CALIFORNIA FRUIT TREE, NUT TREE AND, GRAPEVINE IMPROVEMENT ADVISORY BOARD

2014 IAB NEWSLETTER

IAB NEWSLETTER

DECEMBER 1, 2014

California DROUGHT AND RESOURCES

INSIDE THIS ISSUE:

SPECIAL POINTS OF INTEREST:

- CDFA NURSERY ADVISORY BOARD (NAB) MEETING IN FEBRUARY/ MARCH 2015
- NEXT IAB MEETINGS IN APRIL, MAY & NOV. OF 2015
- MEETING POSTING WEBSITE: WWW.CDFA.CA.GOV/ PLANT/MEETINGS.HTML
- DROUGHT INFO WWW.CDFA.CA.GOV/ DROUGHT/

Governor Brown has convened an interagency Drought Task Force to provide a coordinated assessment of the State's dry conditions and provide recommendations on current and future state actions. The response to this statewide disaster requires the combined efforts of all state agencies and the state's model mutual aid system to address.

In support of this unified effort, all state agencies with a role in supporting drought mitigation and relief efforts are organized under the Incident Command System and will continue provide emergency planning, response, and mitigation support as long as needs exist.

Current Drought Task Force Group includes Governor's Office of Emergency Services (CalOES), California Department of Food and Agriculture (CDFA), California Environmental Protection Agency State Water Resources Control Board (SWRCB), and California Department of Water Resources (DWR).

Visit CDFA website for more information:

www.cdfa.ca.gov/drought/

- Drought Resources for Farmers, Ranchers, and Farmworkers.
- Federal/State Agencies & Assistance Programs

SERIOUS DROUGHT

HELP SAVE WATER

cdfa

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE

WHAT IAB PROVIDES TO THE INDUSTRY

The California Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB), element of the Nursery program, administers an industry requested assessment (Food and Agricultural Code, Section 69814) on the production of nursery plants such as deciduous pome and stone fruit trees and grapevines. The mission of the IAB is to improve the quality of fruit trees and grapevines nursery stock offered for sale. The assessment is used to fund research on plant pests, breeding varieties that are resistant to plant pests, plants pest diagnostics, varietal identification and disease elimination. The University of California, Foundation Plant Services (FPS) provides support and serves as a source of clean planting stock. FPS carries out activities related to the development of planting materials for pome and stone fruit trees, nut trees and grapevines. IAB subvents the costs to carry out Department programs concerning the Registration and Certification (R&C) Program of pome and stone fruit trees, nut trees and grapevines. The assessment is collected annually with the nursery license renewal.

2014 FUNDING DISTRUBUTIONS

In 2014, the IAB approved funding for 16 research/service proposals totaling $438,033, funding for Foundation Plant Services in the amount of $638,452, and payment to the Nursery Program to subvent Registration and Certification (R&C) activities in the amount of $261,970. The total budget approved was $1,781,425. As revenues increased for the past two years, the Board has recommended increasing funding for research, FPS and the R&C programs.

RESEARCH AND SERVICE PROJECTS FUNDED IN FY 2014-15

I. Adapting New Technology to Facilities Virus Testing of Fruit Trees

II. Etiology and Management of Cryptic Canker Pathogens in Cold-store Bare Root and Containers Propagated Stone Fruit Seedlings

III. DNA Methylation as a Diagnostic Marker for Clonal Ageing and Non-infectious Bud-failure (BF) in Almond

IV. Cold Storage of clonal Rootstock Liners and Graft Wood Stock Plant Manipulation to Facilitate Production of Grafted Walnut Trees in One Year

V. Anaerobic Soil Disinfestation Against Soilborne Phytopathogenic Agents

VI. Evaluation of Phloem-feeding Hemipteran Insects in the Spread of Grapevine Red Blotch-Associated Virus

VII. Study the Lethal Effect of the Mixed Infection of Vitiviruses and Leafroll Associated Viruses on Selected Grapevine Rootstocks

