Report on August 22-23, 2006 Workshop to Review Pierce's Disease and Glassy-winged Sharpshooter Research Priorities

Background and Introduction

After six years of intensive effort, there have been considerable successes in the Pierce's disease research program, such as in area-wide management and the treatment of nursery plants before shipment to inhibit Glassy-winged Sharpshooter (GWSS) from spreading to new areas of the state. There have also been considerable scientific advances in understanding of the disease and its vectors. Many ideas have been proposed and tested.

Various initiatives and reports have focused on or contributed to elucidating specific research needs and priorities for this plant disease problem.

Nonetheless, there are continuing pressures to update priorities and to strategically refocus the research program to accelerate the most promising strategies to practical field application. The U.S. Senate, for example, requested this year a report for the USDA funds administered by the University of California Statewide IPM Program that among other things asked for information to "determine when the research objectives will be met, and the expected date of completion."

With those factors in mind, Rick Roush, Tom Esser and Nancy Irelan organized a scientific workshop ("summit") to review the status and priorities of PD/GWSS research program, its request for proposals (RFP), and other practices, and consider whether new approaches or other changes should be adopted.

For simple reasons of logistics, this meeting was kept reasonably small, and therefore was organized on an invitation-only basis. However, the invitees (listed below) represented a diverse range of public and private sector scientists. The meeting was held on August 22-23 at UC Davis.

Prior to the meeting, invitees were provided one-page summaries of all projects funded in the most recent grant round, and a spreadsheet of titles and expenditures for all projects funded over the last 3-5 years.

As described further below, the working group agreed as a broad consensus that there was a need to refocus the program, and even on a relatively short list of high priorities. We all recognized that significant funding for Pierce's Disease research and implementation will not last indefinitely, and at current levels may last for only another 4 years or so. Further, successfully focused research and delivery of practical solutions to the problem of Pierce's Disease was seen as critical for the continuation of federal and grower funding for future research and implementation programs.

A steering committee of Nancy Irelan, Tom Esser, George Bruening, Rick Roush, Bob Webster, Cliff Ohmart, and Charlie Hossom agreed to take the points and recommendations from this workshop and refine them.

This report is intended to inform the wider community of scientists and industry leaders about our conclusions, and to invite further comment. The principal outcome of the workshop is a significantly revised RFP, also included with this report. A**fter incorporating as appropriate any other public comments received by October 1, the steering committee will recommend the new RFP to the Pierce's Disease Board on October 11 for approval.**

We thank Joyce Strand, Melanie Caruso, Rachel Warren and Cheryl Morris of the University of California Statewide IPM Program for their assistance in organizing the meeting and in preparing this report.

Major themes of discussions at the Workshop

Although grant applications and discussions at the annual Pierce's Disease (PD) Symposium have been organized along the lines of disciplines such as vector biology, an alternative and useful way of thinking of all PD research is how it fits into tactics that can be applied to practical management of PD in the field. In broad terms, tactics to management PD can be classified as follows, which helps to focus attention on the outcomes expected from the research program.

1. Reduction of ***Xylella fastidiosa*** (Xf) natural reservoirs (eliminate host plants or reduce their disease load).

2. Control of insect vectors
2a. slow spread of vectors, especially into new regions of the state
2b. chemical control, including in area-wide management programs
2c. biological control
2d. interference with Xf/PD acquisition, transmission, retention and disease expression by vectors

3. Crop protection from Xf
3a. chemical (e.g., injected or sprayed) to prevent or cure infections
3b. discover and deploy conventional resistance genes
3c. discover and deploy resistance transgenes
3d. biological control of Xf (e.g., competition from less virulent strains of Xf)

4. Diagnostics or monitoring tools for both insects and diseases that help to inform application of the above tactics.

It was readily agreed after considerable discussion among those present that despite years of research and practical application, there was little evidence that reduction of Xf natural reservoirs can help in management of PD, or at least there was little that could be practically done of which growers weren't already aware. It may be possible that more can be done on Xf reservoirs that affect other crops, such as almonds, but the principal focus of this meeting was on PD in grapes and its funding.

