
0	1	2	3	4
Petit Verdot	Cabernet Franc	Albariño	Aramon	Alicante
	Merlot	Mourvedre	Barbera	Alicante Bouchet
	Rousanne	Vermentino	Cabernet Sauvignon	Chardonnay
	Sauvignon blanc		Cinsault	Malbec
	Syrah/Shiraz		Dolcetto	Pinot gris
	Viognier		Lemberger	Pinot noir
	American types		Petit Manseng	
	(eg., Norton)		Pinot blanc	
	Hybrids		Riesling	
	Wild grape		Sangiovese	
	(Vitis riparia, V. cordifolia)		Tannat	

Figure 4. Relative susceptibility of *Vitis vinifera*, hybrid varieties, and wild *Vitis* spp. to North American grapevine yellows (NAGY). Legend:

(0) Does not show symptoms, but may harbor phytoplasmas.

(1) In mixed plantings, these cultivars rarely, but occasionally show symptoms.

(2) In mixed plantings, these cultivars will occasionally express symptoms, particularly if vines are planted in a known NAGY area. Affected vines usually die within one to three years, but the overall incidence is less than 1 percent of original planting lost per year.

(3) In mixed plantings, these cultivars often express symptoms, particularly if vines are planted in a known NAGY area; the annual incidence may rarely exceed 2 percent of original planting. Affected vines usually die within one to three years.

(4) In mixed plantings, these cultivars are the most frequently affected and symptoms progress rapidly to vine death, often by the second year of symptom expression. Chardonnay is the representative cultivar of this group. Annual frequency of newly infected vines often exceeds 2 percent of original planting (T. K. Wolf, personal observation; C. Y. Olivier, personal communication).

Insect Vectors

Leafhoppers and planthoppers (Hemiptera) are phloem-feeding (plant-sap-feeding) insects with piercing-sucking mouthparts and rows of spinelike hairs on their hind legs. Leafhoppers are much more abundant and diverse than planthoppers in Virginia vineyards (Beanland, Noble, and Wolf 2006), and have been the focus of NAGY vector identification efforts. Leafhoppers are small insects (2 to 30 millimeters, but often smaller than 6 millimeters or 0.25 inch in body length) that can be seen hopping on the vineyard floor or resting on grapevines and other vegetation.

Of the approximately 20 species of leafhoppers common in Virginia vineyards, several have tested positive for the causal phytoplasmas of NAGY using DNA-based testing (DNA extraction and polymerase chain reaction, or PCR). However, insects that have fed on a phytoplasma-infected plant may ingest phytoplasma cells, causing them to test positive, but they may not necessarily be able to transmit those phytoplasmas to new plants. For the insect to infect a healthy plant, phytoplasma cells must be able to multiply in the insect's salivary glands (Weintraub and Jones 2010). Therefore, transmission studies, where the saliva exuded from insects feeding on infected plants is tested for phytoplasmas in the laboratory using PCR are required to establish vector identities. Once insect vector identities are known, an understanding of their behavior and population demography (e.g., phenology, movement patterns, host plant preferences, natural enemies) is critical to effective management.

To date, several species of leafhoppers have been identified as potential vectors in Virginia, including *Scaphoideus titanus* (fig. 5), a known vector of flavescence dorée in Europe. Further testing is required to understand the efficiency of *Scaphoideus titanus* and additional species in vectoring phytoplasmas and to determine levels of vector specificity in transmitting the two strains of phytoplasmas implicated in NAGY.

Figure 5. The North American leafhopper, *Scaphoideus titanus* (Cicadellidae: Hemiptera) is the vector of the European grapevine yellows disease, flavescence dorée. *Scaphoideus titanus* occurs in Virginia, where it is a specialist on grape hosts and a suspected vector of the causal phytoplasmas of North American grapevine yellows. Photo by Michael Maixner.

Alternative Host Plants

In addition to cultivated grapevines, the causal phytoplasmas of NAGY have been detected in a broad range of woody and herbaceous plant species. To date, alternative host plants that have tested positive for the causal phytoplasmas include wild grape (e.g., *Vitis cordifolia* and *V. riparia*), black cherry (*Prunus serotina*), American elm (*Ulmus americana*), American sycamore (*Platanus occidentalis*), dandelion (*Taraxacum* sp.), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), and *Lespedeza* sp. (T. K. Wolf and L. Beanland, unpublished data). Unlike infected cultivated grapevine, these plants are usually asymptomatic. However, we caution that transmission experiments are needed to clarify the role of these alternative host plants as disease reservoirs (i.e., to determine whether leafhopper vectors can transmit phytoplasmas from alternative host plants to cultivated grapevines).

