EPIDEMIOLOGICAL APPROACHES TO THE CONTROL OF WALNUT BLIGHT DISEASE

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ABSTRACT

Buds of untreated trees were dissected starting about bud break and continuing until shoot expansion to determine the process by which inoculum of *X. c. juglandis*, which is primarily found within buds, moves to developing shoots and nuts. The highest numbers of cells of the pathogen were found in the outermost bud scales, cataphylls, as well as the outmost immature leaves within buds. Those innermost undeveloped leaves within walnut buds and the meristematic site were usually free of pathogen until buds opened and had very low populations for several days after bud opening. Unlike in previous years where the incidence of contamination of newly emerging leaves only gradually increased with time after bud break on untreated trees, in 2006 colonization increased progressively from about 50% of the leaves colonized at bud break to most leaves being colonized by mid-April. This process of contamination was delayed on buds from trees treated at the time of bud break with Kocide + Manex. While in all earlier years except the wet year of 2005 the incidence of leaves contaminated with *X.c. juglandis* increased only slowly with time, and the population size on those leaves that became contaminated did not increase appreciably, in 2005 and in our sprinked plot in 2006 relatively large population sizes of *X.c. juglandis* developed by late April (> 10^5 cells/leaf). This suggests that while infestations of the outermost leaves within a bud supplies inoculum for epidemics of walnut blight, this inoculum must move to the developing shoot tip and nuts at the time of (or after) bud break, providing evidence as to why the disease is somewhat dependent on moisture. Artificial rainfall applied in 2006 may be responsible for the enhanced colonization seen in this year compared to previous years of observation. In another study, thousands of buds were tagged as they opened and we applied a single bactericide spray at various times relative to bud opening. Since we knew the stage of bud/shoot development relative to the time of spray we could follow pathogen populations on buds of different phenological states. Pathogen populations were much more reduced in April on leaves and nuts developing from buds that were sprayed immediately after they opened compared to buds that opened either before or after spraying. Inoculum of *X.c. juglandis* can rapidly move onto developing leaves and nuts after bud break and protective bactericide sprays are best applied at this critical time. Leaf scales and cataphylls, unlike leaves, were observed to imbibe large quantities of suspensions of Kocide+Manex when applied in 0.5% Breakthru, suggesting that the effectiveness of early-season sprays may be due at least partly to the ease with which the pathogen can be exposed to the bactericides in such tissues. Those buds that opened earliest in the Chandler orchard were far more likely to produce a nut than those buds that opened even a week later. This suggests that we might achieve best control of nut infections by timing early season sprays based on the earliest buds that open in an orchard.

OBJECTIVES

1) Detailed examination of location of *X.c. juglandis* inoculum in current season buds and examination of the process by which it moves onto distal leaves and nuts.

2) Determine efficacy of early-season bactericides applied at different phenological stages for disease control.

3) Determine the fruitfulness of walnut buds that open at different times during the spring to ascertain the intensity of disease control efforts justified for early vs. late emerging buds.

PROCEDURES AND RESULTS

Rationale for study:
Our investigation of the epidemiology of walnut blight disease had suggested that processes that occurred shortly after bud break were of primarily importance in determining whether disease would occur if inoculum was present in an orchard. Specifically, our preliminary results had indicated that the pathogen was present within walnut buds and was responsible for causing infection on the nuts that arose on shoots that emerged from those buds. Furthermore, our preliminary data indicated that the immature leaves and meristematic tissues within the buds were not usually contaminated before the bud opened. Instead, the inoculum appeared to move relatively rapidly from the outer parts of the bud to the developing leaves and nuts as they emerged from the bud. Thus protective bactericides applied shortly after bud break were expected to have a much larger effect on the likelihood that a nut would become infested with the pathogen than bactericides applied at a later date. It is also known that bactericides generally act much more effectively as a protectant, in preventing the growth of bacteria than in killing established bacterial populations. Thus we expected that later bactericide sprays might have substantiall less impact on impact on the disease than initial bactericide sprays. The large numbers of the walnut blight pathogen commonly found in dormant walnut buds and developing shoots, even on trees treated with copper bactericides, suggested that many of the cells are within the buds and not subject to topical bactericide applications. Our earlier studies had indicated that applications of copper hydroxide, streptomycin or other materials without surfactants before bud break did not reduce the population sizes of *X.c. juglandis* on dormant buds or lead to disease control. We have consistently found that an application of a mixture of Kocide 101 and Manex with Breakthru is effective in reducing disease when applied shortly after bud break. Presumably, after buds begin to open the bactericide can more easily reach the internal parts of the bud that are then exposed. More importantly, a single application of these bactericides with Breakthru shortly after bud break has often been nearly as effective in control of walnut blight as repeated applications of a Kocide + Manex mixture without high concentrations of Breakthru applied by cooperating growers later in the growing season. The earlier work had also shown that disease control with a combination of Kocide+Manex with penetrating surfactant was much more effective if applied very soon after bud break and lesser amounts of surfactant were required. Presumably if the bactericide application were delayed until slightly later, such that most buds were open but not so late that the protective benefit from having an early application of Kocide+Manex was not lost, sprays might require much less or no surfactant. Such “late” but still early-season applications of the bactericides had not been evaluated. The benefit of such “later” early-season bactericide applications is that there may be a lesser need for surfactant, but the negative aspect is that there may be a very narrow “time window” when such sprays might best be applied; i.e., one might apply the bactericides too late after bud break (enabling the pathogen to move from within the bud to newly-emerging tissues), thus negating the protective effect of the bactericides.

One of the main issues related to the sufficiency of early-season copper applications for disease control is the varied phenological development of the trees. Bud break does not occur simultaneously on a tree, and in fact often occurs over a period of up to 3 or more weeks. Thus it is impossible to state that there is a particular day on which bud break has occurred, and instead we must consider a period of time over which this occurs. The timing of application of the bactericide relative to that of bud break is particularly important. Bactericides applied very early (unless applied with penetrating surfactants) will not access the pathogen and developing green tissues, while bactericides applied after most buds have opened will not have protected green tissues from the buds that opened the earliest to contamination by the pathogen. Our earlier studies of spray timing did not accounted for the precise status of bud opening in relation to disease control. In 2005 we initiated a very ambitious project in which we tagged thousands of buds as they opened and applied a single bactericide spray at various times relative to bud opening; this design was again used in 2006. Since we knew the stage of bud/shoot development relative to the time of spray we could follow pathogen populations on buds of different phonological states. Our work thus addressed the temporal pattern of colonization of various parts of the developing walnut shoot to determine the "window of time" over which bactericide applications must be made (Objective 1) and determined the effects of bactericide sprays at various phenological stages of development to better determine how a phonologically-based spray program might be implemented (Objective 2). In 2005 exceptionally heavy and late spring rains had resulted in very high levels of disease, and patterns of bacterial growth and apparent spread that we had not observed in any previous year. The study in 2006 thus addressed bacterial growth and movement and effects of bactericide sprays under a different rainfall regime. We also had observed that buds that opened early in the spring were much more likely to bear nuts than buds that opened later in the spring, and again addressed this issue in Objective 3.

