END OF FISCAL YEAR PROGRESS REPORT
July 31, 2014

Virginia Wine Board

CHARACTERISTICS OF GRAPEVINE YELLOWS-SUSCEPTIBLE VINEYARDS AND POTENTIAL MANAGEMENT STRATEGIES

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Start date: 1 July 2013
Type of Project: Research

ACCOMPLISHMENTS TO DATE

Overall Project Objectives

i. 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
ii. Evaluate efficacy of potential Grapevine Yellows management practices

Summary

To address Objective 1, in May - September 2013, we conducted a survey of 30 vineyards ultimately aimed at linking ecological factors to North American Grapevine Yellows (NAGY) disease incidence. In a subset of three of these vineyards with historically high incidence of NAGY, we conducted weekly, season-long (April – October, 2013) surveys of the leafhopper community to understand the phenology and population dynamics of potential vector species. Vineyard NAGY infection incidence ranged from 0 – 10% in susceptible *Vitis vinifera* varieties (primarily Chardonnay). We collected 72 species of leafhoppers and approximately 10 species of planthoppers across the 30 vineyards studied. Of these, 7 species of leafhoppers have been identified as candidate vectors based on preliminary transmission studies and subsequent phytoplasma DNA sequencing in 2012 - 2013. As we confirm these species as vectors of NAGY with greater sample sizes and using Chardonnay vines in our ongoing transmission trials, we can examine the phenology data of each species to aid in preparing specific management recommendations for wine growers.

With respect to Objective 2, Dr. Tony Wolf and assistants began an ongoing, replicated insecticide trial at two cooperating vineyards, Linden and Rutger deVink, to test the efficacy of a season-long spray program targeting leafhopper control in spring 2013. This experiment is being repeated in the 2014 season. Season-long insecticide cocktails have had the intended effect of reducing overall leafhopper abundance, however, we do not yet know if this will result in reduced NAGY infections.

Teresa and Tony published a peer-reviewed extension article reviewing what is known about NAGY and outlining management strategies through the Virginia Cooperative Extension in September 2013 and Teresa presented her NAGY research orally at a conference at UC Riverside and to the public at the 2014 USA Science and Engineering Festival in Washington, DC (see **Appendix**).

Finally, Teresa will be leaving the position in August 2014 to begin a science policy fellowship in Washington, DC. We have hired a replacement research associate, Dr. Paolo Lenzi, a molecular biologist, who will pick up the research where Teresa left off beginning in late July 2014.

Objective 1: Vineyard survey and vector identification

NAGY vineyard survey—To understand the ecological factors that predict NAGY incidence, we conducted an extensive survey of 30 mid-Atlantic vineyards (primarily in VA) in which we mapped NAGY incidence in susceptible wine grape varieties (primarily Chardonnay) and measured the abundance and diversity of leafhoppers in the vineyard floor, canopy, and bordering vegetation using a sweep net and yellow sticky card traps **(Figure 1).** In a subset of 3 of these vineyards with historically high NAGY incidence, we sampled leafhoppers weekly to

understand the seasonal phenology of candidate vector species **(Figure 1).** We surveyed 62,725 vines across the 30 vineyards and found 511 that were infected with NAGY (~1%). While this overall incidence of NAGY is low, these vineyards were purposely chosen as representative of a historically broad sample of typical NAGY infection rates, ranging from never observed to extremely high, to allow us to begin to understand which environmental factors determine disease risk. We found at least one NAGY-infected vine in 21 out of the 30 vineyards surveyed, with infection rates in susceptible varieties ranging from 0 – 10%. We observed NAGY farther south than has previously been documented – in Pinot Noir in southern Virginia (Washington County) and in Chardonnay in the Yadkin Valley of North Carolina. Nevertheless, 2013 appeared to be a "low incidence" year for NAGY overall, with low incidence observed in many vineyards that have historically had problems with NAGY. The reason for this year-to-year variability is unknown and requires further long-term study.