VIII. Development and Application of Next Generation Sequencing to Facilitate the Release of New Grapevine Accessions in Quarantine and Certification Programs

IX. Study of the Effects of Red Blotch Disease on Different Grapevine Rootstocks and Different Vitis Vinifera Plants

X. Search for, and Development of Nematode Resistance in Grape Rootstocks

XI. Development of Next Generation Rootstocks for California Vineyards

XII. Ecology of Leafroll Disease

XIII. Sampling Plans and In-field Diagnostics to Help Remove Red Blotch Associated Virus from the Grapevine Value Chain

XIV. Support to Foundation Services

XV. Heat Therapy and Indexing of Stone and Pome Fruit Cultivars

XVI. Methyl Bromide CUE

XVII. Support to R&C Programs at CDFA

Total Research & Service Projects Funded: $1,781,425

WHO PAYS THE NURSERY STOCK ASSESSMENT AND HOW IT IS USED?

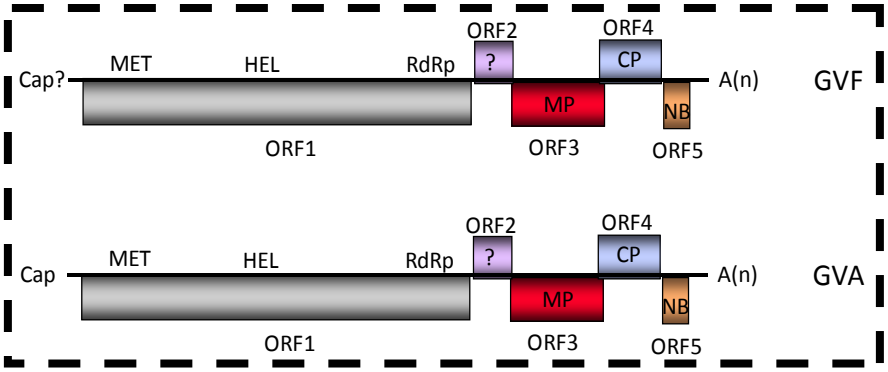
The California Fruit Tree, Nut Tree and Grapevine Improvement Advisory Board (IAB) was established in 1988 to promote production of high quality tree and grapevine nursery stock, and to help develop and promote consumer education. The IAB is funded through a 1% annual assessment on gross sales paid by producers of deciduous pome and stone fruit tree, nut tree, olive tree and grapevine nursery stock. Section 44 of the Food and Agricultural Code defines sell to include, "offer for sale, expose for sale, possess for sale, exchange, barter, or trade." Therefore you are required to pay the assessment on any qualifying nursery stock disposed of by any of these means. The IAB uses these funds to support Foundation Plant Services (FPS), to subsidize Prunus Necrotic Ringspot and Prunus Dwarf Virus testing in registered trees, to subsidize virus testing and clean-up of new fruit and nut tree varieties, to subsidize fanleaf, leafroll, and tomato ringspot sampling, vine mealybug trapping in grapevines, support research into disease and pest-resistant varieties and alternatives to fumigants currently in use.

	Call for Research Proposal FY 15-16
Categories	Virus elimination research; developing new or improving existing detection methods for virus and virus like diseases; disease and genetic disorders for Fruit Tree, Nut Tree and Grapevine
RFP Instructions	http://www.cdfa.ca.gov/plant/pe/nsc/docs/iab/RFP_instructions_15-16.pdf
Due February 2, 2015	Submit your proposal(s) to: CDFA 1220 N. Street Room 344, Sacramento CA 95814 Attention: Sean Dayyani or email sean.dayyani@cdfa.ca.gov

