END OF FISCAL YEAR PROGRESS REPORT
July 31, 2013

Virginia Wine Board

CHARACTERISTICS OF GRAPEVINE YELLOWS-SUSCEPTIBLE VINEYARDS AND POTENTIAL MANAGEMENT STRATEGIES

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Start date: 1 July 1 2012
Termination date: 31 June 2013 (but funding continued through 31 June 2014)

ACCOMPLISHMENTS TO DATE

Overall Project Objectives

1. Identify phytoplasma alternative hosts in and around Grapevine Yellows-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

Summary

The postdoctoral associate, Dr. Teresa Stoepler, was hired on August 1, 2012. From August 2012 – January 2013, Teresa learned to identify North American grapevine yellows (NAGY) disease symptoms in grapevines in the field, established working relationships with local vineyard owners and managers who continue to welcome our work in their vineyards, established diagnostic lab protocols for detecting phytoplasmas in both plant and insect feeding media (sucrose solution) samples, collected and identified potential vector insects, and conducted preliminary laboratory studies to establish the identities of the insect vectors of NAGY. Teresa also collected samples of potential alternative host plants and tested them for phytoplasmas.

In June 2013, Teresa began conducting a survey of 30 vineyards aimed at linking ecological factors (potential leafhopper vector composition and abundance, surrounding vegetation, weather patterns) to NAGY disease incidence with a focus on Chardonnay. In addition, in a subset of 3 of these vineyards (all with historically high incidence of NAGY), Teresa and assistants conduct weekly, season-long surveys of the leafhopper community to understand the phenology and population dynamics of potential vector species. Leafhoppers collected at these high incidence sites are returned to the lab to be tested for their potential to transmit the causal phytoplasmas of NAGY using transmission studies. A part-time (1450 hr) research assistant, Brinton Domangue (M.S. Biology, James Madison University) was hired onto the NAGY project in April 2013. Dr. Tony Wolf is conducting 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. Teresa and Tony presented their NAGY study plans for the 2013 season at the Virginia Vineyards Association Meeting in February 2013. Finally, Teresa and Tony have a peer-reviewed extension article reviewing what is known about NAGY and outlining management strategies that is in press with Virginia Cooperative Extension (see **Appendix**).

Laboratory setup and testing

In August 2012, Teresa engaged in a three-day, intensive laboratory training with Ellen Dally of the Molecular Plant Pathology laboratory headed by collaborator, Dr. Robert Davis at the USDA-Agricultural Research Service in Beltsville, MD. During this training, Teresa learned updated DNA extraction and polymerase chain reaction (PCR) protocols specific to phytoplasma detection in plant and insect samples. Teresa returned to the AREC and purchased necessary molecular lab supplies to begin diagnostic testing for NAGY-causal phytoplasmas, set up the lab at the AREC, and adapted USDA lab protocols to available AREC equipment. Using these protocols, Teresa successfully conducted DNA extractions, PCR and gel electrophoresis on grapevine and alternative host plant tissue, and sucrose-solutions fed on by leafhoppers for the causal phytoplasmas of NAGY.

Insect collection and preliminary transmission studies: Aug – Oct. 2012

Leafhoppers and planthoppers were collected from vineyards and nearby vegetation using a sweep net and then are aspirated from the net into a vial. To date, Teresa has collected and tentatively identified to species approximately 50 species of leafhoppers in VA vineyards. The more common species are being evaluated for their potential to vector the causal phytoplasmas of NAGY. In 2012, Teresa visited and collected samples from a total of 18 vineyards (16 in VA and 2 in PA). Live insects were identified, sorted by species and collection site, and their vector capabilities were tested using transmission assays in the lab (using sucrose solution). Transmission studies are needed to determine insect vector identity because insects may test positive for phytoplasmas, but are unable to transmit them to healthy plants unless the phytoplasmas can replicate in their salivary glands.

