AES/CE MAR 84

Commodity: Walnut Board
Department: Plant Pathology

University of California
Division of Agricultural Sciences
PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2009 Anticipated Duration of Project: 2nd of 3 years

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Project Title: Epidemiology and Management of Walnut Blight

Keywords: Disease Modeling, Disease Forecasting, Wetness Periods, Bacterial Disease, and Disease Control
Relevant AES/CE Project No. ______

Problem and Significance

Walnut blight, caused by the bacterium *Xanthomonas axonopodis pv. juglandis*, attacks catkins, female blossoms, fruit, green shoots, leaves, and buds of English walnut. Fruit infections occur soon after flowering and these infections account for most of the economic loss. The extended period of host susceptibility is one of the chief obstacles in controlling this disease. The pathogen survives from one year to the next in twig lesions, buds (living and dead), and diseased fruit that remain on the tree. The optimum temperature for bacterial growth is 28 to 32C, and the minimum is about 1C, but lesions on immature fruit generally do not occur below approximately 12C or above 25C (Adaskaveg et al. 1994, 1998, 1999). Furthermore, disease increases with increased wetness duration within this temperature range (Adaskaveg et al. 1998, 1999).

Walnut blight continues to cause crop losses in many central and northern California orchards with varying incidences from year to year. Based on analysis of environmental data and actual disease progression over several seasons at several locations, we developed and in 2000 we initiated XanthoCast™ as a model to predict infection periods for walnut blight. This accumulation model utilizes wetness period duration and temperature (the two micro-environmental parameters that were shown to be critical for disease development in growth chamber and field studies) for calculating the risk of disease based on current ambient conditions for each field weather station. Irrigate.net (located at www.irrigate.net) currently provides the basic XanthoCast™ information with a up to five-day weather forecast, and prediction of XanthoCast indices for individual weather stations located between Red Bluff and Davis as a service on the website to any grower, PCA, or farm advisor. The XanthoCalculator allows individual web-based forecasting for different grower fields and locations. As confirmed by IPM specialists, the forecasting model reduces the total number of applications while maintaining the same level of management as a calendar-based program. Thus, the system has been successfully used in less conducive (e.g., 2001-02) and very favorable environments (e.g., 2003-05) for predicting disease. In 2007, XanthoCast was initiated with UC IPM and we will use CIMIS environment data for the 2008-09 seasons to validate the model in this system. The XanthoCast system offers flexibility to changes in the micro-environment during each season and is a very robust program that provides regional- or grower-specific forecasts, judicial use of pesticides, and disease control similar to calendar-based programs.

In the last few years, our research on the XanthoCast Model focused on time of initiation of the model in the spring time. Our timing studies indicated that early flower emergence applications followed by applications based on the XanthoCast environmental model proved the most consistent disease control. We clearly showed that a catkin bactericide treatment was needed when high precipitation (favorable environments) occurred in an orchard with a history of high disease levels (*See* Fig. 12, JEA- Annual Walnut Blight Report 2005). When precipitation was low during catkin emergence, then no difference occurred between catkin plus pistillate flower treatments and the pistillate flower alone treatment (*See* Figs. 10 and 11, JEA- Annual Walnut Blight Report 2006 and Table 7, Walnut Blight Report 2007) regardless of inoculum levels. Furthermore, in simulated rain studies at the KAC trial (Fresno Co.) and under natural precipitation

