EPIDEMIOLOGY AND MANAGEMENT OF WALNUT BLIGHT

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ABSTRACT

With low rainfall in the spring of 2014, the incidence of walnut blight was low in most walnut production areas in the state. Still, in some orchards moderate levels of disease developed even with properly timed applications of copper-mancozeb. In a survey of 17 of these latter orchards in the Sacramento and San Joaquin valleys, 93 of the 139 strains collected (=67%) were copper resistant at 50 ppm and 41% of the orchards had strains resistant to copper at 75 ppm or higher. Strains with reduced copper sensitivity were also less sensitive to copper-mancozeb. Thus, a shift to higher levels of copper resistance in pathogen populations is occurring that will necessitate a change in management strategies: higher rates of copper and immediate registration of alternative treatments with different modes of action. For blight management, we continued to evaluate copper products, mancozeb, antibiotics, biocontrols, stimulators of plant host defense mechanisms, and other products with potential direct or indirect effects on the pathogen. Dithane, registered on walnuts in 2014 as another mancozeb product, will be available in 2015. In orchards with copper-resistant populations of the pathogen, copper (e.g., Kocide 3000) was ineffective, but was very effective when mixed with mancozeb. In research trials comparing copper products mixed with Manzate, Cuprofix Ultra Disperss, Badge X2, Kocide 3000, and ChampION++ were statistically similarly effective, but the lowest disease incidence was observed with ChampION++. Numerically, ChampION++-Manzate, MagnaBon-Manzate were highly effective at two locations each and Cuprofix-Manzate was most effective at another location. At one location, however, MagnaBon caused some marginal leaf burn. Adding Gavicide (an agricultural oil) to Kocide-Manzate resulted in a trend for improved efficacy; whereas another oil did not. Copper activity was also increased in mixtures with Tanos, Stout, or the thiadiazole derivatives Terrazole and ATD. In lab assays, ATD also increased copper-sensitivity of copper-resistant *Xaj* strains. In field trials evaluating other alternatives, kasugamycin (Kasumin) used by itself showed moderate efficacy, but in mixtures with Manzate, ATD, MagnaBon, or the antibiotic oxytetracycline (Fireline), efficacy was improved and was similar to copper-mancozeb treatments. Fireline used by itself also performed very well. The biocontrols Actinovate and Botector were very effective at low disease pressure and still gave intermediate levels of control at higher disease pressure. In epidemiological studies on bacteria colonizing walnut buds, we again found that *Xaj* commonly occurs together with other yellow-pigmented and non-yellow-pigmented bacteria. In studies on the prediction of walnut blight disease risk, disease levels in 35 orchards based on fruit evaluations (including summer nut drop) in the previous season was a more accurate predictor with an average error of 8.6% than walnut bud populations of *Xaj* collected in the previous fall season with an average error of 21.3% for 2 yr. The best strategy to optimize management efforts is to identify high-risk orchards based on disease levels in previous season and to use environmental forecasts (i.e., XanthoCast) after the pistillate-flower-emergence application of a bactericide.

OBJECTIVES

I. New treatments - Evaluate the toxicity of alternative copper and non-copper based materials against *Xaj* in the laboratory and the efficacy of these materials for managing walnut blight under ambient and simulated rain conditions at Kearney Ag Research and Extension Center (KARE), UC Davis, and in commercial orchards.

A) Compare the efficacy of new copper bactericides such as reduced-MCE products (Kocide 3000, Badge X2) and other copper products (e.g., Magna Bon CS-2005, Cueva)
- Evaluate copper persistence with and without adjuvants under simulated rain conditions
- ATD chemistries as activators of copper toxicity
- Conduct surveys to determine the distribution and incidence of isolates of the pathogen with reduced sensitivity to copper-mancozeb.

B) Optimize the performance of kasugamycin and other antibiotics in mixture rotations with copper, EBDC compounds (e.g., Manzate, Dithane) and other fungicides (e.g., Captan, Quintec) or systemic acquired resistance materials (e.g., Actigard) for sustained usage and stewardship of the antibiotic.
- Support the full-registration of Kasumin in copper or mancozeb mixtures
- Evaluate new formulations of oxytetracycline and other antibiotics
- Evaluate antibiotic persistence with and without adjuvants under simulated rain conditions

C) Investigate the use of novel chemistries and biologicals in mixtures and rotations to protect trees for extended periods.
- Evaluate biocontrols (e.g., Actinovate, Blossom Protect, Double Nickel 55)
- Evaluate inducers of host-defense mechanisms (e.g., SARs - Actigard, Stout, phosphonates)

II. Epidemiology - Continue to evaluate disease development throughout the spring and monitor environmental parameters (e.g., leaf wetness, precipitation, temperature, and relative humidity) that are conducive to bacterial infection of walnut tissues and evaluate populations of the bacterial pathogen using molecular approaches

A) Evaluate and characterize bacterial species in walnut buds (commensals and pathogens)

B) Compare and determine if *Xaj* populations in dormant buds or early summer disease levels (e.g., June drop) can be used to forecast disease risk in orchards in the subsequent spring season (recommended by PRAC – Nov. 2013).

C) Evaluate diversity within *Xaj* populations and rapid identification and quantification of the pathogen using molecular methods (e.g., qPCR).

III. Natural host resistance of new walnut genotypes against walnut blight. New genotypes to be evaluated include Forde, Ivanhoe, and others at KARE.

A) Evaluate if different walnut genotypes support higher or lower populations of *Xaj* in buds.