It was next agreed that relatively little more research was needed on topics 2a and 2b, which have been intensively studied. That is, some new research may be needed from time to time to

refine the use of chemical insecticides, for example, but this no longer needed to be a major theme of the research program. On the other hand, there was a need for more discussion on topics 2c and 2d, and breakout sessions were assigned to these.

There was also agreement that the current prospects were not promising for chemicals that could be applied to the plant to prevent or cure PD (topic 3A). However, breakout sessions were assigned to 3b and 3c, collectively entitled Grapevine Genetics, to 3d,.biological control of Xf, and to topic 4, Diagnostics or monitoring tools that help to inform application of the above tactics.

The breakout sessions were arranged so that all participants could attend at least two of the groups, to encourage cross-disciplinary discussions and sharing of information that is relevant to multiple pest management applications. The complete and unabridged notes from the discussions from each breakout are appended at the end of this report.

The whole group then further discussed the results of the breakouts and from those identified its overall priorities. These are partly reflected in Attachment A in the revised draft request for proposals (RFP). However, there were also two recommendations for how results are currently communicated and implemented, as follows.

First, rather than rely entirely on the PD Symposium as an event to communicate results to growers, a regular session should be established at the Unified meetings in Sacramento in late January to communicate key results to growers at an appropriate technical level. The PD Symposium is not at a convenient time or place for growers.

In addition, several areas surfaced during discussion where the research has been completed but where there is a need for implementation. The UC can only recommend grants for research funding to USDA, but CDFA has the legal capacity to offer grants for implementation. Thus, the second recommendation was to develop a new grant category of grants designed solely for implementation.

Implementation grants might take the form of written information to the vineyard industry or other audiences. For example, research completed under the program indicated that commercially available GWSS traps are not optimally designed, but should be round. However, the current manufacturer of the traps has not changed the design.

Other recommendations are summarized in below.

PIERCE'S DISEASE SUMMIT AGENDA

AUGUST 22 Buehler Alumni Center Founders Board Room

8:30 AM arrival and casual introductions

9:00 AM: Overview of the issues: Where should we go from here?

Focus on bringing solutions to practical and commercial use, while continuing with strong peer review

US Senate asking serious questions about continuing the UC's USDA-funded program; there is a limited time line for funding for this work.

Disappointed applicants who have had work cut off; perhaps inadequate guidance on priorities and expectations

Excessive competition or overlapping proposals in some research areas?

More than a $ million in funds uncommitted this year

Do we need a new approach to building larger teams and larger grants in everything from biocontrol to transgenics or a different RFP?

Will larger teams work and will people be willing to contribute to them? What incentives are needed?

What and who are we missing that we need to streamline a timeline to delivery of control tactics and in an integrated package?

10:30 AM What are the major research themes of significant promise, and subdivide into groups to consider them?

What areas are essentially completed or no longer viable from a research perspective (e.g., area-wide management is working; does it need more refinement and, if so, how?)

Summary of any very new research results?

Is there something we have missed? Are we asking the right questions?

What are the key priorities across whole effort?

Possible themes/core research areas: Transgenics, Biocontrol of insects, Biocontrol of Xylella, etc.

Noon: Lunch break (catered)

1 PM Breakouts

Each Breakout to consider:

What is the state of the art in this area? Discussion of any breaking new research results?

What's the most promising approach or approaches to for success, or how do we decide or test it?

How do we move forward?

For this particular area, what changes in the program or RFP are needed?

How can a team or teams be formed if appropriate?

What key scientists should be involved but whom we have not reached?

3:30 PM Breakouts report to whole group in detail

Reception/Dinner

AUGUST 23

8 AM: Building on the breakouts to identify and draft any proposals for change

Is there something we have missed? Are we asking the right questions?

What are the key priorities across whole effort?

What key scientists should be involved but whom we have not reached?

Are there consolidated changes to the RFP or an approach to team building, or do these separately by topic area?

Review the current RFP for any changes

How should we report back to the larger PD community on any proposed changes? Who should report? E.g., should we redraft the RFP? Write an alternative form of a report?

