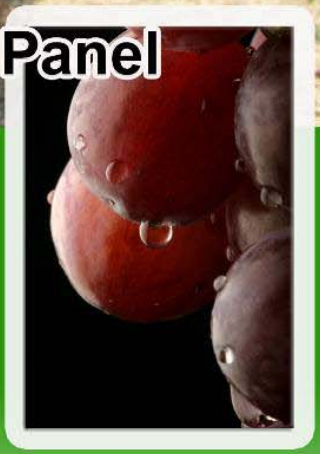
PD/GWSS RESEARCH SCIENTIFIC REVIEW

FINAL REPORT

August 2007

CDFA Research Scientific Advisory Panel



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A. Introduction

For the past seven years, a collaborative effort has been underway between the California wine and grape industry, government funding agencies and the PD/GWSS research community to better understand Pierce's disease and its insect vectors. The expectation is that this effort will lead to the development of a portfolio of innovative and sustainable tools and technologies to control the disease, avoid additional spread, and prevent future infections and outbreaks. This effort is also expected to yield information helpful for protecting other commodities (including citrus, almonds, stone fruits, alfalfa, and ornamentals) from serious plant diseases caused by closely related strains of the Pierce's disease bacterium. During the earlier years of this effort, the CDFA PD/GWSS Board commissioned the National Academy of Sciences (NAS) to conduct an independent scientific review to provide a historical background on this disease and its insect vectors, and to assess the research completed at that time. As an outcome of this work, the NAS panel set forth a list of Pierce's disease research priorities (research category areas) in their final report (published in 2004) to be targeted for subsequent research and funding activities.

Now, three years after the publication of the NAS report, the CDFA PD/GWSS Board recognizes that extensive research efforts have taken place to better understand and thereby control PD and its insect vectors. This is evident by the number of peer-reviewed papers relating to Pierce's disease published in the last four years (Fig. 1).

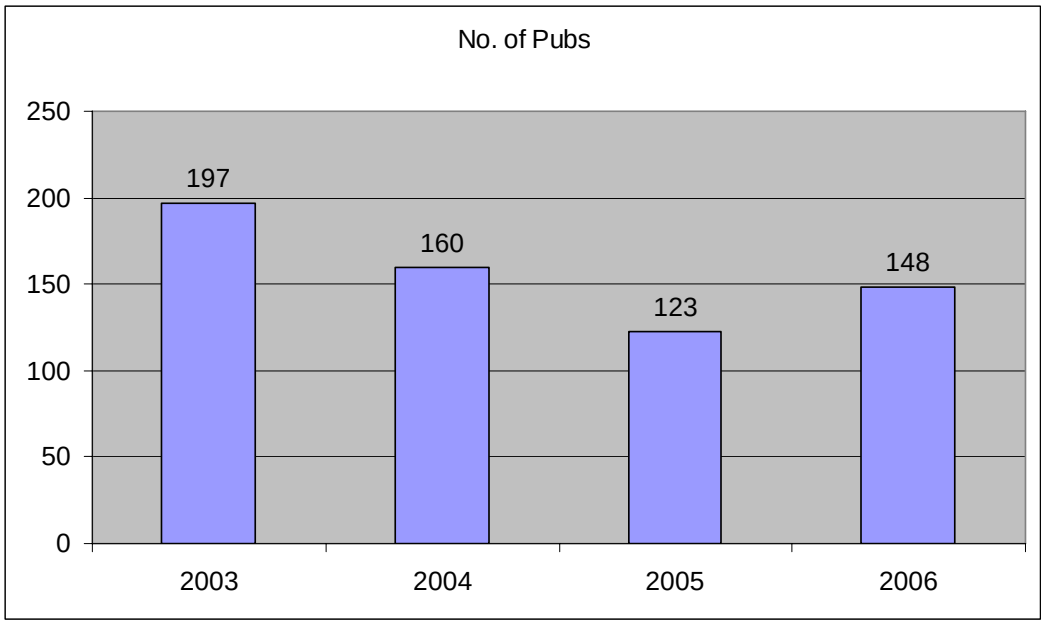


Figure 1. Numbers of peer reviewed publications relating to PD/GWSS.

The industry continues to provide support for these scientific efforts through the California Statewide Winegrape Assessment Program, which is administered by the California Department of Food and Agriculture. Between 2001 and 2005, a little over

$11.3 million was committed to supporting the CDFA PD/GWSS Competitive Grant Program. All of this investment was provided directly by the California winegrape industry through the assessment program. This industry levy, however, is scheduled to "Sunset" in March of 2011. Given that there are only three years remaining in the current funding cycle, the industry is keen to gain a clear picture of what has been accomplished and what tools they may have to work with in the future to control the disease.

The California winegrape industry is not alone in their desire to gain a better understanding of the potential benefits derived from the financial investments made in PD/GWSS research activities. Last year, the US Senate requested a report about the PD/GWSS USDA funds administered by the University of California Pierce's Disease Research Grant Program (UCPD) to determine if, and when, the research program objectives will be achieved.

Justification of PD/GWSS research investments with evidence of potential commercially viable outcomes has become increasingly important. PD/GWSS funding programs continue to face increasing competition for resources. During 2006, the USDA PD/GWSS research funds administered by the UCPD Research Grant Program were discontinued and then eliminated through the federal appropriations process. The research community may be running out of time and money to find a solution to this devastating disease.

Given the persistent nature of Pierce's disease and its insect vector and the scientific commitment and financial investment currently devoted to control of this disease, there is constant pressure to: 1) maintain a clear and up-to-date understanding of research progress; 2) continually update priorities; and 3) strategically refocus the research program on an on-going basis to accelerate the most promising strategies to achieve practical field application. As a consequence, the CDFA PD/GWSS Board requested a thorough review, assessment and interpretation of all research activities previously and currently funded by the CDFA PD/GWSS Research Program since the last independent scientific review (the 2004 NAS report). In particular, the Board requested that research progress be examined within the context of the remaining three-year funding time limit (grape levy) and the recommendations put forward by the National Academy of Sciences Report. The Board is seeking a better understanding of research progress made, and anticipated timelines toward achieving sustainable solutions and prevention strategies that address Pierce's disease and associated insect vector infestations in commercial vineyards. The ultimate intent of the program review is to create a knowledge foundation that can be used in the development of an updated strategic plan for the CDFA PD/GWSS Research Program. The plan will outline strategies for optimizing research activities to accelerate the development of viable solutions for control, prevention and elimination of Pierce's disease.

The CDFA PD/GWSS Board contracted with Dr. Nancy Irelan to provide leadership in conducting the PD/GWSS Research Program review and development of a strategic research plan. Based on Dr. Irelan's initial recommendations, the Board established a Research Scientific Advisory Panel (RSAP) to:

- Greatly accelerate the research program review, gap analysis and strategic planning;

- Assess research program progress against stated goals and recommendations made by prior program evaluations;

- Generally enhance the objectivity and transparency of the research assessment and funding recommendation process; and

- Generally augment the scientific rigor and broaden the technical perspective of the program.

Individuals were selected to serve on the RSAP based on their research/innovation track record and international reputation within their field of expertise. To avoid any potential conflicts of interest, scientists that had been funded previously by the CDFA PD/GWSS Board were not eligible to serve on the RSAP. In combination, these individuals represent the breadth of technology and scientific discipline that encompass the current CDFA PD/GWSS Research Program. Descriptions of each RSAP member including a photo, core expertise and brief bio, are provided in Appendix A.

While the CDFA PD/GWSS Board is primarily concerned with the progress and productivity of research generated through the CDFA PD/GWSS Research Program, the Board recognized the need to obtain a full understanding of all relevant PD/GWSS research activities, and to collaborate when possible across all avenues of funded, on-going PD/GWSS research and innovation. The Texas and California APHIS PD/GWSS Research Programs, the ARS PD/GWSS Research Program, and the University of California Pierce's Disease Research Grant Program share this perspective and consequently participated in the review process and assisted in solicitation of research project information from their respective PD/GWSS scientists. Collection of this project information was critical to successfully complete the research program review and assessment process.

Given the extensive amount of data and materials to be reviewed by the RSAP, it was clear that an efficient and unified process was required to generate, consolidate and organize the review materials. To this end, PIPRA —the Public Intellectual Property Resource for Agriculture [www.pipra.org], was recruited to create a web-accessible electronic database. PIPRA populated this database with project numbers, titles, funding information and the names of the Principal Investigator(s) and collaborators associated with each research project. Principal Investigators (PI) were then asked to provide succinct information for each of their projects outlining the project goal, key objectives, overall experimental game plan and anticipated deliverables relating to control/prevention of PD. These project summaries were submitted by project PI's directly into the database via a web-based interface. This summary information was combined with a list of abstracts for each peer-reviewed publication that the respective researcher had produced post-NAS (National Academy of Sciences) report. In addition,

most summaries were linked to a more detailed project description provided in the proceedings of the annual PD Research Symposium.