MOLECULAR CHARACTERIZATION AND DETECTION OF A NOVEL VITIVIRUS ISOLATED FROM A GRAPEVINE IN CALIFORNIA

DR. ADIB ROWHANI, UC DAVIS

In a field trial using GLRaV-1 isolates on different rootstock combination we found that a GLRaV-1 isolate from an AUD46129 grapevine accession showed scion/rootstock incompatibility on rootstocks 101-14 and Freedom. All traditional molecular methods failed to determine the basis of this behavior. In order to diagnose the pathological reaction, dsRNA was extracted from AUD46129 accession, and cDNA libraries were prepared and analyzed using high throughput sequencing. The sequencing data showed the presence of multiple strains of GLRaV-1, GVA and partial sequences (multiple fragments of ~3100 nt) of an entirely novel vitivirus, which has tentatively been named Grapevine virus F (GVF). Total nucleic acid extracted from the Audibert grapevine was used as template for specific primers that were used to bridge the gaps between the fragments. The complete viral genome was assembled from this data, with a final genome size of 7539 nt. The genome structure revealed five open reading frames (ORFs) organized similarly to *Grapevine virus A* (GVA). RT-PCR primers were designed based on the available sequences for virus detection. The primers were used to test 454 plants in the UC Davis virus collection and USDA National Clonal Germplasm Repository. Total of 32 plants from these two collections tested positive for GVF. The PCR products from the positive samples, were recovered from agarose gel electrophoresis and sequenced. PCR primers designed from the GVF sequence have been used to generate a SYBR green assay, which will allow for routine diagnosis of the virus in multiple field samples. Studies using *Planococcus ficus* mealybugs have been initiated, but plant-to-plant transmission of the virus has not yet been demonstrated.



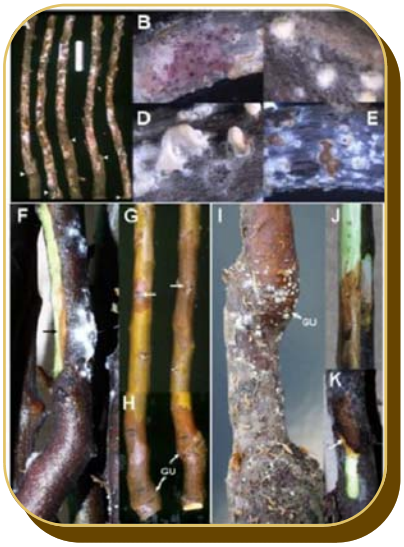
"Grapevine Virus F vs Grapevine Virus A"

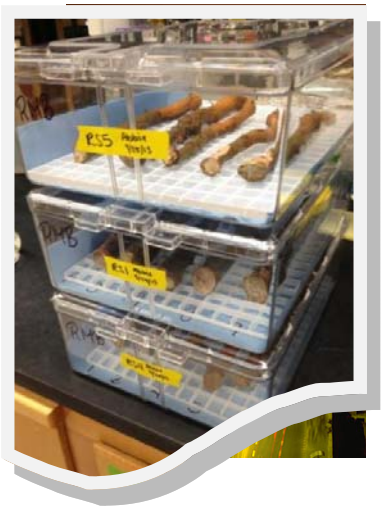
ETIOLOGY AND MANAGEMENT OF CRYPTIC CANKER PATHOGENS IN COLD BARE ROOT AND CONTAINER-PROPAGATED STONE FRUIT SEEDLINGS

DR. RICHARD BOSTOCK,UC DAVIS

The focus of this project is a canker disease that can emerge in cold-stored bare root and container-propagated stone fruit seedlings that can arise in storage or after planting in the field. Our previous research demonstrated the involvement of four opportunistic fungal pathogens: Fusarium avenaceum, Fusarium acuminatum, Cylindrocarpon obtusiusculum, and Ilyonectria robusta. The purpose of this project is to provide the orchard nursery industry with more precise information about disease incidence and potential inoculum sources, as well as provide science-based cultural and chemical treatment options for disease management. The specific objectives are to i) assess incidence of canker pathogens in bare root seedlings and budwood; ii) develop molecular markers to conduct forensic analyses to trace inoculum sources and pathways for infection; and iii) evaluate chemical treatments for efficacy in managing seedling canker disease.

