EPIDEMIOLOGY AND MANAGEMENT OF WALNUT BLIGHT

J. E. Adaskaveg, H. Förster, J. Dieguez-Uribeondo, E. Erickson, C. Thomas, R. Buchner, B. Olson, C. Pickel, T. Prichard, and J. Grant

ABSTRACT

Field studies focused on evaluating the efficacy of new chemical bactericides and biopesticides for reducing the incidence of walnut blight under ambient and simulated-rain conditions. In these studies, the only consistent alternative to copper-Manex was the non-registered DBNPA bactericide formulation 918-48C. Improved agricultural formulations of DBNPA have been developed with decreased water solubility and increased persistence on plants without phytotoxicity. No other new material than copper-Manex is immediately available for management of copper-resistant populations of the walnut blight pathogen in California. The antibiotic Starner and the silver metal-based compounds Axenohl showed some efficacy against walnut blight but regulatory policies toward these materials need to be determined before agricultural registrations will proceed. Serenade showed good results in our trials this year, but again under very low disease levels. In the last two years, we demonstrated that simulated rainfall studies were very effective in developing efficacy data during low rainfall years. Accumulated simulated rain of as little as 20 h duration over 5 weeks showed significant increases in disease incidence. Alternative materials including oxidants (e.g., Zerotol), biopesticides (e.g., Serenade Organic), and silver-based bactericides (e.g., Axenohl) were tested under simulated rain conditions and showed promise for managing the disease. In the Fresno Co. trial, DBNPA formulations 918-48C and 918-54A and Zerotol were similar in performance to copper-maneb treatments (Cuprofix-Manex and NuCop-Manex). In the Solano Co. trial, DBNPA 918-48C and 117-2 reduced the disease to zero levels, whereas treatments with Serenade applied alone or in mixture/rotation with Kocide-Manex were statistically intermediate between the control and the two DBNPA treatments. Trials using inoculum-reduction strategies were done under ambient and simulated rain-conditions. We demonstrated for a second year that the pathogen has a high reproduction potential and disease was significantly higher in the simulated rain treatment than in the ambient treatment for both the treated (Copper-Manex-0.5% Breakthru) and non-treated trees and thus, this strategy is questionable under conducive environments. Lastly, XanthoCast™, the internet-based walnut-blite forecasting program, was available commercially for a second year for growers. In general, the XanthoCast cumulative index followed the actual disease progress in each location. Thus, disease was accurately predicted for different specific microclimates recorded for each weather station. Correlations of observed and predicted XanthoCast values that were based on 3- and 5-day weather forecasts gave coefficients of determination or $R^2$ values mostly between 0.73 and 0.88. Thus, walnut blight infection periods can be quite accurately forecasted using the XanthoCast model and Fox Weather’s proprietary microclimate forecasts for specific weather stations throughout the northern walnut growing areas. Furthermore, the model and the forecast system allow for targeted bactericide applications based on walnut blight infection periods at each weather station location. Disease was low throughout the walnut growing regions of the state this year and XanthoCast called for two to three applications as compared to six weekly calendar-based applications. The model was experimentally verified in simulated rain studies. Regression
models of wetness duration on blight incidence were significant and had $R^2$ values of 0.97 in 2001 and 0.99 in 2002. Total natural rainfall was 15 mm in 2001 and 8.5 mm in 2002. The two regression lines were shown to be statistically different in slope and in midpoint. Thus, natural rainfall and temperature differences between the two growing seasons and the simulated rain treatments within each season explained most of the observed variation in disease incidence.

**INTRODUCTION**

Walnut blight, caused by *Xanthomonas juglandis*, is a major disease of walnut in central and northern California. The pathogen attacks catkins, female blossoms, green shoots, leaves, buds, and fruit of English walnut. Fruit infections account for most of the economic loss in California. These infections commonly occur in the spring under wet conditions. The bacterium survives from one year to the next in buds (healthy and diseased), diseased fruit that remain on the tree, and possibly in twig lesions (Miller and Bollen 1946; Mulrean and Schroth 1982; Teviotdale et al. 1985, Ogawa and English 1991).