(2005) when high rainfall naturally occurred in late spring (May), incidence of blight was significantly lower when bactericide treatments were based on the calendar or based on XanthoCast forecasts than when only bud break treatments were applied. Thus, we plan to continue with development of the XanthoCast model with trials focused on the initial timing of bactericide treatments in relation to forecasted weather forecasts. Additionally, we potentially can look at the previous years' incidence of disease as an indicator for disease risk and timing of the first spray. Still, we demonstrated in Butte Co. that in an orchard with 80% incidence in 2005 that the disease was reduced to 5.5% (2006) and 2.5% (2007) with nine or seven (the last two treatments were not justified because they were done in June) weekly applications starting at pistillate flower bud break (30-40% flower emergence). In 2008, no data was obtained because the natural incidence of disease was low due to the lack of rain in April and May. Because we also showed that bacterial populations can exponentially increase on diseased fruit in the field under favorable temperature and wetness conditions and can reach very high levels within 3 to 6 hours starting from a low or medium initial population size, inoculum levels will not be further considered in the optimization of the XanthoCast model after the initial treatment for blight management. Early bloom treatments can be important in reducing inoculum levels, but they are not effective as stand-alone treatments under high disease pressure conditions during high-rainfall years.

In our studies on the timing of bactericide applications, we have evaluated and plan to continue to evaluate single "eradication" or inoculum reduction treatments that are done at pistillate flower bud break along with catkin flower and fruit development treatments in April and May based on XanthoCast forecasts. This approach verifies the importance of early, mid-, and late-season bactericide treatments based on environmental events namely temperature and leaf wetness, as well as precipitation during the spring season. In addition to environmental events, we will investigate another phenological character in addition to catkin and pistillate flower emergence (i.e., bud break). This character is "number of pistillate flower bloom cycles". In 2007, we had a staggered bloom and pistillate flowers blossomed over several weeks. At least four cycles were observed while the percent bloom in each cycle diminished. The implications are that there could be multiple start dates for pesticide application and for initiating and running the XanthoCast model. Bactericide treatments have been demonstrated as prophylactic programs and the initial treatment in relation to the primary bloom and concurrent environment is very important in protecting the developing female flower and subsequent fruit. Thus, "pistillate flower bloom cycle" and environmental conditions during each cycle will be evaluated experimentally for the XanthoCast system.

Our research project on the management of walnut blight since 1994 has assisted in the development of copper-Manex treatments and it has demonstrated the efficacy of zinc compounds and zinc-Manex combinations for disease control and maximum yields. We have also initiated the alternation of copper- and zinc-based bactericides or use of copper-zinc mixtures (e.g., Kocide 20/20, Nordox 30/30) to minimize the development of copper-resistant populations of the pathogen. In addition, in recent years a new lower-concentration copper product (i.e., Kocide 3000 - DuPont) was shown to be highly effective. Other new copper materials that should be further evaluated include copper hydroxide (Kentan DF – Isagro) and a pre-mix of copper hydroxide with copper oxychloride (i.e., Badge – Isagro-USA). In 2006-08, we demonstrated that several EBDC fungicides/bactericides other than Manex can be effectively used in mixtures with copper and the antibiotic Kasumin that we are developing for the walnut industry. Although the EPA has prompted the cancellation of maneb registrations in the United States, full registrations other EBDC fungicides/bactericides such as Manzate are expected by EPA in the coming years. Manzate/Kocide mixtures were similarly effective as Manex-Kocide treatments over the past three years. Manzate is formulated as a flowable liquid (F) or a dispersible granular (DF) material. The latter formulation has better shelf-life and disposability of packaging. Evaluations of other EBDC fungicides included Dithane (Dow AgroSciences) and Maneb (UPI – Ag Products formerly Cerexagri). We have evaluated these materials for the last three years and additional trials are needed for a seamless transition of the industry to the mancozeb line of products.