Isolations from nursery materials. We are focusing our effort on F. avenaceum and F. acuminatum, which appear to ne the more common and aggressive pathogens that can cause this disease. In May and November 2013 we retrieved materials from one to the nurseries. This included discarded plants (almonds and cherry seedlings, with and without symptoms, and budwood), wheat stubble, and soil samples. In addition surfaces of storage pallets and walls within and adjacent to the cold-storage facility were samples for presence of the pathogens. The pathgenic Fusaria were isolated using selective media from all the materials and locations that we samples. These isolated were purified and their species identity confirmed by their morphology in culture and by molecular identification. These materials and the isolated contained have provided a focus for most or our effort. We will continue to sample field sites, storage facilities, and budwood at several of the major nurseries and will continue to add selected isolates to our collection.





Caption describing picture or graphic.

"What causes Canker disease in cold storage?"

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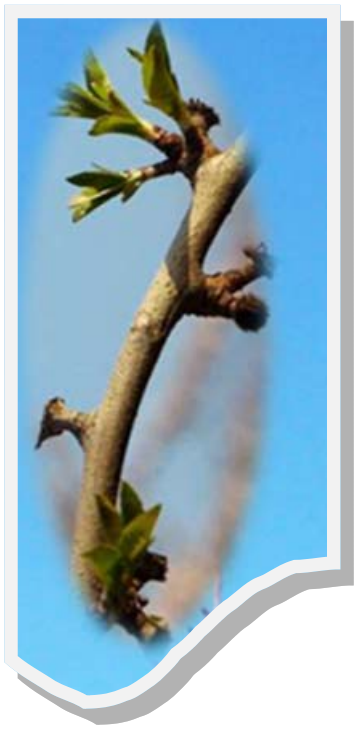
MOLECULAR MARKER BASED DIAGNOSTICS FOR ALMOND BUD-FAILURE

DR. THOMAS GRADZIEL, UC DAVIS



This research is a continuation of a project jointly funded by the Almond Board of California (ABC) and the California nursery industry (IAB). It advances previous UC Davis (UCD) studies which have led to an understanding of the pattern of Non-infectious Bud-failure (BF) development within propagation sources (clones) of commercially important almond cultivars including Nonpareil and Carmel, which allow effective selection of clonal sources with lower probabilities of expressing BF during the crucial early years of orchard growth. Attempts to develop molecular markers as indicators of BF-potential have proven unsuccessful, presumably because BF genetic deterioration is not associated with changes in the marker-targeted DNA sequence of the gene(s) involved, but rather involves suppression of gene activity through still poorly understood epigenetic mechanisms. This project is thus pursuing epigenetic markers based on the methylation patterns for individual genes from clones of Nonpareil and other important almond cultivars which differ in the level of BF expression and/or the clone age (since it is known that the potential for BF-expression increases with age of susceptible cultivars). We have now identified a number of methylation-markers associated with the level of BF expression, as well as with the age of the clone.

Because of the large number of potential markers and the inherent difficulties in accurately scoring both BF-potential and clone age, we are now analyzing the data through both large-scale statistical analysis and individual assessment of putative candidate gene function to identify epigenetic markers associated with BF expression. A strong association might then be used as predictor of the ultimate level of BF expression in vegetative progeny from different nursery source trees and, if highly correlated, may help identify the gene(s) controlling this disorder, which in turn might lead to a better understanding of BF development as well as its control.

"What is the cause for Almond Bud Failure?"

