Virginia Polytechnic Institute and State University
Proposal Cover Sheet

PROPOSAL INFORMATION

Virginia Winegrowers Advisory Board
Sponsor

Solicitation No.

Reducing The Spread Of Grapevine Yellows In Virginia Vineyards
Proposal Title

$31,896.00	7/1/2004	6/30/2005	☐ New or ☒ Renewal of 447108
Amount	**Begin Date**	**End Date**	

PRINCIPAL INVESTIGATOR(S)

Tony K. Wolf	Leann Beanland	
Principal Investigator	Co-Principal Investigator	Co-Principal Investigator
Signature	Signature	Signature
540-869-2560	540-869-2560	
Phone	Phone	Phone
vitis@vt.edu	lbeanland@vt.edu	
Email	Email	Email
Professor	Research Associate	
PI Title	Co-PI Title	Co-PI Title
AHS Jr AREC	AHS Jr. AREC	
Department / Center	Department / Center	Department / Center

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VIRGINIA TECH ENDORSEMENT

	3-30-04	04-1907-03
David W. Richardson **Director of Sponsored Programs**	**Date**	**VT Proposal No.**

A proposal submitted to:

Virginia Winegrowers Advisory Board

for continued funding of

REDUCING THE SPREAD OF GRAPEVINE YELLOWS IN VIRGINIA VINEYARDS

Principal Investigators:

Dr. Tony K. Wolf
Professor
AHS Agricultural Research and Extension Center
Virginia Tech
595 Laurel Grove Rd.
Winchester VA 22602
Tel: 540-869-2560 x 20
E-mail: vitis@vt.edu

Dr. LeAnn Beanland
Research Associate
AHS Agricultural Research and Extension Center
Virginia Tech
595 Laurel Grove Rd.
Winchester VA 22602
Tel: 540-869-2560 x 23
lbeanlan@vt.edu

Start date: 1 July 2004

Amount requested: $31,896 (current year)

A. Title: Reducing the spread of grapevine yellows in Virginia vineyards

B. Date: 1 July 2004

C. Estimated duration: Proposal is for third year of multi-year project

D. Objectives:
The long-term goal of this work is to understand the ecology of grapevine yellows (GY) disease in order to develop disease control strategies. To accomplish this goal, we are proposing the following objectives:
- Identify specific vectors of GY phytoplasmas and the seasonal nature of their infectivity
- Identify alternative host flora of GY phytoplasmas in and around GY-affected vineyards
- Investigate possible control measures (long-term)

E. Justification/Practical importance:
Grapevine yellows (GY) is a destructive disease of grapes throughout the world and is widespread in Virginia vineyards. Losses of infected vines can exceed 30% over a 10-year period. The disease is particularly destructive and widespread in Chardonnay vines, which account for over 30% of the state's grape acreage. Other varietals can be infected, although typically at lower rates. GY incidence tends to be greatest in isolated vineyards that border woods and those located near the Blue Ridge Mountains. More than 40% of vines in one of our test vineyards has been removed due to high levels of GY disease. GY is caused by a bacteria-like organism called a *phytoplasma*. Research elsewhere has shown that insects are responsible for transmitting GY phytoplasmas to cultivated vines. There are at least two strains of phytoplasma present in Virginia vineyards that may be transmitted by different insect species.

Control of GY is difficult with our current understanding of the disease. There is no 'cure' for affected vines. *Potential* control measures are to target the insects that transmit the phytoplasmas with insecticides, remove alternative hosts around the vineyard, or to avoid planting susceptible vines in areas where GY pathogens and vectors are endemic. We do not know which leafhopper species are responsible for pathogen transmission, nor do we know when they are infective. The indiscriminant use of insecticides has the potential of increasing secondary pests and is an expensive and environmentally insensitive approach. Although we are aware that wild grapevines serve as a host for phytoplasmas, other hosts may similarly serve as reservoirs.

