EPIDEMIOLOGY AND MANAGEMENT OF WALNUT BLIGHT

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ABSTRACT

Wetness periods and temperature effects on the development of walnut blight infection caused by Xanthomonas campestris pv. juglandis were evaluated. Wetness durations longer than 5 minutes for fruit in the prebloom or early postbloom stage of development as reported by Miller and Bollen (1946) were required for infection of maturing walnut fruit under field conditions. In field inoculation studies, incidence and severity of walnut blight on developing fruit (5-10 weeks after bloom) and leaves (10-14 weeks old) increased with increasing wetness periods that extended from 12 to 24 hours for fruit of mature trees and 24-48 hours for mature leaves of potted plants. As Miller and Bollen (1946) indicated, older tissue is less susceptible to infection. In disease monitoring studies in commercial orchards, differences in disease incidence between two orchards was correlated with the accumulation in hours of wetness (above a critical value of 10 on a scale of 0 to 100). A quadratic regression model was significant and explained the data with a R² value of 0.95. In the future, this information may be utilized in developing new cultural practices and bactericide treatments for the management of walnut blight.

Because of the development of copper-resistant populations of the bacterial pathogen, evaluations of non-copper-based materials were conducted. Alternative treatments that were effective in reducing disease incidence included copper-EBDC mixtures such as copper hydroxide (Kocide 101®)-maneb+zinc (Manex®), selected zinc-based compounds used alone or in combination with EBDC fungicides, and new experimental bactericides (e.g., PHMB) that are already registered by the United States Environmental Protection Agency for non-agricultural use. In large scale field tests in 1994, the most efficacious treatments immediately available to growers were tank mixtures of copper hydroxide-maneb+zinc (8 lb-2 qts-100 gals/A), zinc sulfate-calcium hydroxide (12-12-100/A), and alternate weekly applications of copper hydroxide and zinc sulfate-calcium hydroxide. Yields were significantly higher in the alternate application program than in the non-treated check, whereas the other treatments evaluated formed intermediate statistical groups. Interestingly, zinc sulfate-calcium hydroxide is immediately available and if the Section 18-Emergency registration is approved for a second year, copper-maneb+zinc combinations will be available for use in 1995. Although some zinc compounds were highly effective such as zinc lignosulfonate and zinc citrate, other zinc compounds like zinc-EDTA were not effective. In 1995, research will need to be continued to determine the effectiveness of these new alternative
treatments against both copper-sensitive and copper-resistant strains of the walnut blight pathogen. Ideally, we hope to develop a walnut blight management program based on bacteriocides with different modes of action that can be alternated in application during the growing season to prevent the development of chemical resistance in populations of *Xanthomonas campestris pv. juglandis*.

INTRODUCTION

In 1993, walnut blight caused losses approaching 60% in many northern California orchards. Less extensive losses, were experienced in other regions of California. One year later, walnut blight did not reach epidemic proportions, however, the disease still caused losses in many orchards, especially those orchards with previous blight losses throughout California. The causal bacterium, *Xanthomonas campestris pv. juglandis* (Pierce) Dye, survives from one year to the next in buds, diseased fruit that remain on the tree, and possibly in twig lesions (Ogawa and English 1991). Furthermore, a considerable percentage of overwintered healthy buds and catkins have epi- and endophytic populations of the bacterium (Miller and Bollen 1946; Mulrean and Schroth 1982). 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. Fruit infections, however, may occur at any time after fruit formation until harvest (Ogawa and English 1991) provided that environmental conditions are conducive for bacterial growth and infection. This extended period of host susceptibility is one of the chief obstacles in control of this disease (Ogawa and English 1991).

Critical information on field environmental conditions required for infection and disease development are not available except that extended wetness periods enhance infection. The optimum temperature for bacterial growth is 28 to 32 C with a minimum of about 5 C and a maximum of 37 C (Breed et al. 1957). Infection of walnut tissues can occur between 5 and 27 C. The rate of disease development on nuts can occur in 4 days at 27 C and 8 days at 15 C, whereas in leaves, infections develop in thirteen days at 16 C and six days at 21 C (Miller and Bollen 1946). Miller and Bollen (1946), using a humidity chamber, also studied the effects of wetness on potted plants with a small number of flowers and fruit that were inoculated and evaluated at the prebloom and early postbloom stages of development. From these studies they concluded that wetness periods of only 5 min for fruit and 15 min for leaves was sufficient for infection of very young tissue that were “water-congested”. From their field research, Miller and Bollen (1946) concluded that rainfall is the most significant factor in the dissemination of inocula and that the greater the rainfall (amount in inches) the greater the disease incidence. We believe that extended and periodic wetness periods were responsible for the epidemic outbreak of walnut blight in 1993. Wetness periods, temperature, wind, and relative humidity are weather parameters that need to be more critically evaluated in the field using modern equipment (dataloggers) to determine their role in establishing bacterial infection of young and older fruit. Based on our preliminary data obtained in 1993 and our 1994 data, both the duration of wetness and temperature can affect the onset and severity of walnut blight, but durations longer than 5 min are required. Thus, research is needed to determine the critical environmental parameters for disease development under field
conditions. This information may then be utilized in determining appropriate cultural practices and bacteriocide treatments to minimize disease losses.

Chemical sprays and dusts have been the most commonly used control practices for walnut blight. Materials recommended to date include copper-based compounds and antibiotics (streptomycin). The effectiveness of these treatments has been related to the number and duration of wetness periods during the development and maturation of the crop. Failures in blight control are related to lack of protection during environmental conditions conducive to infection and disease development, as well as to copper-resistant populations of the bacterial pathogen.

