Final Progress Report
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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

Introduction: North American Grapevine Yellows (NAGY) is a lethal disease of grapevine, caused by at least one genetically unique phytoplasma (bacteria-like organism), vectored from alternative flora to our cultivated grapevines by one or more insect vectors. In collaboration with colleagues at the USDA/ARS in Beltsville, MD, we have studied the biology and ecology surrounding NAGY in Virginia vineyards since the early nineties (Beanland et al., 2006; Davis et al. 1998, 2015; Prince et al., 1993; Wolf et al. 1994) and have provided current management recommendations to industry (Stoepler et al., 2013). While much was learned about grape varietal susceptibility, ecological factors predisposing vineyards to NAGY, alternative hosts, the taxonomy of the causal phytoplasmas, and the analytical protocols necessary for reliable and accurate pathogen detection, we still lacked positive identification on the vector(s) involved in NAGY. Nor could we confidently advise growers on potential management strategies where the disease is prevalent. The overarching goal of this project was to fill some of those knowledge gaps. While omitting here the analytical details involved, previous year-end project reports found, in summary:

- Additional alternative hosts for NAGY phytoplasmas include American elm, sycamore, poison ivy, as well as the previously recognized black cherry and wild grapevine. There may also be certain herbaceous plants that serve as season inoculum reservoirs. The relative abundance of alternative hosts in the vineyard environment may have a bearing on the frequency of NAGY within the vineyard.
- 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 of 30 mid-Atlantic vineyards (primarily VA) in 2013**.** In a subset of 3 of those vineyards with historically high NAGY incidence, we sampled leafhoppers weekly to understand the seasonal phenology of candidate vector species**.** We surveyed 62,725 vines across the 30 vineyards and found 511 that were infected with NAGY (~1%). While this overall incidence of NAGY might be considered 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 but was also observed in our earlier work with NAGY (Beanland et al., 2006).

- Season-long insecticide spray programs were evaluated (against control blocks) in 2013 and 2014 to determine whether multiple insecticide sprays would reduce leafhopper populations. Season-long spraying (5 - 7 sprays) effectively reduced leafhopper abundance, as illustrated by the data of **Figure 3**; however, we could not say that this reduced the incidence of NAGY in the subsequent (2014 or 2015) seasons because we found no new NAGY-infected vines at either vineyard in either year with either the treated or control part of the vineyard blocks.
- 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). We found some 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 2015. A summary of our results was presented in the 2015 final project report. Surprisingly, we have seen some situations where vines that were severely pruned – such as the removal of one or both cordons and much of the corresponding trunks – have remained apparently free of NAGY symptoms for over 2 years. For example, 7 of 10 Tannat vines have responded in this fashion. On the other hand, 7 of 18 Cabernet Sauvignon vines at Willowcroft Vinyards appeared to "recover", or failed to show NAGY symptoms in 2014, despite showing symptoms in 2013 and not having removed affected organs from those vines when the symptoms were first observed. We now recommend that affected vine parts (e.g., an entire cordon) be removed from the vine (or at least severed from the vine and allowed to dry on the trellis) as soon as symptoms are first conclusively identified. This minimal amount of work may likely save some, but not all affected vines.

Progress in late-2015/early 2016:

Phytoplasma transmission experiments: Chardonnay seedlings and grafted grapevines which had been used in insect transmission trials of 2013 and 2014 were maintained in insect-exclusion cages in a greenhouse range of the AHS Jr. AREC. These plants were maintained and monitored for possible NAGY symptoms throughout the 2015 season and through June of 2016. This long-term monitoring was necessary due to the long incubation period of phytoplasmas in vines, and the fact that NAGY symptoms are not usually expressed in the field until after bloom (June). None of the grafted Chardonnay vines used in transmission studies of 2013 developed NAGY symptoms. We did observe some leaf curling in some of the seedlings from the 2014 transmission attempts, which could have been mistaken for NAGY symptoms. Considering that only one to several leafhoppers were placed on each test vine, which would have provided relatively 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 NAGY primers. Several of these indicator plants produced what we *initially* interpreted as positive transmission capacity with two of the leafhopper species; however, none of

these transmission experimental plants conclusively showed NAGY symptoms, nor test positive for NAGY phytoplasmas **(Table 1**)