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DEVELOPMENT AND APPLICATION OF NEXT GENERATION SEQUENCING (NGS) TO FACILITATE THE RELEASE OF NEW GRAPEVINE ACCESSIONS IN QUARANTINE AND CERTIFICATION PROGRAMS

DR. ADIB ROWHANI, UC DAVIS

Recent developments in molecular technology have led to significant improvements in detection and control of many pathogens. The use of those techniques for pathogen detection in quarantine and certification programs has not yet been universally accepted. This is primarily because of the need to validate these techniques and determine their limitations. We are proposing to supply the data for that validation for the case of grapevine registration and certification. We will make a side-by-side comparison of 1) the classical, currently used technique for the analysis of viral pathogens of grapevine, with 2) the more recently developed technology of Next Generation Sequencing (NGS). In both cases, we will test the same set of fifty selected grapevine accessions infected with one or multiple viruses of importance to the grape industry. In the first case, viral pathogens will be analyzed using biological assays on a standard herbaceous and woody index panel of host plants, as is required by APHIS and CDFA for certification. We will compare the results from that bioassay with a second analysis based on NGS of the total grapevine viruses in each of the selected accessions. The two tests will run concurrently. We expect to show that, for the evaluation of the disease status of grapevine stocks, NGS is superior to biological assay, as well as to ELISA, RT-PCR and real time RT-qPCR in sensitivity, reliability, speed and labor intensity, and cost. We will make the case for the replacement of biological assays with NGS for the certification of novel grapevine accessions. Our data will be useful to federal and state regulatory agencies as evidence supporting the revision of the existing mandated protocols for the testing and release of novel grapevine accessions from quarantine. The improvements brought with the up-date to NGS technology for this application will be of significant benefit to the grape growing industry. The first year objectives of this project have been met. We have identified the first batch of 20 grapevine accessions that carry infections of agronomic importance, for use in the comparative demonstration of the effectiveness of the two techniques evaluated in this project. We have chip-bud grafted material from each of those to the standard four bioassay index hosts, and begun their two year incubation period toward symptom scoring. We have also made total RNA extractions from those infected plants and begun NGS analysis, using BLAST sequence comparisons to subtract the host coded sequences from those of the pathogens of interest. This progress will generate the data for the comparisons between the techniques, which will meet the subsequent objectives of this proposal.

"NGS testing can show all pathogens and diseases"

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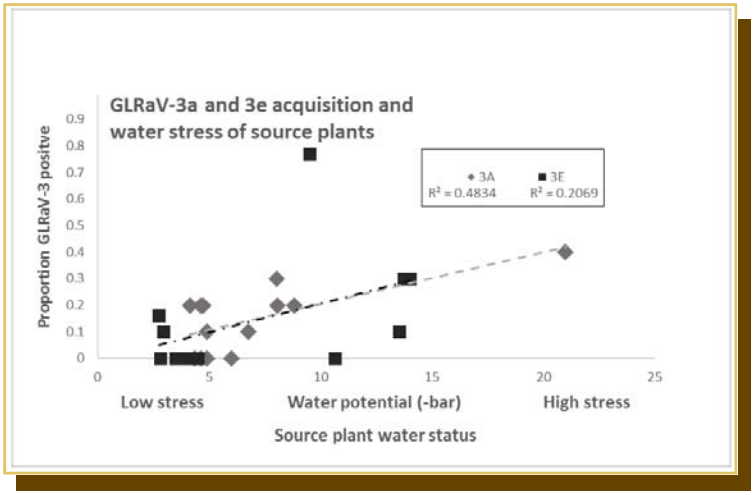
GLRAV-3 TRANSMISSION BY MEALYBUGS

DR. RODRIGO ALMEIDA, UC BERKELEY

Our research focuses on improving the general understanding of spread and symptom development of Grapevine Leafroll Disease (GLD), which will inform management practices for improved disease control. Mealybug vectors and contaminated planting material have been implicated as key driving factors of spread of GLRaV-3. Mealybug-mediated spread has become an increasing concern since the invasive vine mealybug established in California in the 1990s, but it can be difficult to discern the source of new infections because the incubation time of GLD in new vineyards is not well understood. Furthermore, plant water status may affect disease spread by altering pathogen loads in vines, altering mealybug behavior, or other interactions. While there is some information about transmission efficiency of GLRaV-3 based on greenhouse experiments, there is no information on the potential role of plant water status in disease spread. In addition, no work has compared the amount of time after infection until symptom expression for grapevines infected by insects in the field, grafting, or material already infected when delivered from a nursery. In greenhouse trials, we found that virus acquisition by mealybugs increased when infected donor plants received less water. In contrast, we found that well watered plants were more susceptible to new infection than plants that received less water, and third instar mealybugs show preference for well-watered plants. An experimental vineyard was established this year, using the indicator variety Pinot Noir, which includes nursery-infected, graft-inoculated, and mealybug-inoculated vines, with all infection treatments subject to two levels of irrigation. Data collection is ongoing in the experimental vineyard. Our study will lead to a better understanding of disease incubation time and disease source, and to an understanding of the effects of plant water status on disease spread.