Dependence on any one chemical treatment for management of a disease, potentially can lead to the loss of efficacy of the treatment due to the development of resistance in the 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). Thus, research is needed to find alternative materials that are more efficacious than copper-based compounds and antibiotics for the control of walnut blight. Materials that could be used immediately and that need to be evaluated include zinc-based compounds and EBDC fungicides such as maneb+zinc, mancozeb, and zineb that can be used alone or in combination with copper- or zinc-containing compounds. Conover and Grenhold (1981) have indicated that copper-maneb or copper-mancozeb mixtures were more effective than copper alone for managing bacterial spot of tomato caused by Xanthomonas campestris pv. vesicatoria. Others have also indicated this and that these mixtures were effective for bacterial speck of tomato caused by Pseudomonas syringae pv. tomato. The enhanced activity of these combinations was later attributed to an antibacterial component of EBDC fungicides, ethylenethiram monosulfide. In 1993, Olson et al. (Walnut Research Reports) reported that a copper-maneb+zinc (Kocide 101*-Manex*) mixture was the most effective treatment for control of walnut blight in their previous year trials. Based on this research, a Section 18-Emergency Registration was granted for Kocide 101-Manex mixtures for counties in northern California. Additional research is needed to confirm and compare Kocide-Manex treatments to other bacteriocidal treatments.

Based on research for control of bacterial spot caused by either Cu-sensitive and Cu-resistant populations of Xanthomonas campestris pv. vesicatoria, Adaskaveg and Hine (1985) indicated that zinc-based compounds such as zineb were effective. Others have also observed that basic zinc sulfate (Bertrand et al. 1985; Miller and Gorsuch 1985) and ziram (Zehr et al. 1991) were effective for control of bacterial spot of peach caused by Xanthomonas campestris pv. pruni. Miller and Bollen (1946) also indicated that zinc sulfate-calcium hydroxide mixtures were efficacious for control of walnut blight. Because copper-based treatments were generally more effective than zinc compounds and Cu-resistant strains were not previously reported, most growers used copper treatments. Thus, zinc-based compounds and new experimental bacteriocidal compounds (e.g., PHMB) that are already registered with the United States Environmental Protection Agency for non-agricultural, water purification use need to be evaluated as potential materials that can be used in a management program of walnut blight.
OBJECTIVES

I. Develop critical data on environmental conditions conducive to walnut blight on young and developing fruit from early to late spring (April-June), as well as on leaves of potted plants

A) Growth chamber and greenhouse studies using leaves of potted plants to determine the role of wetness periods and temperature on disease development

B) Bagging studies using fruit on trees to determine the role of wetness periods on disease development under ambient temperatures and other environmental conditions

C) Environmental monitoring under field conditions throughout the spring to determine the effect of microclimates (wetness periods, temperatures, relative humidity, wind, and rainfall) on walnut blight development

II. Compare alternative bacteriocides and other chemical treatments to copper-based compounds for management of walnut blight. Chemicals to evaluate include: zinc-based compounds (zinc sulfate, zinc-sulfate-calcium hydroxide mixtures, zinc oxide, ziram, and zinc lignosulfonates), EBDC fungicides (mancozeb, mane+zinc, zineb) alone or in mixtures with copper or zinc-based compounds, and experimental compounds (hydroxyquinolate, aluminum tris-ethyl-phosphonate, and polyhexamethylene biguanide or PHMB).

A) Laboratory evaluations of bactericide assays including: Direct Dilution, Disk Assay, and Amended Media

B) Small scale field trials with registered and non-registered, experimental compounds

C) Large scale field trials with registered and experimental studies (large scale trials included efficacy of bacteriocides for disease management and effect of bactericide spray programs on crop yield

PROCEDURES

Identification and pathogenicity of isolates of Xanthomonas campestris pv. juglandis. Isolates of Xanthomonas campestris pv. juglandis were isolated from infected walnut fruit. For this, tissue was excised from the margin of a lesion, surface sterilized for 1 min in 400 ug/ml Cl that was prepared from 5.25% sodium hypochlorite, the tissue was rinsed in sterilized distilled water (SDW) for 1 min, blotted dry, placed in 1-2 ml of SDW, ground using a mortar and pestle, and a loopful of the aqueous suspension was struck onto nutrient agar (NA). Yellow bacterial colonies were transferred and grown on brilliant cresyl-blue starch medium (BSM) and characteristic colonies were re-transferred to NA. Selected isolates were also Gram stained and grown on SQY, YDCP and LB media for positive cultural identification of the bacterium. Additionally, isolates were characterized using the Biolog GN Microlog assay. Four copper (Cu)-sensitive and four Cu-resistant isolates were also shown to be pathogenic in the inoculation studies described below. Positively identified isolates were stored in 10 ml of SDW at 5 C.
Inoculation studies of fruit to determine the effect of wetness periods on the development of walnut blight in the field. Inoculum was prepared for each isolate evaluated by suspending a 48-hr-old culture that was grown on NA in sterile distilled water at pH 7.0. A Bausch & Lomb Spectronic 20 spectrophotometer set at 620 nm was used to adjust the inoculum concentration. A bacterial suspension with a transmittance reading of 70% was found to contain 1 x 10^8 colony forming units (cfu) per milliliter as determined by dilution plating. Bacterial suspensions containing 1 x 10^6 cfu/ml were made by dilution and the suspensions were hand sprayed until runoff on fruit of terminal branches of mature walnut trees cv. Hartley. Immediately after inoculation, selected branches with inoculated fruit were covered with plastic-lined, brown paper bags that contained a wet paper towel, and the bag ends were tied around the branch with wire to seal the bag. Treatments included non-inoculated, non-inoculated & bagged, inoculated (0 hr inoculation), and inoculated & bagged for 1, 6, 12, 24, or 48 hours of wetness. Fruit required approximately 30 minutes to dry after bags were removed. After 10-14 days, disease incidence was evaluated as the number of fruit infected of the total number fruit inoculated; whereas, disease severity was determined as the average number of lesions per fruit. These experiments were done once in mid-May and once in early June. Temperature and wetness periods were recorded in the field using electronic weather sensors and a datalogger (Campbell 21X). Four replications of 4-7 fruit (1 replication / treatment / tree) were used and the experiment was done twice. Data were analyzed using regression, analysis of variance, and general linear model procedures of SAS 6.04.

