EPIDEMIOLOGICAL APPROACHES TO THE CONTROL OF WALNUT BLIGHT DISEASE

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ABSTRACT

Buds of untreated trees, untreated trees exposed to artificial rainfall, and trees exposed to artificial rainfall and also treated with Kocide + Manex with Breakthru at bud break and again with Kocide + Manex alone one week later were dissected starting about 2 weeks prior to 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 to only about 50% incidence of colonization, in 2005, colonization increased progressively from about 35% 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 2004 the incidence of leaves contaminated with *X .c. juglandis* increased with time, the population size on those leaves that became contaminated did not increase appreciably even though leaves were wetted repeatedly with artificial rain, in 2005 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. More frequent rainfall events in 2005 may be responsible for the enhanced colonization seen in this year compared to all 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 phonological 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 of after spraying. Inoculum of *X.c. juglandis* can rapidly move onto developing leaves and nuts after bud break and that 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. Large increases in population sizes of *X.c. juglandis* were observed on both treated and untreated leaves and nuts in May, associated with frequent rainfall events; while populations of the pathogen were generally lower on treated trees than on untreated trees, such increases in pathogen abundance throughout the growing season had never been seen before and was probably related to the exceptionally frequent rainfall events in late April and in May. 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. The proportion of nuts that were evaluated on trees in early June that exhibited walnut blight disease was very high (>60%) on untreated control trees, and single applications of Kocide + Manex provided little apparent disease control. However, since equal numbers of buds had been tagged on treated and untreated trees we could account for nuts that had apparently dropped from the trees after infection but before rating; trees treated once with Kocide + Manex near bud break harbored about 40% more nuts than did untreated control trees. Thus early-season bactericide treatments apparently provided much more disease control that was estimated from the incidence of infected nuts due to the high numbers of infected nuts that apparently dropped from trees before rating.

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.

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 primary 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. Consequently, 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 actually killing established bacterial populations. Thus, we expected that later bactericide sprays might have relatively little impact on the disease. 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, 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 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 2 or more weeks. As a result, 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 account 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. 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 in 2005 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).

Objective 1

<u>Spatial location of *Xanthomonas campestris* pv. *juglandis* in developing walnut shoots</u>

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. 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. This data clearly showed that most leaves were colonized by *X.c. juglandis* shortly after they formed. We thus needed to know how fast these plant parts

became contaminated after emergence 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. If we knew the temporal pattern by which leaves and nuts became contaminated with the pathogen we could also better know when to apply protective bactericides. We therefore, in 2005, performed detailed spatial analysis of the distribution of *X.c. juglandis* in the various parts of walnut buds both before and during opening to determine if buds were uniformly infested. To assess how bactericide applications affected the distribution of inoculum in buds of untreated trees, samples from untreated trees exposed to artificial rainfall, and trees exposed to artificial rainfall and also treated with Kocide + Manex at bud break were dissected starting about 2 weeks prior to bud break and continuing until shoot expansion to determine the process by which inoculum of *X.c. juglandis* moves to developing shoots and nuts.

Walnut buds were carefully dissected to separate the various "bud scales" and the somewhat more internal cataphylls and the embryonic leaves present in walnut buds. In the Ashley variety that was sampled from trees in a commercial orchard in Butte County, most buds had 3 relatively hard, non-fleshy "bud scales" and 4 cataphylls (Fig. 1). Cataphylls are the outermost layers of the bud and, while fleshy, are not green nor do they develop into leaves upon bud break. Instead, these cataphylls enlarge somewhat but fall from the developing shoots by about mid-April. Most buds also contained about 6 embryonic leaves, which surrounded a tiny flower meristem at the apex of the bud. The number of leaf embryos varied somewhat between buds, but all buds had at least 6 leaf embryos. The outermost leaf embryos (destined to be the largest and most basal leaves on the stem) were relatively large, but the innermost leaf embryos (destined to be the most distal leaves on the shoot) were very tiny within the buds and did not always develop into fully expanded leaves. About 80% of the buds harbored *X.c. juglandis* when buds were sampled at the time of opening (Figs. 2-8). This high level of infestation made it possible to dissect buds with the assurance that a high proportion would yield data on the localization of *X.c. juglandis*.

There was a very high degree of spatial segregation of *X.c. juglandis* populations within infested walnut buds. The highest level of contamination was observed on bud scales; about 80% of all bud scales from untreated trees were contaminated, and the average population size on contaminated bud scales was about 1000 cells/bud scale (Figs. 2-3). Similar fractions of bud scales were contaminated with *X.c. juglandis* and average pathogen populations were similar irrespective of their relative position on the bud; i.e. the most exterior bud scales were not more likely to be contaminated that those somewhat more interior to the bud (Figs. 2-3). The fraction of bud scales that harbored *X.c. juglandis* and the average population size on infested bud scales was both substantially lower on trees treated a single time with Kocide + Manex in water alone than on untreated trees (Figs. 2-3). This suggests that the topical application of Kocide + Manex had effectively reduced the level of contamination of the bud scales. 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.

Presumably, cells of *X.c. juglandis* are more exposed to copper compounds when applied in water alone compared to leaf tissues.

The incidence of contamination of cataphylls by *X.c. juglandis* on untreated trees was somewhat lower than that of the bud scales (Figs. 4-6). The average population size of *X.c. juglandis* on the cataphylls that were colonized by the pathogen was generally similar (about 100 to 300 cells/cataphyll) and lower than on bud scales (Figs. 4-6). The incidence of contamination of cataphylls by *X.c. juglandis* on trees treated with early-season bactericides was always lower than on untreated trees (Figs. 4-6). Likewise, the average population size of *X.c. juglandis* on those cataphylls that were contaminated with this pathogen on bactericide-treated trees was always lower than that of untreated trees (Figs. 4-6).