At the time of the RSAP review in April 2007, the PIPRA database contained information on 225 projects. Of these, the RSAP focused on 146 projects that received funding after the publication of the NAS report. These projects were divided among the RSAP according to panel member expertise. Most projects were reviewed by at least two panel members. When evaluating projects, panel members were asked to assess the following:

1. Where does each project fall within the research pipeline (basic knowledge generation (1), application development (2), feasibility study (3), scale-up (4), and commercialization (5))?

2. What are the expected outcomes/deliverables as they relate to control/prevention of Pierce's disease and GWSS vectors?

3. What are the expected timelines for these outcomes/deliverables in terms of providing a sustainable solution to PD/GWSS (short: less than two years, medium: two to five years, long: more than five years)?

4. Does the work address the priorities identified by the NAS report? If so, indicate priority number(s) as defined in the NAS report.

5. Is there concrete evidence of progress being made on the project (e.g. journal articles, databases, patent applications, quantifiable data in a yearly report, etc.)? Rank this progress on a scale of 0-5, with 5 being excellent progress and 0 being no progress.

Each panel member recorded these assessments, along with freeform comments, in an Excel spreadsheet. Spreadsheets from individual panel members were then combined to generate summary statistics pertaining to numbers of projects addressing each NAS recommendation, stage in the research pipeline, and timelines for providing a viable control strategy (see Section B).

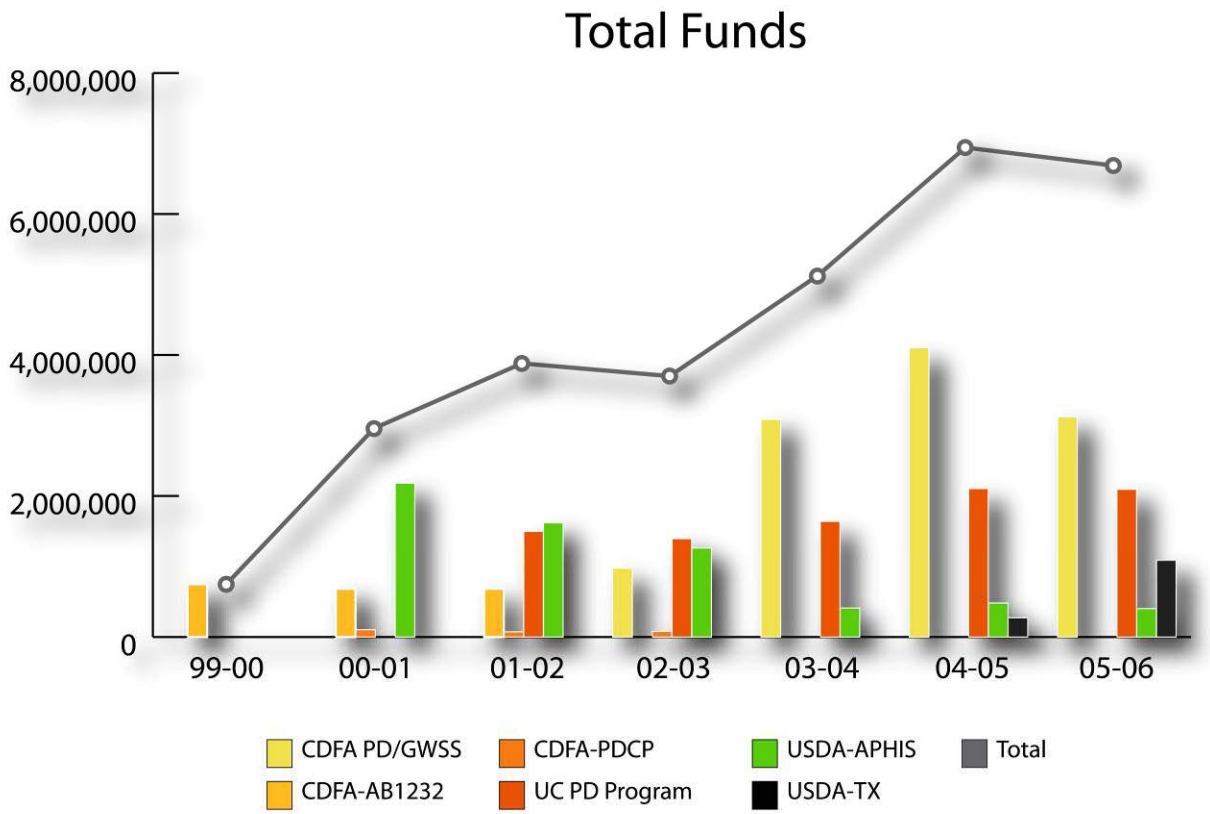
Although useful for generating an overview of where PD/GWSS research dollars have been targeted, the primary purpose of these spreadsheets was to identify projects that were making significant progress toward sustainable PD/GWSS control strategies. Each panel member was asked to assemble a list of research tracks (i.e. specific projects or combinations of research projects) that they felt held the most promise for providing a control strategy in the short-medium time frame (less than five years), and a second list of projects that might provide a solution in five to 10 years. Panel members were also asked to identify gaps in our current knowledge base of PD/GWSS that needed to be addressed to enable new control strategies.

Each panel member completed these tasks prior to a face-to-face meeting of the RSAP held in Bakersfield, California on May 10 and 11, 2007. At this meeting, panel members discussed their individual findings, explaining why specific research tracks were found particularly promising, or not promising, and a panel consensus was reached as to which approaches held the most promise, which were not progressing

well, and what knowledge gaps remained. The following sections of this report describe these findings and provide details on why the RSAP reached these conclusions.

B. Overview of PD/GWSS Research Funding

PIPRA obtained funding data for PD/GWSS research projects from all of the participating PD/GWSS Research Funding Agencies, except for the USDA/ARS, which for technical reasons was unable to provide these data. Not counting the USDA/ARS, over $30 million have been awarded for PD/GWSS research since 1999 (Fig. 2). The CDFA PD/GWSS Board alone awarded $11.3 million during this period, and the UCPD Research Grant Program awarded $8.7 million, which combined, accounted for two-thirds of the total funding.



Funding Source	1999-2000	2000-2001	2001-2002	2002-2003	2003-2004	2004-2005	2005-2006	Total by Source
CDFA PD/GWSS Board			$19,000	$974,389	$3,086,870	$4,102,045	$3,120,661	$11,302,965
CDFA-AB1232		$680,901	$680,836					$2,109,136
CDFA-PDCP	$747,399	$102,807	$73,296	$79,532				$255,635
UC PD Program			$1,498,819	$1,394,721	$1,640,209	$2,105,906	$2,095,127	$8,734,782
USDA-APHIS		$2,182,843	$1,618,811	$1,261,234	$410,687	$480,705	$402,336	$6,356,616
USDA-TX						$272,727	$1,087,531	$1,360,258
Total by Year	$747,399	$2,966,551	$3,890,762	$3,709,876	$5,137,766	$6,961,383	$6,705,655	$30,119,392

Figure 2. Total Funding for PD/GWSS research projects funded by agencies other than USDA/ARS.

The CDFA PD/GWSS Board has subdivided PD/GWSS research into seven priority areas (Host resistance via traditional breeding and transgenics, Biological control of *Xylella fastidiosa* (*Xf*), Understanding transmission of the disease, Biological control of GWSS, Monitoring GWSS population densities and dispersal, Monitoring *X. fastidiosa*, and Other). When looking at the total funding by all agencies (other than USDA/ARS), the two largest areas of research are host resistance and understanding transmission of the disease (Fig. 3A). CDFA PD/GWSS Board funding shows this same pattern, but with a higher proportion going to host resistance (43%; Fig. 3B).

The RSAP was asked to evaluate how currently funded research projects aligned with the recommendations of the NAS report. The NAS report listed 32 explicit research areas that should be supported. Based on the project summaries, the RSAP assigned each project to one or more of these research areas, or if a project did not address any of the NAS-recommended areas, it was categorized as either “not a recommended area” or “epidemiology”. The latter category was not listed as an independent research area by the NAS report, although it is clear that epidemiological information is needed to evaluate the progression of PD, the effectiveness of control strategies, and to determine the sources of GWSS and *Xf.* The RSAP felt that the failure to list epidemiological research as an explicit research area in need of funding was an oversight of the NAS report. Figure 4A shows the number of projects assigned to each NAS-defined research area, plus “epidemiology” and “not recommended”. For comparative purposes, Figure 4B shows the research funding allocation against each NAS research category.