Towards the end of the 2015 season, we focused our transmission attempts on trials based on 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. 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 feeding vials where the insects had access to 5% sucrose solutions described in previous years' reports. In 2015, we placed 5 individuals per vial, instead of 1, in order to increase the amount of phytoplasma DNA potentially released into the sucrose solutions. A total of 38 sucrose trials were carried out during the 2015 season (**Table 2**), including species that were not present in our 2012 and 2013 sucrose transmission trials (**Table 3**), but commonly found in Virginia. We also extensively re-evaluated the PCR protocols and primers that we were using for NAGY phytoplasma detection, particularly in light of new findings (Davis et al., 2015) about the genomic classification of the principal phytoplasma found in the NAGY disease. The putative positive results reported in the mid-year (January 2016) progress report were not confirmed during the last six months using the more robust PCR protocol. Samples that resulted positive with the routinely used 16S PCR protocol, in fact, were negative when other PCR protocols were used. To avoid dealing with false positives, we introduced other PCR protocols, using different PCR primer sets. In order to avoid any possibility of contamination, we used the primer set P1-P7 for the first round of amplification, followed by P1A-16Sr primer set for the nested PCR.

Using these refined methods, we could obtain reliable results that allowed us to discard the false positive samples. We then did an extensive re-evaluation of the ~1800 sucrose feeding solutions that had been produced by Dr. Teresa Stoepler in 2013. Of those, only 4 samples, used to feed four *Coelidia oelitoria* individuals, were confirmed to be positive for the one NAGY phytoplasma. Amplification of both the *SecY* gene region as well as the *Tuf* gene region provided definitive detection of one of the 2 known NAGY phytoplasmas (**Figure 1**). Since this is the first time we were able to PCR amplify phytoplasma genes other than the 16S, these results show that *Coelidia oelitoria* is a NAGY vector, since it was shown to be able to release phytoplasma in the artificial diet. To further confirm these results, we sequenced all the amplification products for all the four samples (DNA nucleotide sequencing). There was no difference among the sequencing results of the four samples with the SecY reference (Davis et al., 2015), which confirmed that the phytoplasma present (and transmitted) was indeed one of NAGY phytoplasmas. *Coelidia oelitoria* (**Figure 2**) is a large (6.0 to 8.0 mm) leafhopper, by leafhopper standards and, despite extensive sampling (ground and brush sweeps, yellow sticky trap cards), we do not routinely find this species within vineyards. It is more commonly found in brush and trees outside the vineyard. This low occurrence, however, possibly partly explains the distribution of NAGY within Virginia vineyards. The occurrence of NAGY-affected vines is relatively low in any given year (e.g., 10 vines in a 1000-vine planting = 1%), although the cumulative attrition rate can be an economic challenge. If abundantly found leafhoppers such as *Agallia constricta*, or *Endria inimica* (**Tables 2 and 3**) were competent NAGY phytoplasma vectors, we would anticipate observing more NAGY-infected vines, given the abundant nature of these leafhoppers. The relatively greater occurrence of *C. oelitoria* outside the vineyard could also explain the *generally* observed greater distribution of NAGY-affected vines at the vineyard edges, as opposed to deep within the vineyard. Interestingly, *C. oelitoria* was the leafhopper species which resulted in the greatest percentage of positive transmissions into sucrose solution in our initial transmission studies in 2013 (**Table 3**).

Alternative hosts: We had proposed a more thorough survey of potential alternative hosts in and around vineyards in the 2015 season; however, this part of the project was dropped when the 33% reduction in funding of the proposed project led to the loss of our wage employee, D. Melby.

Phytoplasma titer in infected plants: We were 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 NAGY symptoms to phytoplasma titer. In 2015 we used quantitative PCR (qPCR) to determine the titer of phytoplasma DNA in grapevine samples. The amount of NAGY 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 different, despite the difference in susceptibility of the varieties to NAGY.
- 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.

Collectively, our results suggest that the actual concentration of NAGY phytoplasmas is linked to the manifestation of symptoms. These data were presented as a poster at the American Phytopathological Society annual meeting in the summer 2015.