Insect vector identification— In 2013, we found higher levels of leafhopper diversity than expected; we collected more than 72 species of leafhoppers and 10 species of planthoppers across the 30 vineyards surveyed. Of these, approximately 20 – 30 species of leafhoppers and 3 species of planthoppers are common in Virginia vineyards. The only leafhopper species whose abundance was found to be positively correlated with the incidence of NAGY in 2013 is *Exitianus exitiosus* **(Figure 2).** As this species was also preliminarily identified as a vector in our transmission assays (see below), this species warrants further attention.

To identify the insect vectors of NAGY, we conducted transmission assays by collecting 1840 leafhoppers and planthoppers from high-incidence NAGY vineyards throughout the growing season and placing them in individual tubes with a sucrose solution. The insects feed on the sucrose, which is then tested for the presence of the causal phytoplasmas of NAGY using polymerase chain reaction (PCR) in the laboratory. Insects whose sucrose/saliva mixtures tested positive had fed on phytoplasma-infected plants naturally in the field and because they were able to transmit these phytoplasmas into sucrose (similar to a plant cell), they are candidate vectors of NAGY in wine grapes. Using this sucrose tube method, we have preliminarily identified 7 candidate vector species which will be the focus of our experiments going forward **(Table 1)**. We note that the leafhopper, *Scaphoideus titanus*, is also a suspected vector based on previous work, but was captured in extremely low abundances in our 2012-2013 sampling and *Scaphoideus* species never transmitted phytoplasmas to sucrose in our transmission assays (data not shown).

In addition to these transmission assays with sucrose, we also caged candidate vector leafhoppers collected from high-incidence vineyards onto healthy, potted Chardonnay plants as a second method to confirm vector identities in real plants. We will continue to monitor these Chardonnay vines (n=100) for the development of NAGY symptoms until next summer, and they will be tested for phytoplasmas using DNA extraction and PCR to confirm their infection status.

Objective 2: Grapevine Yellows management practices evaluation

In summer 2013, Dr. Tony Wolf conducted a season-long spray program to determine whether this approach effectively reduces leafhopper populations. This study was conducted at two local cooperating vineyards in Fauquier County. 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 **(Figure 3);** however, it remains to be determined whether this leads to a concomitant

reduction in the incidence of new NAGY infections. This insecticide experiment is currently being repeated for the 2014 season.

As proposed, trials were initiated in two vineyards during 2013 to survey and remove NAGY symptomatic portions of vines to determine if this severe pruning delayed or arrested symptom development in the subsequent year (2014). Approximately 25 vines were heavily pruned in this fashion during 2013 and these vines are being closely monitored for NAGY symptoms and for vine development this season.

Work underway this growing season (2014)

Based on our 2012-2013 transmission results **(Table 1)**, we are focusing on these 7 candidate vector species. In 2014, we are repeating our transmission assays as described above, except with several modifications aimed at increasing the rate of transmission in effective vectors. In previous transmission assays, transmission rates were sometimes very low and were inconsistent between years because these field-collected insects may not have fed on infected plants, or may have fed on infected plants too soon before collection to transmit phytoplasmas (insufficient time for phytoplasmas to replicate in insect tissues). Further, insects were collected throughout the growing season in 2013 (May – October), yet only those insects collected in late July – September when NAGY-infected wine grape symptoms are evident and phytoplasma concentration (titer) is presumably at its peak, yielded positive results. Therefore, in 2014, we will greatly increase our positive transmission sample sizes by (1) Focusing on the 7 species which have yielded positive results, especially the most abundant ones **(Table 1),** (2) Ensuring that insects have fed on phytoplasma-infected plants and that sufficient time has passed to allow phytoplasma replication in the insect's salivary glands (insects will first be caged onto NAGY-infected plants and allowed a latent period for phytoplasma replication prior to testing), and (3) Focusing transmission assays in the late summer (July – September) when NAGY symptoms are observed in infected *V. vinifera* and phytoplasma concentrations are thought to be highest, resulting in higher probability of transmission.