The two research areas with the largest number of projects are promotion of GWSS natural enemies (NAS recommendation 3.11) and pathogenicity of *Xf* (NAS recommendation 4.1), with 30 projects each. In contrast, several NAS-recommended areas have received very little attention, such as use of covercrops to reduce sharpshooters (recommendation 3.5), manipulating alternative hosts to control *Xf* (recommendation 4.6), and the effect of *Xf* on GWSS fecundity (recommendation 5.3). The RSAP discussed whether these areas should still be considered high priority and concluded that none of these three areas held great promise for leading to effective control strategies, thus should be removed from the high priority list.

The most surprising lack of research effort, however, was the absence of projects focused on NAS-recommended areas related to economic studies of PD/GWSS and PD/GWSS control measures (Recommendations 2.3, 2.4, 3.10, 3.13, and 3.14). This gap will be discussed in more detail in Section E.

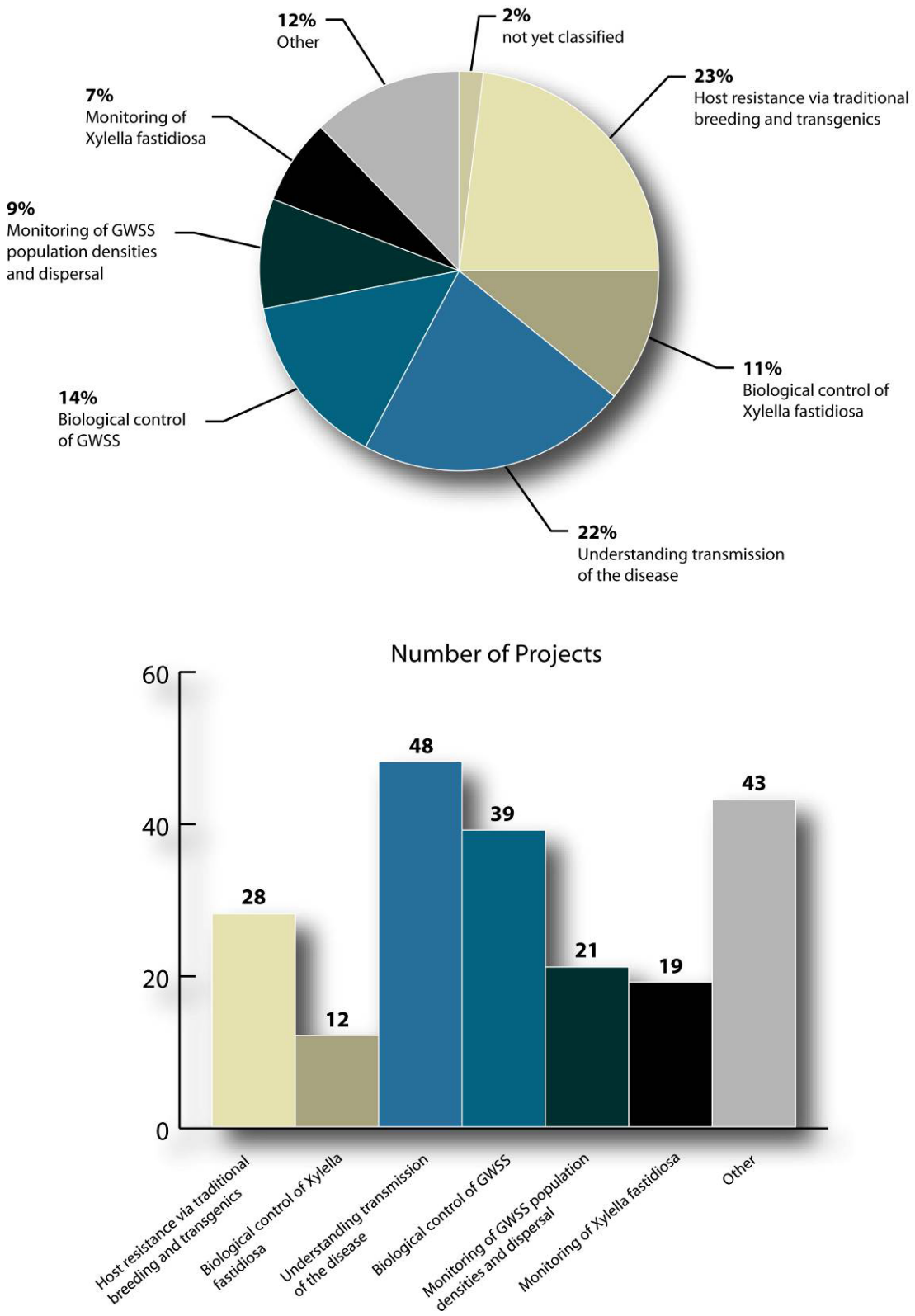


Figure 3A. Total PD/GWSS research funding by priority areas. The pie chart indicates percentage of total dollars targeted to each area. The bar chart indicates number of projects targeted to each area.

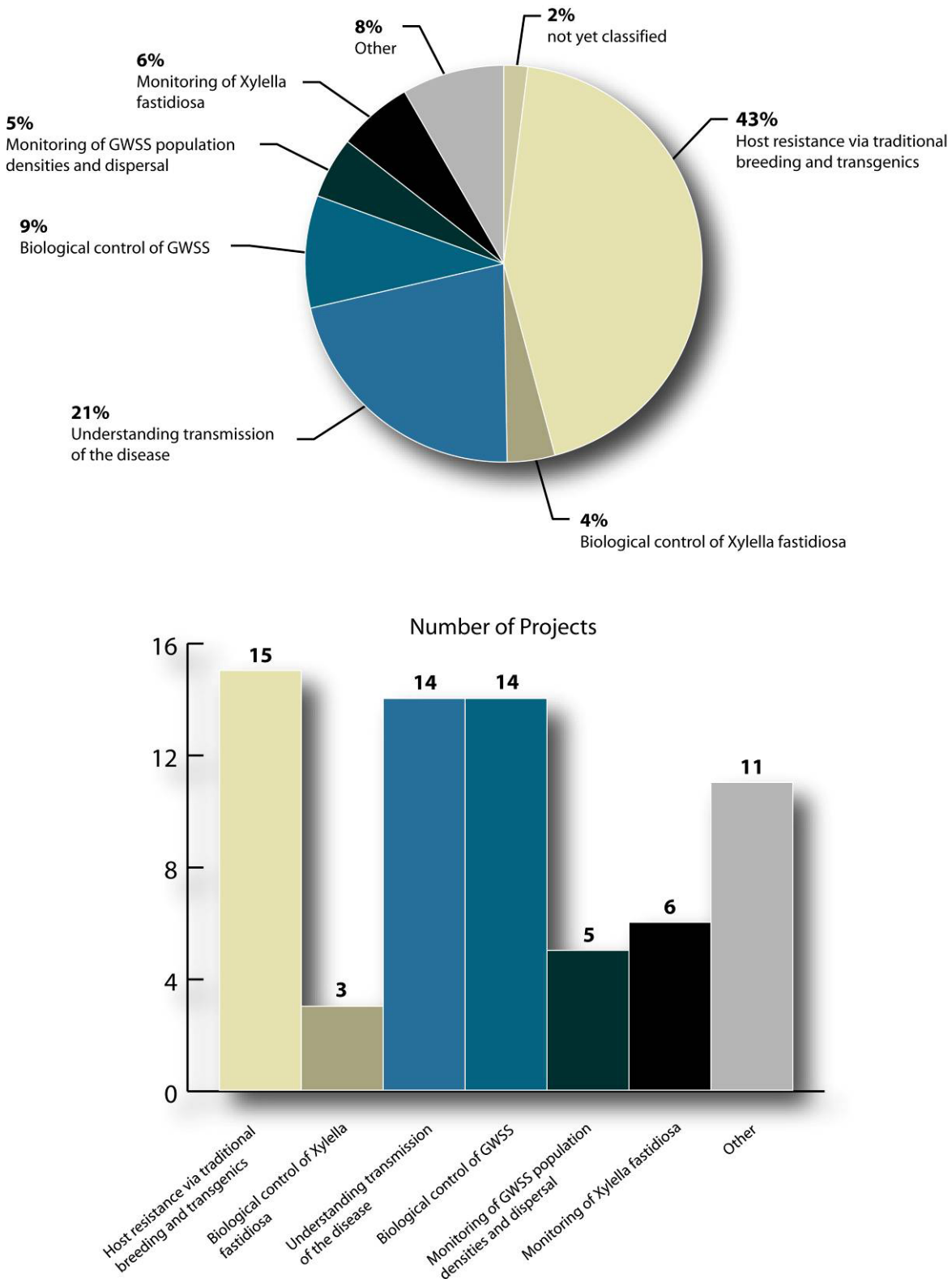


Figure 3B. PD/GWSS Board funding by priority area. The pie chart indicates percentage of total dollars targeted to each area. The bar chart indicates number of projects targeted to each area.