Management Recommendations

The occurrence of NAGY varies among vineyards and years, possibly due to variations in the populations of insect vectors of the phytoplasmas, the distribution of alternative host plants of the phytoplasmas, and the insect management programs utilized in those vineyards. Due to the complexity of this disease, the potential number of insect vectors and alternative host plants, and the range of environmental variables likely to affect disease risk, NAGY management is challenging. With our current knowledge, we recommend a multifaceted approach to NAGY management in vineyards, involving both proactive and active tactics. Proactive management practices include careful vineyard site selection and planting of cultivars that are relatively tolerant of NAGY in areas where the disease is known to occur.

As discussed previously, the highest incidence of NAGY appears to occur close to wooded areas, particularly in the northern Piedmont and Blue Ridge Mountain regions of Virginia. This information should be taken into consideration when new vineyard sites are chosen, especially if the grower plans to grow highly susceptible varieties (fig. 4). Although it is not known exactly how much spatial separation is necessary to prevent any insect movement into vineyards, a general recommendation would be to separate vineyards from wooded areas and wooded fence rows by at least 30 meters (approximately 100 feet; Beanland, Noble, and Wolf 2006) or as far as is feasible.

The removal of wild grapevines (e.g., *Vitis riparia* and *V. vulpina*) from the vineyard environment may be beneficial because they are known alternative hosts of the causal phytoplasmas, and because wild grape is the only natural host of the known phytoplasma-transmitting vector (*Scaphoideus titanus*; fig. 5), which occurs in Virginia. However, further transmission experiments are necessary to determine whether wild grapevines and/or other alternative hosts are effective reservoirs of phytoplasmas when they occur near vineyards.

Active management tactics include the use of insecticides targeted at potential insect vectors. Unfortunately, because leafhopper vectors may be present throughout the growing season, spraying throughout the entire season may be necessary. The repercussions of season-long spray programs include secondary outbreaks of other pests (e.g., mites) and reductions in natural enemy populations that may help to control vector populations (e.g., parasitoid wasps, green lacewings, predaceous flies, nabid bugs, and ladybird beetles; Daane and Costello 2000). Once infected, susceptible grapevines typically die and there are no practical, therapeutic measures that can be used to treat infected vines.

Current Research

There is much that remains unclear about NAGY, particularly with respect to vectors and alternative hosts. At more fundamental levels, we also do not understand how phytoplasmas cause the disease symptoms they do or how the pathogens are distributed throughout the plant. Future insect monitoring and transmission studies will help to determine which vector species are the most abundant and effective and these species should be the target of insecticide programs (i.e., the timing of sprays should be based on knowledge of the target species' biology and phenology).

Our current research includes:

1. Further identifying insect vectors and their relative efficacy.

2. Monitoring vector behavior and phenology as the starting point for a more efficient management program.

3. Understanding the role of alternative host plants as disease reservoirs in and around NAGY-affected vineyards.

4. Identifying the common landscape and ecological features of high-risk vineyards.

5. Evaluating of the efficacy of potential NAGY management practices.

Given the slow disease cycle and the complexity inherent to the NAGY system, such research will require a multiyear approach. Progress in our understanding of NAGY will benefit from the cooperation of growers in keeping accurate records of spray regimes and vine removal, as well as active participation in evaluation of new management techniques.

Growers and vineyard managers should familiarize themselves with NAGY disease symptoms and be able to distinguish them from those of other diseases and nutritional deficiencies (figs. 1-3). Confirmed infected vines displaying the combination of the three NAGY diagnostic symptoms described above should be removed from vineyards to avoid the risk of those vines serving as a disease reservoir. The location, date, and symptoms of removed vines should be recorded, if possible, to allow quantification of NAGY impacts and to enable future analyses of compiled NAGY incidence reports from multiple vineyards.

Conclusions

North American grapevine yellows is a complex disease due to the number of potential insect vector species and alternative host plants, the prolonged disease cycle, and the inability to isolate the phytoplasmas for study in the laboratory. Unfortunately, there are no easy management solutions. Growers with high-risk NAGY vineyard sites should avoid planting highly susceptible varieties. It is critical that growers are able to recognize disease symptoms and to distinguish them from other diseases and deficiencies to prevent unnecessary vine removal. Further research is necessary to understand which vector(s) and alternative host plant(s) are important in the NAGY disease cycle.

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