Given the expansion of Virginia vineyards into areas at risk of GY, it is essential that control measures be explored; control of GY was the second most highly prioritized project in Virginia in a 2001 industry-wide survey. The research proposed here continues work funded by the VWAB over the last five years. We've surveyed the insects present in GY-affected vineyards, but identification of vectors has not been completed. We will also characterize the role that alternative host plants play in the spread of GY.

F. Background:
Grapevine yellows diseases were first reported and described in the 1950's, first in and later in the U.S.A. and other countries (reviewed in Caudwell, 1990). Symptoms of GY in the mid-Atlantic include vein yellowing, fruit abortion, leaf curling, lack of shoot lignification, general loss of vigor, and vine death within one to three years of symptom onset (Wolf et al., 1994; Wolf, 2000). Vineyard surveys have shown GY to be concentrated in the Piedmont area of the state, which coincides with the major concentration of existing as well as new vineyards. Rates of spread within affected vineyards are 1- 6% of vines per year (Wolf *et al.*, 1994) that has led some vineyards to experience more than 30% vine loss in 10 years.

Phytoplasmas are the pathogens that cause grapevine yellows and numerous other plant diseases (Davis and Lee, 1991). These organisms are cell wall-less obligate parasites that live in the phloem of plants or in the organs of insect vectors. They are transmitted from plant to plant by vector insects during feeding. Two different phytoplasmas are present in Virginia vineyards. One phytoplasma is related to peach X-disease (Prince *et al.*, 1993) the other is an aster yellows-type (Davis *et al.*, 1998). Phytoplasmas cannot be cultured outside of their plant or insect hosts. Hence detection and characterization of these pathogens has been accomplished using molecular (DNA based) techniques, such as polymerase chain reaction

(PCR) and RFLP analysis (Deng and Hiruki 1991; Lee *et al.*, 1992a; Ahrens and Seemuller, Lee *et al* 1993; and Davis *et al.*, 1998).

Leafhoppers, planthoppers and occasionally psyllids have been identified as vectors of phytoplasmas that cause numerous plant diseases. For instance, *Scaphoideus titanus* Ball is the vector of GY (Flavescence dorée) in France (Caudwell *et al.*, 1971) and a planthopper transmits another GY in Germany. *S. titanus* is one of several leafhopper species captured in Virginia that may be involved in GY spread.

Alternative hosts for the pathogens that cause GY are hypothesized to be wild *Vitis spp.* (Caudwell and Dalmasso, 1985), *Prunus spp.* (e.g., chokecherry), black locust (R. Davis pers.com) or herbaceous plants infected by aster yellows (Prince *et al.*, 1993; 1994). A phytoplasma, indistinguishable from a strain found in GY-affected 'Chardonnay', has been detected in chokecherry (Prince *et al.*, 1993). (Chen *et al.* (1993), Maixner *et al.* (1993), and Davis *et al.* (1998) detected phytoplasmas in NY and VA wild grapevine.

Summary of 2003 Accomplishments

- Determined the incidence and abundance of candidate vector species at two Virginia survey vineyards (Glen Manor and Blue Ridge Chase)
- Identified 23 captured insect species that were positive for phytoplasma
- Identified 6 insect species that shed phytoplasma into sucrose solutions while feeding
- Performed transmission experiments using 12 insect species (522 individual insects)
  * Test Chardonnay plants used in 2003 experiments will be tested for Grapevine Yellows once symptoms of disease are expressed in July and August 2004.
- Found positive expression of Grapevine Yellows in Pennsylvania and Finger Lakes, New York vineyards

Executive summary of project importance, procedural status, expected results, and timeframe for completion of vector studies.

Grapevine Yellows continues to be a destructive disease in Virginia and other mid-Atlantic vineyards. Our survey efforts have shown a close association between affected vineyards and closely adjacent, wooded habitat. Chardonnay is particularly sensitive, but many other varieties including Riesling, Cabernet Sauvignon, Merlot, Malbec, Sauvignon blanc, and Petit Manseng, show symptoms and die in affected vineyards. Partnered funding from the Viticulture Consortium:East in 2003 was contingent upon expanded surveys of Chardonnay vineyards in Pennsylvania and the Finger Lakes region of New York State. Those efforts revealed a widespread occurrence of GY; however, the frequency of infected vines within a vineyard decreases as one moves from Virginia north to the Finger Lakes.