Chemical treatments have been the most commonly used management practices for walnut blight. Copper-based compounds have historically been the most efficacious and the most widely used. The effectiveness of copper treatments has been related to the number of applications and to frequency and duration of wetness periods during the development and maturation of the crop. Failures in blight control are related to lack of protection during conducive environments for disease development, as well as to the development of populations of the bacterial pathogen that are less sensitive to copper bactericides. In any disease management program, dependence on any one chemical treatment potentially can lead to the loss of efficacy of the treatment due to the development of resistance in a pathogen population to that chemical. In the case of management of walnut blight in California, the extensive use of copper in California for more than 25 years is attributed to the development of resistant populations of the walnut blight organism (Lee et al. 1993). Because of the development of copper-resistant populations, management of the disease is dependent on applications with higher rates of copper, addition of other bactericidal compounds to copper treatments (e.g., Kocide®-Manex®), or on new bactericidal treatments. New treatments with different modes of action from that of copper or antibiotics need to be evaluated to develop management practices for walnut blight in California to decrease the potential for resistant populations from developing (Adaskaveg et al. 2001). Several new alternatives are available and include natural bactericidal products (Serenade), bactericidal sanitation treatments (e.g., DBNPA, Zerotol, Oxidate, etc.), systemic acquired host resistance (SAR) compounds (e.g., Milsana®), and antibiotics (Starner). Starner is currently registered in Japan for control of bacterial diseases including those caused by *Xanthomonas* species. Based on previous research (1998-2000), the DOW bactericide was selected as the most likely compound to be developed by its manufacturer. The formulations that were supplied previously (DOW 117-1, 117-2) are very water-soluble and do not persist well on the plant surface. In addition, phytotoxicity was observed in some trials. Some phytotoxicity was also observed with the EC formulation of the material that was provided to us for our 2001 trials. Two new formulations given to us in 2002 (DB 918-48C and DB 918-54A) were designed for improved plant persistence and minimal phytotoxicity. Additional applications of bactericidal compounds could provide added control as new plant growth emerges during the spring. In field studies in 2002, we evaluated the DOW bactericide, an acidified hydrogen peroxide formulation (Zerotol-BioSafe, Inc., Glastonbury,
CT), the antibiotic Starner (Valent Biosciences), formulations of copper (NuCop-MicroFlo Co., Cuprofix-Cerexagri Inc., and Kocide-Griffen Co.) mixed with Manex, a new formulation of copper in gelatin (Bioacumen-Business Generation, North Sydney, Australia), a copper/silver-based bactericide (Axenohl-ETI-H2O, Inc., El Cajon, CA), and the QRD137WP formulation of the biopesticide Serenade applied either alone or in a mixture and rotation with copper.

In 1998, severe epidemics of walnut blight caused substantial yield losses in northern California (Adaskaveg et al., 1998). In 1999 to 2002, however, low rainfall and cool temperatures were not conducive for the development of walnut blight (Adaskaveg et al. 2001). In each low-disease year, the incidence of disease has decreased in most orchards where trials were conducted regardless of bud populations of the pathogen observed during the dormant period. The concept of the disease triangle is essential for the development of the disease. A microclimate model to predict walnut blight in a forecasting system that is developed with all components of the disease triangle (host, pathogen, and environment) will help in the management of this potentially destructive disease of walnut. Inoculum appears to be the most predictable parameter to estimate in commercial orchards. This is because the pathogen is endemic in walnut orchards throughout California, the previous year’s disease incidence is an excellent indicator of inoculum potential in the orchard for the next season, and the pathogen has a high reproductive potential under conducive environments (as we have shown in our irrigation studies).

In our epidemiological studies, we developed critical information on field environmental conditions required for infection and disease development. The interaction of daily wetness period duration and temperature during the wetness period is important. The optimum temperature for in vitro bacterial growth is 28 to 32 C with a minimum of about 5 C and a maximum of 37 C, whereas infection of walnut tissues can occur between 5 and 27 C (Miller and Bollen 1946). In inoculation studies, disease development on nuts occurred in 4 days at 27 C and 8 days at 15 C (Miller and Bollen 1946). Using a humidity chamber, Miller and Bollen (1946) also studied the effects of wetness on potted plants with a small number of flowers that were inoculated and evaluated at early bloom stages of development. From these studies they concluded that rainfall was involved in dissemination and wetness periods of only 5 min were sufficient for fruit infection of very young tissues that were “water-congested”. As we initially indicated in 1994-95, we are demonstrating that extended wetness periods are critical in the development of walnut blight epidemics. Based on our data obtained in 1994-95 and 1997-2002, both wetness duration and temperature can affect the onset and severity of walnut blight. Wetness duration of much longer than 5 min, however, is required. Other parameters such as wind and relative humidity are weather parameters that need to be more critically evaluated in the field under natural inoculum levels. Thus, research is being conducted to determine the critical environmental parameters for disease development under greenhouse and field conditions. A microclimate accumulation model known as XanthoCast™ is based on accumulated wetness periods (hr/day) at selected temperature ranges.

OBJECTIVES

1. Evaluate the toxicity of alternative, non-copper based chemicals to X. juglandis. Compare, in laboratory, greenhouse, and small-scale field tests, the toxicity and efficacy of protective treatments including experimental natural products (antibiotic Starner),
and bactericidal treatments (e.g., re-formulated DOW-01) for control of walnut blight as compared to zinc-containing and fixed-copper compounds.

A) Comparative efficacy of new bactericides using handgun and air-blast spray application methods in field trials on walnut.

B) Greenhouse evaluations of new materials using pepper/walnut plants inoculated with *X. vesicatoria*/*X. juglandis* and incubated on misting benches or in growth chambers.

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

A) Determine reproduction potential of pathogen on plant surface for potential incorporation in existing model that is based on leaf wetness and temperature. A spiral planter was recently purchased for this research.

B) Evaluation of an improved detection method of the bacterial pathogen using a commercially available ELISA system from Agdia (previous kit from Adgen was accurate for pure cultures but was not accurate when using plant tissue) as compared to a DNA-based molecular method (PCR) and bacterial isolation.

III. Continue to develop and evaluate XanthoCast as a model to forecast the incidence of walnut blight.

A) Evaluate the automated model of XanthoCast with up to a 5-day forecast included in this year’s version.

B) Apply bactericide treatments based on the forecasting model to determine if the total number of applications could be reduced as compared to a weekly calendar-based program.

C) Continue experimental validation of the model in irrigation plots at Kearney AgCenter and at UC Davis.

