MID-TERM PROGRESS REPORT
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Title: Characteristics of Grapevine Yellows-susceptible vineyards and potential management strategies

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Overall Project Objectives

1. Identify phytoplasma alternative hosts in and around North American Grapevine Yellows (NAGY)-affected vineyards and attempt to identify the characteristics of vineyards that predispose them to increased risk of NAGY
2. Evaluate efficacy of potential Grapevine Yellows management practices

Objective 1:

a. NAGY transmission assays:

During 2015 we maintained and visually monitored the Chardonnay seedlings and grafted grapevines used in our previous transmission trials. Due to the incubation period, Grapevine Yellows (GY) symptoms tend to appear late in the season. We noticed some leaf curling in some of the seedlings, which could have been mistaken for GY symptoms. Those symptoms, however, disappeared in the new leaves. Considering that a few leafhoppers were placed on each vine, which could have provided a little *inoculum* for transmission, we decided to determine the presence or absence of phytoplasma with molecular analysis, despite the lack of symptoms. Through the fall and winter (2015/2016) DNA was extracted from the leaf veins of all 65 seedlings and PCR analysis was carried out using specific 16S GY primers. Several of these indicator plants produced positive results for NAGY phytoplasmas, indicating a positive transmission capability of with two of the candidate vectors which we have focused on over the last several years. The very low rate of transmission/disease expression may highlight the very low transmission rate that occurs with the insect species/host combination in the field, or it might reflect the conditions (hotter greenhouse temperatures) under which the indicator vines are maintained; high temperatures are known to suppress expression of Yellows diseases in some other systems.

Towards the end of the season, we have focused our transmission attempts using an artificial diet, rather than plants. We collected insects from high NAGY incidence vineyards and forced them to acquire phytoplasma by caging them on NAGY-infected grapevine shoot stems for 1 to 2 days. This Acquisition Access Period (AAP) is the minimum time required by a hopper to become virulent upon feeding on an infected source. We switched from 2 to 1 days after noticing a high rate of mortality of the insects caged on the vines. Surviving insects were transferred to pots of barley and clover plants for 21 days, the latency period necessary for hoppers to become infective. Finally, insects were separated by species and transferred to 5% sucrose solutions. This year, we placed 5 individuals per vial, instead of 1, in order to increase the amount of phytoplasma DNA potentially released in the solutions. 38 sucrose trials were

carried out during the season, including species that were not present in our previous table of reference (Table 1), but commonly found in Virginia. Subsequent PCR analysis of the sucrose solutions with 16S primers has confirmed the ability of several very common leafhopper species to release GY phytoplasmas while feeding (Table 2, Appendix). In particular, these experiments have suggested that species like *Graminella nigrifrons*, *Exitanius exitiosus, Latalus sayii* and *Deltocaephalus flavicosta* could be considered as potential NAGY vectors, along with the *Endria inimica* and *Agallia constricta*, whose ability to release GY phytoplasma was already known from last year. Moreover, for the first time some *Exitanius exitiosus* and *Deltocaephalus flavicosta* samples resulted positive to PCR even when *SecY* primers were used. Our failure to detect a *SecY* amplification product has always been an issue with our sucrose trials, because of its inconsistency. This time, it has also been possible to confirm the DNA sequence for a *SecY* PCR product of one sucrose vial used to feed *Exitanius exitiousus* (NAGY Group III). Interestingly, the leafhopper species that are most commonly found to secrete phytoplasmas into the sucrose solution are not considered important, direct feeders on grapevines. They are, however, commonly found on the vegetation of the vineyard floor.

b. Alternative hosts: To assess whether non-*Vitis vinifera* species can be considered as alternative NAGY hosts, we need to demonstrate not only the presence of GY DNA, but also the ability of insects to transmit phytoplasmas from the alternative host to grapevine. For this kind of experiment, we need insects that have never been exposed to phytoplasma. We are rearing colonies of potential candidate vectors to be used for these experiments in the spring of 2016.

c. Phytoplasma titer in infected plants: We are also interested in determining the concentration of phytoplasmas in different grapevine varieties and in different plant tissues, in order to better understand how fast phytoplasmas move within the plant and to relate the manifestation of GY symptoms to phytoplasma titer. In 2015 we used quantitative PCR (qPCR) to determine the titer of phytoplasma DNA in grapevine samples. The amount of Yellows DNA was normalized to a plant gene, to make sure that the final results were not affected by different amounts of DNA.