Impact statement:
Although funding for this project has ceased, we are still involved in developing a Grapevine Yellows Risk Management tool that growers can use pre-plant through the life of the vineyard to reduce the economic impact of NAGY on their operation. The identification of *C. olitoria* as a competent NAGY phytoplasma vector could be used in such a management program; however, it would require the grower to take the time to monitor for, and accurately identify the leafhopper before a management program was implemented. Infected vine "salvage" strategies could be implemented to slow the loss of infected vines. And some growers have taken the approach of removing known alternative NAGY hosts from their vineyard environment.

Presentations in this period:
Lenzi P, Stoepler T, Melby D, Wolf T. 2015. Characterization of North American Grapevine Yellows (abstract). Paper presented at the 2015 APS Annual Meeting, Pasadena, CA, USA (http://www.apsnet.org/meetings/Documents/2015_meeting_abstracts/aps2015abP320.htm)

Tables and figures:
Detailed data can be found in appendices on pages 6-9 of this report.

Literature cited (* references work funded by the VA Wine Board):

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Prince, J. P., R. E. Davis, T. K. Wolf, I.-M. Lee, B. D. Mogen, E. L. Dally, A. Bertaccini, R. Credi, and M. Barba. 1993. Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. Phytopathology 83:1130-1137.

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Wolf T.K. 2015. North American Grapevine Yellows. pp. 111-113, In: Compendium of Grape Diseases, Disorders, and Pests; 2nd ed., The American Phytopathological Society; W.F. Wilcox, W.D. Gubler, and J.K. Uyemoto (eds.).

Appendix 1. Record of leafhopper transmission trials during 2014 onto Chardonnay grape seedlings, which were then evaluated for visual symptoms and PCR-tested for presence of NAGY phytoplasmas. None of these transmission trials resulted in positive transmission of NAGY-causal phytoplasmas.