9:30 AM breakouts as needed to draft any recommendations

11 AM Report back

Identify key priorities across the whole research program

Noon Lunch

1 PM Wrap up and next steps

PD "SUMMIT" (WORKSHOP) PARTICIPANTS

Alan Bennett, UC Davis
George E Bruening, UC Davis
Edwin L Civerolo, USDA, ARS
Doug Cook, UC Davis
Bob Curtis, California Almond Board
Tom Esser, CDFA
Goutam Gupta, Los Alamos National Lab
Harvey Hoch. Cornell University
Mark Hoddle, UC Riverside
Donald Hopkins, University of Florida
Charles Hossom, Stags Leap Winery
Nancy Irelan, Redtail Ridge Winery ??
John N Kabashima, UC Cooperative Extension, Orange County and South Coast Research & Extension Center
Bruce Kirkpatrick, UC Davis
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Judy Stewart-Leslie, Manager, Consolidated Central Valley Table Grape Pest and Disease Control District
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Kim Waddell
Andrew Walker, UC Davis
Robert K Webster, UC Davis
Bob Wynn, CDFA

Breakout groups

Grapevine Genetics
Alan Bennett
George E Bruening
Goutam Gupta
Charlie Hossom
Bruce Kirkpatrick
John M Labavitch
Andrew Walker
Donald Hopkins
Doug Cook

Monitoring/Diagnostics/Sampling
Bob Curtis

Goutam Gupta
Sandy Purcell
Richard A Redak
Beth Stone-Smith
Nick Toscano
John Kabashima
Judy Stewart-Leslie
Doug Cook

Biocontrol of GWSS
Mark Hoddle
Richard A Redak
Beth Stone-Smith
Nick Toscano
John Kabashima
Judy Stewart-Leslie
Cliff Ohmart
Kim Waddell
Ed Civerolo

Biocontrol of PD within Plant
Harvey Hoch
Ed Civerolo
Kim Waddell
Bruce Kirkpatrick
Donald Hopkins

Interference with Xf/PD acquisition, transmission retention and disease expression
Cliff Ohmart
Sandy Purcell
Charlie Hossom
Harvey Hoch
Andy Walker

SUMMARIES OF BREAKOUT DISCUSSIONS

Grapevine genetics

Conventional genetics

Currently PD programs invest >$400K in conventional breeding for resistance to Xf
Limiting factors are time and money; the sources of resistance, breeding methods and markers all are available and in use
One single-locus resistance is in an advanced breeding state, having been introgressed to 87% *Vitis vinifera*, which likely will be 94% vinifera next year

Other apparently single-locus sources of resistance also are available but are in a less advanced state of introgression

Mechanisms and gene identity are unknown for these natural sources if resistance; should these be investigated?

The first application of vinifera with introgressed resistance could be replanting of vines lost to PD or to roguing for prevention of PD spread, rather than for new plantings, which would be expected to be of accepted varietals (i.e., it may be better to restore production after PD attack or threat, even with non-varietals, rather than having no production)

Advancement to varietal or near varietal state will require 4+ years of additional breeding and several years of wine testing

Transgenic PD resistant grapevine

Public acceptance of transgenics remains questionable, except possibly in the face of extensive loss of production as the alternative

Offers the opportunity to introduce resistance without significant alteration of the traits of classical varietals; offers the possibility of introducing particularly robust and/or multiple resistances for greater durability

One possibility is to transform only the rootstock, to minimize the chances of change to the scion and possibly increase public acceptance

In general, there are very few candidate genes that are available as transgenes at present; proposed candidate transgene proteins or transgene protein targets: hemagglutinin-like protein, type I secretion protein, major outer membrane protein

An alternative to the identification specific targets and potential transgenes specific for those targets is high throughput selections that are not dependent on knowledge of the specific target of the Xf cell; such a selection may be *in vitro* or conceivably in a model plant system.

Additional effort in identification of plant-based resistance, both within *Vitis* and unrelated plant species, may well uncover useful target resistance genes of use in either conventional breeding or as transgenes.