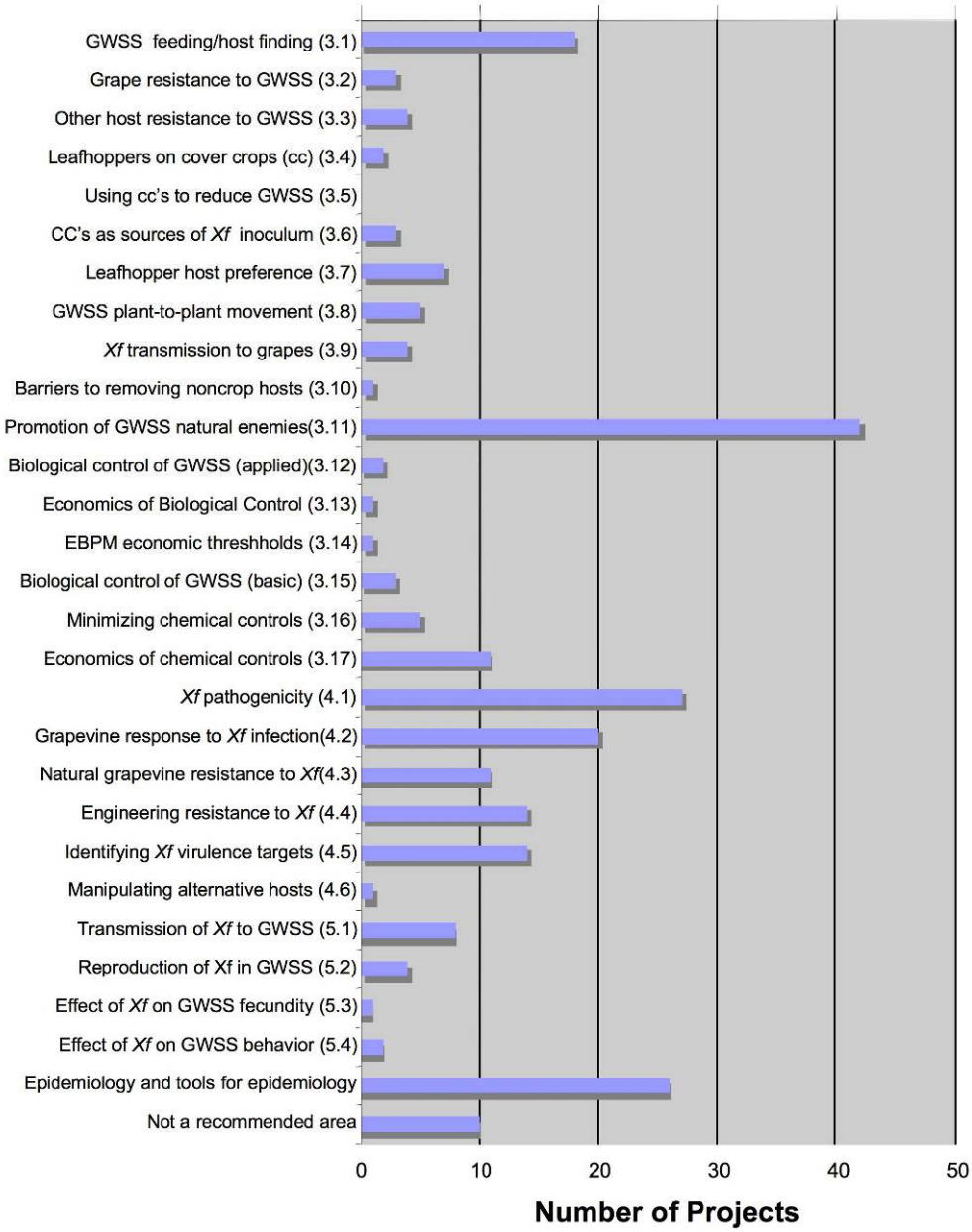


Figure 4A. Distribution of PD/GWSS research projects by NAS-recommended research areas.

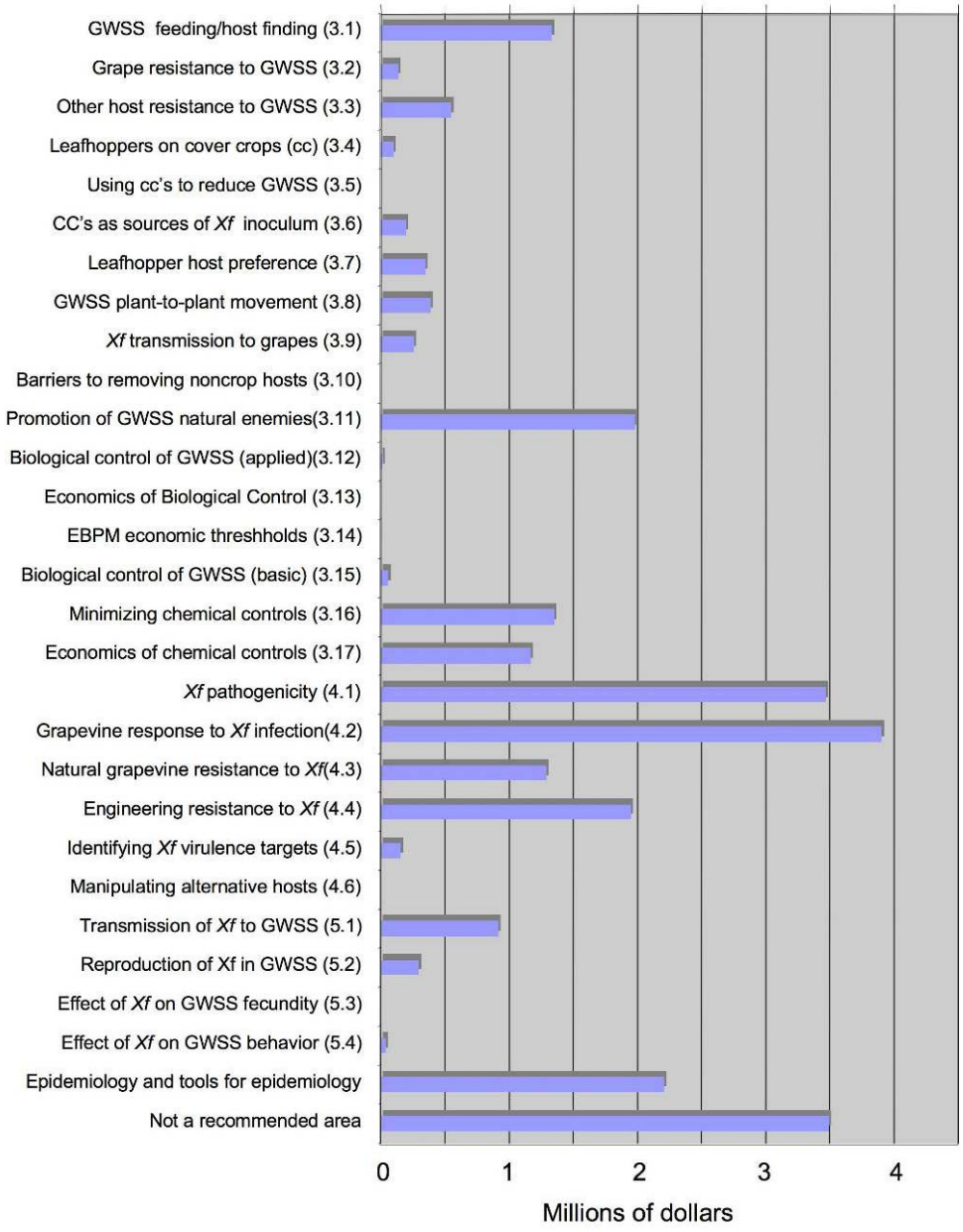


Figure 4B. Distribution of PD/GWSS research funding by NAS-recommended research areas.

The RSAP also categorized each research project in terms of how long it would take to develop a commercially viable PD/GWSS control strategy following that line of research. Figure 5 shows the breakdown of projects in each category. Not surprisingly, the great majority of projects were assigned to the long-term category, meaning that the RSAP felt it would be at least five years before such research would contribute to a PD/GWSS control strategy. There were a significant number, however, that fell into the medium (two to five years) and short-term (one to two years) categories.