SIGNIFICANT FINDINGS

With low to moderate rainfall in the spring of 2014, the incidence of walnut blight was generally low to moderate at many locations. Our trials were conducted in orchards with a history of high disease levels or trees were inoculated with the walnut blight pathogen (i.e., at KARE) and thus, efficacy data were obtained for registered and new treatments. We evaluated the copper products Kocide 3000, Badge X2, Cuprofix Ultra Disperss, ChampION++ (previously coded as NUP 12032), and MagnaBon (CS2005) alone or in mixtures with Manzate, oil, antibiotics (Kasumin, Fireline), or new potential enhancers of copper activity such as Tanos and the thiadiazole derivatives ATD and Terrazole. Additionally, we evaluated biocontrols (Actinovate, Botector), stimulators of plant host defense mechanisms (Quintec, Stout, Actigard) alone or in mixtures with other materials, and other products with potential direct or indirect effects on the pathogen (e.g., LWB-2014 and the bacterial membrane disruptor ceragenin). The fungicide Captan that is known to have some antibacterial activity was also mixed with Kasumin. The results can be summarized as follows:

1. Copper-Manzate was highly effective in all trials conducted although a reduced efficacy of this treatment has been reported by growers at some locations.
    - Mancozeb received full registration for use on walnuts in 2013, and Dithane was registered on walnuts in the fall of 2014 based on our research.
    - Kocide 3000 (representing a reduced-copper product) when used by itself was not effective in the presence of copper-resistant populations of the pathogen, but was very effective when mixed with Manzate, as well as with Tanos, Stout, or the thiadiazole derivatives Terrazole and ATD. In laboratory tests, ATD also increased copper-sensitivity of copper-resistant *Xaj* strains.
    - In comparisons of copper products mixed with Manzate, Cuprofix Ultra Disperss, Kocide 3000, ChampION++, and MagnaBon (CS2005) were statistically similarly effective. Numerically, ChampION++-Manzate and MagnaBon-Manzate were most effective at two locations each, and Cuprofix-Manzate at one location. In a comparison of Manzate DF and Manzate Max, both mixed with Cuprofix, Manzate Max resulted in a numerically lower disease incidence.
2. Kasumin used by itself showed moderate efficacy. When mixed with other products such as Manzate, ATD, MagnaBon, or Fireline, efficacy was improved and was similar to copper-Manzate.
3. Among other alternative treatments, the antibiotic oxytetracycline (e.g., Fireline) performed very well. The biocontrols Actinovate and Botector were very effective at low disease pressure and still gave intermediate levels of control at higher disease pressure.
4. Copper resistance was evaluated in 139 *Xaj* strains from orchards in the Sacramento and San Joaquin Valleys and was present at some level in 15 of the 17 locations tested. A high proportion of copper resistant isolates still grew at 75 ppm MCE (as compared to the previously tested 50 ppm) indicating an increase in resistance. Furthermore, many of the high-resistant copper strains, showed a decreased sensitivity to copper-mancozeb, but remained sensitive to mancozeb. This confirms our observations from last year.
5. In epidemiological studies on bacteria colonizing walnut buds, we again found that *Xaj* commonly occurs together with other yellow-pigmented and non-yellow-pigmented bacteria.
6. Similar to last year's report, we again conducted studies to predict walnut blight disease risk and disease levels in the current season. With an average error rate of 8.6% over two years, prediction based on summer fruit drop between mid-June and early July was a better indicator of disease risk than prediction based on bud populations determined at the end of the previous season (late fall) with a 21.4% error over two years. We concluded that the best way to estimate disease risk in the current season and to provide optimum management is to integrate risk assessment based on nut drop in the previous season with forecasting of infection periods in the current season after pistillate-flower-emergence application of a bactericide based on weather forecasts (i.e., XanthoCast).

Walnut blight caused by the bacterium *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is a continuous threat for California walnut growers, especially in central and northern locations where precipitation in the springtime is generally higher than in southern production areas of the state. The incidence of disease varies widely among years, and crop losses can be very high. The pathogen infects catkins, female blossoms, fruit, green shoots, leaves, and buds of English walnut and survives from one year to the next in twig lesions, living and dead buds, and diseased fruit that remain on the tree. Fruit infections occur soon after flowering and these infections account for most of the economic loss. The extended period of host susceptibility and the availability of few effective treatments are the main challenges in controlling walnut blight.

Knowledge on population growth potential and environmental requirements of the pathogen, analysis of wetness period duration and temperature data from field weather stations, as well as actual disease progression led to the development of XanthoCast™ as a model to predict infection periods for walnut blight. The website www.agtelemetry.com provides the basic XanthoCast™ information with XanthoCast indices for weather stations in the northern portion of the California walnut production region where environments are most favorable for the disease. Our timing studies indicated that early female flower emergence applications followed by treatments based on XanthoCast provide the most consistent disease control. A catkin bactericide treatment is only needed when high precipitation occurs in an orchard with a history of high disease levels.

For forecasting disease pressure and the intensity of bactericide management programs in the coming season, others have proposed that the magnitude of female bud pathogen populations as determined between late summer/fall of the previous season and winter prior to bloom can be used (Lindow et al., 2014). We are challenging this concept based on our epidemiological understanding of the disease where development of epidemics is more dependent on environmental conditions than on inoculum levels because *Xaj* populations can very rapidly increase during favorable environments. We are suggesting that disease levels in the previous season (that can be can much more easily obtained than levels of bud populations) can be a reliable indicator for disease risk in the coming season, and that management programs have to be based on environmental conditions. To experimentally support this strategy, we have been comparing bud population sizes and actual disease levels from a large number of orchards in the previous season with disease levels in the current season. In last year's report, we indicated that disease levels in the previous season were the more accurate indicator, and these investigations were continued in 2014.

Our research on the management of walnut blight since 1994 has assisted in the development of copper-EBDC treatments and demonstrated the efficacy of zinc compounds and zinc-EBDC mixtures that can be used to minimize the development of copper-resistant populations of the pathogen. We evaluated numerous copper compounds including copper hydroxide, cuprous oxide, and copper oxychloride, as well as reduced-copper products (e.g., Kocide 3000, Badge). We also showed that products containing mancozeb (Manzate, Dithane, Penncozeb, etc.) instead of maneb are effective in mixtures with copper or the antibiotic Kasumin to control disease caused by copper-resistant populations of *Xaj*. An important outcome of this was the full registration of mancozeb (i.e., Manzate in 2013 and Dithane in 2014) for use on walnuts.