Experimental Design and Results

Spatial location of *Xanthomonas campestris* pv. *juglandis* in developing walnut shoots

The process of contamination of distal parts of the developing shoot appears to involve a superficial contamination of the developing leaves at the time of bud break. Since our earlier studies had shown that young developing leaves were usually contaminated with pathogen shortly after emergence from the bud, the process of contamination must be most important at the time of bud break, and perhaps moisture, the most logical mechanisms of moving inoculum to the innermost bud parts (most distal leaves), is most important at this time. These findings also explain why early-season applications of bactericides are effective in controlling walnut blight. The applications of mixtures of Kocide + Manex that were most effective in controlling walnut blight were those made at the time of bud break, and thus appear to be killing inoculum of the pathogen as it is exposed to the exterior of the bud, but before inoculation of new leaves has occurred. The application of early-season bactericides will thus benefit from more detailed studies of how asynchronous bud break/shoot development is in walnut – i.e. if bud break was completely synchronous then the window for protective bactericides may be very short and the width of the spray window is probably dictated by the extent to which buds continue to open over an extended period of time – thus we might develop a better phenologically-based spray program by understanding what the phonological targets of the sprays are and how to identify them in different years. We therefore established a

complex plot in Tehama County where thousands of buds were tagged as they opened and we applied a single bactericide spray at various times relative to bud opening. Since we knew the stage of bud/shoot development relative to the time of spray we could follow pathogen populations on buds of different phenological states. The phenological state of 500 buds per bud cohort to receive a given spray treatment was noted at short intervals starting at the time of first bud break by placing small colored plastic ties on the base of each bud every 3 to 4 days during the 15 day period over which bud break occurred. Ties were applied to buds that were newly cracked open. Thus 4 different phenological ages of buds were be defined. Ties were applied to buds that opened on April 15, 18, 24, and 28. By this method we were be able to determine the time when individual buds emerged and the permanent tags allowed us to monitor pathogen populations in each cohort of buds as well as eventual disease development on nuts resulting from these buds. At each interval a replicate set of 5 trees was sprayed with a mixture of Kocide + Manex containing 0.5% Breakthru. Sprays were applied on April 18, 24, and 28. We thus had several hundred tagged buds, yielding several hundred nuts that received bactericide application each at different times relative to bactericide sprays or that were left untreated. The plot areas were both equipped with overhead sprinklers to provide artificial rainfall. The design and operation of the artificial rainfall system is described in the 2006 report of Buchner.

Given that a majority of the cells of the walnut blight pathogen appear to be within walnut buds before bud break, the question remains as to how the pathogen makes its way to the developing new leaves and nuts of the developing shoot after bud break. As in earlier studies we made detailed measures of the exact location of the pathogen on shoots by dissecting walnut tissues at various stages of development after bud break. We thus needed to know how fast these plant parts became contaminated after mergence from the bud and whether such a process involves moisture that may be important in allowing colonization of the distal shoot parts such as nuts even if initial inoculum existed only in the bud. Knowledge of the location of the pathogen in walnut buds would also be useful in understanding the process involved in the early-season bactericide strategies discussed in Objective 2. We therefore in 2006 performed detailed spatial analysis of the distribution of *X. c. juglandis* in the various parts of walnut buds both shortly before and during opening to determine if buds were uniformly infested. About 75% of the buds in this Chandler orchard harbored *X.c. juglandis* when sampled in March and most had populations of *X.c. juglandis* of over 3,000 cells/bud (Figure 1). On average about 50% of the cataphylls sampled from buds that opened at different times also harbored detectable *X.c. juglandis* at the time of opening and these populations remained approximately constant over the next two weeks before cataphylls dropped from the shoots (Fig. 2). The population size of *X.c. juglandis* in those buds that were contaminated with the pathogen was about 1000 cells/cataphyll (Fig. 2). The fact that a similar proportion of buds harbored inoculum in winter and later in spring, suggests strongly that the inoculum resides permanently in the buds and thus that the initial inoculum in buds is predictable well before bud break and that the *X.c. juglandis* found in developing shoots originated in the buds.

On average, the incidence of contamination of the first (basal leaf) in walnuts buds were lower than that of the associated cataphylls (about 30%) (Fig. 3). The incidence of colonization of these older leaved gradually increased from later April though early May, when nearly all leaves harbored at least some cells of *X.c. juglandis* (Fig. 3). The increasing incidence of contamination of the basal

leaves on shoots with time after open was also associated with an increase in average population size of *X.c. juglandis* from about 10^3 /cells/leaf to about 10^6 cells/leaf. Interestingly the population sizes on leaves emerging from buds that opened early in the growing season were always higher than those from buds that open only on April 24 or April 28 (Fig. 3). Very few of the most distal leaves on a shoot harbored detectable populations of *X.c. juglandis* shortly after opening (Fig. 4), although the incidence of infestation gradually increased from less than about 10% to more than 90% infestation between mid-April and Mid-May (Fig. 4). These increases in the frequency of infestation were also associated with a gradual increase in the average population size of *X.c. juglandis* on contaminated leaves from less than about 100 cells/leaf to over 10^5 cells/leaf over this same time period (Fig. 4). The pattern of colonization of nuts was similar to that of the distal leaves (closest to the nut); very few nuts were contaminated with *X.c. juglandis* shortly after their formation in the spring, but the frequency of infestation increased to over 80% by mid-May (Fig. 5). Likewise, the average population size of *X.c. juglandis* on developing nuts increased from less than 100 cells/nut shortly after their formation in Mid-April to over10^5 cells/nut by mid-May. When considered over all of the shoot parts, the populations of *X.c. juglandis* increased dramatically on untreated trees during the spring in this plot; the average population size of *X.c. juglandis* increased from about 10^3 cells/sample to over 10^6 cells per sample between April 18 and May 15 (Figs. 3-5). Such increases are unlike any other site in over 10 years of sampling walnut except for 2005 when very late and heavy rains had occurred. Thus there was a very high degree of spatial segregation of *X. c. juglandis* populations within infested walnut buds, especially early in the spring. These results suggest that the pathogen is not systemic in the tree, and that the colonization of the buds is a result of an "invasion" of the bud from the exterior, probably as it is forming. The cells of this pathogen thus are apparently superficial on bud scales and embryonic leaves. The interior of the bud might shield the cells from the stress of the surface of the plant, e.g. it is probably relatively moist within buds. Upon bud break, however, many of the cells might succumb to the desiccation stress and stresses due to UV irradiation on the surface of leaves. The fact that the innermost embryonic leaves are not as likely to be colonized by *X. c. juglandis* suggests that the pathogen initially colonizes the buds in a somewhat superficial manner, apparently from exterior sources of inoculum.