The pattern of colonization of embryonic and developing walnut leaves by *X. campestris pv. juglandis* strongly suggests that the leaves become inoculated with the pathogen shortly after emergence from the bud. A much lower proportion of the immature leaves in buds that were about to open harbored any detectable pathogen than on untreated trees (Figs. 7-11). Generally, less than about 40% of the individual immature leaves were contaminated with *X.c. juglandis* before about March 30, when bud break was initiated, and less than about 100 cells of *X.c. juglandis* was observed on any of those few leaves that were contaminated (Figs. 7-11). For example, leaves 1 and 2, which would become the two oldest leaves on the developing shoot, seldom were contaminated with *X.c. juglandis* before bud break on untreated trees (Figs. 7-8). Unlike in 2004 where the incidence of contamination of leaves remained below 50%, even though the trees were receiving large amounts of artificial rainfall each week, both the incidence of colonization and the number of cells of *X.c. juglandis* increased dramatically on untreated leaves between about April 1 and the end of April (Figs. 7-11). By late April nearly all leaves on untreated trees were colonized by *X.c. juglandis* and average populations of the pathogen were more than 10^5 cells/leaf (Figs. 7-11). The differences in colonization of untreated leaves between 2005 and 2004 were striking; while there was more frequent rains in 2005 than in 2004, there were several rain events and repeated artificial wetness events in 2004 that had not led to such increases in *X.c. juglandis* populations as seen in 2005. In fact, no such increases in populations of *X.c. juglandis* had ever been seen in the 10 years of our studies, and we are continuing to examine weather records to determine if some particular weather condition might be associated with such large increases in pathogen populations. Since *X.c. juglandis* is apparently not a particularly fit epiphyte on walnut leaves, the higher frequency of rain in 2005 in might have enabled it to persist better between rain events, whereas in all other years it did not persist. The application of Kocide + Manex, but without Breakthru early in the spring had little effect on the colonization of leaves by *X.c. juglandis* (Figs. 7-11). The largest effects of bactericide applications were observed on the most distal leaves where average *X.c. juglandis* populations were reduced about 10-fold and the incidence of colonization by the pathogen was reduced about 20% (Figs. 7-11). The relatively small effect of the bactericide application in 2005, compared to similar studies done in 2004 may be attributable to the fact that no surfactant was added to the bactericide, hence reducing its ability to penetrate into the bud when applied early in the spring, thus reducing the amount of protectant bactericide that would be present on newly-emerging leaves as the bud opened. These results do 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 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.
Substantial numbers of the pathogen was observed in the so-called "stem" material remaining after leaves and nuts had been excised from developing buds/shoots (Fig. 12). While a relatively low incidence of infestation of this material was observed before bud break, the incidence of infestation increased on untreated trees increased to close to 100% by early April, and remained high for the remainder of the spring (Fig. 12). The application of bactericides did not appreciably affect either the incidence or population size of *X.c. juglandis* compared to untreated trees (Fig. 12). Given that the most exterior bud parts are most heavily contaminated with *X.c. juglandis* and that the "stem" contains the plant part that is in direct contact with the base of the bud before emergence, the "shoot" is probably contaminated early in the process of bud expansion.

Nuts on untreated trees became infested with *X.c. juglandis* very soon after their formation on trees in 2005 (Fig. 13). By early April over 80% of the nuts had detectable populations of *X.c. juglandis*, although population sizes were generally less than about 10^3 cells/nutlet (Fig. 13). Early-season bactericide applications reduced the incidence of infestation of nuts with *X.c. juglandis* and average pathogen populations on most sampling dates (Fig. 13).

Dissections of buds and shoots were also made from trees that received no artificial rainfall so that the effect of augmented rainfall on the process of colonization of walnut plant parts could be monitored. We then could compare directly the incidence of contamination of a given plant part on untreated trees that were exposed to only natural rainfall with those that had also received artificial rainfall. Probably because natural rainfall was so frequent, we did not observe an elevated incidence of contamination of a given plant part with increasing amounts of rainfall (Figs. 2-13). For example, the incidence of contamination of a given leaf cohort was as usually as high or higher on "unsprinkled" trees as on trees that had received artificial rainfall (Figs. 2-13). Likewise, average *X.c. juglandis* populations on infested leaves were usually similar on trees that received normal and augmented rainfall (Figs. 2-13).

Objective 2
Effect of bactericide sprays made at different phenological stages on control of *X.c. juglandis*

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 (youngest leaves), is most important at this time. These findings also explain why early-season applications of bactericides are so 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 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. 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 up to 500 buds per bud cohort to receive a given spray treatment was noted at 7 day intervals starting at the time of first bud break by placing small colored plastic ties on the base of each bud every 7 days during the 21 day period over which bud break occurred. Ties were applied to buds that were newly cracked open. As a result, 5 different phenological ages of buds were defined. 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 in nuts. At each 5 day interval a replicate set of 3 trees was sprayed with a mixture of Kocide + Manex containing 0.5% Breakthru. We thus had several hundred tagged buds, yielding several hundred nuts that received bactericide application each at different times relative to bactericide sprays. The plot areas were both equipped with overhead sprinklers to provide artificial rainfall. The design and operation of the artificial rainfall system is described well in the 2005 report of Buchner.

About 70% of the buds in this Chandler orchard harbored *X.c. juglandis* when sampled in February (Fig. 14). Quite significantly about 60% of the buds sampled on April 6 on untreated trees also harbored *X.c. juglandis* (Fig. 15). 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.