Copper-mancozeb applications are currently the most effective treatment for walnut blight. In recent years, some growers suffered high disease losses although copper-mancozeb treatments were applied. This led to an investigation if shifts in sensitivity to mancozeb could have developed in some *Xaj* populations. In 2013, we confirmed the presence of strains that were less sensitive to copper-mancozeb and we continued our surveys on bactericide activity in 2014. With full registration of mancozeb postponed for so many years and with continuing safety concerns with this compound, we started evaluating potential alternatives for mancozeb in laboratory and field studies. Alternatives include Tanos, Stout, and the thiadiazole derivatives Terrazole and ATD. Several thiadiazoles were compared in laboratory tests in 2013 and the most effective one, ATD, was included in this year's field studies. Additional compounds were tested in 2014, and data are presented in this report.

Over the years, we evaluated many possible alternatives to copper-EBDC treatments. Among them, kasugamycin (Kasumin, Arysta Life Science) has the greatest potential in walnut blight management. This antibiotic is used in agriculture in other countries but not in human or animal medicine. Kasumin alone significantly reduced walnut blight incidence and, under low rainfall, it was highly effective. Under high rainfall, mixtures of the antibiotic with EBDC or copper resulted in an increased efficacy and outstanding performance. Moreover, in most trials, Kasumin-EBDC or Kasumin-copper mixtures were similar to or higher in efficacy than copper-EBDC mixtures. Kasumin was federally registered on pome fruit for management of fire blight in 2014 and is expected to soon be registered on walnut. Ideally, we would like to have some research-authorized demonstrations once a federal registration has been established on walnut.

Additional research is still needed on other possible alternative treatments such as natural compounds (e.g., Citrox, Regalia, Serenade Optimum - evaluated in previous years), biocontrols (e.g., Actinovate, Blossom Protect, Double Nickel 55, Botector), enhancers of host resistance (e. g., Actigard, Quintec, Stout), antibiotics registered for other bacterial diseases (e.g., oxytetracycline – Fireline/Mycoshield), and phosphonates (e.g., ProPhyt, K-phite). Some of these compounds demonstrated promising results in our trials previously. Thus, Actinovate was registered for use in 2011 based on our efforts. We are continuing studies to improve its efficacy and evaluate other products. Our overall goal for management of walnut blight is to ultimately have multiple treatments available that provide equal or improved efficacy to that obtained with traditional copper-containing compounds. New treatments will need to be effective against copper-sensitive and -resistant populations of the walnut blight pathogen. This strategy will result in a lower usage and decreased environmental impact of any one material and a lower potential for selecting resistant populations of the pathogen.

PROCEDURES

Isolation of* Xaj *from diseased walnut fruit and determination of in vitro sensitivities against copper, mancozeb, and new bactericides. Samples of buds and blighted walnut fruit were collected by farm advisors, PCAs, and chemical industry representatives throughout the 2014 season. Tissue samples were suspended in sterile water, aliquots were streaked onto yeast-dextrose-calcium carbonate (YDC) agar amended with cycloheximide, cephalexin, 5-fluorouracil, and tobramycin, and single colonies were obtained. Colonies were characterized by PCR using our *Xanthomonas*-specific primers and for their sensitivity to bactericides.

Copper sensitivity was determined by streaking isolates onto nutrient agar amended with 10, 50, 75, or 100 ppm copper ion using copper sulfate pentahydrate. Growth at 50 ppm copper after 2-3 days of incubation at 25C was considered copper-resistant and growth at 75 ppm or higher was considered increased resistance. Sensitivity to mancozeb and ATD was tested with the spiral gradient dilution method using nutrient media amended or not amended with 50 ppm copper. Bacterial suspensions were streaked radially onto the agar plates and incubated for 2 days at 25C. Inhibitory concentrations where a reduction of bacterial growth was observed were determined by measuring radial growth and then using a computer program with an algorithm based on the molecular weight of the test substance and diffusion constants.

Evaluation of alternative bactericides for management of walnut blight in field studies. Trials were established in experimental orchards at KARE (Fresno Co.; cvs. Vina, Chandler, Hartley; *Xaj* copper-sensitive) and in commercial orchards in Solano (cv. Tulare; *Xaj* partially copper-resistant) and Sutter/Yuba Co. (cv. Tehama; *Xaj* partially copper-resistant). The Fresno Co. trial was conducted with simulated rain applied for 5-7 h on selected days as indicated in the figures of the results. The other trials were conducted under natural rainfall conditions with bactericide applications based on XanthoCast, and four or five treatments were done with treatment timings indicated in Figs. 2-5.

Copper compounds evaluated included the microencapsulated copper hydroxide Kocide 3000 (DuPont Chemical Co. sold to Mitsui & Co.), copper hydroxide (ChampION++, previously coded as NUP 12032; NuFarm Americas, Inc.), a pre-mixture of copper hydroxide/oxychloride (Badge X2; Gowan Co.), basic copper sulfate (Cuprofix Ultra Disperss; United Phosphorus, Inc.), and copper sulfate (MagnaBon CS2005; MagnaBon International, LLC). The EBDC Manzate ProStick 75DF or Manzate Max, the phthalimide Captan (Arysta Life Science), the antibiotics kasugamycin (Kasumin; Arysta Life Science) and oxytetracycline (Fireline; AgroSource, Inc.) were used either alone or in selected mixtures. Copper products were used together with new potential enhancers of copper activity such as Tanos and the thiadiazole derivatives ATD and Terrazole. Biocontrols evaluated included Actinovate (a fermentation product of *Streptomyces lydicus*; Natural Industries, Inc.) and Botector (*Aureobasidium pullulans;* Westbridge Agricultural Products). Other treatments evaluated were stimulators of plant host defense mechanisms including Quintec (quinoxyfen; DowAgro), Actigard (acibenzolar-S-methyl; Syngenta Crop Protection), Stout (registrant is proprietary information), the experimental LWB-2014, and the bacterial membrane disrupter ceragenin. Incidence of disease was based on the number of infected fruit of 50-150 fruit evaluated for each of four or five single-tree replications. Data were evaluated using analysis of variance or general linear model procedures and least significant difference (LSD) mean separation procedures of SAS 9.4.