What other approaches to gene discovery should be explored?

Proof of concept experiment is lacking with possible exception of Dennis Gray experiments in Florida

First application may be production of *Vitis vinifera* in Florida or other locations in which the disease pressure currently makes wine grape production impossible

Management of transgenic approaches:

can teams be assembled that will result in a synergistic application of capabilities from different laboratories?

what arrangements could result in periodic sharing of information (not limited to the PD symposium)?

research in this area requires stable funding because of the need to retain skilled personnel

Biological control of Xf

Three categories of biological control of a plant-infecting bacterium

1. Classical biocontrol with an unrelated endophytic bacterium
2. Prior inoculation of an avirulent variant of Xf

3. Endophyte-based transgene expression of an anti-Xf protein or other compound

Arguments in favor of biocontrol approaches for Xf diseases:

Possibly could be initiated in a producing grapevine at risk to PD
Should not alter quality or agronomic traits of the grapevine
Depending on the approach, possibly easier public acceptance than for trangenes

Arguments against biocontrol approaches for Xf diseases (primarily feasibility):

No relevant precedents demonstrating biological control of other plant pathogenic bacteria
General lack of support for approach (e.g., low ranking in NAS review)
At least some endophytes appear not to overwinter or are severely diminished in cold weather
Some protective endophytes might gain (or regain) virulence

Current research

Don Cooksey – culture independent endophytes
Steven Lindow – search for a systemic colonizer to deliver an agent for interfering with diffusible signal factor
Bruce Kirkpatrick – catalog of grapevine endophytes to be screened for interference with Xf movement or colonization; similar complexes of bacteria were recovered in the spring and in the fall, suggesting authenticity of the isolates identified by 16S rDNA markers; isolates were scored for 15cm movement (not enough to confirm systemic spread) and later for 30cm movement (indicative of systemic spread) and for inhibition of Xf on plates (1 in 10 showed inhibition); none of the isolates (*Cellulomonas* and *Bacillis* primarily) protected plants against subsequently inoculated Xf but did show some symptom reduction; in the field, the endophytes disappeared in the second winter. Results suggest that naturally occurring endophytes may not be very effective in protecting against PD Xf. However, one endophyte, *Pseudomonas viridiflava*, moves well in grapevine, induces no symptoms, and is transformable, i.e., is a candidate for category 3 biological control
Don Hopkins – apparent cross protection of cabernet by prior inoculation of elderberry strain Xf and subsequent inoculation of PD Xf (i.e., a positive result contrary to the lack of demonstrated biological control of other plant pathogenic bacteria as cited above); protection is effective in the greenhouse and field; infection by PD Xf is not prevented but degree of PD Xf colonization is greatly reduced

Consensus

The goal of biological control through inoculation of endophyte(s) has shown sufficient positive results that this approach should not be abandoned
This research area remains in the discovery phase; some promising biocontrol agents should be re-tested
There is a need to investigate the differences between virulent and non-virulent but protective Xf
Test endophyte-bearing plants by placing them in a high disease pressure environments rather than by inoculating them in the greenhouse, which may provide too stringent a test

Inoculate axenic grapevine with candidate protective endophytes in order to reduce the confusion in results that otherwise may arise from the effects of endogenous endophytes

Interference with Xf acquisition, transmission retention and disease expression

State of the art
-presence of bacteria in insects and its ability to transmit is very high
--unknown how to manipulate vector transmission
-basics of acquisition known

Don't know
If vector acquires, will it transmit?
What is most important reservoir for acquisition?