**PROCEDURES**

*Evaluation of alternative bactericides for management of walnut blight - field studies.* Trials were established in two experimental orchards in Solano (UC Davis) and Fresno Co. (Kearney Agricultural Center) and in one commercial orchard in Butte Co. In the Solano Co. trial on Hartley walnuts, applications were done on April 12, April 19, April 26, May 3, May 9, May 17, May 23, and May 29. In the Fresno Co. trial on Chico walnuts applications were done on April 17, April 24, May 2, May 8, May 14, and May 21. In the Butte Co. trial on Vina walnuts applications were done on April 4, April 10, April 18, April 23, April 30, May 8, and May 21. Treatments were applied using an air-blast sprayer (100 gal/A). In the Solano Co. trial, trees were irrigated with high-angle sprinklers for 4 h, 4 to 48 h after each treatment application. Irrigation dates were May 3, May 10, May 17, May 24, and May 31. In the Fresno Co. trial, trees were irrigated with high-angle sprinklers for 4 h the following day after each treatment application. Incidence of disease was based on the number of infected fruit in a sample of 50-200 fruit for each of four single-tree replications. Phytotoxicity evaluations were based on a scale of 0 (healthy) to 4 (extensive injury). Data for efficacy studies were evaluated using analysis of
variance and least significant difference mean separation procedures or general linear model and LSD mean separation procedures of SAS 6.12.

Evaluation of a single bud-break application with Kocide-Manex plus 0.5% BreakThru. A trial was set up in Solano Co. using two walnut varieties, Hartley and Chico, to evaluate a single bud-break treatment of Kocide-Manex (8 lb-58 fl oz) plus 0.5% BreakThru at 100 gal/A for walnut blight control. In addition, sub-plots of the orchard were established: disease in one subplot was allowed to develop under the natural local weather conditions, whereas the other subplot was irrigated once a week for 4 h for 5 weeks starting on 5/3/02 and ending on 5/31/02. These irrigations were done to increase disease development. The bud-break treatment was applied on April 9, 2002 using a tractor-pulled air-blast sprayer. Fruit evaluations for disease on June 12 were based on 100 fruit for each of the four single-tree replications. Data were evaluated using analysis of variance and least significant difference mean separation procedures and LSD mean separation procedures of SAS 6.12.

Evaluation of alternative bactericides in greenhouse and lathhouse studies. The biocidal oxidants Zerotol and TM 443 were evaluated in the greenhouse on 35-40 day-old sweet pepper plants (cv. Yolo Wonder) for control of bacterial leaf spot caused by X. vesicatoria. Pepper plants were used for chemical screening because of their rapid growth and because X. vesicatoria is closely related to the walnut blight pathogen. Plants were treated with bactericidal compounds (50 and 500 ppm a.i. Zerotol; 50 and 200 ppm a.i. TM-443) to runoff using an air-nozzle sprayer (equivalent to 400 gal/A). Because of the low pH of Zerotol (pH 3), one Zerotol treatment at 500 ppm was adjusted to pH 6 using calcium carbonate. Plants were allowed to air-dry for at least 4 h. For inoculation, bacterial suspensions containing 10^8 cfu/ml were sprayed to runoff on treated plants. The plants were then covered with plastic bags for 24 h. After 14 days of incubation in the greenhouse at 22-27 C, plants were evaluated for disease and for phytotoxicity. Disease severity was assessed as number of spots on each of the five most diseased leaves per plant. For comparisons between experiments, results were expressed as percent reduction in disease as compared to the non-treated control. Evaluation of phytotoxicity was based on the number of necrotic spots and leaf curling. Leaf necrosis ratings ranged from 0 = No necrosis to 5 = Severe toxicity, more than 2 necrotic spots per leaf. Leaf curling ratings ranged from 0 = No curling to 5 = more than 20 % of leaves showing leaf curling. Experiments were conducted 3 times in a completely randomized fashion with 3 replicates.

On walnut, possible phytotoxic effects of selected formulations of the DOW bactericide were evaluated on Hartley plants growing in a lathhouse. All treatments were applied to run-off at 1000 ppm a.i. using an air-nozzle sprayer. Experiments were conducted twice in a completely randomized fashion with 3 replicates. Phytotoxicity rating was done 14 days after treatment and was based on leaf and vein necrosis. Leaf necrosis ratings ranged between 0 = no necrosis to 5 = Severe toxicity, more than 2 necrotic spots per leaf. Vein necrosis ratings ranged between 0 = no vein necrosis to 5 = more than 20 % of leaves showing vein necrosis. Data were evaluated using analysis of variance and least significant difference mean separation procedures or general linear model and LSD mean separation procedures of SAS 6.12.

Evaluation of the biocidal oxidant Zerotol in laboratory assays against isolates of Xanthomonas species. In a direct contact assay, bacterial suspensions (10^8 cfu/ml) of X.
**vesicatoria** (isolates X97 and X105; X-105 is a copper-resistant isolate) or *X. juglandis* (isolate X91) were incubated in selected concentrations of Zerotol (0, 0.5, 2.5, 5, 10, and 50 ppm) for 10 min or for 4 h. After incubation the bacterial-test substance mixture was diluted 1:1000 with sterile water and the resulting suspension was plated out onto nutrient agar using a spiral plater. Numbers of bacterial colonies on the agar plates were counted after 48 h of incubation at 25C. Colony numbers of the chemical treatments were compared to those of the water control.