Shoot samples were collected on April 7, April 26, and May 10 from trees treated at different times relative to bud opening with Kocide + Manex in 0.5% Breakthru, as well as from untreated trees, and the population size of *X.c. juglandis* estimated. At each sampling time the populations of *X.c. juglandis* was estimated on the various parts of the walnut shoot (from bud scales to terminal leaves, etc.). 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 7 and May 10. Such increases are similar to that seen in the Butte county site in 2005 (Figs. 2-7), but unlike any other site in over 10 years of sampling walnut. Much lower amounts of multiplication of *X.c. juglandis* was observed on walnut tissues sprayed at various times relative to bud break. For example, when considered over all parts of the shoot, the

populations of *X.c. juglandis* increased from only about 100 cells/sample to about 10^5 cells/sample on shoots developing from buds that opened on March 25 and sprayed on that same day (Fig. 17) when measured on April 7 and May 10, respectively. Likewise, similar populations were observed on shoots that developed from buds that opened on March 31 and were sprayed on the same date (Fig. 18). However, populations increased from about 10^3 to nearly 10^6 cells/sample on shoot parts developing from buds that opened on March 25 but that were not sprayed until March 31 over the time period of April 7 until May 10 (Fig. 19). Thus, application of Kocide + Manex in 0.5% Breakthru decreased *X.c. juglandis* populations most when applied to buds on the same day they opened compared to a delay of a week before application.

More insight into the process of colonization of walnut shoots and the efficacy of bactericide sprays could be gained from detailed examination of the populations of *X.c. juglandis* on buds and shoot parts from buds that were sprayed with bactericide at different times relative to bud opening and sampled at a given subsequent time. For example, when sampled on April 7, populations of *X.c. juglandis* on untreated trees ranged from about 100 to 10^4 cells/sample, being highest on cataphylls and basal leaves, and were similar on shoots developing from tagged cohorts of buds that were closed on March 25, opened March 25, or opened March 31 (Fig. 20). Thus while these buds opened over a 2 week period, the process of colonization of developing shoot tissues was similar on all of the shoots, suggesting that weather conditions, if they affected the colonization process, were similar over this period. The populations of *X.c. juglandis* were much lower on shoot parts developing from buds that opened on March 25, if the trees were sprayed on March 25, than if sprayed on March 31 (Fig. 21). In fact, pathogen populations were similar for a given plant part from buds opened March 25 whether it was sprayed a week after bud opening or whether it was left unsprayed (Fig. 21). The proportion of tissues that harbored any *X.c. juglandis* was much lower on samples from shoots developing from buds that opened on March 25 and were sprayed on this same day than from shoots from trees sprayed on March 31 or from untreated trees (Fig. 33). Similarly, the populations of *X.c. juglandis* were much lower on plant parts on trees sprayed on March 31 that developed from buds that opened on March 31 compared to buds that opened on March 25 (Fig. 23). The populations of *X.c. juglandis* assayed on April 7 were somewhat lower for a given plant part on trees that were sprayed on March 25 when developing from buds that were open on March 25 when spraying was done compared to buds that were closed at the time of spraying (Fig. 22). The reductions of X.c. juglandis populations conferred by bactericide sprays made near the time of bud break were much larger on nuts and stems than that observed on leaves sampled at this time (Figs.21-23). Thus on this early sample date, it is clear that much better control of *X.c. juglandis* populations were achieved on tissues that emerged from buds that were sprayed on the day that they opened than from buds that were closed at the time of spray , and particularly from buds that had been open for a week before spraying.

When sampled on April 26, further evidence was provided for superior control of populations of *X. c. juglandis* when bactercides were applied close to the time of bud opening. Populations of *X. c. juglandis* on untreated trees were about 10^5 cells/sample, irrespective of whether they were from shoots developing from tagged cohorts of buds that were closed on March 25, opened March 25, opened March 31, or opened April 7 (Fig. 24). Thus while these buds opened over a 2

week period, the process of colonization of developing shoot tissues was similar on all of the shoots. In contrast, populations of *X.c. juglandis* on plant parts developing from buds that opened on April 7 were about 100-fold lower on trees treated with bactericide on this date compared to untreated control trees (Fig. 25). The largest reductions of pathogen populations were observed on the most distal leaves and nuts (Fig. 25). Populations of *X.c. juglandis* were reduced somewhat less (from 10-100 fold) on tissues developing from buds that opened on March 25 but which were not sprayed until April 7 (Fig. 26), or from buds that opened March 31 but which were not sprayed until April 7 (Fig. 27). In fact, when sampled on April 26, populations of *X.c. juglandis* on trees sprayed with Kocide + Manex on April 7 were on average, about 10-fold lower on a given plant part on shoots emerging from buds that opened on April 7 compared to those that opened March 25 or March 31 (Fig. 28). Thus on this sample date, like the earlier sample date, it is clear that much better control of *X.c. juglandis* populations were achieved on tissues that emerged from buds that were sprayed on the day that they opened than from buds that had been open for a week or two before spraying.

When sampled on May 10, populations of *X.c. juglandis* on leaves and nuts of untreated trees had increased to about 10^5 cells/sample, irrespective of whether they were from shoots developing from tagged cohorts of buds that were closed on March 25, opened March 25, opened March 31, or opened April 7 (Fig. 29). Thus while these buds opened over a 2 week period, the process of colonization of developing shoot tissues was similar on all of the shoots. While *X.c. juglandis* populations on tissues emerging from buds treated at the time of bud break were reduced more than when bactericides were applied after bud break at the sampling time, differences in population sizes were much smaller than at earlier sample times. For example, populations on tissues developing from buds that opened on March 25 and sprayed on that date were only slightly lower than that from buds that opened on March 25 but sprayed on March 31; both treatments reduced *X.c. juglandis* populations between 10 and 100-fold compared to untreated trees (Fig. 30). Likewise, *X.c. juglandis* populations on tissues developing from buds that opened on March 25 and March 31 and sprayed on March 31 were similar (Fig. 31). In contrast, little difference in pathogen populations were observed on tissues from buds that opened on March 31 and treated on that same date compared to untreated trees (Fig. 32). While the incidence of colonization of plant parts by the pathogen was reduced to relatively low frequencies (<50%) by bactericide sprays when assayed earlier in the spring (Fig. 33), nearly all tissue samples collected on May 10 harbored some detectable *X.c. juglandis* (data not shown).