We anticipate that the 2004 season will be the final year of general survey efforts, both for alternative host plants and for candidate insect vectors. We feel that it is important to repeat the weekly insect sampling for one final season due to the very different population numbers revealed between 2002 and 2003. The two previous seasons also differed dramatically in precipitation. Moreover, it is necessary to repeat transmission experiments to determine the likelihood that a given insect vector can transmit grapevine yellows phytoplasma.

Developing Management Strategies: Once vector species have been identified from transmission experiments, we will use the 2002-2004 field data we have collected to determine when these species are present and when control measures should be implemented. We also expect to generate an expanded list of alternative hosts of phytoplasma that should be avoided when establishing vineyard sites and which growers may consider removing from the vicinity of existing vineyards.

See Appendix A (2003 Progress Report) for a detailed explanation of the 2003 activities and findings.

The Viticulture Consortium:East has already granted almost 60% of the funding required for 2004-2005 period.

G. PROCEDURES FOR PROPOSED RESEARCH IN 2004:

Objective 1: Identify specific insect vectors of GY phytoplasmas (2002 -2004 activity): Based on our preliminary data, and upon other GY diseases, we hypothesize that one or more leafhopper or planthopper species are responsible for transmission of phytoplasmas in GY-affected vineyards of the mid-Atlantic. Insect survey results of 2002 and 2003 have shown that a diverse range of planthoppers and leafhoppers occur in GY-affected vineyards, particularly as collected from the vineyard floor. Some of these insects tested positive for GY phytoplasmas. To demonstrate vector identity, an insect species must transmit GY phytoplasma from infected plant material to uninfected indicator plants. Definitive transmission studies, to prove vector competence, have begun and preliminary results obtained. Vector identification will continue using the following:

***a)* Continued surveys of candidate vector species in two GY infected vineyards.** Beginning in early May, sweep net samples will be taken from two vineyards with grapevine yellows infected vines. One hundred sweeps will be taken from within the vineyards and another 100 sweeps will be made in the vegetation around both vineyards. Samples will be collected and stored separately. Insects will be transported to the laboratory, and placed in a -80ºC freezer. After at least 24 hours, insects from each sample will be counted and identified to species. Insects will be bulked by species by date and subjected to PCR analysis for the presence of grapevine yellows phytoplasma(s). Sticky traps will be erected at both vineyards in 3 transects of 5 traps each to determine the distribution and movement of candidate vector species. Traps will be replaced weekly through mid-June and then will be replaced bimonthly.

***b)* Transmission from field collected candidate vector species to rooted Chardonnay cuttings:** We will live-capture leafhopper and planthopper species from around GY-infected vines in test vineyards focusing on those species that are abundant, have tested positive for phytoplasma, or have transmitted phytoplasma to test solutions in membrane feeding trials. Batches of up to 50 individuals of the same species (depending on availability) will be confined on rooted Chardonnay cuttings for an inoculation access period during which transmission can occur if test species are GY phytoplasma vectors. Insects will be allowed to feed until they die. Recovered leafhoppers will be analyzed for GY phytoplasmas. Indicator plants will be placed in an "insect free" space in the greenhouse and monitored for yellows symptoms and similarly analyzed for GY phytoplasmas.

c*)* Transmission from cultivated GY-positive wild grapevine to indicator plants*:* Chardonnay cuttings will be used as indicator plants. Cuttings will be maintained in an insect-exclusive greenhouse and routinely tested for phytoplasmas. Only uninfected vines will be used in experiments. Five to 30 individuals of candidate vector leafhopper species will be confined on GY-positive wild grapevine for a 2-7 day acquisition access period (AAP). After the AAP test insects will be caged on indicator plants for a 10-day to 3-week incubation period and an inoculation access period during which transmission can occur if test species are GY phytoplasma vectors. Inoculation access periods will continue until test insects die. Any

recovered leafhoppers will be analyzed for GY phytoplasmas. Indicator plants will be monitored for yellows symptoms and similarly analyzed for GY phytoplasmas.