"The effects of plant water status on disease spread"



SYMPTOMATOLOGY AND DISTRIBUTION OF GRAPEVINE RED BLOTCH-ASSOCIATED VIRUS IN RED AND WHITE WINE GRAPE VARITIES AND ITS EFFECT ON FRUIT MATURITY

DR. MYSORE SUDARSHANA, USDA

Cabernet Sauvignon

We conducted surveys in a Chardonnay and a Cabernet Sauvignon block of a commercial vineyard in August 2013. All 18 samples of Chardonnay and 8 samples of Cabernet Sauvignon tested positive for Grapevine red blotch-associated virus (GRBaV). Due to non-availability of uninfected grapevines studies on impact were abandoned. However, symptoms were recorded on Chardonnay, Malbec, Petit Verdot, Pinot Noir and White Riesling in this vineyard. A Cabernet Franc vineyard was visited and the impact on sugar accumulation was assessed at maturity in October. In GRBaV-infected grapevines, the mean reduction in total soluble solids was 5 °Brix when compared to normal vines. Another vineyard in San Luis Obispo County was visited in November 2013 and symptoms on Rousanne and Grenache were recorded. In Grenache block, grapevines infected with GRBaV were identified after laboratory tests and tagged for further observations in 2014. Laboratory tests using qPCR have confirmed that GRBaV is well-distributed in grapevines and the virus can be detected in green shoot and petioles of leaves on new growth when the buds start pushing in March/April. We have standardized a PCR method that uses simple nucleic acid extraction methods which can reduce sample processing time considerably.

"Red Blotch has flat margins and pink veins"

Chardonnay

STUDY THE LETHAL EFFECT OF THE MIXED INFECTION OF VITIVIRUSES AND LEAFROLL ASSOCIATED VIRUSES IN SELECTED GRAPEVINE ROOTSTOCKS

DR. ADIB ROWHANI, UC DAVIS

Grapevine leafroll disease causes poor color development and non-uniform maturation of fruits in *Vitis vinifera*. It also reported delay in fruit maturation from 3 weeks to a month in diseased vines. Other symptoms included downward rolling of basal leaves followed by rolling of the leaves near the shoot tips, color change of the leaves, and phloem disruption. This condition has been associated with yield losses of as much as 20 to 40%. The grapevine-infecting vitiviruses are reported to be associated with the rugose wood (RW) disease complex which includes several important diseases that result in woody cylinder modifications. Our observation in California has shown that the Cabernet Franc plants propagated on Freedom, 420A, 3309C or 101-14 when inoculated with a virus source containing Grapevine leafroll associated virus 1 (GLRaV-1) and GVA, the plants died within 1-2 years after inoculation. In this project we are investigating whether there is a synergistic interaction between GLRaV-1 and GVA that killed the plants on these 4 rootstocks or the presence of a different GLRaV-1 strain in this isolate was the cause. To pursue our hypothesis, we produced Cabernet Franc plants on 6 different rootstocks of 3309C, 101-14, Kober 5BB, Freedom, 420A and St. George 15. These plants have been planted in the field and inoculated with different viruses and virus isolates including: 5 different isolates of GLRaV-1, 2 different isolates of GLRaV-2, one isolates each of GVA and GVB and a combination of two of GLRaV-1and -2 isolates with each one of the GVA or GVB. These inoculated plants will be monitored for virus movement and symptom expression and severity. To investigate the role of the strains of GLRaV-1 in the lethal interaction between the scion and the rootstocks, dsRNA has been extracted from the samples collected from the isolate LR132 and the Audibert cultivar and used for cDNA synthesis. The cDNA has been enriched and sequenced by an Illumina platform. Sequence analysis is in process.