In sucrose transmission assays, leafhoppers are placed in microcentrifuge tubes individually and tubes were capped with 5% sucrose solution. Parafilm is stretched under the cap to form a membrane through which the insect can feed on the sucrose. After two days, the remaining sucrose solution/leafhopper saliva mixture is removed from each tube and tested for phytoplasmas using direct and nested PCR. The presence of phytoplasmas in the sucrose solution indicates that the leafhopper species in that tube is an effective vector. In 2012, sucrose-saliva samples were collected from a total of 138 leafhoppers representing at least 10 species collected from 4 vineyards. Eight of these preliminary transmission samples have tested positive for phytoplasmas (5.8%) (see below). Much more extensive sampling of potential vectors using this method is currently underway (see Objective 1: Vineyard survey and vector identification).

Alternative hosts

Teresa collected potential alternative host plant samples from vegetation surrounding various vineyards in VA and PA and tested them for the causal phytoplasmas of NAGY. These samples included several new species of potential alternative host plants, including American Elm and Poison Ivy, which tested positive for phytoplasmas. Other alternative hosts which have previously tested positive for phytoplasmas, including black cherry and wild grape, also tested positive. These alternative host samples were DNA sequenced to confirm phytoplasma group and strain identities (see below).

DNA sequencing of phytoplasmas

Plant and transmission (leafhopper saliva/sucrose solution) samples which test positive for the presence of phytoplasmas in the laboratory using PCR are sent to the Virginia Tech Bioinformatics Institute for DNA sequencing. This is necessary to confirm the identity of the phytoplasmas present (e.g., group and strain) and to confirm that they are the causal phytoplasmas of NAGY. We successfully sequenced phytoplasma-positive transmission samples and Dr. Chris Dietrich (Illinois Natural History Survey) has confirmed the identity of eight individual leafhoppers of five different species. A second round of PCR and DNA sequencing will now be performed on these samples using a different set of primers (targeting the secY region of phytoplasma ribosomal DNA) to further confirm the identity of the phytoplasmas transmitted into the solution. In addition, further testing of these leafhopper species and others using both sucrose assays as well as Chardonnay grapevines are required to ultimately determine the vector status of these and other common leafhopper species found in Virginia vineyards.

Objective 1: Vineyard survey and vector identification

To understand the ecological factors that predict the incidence of NAGY in commercial vineyards, we are conducting a survey of 30 vineyards (24 in VA, 3 in NC, 1 in MD, and 2 in PA) including mapping of infected vines, vineyard site characteristics (weather, surrounding habitat and vegetation type), and potential leafhopper vector abundance and species composition. Leafhopper abundance and composition data will be collected at least 8 times each during the peak leafhopper season (June – Sep.) using sticky traps and sweep netting methods. Because there is a year-long lag in the expression of NAGY symptoms following infection, this study is expected to continue for at least another year (2014). A subset of 3 of the "high incidence" VA vineyards are sampled intensively (weekly) throughout the entire growing season (April – Oct) to understand the population dynamics of potential vector leafhoppers.

To identify the vector leafhopper species implicated in NAGY transmission, leafhoppers collected from the high incidence vineyards are tested for their ability to transmit the causal phytoplasmas of NAGY using transmission assays as described above. So far this season, we have collected over 900 sucrose-leafhopper saliva samples total from more than 15 species to be tested using PCR in fall 2013.

In addition to the sucrose solution media, potted Chardonnay vines are also being used to confirm suspected vector leafhoppers, focusing on three species: *Scaphoideus titanus, Agallia constricta*, and *Graphacephala versuta*. Field-collected leafhoppers are caged onto healthy Chardonnay vines to test their potential as NAGY vectors. These vines will be protected from further insect feeding and monitored for at least one year for the development of NAGY symptoms. If successful, NAGY vector identification will represent a significant contribution to our understanding of NAGY infection dynamics and will serve as a major deliverable to the wine grower and extension community.

Objective 2: Grapevine Yellows management practices evaluation

Two potential management practices are being evaluated during the 2013 growing season. One involves a season-long insecticide spray program to determine whether insecticides are (**1**) effective in reducing leafhopper numbers and (**2)** if so, whether the depression in leafhoppers translates into a reduction in the number of Yellows-affected vines observed in the following year. This study is being conducted by Tony Wolf at two local cooperating vineyards, Linden and RdV. Control and insecticide plots were established in two different blocks at RdV and one block at Linden; blocks are approximately one acre in size. As of mid-July 2013, five insecticide sprays were made to the respective insecticide sections of each of the three blocks. Leafhopper populations are being monitored weekly using both sticky traps and ground sweep netting. These sprays have generally been very effective in substantially reducing leafhoppers in the insecticide section. We can't judge the impact of the insect reductions until the following year, when NAGY symptoms would be expressed from infections that occur this summer.