Objective 2, to identify phytoplasma alternative host flora in and around vineyards (2004 growing season): We hypothesize that other, non-*Vitis* spp., may serve as natural reservoirs of GY phytoplasmas. Common woody and herbaceous alternative host flora, growing in and within 100 m of two GY-affected survey vineyards will be sampled monthly from May to September and PCR analyzed for GY phytoplasmas. Woody specimens, including *Vitis, Prunus*, *Robinia pseudoacacia* and *Ulmus* spp. will be individually identified and sampled. Herbaceous flora, including wild asters, will be sampled by area both within and outside the vineyard, collected and identified to species. Tissue samples will consist of 5-6 mature leaves and petioles per specimen. Identification of GY phytoplasmas in the flora will reveal which species serve as hosts for specific grapevine-infecting phytoplasmas. Results of these surveys will provide baseline information on the ecology of GY in the affected vineyards and may provide a rationale for eliminating certain alternative hosts from areas adjacent to susceptible vineyards.

A sub-objective of 2 will be a repeat survey of six Chardonnay vineyards in southeast Pennsylvania, and in the Finger Lakes Region of New York during 2004 to compare the distribution and incidence of GY in these vineyards with that in the more intensively studied Virginia vineyards. The same vineyards that were surveyed in 2003 will be surveyed in 2004. The commonalties that we are seeking among these vineyards are: *i*) taxonomic identity of phytoplasmas in symptomatic Chardonnay vines (is it the same agent as found in Virginia?); *ii*) presence of identified (from VA) alternative hosts in vineyard environ; *iii*) and presence of known vectors from vineyard sweeps.

Objective 3, to investigate possible control measures (2004): Design of control strategies will depend upon the information previously generated in project. At minimum, we anticipate learning which insects and which alternative hosts are implicated in the maintenance and spread of GY in this region. Knowledge of specific leafhopper vectors may allow the targeting of those insects with insecticides, which is the standard means of FD control in France (Caudwell, 1990). If alternative hosts are shown to be the principal source of phytoplasmas, we may be able to manage infection via elimination of these plant species from the vineyard vicinity. Our results may also suggest that the most efficient means of control is to avoid planting susceptible cultivars in GY-prone areas. Unfortunately, this is the only current means of management that we can currently endorse.

H. Personnel and facilities:
Project is under the general direction of Dr. Tony Wolf. Field and laboratory procedures are conducted or directed by Dr. LeAnn Beanland (vector entomologist).

I. Other entities:
None

J. Source of other funds:
An identical proposal was submitted to the Viticulture Consortium:East, (VC: East) for cost-sharing as indicated in the Budget, section K. The Viticulture Consortium did award $40,000 in support of this project for 2004-2005.

K. Budget:
Proposed budget is an estimate of the total funding needed to complete the 2004 proposal objectives.

	Funds sought from the VWAB	Funds sought from the VC: East
Salary	**19,656**	19,656
Faculty fringe (23%)	**4,521**	4,521
Technical assistance (wages)	**3,000**	3,000
Wage fringe (7.3%)	**219**	219
Supplies	**3,200**	14,200
Domestic travel	**1,300**	2,850
Total	**31,896**	*44,446**

$40,000 has been granted from the Viticulture Consortium (March, 2004).

Budget justification:
Salary: post-doctoral research associate (Dr. LeAnn Beanland) at annualized rate of $39,312. The Viticulture Consortium – East proposal seeks one-half of base salary with benefits, with the balance sought from Virginia Winegrowers Advisory Board (VWAB). Fringe benefits of 23% and 7.3% are Office of Naval Research negotiated rates for Virginia Tech faculty and part-time wage employees, respectively.

Technical assistance: part-time laboratory assistant for extraction of plant and insect DNA and field collection of insects and plant material.