> "downward rolling of leaves, and color change of leaves "

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HEAT THERAPY AND INDEXING OF STONE AND POME FRUIT TREE CULTIVAR

DR. KEN EASTWELL, WSU

Requests for submission of fruit tree selections to the program from the 2013-14 fiscal year are received in January, therefore the receipt of new selections for the current granting cycle are occurring at the time at which this report was prepared. Activities from the agreement approved in the 2012-13 fiscal year are continuing and we are meeting project goals established for that period. In January-February 2013, the program received nine stone fruit selections representing peach, cherry and plum selections from six different nurseries. All of these selections were infected with viruses. Two additional almond selections were received for indexing and heat therapy. Both were infected with viruses. Heat therapy was initiated on trees propagated from the peach budwood received in February 2013. Four shoot tip grafts were made after heat therapy. Two grafted plants survived and are currently undergoing testing as per our standard operating procedures. Although incomplete at this time, one of the propagated trees has yielded negative results for the first eight pathogen tests.

The California Department of Food and Agriculture recently indicated that it would not accept material infected with *Cherry virus A*. This virus was discovered just over a decade ago, but still very little is known about its biological consequences. Several comments, unsubstantiated by data, appear in the literature regarding infections symptoms. Additionally, observations from uncontrolled experiments suggest that *Cherry virus A* may increase the severity of disease caused by other viruses. Since this is still an emerging issue, and since there are no scientifically rigorous reports documenting the economic significance of *Cherry virus A*, the response from regulatory agencies internationally has been mixed. Until recently, the protocols used by international fruit tree quarantine and certification programs did not detect this virus. However, the protocols at WSU/CPC-FT were enhanced and approved by USDA-APHIS in 2010 to include a molecular assay for *Cherry virus A*. Results emerging from our program and from those at other facilities suggest that *Cherry virus A* is common in *Prunus*. Current tests indicate that typically more than 40% of the material entering these virus testing programs are infected with *Cherry virus A*. Of course the historical levels of *Cherry virus A* in *Prunus* species is not known. In order to comply with increased testing requirements, WSU/CPC-FT tested 26 stone fruit selections of interest to the CDFA-IAB. The selections included peach, plum, nectarine and cherry trees. Of these selections, 17 or 65% were infected with *Cherry virus A*; infections with *Cherry virus A* were detected in each of the four *Prunus* species included in this series of tests.

"Heat Therapy requires multiple years of testing"

WASHINGTON STATE UNIVERSITY

IAB Mission Statement:

Enable production of the highest possible quality grapevine and deciduous fruit and ornamental nursery stock and to help develop and promote consumer education.

Board Members:

1. Nicholas Podsakoff - Chair - Vintage Nurseries - Grapevine
2. Chuck Fleck - Vice Chair - Fowler Nursery - Tree
3. Alex Brody - Burchell Nursery - Tree
4. Cliff Beumel - Sierra Gold Nursery - Tree
5. David Cox - L.E. Cooke - Pome
6. Ray Tonella - Public
7. Benjamin Kaesekamp - Knights Grapevine Nursery Inc. - Grapevine
8. Tom Burchell - Burchell Nursery - Olive
9. Andrew Jones - Sunridge Nursery - Grapevine
10. Ernie Bowman - Martinez Orchard - Grapevine
11. Mike Farris - Dave Wilson - Tree

Sean Dayyani - IAB Manager

Membership on the Board consists of 11 representatives:

* Two each from stone fruit and nut tree industries;
* Four from grapevine industries
* One each from pome fruit and olive tree industries
* One public member

CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

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www.cdfa.ca.gov/plant/pe/nsc/iab/index.html

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