Transmission Experiments, 2014. Insects collected from high incidence vineyards, caged on NAGY infected vines, transferred to wheat/barley/clover, then to grape seedlings to test as vectors.													
Grape seedling plant prefix	Grape host #	Inoculation #	Insect id	Date insect collection	Vineyard	NAGY acquisition host vine location (Row-Panel-Vine)	Acquisition Vine Variety	# days on acquisition host	N insects transferred to grape seedling	Date insects transferred to grape seedling	Insect collection location	Collection type (vine canopy, floor, forest)	Collection plant
G2014	1	1	*Agallia constricta*	7/22/2014	Williams Gap	R52-P18-V5	Tannat	3	4	8/20/2014	Williams Gap	vineyard floor	Tannat
G2014	2	2	*Agallia constricta*	7/22/2014	Williams Gap	R52-P18-V5	Tannat	3	5	8/20/2014	Williams Gap	vineyard floor	Tannat
G2014	3	3	*Agallia constricta*	7/22/2014	Williams Gap	R52-P19-V2	Tannat	3	11	8/20/2014	Williams Gap	vineyard floor	Tannat
G2014	4	4	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	5	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	5	4	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	5	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	6	4	*Endria inimica*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	4	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	7	4	*Exitanus exitiosus*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	3	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	8	4	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	4	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	9	5	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	5	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	10	5	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	5	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	11	5	*Agallia constricta*	7/29/2014	Williams Gap	R61-P16-V1	Tannat	2	5	8/21/2014	Williams Gap	vineyard floor	Tannat
G2014	12	6	*Endria inimica*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	13	6	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	14	6	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	15	6	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	16	6	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	17	6	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	18	7	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	19	7	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	20	7	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	21	7	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	22	7	*Agallia constricta*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	23	7	*Endria inimica*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	24	7	*Endria inimica*	7/29/2014	Williams Gap	R63-P8-V5	Tannat	2	5	8/22/2014	Williams Gap	vineyard floor	Tannat
G2014	25	14	*Endria inimica*	8/26/2014	Wild Meadow	R16-P5-V4	Chardonnay	1	5	9/15/2014	Wild Meadow	vineyard floor	Chardonnay
G2014	26	16	*Graphocephala verasuta*	8/26/2014	Wild Meadow	R16-P10-V4	Chardonnay	1	1	9/15/2014	Wild Meadow	forest/floor	wild grape
G2014	27	19	*Exitanus exitiosus*	8/27/2014	Wild Meadow	R21-P7-V2	Chardonnay	1	4	9/18/2014	AREC	vineyard floor	
G2014	28	18	*Exitanus exitiosus*	8/27/2014	Wild Meadow	R23-8th to la	Chardonnay	1	3	9/18/2014	AREC	vineyard floor	
G2014	29	18	*Endria inimica*	8/27/2014	Wild Meadow	R23-8th to la	Chardonnay	1	2	9/18/2014	AREC	vineyard floor	
G2014	30	21	*Agallia constricta*	9/3/2014	Wild Meadow	R7-P2-V1	Chardonnay	1	3	9/25/2014	RdV	vineyard floor	
G2014	31	22	*Exitanus exitiosus*	9/3/2014	Wild Meadow	R10-P6-V5	Chardonnay	1	4	9/25/2014	AREC	Lawn	
G2014	32	24	*Exitanus exitiosus*	9/3/2014	Wild Meadow	penultimate	Chardonnay	1	5	9/25/2014	AREC	vineyard floor	
G2014	33	26	*Exitanus exitiosus*	9/3/2014	Wild Meadow	R20-P6-V3	Chardonnay	1	5	9/25/2014	AREC	Lawn	
G2014	34	29	*Amblysellus curtisii*	9/10/2014	Wild Meadow	R28-6th to la	Chardonnay	1	1	10/6/2014	AREC	forest/floor	
G2014	35	30	*Exitanus exitosus*	9/10/2014	Wild Meadow	R31-4th to la	Chardonnay	1	3	10/6/2014	AREC	vineyard floor	
G2014	36	30	*Amblysellus curtisii*	9/10/2014	Wild Meadow	R31-4th to la	Chardonnay	1	5	10/6/2014	AREC	vineyard floor	
G2014	37	30	*Endria inimica*	9/10/2014	Wild Meadow	R31-4th to la	Chardonnay	1	5	10/6/2014	AREC	vineyard floor	
G2014	38	31	*Amblysellus curtisii*	9/10/2014	Wild Meadow	R31-7th to la	Chardonnay	1	2	10/6/2014	AREC	vineyard floor	
G2014	39	31	*Endria inimica*	9/10/2014	Wild Meadow	R31-7th to la	Chardonnay	1	5	10/6/2014	AREC	vineyard floor	
G2014	40	33	*Exitanus exitosus*	9/22/2014	Willowcroft	R4-P7-V1	Cabernet sauvign	1	5	10/16/2014	AREC	vineyard floor	
G2014	41	34	*Amblysellus curtisii*	9/22/2014	Willowcroft	R5-P10-V2	Cabernet sauvign	1	1	10/16/2014	AREC	forest/floor	wild grape
G2014	42	35	*Amblysellus curtisii*	9/23/2014	Willowcroft	R6-P6-V3	Cabernet sauvign	1	1	10/16/2014	AREC	forest/floor	wild grape
G2014	43	36	*Endria inimica*	9/23/2014	Willowcroft	R7-P10-V2	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	44	36	*Exitanus exitosus*	9/23/2014	Willowcroft	R7-P10-V2	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	45	36	*Endria inimica*	9/23/2014	Willowcroft	R7-P10-V2	Chardonnay	1	4	10/16/2014	AREC	vineyard floor	
G2014	46	36	*Exitanus exitosus*	9/23/2014	Willowcroft	R7-P10-V2	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	47	36	*Exitanus exitosus*	9/23/2014	Willowcroft	R7-P10-V2	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	48	37	*Endria inimica*	9/23/2014	Willowcroft	R7-P11-V1	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	49	37	*Exitanus exitosus*	9/23/2014	Willowcroft	R7-P11-V1	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	50	37	*Exitanus exitosus*	9/23/2014	Willowcroft	R7-P11-V1	Chardonnay	1	5	10/16/2014	AREC	vineyard floor	
G2014	51	38	*Exitanus exitosus*	9/30/2014	Willowcroft	R9-P9-V1	Chardonnay	1	2	10/23/2014	Willowcroft	vineyard floor	
G2014	52	39	*Amblysellus curtisii*	9/30/2014	Willowcroft	R12-P13-V1	Riesling	1	1	10/23/2014	AREC	forest/floor	
G2014	53	41	*Exitanus exitosus*	10/6/2014	Willowcroft	R15-P8-V2	Riesling	1	5	10/30/2014	Willowcroft	vineyard floor	
G2014	54	42	*Endria inimica*	10/6/2014	Willowcroft	R15-P5-V3	Riesling	1	1	10/30/2014	Willowcroft	vineyard floor	
G2014	55	42	*Exitanus exitosus*	10/6/2014	Willowcroft	R15-P5-V3	Riesling	1	2	10/30/2014	Willowcroft	vineyard floor	
G2014	56	43	*Amblysellus curtisii*	10/9/2014	Willowcroft	R16-P3-V3	Riesling	1	1	10/30/2014	Willowcroft	vineyard floor	
G2014	57	43	*Exitanus exitosus*	10/9/2014	Willowcroft	R16-P3-V3	Riesling	1	4	10/30/2014	Willowcroft	vineyard floor	
G2014	58	45	*Exitanus exitosus*	10/9/2014	Willowcroft	R17-P8-V2	Chardonnay	1	4	10/30/2014	Willowcroft	vineyard floor	
G2014	59	47	*Exitanus exitosus*	10/23/2014	Willowcroft	R5-P10-V2	Cabernet Sauvign	1	5	11/17/2014	Willowcroft	vineyard floor	
G2014	60	49	*Exitanus exitosus*	10/27/2014	Willowcroft	R4-P3-V2	Cabernet Sauvign	1	5	11/17/2014	Willowcroft	vineyard floor	
G2014	61	54	Amblysellus curtisii	11/5/2014	Willowcroft	R17-P8-V2	Riesling	1	2	11/24/2014	Willowcroft	vineyard floor	
G2014	62	54	Exitanus exitosus	11/5/2014	Willowcroft	R17-P8-V2	Riesling	1	5	11/24/2014	Willowcroft	vineyard floor	
G2014	63	54	Exitanus exitosus	11/5/2014	Willowcroft	R17-P8-V2	Riesling	1	5	11/24/2014	Willowcroft	vineyard floor	
G2014	64	55	Endria inimica	11/5/2014	Willowcroft	R17-P8-V2	Riesling	1	2	11/24/2014	Willowcroft	vineyard floor	
G2014	65	56	Exitanus exitosus	11/5/2014	Willowcroft	R17-P8-V2	Riesling	1	5	11/24/2014	Willowcroft	vineyard floor	