When summarized 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 (Table 1). Much less control as observed when bactericides were applied either before or after bud break.

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 2). A very high incidence of walnut blight infections were observed in the plot area, and most nuts exhibited very severe infection (many blackened sites per nut). While the incidence of walnut blight disease was less in

all treatments in which bactericides had been applied, the differences in incidence of infection was low (Table 2). It was apparent that many nuts had dropped from the trees. While the reason for the dropped nuts could not be ascertained since the nuts quickly degraded on the soil we presume many nuts had dropped because of the severe infections that had occurred. However, since equal numbers of buds had been tagged on treated and untreated trees we could account for nuts that had apparently dropped from the trees after infection but before rating. Trees treated once with Kocide + Manex harbored about 40% more nuts than did untreated control trees (Table 3). Thus early-season bactericide treatments apparently provided much more disease control than was estimated from the incidence of infected nuts due to the high numbers of infected nuts that apparently dropped from trees before rating. It was also apparent from examination of the number of nuts remaining on the tree by mid-June, when disease ratings were taken from equal numbers of buds that had been tagged at the time of bud opening, that different cohorts of buds had VERY different likelihood of producing a nut. For example, those buds that opened on March 25 produced nearly twice as many nuts as those that opened on March 31 (Table 3). Furthermore, those buds that opened on April 7 produced only about 20% as many nuts as the first buds to open on March 25, and those that opened after April 7 produced almost no nuts. 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.

Table 1
Fold-reduction in population size of *Xanthomonas campestris* pv. juglandis populations on walnut trees treated once at different times relative to bud break with Kocide + Manex in 0.5% Breakthru

	Sample date			
Spray timing	April 7	April 26	May 10	Mean
Before bud break	6.0		0	1.9
At bud break	44.7	16.6	9.8	25.1
7 days after bud break	1.4	8.7	35.5	15.2
14 days after bud break		9.5		9.5

Table 2
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)	
Relative spray date	Sprayed	Control
Before bud break	0.62	0.77
At bud break	0.72	0.80
7 days after bud break	0.71	0.77
14 days after bud break	0.66	0.80

Table 3
Number of nuts harvested from buds tagged on walnut trees as they opened at different times

Spray date	# Nuts/500 buds				
		Bud opening date			
	Closed March 25	March 25	March 31	April 7	April 14
March 25	108	290			
March 31	16	450	256		
April 7	10	373	260	148	
No Spray	49	267	192	87	7

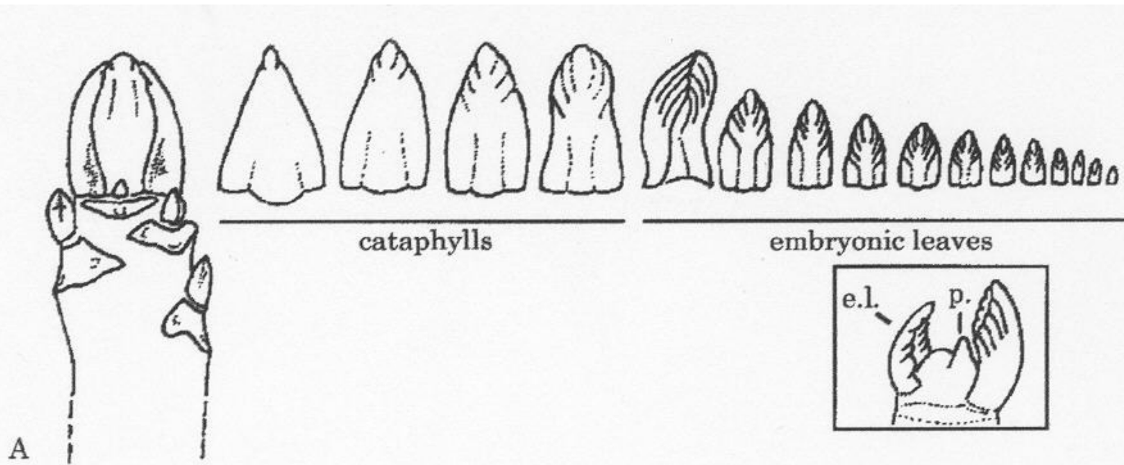

Figure 1. Depiction of the plant parts in a dissected walnut bud. Numbered from left to right are the cataphylls and embryonic leaves in a typical walnut bud.