The pattern of colonization of embryonic and developing walnut leaves and nuts by *X. c. juglandis* strongly suggests that the leaves and nuts become inoculated with the pathogen shortly after emergence from the bud. This is perhaps most obvious when the populations on different tissues developing from a given cohort of buds (buds opening on a given day) are compared. For example, the incidence of infestation of cataphyls and average population size of *X.c. juglandis* on infested cataphyls was generally much higher than on embryonic distal leaves or developing nuts on buds from untreated trees that had opened on either April 15 or April 18 when sampled on April 18 (Fig. 6). Likewise, the incidence of infestation of cataphyls and basal leaves and average population size of *X.c. juglandis* on infested cataphyls and basal leaves was generally much higher than on embryonic distal leaves or developing nuts on buds from untreated trees that had opened on either April 15 or April 18 when sampled on April 24 (Fig. 7). However, presumably due to the extensive moisture supplied as artificial rain throughout the spring, both the incidence of contamination of both basal and distal leaves as well as nuts and average *X.c. juglandis* population size on contaminated shoots increased substantially by May 15 (Fig. 8). Thus it appears that, as expected, the most interior tissues in walnut buds remain un-colonized by *X.c. juglandis* until the time of bud

break and then become contaminated with *X.c. juglandis* within a few days. The process of contamination presumably is aided by the presence of moisture, which was in abundance in this experiment. With continuing abundant moisture the pathogen can continue to move not only within leaves/nuts on a given shoot (as appears to be the case in most "normal" springs without abundant rainfall) but also between shoots when moisure is abundant, leading to high levels of contamination of most leaves and nuts.

Efficacy of early-season bactericides applied at different phenological stages for disease control

Shoot samples were collected at various times in the spring of 2006 from trees treated at different times relative to bud open with Kocide+Manex in 0.5% Breakthru to compare populations of X.c. juglandis with that on untreated trees. At each sampling time the populations of *X.c. juglandis* was estimated on 4 parts of the walnut shoot (from cataphylls, first (basal) true leaf, most distal true leaf, and from developing nuts). In general, over all sample dates and all shoot position samples for bactericide sprays applied at different times relative to bud opening, the greatest reductions in *X.c.juglandis* populations were achieved by Kocide+Manex sprays applied with 0.5% Breakthru at the time of bud opening. Much less control of *X. c. juglandis* populations was observed when bactericides were applied either before or after bud break. For example, when sampled on April 24 the incidence of contamination and average population size of *X.c. juglandis* on all parts of walnut shoots that had emerged from buds on April 18 were lower on trees sprayed with Kocide+Manex on April 18 than that from shoots that emerged from buds that opened earlier (April 15) or later (April 24) (Fig. 9); the average population size of *X.c. juglandis* on leaves and nuts was up to 100-fold lower on shoots that had been sprayed the day that they opened rather than either before or after they had opened (Fig. 9). Bactericide applications made at the time of bud opening had the largest effect on *X.c. juglandis* populations when measured within about 10 days of spraying. For example, when sampled on April 28 the populations sizes of *X. c. juglandis* and the incidence of infestation with *X.c. juglandis* was much lower on the most distal leaves of walnut that had emerged from buds on April 24 (the day of the Kocide+Manex spray) compared to buds that had emerged either before or after the bactericide spray (Fig. 10). The population sizes of *X.c. juglandis* and the incidence of infestation with *X. c. juglandis* however, increased with time, and by mid-May, nearly all distal leaves were colonized by *X. c. juglandis*, with a mean population size of about 10^6 cells/leaf (Fig. 10). A similar pattern of colonization of the emerging nuts on trees sprayed with Kocide + Manex on April 24 was observed. When sampled on April 28 the populations sizes of *X. c. juglandis* and the incidence of infestation with *X.c. juglandis* was much lower on developing nuts of walnut that had emerged from buds on April 24 (the day of the Kocide+Manex spray) compared to buds that had emerged either before or after the bactericide spray (Fig. 11). A similar pattern was observed on trees sprayed on April 28. Bactericide applications made at the time of bud opening had the largest effect on *X.c. juglandis* populations when measured within about 10 days of spraying. For example, when sampled on May 10 the populations sizes of *X. c. juglandis* and the incidence of infestation with *X.c. juglandis* was much lower on the most distal leaves of walnut that had emerged from buds on April 28 (the day of the Kocide+Manex spray) compared to buds that had emerged either before or after the bactericide spray (Fig. 12). The population sizes of *X.c. juglandis* and the incidence of infestation with *X. c. juglandis* on distal leaves however,

increased with time, and by mid-May, nearly all developing distal leaves were colonized by *X. c. juglandis*, with a mean population size of about 10^5 cells/leaf (Fig. 12).
The maximum suppressing effect of bactericide sprays made at the time of bud opening is perhaps most apparent by comparing *X.c. juglandis* populations on walnut plant parts over time with that developed from buds that opened at a given time and which were sprayed at different times relative to the time of bud break. For example, the incidence of contamination of basal walnut leaves was much lower throughout April and early May on shoots that emerged from buds that were sprayed with Kocide + Manex on April 18 (the day that they opened) compared to that on shoots that emerged either before or after the time of the bactericide spray (Fig. 13). Likewise, the incidence of contamination of distal walnut leaves was much lower throughout April and early May on shoots that emerged from buds that were sprayed with Kocide + Manex on April 18 (the day that they opened) compared to that on shoots that emerged either before or after the time of the bactericide spray (Fig. 14). Thus, in a more "normal" year in which heavy rainfall (or artificial rain) was not encountered in May to facilitate growth and/or movement of *X.c. juglandis* from shoot to shoot on the tree we would have expected that bactericide applications made on the day of bud break would have yield persistent reductions in *X. c. juglandis* populations that would have persisted throughout the spring. Such reductions in *X. c. juglandis* populations had lead to substantial reductions in the incidence of walnut blight infections.