To estimate the relative importance of candidate vectors, we are recording the number of candidate vector leafhoppers in ongoing diurnal (10 am – 3 pm), crepuscular (4 – 7 pm) and nocturnal (8 pm – 12 am) vineyard sweep samples (floor, canopy, and bordering vegetation) at high-incidence vineyards (Williams Gap, Wild Meadow, and at the AREC) weekly, July – September, 2014. Collecting insects during crepuscular and nocturnal periods ensures that species abundances are not biased by collection time, as several of the candidate vectors have been collected at night. We will estimate the relative importance of each vector with the transmission risk index: (transmission rate) × (total N. leafhoppers/100 sweeps).

Reports and Presentations

1) Drs. Stoepler and Wolf co-authored a Virginia Cooperative Extension publication on our current knowledge of NAGY which was published in September 2013:

> **Stoepler, T.M.** and Wolf, T.K. **2013.** North American grapevine yellows: Current knowledge and management recommendations for wine growers. *Virginia Cooperative Extension*, publication AREC-48P.
> **Available at: http://pubs.ext.vt.edu/AREC/AREC-48/AREC-48.html**

2) Dr. Stoepler presented her NAGY research to children and their families at the 2014 USA Science and Engineering Festival, 'Meet a Scientist' Event sponsored by the American Association for the Advancement of Science (AAAS), April 25, 2014, Washington, DC.

3) Dr. Stoepler presented a poster at the Hemipteran-Plant Interactions Symposium at UC Riverside, June 22-25, 2014:

   Stoepler, T.M. and Wolf, T.K. **2014.** Identification of candidate vectors of North American grapevine yellows, a lethal wine grape disease. Hemipteran-Plant Interactions Symposium, UC Riverside, Riverside, CA.

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APPENDIX

i. 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.

ii. Publications and presentations

Stoepler, T.M. and Wolf, T.K. **2013.** North American grapevine yellows: Current knowledge and management recommendations for wine growers. *Virginia Cooperative Extension*, publication AREC-48P. **Available at: http://pubs.ext.vt.edu/AREC/AREC-48/AREC-48.html**

Stoepler, T.M. and Wolf, T.K. **2014.** Identification of candidate vectors of North American grapevine yellows, a lethal wine grape disease. Hemipteran-Plant Interactions Symposium, UC Riverside, Riverside, CA.

iii. Tables and Figures

Table 1. Candidate insect vectors (Order Hemiptera) of Group I and III phytoplasmas that cause North American grapevine yellows in preliminary 2012-2013 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 (Subfamily)	Abundance rank	2012 Sep. 10 – 17		2013 Jul. 12 – Sep. 20		Phytoplasma ID
		N.	% +	N.	% +	
Agallia constricta (Agallinae)	1	5	0	276	1.4	16SrIII-A
Graphocephala versuta (Cicadellinae)	4	40	2.5	64	0	16SrIII-A
Exitianus exitiosus (Deltocephalinae)	6	49	4.1	38	0	16SrIII-A
Coelidia olitoria (Coelidiinae)	32	0	-	24	16.7	16SrIII-A
Endria inimica (Deltocephalinae)	10	14	7.1	21	0	16SrI-B
Amblycellus curtisii (Cicadellinae)	15	5	40.0	20	0	16SrI-B
Scaphytopius magdalensis (Deltocephalinae)	65	2	50.0	1	0	16SrI-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.