**Identification of isolates of Xanthomonas juglandis, X. fragariae, and X. vesicatoria using selective media, physiological assays, and ELISA methods.** *X. juglandis* was isolated from infected walnut fruit. For this, tissue was excised from the margin of a lesion, surface sterilized for 1 min in 400 μg/ml HOCl, rinsed in sterilized distilled water (SDW) for 1 min, blotted dry, placed in 1-2 ml of SDW, and ground using a mortar and pestle. A loop of the aqueous suspension was streaked out onto nutrient agar (NA). Yellow bacterial colonies were transferred and grown on brilliant cresyl-blue starch medium or BSM (Mulrean and Schroth 1981) and characteristic colonies were re-transferred to NA. Selected isolates were also Gram stained for positive cultural identification of the bacterium or identified using the BIOLOG system. For cultural identification we also evaluated another ELISA kit from Agdia, Inc. (Elkhart, ID) specific to the genus *Xanthomonas* following the manufacturer's instructions. Strains of *X. juglandis* (3 strains), *X. fragariae* (2 strains), and *X. vesicatoria* (3 strains) were grown on NA and bacterial concentrations of 10^7 and 10^8 were evaluated in three separate trials.

**Disease development in an orchard in response to different wetness durations that are provided by overhead sprinklers.** Three different irrigation schedules were established in an experimental Chico orchard at Kearney Agricultural Center. For this, overhead sprinklers were installed and trees were either not irrigated (control), irrigated monthly (two 4-hour simulated rain periods), or irrigated weekly (five 4-hour simulated rain periods) from May 7 to June 10. Disease incidence was evaluated on 2 trees for each of 4 replications (8 trees total) of each treatment on June 18. Data for were compared using analysis of variance and regression procedures of SAS 6.12.

**Disease evaluations and environmental monitoring using dataloggers in commercial walnut orchards and weather data from Fieldwise and UCIPM-CIMIS.** In commercial orchards located in Butte, Tehama, Sutter/Yuba, and San Joaquin counties approximately 100 fruit in each of three single-tree replications were tagged and monitored periodically (every 7-10 days) for the development of walnut blight from mid-April to late June 2002. Fruit were carefully examined for lesions and positive evaluations were re-checked in subsequent evaluations and in isolations of sub-samples of infected fruit as described previously. Disease incidence was determined as the number of infected fruit per total fruit sample minus the missing fruit. Percent new walnut blight per day (PNB) was calculated as:

\[ PNB = \frac{D}{t*(C-M)}*100 \]

where D = the number of newly diseased fruit, t = time expressed as the number of days since the previous evaluation, C = number of healthy fruit at the beginning of the evaluation, and M = the number of fruit missing in the evaluation period not previously recorded as blighted. Disease incidence as an accumulation of PNB was plotted with Xanthocast indices (1-day, 7-day, and
seasonal accumulation) for the calendar dates of evaluation. In general in Butte and Tehama Co., physiological time (days after pistillate flower emergence) occurred approximately on April 1 for cv. Vina and Ashley. In over 50 sites of the Fieldwise Inc. network in the Sacramento valley, electronic sensors and dataloggers (Campbell Scientific or Adcon Telemetry) were used to monitor leaf wetness, temperature, relative humidity, and rainfall. Dataloggers were programmed to make readings every minute and to calculate quarter-hourly, hourly, and daily averages for each micro-environmental parameter throughout the evaluation period. Environmental data were downloaded and summarized as hourly and daily summaries. CIMIS environmental data was also downloaded from Butte and Tehama county stations for the same time period. Disease progress curves for 2002 were developed and compared to weekly and seasonal disease index values obtained from our model - XanthoCast™ V.481. A disease index for the season or for a re-setting weekly index was determined using parameters of the model and plotted over time (Gregorian date). XanthoCast™ V.481 develops a forecasting index based on duration of leaf wetness for three temperature scales. The forecast is 14 to 21 days in advance of actual disease based on a latent period for disease expression after infection has occurred. This is the accumulation model described in previous reports.

**Evaluation and utilization of XanthoCast for forecasting and management of walnut blight.**
The walnut blight forecasting model XanthoCast™ was launched as a commercial product in 2001 in collaboration with Fieldwise, Inc. The model is one of two that has been developed. The XanthoCast™ model is a host phenology, temperature mediated-wetness accumulation model that has been incorporated into the Fieldwise website (www.fieldwise.com) for evaluation. This system uses on-line microclimate telemetry data to update the XanthoCast index daily throughout the Sacramento valley. Several base stations are located in selected counties. Thus, microclimate conditions were monitored daily and disease evaluations were made weekly in test orchards throughout the spring from pistillate flower emergence (late March) to fruit maturity (late June). This was done in cooperation with the PMA program. Daily, seven-day, and seasonal summations were maintained and used for walnut blight forecasts and timing of bactericide applications. As infection periods occurred, bactericide treatments were applied and accumulation was delayed for 7 days. If no infection periods occurred, bactericides were not applied. This program was compared to a grower program (calendar-based) and an early application followed by applications based on the model. The treatments were designed to evaluate and improve timing and to reduce the total number of applications of bactericide treatments. A dilute, handgun (400 gal/A) or an air-blast sprayer (100 gal/A) application of copper-maneb (i.e. Kocide-Manex) was used for treatments in the XanthoCast and calendar-based programs. Disease management results for this program are presented in the PMA and BMP projects summarized in the Buchner et al. Annual Report and in part in this report.