Observations of walnut blight infections of nuts in late May suggested that the numerous infections of nuts that were observed were due to infections that had occurred at different times in the spring. It was common to observe nuts with symptoms similar to that shown in Figure 15. Such nuts had one (or occasionally more) large necrotic infection sites, typically at the flower end of the fruit which were typical of walnut blight infections seen in most years. However, many nuts such as the one depicted in Figure 15 also had numerous, small infections that occurred on the flanks of the nut, and which appeared to be very recent, as evidenced by their small size, lack of necrotic tissue, and water-soaked appearance, typical of a very young bacterial lesion. We thus believe that the epidemic of walnut blight that occurred in our plot area which received frequent overhead sprinkling, even late into May during warm days, was characterized by infections that occurred due to inoculum present in mid to late April, resulting primarily from movement of cells from infested buds onto the developing leaves and nuts. However, with the imposition of heavy overhead sprinkling during warm periods in May it seems likely that inoculum that arose from nuts infected earlier spread inoculum throughout the tree, resulting in many "secondary" infections. We believe that this process of secondary infection of trees is very uncommon in California, and associated with the exceptionally heavy infection pressure that we placed on these trees by the use of overhead sprinklers used well into late spring.

The incidence of walnut blight disease was similar on trees treated at various times relative to bud break with Kocide+Manex compared to untreated control trees (Table 1). A very high incidence of walnut blight infections was observed in the plot area, and most nuts exhibited very severe infection (many infected sites per nut). It was not possible for us to distinguish unambiguously the presumptive "early" infections from those small lesions that we presume to be "secondary"

infections. We presume that the incidence of "early" infections was reduced due to the relatively large effect of the early-season bactericide applications on *X. c. juglandis* populations, but given the relatively large and unprecedented increases in *X. c. juglandis* populations and its distribution in the orchard (presumably due to late season sprinkling and rain events) the numerous "secondary" infections events associated with a large and uniform *X. c. juglandis* population by mid-May overwhelmed the nuts. We plan on repeating the study in 2007 but to not apply overhead sprinkling extensively after the time most buds are open, to better mimic the conditions under a more "normal" season. In such a setting we expect that the application of bactericide sprays at the time of bud opening will be demonstrated to provide much better disease control that when it is applied either before or after buds have opened.

The number of nuts produced by a given walnut bud was very strongly influenced by the time of the spring in which it opened. Those buds that opened early in the spring produced MANY MORE nuts than those that opened later in the spring (Table 2). For example, those buds that opened on April 15 produced nearly twice as many nuts as those that opened on April 24 (Table 2). Likewise, the many buds that opened on April 28 produced only about 10% as many nuts as those that opened before April 18 (Table 2). This suggests that we might achieve best control of nut infections by timing early season sprays based on the earliest buds that open in an orchard given that the later buds are unlikely to yield nuts in need of protection. That is, spray timings might best be made to maximize control of *X. c. juglandis* populations in tissues arising from the earliest buds that open rather than waiting for the later buds to open before initiating bactericide sprays.

Table 1
Incidence of walnut blight on nuts developing from buds that opened at various times on trees sprayed with Kocide+Manex in 0.5% Breakthru a single time at various dates

	Disease Nuts (Fraction) Bud Opening Date			
Spray date	April 15	April 18	April 24	April 28
April 18	0.54	0.43	0.56	0.31
April 24	0.61	0.52	0.50	0.44
April 28	0.41	0.43	0.55	0.43
No Spray	0.35	0.58	0.51	0.36

Table 2
Number of nuts harvested from buds tagged on walnut trees as they opened at different times

Spray date	# Nuts/500 buds Bud opening date April 15	April 18	April 24	April 28
April 18	512	366	217	57
April 24	345	275	156	45
April 28	547	394	187	57
No Spray	463	361	226	50

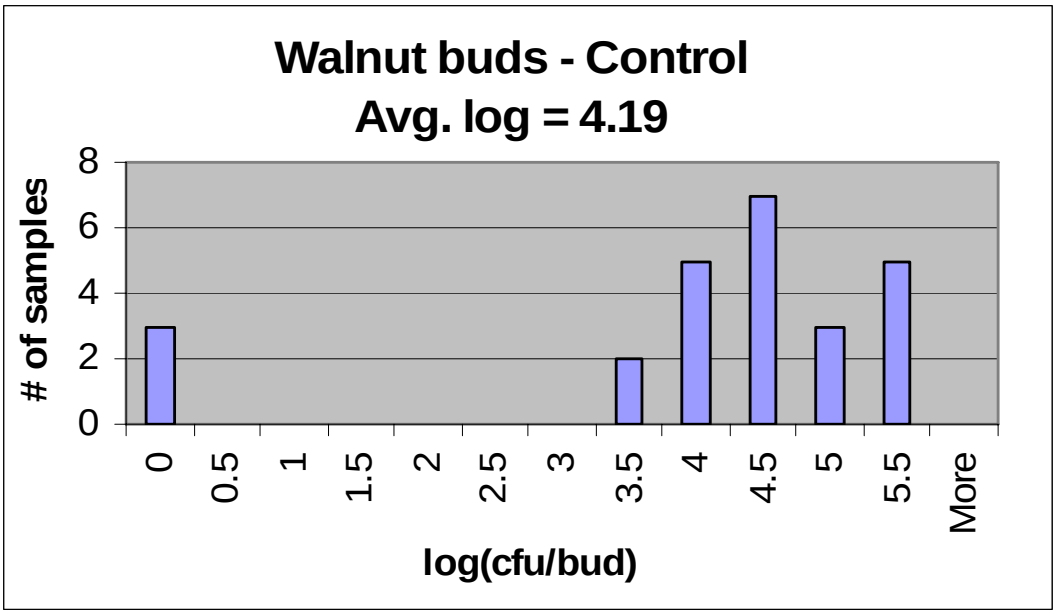

Figure 1. Population sizes of *Xanthomonas campestris pv. juglandis* on individual walnut buds collected in a commercial Chandler walnut orchard in Tehama County in March, 2006.