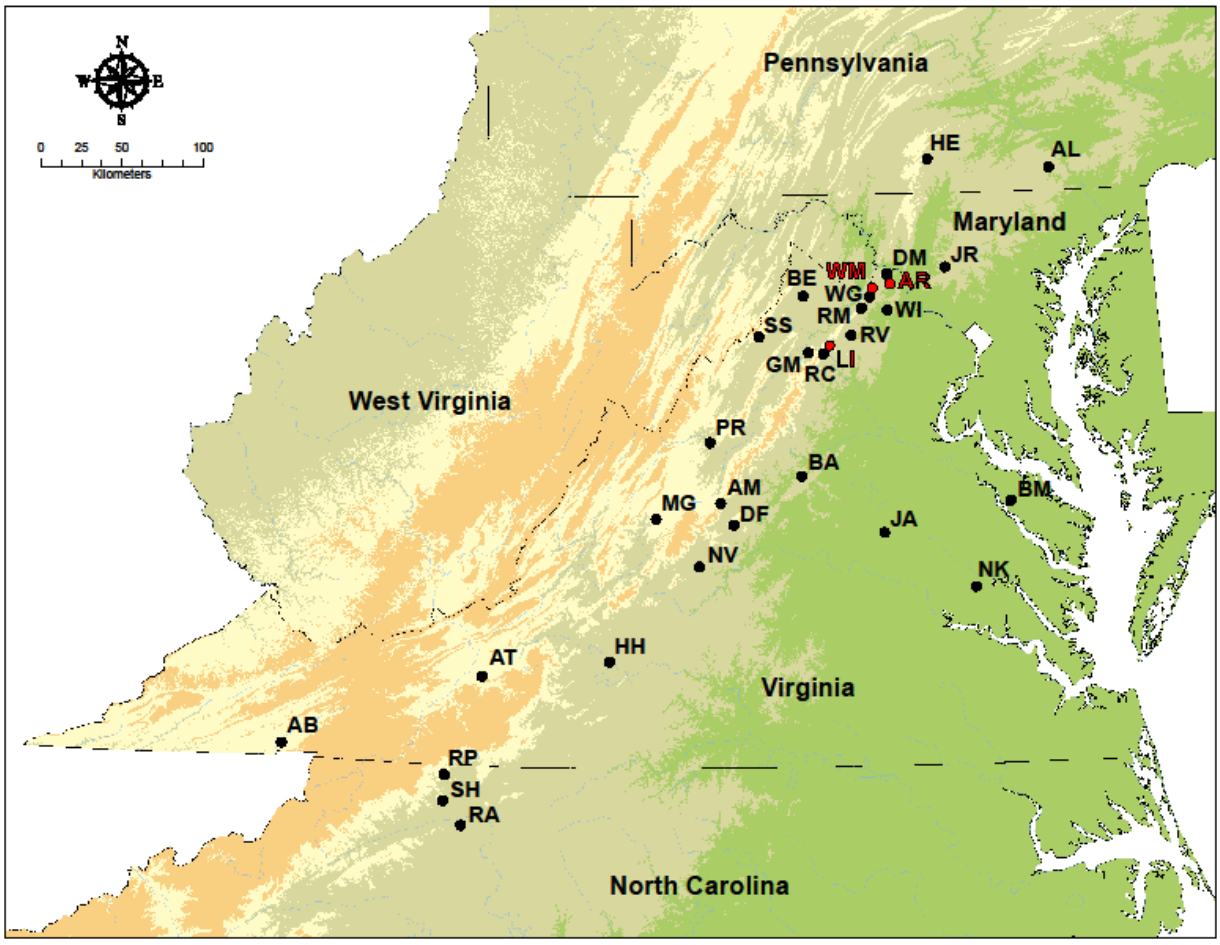
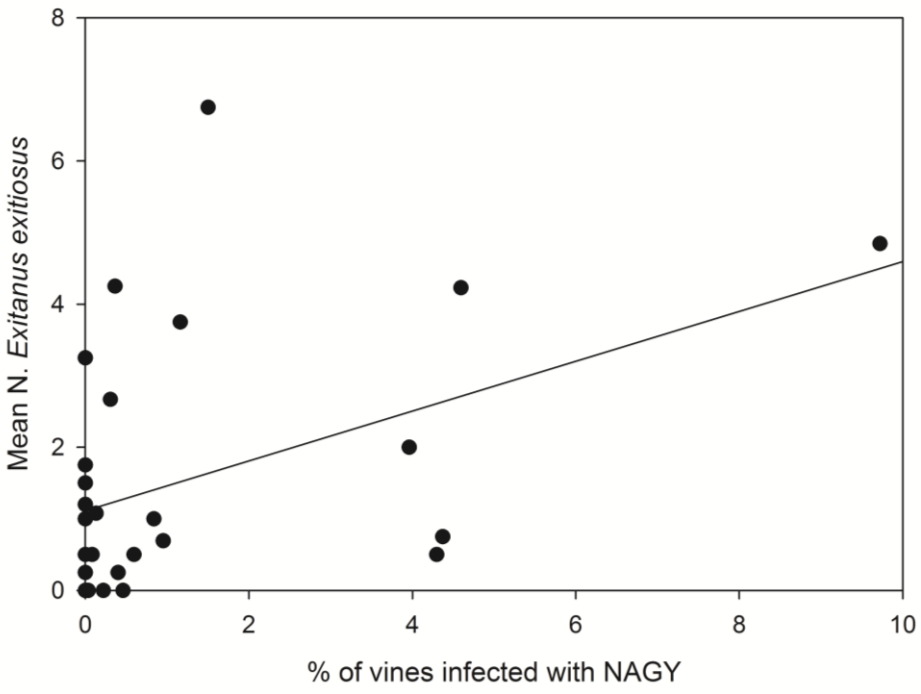