Evaluation of predictors of disease risk in the current season:* Xaj *population sizes in walnut buds in the fall of the previous season vs. actual disease in the previous season. Thirty to fifty female flower buds were collected in the fall of 2012 and 2013 by PCAs in each of 35 orchards that had low (<5%), moderate (5-20%), or high (>20% diseased nuts) disease levels in early summer of 2012 and 2013. Buds were split in half to be used for bacterial isolation and future qPCR amplifications. For bacterial isolation, from each location three bud halves from each of 10 branches were thinly sliced, placed in 0.5 ml of sterile water, and vigorously shaken for 5 min. The suspension was diluted with sterile water (1:100) and then spiral-plated onto YDC agar as described above. Plates were incubated at 25C for 3-4 days and then evaluated for bacterial growth. Growth of *Xaj*, other yellow bacteria, and non-yellow bacteria was rated using a scale from 0 = no growth, 1 = fewer than 100 colonies, 2 = 100-200 colonies, and 3 = plates containing more than 200 colonies. Representative colonies of putative *Xaj* were verified for species identity using specific PCR primers. These ratings were categorized as low (rating <1), moderate (1-1.7), or high (>1.7). Bacterial contaminants were rated using the same scale. These ratings were done for each isolation plate and were then averaged for each location. The incidence of *Xaj* among the total of yellow bacteria recovered was then calculated.

A tabular comparison was done for the two disease predictors (i.e., *Xaj* bud population sizes and disease levels based on nut drop in the previous season). The total number of mis-matches (anytime when low, medium, or high disease was not predicted accurately) and an adjusted number of mis-matches (only those mis-matches were considered where predicted disease was low or medium, but actually developed to high levels). The latter represents the worst scenario in an incorrect forecast, resulting in potential heavy disease losses when risk was assessed as low or medium and proper management practices were not followed. Percent error was then calculated based on the number of mis-matches of the total number of orchards in each disease category and were averaged for the 2012/13 and 2013/14 seasons.

RESULTS AND DISCUSSION

Walnut blight incidence in California in 2014 and sensitivity of* Xaj *isolates to copper, copper-mancozeb, and new bactericides. With low to moderate rainfall in the spring of 2014, the incidence of walnut blight was generally low to moderate at many locations. Our trials were conducted in orchards with a history of high disease levels or trees were inoculated with the walnut blight pathogen (i.e., at KARE) and thus, efficacy data were obtained for registered and new treatments.

As in 2012 and 2013, some growers noted a reduced efficacy of copper-mancozeb treatments. We followed up this observation with in vitro sensitivity assays for strains of *Xaj*. In 2013, in addition to a high incidence of copper resistance (growth at a discriminatory concentration of 50 ppm MCE), copper-resistant isolates from some locations showed a reduced sensitivity to copper-mancozeb mixtures. In our evaluations in 2014, we also tested strains on media amended with 75 ppm MCE and we found that a significant number of strains also grew well at this concentration, and some also showed limited growth at 100 ppm. Thus, among 17 orchards in the Sacramento and San Joaquin Valleys, 7 locations (41.2%) had strains with this increased insensitivity (Table 1) and it appears that copper resistance is increasing. In ongoing evaluations, we are also testing strains collected in previous years at 75 ppm MCE to more accurately define the extent and possibly time of increase in copper resistance. Of the total of 139 strains evaluated in 2014, 41.7% were less sensitive to copper-mancozeb and the majority of these were those with increased insensitivity to copper (Table 1). As determined in 2013, minimum inhibitory concentration values for mancozeb in the presence of 50 ppm copper ion for sensitive isolates were less than 0.06 ppm, whereas those for less sensitive isolates were 0.12 to 0.37 ppm. Although this 2X to 6X shift is rather small, it indicates the potential for adaptation of the pathogen that could lead to further decreased sensitivity. In general, a 10-fold shift in reduced pesticide sensitivity to a multi-site material or to mixtures with different modes of action represents resistance.

Table 1. Sensitivity of *Xanthomonas arboricola* pv. *juglandis* strains from 17 commercial walnut orchards in the Sacramento and San Joaquin Valleys. to copper and copper-mancozeb.

Orchard No.	Growing region*	No. of strains copper-sensitive	No. of strains with growth at 50 ppm Cu**	Growth at 75 ppm Cu***	No. of strains less sensitive to Cu-mancozeb ****	Total Isolates tested
1	SV	2	10	N	0	12
2	SV	8	1	N	1	9
3	SV	2	5	N	3	7
4	SV	4	1	N	0	5
5	SV	3	0	N	0	3
6	SV	0	10	N	0	10
7	SV	4	4	N	0	8
8	SJV	7	0	N	0	7
9	SJV	0	10	N	8	10
10	SJV	6	2	N	2	8
11	SV	0	9	Y	6	9
12	SV	8	2	Y	2	10
13	SV	2	5	Y	4	7
14	SV	0	7	Y	5	7
15	SJV	0	10	Y	10	10
16	SJV	0	9	Y^	9	9
17	SJV	0	8	Y	8	8
	Total	46	93	7/17	58	139
	%	33.1	66.9	41.2	41.7	

* Growing region: Sacramento valley (SV) and San Joaquin Valley (SJV).

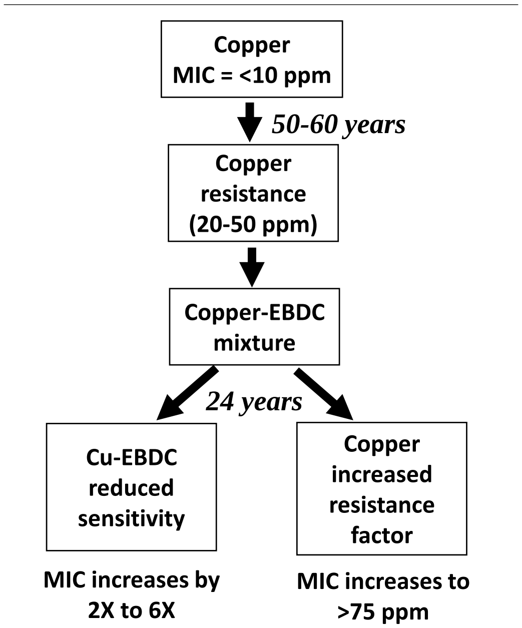
** Growth at 50 ppm metallic copper equivalent or MCE (using $CuSO_4$)