*Supplies:*Supplies include the reagents, enzymes and consumables required for the polymerase chain reaction analysis of insect and plant samples. The cost of DNA extraction and amplification for each plant sample is approximately $10, for insect samples is approximately $4.50. The cost of amplifying each membrane feeding solutions is approximately $4.50. Other materials needed are those necessary for the capture of field insects, growing and maintaining plants in the greenhouse and maintaining insect cultures.

Travel: Funds proposed for travel will provide support for: 1) Dr. Beanland to attend the annual Entomological Society of America meeting in Salt Lake City, UT in Nov. 2004 to present her findings, 2) Dr. Beanland and Dr. Wolf to make two surveys of MD, PA, and Finger Lakes region vineyards for the presence of GY and to collect grapevine samples for PCR analysis. Funds also cover weekly travel to and from field sites in Virginia.

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Appendix A: 2003 Progress Report

Insect Surveys. Beginning in May weekly sweep net samples were taken at two Virginia vineyards with different levels of grapevine yellows disease incidence. Separate sweep net samples were taken from within vineyards and from the native or non-cropped vegetation adjacent to or surrounding the vineyards. Samples were transported to the laboratory and stored at -20º. Leafhoppers, planthopper and psyllids were identified to species when possible and bulked by species by date. In addition, 15 yellow sticky traps were placed in around test vineyards to capture flying insects. Traps were erected in three transects of five traps at each study vineyard. Two traps in each transect were placed in the non-cropped vegetation and 3 were placed in the vineyard, so that movement between the vineyard and surrounding vegetation could be quantified.

In sweep net collections a total of fourteen planthoppers were captured; no psyllids were captured. Numerous xylem-feeding leafhoppers were also captured. The incidence and abundance of leafhopper species are presented in Table 1.

Table 1. Total number of candidate grapevine yellows vector species per sweep in 2002 and 2003 sweep samples.

Species	2002	# per sweep	2003	#per sweep
Agallia constricta	2960	17.3	905	10.3
Exitianus exitiosus	938	5.5	35	0.4
Polyamia weedi	336	1.96	79	0.9
Latalus sayi	311	1.82	90	1
Amblysellus curtisii	234	1.37	26	0.3
Deltocephalus flavicosta	191	1.11	68	0.77
Endria inimica	167	0.98	127	1.44
Sorhoanus orientalis	161	0.94	18	0.2
Chlorotettix galbanatus	122	0.71	92	1
Graminella nigrifrons	85	0.5	191	2.2
Paraphlepsius irroratus	64	0.37	80	0.9
Balclutha abdominalis	54	0.32	27	0.31
Macrosteles quadrilineatus	26	0.15	54	0.61
Doratura stylata	22	0.13	0	0
Jikradia olitorius	15	0.09	22	0.25
Osbornellus auronitens	13	0.08	27	0.31
Scaphytopius nigrifrons	12	0.07	7	0.08
Agalliopsis novella	8	0.05	10	0.11
Athysanus argentarius	8	0.05	1	0.01
Acertagallia sp.	4	0.02	1	0.01
Scaphoideus titanus	3	0.02	2	0.02
Aphrodes flavostrigatus	2	0.01	0	0
Colladonus clitellaris	2	0.01	0	0
Idiodonus kennicotti	1	0	0	0
planthoppers	26	0.15	59	0.67

More insects were captured in the vineyard than in the adjacent or surrounding vegetation in 2003; a total of 1,657 leafhoppers and planthoppers were captured in the vineyard and a total of 960 were collected from around the vineyard.

Table 2. Number of candidate vector species captured in 2003 sticky traps erected in vineyard and in surrounding vegetation.

Species	Total	# per Vineyard Trap	# per Forest Trap
Graphacephala sp.	716	28	71
Jikradia olitorius	274	3.3	41
Agalliopsis sp.	198	4	27
Scaphoideus titanus	195	0.7	31
Delphacodes sp.	80	0.3	13
Osbornellus auronitens	78	0	13
Paraphlepsius irroratus	48	5	1
Draeculacephala sp.	23	2	1
Scaphytopius nirgrifrons	14	0.8	1
Colladonus clitellarius	4	0	0.5
Chlorotettix galbanatus	4	0	0.5
Agallia constricta	2	0	0
Norvellina novica	1	0	0

Testing Insects for GY Phytoplasma. Captured candidate vectors were bulked by species and by date. DNA was extracted and subjected to PCR analysis using nested pairs of universal primer specific for phytoplasmas. The product of positive samples was tested using aster yellows, X disease specific and elm yellows specific primer pairs. To date, hundreds of captured insects have been tested. Eleven leafhopper species were positive for phytoplasma (Table 3).