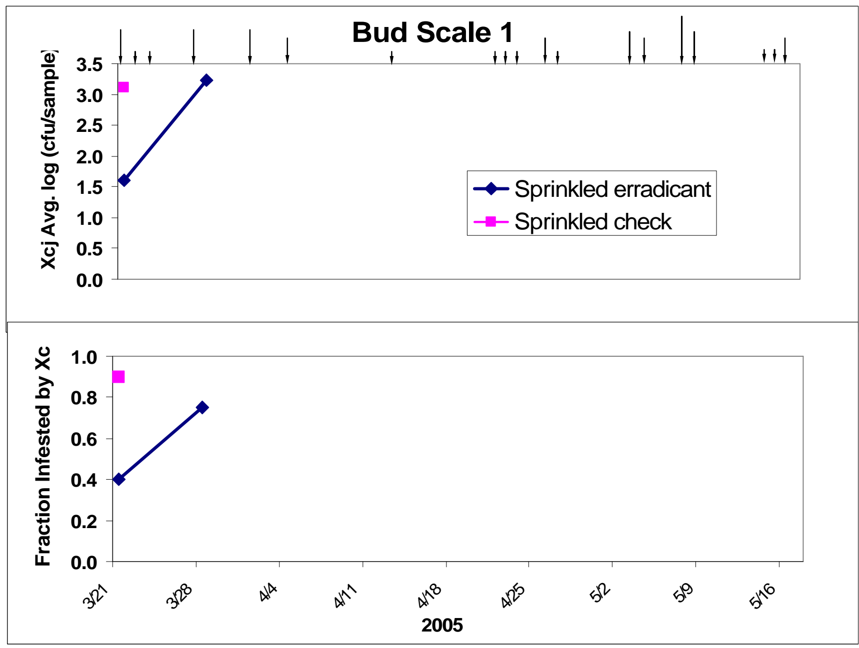

Figure 2. Population size of *Xanthomonas campestris pv. juglandis* on bud scale number 1 (outermost bud scale) of walnut buds dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored <u>detectable</u> pathogen is shown on the top panel while the fraction of bud scales that harbored <u>any</u> pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall or artificial rainfall.

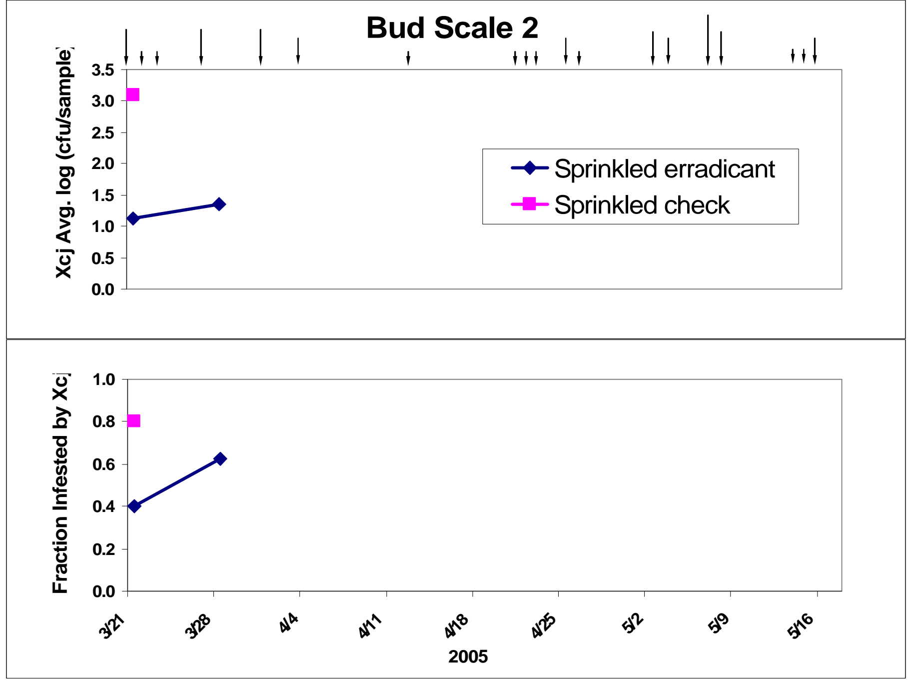

Figure 3. Population size of *Xanthomonas campestris pv. juglandis* on bud scale number 2 of walnut buds dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored <u>detectable</u> pathogen is shown on the top panel while the fraction of bud scales that harbored <u>any</u> pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event or artificial rain.

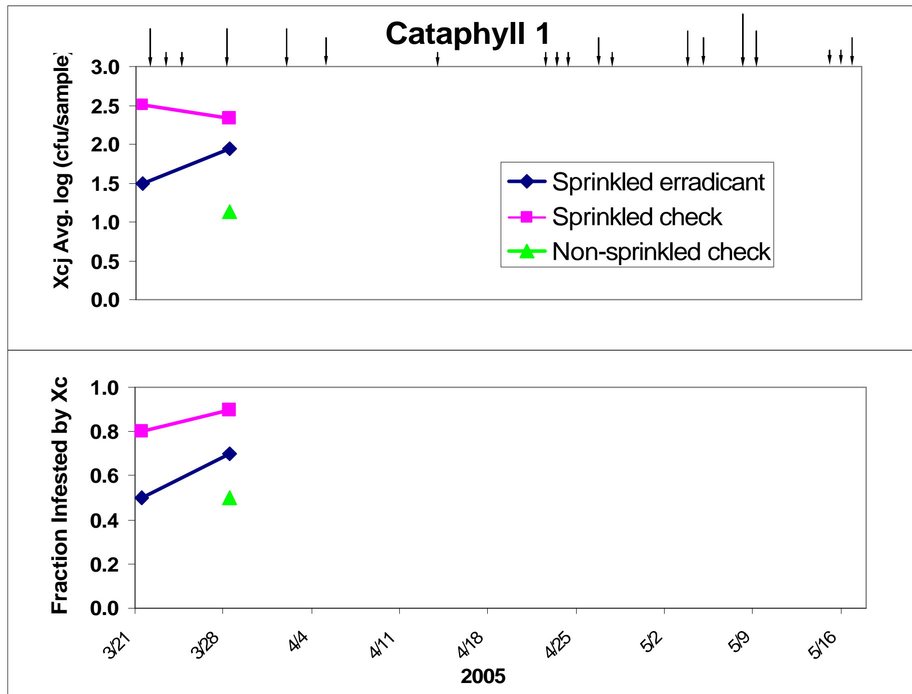

Figure 4. Population size of *Xanthomonas campestris pv. juglandis* on cataphyll 1 (outermost cataphyll) of walnut buds dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event or artificial rain.