Over the years, we evaluated many new alternatives that were either not effective, or will not be developed based on lack of support by the registrants or because of human health issues. In 2005, we identified the potential for kasugamycin (Kasumin, Arysta Life Sciences) in walnut blight management. This is an antibiotic that is used in agriculture in other countries but not in human medicine. In Sept. 2005, a food tolerance was established for Kasumin on imported agricultural products and it was accepted into the IR-4 program (federal program for pesticide registration on minor crops) for establishing a tolerance on pears for fire blight control. Walnut residue trials for kasugamycin were conducted by IR-4 in 2007 and registration is proceeding with a registration target date of 2010 (Federal) and 2011 (California). We are currently demonstrating that this material is a highly-viable rotational treatment to copper-EBDC (i.e., Manzate) when the antibiotic is mixed with either an EBDC or copper material. In our 2007-08 studies, in eight of nine trials, Kasumin significantly reduced walnut blight incidence. Under low rainfall in 2008, Kasumin was highly effective. Under high rainfall (simulated with overhead irrigation or naturally occurring), mixtures of the antibiotic with EBDC or copper bactericides resulted in an increased efficacy. **Moreover, in most trials, Kasumin-EBDC mixtures were similar or higher in efficacy to copper-EBDC mixtures.** Thus, additional studies have to be done with kasugamycin, especially using rotation and mixture programs that include antibiotics, copper materials, and EBDC fungicides.

Additional research is also needed on alternative treatments using natural compounds, biocontrols, and enhancers of host resistance. Two new natural products (e.g., MOI-106, MOI-107) again will be made available to us in 2009 for evaluation against walnut blight by Marrone Organic Innovations. Bio-forge (Stoller-USA) that is thought to increase plant stress responses was evaluated by us in 2007-08 and results warrant further evaluation. Additionally, we will re-evaluate acibenzolar-s-methyl (Actigard, Bion). Previously we showed a moderate reduction in walnut blight after foliar treatments. A report from the University of Florida, however, has indicated that soil application and subsequent root absorption was highly effective in reducing bacterial canker of citrus. A similar approach will be tested on walnuts in small-scale trials. Thus, one focus of our 2009 research will again be the evaluation of field treatments against walnut blight. Our overall goal is to ultimately have multiple bactericides or materials available that are equal to or more efficacious than traditional copper-containing compounds and that could be used in management strategies in orchards with copper-sensitive and copper-resistant populations of the walnut blight pathogen. Multiple materials with high efficacy will mean less environmental impact and less chance for resistant populations of the pathogen to develop.

Objectives

I. New treatments **-** Evaluate the toxicity of alternative copper (i.e., Kentan, Badge, and the low-copper-concentration product Kocide 3000) and non-copper based chemicals (e.g., the antibiotic Kasumin, EBDC fungicides, new natural products – MOI-106, MOI-107) and numbered biologicals, and other experimental materials such as enhancers of plant stress response (e.g., BioForge) and defense mechanisms (e.g., Actigard) against *X. juglandis* and evaluate the efficacy of these materials for managing walnut blight in laboratory and small-scale field tests as compare to fixed-copper compounds. Crop destruction costs will be included in budget.

   A) Comparative efficacy of new bactericides (synthetic and natural products) using air-blast spray application methods in field trials under ambient and simulated rain systems at the Kearney AgCenter (KAC), UC Davis, and in commercial orchards in Butte Co.
   B) Studies using mixtures of copper or kasugamycin with EBDC fungicides (e.g., Manzate, Dithane).
   C) Pre- or during-bloom soil drenches using Actigard in small-scale field trials at UC Davis.

II. Epidemiology **-** Continue to evaluate disease development throughout the spring and monitor environmental parameters (e.g., leaf wetness, precipitation, temperature, and relative humidity) that are conducive to bacterial infection of walnut tissues using dataloggers and CIMIS data. (This will be done in orchards with other ongoing blight research programs).

   A) Develop and evaluate a precipitation-temperature-based version of XanthoCast (ongoing) in cooperation with UC-IPM and CIMIS programs for utilizing leaf wetness data for XanthoCast to be used on university websites.
   B) Evaluate early, mid-, and late-spring timings (e.g., male (catkin) vs. female (pistillate) flower emergence or delayed emergence in respect to "Bloom Cycle", as well as mid- to late spring season timings) under natural and simulated rain environments.
   C) Continue to determine the reproduction potential of the pathogen on the plant surface using spiral plating technology.
   D) Evaluate previous year disease levels as a general indicator for "start time" for using the XanthoCast model.