Additionally, walnut fruit were inoculated on seven dates from May 1 to July 30, exposed to 48 hr wetness periods, and evaluated as described previously. Disease incidence and severity were plotted against inoculation date. Weather data from the Davis, Yolo County, CIMIS station were accessed for temperature and rainfall data for the period of these inoculation experiments.

Inoculation studies using potted plants to determine the effect of wetness periods and temperature on walnut blight. Nuts from walnut cv. Hartley were collected in the fall of 1993, stored in moist peat moss for the winter, and germinated in flats of sterilized potting soil in the spring of 1994. Plants were transplanted into pots and grown in a lathouse at the Department of Plant Pathology Field Station. To evaluate the effect of wetness periods on infection of leaves, potted plants were inoculated as described in the previous section, covered immediately after inoculation with clear plastic bags, and the ends of the bags were secured to the pot using a rubber band. Treatments consisted of non-inoculated, inoculated, and inoculated & bagged for 24, 48, 72, 96, or 120 hr of wetness. All treatments were incubated on a bench in a shaded and temperature-regulated greenhouse set for 20-25 C. After 10-14 days, disease was evaluated on the third and forth leaf of each potted plant. Incidence (no. of infected leaflets) and severity (no. of lesions/leaflet) of disease was determined for the third and forth compound leaves. Data were analyzed using linear regression and analysis of variance procedures of SAS 6.04.

For growth chamber experiments, potted plants were placed in growth chambers set for 15, 20, 25, or 30 C two to three days before inoculation. Plants were inoculated as described above, however the inoculum concentration was 1 x 10^8 cfu/ml. Plants were exposed to 48, 72, 96, or 120 hours of wetness at each temperature evaluated. After 7-10 days, the incidence (no. of leaflets infected) and severity (number of lesions per leaflet) of disease were evaluated for the
third and forth compound leaves. There were four single-plant replications for each wetness period at each temperature and the experiment was repeated twice. Data was analyzed as a factorial experiment using general linear model procedures of SAS 6.04.

For both greenhouse and growth chamber experiments, temperatures were monitored with high/low thermometers and relative humidity was recorded in the greenhouse and in each growth chamber using a motorized psychrometer. Subsamples of lesions from each experiment were re-isolated to verify the causal agent. Isolation procedures described in the isolation, identification, and pathogenicity section were used.

**Disease evaluations and environmental monitoring using dataloggers in commercial walnut orchards and weather data from UCIPM-CIMIS.** In four commercial orchards located in Butte and Tehama counties, 400-600 fruit in 4 to 6 replications (100 fruit/replication) were tagged and monitored periodically for the development of walnut blight from 1 April to 30 June 1994. Fruit were carefully examined for lesions and positive evaluations were double checked in subsequent evaluations. Isolations were made for the bacterial pathogen from subsamples of infected fruit to verify blight incidence. In two of the commercial orchards evaluated, electronic sensors and a datalogger (Campbell 21X) were used to monitor leaf wetness, temperature, relative humidity, rainfall, windspeed, and wind direction. Dataloggers were programmed to make readings every minute and to record hourly averages for each microenvironmental parameter throughout the evaluation period. Environmental data were downloaded and summarized. Disease incidence and the recorded microclimate data were plotted for the calendar dates of the evaluation for each orchard evaluated. Duration of wetness data was summarized as a seasonal accumulation hours of wetness with values greater than a critical value of 10 on a scale of 0 to 100. These data were analyzed using regression and general linear model procedures using SAS 6.04 for correlating disease incidence and wetness duration as a polynomial model.