*** Sub-sample with growth at 75 ppm MCE (using $CuSO_4$)

****Growth at 50 ppm MCE (using $CuSO_4$) + mancozeb in a concentration gradient (0.03 to 4 ppm).

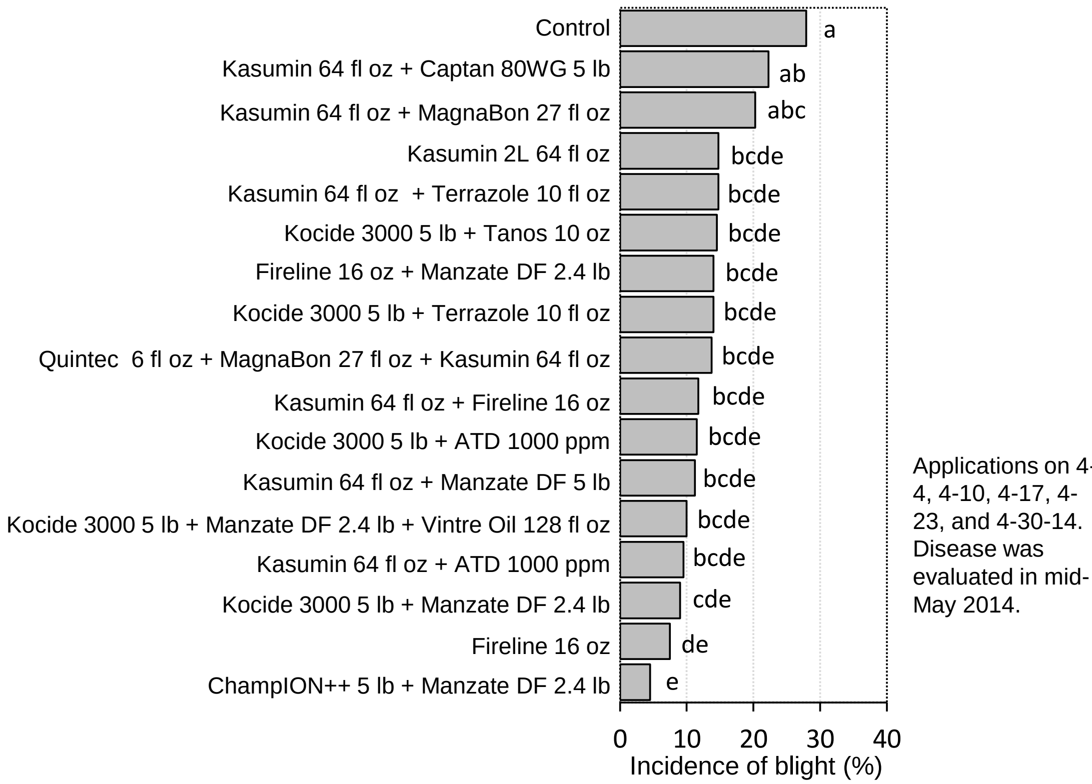
^ Some growth at 100 ppm MCE (using $CuSO_4$).

Fig. 1. Copper use in walnut blight management



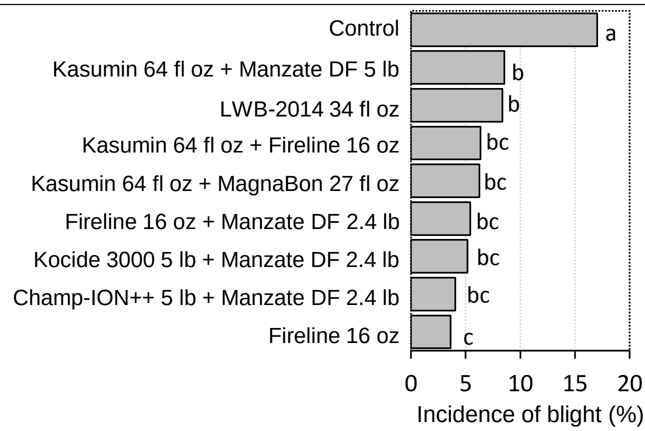
An overview of copper and copper-mancozeb use and resistance development in *Xaj* populations is presented in Fig. 1. Ideally, two modes of action with activity against the pathogen should be mixed from the onset to delay resistance developing to anyone mode of action over an extended period of time or almost indefinitely. Once resistance develops in a pathogen population to a mode of action, the addition of a second compound only slows the continued selection for greater resistance levels to that compound. This is the case with copper use against walnut blight. Resistance to copper developed and then copper-mancozeb was used exclusively for 24 years. The extended use of the combination results in the continued selection of copper resistance to higher levels (i.e., >75 ppm). The immediate, short-term solution to this situation is to use higher rates of copper. More importantly, however, every effort to register kasugamycin and to develop new treatments with different modes of action to manage walnut blight should be made by the California walnut industry. The activity of mancozeb that was finally fully registered on walnut in California in 2013 needs to be protected after over twenty years of emergency registrations for copper-EDBC mixtures. In Florida, where copper-mancozeb was used since the early 1980s, copper-EDBC mixtures are ineffective against copper- resistant strains in some locations. Thus, we are continuing our evaluation of alternative bactericides, as well as of other copper activity-enhancing compounds (*see below*).