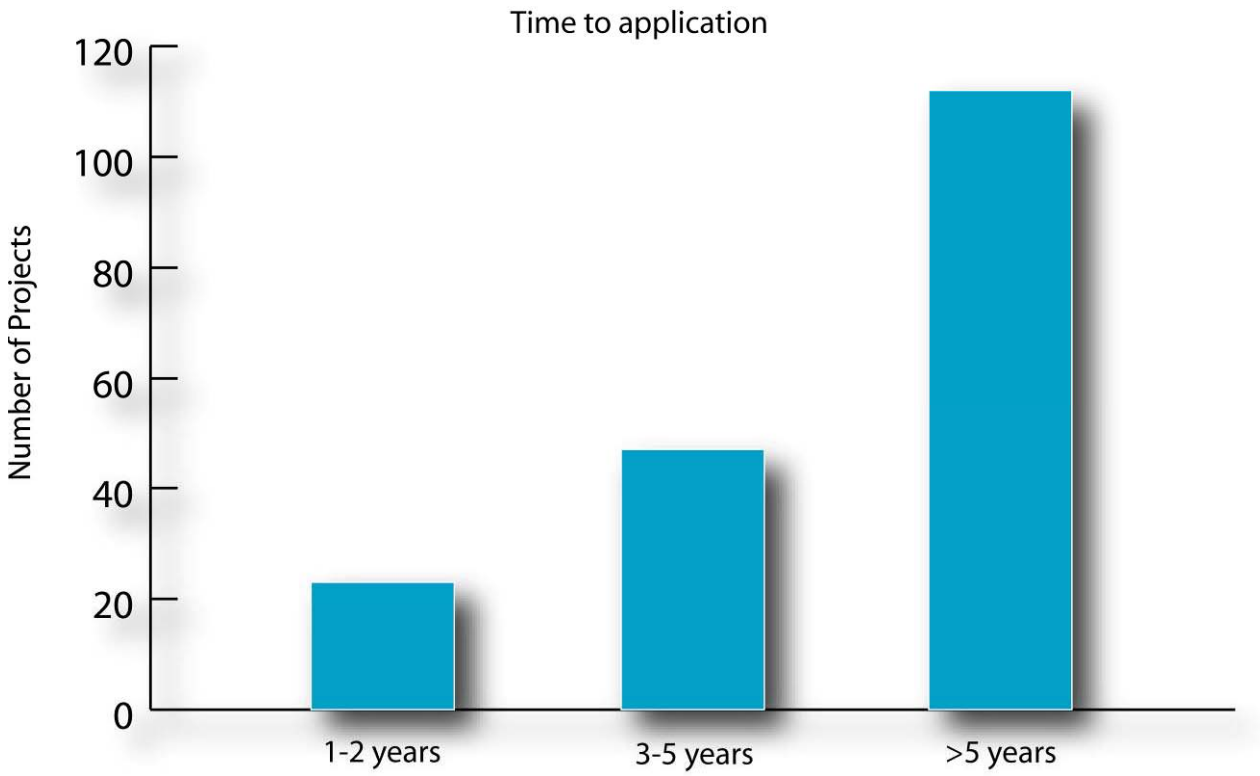


Figure 5. Timelines of funded research projects.

The RSAP recognizes that an ideal distribution would include more projects with short to medium term solutions; however, much of the research that fell into the long-term category is required to provide fundamental knowledge about Pierce's disease that is a prerequisite to more directed and shorter term projects. As our basic understanding of the biology underlying Pierce's disease improves, less funding will be needed for such enabling research, allowing greater funding of well-informed applied research.

C. Significant Progress Since Publication of the NAS Report

One of the questions the RSAP was asked to address was what progress has been made in PD/GWSS research subsequent to the NAS report that is relevant to the development of control methods. The RSAP felt that progress has been significant, especially in terms of understanding the pathogenicity of *Xf*, which in turn leads to better designed strategies for limiting *Xf* infection. Below we highlight particularly noteworthy advances occurring in the last four years. Several of these areas will be discussed in

greater detail in subsequent sections of this report. Omission of certain research areas and projects from this discussion does not necessarily mean that these projects are not making progress, only that the RSAP did not find the information supplied to be indicative of relevant progress in the context of leading to a viable control strategy for PD/GWSS.

Identification of *X. fastidiosa* factors that mediate virulence on grape (*Diffusible signal factor,* polygalacturonase, *tolC, type I and type IV pili, adhesins*). At the time of the NAS report, two grape strains of *Xf* had recently been sequenced. By comparing this sequence to the sequences of better-characterized bacterial pathogens, it was possible to quickly identify a set of genes likely to contribute to virulence. In the last four years, several labs have participated in the effort to knock these genes out and/or overexpress them, then testing the mutant strains for virulence on grape. This work has led to several important insights that can potentially be applied to new PD control strategies.

The most exciting new insights in this category come from Dr. Steve Lindow's lab at UC Berkeley on *Xf* Diffusible signal factor (Dsf), which is a fatty acid employed by *Xf* in cell:cell communication. Significantly, Lindow's group has shown that overproduction of Dsf by either *Xf*, or by a co-inoculated bacterial endophyte, blocks the spread of *Xf* through the grape xylem, thus preventing development of PD. Furthermore, Dr. Lindow's group identified a gene in *Xf*, *rpfF*, that confers the ability to produce Dsf to both bacterial endophytes and plants. They have shown that spraying grape plants with Dsf-overproducing bacteria (e.g. *Erwinia herbicola*) confers protection against PD, as does transgenic expression of *rpfF* in grape plants. These data suggest several exciting strategies for commercial development, which are described in more detail in Section D.

Another new insight is that the *Xf* gene that encodes polygalacturonase (PG) is required by *Xf* to cause PD. PG is an enzyme that breaks down plant pectins. This work, led by John Labavitch at UC Davis, also showed that injection of PG into grape xylem caused the degradation of xylem pit membranes, which would facilitate movement of *Xf* through the xylem. Combined with work in other labs on polygalacturonase inhibiting proteins (PGIP's), it appears feasible to confer resistance to *Xf* infection simply by engineering grape rootstocks to express a PGIP.

Several additional *Xf* genes have also been demonstrated to play important roles in virulence on grape plants. Specifically, *tolC,* and genes encoding type IV pilins and various adhesions are all required for *Xf* virulence on grapes. *tolC* encodes a protein involved in export of various proteins and toxins out of *Xf.* Type IV pilins are required for movement of *Xf* "upstream" within the xylem, while adhesins are required for attachment of *Xf* to xylem cell walls and/or to other bacteria, and thus are critical for formation of biofilms within xylem and within insect vectors. While various transgenic and non-transgenic strategies can readily be envisioned for interfering with the function of these proteins (described in more detail below), and thus conferring resistance to *Xf* infection, the research has not yet advanced to the point of demonstrating such a control method.

Development of strain-specific genetic markers. At the time of the NAS report, complete genome sequences for two *Xf* strains were available, one from citrus and one from grape. Subsequent to the NAS report, two additional *Xf* strains have been sequenced by the DOE Joint Genome Institute, one from almond (the "Dixon" strain) and one from oleander (the "Ann-1" strain). Two additional strains, one from almond and another from citrus, are currently being sequenced by DOE. These additional genome sequences are enabling identification of sequence differences between strains and development and testing of strain specific genetic markers. Such genetic markers will be useful for epidemiological studies aimed at identifying the source(s) of specific PD outbreaks. These genome comparisons also enable studies aimed at identifying determinants of host specificity (e.g. genes or gene variants that enable a strain to infect citrus, but not grapes and vice versa). With recent advances in sequencing technology and a dramatic decrease in sequencing costs, it is expected that additional grape strains will also be sequenced, further empowering comparative genomics-based approaches.

Potential for using transgenic rootstocks. The grape industry has expressed interest in developing rootstocks that confer resistance to PD in the scion. The primary advantage of this would be the ability to continue using current grape varieties, thus avoiding both long-term breeding programs and/or development of transgenic grape scions. A significant advance in the last four years was a "proof-of-principle" experiment in which it was shown that PGIP expressed in the rootstock is transported to the leaves. In a similar vein, transgenic rootstocks that produce Dsf will soon be tested for their ability to inhibit PD in conventional scions. Although intuitively it would seem that wine produced from such chimeric grape plants would be more acceptable to consumers than wine produced from transgenic grapes, it is not clear to the RSAP whether regulatory agencies, the European Union, and anti-GMO groups would consider such chimeric plants different from fully transgenic plants. This is a question that merits further investigation.

Insecticidal-based control of GWSS (area-wide control programs). The USDA-APHIS and CDFA Pierce's Disease Control Program have developed a comprehensive program for monitoring GWSS populations in key areas of California and have worked with citrus and grape growers to apply insecticides at strategic times and places and at a large spatial scale. Currently spread of GWSS in the Central Valley appears to be largely contained, strongly suggesting that the area-wide program is working. However, sharpshooter populations are known to be cyclical, thus it is difficult to predict how much greater GWSS populations would be in the absence of insecticide treatments. None-the-less, the RSAP was impressed with the sophistication of the area-wide control program in terms of monitoring GWSS populations, recruiting farmers to participate, and minimizing pesticide applications.