Figure 1. Map of North American Grapevine Yellows 2013 vineyard sites (n = 30). Sites in red were 'intensively' sampled weekly April – October 2013 (n = 3).

(A)



(B)

Figure 2. (A) The percentage of NAGY-infected vines in susceptible blocks increases with the number of *Exitianus exitiosus* leafhoppers collected from the vineyard floor late in the season. Leafhoppers were collected with a sweep net during Jul. 1 – Sep. 30, 2013 from 30 Mid Atlantic vineyards. This pattern suggests that *E. exitiosus* may be one important vector (transmitter) of NAGY-phytoplasmas. Each point represents one vineyard. **(B)** An adult *E. exitiosus* leafhopper.

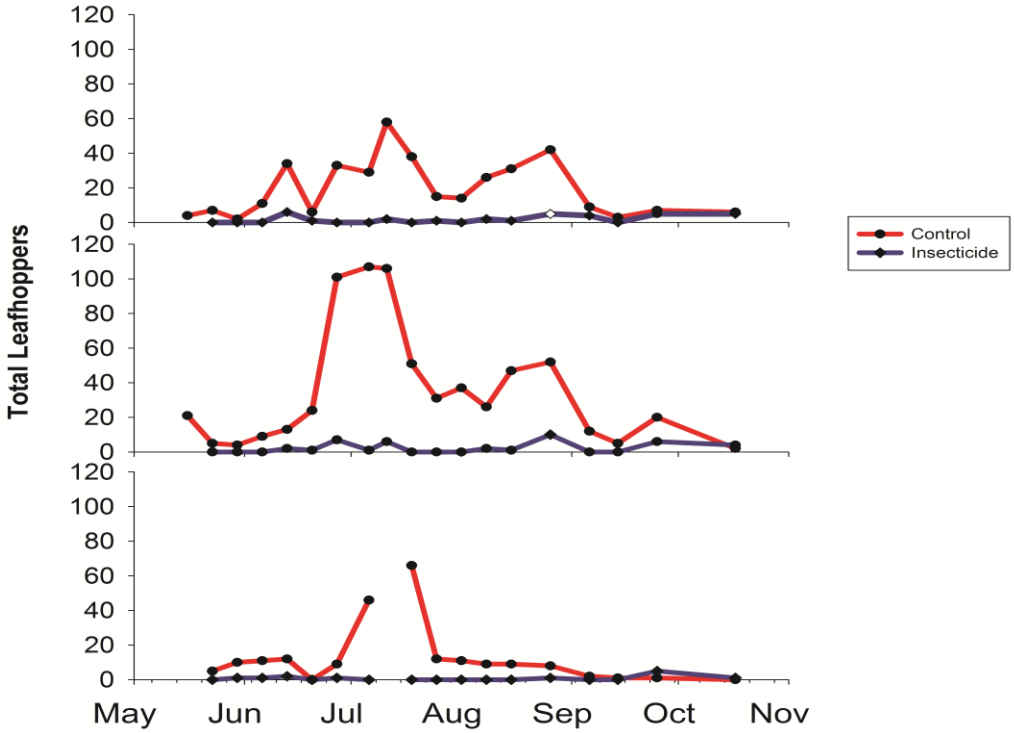


Figure 3. Total number of leafhoppers collected from the vineyard floor from control (red; no or little insecticide application) and insecticide-cocktail treatment plots (navy blue) in Fauquier County, VA, 2013.