Fig. 2. Efficacy of new copper- and non-copper-based treatments for managing walnut blight on cv. Tehama under natural rainfall conditions - Yuba-Sutter Co. 2014
- Populations of *X. arboricola* pv. *juglandis* partially resistant to copper -



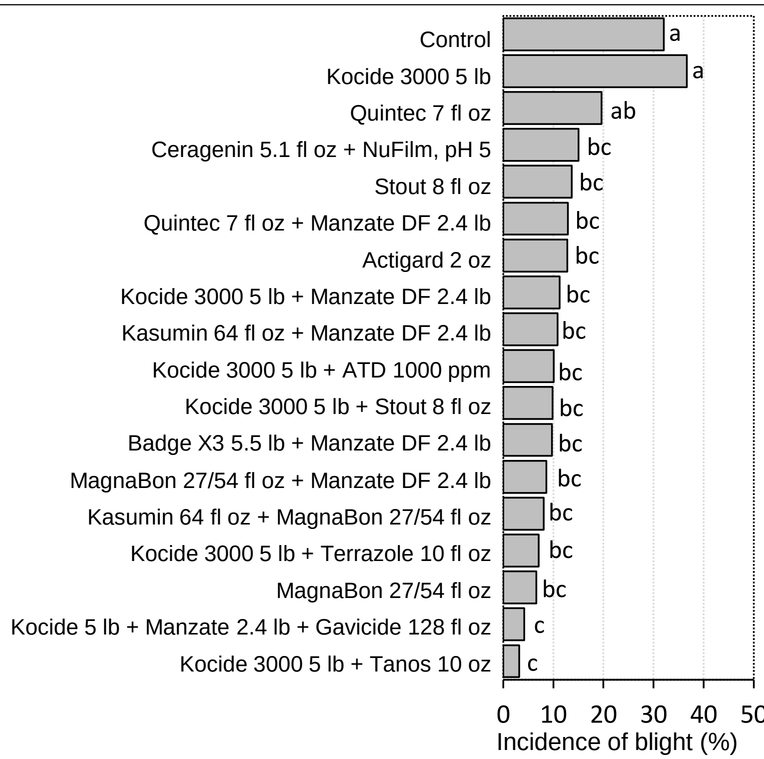
Evaluation of alternative bactericides for management of walnut blight in field studies. Data on new treatments for managing walnut blight were obtained from commercial and experimental orchards. When comparing copper products mixed with Manzate, Cuprofix Ultra Disperss, Kocide 3000, ChampION++-Ion^{2+}, and MagnaBon were statistically similarly effective. Numerically, ChampION++-Ion^{2+}-Manzate and MagnaBon-Manzate were highly effective at two locations each (Figs. 2,3 and Figs. 4,6, respectively), and Cuprofix-Manzate was most effective at another location (Fig. 5). At one location, however, MagnaBon caused some marginal leaf burn, but was not phytotoxic to fruit (trial of Fig. 4). Adding an agricultural oil to Kocide-Manzate resulted in a trend for improved efficacy of this treatment in one of two studies where this was done (Fig. 4). In a comparison of Manzate DF and Manzate Max, both mixed with Cuprofix, Manzate Max resulted in a numerically lower disease incidence (Fig. 5). Thus, Copper-Manzate treatments were highly effective in our trials although a reduced efficacy of this treatment has been reported by growers at some locations (see above). Kocide 3000 (representing a copper product) when used by itself was not effective in the presence of copper-resistant populations of the pathogen, but was very effective when mixed with Manzate (Fig. 4).

Fig. 3. Evaluation of new bactericides for management of walnut blight on cv. Hartley - Kearney Ag Center 2014
Inoculation with a copper-resistant strain of *X. arboricola* pv. *juglandis*



Applications were done on 4/6, 4/14, 4/23, and 5/1/14. Inoculations were done on 4/15, 4/25, and 5/2/14. Simulated rain was applied for 5 to 7 h each on 4/4, 5/5, 5/9, and 5/12/14. Disease was evaluated on 6-6-2014.

Fig. 4. Efficacy of new copper- and non-copper-based treatments for managing walnut blight on cv. Tulare under natural rainfall conditions - Solano Co. 2014
- Populations of *X. arboricola* pv. *juglandis* partially resistant to copper -



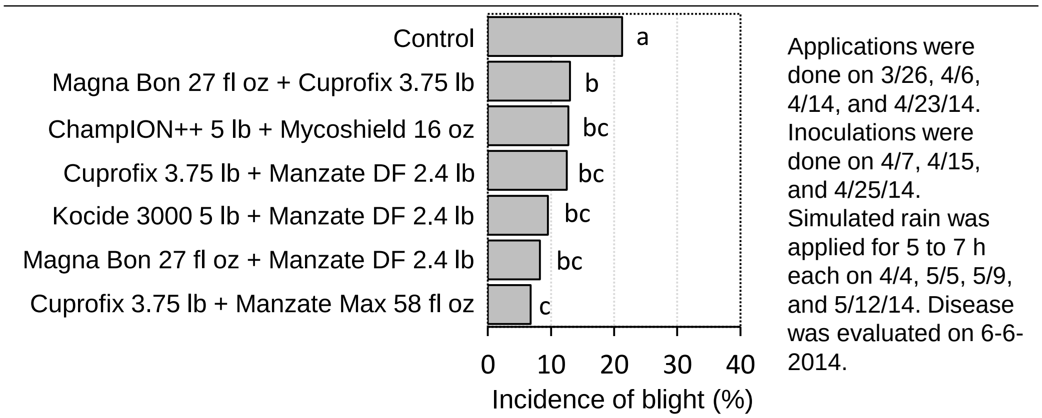
Applications on 4-4, 4-10, 4-17, 4-23, and 5-1-14. Increased Magna Bon rate to 54 fl oz/A on 4-10-14. Changed Magna Bon rate back to 27 fl oz/A for treatment with Kasumin on 5/1 application, due to phytotoxicity showing (kept it at 54 fl oz/A for other treatments). Disease was evaluated in late May 2014.

Copper activity-enhancing compounds were evaluated in laboratory and field studies. In 2013 we identified ATD as the most active one of several thiadiazole derivatives. Copper-resistant isolates that were not inhibited by copper alone, showed greatly reduced growth when ATD was added to the copper-amended agar medium. These observations were confirmed in additional laboratory and field tests in 2014. ATD in mixtures with copper was effective in laboratory assays against numerous copper-sensitive and 50-ppm-copper-resistant strains of the pathogen but at higher rates (1.5 to 50X) than mancozeb. For strains resistant to 100 ppm copper, 50 to 180X rates of

ATD were need in mixtures with copper to inhibit these strains similar to copper-mancozeb mixtures. In field trials, copper (Fig. 4) and kasugamycin (Fig. 2) activity was increased with the addition of ATD. In general, the efficacy of copper was improved in mixtures with Stout or the thiadiazole derivatives Terrazole and ATD (Fig. 4). Thus, ATD when used in combination with copper or kasugamycin has a similar effect as mancozeb and has registration potential as a walnut blight treatment. This thiazole group of chemicals has low human toxicity (ED_{50} values >5000 ppm). ATD in mixtures with copper is used for control of bacterial diseases in some countries and its registration potential in the US is being explored.