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).

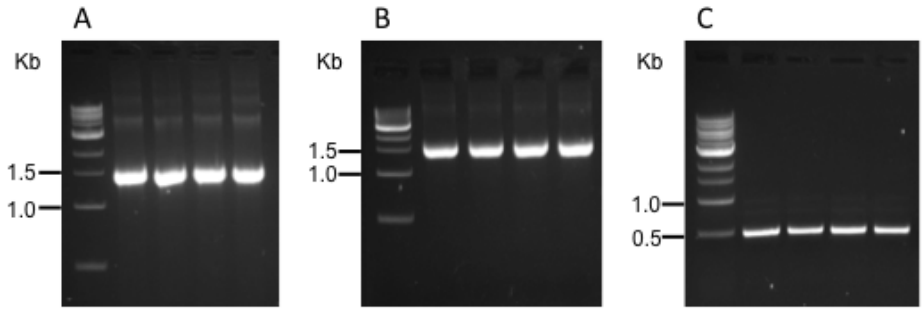
Species	Subfamily	Sucrose media transmission (n positive/n tested) 2014	2015
Agallia constricta	Agallinae	6/13	0/1
Endria inimica	Deltocephalinae	4/6	1/10
Exitanus exitiosus	Deltocephalinae	0/3	2/9
Deltocephalus flavicosta	Deltocephalinae	-	2/3
Latalus sayii	Deltocephalinae	-	1/1
Graminella nigrifrons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

Table 3. 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 2012	2013	NAGY-phytoplasma Group
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.