**Laboratory evaluation on the toxicity of copper and non-copper based bacteriocides to copper sensitive and copper resistant strains of Xanthomonas campestris pv. juglandis.** To determine the toxicity of copper and zinc to copper (Cu)-sensitive and Cu-resistant strains of *X. campestris pv. juglandis*, copper sulfate and zinc citrate (Zinc-All-7.5%) were used in a modified assay of Thompson's direct dilution method. A stock solution of zinc citrate (3600 ug Zn/ml) and a stock solution of copper sulfate (4800 ug Cu/ml) were prepared. Each solution was sterilized by filtration through a sterile 0.1 um Millipore filter, and serial dilutions were made by the sequential 1:1 addition of either chemical solution and a suspension of each strain of the bacterium (prepared at a concentration of 1 x 10^8 cfu/ml). The final concentration ranged from 0-1800 ug Zn/ml for the zinc citrate solution and 0 - 2400 ug Cu/ml for the copper sulfate solution. After 1 hour of incubation at 25 C, the viability of each strain exposed to each chemical concentration was determined by plating 100 ml of the bacterial chemical mixtures onto NA. The number of colonies per plate was determined 48 hr after incubation at 27 C. The concentrations of copper and zinc in the serial dilutions was confirmed by atomic absorption spectrophotometry. The direct dilution assays were done twice with three replications for each dilution. The data were analyzed using regression and analysis of variance procedures of SAS 6.04 and presented as a regression of the log_{10} concentration of the number of bacterial colonies growing on the concentration of either copper or zinc in each dilution.
Four Cu-sensitive and four Cu-resistant strains of *X. campestris pv. juglandis* were evaluated for their sensitivity to several copper- and zinc-containing compounds, as well as other experimental compounds using in vitro, agar disk assays. Chemical concentrations initially tested were based on dosages recommended by respective manufacturers for use in the field. Because copper bacteriocides were being used at the rate of 4 lb Cu/A (based on metallic ion equivalent), in vitro assays of both copper and zinc materials were done for the following concentration: 4800, 2400, 1200, 600 ug metallic ion/ml. The following copper- or zinc-based compounds were evaluated: copper hydroxide (Kocide 101), cuprous oxide (Nordox), zinc citrate (Monterey Zinc All 7.5%), zinc sulfate, zinc sulfate-calcium hydroxide mixtures, zinc oxysulfate (Monterey Micronized Neutral Zinc), zinc-EDTA (Hamp-ene Zinc 9%), zinc lignosulfonate complex (Liquid Zinc 7%), ziram, and zineb. EBDC fungicides evaluated included mancozeb (Dithane DF), maneb+zinc (Manex), and zineb (Zineb). Rates used of these materials were based on the Section-18 for Manex at 2 qts/100 gals/A (2400 ug/ml). Thus, rates of maneb+zinc evaluated in vitro were 2400, 1200, 600, and 300 ug ai/ml; rates for mancozeb (2 lbs/100 gal/A) were 1800, 900, 450, and 225 ug/ml; whereas, zineb was evaluated at 2400, 1200, 600, and 300 ug Zn/ml. Maneb+zinc and mancozeb were also evaluated in selected combinations with copper and zinc compounds at the same rates for each material. Experimental compounds and the rates evaluated were: aluminum tris-ethyl-phosphonate (Aliette - 4800, 2400, 1200, 600 ug ai/ml), hydroxyquinolate (Beltanol - 2000, 1000, 500, or 250 ug ai/ml), and polyhexamethylene biguanide (PHMB - 50, 100, 500, or 1000 ug ai/ml). For the disk assay, analytical paper disks (12.7 mm diam.) were infiltrated with 100 ul of each chemical solution or mixed chemicals before use. One hundred microliters of each bacterial strain (1 x 10^8 cfu/ml) were spread evenly over NA plates (10 ml/plate) with a sterile glass rod, and allowed to dry. Four treated paper disks (one of each concentration were placed on the NA and tapped lightly to ensure even contact. All plates were incubated at 27 C and the size of inhibition zones were recorded after two days from the outer edge of the disk to the margin of bacterial growth. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

**Small scale field trials to determine the efficacy of copper and non-copper based bacteriocides.** Based on the results of the in vitro disk assays, the following chemicals were evaluated in hand spray trials: zinc sulfate-calcium hydroxide, hydroxyquinolate (Beltanol), ziram, copper hydroxide (Kocide 101), copper hydroxide-maneb+zinc (Manex) and PHMB. For this, chemicals were sprayed to runoff on fruit of walnut cv. Hartley and allowed to dry (2-3 hours). Fruit were then inoculated with aqueous suspensions of a Cu-sensitive strain of *X. campestris pv. juglandis* (1 x 10^8 cfu/ml), bagged for a 48-hr wetness period, and allowed to air-dry as described previously. After 10-14 days, disease incidence and severity were determined also as described previously for fruit evaluations. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

**Large scale field trials to determine the efficacy of copper and non-copper based bacteriocides on the incidence of walnut blight.** In four commercial orchards, large scale efficacy trials were established. In two orchards, one in Butte Co. and one in Tehama Co., plots were designed to determine the efficacy of bacteriocides programs and to determine the effect of these programs on crop yield. The other two trials were designed to determine the efficacy of

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bacteriocides. All treatments were applied in 7-10 day intervals for a total of 8 applications using an air-blast sprayer at a rate of 100 gal/A. In the first two plots the treatments were: non-treated check, copper hydroxide (Kocide 101-4 lb Cu/A), copper hydroxide-maneb+zinc (Manex-2 qts/A), zinc sulfate-calcium hydroxide (4 lb Zn/A) mixed in a 1:1 ratio, and an alternate application of zinc sulfate-calcium hydroxide and copper hydroxide (4 applications of each treatment). Applications began at rebloom (late March) and were completed by late May. For the Butte plot, there were six replications (14 trees/replication) for each treatment; whereas in the Tehama plot there were 5 replications (28 trees/replication) for each treatment. Three weeks after the last application, incidence of walnut blight was determine based on a 150 nut sample from three trees in each replication for each treatment. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04

In the second two plots (both in Butte Co.), treatments applied were: aluminum tris-ethylphosphonate (Aliette - 5 lb/100 gal), cuprous oxide (Nordox-4 lb Cu/100 gal), zinc oxide (Monterey Neutral Zinc-4 lb Zn/100 gal), cuprous oxide (4 lb Cu/100 gal )-mancozeb (Dithane DF-2 lb/100 gal), and ziram (12 lb/100 gal). Applications were made every 7 to 10 days and began at rebloom (late March) and were completed by late May. In the first plot, there were five replications (3 trees/replication) for each treatment. In the second plot, there were six replications (1 tree per replication) for each treatment. Three weeks after the last application, incidence of walnut blight was determine based on a 150 nut sample from each replication for each treatment. Data from the two plots were combined and were analyzed using general linear model and LSD mean separation procedures of SAS 6.04