Epidemiology of GWSS. There has been a large amount of effort expended to obtain weekly trapping data for GWSS in areas of Kern, Tulare, and Ventura Counties. These data have been loaded into a geographic information system (GIS) database maintained by the CDFA Pierce's Disease Control Program in Sacramento. For certain areas, crop layers have also been added to this GIS database. This is an extremely valuable resource that should be built upon by the addition of data on PD incidence and the presence of specific *Xf* strains associated with different GWSS host plants adjacent to crops. Project 103: "Epidemiological Analyses of GWSS and PD Date" (PI Thomas Perring) proposes to add data on PD to this GIS database. The RSAP noted that this is an important and highly desirable objective; however, it was not clear from the information submitted to the PIPRA database what progress has been made in this effort.

Taxonomy and general biology of parasitoids. Classical biological control of GWSS using egg parasitoids holds significant promise for reducing overall populations of the vector and thereby reducing disease transmission. This is an on-going effort involving exploration for candidate parasitoids, initial screening for suitability, mass rearing, release and evaluation. A clear understanding of the taxonomy and general biology of egg parasitoids attacking GWSS is critical for making progress and considerable time and effort has been put toward this goal. Molecular techniques developed by Jesse de León and others, coupled with better keys based on adult morphology, now provide relatively quick and accurate methods for distinguishing cryptic species. These enabling technologies are being used to reduce contamination problems in rearing facilities and for evaluating the success of field releases. Sound techniques for mass rearing of parasitoids have been developed, although the process is labor intensive and requires a reliable source of GWSS eggs, which has been problematic.

Breeding for resistance/mapping resistance genes. In annual crop species, the most cost effective and environmentally safe method for preventing disease is breeding for resistance. Such traditional breeding can be dramatically accelerated if the genes controlling resistance have been linked with DNA-based molecular markers that can be scored in a high throughput fashion. Such marker-assisted breeding appears to not be well developed in grapes, presumably because wine grapes are mostly clonally propagated. A noteworthy exception is the work of Dr. Andy Walker at UC Davis, who has mapped a PD Resistance trait, designated *PdR1*, derived from *V. arizonica b43-17* (project 116: "Map-based Identification and Positional Cloning of *Xf* Resistance Genes from Known Sources of PD Resistance in Grape"). This work has been published in *Theoretical and Applied Genetics* and describes several PCR-based markers that can be used to introgress this resistance trait into *V. vinifera* germplasm. Ultimately the goal of this work is to clone *PdR1*, which would allow direct transformation of the *PdR1* gene into premium wine varieties, thus bypassing many years of backcrossing. Resistance to PD is common among wild grape species in areas of high PD pressure, thus there are likely multiple sources of useful resistance genes that could be exploited if breeding programs were expanded.

Grape genomics (EST collection, Affymetrix gene chip, genome sequencing). The International Grape Genome Program (IGGP) is progressing. The current release of the TIGR Plant Transcript Assemblies for *Vitis vinifera* contains 312,911 expressed sequence tags (ESTs), which have been assembled into a total of 62,505 unique gene sequences. These should represent the great majority of expressed genes in grape plants. An Affymetrix *V. vinifera* gene chip was released in June, 2004 that contains probes for 14,000 *V. vinifera* genes. Numerous Bacterial Artificial Chromosome (BAC) libraries from various grape varieties are available. A grape genome sequencing project is underway in Europe and is employing a combination of whole genome shotgun sequencing and BAC end sequencing. All of these sequence resources will greatly facilitate association of specific traits in grapes with specific genes. For example, EST collections can be searched for DNA sequence polymorphisms between grape varieties, which then can be quickly mapped relative to agronomically important traits. This will facilitate both traditional breeding approaches for trait development, as well as transgenic approaches. Although the PD/GWSS Board funded some of the early work on grape EST sequencing in Dr. Doug Cook's laboratory at UC Davis, the majority of grape genomics tools are currently being developed by labs in Europe.

D. Areas to Target Going Forward--Short-term (two to five years)

Very few of the research areas funded by the PD/GWSS Board will lead to commercially viable control strategies within the next five years. A time frame of less than five years would preclude any strategy involving transgenics and/or traditional breeding due to the length of time required to obtain regulatory approval in the case of transgenics, and the time it takes to develop, test and release new varieties in the case of traditional breeding. However, there are extensions of current control strategies that could be applied in the short-term that would likely improve effectiveness.

Expansion and enhancement of the GIS database. The RSAP recommends that the GIS database be expanded and enhanced to enable better tracking of *Xf* strain distribution and spread. This would allow both researchers and disease managers to correlate *Xf* presence, GWSS populations and PD, and thus gain a clearer understanding of where PD outbreaks are originating. Such information would provide insight into whether it is necessary to control GWSS in specific areas, whether specific non-crop *Xf* hosts should be managed, and the likely areas for future PD outbreaks. Additionally, data on actual grower loss to PD (e.g. vine replacement) should be collected and added to the GIS database. Such data would be useful for tracking the spread of PD, and for justifying further investment in the control of this disease. As mentioned above, project 103 proposes to add PD data to the GIS database. Perhaps additional projects aimed at adding *Xf* epidemiology data could be targeted.

PD resistance in existing commercial grape varieties. A second area that merits more attention in the short-term is collection and dissemination of information on PD resistance in existing commercial varieties of grapes. There appears to be significant anecdotal information about which commercial grape varieties are most susceptible to PD, but it was unclear to the RSAP whether any one has performed a

carefully controlled study of commercial grape varieties and disseminated the results. The RSAP envisions greenhouse studies employing both GWSS mediated inoculations in one set of experiments and mechanical inoculations in another set, to distinguish between resistance derived from reduced attractiveness to the vector versus reduced susceptibility to colonization by the bacterium. Data on both PD symptoms and *Xf* growth should be obtained to distinguish also between tolerance and resistance, as tolerant varieties could become problematic reservoirs of the pathogen. If some commercial grape varieties are truly more resistant than others, then growers should be encouraged to replace susceptible varieties with resistant varieties. Potentially this could be subsidized by APHIS with the expectation that costs will be recovered later due to reduced pesticide use, which is currently subsidized by APHIS.

Areas to Target Going Forward--Long-term (five to 10 years)

Diffusible signal factor (Dsf). As mentioned above, the RSAP was quite enthusiastic about the potential applications of Diffusible signal factor (Dsf) to control PD. Priority should be given to field-testing the strategies that appear to work in the greenhouse. In particular, it will be important to determine whether aerial application of Dsf-overproducing phytobacteria can confer protection in a field situation, without negative impacts on plant growth and development. A potential hurdle to such tests may be obtaining permits to release recombinant microbes. Are there naturally occurring bacterial strains that overproduce Dsf? If not, can one select a non-recombinant mutant strain that overproduces Dsf? This might be a rapid way around the use of recombinant organisms. If effective, such an approach could conceivably be developed into a commercial application in less than five years. Another approach would be to synthesize chemical analogs to Dsf that could be applied externally to disrupt the disease cycle. Longer term, priority should be given to testing Dsf-producing rootstocks for their ability to confer PD resistance to non-transgenic scions.

Transgenic expression of Polygalacturonase Inhibiting Proteins (PGIP). Work from several different labs makes the RSAP optimistic that transgenic expression of PGIP could prove to be a very effective strategy for preventing PD. Because the *Xf* polygalacturonase gene has been cloned, and recombinant PG protein has been expressed, it is now possible to screen many different PGIPs for their ability to inhibit the *Xf* PG enzyme. Although it has already been shown that pear PGIP is effective, there likely are PGIPs from other sources that are even more effective. Additional work remains to be done on optimizing delivery of PGIP to the xylem and on maximizing expression of PGIP in the cells surrounding the xylem. Greenhouse tests indicate that PGIP produced in the rootstock is translocated to the scion, but it remains to be shown that the levels of PGIP translocated are sufficient to confer protection against PD. Once this is established in the greenhouse, permits will need to be obtained to commence field trials.

As an alternative to transgenic expression of PGIP, it may be worth investigating the potential for small molecules to inhibit *Xf* PG. Would it be possible to develop an environmentally safe chemical that would effectively inhibit *Xf* PG activity and not grape PG's? Given that crystal structures are available for both bacterial PG's and plant

PGIP's, there seems to be potential for rational design of such a compound. Ideally this compound would be taken up through roots, thus could be added to irrigation water. The availability of recombinant *Xf* PG protein should make it feasible to develop a high throughput screen for testing readily available chemical libraries.