Figure 1. PCR results with feeding solutions of *Coelidia olitoria*. The far left lane is a Kb scale to allow references to the next 4, diagnostic sample lanes, which are all from the leafhopper Coelidia olitoria. The bright bands signify a positive PCR reaction for the specific primer used in that sample (see footnote).



Nested PCR amplifications of sucrose artificial diets used to feed *Coelidia olitoria*.
A. Amplification of 16S-23S region using primers 16S-SrIII-P1 in direct PCR and 16S-SrIII-P1A in nested PCR. **B.** Amplification of SecY region using primers L15-F1A(III)-Map-R1A(III) in direct PCR and primers SecY-F1(III)-SecY-R1(III) in nested PCR . **C.** Amplification of Tuf region using primers Tuf 340-Tuf 890 in direct PCR and primers Tuf 400-Tuf 835 in nested PCR.

Figure 2. Coppery leafhopper (*Coelidia olitoria*).

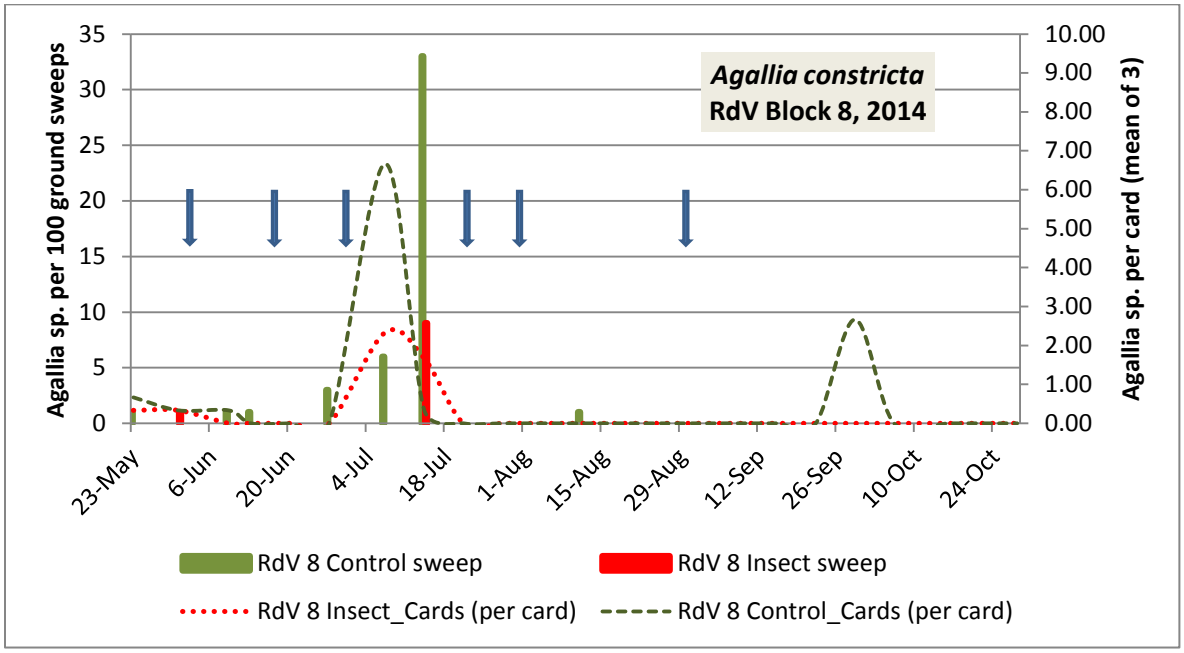


Figure 3. Seasonal abundance of *Agallia constricta* in a vineyard block of one vineyard surveyed and used in a test of insecticide suppression of leafhoppers during the 2014 season. The green bars reflect leafhoppers collected from vineyard floor sweeps in the half of the block that was used as a control (no insecticide), the green dashed line is the mean number (n=3) of *Agallia* leafhoppers found on sticky cards in the control half of the block, the red bars are insects found in ground sweeps of the insecticide half of the block, and the red dashed line is the mean number (n=3) of *Agallia* leafhoppers found on the sticky cards in the insecticide portion of the block. The blue arrows are the dates that the insecticide-treated half of the block was sprayed with an insecticide (6 times in 2014).