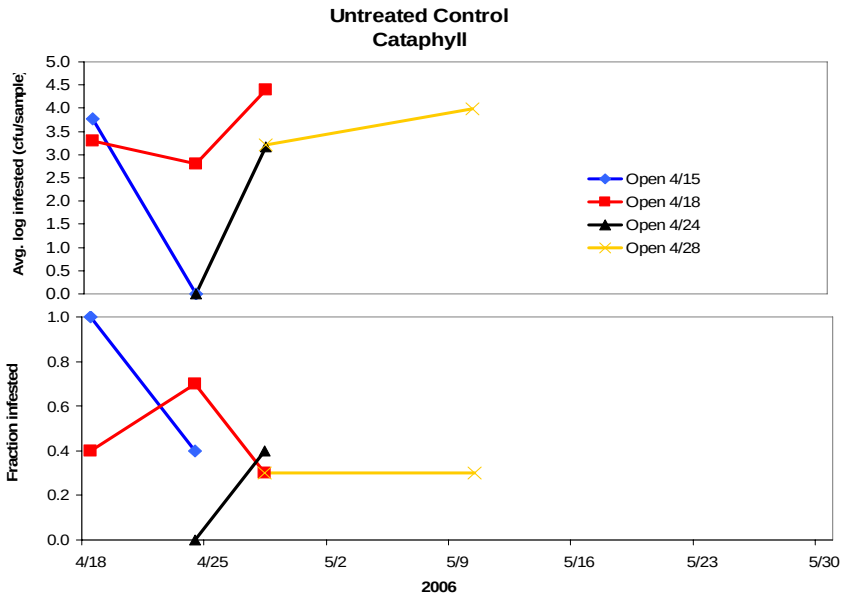

Figure 2. Population size of *Xanthomonas campestris pv. juglandis* on cataphyll 1 (outermost cataphyll) of walnut buds from untreated trees dissected at various times in 2006. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel.

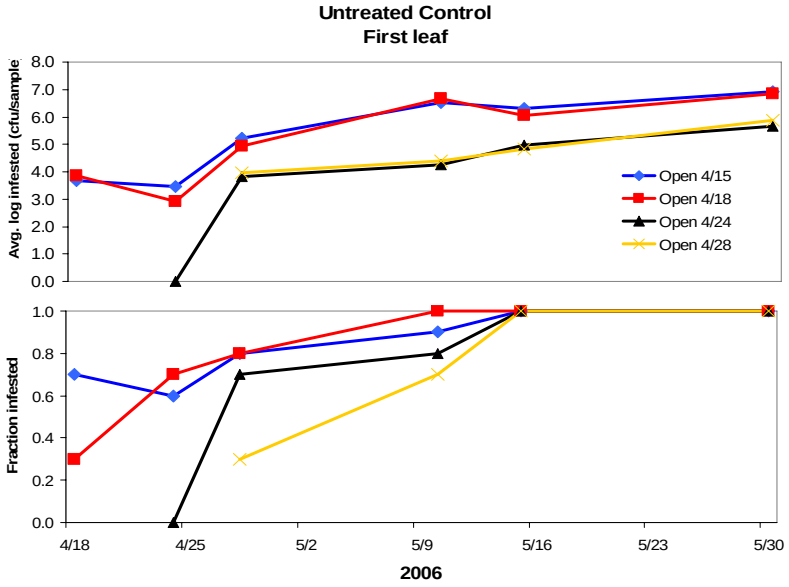

Figure 3. Population size of *Xanthomonas campestris pv. juglandis* on leaf 1 (basal [oldest] leaf) of walnut buds and shoots from untreated trees dissected at various times in 2006. The mean population size of the pathogen on leaves that harbored detectable pathogen is shown on the top panel while the fraction of leaves that harbored any pathogen cells are shown in the bottom panel.

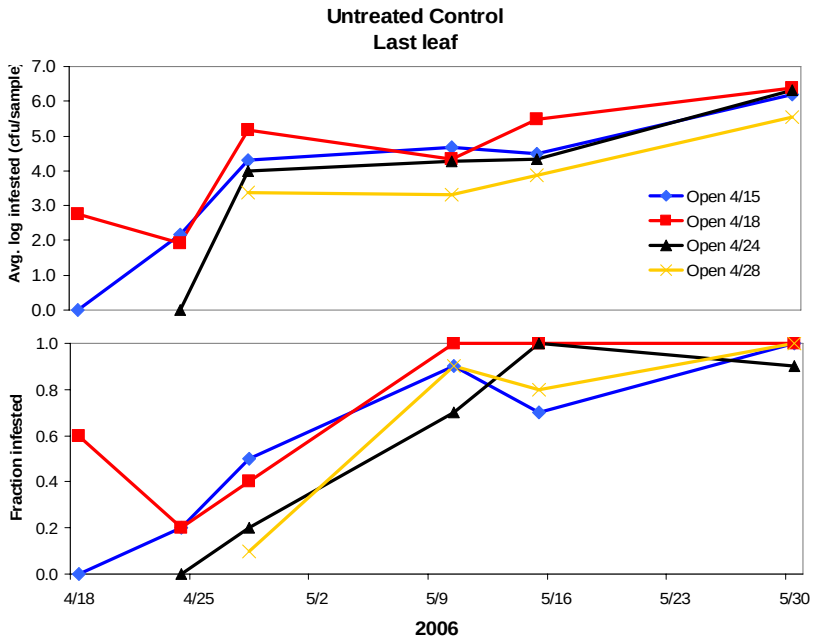

Figure 4. Population size of *Xanthomonas campestris pv. juglandis* on most distal of walnut buds and shoots dissected from untreated trees at various times in 2006. The mean population size of the pathogen onleaves that harbored detectable pathogen is shown on the top panel while the fraction of leaves that harbored any pathogen cells are shown in the bottom panel.

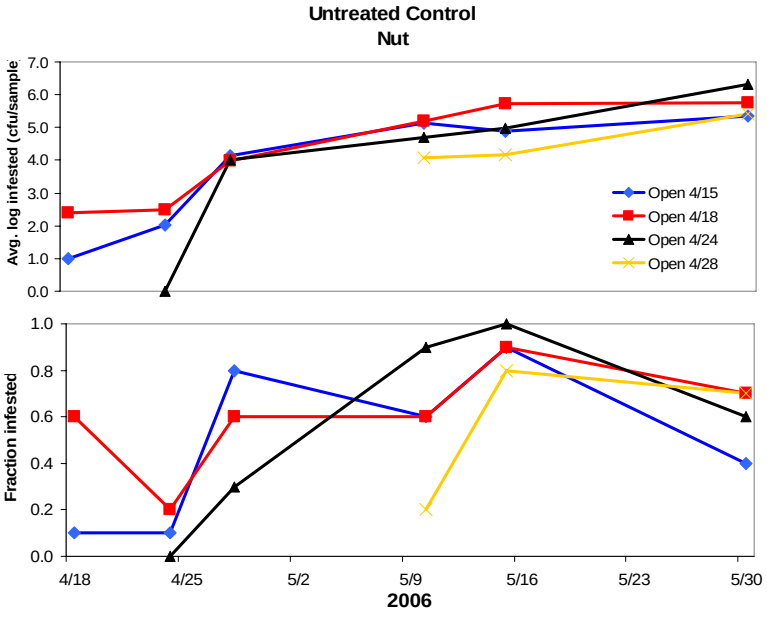

Figure 5. Population size of *Xanthomonas campestris pv. juglandis* on nuts of walnut on untreated trees at various times in 2006. The mean population size of the pathogen on nuts that harbored detectable pathogen is shown on the top panel while the fraction of nuts that harbored any pathogen cells are shown in the bottom panel.