Targeting other *Xf* proteins required for virulence. As described above, several *Xf* proteins have been identified that are required by *Xf* to cause PD. Most of these proteins are secreted or are located on the *Xf* cell surface. This makes them attractive targets for inhibition by either small molecules, or by transgenically produced proteins. For example, recent work has shown that Type IV pili are used by *Xf* for "twitching locomotion", which enables *Xf* to crawl upstream in the xylem and thus spread throughout a plant. For a captivating video clip of this locomotion, see the website maintained by Drs. Harvey Hoch and Thomas Burr at Cornell University (http://www.nysaes.cornell.edu/pp/faculty/hoch/movies/), which was produced as part of project 184: "Evaluating the Roles of Pili in Twitching and Long Distance Movement of *Xylella fastidiosa* in Grape Xylem and in the Colonization of the Sharpshooter Foregut," funded by the UCPD program. It should be possible to select an antibody, small peptide, or small molecule that will bind to these type IV pili, thus preventing them from attaching to surfaces and disrupting movement of *Xf* cells in the xylem. The advantage of developing an antibody, or peptide is that it could be expressed inside grape plants, thus bypassing the need to produce and apply the compound. The disadvantage, of course, is the regulatory and public perception hurdles associated with transgenic crops that would have to be overcome. As with type IV pili, it should be possible to develop small molecule and/or protein-based inhibitors of TolC or *Xf* adhesin proteins (FimA, FimF, XadA, or HxfB), all of which contribute significantly to *Xf* virulence in grape.

Parasitoids. The use of parasitoids to reduce population densities of GWSS continues to show promise, especially in settings where synthetic insecticidal sprays cannot be used (e.g. organic farms, urban areas, or other non-crop habitat). A major limitation of this approach is the labor-intensive methods required to produce parasitoids. Development of better methods of parasitoid rearing is needed. Perhaps the largest obstacle is a reliable, efficient means of mass-producing GWSS eggs or an alternative suitable host for large-scale production of parasitoids.

Marker Assisted Selection-based breeding for resistance. As described above, resistance to PD is common in wild grape species. Clearly there are genes that already exist in grape that will confer PD resistance. Dr. Walker has already demonstrated that it is feasible to map these genes, identify DNA-based molecular markers linked to these genes, and then use these markers to accelerate the introgression of resistance into a *V. vinifera* background. Although the generation time of grape makes such breeding programs long-term endeavors, this is a sure way to develop non-transgenic PD resistant grape varieties and deserves more attention than it is currently getting. The RSAP recommends recruitment of additional breeders so that genes in addition to *PdR1* can be mapped, tagged with molecular markers, and the process of introgression into multiple commercial backgrounds initiated.

E. Gaps Identified

Many of the gaps in our current understanding of PD/GWSS have been pointed out in the previous sections. However, one important area that has not yet been addressed is economic analyses. A major recommendation of the NAS report was to assemble more robust economic data on the impact of PD/GWSS on agriculture, both in terms of real and potential losses due to PD and the costs of current and prospective control measures. Relative to current control measures, who is footing the bill for these, who is benefiting from these, and do the economic benefits outweigh the costs? From what the RSAP was able to discern, this recommendation has not been acted upon. Such economic analyses are needed to assess the potential payoff to continued funding of PD/GWSS research programs and of area-wide PD/GWSS control programs, and to evaluate the commercial viability of proposed control measures. The appropriate approach for such ex ante research evaluation is to develop an industry simulation model and consider the payoff to consumers, producers, taxpayers and the environment from alternative research scenarios. The guidelines for such a study are provided in *Science Under Scarcity*, Alston, Norton and Pardey (CAB, 1998).

A second area that has not been adequately addressed by research to date is the interaction of *Xf* with GWSS. A better understanding of this interaction might enable development of GWSS variants that are poor hosts for *Xf,* yet still competitive with wild-type GWSS. If such a GWSS strain could displace native GWSS populations, it would dramatically slow the spread of PD. An example of this kind of approach applied to malaria was recently published in *Science* (27 April 2007: Vol. 316. pp. 597 - 600). Although there are now a large collection of *Xf* mutants available, it appears that most of these have not yet been tested for their ability to colonize GWSS and to be transmitted by GWSS. This work should not take long to complete, and may be simply a matter of promoting more collaborations among entomologists and microbiologists.

Another important gap is an integrated GIS-based database that includes GWSS population density information, *Xf* genotype distribution, PD distribution, crop information, temperature information and geographical information. As mentioned above, there are efforts underway to create such a resource, but these seem to be mostly University-based. Since the lifetime of University databases is often as short as the funding for a specific project, it is strongly recommended that the CDFA maintain the GIS database, with University researchers contributing layers to the database. Creation of such an integrated database would enable researchers to more directly address the source of *Xf* strains causing PD outbreaks, and determine how it is being vectored.

A fourth perceived gap is in extension rather than research *per se*. There is a lack of up-to-date recommendations specific to each grape growing region on the most effective management strategies to limit PD losses. A significant amount of information regarding “best practices” has been generated in the last six years, yet there is no apparent mechanism, such as a website, for growers to quickly access current information.

F. Recommendations

1. Research areas to target for further funding

In the above sections, we identified research areas that the RSAP felt held the most promise for development of effective PD control strategies. Here we provide a bulleted list of these areas for convenient reference:

- Assessment of PD resistance in existing commercial grape varieties.

- Use of Diffusible signal factor (Dsf) for disrupting *Xf* colonization, including delivery by plant associated microbes, transgenic rootstocks, and application of chemical analogs.

- Inhibition of *Xf* polygalacturonase (PG). This research area includes identification of PGIPs with high activity against *Xf* PG, delivery of PGIP to grape plant scions from transgenic rootstocks, and development of small molecule inhibitors of *Xf* PG.

- Targeting other *Xf* proteins required for virulence. This research area includes development of protein/peptide-based inhibitors of cell surface proteins such as pilins and adhesins, along with identification of chemical inhibitors of these proteins.

- Production of parasitoids, with a particular emphasis on developing efficient means of mass producing GWSS eggs or an alternative suitable host for large-scale production of parasitoids.

- The interaction of *Xf* with GWSS. Collaborations among entomologists and microbiologists should be particularly encouraged.

- Marker Assisted Selection-based breeding for resistance. The RSAP recommends recruitment of additional breeders so that genes in addition to *PdR1* can be mapped, tagged with molecular markers, and the process of introgression into multiple commercial backgrounds initiated.

- Expansion and enhancement of the GIS database to include location of PD-infected vines, location of specific *Xf* genotypes, and location and number of infected vines removed from vineyards.

- Economic analysis of the impact of PD/GWSS on agriculture, both in terms of real and potential losses due to PD and the costs of current and prospective control measures.

- Expansion of extension resources to disseminate information on “best practices” for controlling PD in specific regions of California and elsewhere in the USA.

2. Funding mechanisms

With the exception of the last four items on the above list, these research areas can best be funded through a continuation of the existing competitive grants program, provided that the Request for Proposals (RFP) explicitly describes the areas of research to be targeted. The language of the RFP should not explicitly exclude other areas of research, however, as creative new ideas not anticipated by the RSAP may still be submitted. Successful projects should be considered for multiyear funding (e.g. up to three years) to reduce the burden of submitting a new proposal each year, and to facilitate recruitment of qualified personnel to conduct the research.

The RSAP recommends that a separate RFP be written that targets economic analyses. Because of the nature of economic analyses compared to laboratory and field science, it will be necessary to have a different review panel. Language for such an RFP should include the following:

> Economic analysis of PD/GWSS should respond to the following needs for additional economic information. These specific topics are illustrative and are not listed in priority order: (a) Modeling and measuring the economic effects of the current PD/GWSS disease situation. How much cost have the industries incurred so far and who has incurred those costs among consumers, producers, taxpayers, and other stakeholders by crop? (b) Simulating alternative ex ante scenarios of the costs if PD/GWSS were to continue unabated. How much cost is likely to be incurred, who is likely to incur those costs among consumers, producers, taxpayers, and other stakeholders by crop? (c) Evaluate, in an ex ante sense using simulation models, the likely contributions of alternative investments in PD/GWSS research and development. This research could evaluate the potential contributions of several alternative R&D efforts that have different impacts on control of PD/GWSS and different time horizons. Such a project would not attempt to evaluate the likely scientific merit of alternative research efforts, but rather assess the payoff for the industry, including consumers, if reasonable success is obtained.

To recruit and fund additional grape breeders, it may be necessary to develop some kind of contractual arrangement with existing breeding facilities and/or scientists that are best equipped to embark on such an effort.

Similarly, to build on the current GIS database, it may be necessary to contract with specific qualified scientists to generate the data and then work with CDFA to enter these data in the existing GIS database. To be successful, these

efforts will need to be closely coordinated with the personnel that are maintaining the database.

Lastly, to expand on current extension efforts, it will be necessary to work closely with existing extension personnel to establish what resources are needed to collect information on best practices for PD control and then disseminate this information through existing extension and outreach education channels.