III. Host resistance **-** Continue to evaluate walnut genotypes for natural host resistance to walnut blight under simulated rainfall conditions at the KAC.

Plans and Procedures

I. New Treatment Evaluations

Evaluation of protective bactericidal treatments for control of walnut blight as compared to copper-containing compounds. In small-scale field studies, the relative efficacy of protective bactericidal treatments such as new copper materials (e.g., Kentan), lower-concentration copper products (e.g., Badge, Kocide 3000), the antibiotic kasugamycin (e.g., Kasumin), and new natural products (e.g., numbered products from Marrone Organic Innovations) will be compared to Kocide 3000 and Kocide 3000/Manzate treatments using air-blast spray application methods under ambient and simulated rain systems at the Kearney AgCenter (KAC), at the UC Davis-Plant Pathology Field Station, and selected field sites in cooperation with growers. Products will also include other copper formulations or a mixture of copper and lime, whereas EDBC materials will include Manzate and Dithane. The enhancer of plant stress responses (Bioforge) will also be included in these studies. Due to low rainfall in the spring of some years, we will conduct simulated rain studies in some of our orchards at KAC and UC Davis. In these orchards, wetness periods to tree canopies will be supplied once a week for 4 to 6 hours using high-angle irrigation systems. The field trials will require crop destruction and grower reimbursement. Treatments will be replicated four times on different trees. Data for chemical control will be analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

Additionally, pre- or during-bloom soil drenches using Actigard in small-scale field trials at UC Davis. These treatments will be applied to selected trees at rates to be determined. The trials will be conducted on Hartley and Vina cultivars. Data for chemical control will be analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

In the laboratory, inhibitory effects of selected concentrations and formulations of each compound against *X. juglandis* will be determined. For this, spiral-, disk-, and amended media-assays will be utilized. Emphasis will be on comparative evaluations of interactions between fungicides (EBDCs, Syllit) and toxicants such as copper and Kasumin. Additional studies on the activity of the antibiotic over time will be evaluated in small-scale plant studies. For this treated leaves will be inoculated at selected time intervals (1, 2, 4, and 8 days) after treatment and then the bacterium will be re-isolated to determine viability of the pathogen. Data from toxicity studies will be analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

II. Epidemiology

Phenological, environmental, and disease modeling studies. Environmental and phenological monitoring will be conducted in Butte and Solano Co. where other blight research programs are ongoing. Leaf wetness, temperature, relative humidity, precipitation, and other environmental parameters will be monitored using dataloggers and compared to CIMIS data (we will continue to work with CIMIS representatives for installing leaf wetness sensors at selected stations for the evaluation of a regional XanthoCast system through UCIPM). Additionally, phenological stages (catkin and pistillate bloom) and number of pistillate flower bloom cycles will be evaluated weekly during the growing season. For this and for disease monitoring, lab personnel and personnel working with farm advisors will evaluate tagged walnuts from early April through May (approximately 6 weeks). This will be performed as described in our previous reports. Bloom stage, female flowering cycle, disease data, and concurrent environmental data from test sites will be analyzed using statistical procedures of SAS 9.1.

Determine reproduction potential of the pathogen on plant surfaces and last-season disease history for determining start date of the XanthoCast model. To determine the reproductive potential of the pathogen on leaf surfaces, systematic and frequent sampling of buds and fruit is required. Additionally, with temperature and wetness (leaf wetness and precipitation) factors affecting disease as defined in our model, sampling ideally should be done around wetness events and should be done with some general information as to the levels of disease in the previous year. Furthermore, sampling numbers should be large enough to minimize variation (25-50 per sample). Thus, we will conduct experiments to sample a large number of buds with and without symptoms from orchards with a high or low level of disease in the previous year. Collected plant samples will be assayed and plated on a *Xanthomonas*-selective medium (BSM) to determine population size. Comparisons of populations between sampling times will determine the reproductive potential of the pathogen under a set of environmental conditions for each location. Plots will be set up in orchards with environmental monitoring equipment.