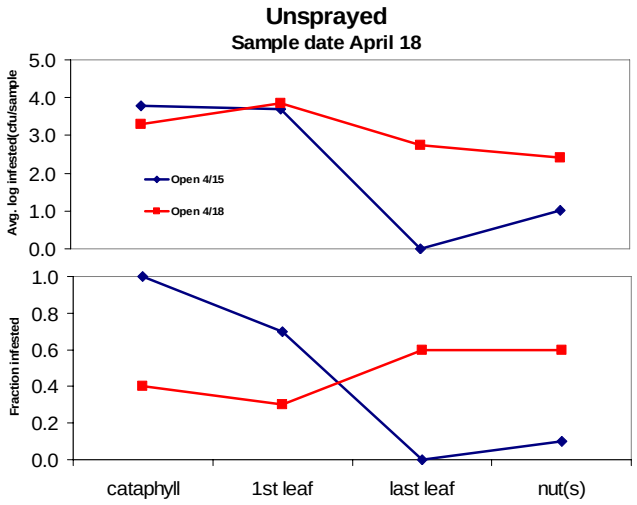

Figure 6. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) of unsprayed Chandler walnut trees that were assayed by dilution plating of tissue macerates on April 18, 2006 from buds that opened on April 15 (diamonds) or April 18 (squares).

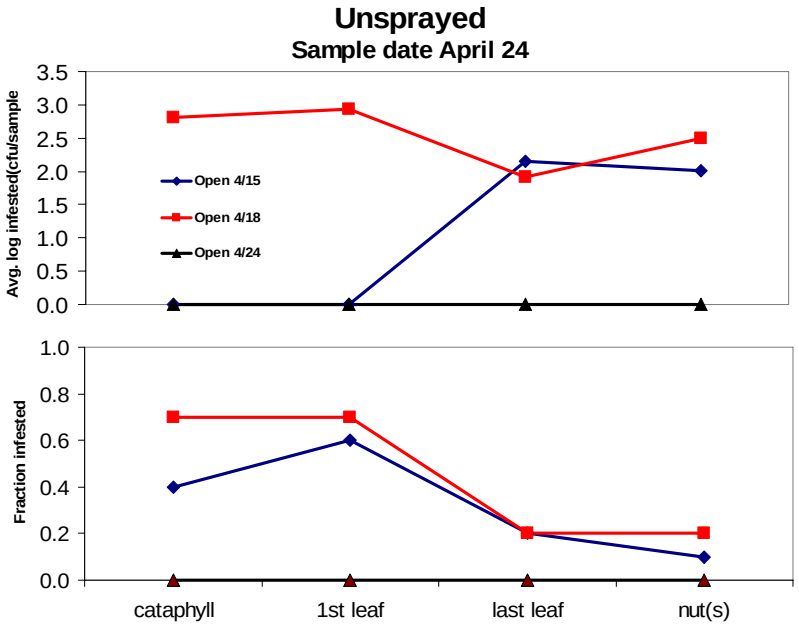

Figure 7. Population size of *X.c. juglandis* on various tissues from developing shoots of trees (shown on abscissa) from buds that opened on April 15 (diamonds) April 18 (squares) or April 24 (triangles) on untreated Chandler walnut when assayed by dilution plating of tissue macerates on April 24.

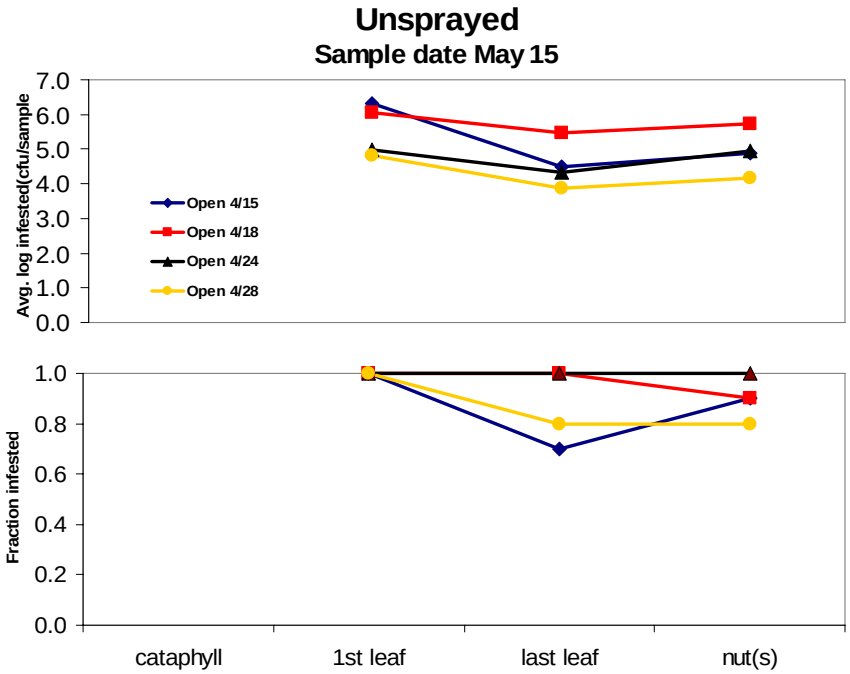

Figure 8. Population size of X.c. juglandis on various tissues from developing shoots (shown on abscissa) developing from buds that opened on April 15 (diamonds), April 18 (squares) April 24 (triangles) or April 28 (circles) on untreated Chandler walnut trees when assayed by dilution plating of tissue macerates on May 15.