As reported in our previous 2015 report, we showed that:

- The difference in phytoplasma titer in four different varieties (Chardonnay, Riesling, Cabernet Sauvignon and Tannat) is not statistically significant, despite the difference in susceptibility of the varieties to GY.
- Phytoplasmas are much more abundant in symptomatic leaves, and nearly undetectable in asymptomatic leaves on the same vine, illustrating the variable distribution of the causal agent within infected vines.
- Phytoplasma concentration is barely detectable in the infected stems, compared to the leaves.
- Phytoplasma titer increases from year to year, suggesting either that the microbes are able to replicate during the winter, or that their population doesn't drop during the winter.

Taken together, our results seem to indicate that the actual presence of phytoplasma is linked to the manifestation of symptoms. These data were presented as a poster at the American Phytopathological Society annual meeting in summer 2015.

Objective 2: Grapevine Yellows management practices evaluation

a. Insecticide programs targeting vectors: Dr. Tony Wolf designed and managed a season-long

insecticide spray program in 2014 to determine whether this approach effectively reduced leafhopper populations. This study was conducted in three vineyard blocks at two cooperating vineyards in Fauquier County, and repeated similar spray programs conducted in those same vineyards during 2013. Weekly leafhopper samples using both sticky traps and sweep netting were compared between insecticide-treated and paired control (non-treated) blocks. We found that season-long spraying does effectively reduce leafhopper abundance, as illustrated by the data of Figure 5 (Linden Chardonnay); however, it remains to be determined whether this leads to a concomitant reduction in the incidence of new NAGY infections. Our hypothesis here was that if we could suppress leafhopper populations for the entire season, we would expect to see a significant reduction in the incidence of NAGY in the insecticide-treated blocks, relative to the unsprayed control blocks, in the subsequent year. This crude "shotgun" approach to leafhopper management ignores the specifics of *which species* and *what timing* might be important, and may well be unsustainable; however, it allows us to ask a general question about whether insect (vector) management may aid NAGY management.

The insecticide treatments were not repeated in 2015; however, weekly monitoring of the vineyard blocks was necessary in order to determine the impact of insecticide sprays vs. no sprays in 2014. Fortunately for the two commercial growers, but unfortunately for our research purposes, there were NO cases of NAGY in any of the 3 vineyard blocks that were differentially treated in 2014 leading one of our cooperators to declare that we had "found a solution to NAGY". Ironic perhaps, but the two vineyards (Linden and RdV) which had experienced heavy losses to NAGY in 2011 and to a lesser degree in 2012, have been relatively unaffected since.

b. Removal of affected vine parts/organs as a tool to manage NAGY: As proposed, trials were initiated in two vineyards during 2013 to survey and remove NAGY symptomatic portions of vines when symptoms became apparent. We did this to determine if this severe pruning delayed or arrested symptom development in the subsequent year (2014). Although it is difficult to explain how removal of an affected cordon or trunk might arrest the development of a systemic pathogen, we had anecdotal evidence that the severe pruning can be used to effectively prolong the life of affected vines *of some varieties, but not others*. Approximately 60 vines were either heavily pruned in this fashion, or were left untreated during the 2013 season. These vines were closely monitored for NAGY symptoms and for vine development in 2014, and will be followed, if still alive, into 2015. The results of this part of the project were presented at the 2015 VVA's Winter Technical Meeting and in our year-end report to the Wine Board (August 2015). The treated vines were revisited on several occasions during 2015 with any change in status noted. Our take-home message from this work is that early intervention of symptomatic vines, as in cordon and/or trunk removal, does have the potential to extend the productive life of a vine. Our inability to detect phytoplasmas in asymptomatic vines (those not showing symptoms), however, does not rule out the possibility that such vines still harbor a low titer of phytoplasmas. We do not, however, feel that asymptomatic vines are effective sources of inoculum for transmission to other grapevines.