3. Evaluating potential hurdles

The above recommendations do not address potential hurdles associated with development of these various approaches to controlling PD. For each of these recommendations, the RSAP is optimistic that technical hurdles will be surmountable. However, the RSAP did not have adequate expertise to address potential hurdles associated with regulatory issues, public acceptance, intellectual property and economics. For example, what is the projected cost of obtaining regulatory approval for commercial use of transgenic rootstocks, who would bear these costs, and would the public accept wine produced from such plants? What intellectual property would need to be licensed to produce transgenic grape plants? Answers to these questions would help inform research funding priorities. If regulatory approval of transgenic grape vines will cost more than the industry is willing to pay, will state or federal agencies be willing to pay these costs? If not, then research that leads to non-transgenic control measures should be given priority over research that requires use of transgenic plants or microbes. On the other hand, transgenic solutions may prove to be economically superior to non-transgenic solutions if they reduce the requirement for insecticide applications and vine replacement. What level of PD pressure would drive adoption of transgenic varieties? **The RSAP recommends that an additional group of experts be assembled to evaluate these issues.** People with expertise in regulatory affairs, intellectual property, tech transfer and/or economics would be appropriate for such an evaluation.

4. Communicating research progress

As described in the introduction, the RSAP worked with PIPRA to develop a web-based database of research projects and reports. To promote communication among researchers, the RSAP recommends the database be expanded and used as a universal reporting system for all PD/GWSS-related research. This would also simplify and facilitate the reporting process. Researchers would submit their reports on-line via a web-based interface. The format for such progress reports should be standardized and include links to publications arising from the funded research. In addition, such a database should be searchable by other PD/GWSS researchers, funding agencies, and other interested parties, and thus facilitate dissemination of research results.

APPENDIX A

CDFA PD/GWSS Research Scientific Advisory Panel:

Background Materials

Dr. Adam Bogdanove

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Department of Plant Pathology
Associate Chair
Interdepartmental Microbiology Graduate Program
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ajbog@iastate.edu

Areas of expertise:

- Molecular biology of plant-bacterial interactions
- Tissue-specificity in bacterial pathogenesis of plants
- Plant disease resistance

Education:

- BS in Biology, Yale University
- PhD in Plant Pathology, Cornell University
- Post-Docs at Purdue and the Boyce Thompson Institute for Plant Research

Brief Bio:

Research in the Bogdanove laboratory focuses on bacterial plant pathogenesis and plant disease resistance mechanisms. Key areas of focus include molecular mechanisms of pathogenesis and plant defense; bacterial type III secretion; plant signal transduction; microbial and plant biotechnology for disease control. The Bogdanove lab utilizes genomic and proteomic approaches to gene discovery alongside molecular biology, genetics, cell biology and biochemical approaches to understanding gene function. The long-term goal is to generate knowledge and tools useful in interfering with disease and in enhancing and extending natural plant defense for better disease control.

For more information:
http://www.public.iastate.edu/~ajbog/research.html

Dr. Michael Davis

Professor
Citrus Research and Education Center
Department of Plant Pathology
University of Florida
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Areas of technical expertise:

- Tropical/subtropical plant pathology
- Fastidious prokaryotic plant pathogens
- Disease diagnostics and applied plant pathology
- Genetic engineering of fruit crops for disease resistance

Education:

- PhD in Plant Pathology, University of California
- MS in Plant Pathology, Colorado State University
- BS in Botany, Colorado State University

Brief Bio:

Dr. Davis's current research is on the etiology, epidemiology and control of diseases of tropical/subtropical crop plants. He has performed a substantial amount of research on plant diseases caused by fastidious prokaryotes. Dr. Davis was the first individual to isolate, in pure culture, a number of xylem-inhabiting plant pathogens, including *Xylella fastidiosa*. He also was one of the first scientists to clone DNA for diagnostic probes to identify plant pathogens. Dr. Davis's current research is focused largely on the development of transgenic papaya and sugarcane for disease control.

For more information:
http://trec.ifas.ufl.edu/Personnel/mdavis.htm

Dr. Roger Innes
(Panel Chair)

Professor and Associate Chair
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Areas of Technical Expertise:

- Genetic and biochemical basis of disease in plants
- Host-response and disease resistance in plants

Education:

- PhD from University of Colorado, 1988
- Postdoctoral Fellow, University of California, Berkeley 1988-91

Brief Bio:

Our primary interest is in understanding the genetic and biochemical basis of disease resistance in plants. Plants are able to specifically recognize pathogens and actively respond. We are investigating how this specific recognition is accomplished and how recognition is translated into a resistant response.

To address these questions we take a molecular genetic approach. We use the small mustard *Arabidopsis thaliana* as our standard host plant, and the bacterial pathogen *Pseudomonas syringae* as our standard pathogen. Recognition of specific *P. syringae* strains by Arabidopsis is mediated by specific disease resistance (*R*) genes of Arabidopsis. These *R* genes are thought to encode receptors that detect a signal produced directly or indirectly by bacterial proteins that are injected into the plant cell. The molecular mechanism of this detection step is poorly understood, however. Understanding this mechanism is a major goal in plant biology, as it will likely lead to new approaches for engineering disease resistance in plants, as well as, provide critical insights into how pathogens evolve to escape recognition and cause disease.

For more information:
http://www.bio.indiana.edu/~inneslab/index.html

Dr. Greg Loeb

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Areas of technical expertise:

- Insect ecology
- Integrated pest management
- Biological control

Education:

- PhD from University of California at Davis
- MS from San Diego State University
- BA/BS from University of California at Davis

Brief Bio:

The overall objective of my program is to develop an understanding of the principal forces that influence the population dynamics and community structure of arthropod herbivores in agricultural and natural ecosystems and use this information to devise and implement multi-tactic pest management programs for grape and small fruit crops. My more basic research is primarily focused on how host plant traits and other environmental factors influence interactions between plant parasites and beneficial arthropods, with the specific applied goal of conserving natural enemies in agroecosystems. As a model system for examining this issue we have been studying the role of leaf morphology in mediating interactions between natural enemy mites (predatory and mycophagous species) and parasites of grapes. A second research area of interest for me is the relationship between host plant resistance and host plant tolerance using strawberry as a model system. My more applied research program focuses on the ecology and integrated control of specific arthropod pests of grapes and small fruit crops. As a grape and small fruit entomologist, 30% of my time is committed to extension activities. Educating growers and pest control advisors as to the proper and effective use of pesticides is an important aspect of my responsibilities. In addition, however, I try to include other approaches to pest control such as the use of predators and parasites, cultural techniques and host plant resistance.

For more information:
http://www.nysaes.cornell.edu/ent/faculty/english-loeb

Dr. George Sundin

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Areas of technical expertise:

- Analysis of bacterial pathogen genes controlling the host infection process
- Pathogen host-specificity and pathogen recognition response
- Comparative genomics

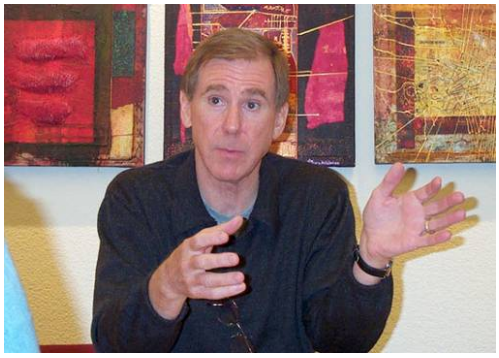
Education:

- PhD from Oklahoma State University

Brief Bio:

Dr. Sundin currently provides oversight for the Phytobacteriology and Tree Fruit Pathology programs. His main research interests are in plant pathogen biology and plant-microbe interactions. Current research projects range from molecular biology-driven basic science to applied field and environmental research aimed at solving immediate problems. Dr. Sundin's long-term goal is to improve the sustainability of tree fruit production by increasing host resistance to bacterial and fungal diseases. This goal can only be achieved through developing an intimate understanding of pathogen-host interactions. He is also a member of the Center for Microbial Ecology.

For more information:
http://cmp.msu.edu/faculty/sundin.htm



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Areas of expertise:

- National and international agricul
  policy, human resources and regulations
- Ag economics and sustainable agriculture
- Wine economic issues

Education:

- PhD in Economics, University of Chicago
- MS from Michigan State
- BS in Agricultural Management, California State Polytechnic University

Brief Bio:

Dr. Sumner contributes to teaching, research and outreach on a wide range of topics concerning the economics of agriculture. He has a special interest in wine economic issues.

Prior to beginning his current position in January 1993, Sumner was the Assistant Secretary for Economics at the United States Department of Agriculture where he was involved in policy formulation and analysis on a whole range of topics facing agriculture and rural America — from food and farm programs to trade, resources, and rural development.

Dan participates in research, teaching, and directs an outreach program related to public issues facing agriculture. His research and writing focuses particularly on the consequences of farm and trade policy on agriculture and the economy.

Agricultural Issues Center – The Center provides research-based and objective information about the full range of public issues affecting California agriculture. For almost 20 years, the Center has served as a forum where important and often controversial trends and issues involving California agriculture--ranging from water transfers to international trade--have been identified, studied and debated.

For more information:
http://sumner.ucdavis.edu/