Priorities
-overwinter field retention condition
-way to know potential for early-season brood to infect

Add to RFP knowledge gaps
-what is a way to identify host-specific pathogenicity from non-pathogenic strains

Biodiversity of the pathogen related to disease ex-host expression
-Identify strains pathogenicity, virulence
-understand phenotype to know if strain is a risk

Need cost-effective technology to identify strain

Diagnostics or monitoring tools

Insect Monitoring
-synpopsis on trap info (pamphlet) (color, size, shape, ….)
-lobby manufacturer to change trap design to match recommendations from research

Finite experiments on what do low trap numbers really mean in relation to population levels?
Model shows risk
-dispersal (inter and intra) of GWSS- go through reports and find out where there are gaps
-degree day info done/in process
-use of PCR to detect Xf in GWSS

Xf monitoring
-nucleic acid-based detection (PCR)
-protein-based detection (ELIZA)
-strain-specific diagnostic
-faster/cheaper/more sensitive diagnostic-pathogen & host markers

Biocontrol of sharpshooters

1. Have numbers of GWSS been reduced significantly?

2. Has PD been reduced (more importantly)
    a. directly
    b. indirectly
3. Is there a better design than current design?

Background

The parasitoids attack eggs of GWSS. *Gonatacerus ashmeadi*, which was self-introduced into California, is the most effective GWSS parasitoid.

Are parasitoids responsible for decrease in GWSS density in UCR citrus orchard?
Parasitoids are not economically feasible for augmentative and inundative release.
An average of 20% parasitism consistently each year may effectively add to suppression of GWSS over years.

Is there a way within 3 years to set up an experiment that tests if decrease in GWSS at UCR is due to parasitoids (or elsewhere?)
Try an "insecticidal check" that uses bait sprays (sugar solution with pesticide that is effective against parasitoids but GWSS, deployed in scattered large droplets, say an average of 2-3 cm apart)

Fungi, bacteria, viruses have very minimal impact
Earwigs possibly feed on eggs; Hagler research

Biocontrol in urban citrus
Augmentative USDA release failed

Neoclassical biocontrol
Is there additional benefit for research on foreign parasitoids, say Argentina, rather than from native associations?

Risk unknown for the possible impact of these less specific parasitoids on native leafhoppers, especially if/when they reach states east of California

Recommendation 1

Need to develop a model as to how to make biocontrol work best, what to expect to get out of biocontrol in urban environments. Biocontrol may already be working there as efficiently as it ever will. Need to attract/recruit an appropriate modeler to the task.

Parasitism very high in urban areas. Previously released parasitoids did not establish in urban areas. No foreign parasitoids seem worth bringing in for urban areas.

Can anything be done to make what has already been done work better?
Buckwheat provides nutrition that allows the parasitoids to live longer, lay more eggs and generally be more effective

Recommendation 2
Investigate the use of buckwheat and perhaps other plants to support parasitoid populations in selected ag areas, especially organic production sites where spraying options are limited.

This sort of conservation biocontrol seems promising. Inundative and augmentative are too costly; too costly to rear the parasitoids in light of the costs to rear or collect GWSS eggs.

Biocontrol in plant nurseries won't be effective. Too disrupted and too many pesticides.

To document or test the effectiveness of biocontrol
What experiments ?
What strategy?

Recommendations 3 and 4
Split the state up into north, south and Bakersfield, consider urban and ag
5 year project on parasitoid rates

Activity
Insecticide exclusion

Is 30% benchmark for minimal levels of parasitism for an effect from biocontrol (from literature survey by Hawkins and Connell) enough to justify biocontrol as successful?

A. Economic impact
Nursery growers will still spray. Biocontrol could be another layer.
Ag areas: Conservation biocontrol; people understand the "system" they are working with.
Urban: Political exper.

Research Committee should review previous year project summaries to understand what's already been done.

Recommendation 5
Urban areas excited about conservation (biocontrol); don't exclude them

Is it reasonable to expect biocontrol will reduce PD ?
No, without threshold modeling

How low do you have to drop GWSS densities to have an effect on PD?
Life table analysis: mortality by stage, with notation of parasitoid contribution of GWSS mortality. Data goes to the modeler.

Recommendation 6
Consider proactive approach to anticipate the next vector
What can we do now?
Should SE potential invader be looked at now?
2-3 years ahead of the game?

Don't really know what the value of biocontrol is.
Should focus on value of current bc work.

Solicit ag economist for assessment; Ag Issues Center
Dan Sumner
Karen Klonsky
Karen Jetter