**Development of 1- to 5-day forecasts using XanthoCast parameters.** Forecasts using the XanthoCast model for predicting walnut blight were developed in conjunction with Fieldwise Inc. and Fox Weather. The goal of this research was to automate this process into one XanthoCast program by showing actual and forecasted indices up to five days ahead of time. One proprietary, microclimate-forecasting model was used by Fox Weather and was evaluated using the XanthoCast parameters to generate daily and seven-day XanthoCast indices for 1-, 2-, 3-, 4-, and 5-day forecasts. The forecasts were qualitatively and quantitatively compared to the actual XanthoCast daily indices for accuracy and precision in predicting the occurrence and
magnitude of infection events during the spring season. Regression statistics were used to compare observed with predicted values. Forecasts were done for all of the Fieldwise, Inc. weather station sites. Six sites in Butte, Tehama, Solano, and San Joaquin Co. are presented in the results below and are compared to observed XanthoCast indices (daily and 7-day) obtained during the walnut growing season.

RESULTS AND DISCUSSION

Disease Management

*Evaluation of a new antibiotic (Starner), new formulations of copper and silver compounds, a hydrogen dioxide compound, different formulations of the organic biocide DOW-DB, and the biopesticide Serenade Organic (QRD 137WP) for managing walnut blight in field studies.*

DOW Chemical, the manufacturer of DBNPA, made new formulations of the compound available for evaluation (DB918-48C, and DB918-54A). The material is registered as a commercial product for disinfecting industrial water. Three copper formulations, Kocide 50DF, NuCop 50DF, and Cuprofix 37DF in mixtures with Manex, a new copper formulation that contains pectin (Bioacumen), and a silver-based bactericide (AxenoHl) were also evaluated. Zerotol, a hydrogen dioxide compound, is registered for suppression of fungal diseases on a wide range of crops. The efficacy of Serenade was evaluated for the QRD 137WP formulation that was also tested in 2001.

In field studies, trials were conducted in Fresno, Solano, and Butte Co. to evaluate the new materials, and efficacies were compared to copper-maneb treatments. Simulated rain from sprinkler irrigation was applied to the Fresno and Solano plots. In the Butte plot, where copper-resistant strains have been identified, disease incidence was very low in 2002 with 1.4% total incidence (infected nuts on the tree and on the ground) in the control. At selected rates, both of the new DOW-DB formulations (918-48C and 918-54A), Starner, and Bioacumen significantly reduced the disease as compared to the non-treated control, similar to Cuprofix-Manex (Fig. 1). The previous year's formulation of DOW-DB (117-2) had no significant effect. No phytotoxicity was observed with any of the treatments. In the other trials (Buchner et al., 2002 Annual Report), Bioacumen was highly phytotoxic when applied using a high-volume sprayer (400 gal/A). In our trials, no significant differences among treatments were observed when only fruit on the trees were evaluated. Although Starner showed promise at the high rate (6.25 lb/A), Valen Biosciences is not willing to pursue registration in the United States due to uncertainties in EPA policy on registration of antibiotics for agricultural use.

The efficacy of the DOW-DB formulations, Zerotol, and the copper (Cuprofix)-maneb (Manex) treatments was compared in a simulated rain trial in Fresno Co. The control and DB 117-2 had the highest incidence of disease with 9 and 10.8 % of the fruit infected, respectively, at the time walnut blight was evaluated in June (Fig. 2). This was not unexpected because the DOW-DB 117-2 formulation is highly water-soluble. There was no significant difference among the remaining treatments with incidence ranging from 0.5 (Cuprofix-Manex) to 3.9% (DOW-DB 918-54A). Additional studies are warranted for Zerotol and DOW-DB 918-48C.
In a third trial in Solano Co. using simulated rain and with only copper-sensitive strains of the pathogen detected in the orchard, Kocide 50DF/Manex, the three DOW-DB formulations, Serenade Organic QRD137WP, and Axenohl were evaluated using eight treatments between mid-April and the end of May. In addition, a mixture-rotation program with Serenade/Kocide and Kocide/Manex was evaluated. Disease incidence was significantly reduced by all treatments, except for Axenohl (Fig. 3). The efficacy of the significant treatments in this trial was similar. Thus, as in 2001, Serenade QRD 137WP was effective with or without the copper mixtures or copper-maneb rotations. Furthermore, the DOW-DB formulations were as effective as the Kocide/Manex treatment. The DB 918-48C formulation was the most consistent and was as effective as the copper-maneb treatment in all the trials conducted in 2002.

**Evaluation of a single bud-break application with Kocide-Manex plus 0.5% BreakThru.** For a second year, a trial was set up in Solano Co. using two walnut varieties, Hartley and Chico, to evaluate a single bud-break treatment of Kocide-Manex plus 0.5% BreakThru at 100 gal/A for walnut blight control. In addition, disease was allowed to develop in one sub-plot under ambient weather conditions, whereas in the other subplot, it developed under simulated rainfall (4 h once/week for 5 weeks). The results in Table 1 show that there was no difference in disease incidence between bud-break-treated and non-treated trees for both varieties and for irrigated and non-irrigated trees. This indicates that overwintering bacteria in buds cannot be eradicated with a single Kocide-Manex-0.5% BreakThru application at 100 gal/A and that under favorable conditions the pathogen populations and the disease will increase after the treatment. Thus, this two-year study demonstrates that walnut blight can only be effectively managed with repeated applications of effective bactericide treatments applied during favorable environments and host susceptibility periods.