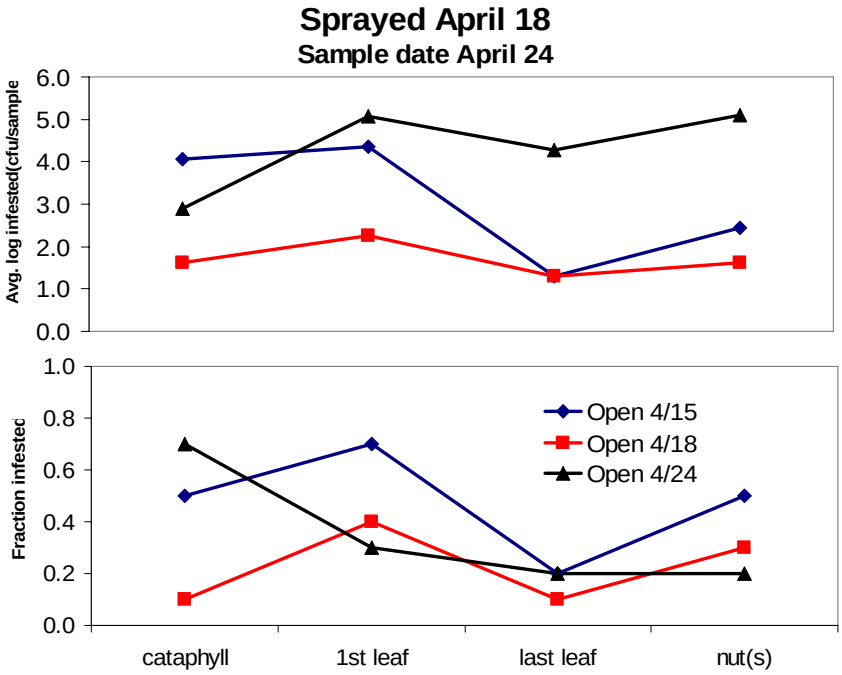

Figure 9. Population size of X.c. juglandis on various tissues from developing shoots (shown on abscissa) from buds that opened on April 15 (diamonds), April 18 (squares), April 24 (triangles) or April 28 (circles) on Chandler walnut trees that were sprayed on April 18 when assayed by dilution plating of tissue macerates on April 24.

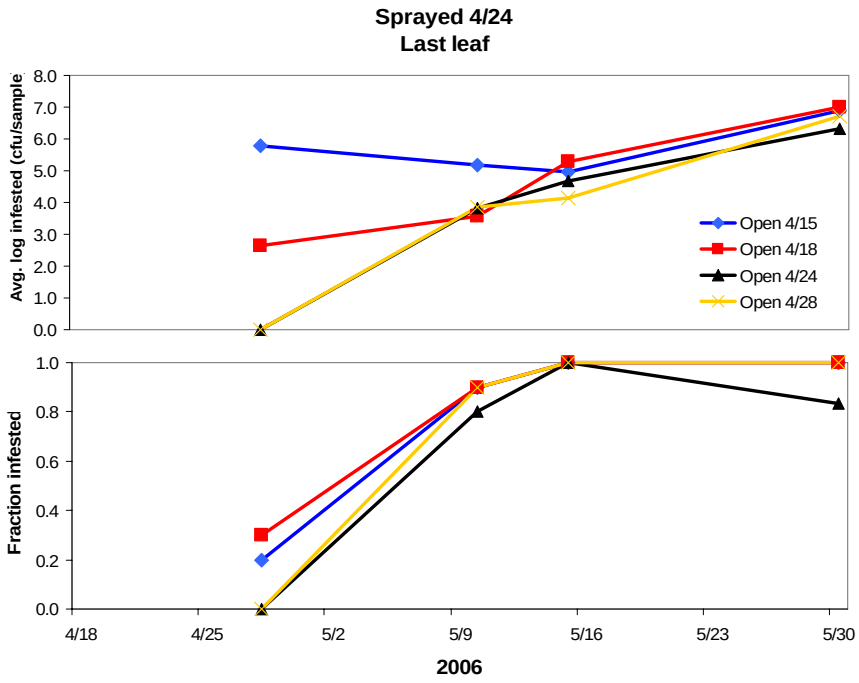

Figure 10. Population size of *X.c. juglandis* on the most distal leaf of walnut shoots from buds that opened on April 15 (diamonds), April 18 (squares), April 24 (triangles) or April 28 (x's) on Chandler walnut trees sprayed with Kocide+Manex on April 24 when assayed by dilution plating of tissue macerates.

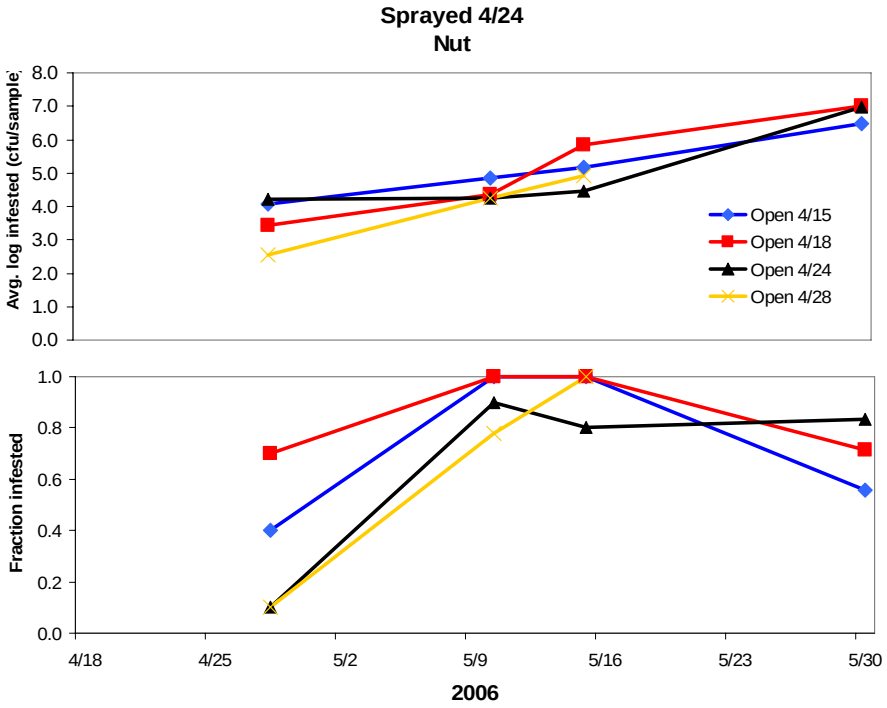

Figure 11. Population size of *X.c. juglandis* on developing nuts of walnut shoots from buds that opened on April 15 (diamonds), April 18 (squares), April 24 (triangles) or April 28 (x's) on Chandler walnut trees sprayed with Kocide+Manex on April 24 when assayed by dilution plating of tissue macerates.