Kasumin used by itself showed moderate efficacy (Fig. 2). When mixed with other products such as Manzate, ATD, MagnaBon, or Fireline, efficacy was improved and was similar to copper-Manzate (Fig. 2). Efficacy of Kasumin mixtures evaluated has been very consistent over numerous years. Kasumin mixtures provide excellent control of walnut blight, are excellent treatments in resistance management programs especially when mixed with mancozeb, and reduce overall copper usage and environmental accumulation. As indicated above, Kasumin was federally registered on pome fruit for management of fire blight in 2014. Residue data for kasugamycin on walnut developed by IR-4 have been reviewed by EPA, and full registration of kasugamycin is pending. The Walnut Commission of California should encourage CDFA and EPA to register Kasumin in a timely manner.

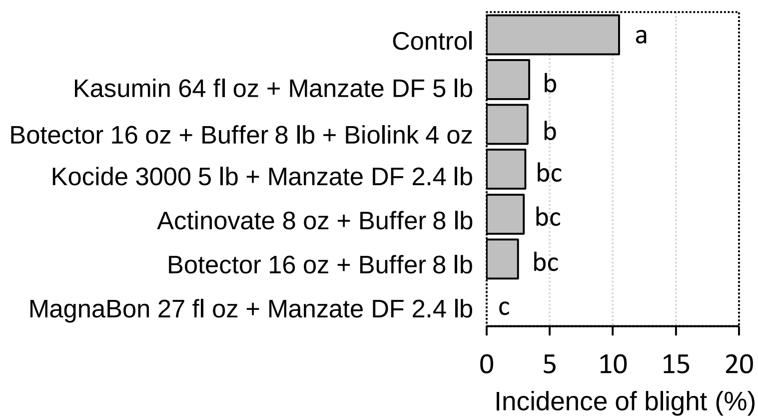
Fig. 5. Evaluation of new copper products for management of walnut blight on cv. Vina - Kearney Ag Center 2014
Inoculation with a copper-resistant strain of *X. arboricola* pv. *juglandis*



Among other alternative treatments, the antibiotic Fireline (oxytetracycline) performed very well by itself (Figs. 2,3), but there was a trend for reduced efficacy in mixtures with Kasumin or Manzate (Figs. 2,3). No phytotoxicity (leaf symptoms or nut drop) was observed as with streptomycin in previous studies. The experimental LWB-2014 (Fig. 3) and the bacterial membrane disrupter ceragenin (Fig. 4) showed intermediate efficacy. Ceragenin showed activity in preliminary laboratory studies. Stout used by itself also showed some efficacy (Fig. 4). This is a systemic acquired resistance compound, and numerically lowered disease incidence in a mixture with copper (Fig. 4). Additional studies are warranted with oxytetracycline, ceragenin, and Stout.

The biocontrols Actinovate and Botector were very effective at low disease pressure with a blight incidence of 7.1 to 10.5% in the untreated control (Figs. 6,7B) and still gave intermediate levels of control at higher disease pressure with 33.5% blight in the control (Fig. 7A). In trials for managing bacterial diseases of other crops, Actinovate was mixed with a growth enhancer that statistically improved the activity as compared to when Actinovate was used alone. Thus, additional trials are planned on walnut for Actinovate and Botector using growth enhancers.

Fig. 6. Evaluation of new antimicrobial and biological products for management of walnut blight on cv. Chandler - Kearney Ag Center 2014
Inoculation with a copper-resistant strain of *X. arboricola* pv. *juglandis*



Applications were done on 4/6, 4/14, 4/23, and 5/1/14. Inoculations were done on 4/15, 4/25, and 5/2/14. Simulated rain was applied for 5 to 7 h each on 4/4, 5/5, 5/9, and 5/12/14. Disease was evaluated on 6-6- 2014.

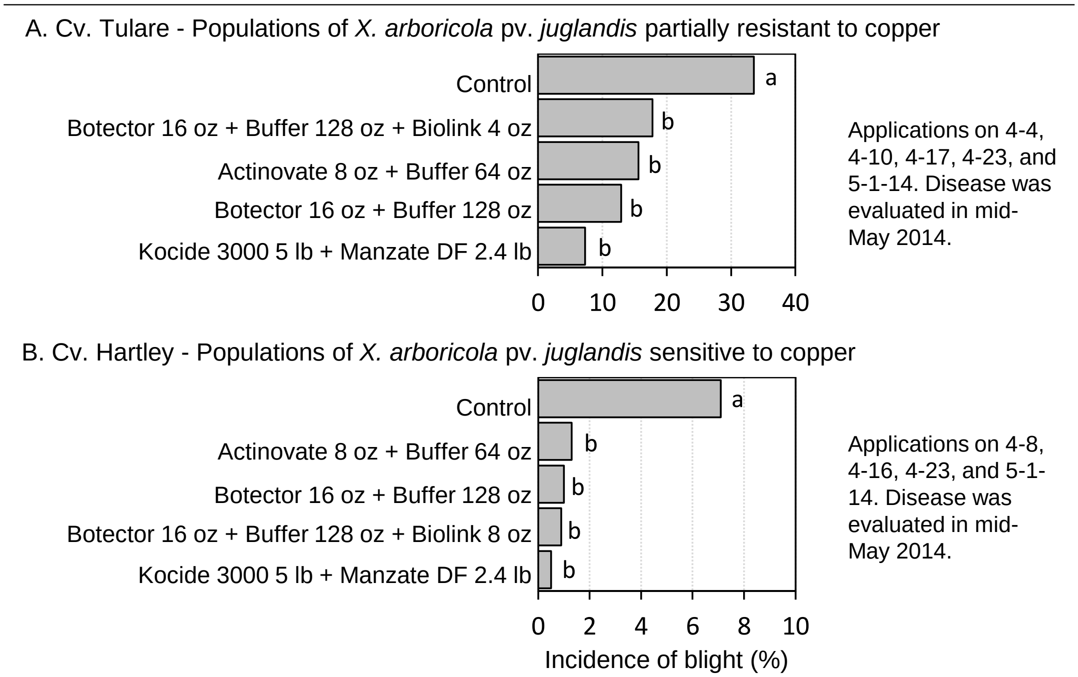
In conclusion of the management studies, among currently available treatments, copper-mancozeb (EBDC) combinations were fully registered (Section 3) in 2013 and 2014 and are the most consistent in controlling walnut blight in California. Kasumin again performed very well. Although moderately effective by itself, it performed very consistently in combination with other treatments. As in previous years, our research demonstrated that applications of copper-mancozeb at pistillate flower emergence followed by subsequent sprays based on temperature and rainfall (as summarized in the XanthoCast model) is currently the most effective strategy in managing the disease.