**Greenhouse/lathhouse and laboratory toxicity studies using alternative bactericides.** In greenhouse studies using *X. vesicatoria* on pepper as a model for evaluating bactericides, two biocidal oxidants, Zerotol and the numbered compound TM 443, were evaluated (Fig. 4B). In these studies, Kocide reduced the severity of disease significantly more than any other treatment. Zerotol at 500 ppm, pH 3 was also very effective; whereas Zerotol at 50 ppm, pH 3 was the least effective. Treatments with intermediate effectiveness were Zerotol at 500 ppm, pH 6, and TM 443 at 40 and 200 ppm (Fig. 4B). Phytotoxicity was observed as minor leaf necrosis and leaf curling in only the Zerotol treatments (Fig. 4A). In a lathhouse study with DBNPA formulations on potted walnut trees, all of the formulations tested showed minor leaf necrosis, whereas only the 117-2 formulation also showed leaf vein necrosis (Fig. 5).

Laboratory studies confirmed the high toxicity of the DBNPA formulations and Zerotol against *X. juglandis*. Technical grade of the DOW bactericide was used in direct-exposure studies for comparison. A 30-sec exposure of the bacteria to a 100-μg/ml solution of DBNPA or a 5-min exposure of the bacteria to a 20-μg/ml solution both resulted in no growth of the organism. After a 10 min exposure of three isolates of *Xanthomonas* spp. to Zerotol in 50 ppm, no growth was observed (Fig. 6A). Longer exposures (e.g., 4 h) resulted in higher mortality at lower rates (e.g., 5 ppm). Thus, these materials look very promising and additional field-testing should be continued.
Identification assays using selective media, physiological methods, and ELISA techniques. Selective media and physiological assays were the most accurate means for positive identification of the Xanthomonas pathogens. The ELISA assay was variable when used with pure cultures of each bacterial species. For X. juglandis, two of the three strains were positive, whereas one strain was negative in one experiment and positive in other experiments. For X. fragariae, one of the two strains was always positive, whereas one strain was positive in one experiment and negative in other experiments. The three strains of X. vesicatoria were always positive but variable in the intensity of the ELISA reaction (weak to strongly positive). The method was ineffective when plant tissue (walnut, strawberry, or pepper for each pathogen, respectively) was used in direct identification assays without prior culturing the bacterium. Thus, we will continue to evaluate other molecular assays in an effort to make accurate identifications more rapid.

Evaluation of the XanthoCast model and the 3- and 5-day forecasts from Fox Weather. The XanthoCast forecasting model was widely publicized in the spring and early summer of 2001 and 2002 to make growers, farm advisors, and PCAs aware of this free service and to help them understand the program on the website (www.fieldwise.com). The model was intentionally left unchanged in the first two years of commercial access to allow users to become familiar with the model and its consistent performance. In 2002, we continued to conduct field trials to evaluate the accuracy of the model over a wide geographical range using a number of weather stations. In these studies, temperature, leaf wetness, precipitation, and relative humidity were monitored using field dataloggers and walnut blight incidence during the spring season was evaluated. Databases were evaluated using XanthoCast. The automated program calculated the accumulation of wetness duration (hours per day) greater than a critical value of 2 from the leaf wetness sensor for three temperature regimes (6-12 C, 12-17 C, and 17-27 C). These data are shown for eight orchards in Figs. 7-14. Orchard locations ranged from the northern Sacramento Valley to the northern San Joaquin Valley. In these graphs (Figs. 7A-12A and Figs. 13-14), a seasonal accumulation index (XanthoCast-Cumulative), a daily index (XanthoCast-day), and a re-setting 7-day (XanthoCast-7 day) index for forecasting walnut blight were plotted and compared to the actual disease progress (Percent blight). In general the XanthoCast cumulative index follows the actual disease progress in each location. Thus, disease was accurately predicted for different specific microclimates recorded for each weather station. Additionally, the model is approximately 5-10 times more sensitive than observed values. Thus, in the graphs, 5-10 index points are approximately equivalent to one percent observed disease. This designed characteristic of the model is consistent with last year’s data. In each of the Figs. 7B and C-12B and C, actual XanthoCast 7-day indices are compared to indices that were generated from 3- or 5-day weather forecasts. The coefficients of determination or \( R^2 \) values mostly ranged from 73% to 88% with only one of the six sites having an \( R^2 \) value of 45% for the 3- and 5-day forecasts. All 1-day forecasts had \( R^2 \) values greater than 72% (data not shown). Thus, walnut blight infection periods can be quite accurately forecasted using the XanthoCast model and Fox Weather’s proprietary microclimate forecasts for specific weather stations throughout the northern walnut growing areas. Furthermore, the model and the forecast system allow for targeted bactericide applications based on walnut blight infection periods at each weather station location.
The XanthoCast model was used for timing of foliar bactericide applications (copper-maneb+zinc complex) for management of the disease. Disease incidence was compared between model-based applications, an early spray plus model-based applications, a grower standard program, and the untreated control at several field sites. In the Butte and Tehama plots, the disease incidence was extremely low and no statistical comparison of the data could be made between the treatment programs. Additional details of bactericide applications in 2002 based on monitoring leaf wetness and temperature or weekly calendar-based programs are also presented in the Buchner et al. Annual Report. In the San Joaquin plots, Escalon and Farmington-Dondero, the three application programs performed similarly and had lower disease incidences than the untreated control. Because of low rainfall, the grower and model programs had similar number of applications and a benefit of using the model was not realized. Still, XanthoCast called for fewer spray applications (2-3) than a traditional calendar-based program that historically used 6 weekly applications. Thus, the XanthoCast program should prove to be an effective tool in managing walnut blight in low and high rainfall years and demonstrates effective stewardship of pesticide usage.