**Evaluation of alternative-bactericde spray programs on crop yield.** In the first two plots, established in two commercial orchards described in the previous section, yields of each replication for each treatment were obtained. In the Butte Co. plot, each replication was 14 trees long by 5 rows wide. The center test row was bordered on either side by two rows that also received the same treatment. This was to prevent spray drift from affecting adjacent treatments. In the Tehama plot, only a single row was treated. Two adjacent rows on either side of the treated row were left untreated and served as buffer rows to prevent spray drift from effecting adjacent treatments. For obtaining the yield, trees in the center row were shaken, the fallen nuts were wind-rowed, and harvested into special trailers equipped with a electronic scale and sensors to determine the gross weight from each replication. A subsample was obtained for each replication of each treatment, each subsample was weighed, hulled, dried, and re-weighed. Net weight of harvested walnuts was corrected for water loss. Subsamples (150 nuts) of each replication of each treatment were sent to Diamond Walnut in Stockton, CA and the Dried Fruit Association evaluated nut quality. Gross weights, corrected net weights, and numbers of nut kernels scored as damaged in quality evaluations were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

**Preliminary evaluation of commercial orchards for the presence of copper-resistant strains of Xanthomonas campestris pv. juglandis.** Fifteen orchards were surveyed in Yuba Co. in early spring to evaluated if Cu-resistant populations of *X. campestris pv. juglandis* were present. Samples were collected from three trees in fifteen commercial orchards. Three to five female buds were excised from branches of each tree, sterilized in 400 ug Cl/ml for 1 min as
described above, rinsed in SDW for 1 min., and blotted dry. Buds were then ground using a mortar and pestle in 1-2 ml of SDW, and a loopful of the liquid was struck onto NA. Three isolates (one per sampled tree) were obtained, each were evaluated on the BSM medium, re-grown on NA, and evaluated for Cu-resistance on NA using the disk assay and copper hydroxide (Kocide 101-4800, 2400, 1200, or 600 ug/ml) as described previously. Isolates with no inhibition zones at a 1000 ug/ml were considered moderately Cu-resistant.

**Determination of potential changes in sensitivity of copper-resistant strains of the bacterium after one or multiple copper applications.** In two orchards considered to have Cu-resistant populations of *X. campestris pv. juglandis*, six isolates were obtained from non-treated trees (checks) and six isolates were obtained from trees sprayed with copper hydroxide (Kocide 101). Sampling times were after one spray application and after eight spray applications. In a third orchard, with no copper resistance six isolates were also obtained from non-treated trees. Isolates were evaluated for Cu-resistance on NA using the disk assay and copper hydroxide (Kocide 101-4800, 2400, 1200, and 600 ug/ml) as described previously. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.04.

**RESULTS AND DISCUSSION**

**Wetness periods and temperature effects on walnut blight Infection in experimentally inoculated trees.** Incidence and severity of walnut blight on fruit increased with increasing wetness periods (Fig. 1A, B). The increase in disease on fruit could not be described adequately using a simple linear regression and thus, polynomial models were evaluated. Quadratic equations were significant (*P* < 0.01) and described the data with *R*² values of 0.92 for the incidence data and 0.85 for the severity data. The disease increased dramatically between the 12 and 24 hr treatments. No disease developed in the 0 hr-wetness period and less than 0.5 lesions per nut developed on less than 25% of the inoculated nuts in the 1 hr-wetness period treatment.

Considering that the inoculum level was much higher than levels immediately encountered at the onset of a wetness event (e.g., rainfall) in the field, wetness periods less than 12 hr probably do not represent an infection period under natural levels of inoculum. As indicated in the procedures, the experiments were done in May and early June when fruit were approximately 20-30 mm wide. This could also explain the differences in our results compared to those of Miller and Bollen (1946). In their research, the fruit tissue evaluated was “water-clogged” in the prebloom and early postbloom stage and they noted that older fruit were less susceptible to infection. In our studies, we also observed a dramatic decrease in the incidence and severity of disease in inoculation studies from May to July (Fig. 2A,B). As Miller and Bollen (1946) indicated, these differences in tissue susceptibility could be attributed to stomatal function and age of tissue. Other factors that could also influence bacterial infection of walnut are environmental conditions observed from mid-June through July (Fig. 3). Interestingly, daily high temperatures were commonly greater than 29 C (85 F) from about mid-June. These temperature are higher than the range of 5-27 C reported for infection of walnut by *X. campestris pv. juglandis*.

In greenhouse studies, leaves of potted plants were infected in wetness period treatments greater than 24 hours (Fig. 4). For the 48-120 wetness periods, the disease incidence and severity was linear with *R*² values greater than 0.93. In growth chamber studies, optimal conditions were
between 72 and 96 hr wetness periods at 20 C for maximum incidence and severity of disease on leaves (Fig. 5). Again these wetness period values are much higher than those reported for leaf infection by Miller and Bollen (1946), but leaf age between the two studies was dramatically different. In our studies, the leaves were approximately 10-14 weeks old. Evaluating the range of temperatures, little to no infection (0.4 lesions per leaf) occurred at 30 C. Although some infection did occur at 30 C, these results are probably a result of the experimental technique. Furthermore, the potted plants were stressed at 30 C because mesophyll breakdown was observed in some leaves. Thus, temperatures of 15 to 25 are conducive to disease development. Based on inoculation results from fruit and leaves, the critical factors influencing disease development are duration of wetness periods and probably initial inoculum concentration and the function of stomata before and during potential infection periods. These parameters need to be continued to be evaluated.