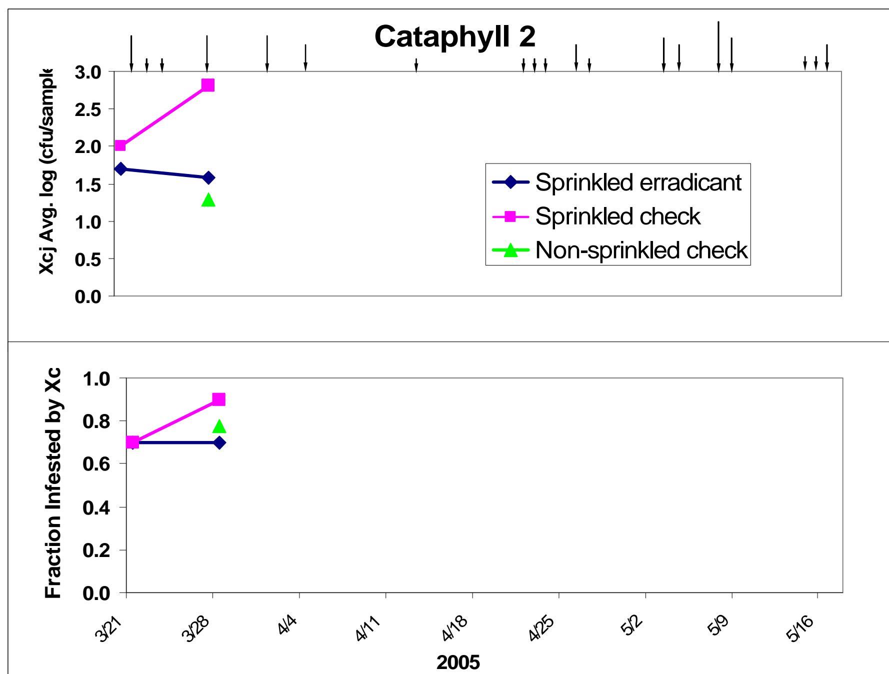

Figure 5. Population size of *Xanthomonas campestris pv. juglandis* on cataphyll 2 of walnut buds dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the middle panel. Vertical arrows refer to natural rainfall event or artificial rain.

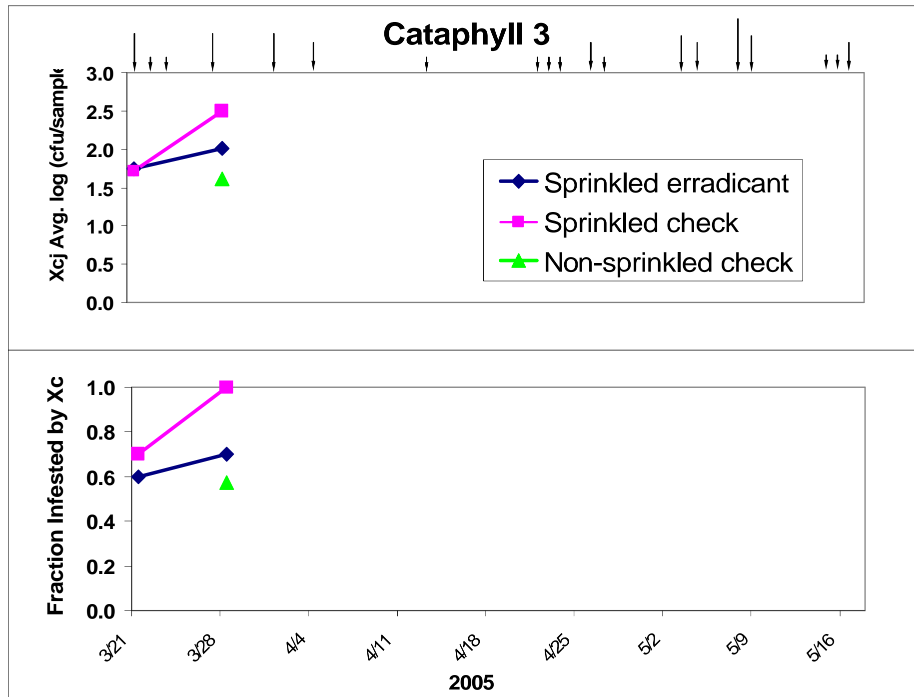

Figure 6. Population size of *Xanthomonas campestris pv. juglandis* on cataphyll 3 of walnut buds dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event or artificial rain.

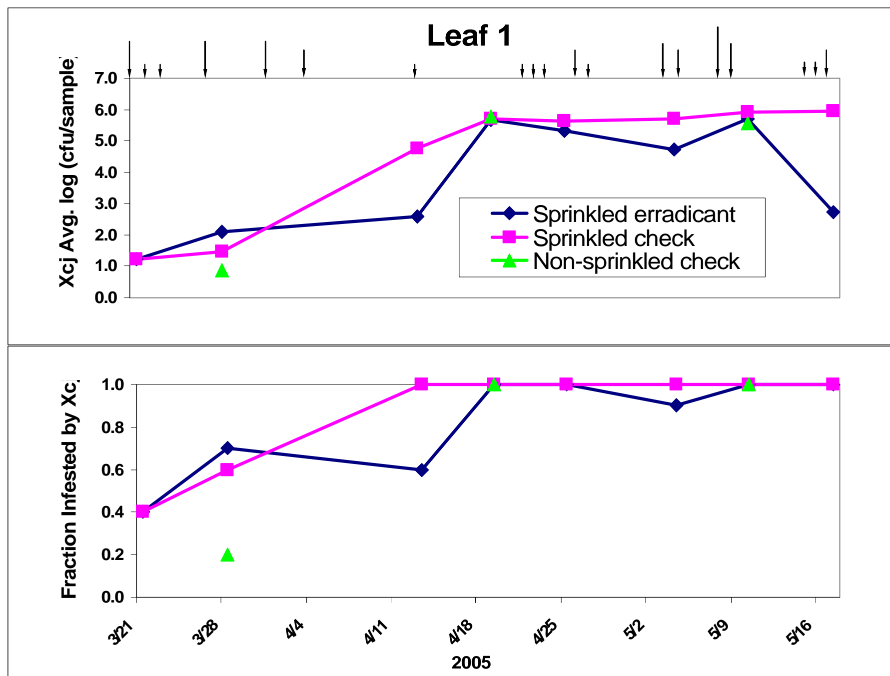

Figure 7. Population size of *Xanthomonas campestris pv. juglandis* on leaf 1 (outermost [oldest] leaf) of walnut buds and shoots dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