Experimental verification of the model was done in a simulated rain trial in Fresno Co., CA. In both 2001 and 2002, as the frequency (weekly as opposed to monthly simulated rain applications) and total hours of rainfall (0, 8, and 20 h) increased, disease incidence increased linearly with a positive slope. Thus, regressions of wetness duration under ambient temperatures and rainfall on disease incidence were significant and had $R^2$ values of 0.97 in 2001 and 0.99 in 2002 (Fig.15). Total natural rainfall was 15 mm in 2001 and 8.5 mm in 2002. Using general linear model procedures to compare the regressions, the lines were shown to be statistically different in slope and in midpoint. Thus, natural rainfall and temperature differences between the two growing seasons and the simulated rain treatments created within each season explained most of the observed variation in disease incidence. This study also confirmed that two of most critical environmental parameters for forecasting walnut blight are leaf wetness (including rainfall) and temperature, the two parameters of the XanthoCast model.

ACKNOWLEDGMENTS

Special thanks to the growers in Butte, Tehama, Sutter-Yuba, and San Joaquin Co. who allowed us to conduct our research in their orchards, to Janine Hasey, Carolyn Pickel, Terry Prichard, and Sara Goldman for evaluating the Butte, Sutter-Yuba, and San Joaquin PMA sites where XanthoCast was tested, Joe Grant for his evaluations of XanthoCast in San Joaquin Co., Dr. Beth Teviotdale for assisting in our research at the Kearney AgCenter, and to the chemical industry representatives who cooperated with us with during this research.
REFERENCES

Fig. 1. Efficacy of new bactericides and antibiotics for management of walnut blight on Vina walnuts in Butte Co. 2002

Control
DB 918-48C 32 fl oz + Sticker 13 fl oz
DB 918-48C 128 fl oz + Sticker 52 fl oz
DB 117-2 16 fl oz + Sticker 13 fl oz
DB 117-2 64 fl oz + Sticker 52 fl oz
DB 918-54A 29 fl oz + Sticker 13 fl oz
DB 918-54A 116 fl oz + Sticker 52 fl oz
Starner 20WP 6.25 lb
Bioacumen 5 gal
NuCop 50DF 8 lb + Manex 58 fl oz
Cuprofix 37DF 15 lb + Manex 58 fl oz

Disease incidence (%)

Treatments were applied on: 4-4, 4-10, 4-18, 4-23, 4-30, 5-8, and 5-21. Disease was evaluated on 6-7-02. Disease incidence is the number of infected nuts per 50-200 nuts evaluated on each of four single-tree replications. No phytotoxicity was observed in any treatment except the Bioacumen treatment, where marginal leaf burn resulted.

Fig. 2. Efficacy of new bactericides and antibiotics for management of walnut blight on Chico walnuts in Fresno Co. 2002.

Control
DB 918-48C 128 fl oz + Sticker 52 fl oz
DB 117-2 64 fl oz + Sticker 52 fl oz
DB 918-54A 116 fl oz + Sticker 52 fl oz
ZeroTol 3.77 L
NuCop 50DF 8 lb + Manex 58 fl oz
Cuprofix 37DF 15 lb + Manex 58 fl oz

Disease incidence (%)

Treatments were applied on: 4-17, 4-24, 5-2, 5-8, 5-14, and 5-21-02. Trees were irrigated with high-angle sprinklers for 4 h the following day after each treatment application. Disease was evaluated on 6-18-02. Disease incidence is the number of infected nuts per 50-100 nuts evaluated on each of four single-tree replications. No phytotoxicity was observed in any treatment.
Fig. 3. Efficacy of new bactericides and biological treatments for management of walnut blight on Hartley walnuts in Solano Co. 2002.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Simulated Rain</th>
<th>Hartley</th>
<th>Chico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.4 b</td>
<td>0.9 b</td>
</tr>
<tr>
<td>Kocide-Manex + Breakthru</td>
<td>-</td>
<td>0.3 b</td>
<td>0.2 b</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>3.62 a</td>
<td>6.67 a</td>
</tr>
<tr>
<td>Kocide-Manex + Breakthru</td>
<td>+</td>
<td>3 a</td>
<td>6.81 a</td>
</tr>
</tbody>
</table>