Development and implementation of XanthoCast – An accumulative, wetness duration and temperature model for forecasting blight. The walnut blight risk model, XanthoCast™, will continue to be provided and field-validated in 2009. Irrigate.Net will change its URL from www.irrigate.net to probably www.agtelemetry.com and this site will continue to provide XanthoCast for the Sacramento Valley. Fox Weather Services will continue to work with us to implement the model. XanthoCast indices and the XanthoCalculator will be offered again. The up to five-day forecasts of XanthoCast based on satellite rain analysis will be provided through Fox Weather. This information can be accessed by the walnut industry to assist in making spray decisions.

Assessment of timings. Data over several years continues to indicate that early wetness periods influence the disease progress curve. In 2009, data will be collected and evaluated for comparisons of timings (e.g., male (catkin) vs. female (pistillate) flower emergence). Below is an outline of our proposed treatments in the simulated–rain plot where treatment start dates, treatment timings, and irrigation schedules that reflect early or late rains will be evaluated:

Irrigation	No.	Treatments	Catkin	Irrig.	Pistillate	Irrig.	2-wk after	Irrig.	4-wk after	Irrig.	6-wk after
Early	1	Control	---	@	---	@	---	---	---	---	---
Early	2	Kocide-Manzate	@	@	---	@	---	---	---	---	---
Early	3	Kocide- Manzate	---	@	@	@	---	---	---	---	---
Early	4	Kocide- Manzate	@	@	@	@	@	---	@	---	@
Early	5	Kocide- Manzate	---	@	---	@	@	---	@	---	@
Late	1	Kocide- Manzate	@	---	---	---	---	@	---	@	---
Late	2	Kocide- Manzate	---	---	@	---	---	@	---	@	---
Late	3	Kocide- Manzate	@	---	@	---	@	@	@	@	@
Late	4	Kocide- Manzate	---	---	---	---	@	@	@	@	@

Application of bactericide treatments based on the forecasting model to determine if the total number of applications can be reduced as compared to a weekly calendar-based program. In 2008, we plan to again use XanthoCast to determine the optimal timing of bactericides. A spreadsheet model called XanthoCalculator that is available on the Irrigate.Net website will be used to chronologically follow the model as described in previous reports. Integration of the 5-day forecast and Satellite Rain Analysis will also be used in the decision-making process. Four to five single-tree replications will be used for each treatment and will be compared to a calendar-based program and an untreated control.

III. Host Resistance to Walnut Blight

Evaluation of walnut genotypes for natural host resistance to walnut blight under simulated rainfall conditions at the KAC. Host resistance among walnut genotypes in simulated rain studies will be evaluated. Dr. McGranahan established a genotype plot at KAC and we assumed responsibility for this orchard five years ago. In the last five years we have evaluated the genotypes for their susceptibility to walnut blight under repeated rain events over a range of ambient temperatures during the spring. As expected, the incidence of disease increased from not being observed in the orchard in 2002 to 16% blighted fruit in the most susceptible genotype after just two seasons of simulated rain in 2003. Between 2004 and 2008 the disease reached up to 48% incidence on the most susceptible cv. Payne and significant differences were observed among cultivars that were quite consistent over the years. In 2009, we will again apply simulated rain weekly for 6 hours from April to May as described previously and evaluate the natural incidence of blight on four to five replications of each walnut genotype in June. New genotypes will be planted in a new orchard as Dr. McGranahan's program supplies genetic material. In addition, we will evaluate inoculum levels in buds of selected genotypes (most susceptible, moderately susceptible, and least susceptible genotypes). Data will be evaluated using an analysis of variance and mean separation procedures of SAS 9.1.