**Epidemiology of walnut blight in the field.** Incidence of walnut blight and environmental parameters during the spring are shown for the large-scale Tehama Co. plot (Fig. 6), the large-scale Butte Co. plot (Fig. 7), and the two small scale Butte Co. plots (Fig. 8). Rainfall and maximum/minimum temperatures from CIMIS stations in Butte and Tehama Co. are shown in Fig. and 9, respectively. Regressions of the incidence of blight on the accumulation of wetness periods greater than the critical value of 10 are shown for the large scale Tehama plot (Fig. 10), for the Butte plot (Fig. 11), and for both plots combined (Fig. 12). On an arbitrary scale of 0-100 with 0 being dry and 100 being completely wet, the critical value of 10 (LW10) was selected because at that value a film of wetness was visible on fruit surfaces. The incidence of blight at the end of spring in the Tehama and Butte plots was approximately 18% and 52%, respectively. Comparing the two plots, both had the same number of rainfall events and similar total rainfall, however the Tehama plot had only 186 total hours of wetness, while the Butte plot had 271 hr of wetness. The data for both plots (Fig. 12) fit a linear regression, however the quadratic equation was significant and had a higher \( R^2 \) value. Theoretically, if we consider a 48-hour wetness period as an infection period for blight based on our inoculation studies, we could determine that the Butte plot had 5.6 infection periods, whereas the Tehama plot had only 3.8 infection periods. Although this is preliminary model, this information can possibly be utilized toward the development of a predictive management model for walnut blight.

**Laboratory evaluations of alternative bacteriocides to copper.** The toxicity of copper and zinc compounds against Cu-sensitive and Cu-resistant populations of *X. campestris pv. juglandis* using the direct dilution method is shown in Fig. 13A, B. Generally, the Cu-sensitive strain was extremely sensitive to copper reducing bacterial colonies to only a few per plate at concentrations of 9-37 ug/ml and copper was completely inhibitory at 150 ug/ml (Fig. 13B). The Cu-resistant strain evaluated had numerous colonies even at concentrations greater than 900 ug/ml. Regressions of \( \log_{10} \) of the number of bacterial colonies on the concentration of copper were significant \( (P < 0.05) \) and linear. Equations were: \( Y = 1.4 - 0.01X \), \( R^2 = 0.70 \) for Cu-sensitive and \( Y = 2.6 - 0.01X \), \( R^2 = 0.75 \) for Cu-resistant strains. Statistical comparisons of the two regressions were significant \( (P < 0.01) \) with midpoints significantly different \( (P < 0.01) \) but not slopes \( (P > 0.21) \). Thus, the toxicity of copper to the Cu-sensitive and Cu-resistant strains was significantly different.
In evaluating the toxicity of zinc to the Cu-sensitive and Cu-resistant strains, both strains were equally sensitive to zinc, reducing bacterial colonies to an average of less than two per plate at 450 ug/ml. The regression equations were significant (P < 0.05) and were: \( Y = 2.7 - 0.004X \), \( R^2 = 0.94 \) for Cu-sensitive and \( Y = 2.5 - 0.004X \), \( R^2 = 0.86 \) for Cu-resistant strains. Statistical comparisons of the two regressions were significant (P < 0.01) with midpoints and slopes not significantly different (P > 0.98). Thus, the toxicity of zinc to the Cu-sensitive and Cu-resistant strains was not significantly different. This data confirmed that Cu-sensitive strains are more sensitive to copper than zinc and that Cu-resistant strains were more sensitive to zinc than copper.

In disk assays, copper hydroxide (Kocide 101) and copper hydroxide-maneb+zinc (Kocide-Manex) had significantly larger inhibition zones than copper hydroxide and cuprous oxide alone and cuprous oxide-mancozeb (Nordox-Dithane DF) treatments (Fig. 14 A). Perhaps the lower rate of mancozeb used 1800 ug/ml compared to the manebl+zinc (2 lb/A - 2400 ug/ml) significantly influenced the size of the inhibition zone. Regardless of differences in inhibition zone size, all materials were inhibitory to the Cu-sensitive strains. Copper-resistant strains were not sensitive to copper hydroxide or cuprous oxide (Fig. 14B), however, all other materials evaluated were significantly more inhibitory than copper compounds using this assay.

Zinc-containing compounds and mixtures with EBDC fungicides were evaluated and compared to copper hydroxide (Kocide 101) using the disk assay (Fig. 15). In summary, some zinc formulations were not effective whereas, other formulations were very inhibitory. For example, Zn-EDTA and zinc 9% (Hampene zinc) were not effective but zinc 7% (zinc lignosulfonate complex) and zinc citrate were very inhibitory. Zinc compounds toxic to Cu-sensitive strains were also toxic to Cu-resistant strains (Fig. 15A, B). As indicated above, Cu-sensitive strains were inhibited by copper hydroxide, whereas, Cu-resistant strains were not. Interestingly, zinc compounds that were toxic were more toxic in mixtures with manebl+zinc (Manex) similar to copper -maneb+zinc combinations. These results indicate that the zinc 7% and the zinc 7.5% formulations should be evaluated in large scale plots next season.

Toxicity of experimental compounds to *X. campestris* pv. *juglandis* in the in vitro assays is shown in Figure 16. Aluminum tris-ethyl-phosphonate (Aliette) was not inhibitory in these assays; whereas, PHMB and hydroxyquinolate were significantly more inhibitory than copper hydroxide. Although hydroxyquinolate probably will never be registered, PHMB is all ready registered with the US-EPA for non-agricultural, water purification use at 50 ug/ml. For all the in vitro evaluations, no cross resistance was observed for copper and any other compound evaluated (ie., Cu-resistant strains were sensitive to all alternative bacteriocides tested).