Sticky Trap Sampling. Seasonal distribution across the forest-vineyard ecotone of candidate vectors is species-specific. For instance, the first captures of *Paraphlepsius irroratus* were in the vineyard and this species was consistently captured in larger numbers there than outside the vineyard (Table 4). *Scaphoideus titanus* was found initially and always in larger numbers in the forest but moved into the vineyard later in the season. The distribution of *S. titanus* could account for the edge effects observed in the occurrence of GY in vineyards where much of the disease is observed in vines near forests. The percentage of *Jikradia olitorius* on vineyard sticky traps also increased over the season, but the same trend was not observed in sweep samples where *J. olitorius* became more abundant in the forest. *Delphacodes* sp. were captured in larger numbers in vineyard sweep samples and in larger numbers on sticky traps placed in the forest. Different species in this genus may have different habitat preferences.

Membrane Feeding Trials. Beginning in July, weekly membrane feeding trials were performed using live-captured leafhoppers collected from test vineyards. Leafhoppers were placed individually in 1.5ml tubes and were permitted to feed on a buffered sucrose solution for 48 hours. Phytoplasma shed by feeding leafhoppers into the artificial media was detected and characterized by PCR analysis of the test solution. This techniques permits us to make preliminary assessments about a candidate insect's ability to serve as a vector for GY phytoplasmas. Twenty-two species of leafhoppers were used in membrane feeding trials (Table 5).

Table 3. Captured candidate vector species that tested positive by PCR analysis for phytoplasma.

Insect species that tested positive for phytoplasmas	**# samples**
A. constricta	13
Acertagallia sp.	1
Agalliopsis sp.	1
B. abdominalis	3
C. galbanatus	8
D. flavicosta	4
E. exitiosus	2
E. inimica	5
G. nigrifrons	8
J. olitorius	4
L. sayi	2
M.quadrilineatus	2
O. auronitens	2
P. irroratus	10
P. weedi	4
S. titanus	7 (from sticky traps)
S. orientalis	2
Sharpshooters	
Draeculacephala sp.	21
Graphacephala sp.	2
Planthoppers	
Delphacodes sp.	2
Lieberniella sp.	3
Flatidae	1
Acanalonidae	1

Table 4. Number of candidate vector species captured in 2003 sticky traps erected in vineyard and in surrounding vegetation.

Species	**Total**	**# per Vineyard Trap**	**# per Forest Trap**
Graphacephala sp.	716	28	71
Jikradia olitorius	274	3.3	41
Agalliopsis sp.	198	4	27
Scaphoideus titanus	195	0.7	31
Delphacodes sp.	80	0.3	13
Osbornellus auronitens	78	0	13
Paraphlepsius irroratus	48	5	1
Draeculacephala sp.	23	2	1
Scaphytopius nirgrifrons	14	0.8	1
Colladonus clitellarius	4	0	0.5
Chlorotettix galbanatus	4	0	0.5
Agallia constricta	2	0	0
Norvellina novica	1	0	0

Table 5. The Leafhopper Species and the Number of each Used in 2003 Membrane Feeding Trials.