IV. Benefits to the industry

New treatments including several new experimental and newly introduced products will be compared to currently recommended compounds (e.g., copper, copper-mancozeb) for their protective action of walnut tissues. This goal is directly beneficial to growers for blight management in developing new, environmentally safer materials with low human health risks. For example, Kocide 3000 with a 30% and Badge with 27% metallic copper equivalent (MEC) represent major steps towards reducing copper rates for a more environmentally oriented management program. At 3.5 to 4 lb Kocide 3000 per A, the total MEC is less than half of previous formulations, while an equivalent efficacy is maintained. In addition, kasugamycin (i.e., Kasumin) potentially represents the first new bactericide for management of walnut blight since the introduction of copper with the highest efficacy in some trials when mixed with copper or EBDC bactericide/fungicides. Copper and antibiotic treatments will be evaluated with potential new full registrations of EBDC bactericide/fungicides (e.g., Manzate, Dithane) that will result in products that are easier to use (long shelf life, safer disposal of packaging, etc.). We are also developing natural products for organic growers to reduce the total use of copper during a growing season. Thus, our research continues to identify potentially effective treatments for walnut blight for agricultural uses under favorable environments for disease. We also continue to demonstrate XanthoCast as a disease forecasting model based on both temperature and wetness period (including precipitation) parameters. We will continue to explore additional factors to improve the model and thus, XanthoCast is also serving as the scientific basis for explaining the epidemiology of walnut blight. XanthoCast will again be available in 2009 to growers, PCAs, and farm advisors on a commercial web site. As demonstrated in selected plots in 2001-2008, the use of a forecasting system can more accurately determine the appropriate timing of bactericide treatments and reduce the number of applications that are currently used in a calendar/phenology-based program. In summary, this research is providing highly effective disease management programs for controlling walnut blight that are cost-effective and environmentally sound. With a goal of having multiple materials available (with high efficacy) and an environmentally-based program for determining their application, the walnut industry is moving to less dependency on any one product, environmentally sound programs with minimal over-use of any one bactericide, and management programs that minimize the risk for selecting resistant populations of the pathogen to applied treatments.

References

1. Adaskaveg, J. E. and Hine, R. B. 1985. Copper tolerance and zinc sensitivity of Mexican strains of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. Plant Dis. 69:993-996.
2. Adaskaveg, J. E., et al. 1994-2006. Epidemiology and management of walnut blight. Annual Walnut Research Reports.
3. Osorio et al., 1994. Phytopathology 84:656-660.
4. Turner, N.C. 1991. Measurement and influence of environmental and plant factors on stomatal conductance in the field. Agricultural and Forest Meteorology 54:137-154.

Budget Request:
Budget Year: 2009
Funding Source: Walnut Board of California

Research Budget

Salaries and Benefits:	Post-Docs/RA's/Farm Advisor Support	23,000	23,000
	Lab/Field Ass't		
	Subtotal	23,000	23,000
	Employee's Benefits	Sub 1	9,200
		Sub 2	
		Subtotal	32,200
Supplies and Expenses* (orchard costs)		Sub 3	5,000
Irrigate.Net (XanthoCast) and Fox Weather (4-day forecasts)**			7,000
Farm Advisor Support (J. Connell – weekly evaluation of blight)			
Equipment		Sub 4	0
Operating Expenses/Equipment Travel (UC Davis only)		Sub 5	0
Travel		Sub 6	3,000
Department Account No. ________		Total	47,200

* - These costs partially pay for maintaining 3 orchards at the Kearney AgCenter that include new bactericide trials (Chico variety), simulated-rain trials (Vina, Hartley, and Chandler varieties), and genotype evaluation trials (formerly G. McGranahan's), as well as for maintaining two orchards at the Plant Pathology field station (Armstrong), UC Davis, for evaluating new bactericides (Hartley variety) and conducting timing studies (Chico/Hartley varieties). These orchards are financially supported by research projects of the principal investigator.

**- Using Irrigate.Net data, Fox Weather is currently developing in cooperation with us an automated model to forecast XanthoCast up to four days in advance.

____ Date: 12/12/08
Originator's Signature

Agricultural Experiment Station/Department Chair ____________________ Date: 12/12/08

Liaison Officer ____________________ Date: __________