Potentially in the future, kasugamycin-copper, -mancozeb, or other combinations in rotation with copper-EBDC treatments can minimize the total amount of any bactericide being used while maintaining high levels of disease control and providing resistance management by alternating different modes of action. Neither kasugamycin nor other compounds like ATD should be considered replacements but rather should be considered complementary rotational treatments. Copper-mancozeb mixtures have an over twenty-year history of proven consistent performance for managing walnut blight under low to highly favorable conditions for disease. Considering shifts in copper-mancozeb sensitivity in sub-populations of the pathogen, alternatives to copper should be registered immediately to reduce the total number applications in a given season and to ensure the continued activity of mancozeb for years to come.

Isolation of* Xaj *from walnut buds and prediction of walnut blight. In isolations from walnut buds, as in last years' studies, a range of yellow-pigmented and other bacteria were recovered indicating that numerous microorganisms can colonize and survive on the protected tissues between the bud scales. *Xaj* was identified by cultural characteristics and by using specific PCR primers.

To predict walnut blight disease risk and actual disease levels in the current season for 35 orchards, disease levels based on summer nut drop in the previous season and size of *Xaj* populations in walnut buds collected in the previous fall were again determined as possible predictors as we did for the 2012/13 seasons. Errors for the two predictors for the 2012/13 and 2013/14 seasons were averaged. The total and adjusted numbers of mis-matches (in the adjusted number, only those mis-matches were considered where predicted disease was low or medium, but actually developed to high levels) were both higher when using bud populations as predictors (Table 2). The adjusted number of mis-matches represents the worst scenario in a wrong forecast resulting in potential

Fig. 7. Efficacy of biological and copper treatments for managing walnut blight under natural rainfall conditions - Solano Co. 2014



heavy disease losses when due to prediction of a low risk, proper management practices are not followed. Average percent error was 8.6% when disease in the previous season was used as a predictor and was 21.4% when bud populations were used (Table 2). Thus, considering the time and cost involved, the bud population method cannot be recommended. In contrast, predicting risk by determining disease levels based on nut drop in the field can be rapidly done by scouting and it is relatively inexpensive. Because both methods resulted in errors, they cannot be relied upon by themselves in the design of disease management programs for the following season. The potential for a disease outbreak to occur or to be realized is still based on favorable environmental spring conditions (i.e., wetness and temperature) due to the pathogen's high reproduction potential. Integration of risk assessment based on nut drop in the previous season with forecasting of infection periods using XanthoCast is currently the best way to forecast and manage the disease in the current season. Several PCAs have utilized this combination method and incorporated the information in their recommendations to growers. High-risk orchards (high nut drop) are prioritized for intense management programs in the subsequent spring.

Table 2. Evaluation of disease levels in the previous season or bud population sizes of *Xanthomonas arboricola* pv. *juglandis* in the previous fall as predictors for disease level in the following season for 35 commercial walnut orchards (*see text for details*)

A. Comparison 1: Fruit disease (1st year) and fruit disease (2nd year) over a 2-year period							
Fruit disease summer 2012 & 2013	**Fruit disease summer 2013 & 2014**	**No. of cases**	**No. of orchards with L, M, or H**	**No. of mis-matches total**	**% error**	**No. of only L or M to H mis-matches**	**% error**
L	L	18	20	2	10	2	20
L	M	2		0			
L	H	0		0			
M	L	7	19	11	57.9	4	46.6
M	M	8		0			
M	H	4		0			
H	L	5	31	13	41.9	0.0	0.0
H	M	8		0			
H	H	18		0			
Total/average		70		26		6	**8.6**

B. Comparison 2: Bud population fall (1st year) and fruit disease (2nd year) over a 2-year period							
population fall 2012 & 2013	**Fruit disease summer 2013 & 2014**	**No. of cases**	**No. of orchards with L, M, or H**	**No. of mis-matches total**	**% error**	**No. of only L or M to H mis-matches**	**% error**
L	L	22	29	7	24.1	7	49.0
L	M	5		0			
L	H	2		0			
M	L	6	19	14	73.7	8	87.8
M	M	5		0			
M	H	8		0			
H	L	2	22	10	45.5	0.0	0.0
H	M	8		0			
H	H	12		0			
Total/average		70		31		15	**21.4**

In summary, a comparison of both prediction methods was the best way to come to a scientific conclusion as to the method with the lowest error. When considering the value of the walnut crop, error rates greater than 10% are considered statistically and economically unacceptable. Only the nut drop method falls under 10% error for predicting disease levels in the subsequent season. Determining incidence of disease in a several-hundred nut sample in June or July is inexpensive and much easier than collecting buds, shipping to a laboratory, and culturing, as well as correctly enumerating the pathogen among all bacteria isolated (including other yellow-pigmented species) from buds. Currently, the best strategy to optimize treatment timings and treatment intensity (i.e., materials, frequency, rates, etc.) is to identify high-risk orchards based on disease levels (diseased nut drop) in the previous season and to use weather forecasts (i.e., XanthoCast) in the current season (after the pistillate-flower-emergence application of a bactericide).

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