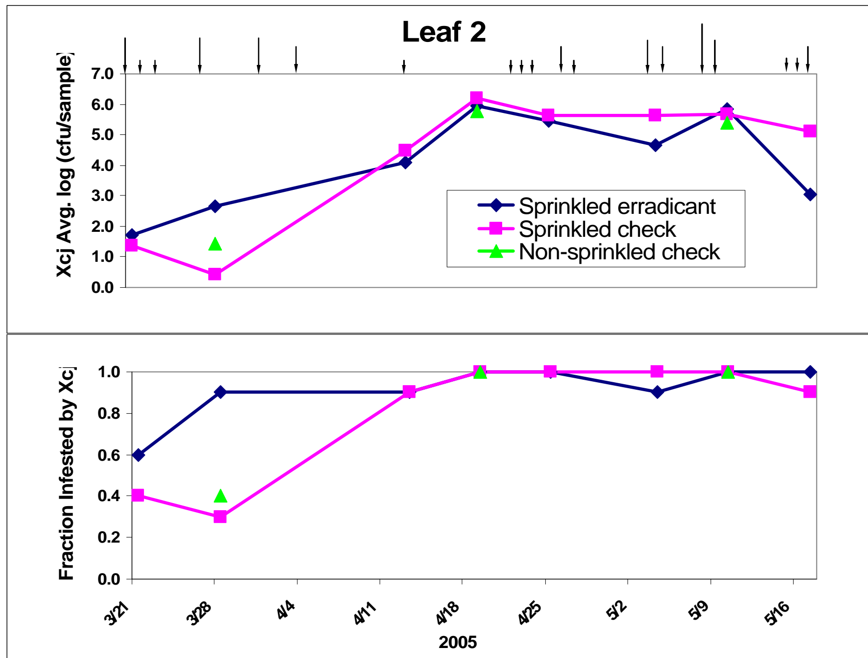

Figure 8. Population size of *Xanthomonas campestris pv. juglandis* on leaf 2 of walnut buds and shoots dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

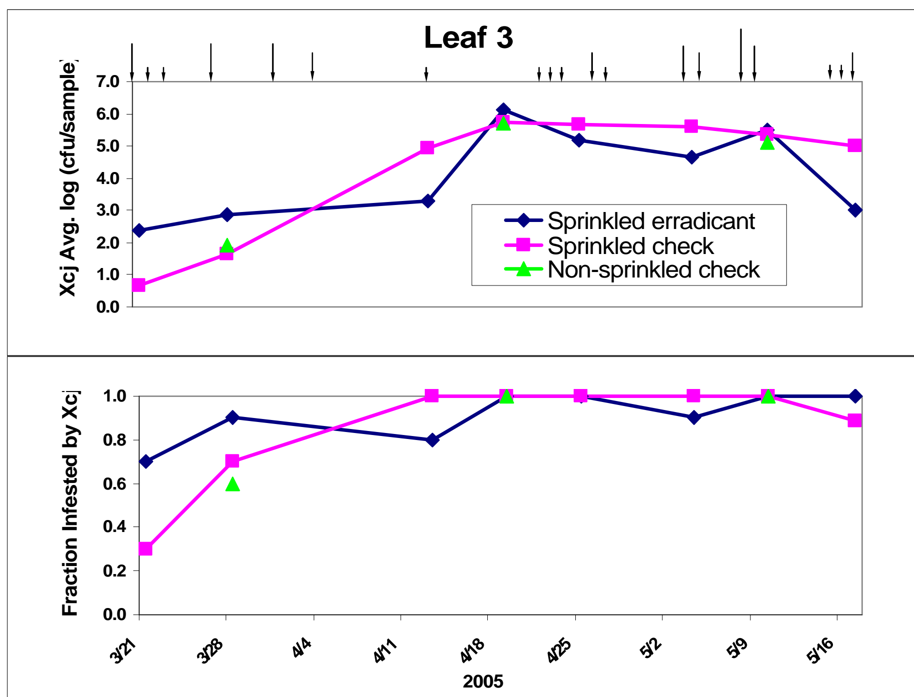

Figure 9. Population size of *Xanthomonas campestris pv. juglandis* on leaf 3 of walnut buds and shoots dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