Agallia constricta	46	*Deltocephalus flavicosta*	5
Endria inimica	21	Flatidae	5
Paraphlepsius irroratus	16	*Graminella nigrifrons*	3
Latalus sayi	14	*Macrosteles quadrilineatus*	3
Exitianus exitiosus	11	*Tylozygus bifidus*	3
Chlorotettix galbanatus	10	*Graphacephala* sp	2
Draculacephala sp.	10	*Osbornellus auronitens*	2
Polyamia weedi	8	*Colladonas clitellarius*	1
Liberniella sp. (planthopper)	7	*Jikradia olitorius*	1
Amblysellus curtisii	5	*Scaphytopius nigrifrons*	1
Delphacodes sp. (planthopper)	5	*Sorhoranus orientalis*	1

Only the solutions of surviving insects were subjected to PCR analysis for phytoplasmas. Test solutions fed upon by *Paraphlepsius irroratus*, *Polyamia weedi* , *Draeculacephala* sp., *Latalus sayi*, and 1 Flatidae planthopper tested positive for phytoplasma.

Transmission Trials. Attempts to establish colonies of leafhopper candidate vector species were met with limited success in 2003. Hence, weekly transmission trials were performed using live-captured leafhoppers from study vineyards collected by sweep sampling. Captured leafhoppers were identified to species and placed in groups of up to 50 individuals on rooted Chardonnay cuttings. Leafhoppers were allowed to feed on the Chardonnay until they died. Recovered leafhoppers were subjected to PCR analysis to determine which may have acquired phytoplasma in the field. A total of 520 individuals in 12 species were used in transmission trials. Forty test Chardonnay cuttings used in these transmission trials are under observation for development of symptoms or are stored at -80ºC awaiting PCR analysis. We must observe the Chardonnay cuttings for up to one year, as the incubation time for symptom expression is not clear.

Table 6. The leafhopper species used in 2003 transmission experiments.

Species	**Number used in trials**
Agallia constricta	428
Ambysellus curtisii	1
Chlorotettix galbanatus	1
Draeculacephala sp.	30
Endria inimica	14
Macrosteles quadrilineatus	14
Norvellina novica	1
Osbornellus auronitens	11
Paraphlepsius irroratus	14
Polyamia weedi	2
Delphacodes sp. (planthopper)	2
Scaphoideus titanus	2

Alternative Plant Host Surveys. Leaf and stem collections from trees, shrubs, vines, forbs and grasses found in and around the two test vineyards were taken on four, roughly monthly, dates in 2003, concentrating on perennial plants that might serve as year-to-year sources of inoculum of GY pathogens. Samples were also collected on single dates from 2 other Virginia vineyards. In July and again in September 5 vineyards in New York and Pennsylvania were visited and leaf and stem samples from wild plants were collected. Plant tissue samples were stored at -20ºC until DNA was extracted from each plant and then subjected to PCR analysis. DNA has been extracted from samples collected on the first collection dates and was subsequently tested by PCR analysis for the presence of GY phytoplasmas. In Virginia, several wild grapevine samples, two cherry trees, one elm tree, one dandelion plant, one white clover and one red clover sample have tested positive for phytoplasma. To date two wild grapevine samples collected In New York have tested positive for phytoplasma. Of the samples for which strain-specific tests have been performed all were found to be infected with aster yellows phytoplasma except the red clover which was infected with an unidentified phytoplasma. Processing of the remaining samples is currently progressing.

Propagation of Grapevine Yellows infected Plant Material. Attempts were made to propagate infected plant material for use as acquisition access plants in transmission experiments. Cuttings were taken from a dormant wild grapevine that had tested positive for phytoplasma in autumn 2002. Aster yellows phytoplasma was detected in one of the 4 surviving cuttings. This plant has been used in transmission experiments. Dormant cuttings have been collected from wild grapevine at both test vineyards in February 2004 and have been planted in vermiculite. Surviving cuttings will be tested for the presence of phytoplasma in mid-summer, 2004.

Distribution of Grapevine Yellows. In order to determine the incidence of the VA grapevine yellows phytoplasmas in other states, leaf and stem samples were taken from grapevines exhibiting symptoms of grapevine yellows disease at several vineyards in New York and Pennsylvania in July and September 2003 (project was roughly 50% federally-funded in 2003). Tissue samples have been stored at -20ºC and will be PCR-analyzed in spring 2003. Three Chardonnay samples and two wild grapevine sample collected from Pennsylvania and New York have tested positive for phytoplasma.