Plans for 2016

The transmission studies facilitated with this project have yielded data that implicate 2 or more leafhopper species as vectors of the phytoplasmas that cause NAGY in Virginia vineyards. While our results do not rule out the potential that other leafhoppers are also competent vectors, the research has allowed us to focus on the ecology and potential management of these positive vectors. We do have more lab work to do between January and July of 2016 when the project will formally end. We are also in the process of preparing information that can be shared with the industry on our findings, including when the use of insecticides might be warranted to manage leafhopper levels, and when the removal of affected plant material might be well advised.

Appendices

Impact Statement

North American Grapevine Yellows is a lethal, insect-transmitted disease of grapevines caused by phytoplasmas (bacteria-like organisms). NAGY is a statewide threat in Virginia, but is particularly severe in the Blue Ridge and Piedmont regions where the highest vineyard densities occur. The goal of our research is to increase understanding of this complex disease and to inform management practices to mitigate vine losses. We anticipate that our research will identify vectors, which may allow temporally-targeted insecticide sprays. We may also identify important alternative hosts of the causal agents of the disease, which might allow removal of the alternative hosts from the vineyard environment. Our preliminary results also suggest that removal of affected organs (e.g., cordons or trunks) from less susceptible varieties may extend the productive lifespan of such vines.

Publications and presentations

Lenzi P, Stoepler T, Melby D, Wolf T. 2015. Characterization of North American Grapevine Yellows. In 2015 APS Annual Meeting, Pasadena, CA, USA

Davis RE, Dally EL, Zhao Y, Lee I-M, Wei W, Wolf TK, Beanland L, LeDoux DG, Johnson DA, Fiola JA, Walter-Peterson H, Dami I and Chien M. 2015. Unraveling the etiology of North American Grapevine Yellows (NAGY): Novel NAGY phytoplasma sequevars related to '*Candidatus Phytoplasma pruni*'. Plant Dis. 99:1-11.

Table 1. Candidate insect vectors (Order Hemiptera) of Group I and III phytoplasmas identified in 2012-2013 sucrose transmission assays. Insects were collected from commercial vineyards in Virginia and fed a 5% sucrose solution in individual tubes. The sucrose solution/saliva mixture was subsequently tested for phytoplasmas with nested polymerase chain reaction (PCR).[1] Species abundance ranking is based on season-long sweep net samples of 72 species of leafhoppers in 27 mid-Atlantic vineyards in 2013; 1= most, 65 = least abundant).

Species	Species abundance rank	Subfamily	Sucrose media N positive/tested		NAGY-phytoplasma Group
			2012	2013	
Family Cicadellidae - Leafhoppers					
Agallia constricta	1	Agallinae	0 / 5	4 / 585	III-A
Graphocephala versuta	4	Cicadellinae	2 / 40	0 / 91	III-A
Exitianus exitiosus	6	Deltocephalinae	2 / 49	0 / 102	III-A
Endria inimica	10	Deltocephalinae	1 / 14	0 / 61	I-B
Amblycellus curtisii	15	Cicadellinae	2 / 5	0 / 25	I-B
Coelidia olitoria	32	Coelidiinae	0 / 0	4 / 24	III-A
Scaphytopius magdalensis	65	Deltocephalinae	1 / 2	0 / 1	I-B

[1]Species that were tested but did not yield any positive results in either 2012 or 2013 were omitted from this table (N = 38 species). In 2012, all insects were collected and tested during September only. In 2013, although insects were tested throughout the growing season (May – Oct.), only insects collected late in the season (late July – late Sep. 2013) yielded positive results.

Table 2. Candidate insect vectors identified in 2014-2015 sucrose trials. Insects were collected from commercial vineyards in Virginia and fed a 5% sucrose solution in individual tubes. The sucrose solution/saliva mixture was subsequently tested for phytoplasmas with nested polymerase chain reaction (PCR).

Species	Subfamily	Sucrose media transmission (n positive/n tested)	
		2014	2015
Agallia constricta	Agallinae	6/13	0/1
Endria inimica	Deltocephalinae	4/6	1/10
Exitanus exitiosus	Deltocephalinae	0/3	2/9
Deltocephalus flavicosta	Deltocephalinae	-	2/3
Latalus sayii	Deltocephalinae	-	1/1
Graminella nigrifrons	Deltocephalinae	-	3/5