**Field evaluations of alternative bacteriocides to copper.** Efficacy of selected compounds that were toxic to Cu-sensitive and Cu-resistant strains of *X. campestris* pv. *juglandis* in vitro, were evaluated in hand-sprayer trials (Fig. 17). The most promising materials included PHMB, copper hydroxide-maneb+zinc (Kocide 101-Manex), ziram, and zinc sulfate-calcium hydroxide. All of which significantly reduced both the incidence and severity of walnut blight.

In large scale efficacy trials in two commercial orchards in Butte and Tehama Co., copper hydroxide-maneb+zinc (Kocide 101-Manex), zinc sulfate-calcium hydroxide, and alternate
applications of Kocide and zinc sulfate-calcium hydroxide had significantly lower disease incidence than non-treated and copper hydroxide (Kocide 101 alone) treatments. In June evaluations of both plots, the copper-maneb+zinc treatment had significantly lower incidence of disease than all treatments (Figs. 18, 19). In smaller scale studies in commercial orchards in Butte Co., aluminum tris-ethyl-phosphonate (Aliette) was not significantly different than the check, whereas cuprous oxide (Nordox), cuprous oxide-manozeneb mixtures, zinc oxide, and ziram had significantly less disease than the check treatment. Of the materials that were effective, ziram had the lowest incidence of disease (Fig. 20).

In the two large scale trials in Butte and Tehama Co., yield data was also obtained for each of the bactericide programs (Fig. 21). Of the treatments evaluated in each plot, only the program of alternating copper hydroxide and zinc sulfate-calcium hydroxide had significantly higher yields than the non-treated check. The other treatments including the copper hydroxide-maneb+zinc treatment, the zinc sulfate-calcium hydroxide, and the copper hydroxide treatment formed intermediate-yield groups. No difference were detected between treatments in quality evaluations of harvested nuts determined by the DFA of California.

Monitoring orchards for copper resistant strains of X. campestris pv. juglandis. From 31 isolates of Xanthomonas campestris pv. juglandis from 15 orchards in Yuba Co., 14 were considered moderately resistant to copper, whereas 17 were sensitive to copper (Fig. 22). Evaluation of the data by orchard indicated that 7 orchards did not have Cu-resistant populations of the organism, whereas, 8 had one or more Cu-resistant isolates (Fig. 22). Orchards that had never been sprayed with copper did not have Cu-resistant populations. In two orchards with Cu-resistant populations and in one orchard with Cu-sensitive populations of the walnut blight organism, no changes were observed in sensitivity of isolates collected after one copper application or after eight applications of copper hydroxide (Fig. 23). Thus, Cu-resistant isolates remained resistant to copper and the Cu-sensitive isolates remained sensitive to copper even after multiple applications.

REFERENCES


Fig. 1. Curvilinear Regressions of Walnut Blight Incidence on Wetness Period Duration of Fruit Inoculated with Xanthomonas campestris pv. juglandis

A. Spring 1994 -

B. Spring 1994 -

Fruit were inoculated with suspensions of X. c. pv. juglandis (1 x 10^6 cfu/ml), exposed to defined wetness periods, and evaluated after 10-14 days.

Fig. 2. Incidence and Severity of Walnut Blight of Fruit Inoculated with Xanthomonas campestris pv. juglandis During the Growing Season

A. B.

Fruit were inoculated with suspensions of X. c. pv. juglandis (1 x 10^6 cfu/ml), exposed to 48 hr wetness periods, and evaluated after 10-14 days.
Fig. 3. Rainfall and Temperatures in Yolo Co. from May - July 1994
- CIMIS Data -
Fig. 4. Linear Regressions of Walnut Blight Incidence on Wetness Duration of Leaves of Potted Plants Inoculated with Xanthomonas campestris pv. juglandis - Shaded Greenhouse Studies -

A.

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Blight Incidence (%)
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Linear:
Y = 55 + 0.3X, R^2 = 0.94, P>F 0.03
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Leaf Wetness (hr)
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B.

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Severity
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Linear:
Y = -32 + 0.3X, R^2 = 0.93, P>F 0.03
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Leaf Wetness (hr)
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Potted plants were inoculated with suspensions of X. c. pv. juglandis (1 x 10^6 cfu/ml), bagged for 48, 72, 96, or 120 hrs of wetness, incubated on a greenhouse bench (Max. 25 C), and evaluated after 10-14 days. No infection occurred in wetness periods less than 24 hours.

Fig. 5. Effects of Leaf Wetness Period and Temperature on the Incidence and Severity of Walnut Blight on Leaves of Hartley - Growth Chamber Studies -

A.

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Incidence (%)
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48 72 96 120 30
0 10 20 30 40 50 60
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B.

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Severity
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48 72 96 120 30
0 1 2 3 4 5
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Potted plants were inoculated to run-off with cell suspensions of X. campestris pv. juglandis (1 x 10^8 cfu/ml), exposed to defined wetness periods, incubated for 7-10 days at each temperature and evaluated for leaflet infection. Sub-samples of lesions were surface sterilized and re-isolated to verify the bacterial pathogen. Values are the average of 8 leaves (2 leaves/plant) for each of three experiments.
Fig. 6. Incidence of Walnut Blight and Environmental Parameters in a Commercial Orchard - Tehama Co.

Incidence of walnut blight is based on infected nuts per 100 nut sample for each of 4 replications.

Fig. 7. Incidence of Walnut Blight and Environmental Parameters in a Commercial Orchard - Butte Co.