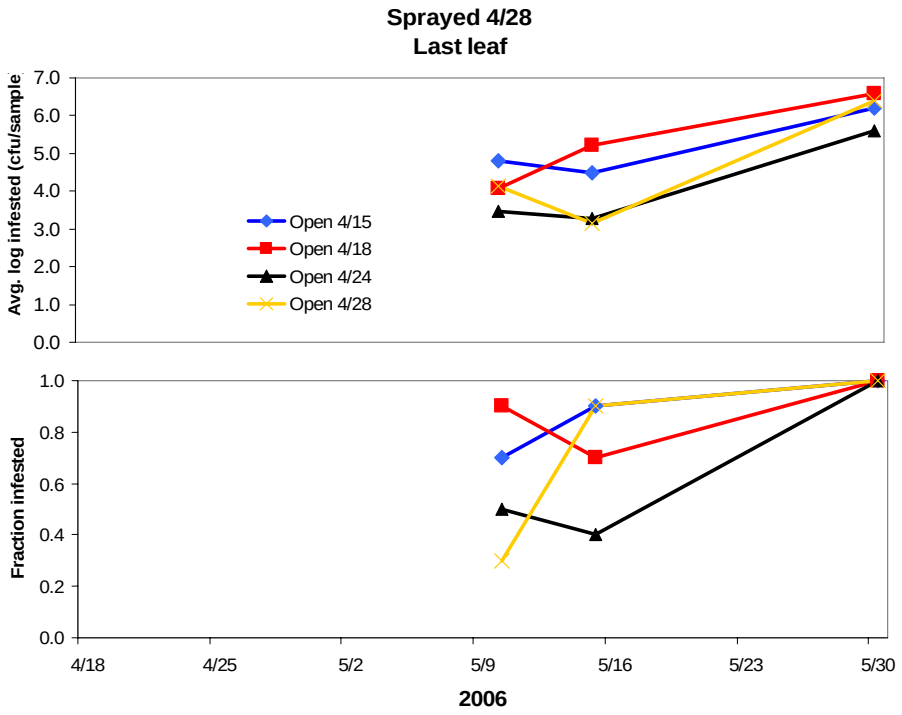

Figure 12. Population size of *X.c. juglandis* on the most distal leaves of walnut shoots from buds that opened on April 15 (diamonds), April 18 (squares), April 24 (triangles) or April 28 (x's) on Chandler walnut trees sprayed with Kocide+Manex on April 28 when assayed by dilution plating of tissue macerates.

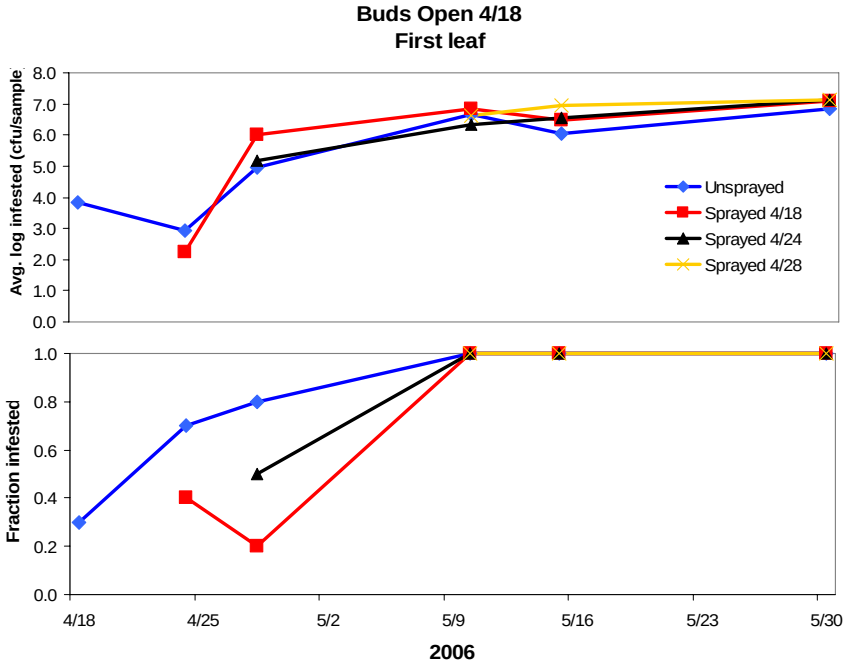

Figure 13. Population size of *X.c. juglandis* on the most basal leaves of walnut shoots from buds that opened on April 18 on unsprayed Chandler walnut trees (diamonds) or sprayed with Kocide+Manex on April 18 (squares), April 24 (triangles) or April 28 (X's) when assayed by dilution plating of tissue macerates.

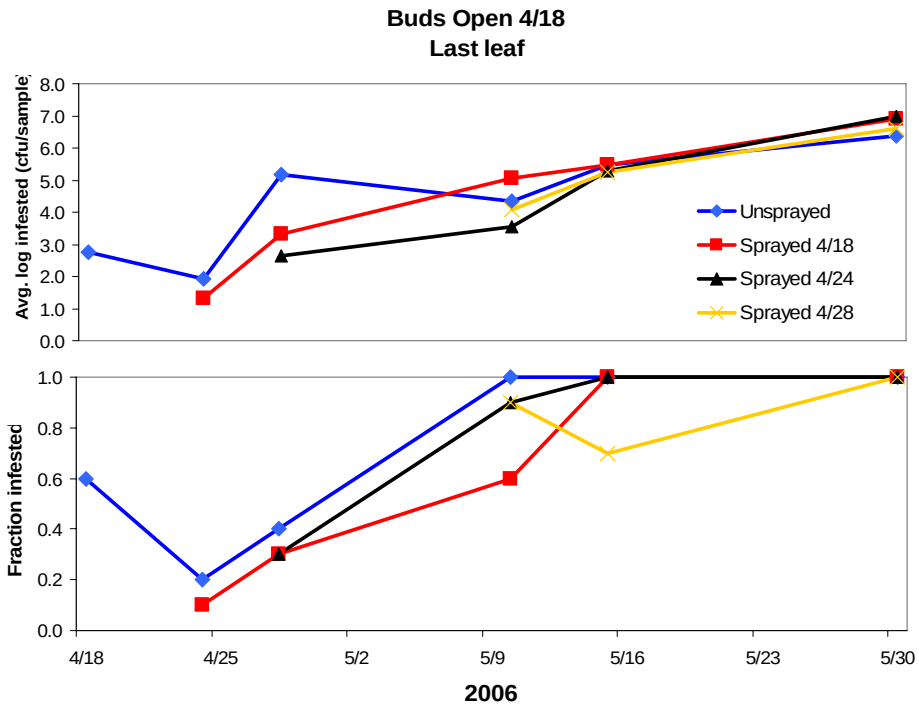

Figure 14. Population size of *X.c. juglandis* on the most distal leaves of walnut shoots from buds that opened on April 18 on unsprayed Chandler walnut trees (diamonds) or sprayed with Kocide+Manex on April 18 (squares), April 24 (triangles) or April 28 (X's) when assayed by dilution plating of tissue macerates.

Figure 15. Symptoms of walnut blight on Chandler nuts on May 28, 2006. Note the large, apparently older infection at the flower end of the nut with more numerous, and apparently newer infections on the sides of the nut.