Trees were sprayed with Kocide-Manex (8 lb-58 fl oz) + 0.5% BreakThru in 100 gal/A at bud break (on 4-9-02). Simulated rain was obtained using a high-angle sprinkler irrigation system. Irrigations were made once a week for 4 h for 5 weeks starting on 5/3/02 and ending on 5/31/02. Fruit were evaluated for disease on June 12, 2002. Incidence is based on evaluation of 100 fruit for each tree.
Fig. 4. Evaluation of phytotoxicity and control of bacterial leaf spot of sweet peppers caused by *X. vesicatoria* using biocidal oxidants

A. Phytotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Necrosis rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kocide 2400 ppm</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Zerotol (pH 3) 500 ppm</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Zerotol (pH 6) 500 ppm</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Zerotol (pH 3) 50 ppm</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>TM 443 200 ppm</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>TM 443 40 ppm</td>
<td>1 2 3 4 5</td>
</tr>
</tbody>
</table>

B. Disease control

Plants were treated with bactericides using an air-nozzle sprayer. After air-drying, plants were inoculated with bacterial suspensions (10^6 cfu/ml), covered with plastic bags for 24 hr, and incubated for 14 days in the greenhouse. Disease incidence was based on the number of spots on each of the five most diseased leaves per plant. Ratings for phytotoxicity were based on the number of necrotic spots and leaf curling and ranged from 0 to 5.

Fig. 5. Lathouse evaluation of phytotoxicity on walnut plants using selected formulations of DBNPA

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Rating of leaf necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>DB 918-54A</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>DB 918-48C</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>DB 117-2</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>DB EC</td>
<td>1 2 3 4 5</td>
</tr>
</tbody>
</table>

Walnut plants were treated with bactericides using an air-nozzle sprayer and incubated for 14 days in a lathouse. Ratings for phytotoxicity were based on the degree of leaf and vein necrosis. Ratings ranged from 0 to 5.

Fig. 6. Evaluation of the biocidal oxidant Zerotol in a direct contact assay against isolates of two *Xanthomonas* species in the laboratory

A. 10 min exposure

B. 4 hr exposure

Bacterial suspensions were incubated for 10 min (A.) or 4 hr (B.) in selected concentrations of Zerotol. Bacteria were then plated out onto nutrient agar and the number of colonies was determined after 2 days of incubation at 25°C.
In Fig. A, actual disease incidence was 3.1%, whereas XanthoCast predicted 35%. In Figs. B and C the gray line represents the algorithm used by Fox Weather to accurately and precisely predict XanthoCast values either 3 or 5 days prior to observed weather. R² values describe the relationship between observed and predicted values.
In Fig. A, actual disease incidence was 2.9%, whereas XanthoCast predicted 28%. In Figs. B and C the gray line represents the algorithm used by Fox Weather to accurately and precisely predict XanthoCast values either 3 or 5 days prior to observed weather. $R^2$ values describe the relationship between observed and predicted values.
In Fig. A, actual disease incidence was 1%, whereas XanthoCast predicted 14%. In Figs. B and C the gray line represents the algorithm used by Fox Weather to accurately and precisely predict XanthoCast values either 3 or 5 days prior to observed weather. R² values describe the relationship between observed and predicted values.

In Fig. A, actual disease incidence was 2%, whereas XanthoCast predicted 41%. In Figs. B and C the gray line represents the algorithm used by Fox Weather to accurately and precisely predict XanthoCast values either 3 or 5 days prior to observed weather. R² values describe the relationship between observed and predicted values.
Fig. 13. Percent blight, and XanthoCast indices for the Escalon trial site.

Actual disease incidence was 4.5%, whereas XanthoCast predicted 31%.

Table 2: Incidence of walnut blight using different timings for Kocide-Manex applications at the Escalon test site

<table>
<thead>
<tr>
<th>Treatment</th>
<th>21-May</th>
<th>04-Jun</th>
<th>10-Jun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>3.7</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Grower Standard</td>
<td>1.7</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Early + Model</td>
<td>0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Model Only</td>
<td>1</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

All blight treatments were 8 lb Kocide 2000 + 58 oz Manex per acre. Application timings were: Grower Standard (4/15, 4/28); Early + Model (4/8, 4/15, 5/2), Model only (4/15, 5/2).

Fig. 14. Percent blight, and XanthoCast indices for the Farmington-Donnerro trial site.

Actual disease incidence was 16.5%, whereas XanthoCast predicted 23%.

Table 3: Incidence of walnut blight using different timings for Kocide-Manex applications at the Farmington-Donnerro test site

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1-May</th>
<th>15-May</th>
<th>10-Jun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>5.3</td>
<td>4.6</td>
<td>16.5</td>
</tr>
<tr>
<td>Grower Standard</td>
<td>0.5</td>
<td>2.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Early + Model</td>
<td>1.7</td>
<td>1.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Model Only</td>
<td>0.5</td>
<td>0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

All blight treatments were 8 lb Kocide 2000 + 58 oz Manex per acre. Application timings were: Grower Standard (4/15, 4/28); Early + Model (4/8, 4/15, 5/2), Model only (4/15, 5/2).

Fig. 15. Effect of wetness duration using simulated rainfall on the incidence of walnut blight

- Experimental validation of XanthoCast model -

\[ R^2 = 0.97 \]

\[ R^2 = 0.99 \]

Regression lines were not corrected for natural rainfall or ambient temperatures conducive to disease. More natural rainfall and conducive temperatures occurred in 2001 (15 mm rainfall) than in 2002 (8.5 mm).