The second management approach involves scouting and excision of infected vine parts from NAGY-affected vines. This is planned for early August 2013.

Reports

Drs. Stoepler and Wolf co-authored a Virginia Cooperative Extension publication on our current knowledge of NAGY which is in press (expected to be published by Sep. 2013). Stoepler and Wolf submitted and received a USDA APHIS federal permit application for the interstate transport of leafhoppers in the 2013 season.

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 describe useful management practices to mitigate vine losses.

ii. Publications and presentations

Stoepler, T.M. and Wolf, T.K. *In press.* North American Grapevine Yellows: Current knowledge and management recommendations for wine growers. *Virginia Cooperative Extension.*

Stoepler, T.M. and Wolf, T.K. Oral presentation. "Updates and 2013 Plans for Grapevine Yellows Management Studies", Virginia Vineyards Association Annual Technical Meeting, Charlottesville, VA, Feb. 1, 2013.

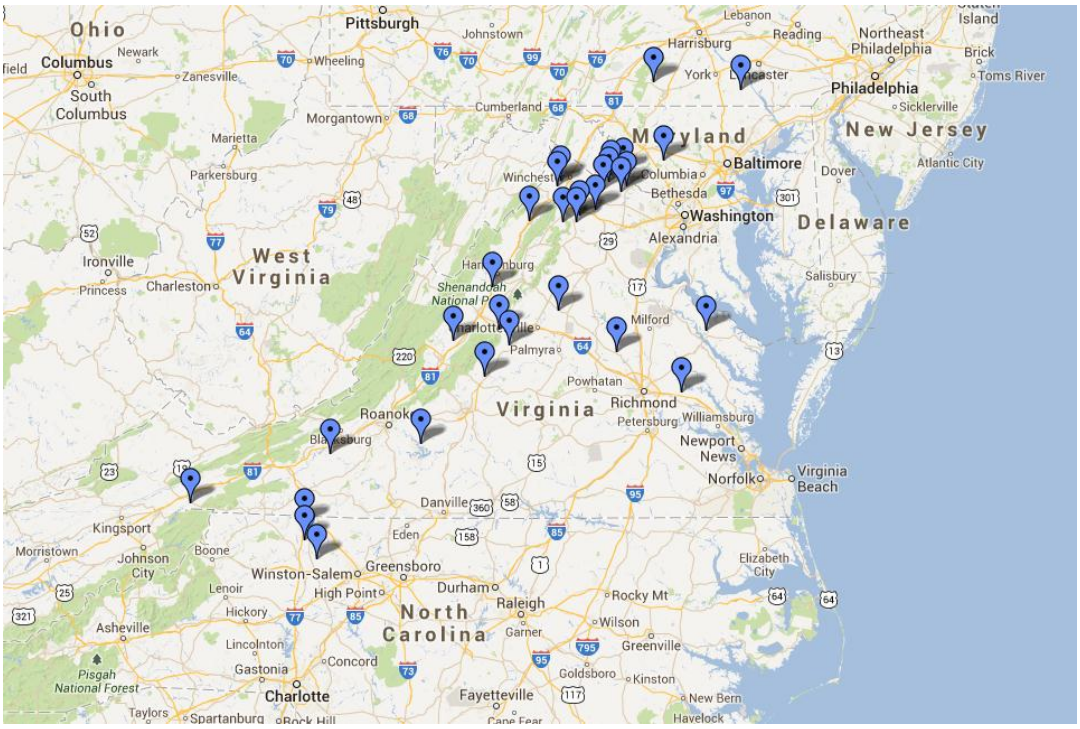

Figure 1. Map of North American Grapevine Yellows 2013 vineyard sites (marked with blue icons).