Incidence of walnut blight is based on infected nuts per 100 nut sample for each of 6 replications.
Fig. 8. Incidence of Walnut Blight in Two Commercial Orchards and Rainfall-Temperature Data for Butte Co. (CIMIS)

Incidence of walnut blight is based on infected nuts per 100 nut sample for each of 6 replications.

Fig. 9. Rainfall and Temperature Data for Tehama Co. (CIMIS)
Fig. 10. Curvilinear Regressions on the Incidence of Fruit Walnut Blight on the Accumulation of Leaf Wetness Hours

- Tehama Plot: Spring 1994 -

Data obtained from a datalogger in the Tehama plot.

Fig. 11. Curvilinear Regressions on the Incidence of Fruit Walnut Blight on the Accumulation of Leaf Wetness Hours

- Butte Plot: Spring 1994 -

Data obtained from a datalogger in the Butte plot.

Fig. 12. Curvilinear Regressions on the Incidence of Fruit Walnut Blight on the Accumulation of Leaf Wetness Hours

- Butte and Tehama Plots: Spring 1994 -

Data from dataloggers in Butte and Tehama plots.
Fig. 13. Toxicity of Copper and Zinc Against Cu-Sensitive and Cu-Resistant Strains of *Xanthomonas campestris* pv. *juglandis* - Direct Dilution Assay -

Copper or zinc was supplied from copper sulfate or 7.5% Zinc All (Monteray Chemical Co.). For the direct dilution assay, $1 \times 10^5$ cfu of *X. c. pv. juglandis* were exposed to each dilution. After 1 hour, 100 µl of the bacterial-bactericide suspension was placed in petri plates containing nutrient agar, incubated for 3 days, and the number of colonies per plate were determined.

Fig. 14. Toxicity of Copper, EDBC, and Cu & EDBC-Mixtures Against Cu-Sensitive and Cu-Resistant Strains of *X. c. pv. juglandis* - In vitro Disk Assay -

A filter disk saturated with each chemical was placed on nutrient agar that was covered with 100 µl of a bacterial suspension ($1 \times 10^5$ cfu/ml). The plates were incubated for 3 days and inhibition zones were measured.
Fig. 15. Toxicity of Zinc, EDBC, and Zn & EDBC-Mixtures Against Cu-Sensitive and Cu-Resistant Strains of X. c. pv. juglandis
- In vitro Disk Assay -

A filter disk saturated with each chemical was placed on nutrient agar that was covered with 100 μl of a bacterial suspension (1 x 10^8 cfu/ml). The plates were incubated of 3 days and inhibition zones were measured.

Fig. 16. Toxicity of Copper Compared to Experimental Compounds Against Cu-Sensitive and Cu-Resistant Strains of X. c. pv. juglandis
- In vitro Disk Assay -

A filter disk saturated with each chemical was placed on nutrient agar that was covered with 100 μl of a bacterial suspension (1 x 10^8 cfu/ml). The plates were incubated of 3 days and inhibition zones were measured.
Fig. 17. Efficacy of Chemical Treatments for Management of Walnut Blight on Inoculated Fruit in the Field
- Copper Sensitive Strain -

Data based on two field experiments. For each chemical treatment, chemicals were applied to four replications of 5-7 nuts, air-dried, inoculated with cell suspensions of *X. c. pv. juglandis* (1 x 10^6 cfu/ml), bagged for a 48 hr wetness period, and evaluated after 10-14 days. Severity equals the number of lesions/fruit (X10).
Fig. 18. Efficacy of Copper and Zinc Treatments for Control of Walnut Blight in a Commercial Orchard

- Field Evaluation -

Incidence of walnut blight is based on the number of infected nuts in a 150 nut sample from three trees per replication. Treatments consisted of six 25-tree replications on Ashley and Chandler walnut varieties.

Fig. 19. Efficacy of Copper and Zinc Treatments for Control of Walnut Blight in a Commercial Orchard

- Field Evaluation -

Incidence of walnut blight is based on the number of infected nuts in a 100/150 nut sample from three trees per replication. Treatments consisted of six 14-tree replications.
Fig. 20. Efficacy of Copper, Zinc, or Foesetyl-AL Treatments for Control of Walnut Blight in Two Commercial Orchards
- Field Evaluation -

Incidence of walnut blight is based on the number of infected nuts in a 150 nut sample from three trees per replication. Treatments consisted of six replications.

Fig. 21. Effect of Walnut Blight Spray Programs on Crop Yield
- 1994 Evaluation -

Walnut plots were treated with eight applications of each bactericide treatment from late March to late May. Yields were based on six replications (14-28 trees/rep) of each treatment in each county and were corrected for water loss.
Fig. 22. Evaluation of Cu-Resistance in Sample Populations of *X. campestris pv. juglandis* from 15 Orchards in Yuba County - Disk Assay -

Three isolates from three trees were evaluated from each orchard. A filter disk saturated with Kocide 101 (10-1000 ug Cu/ml) was placed on nutrient agar that was covered with 100 uF of a bacterial suspension (1 x 10^8 cfu/ml). The plates were incubated for 3 days and inhibition zones were measured. No inhibition at 1000 ug/ml = moderate resistance.

Fig. 23.

Monitoring Changes in Cu-Resistant and Cu-Sensitive Sample Populations After Copper Applications in Three Orchards - Disk Assay -

Three isolates from three replications were evaluated from each orchard and sampling time. A filter disk saturated with Kocide 101 (2400 ug Cu/ml) was placed on nutrient agar that was covered with 100 uF of a bacterial suspension (1 x 10^8 cfu/ml). The plates were incubated for 3 days and inhibition zones were measured.