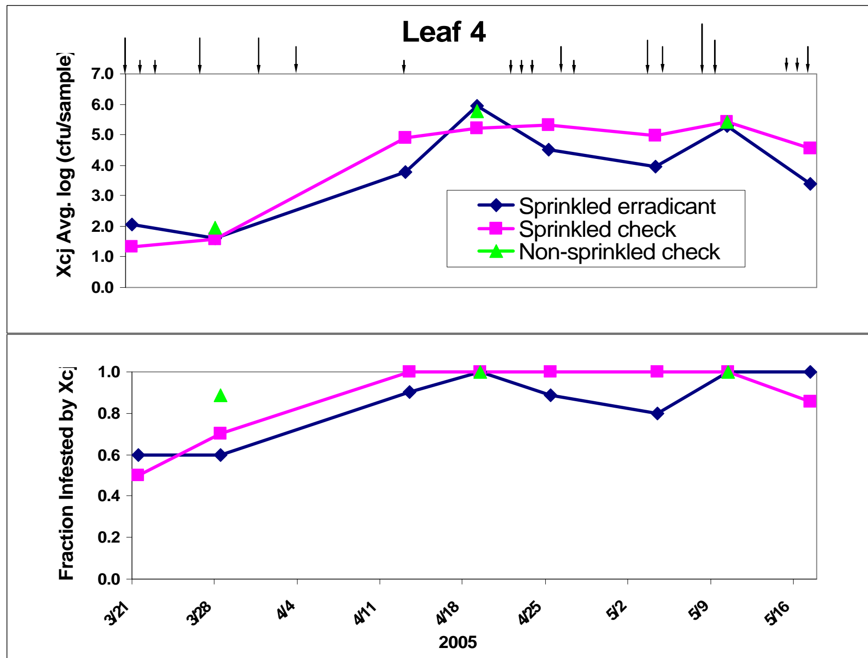

Figure 10. Population size of *Xanthomonas campestris pv. juglandis* on leaf 4 of walnut buds and shoots dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

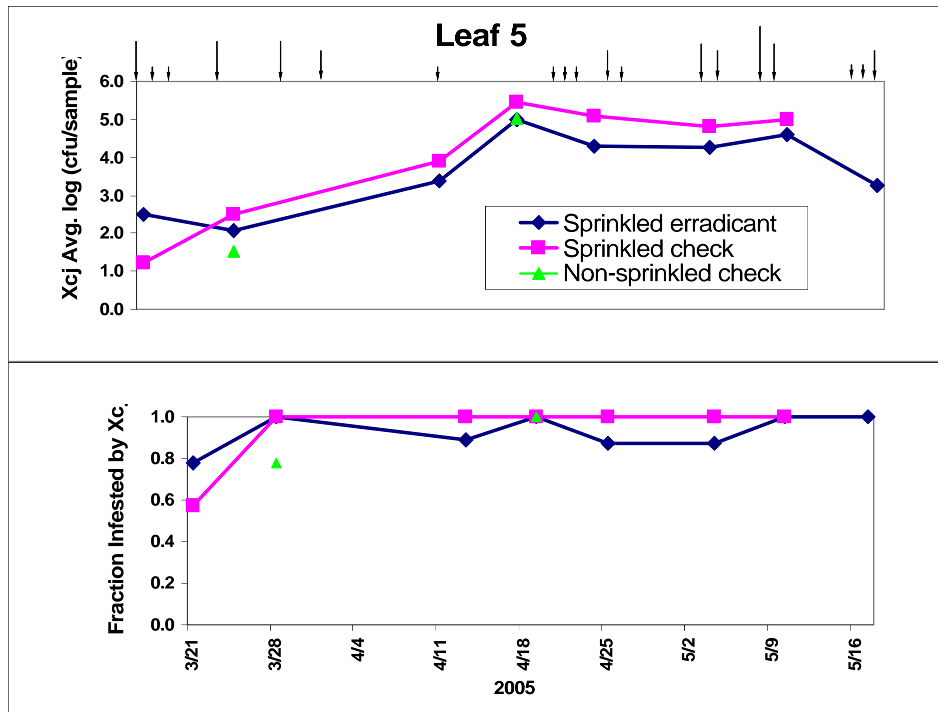

Figure 11. Population size of *Xanthomonas campestris pv. juglandis* on leaf 5 of walnut buds and shoots dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

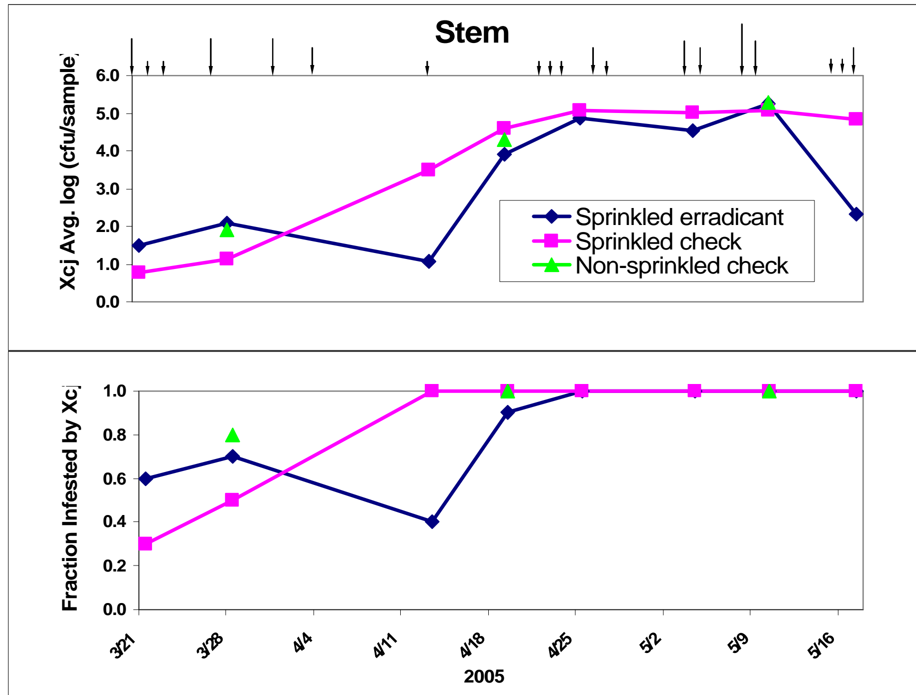

Figure 12. Population size of *Xanthomonas campestris pv. juglandis* on "stem" tissue remaining after removal of leaves and nuts of walnut dissected at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event .

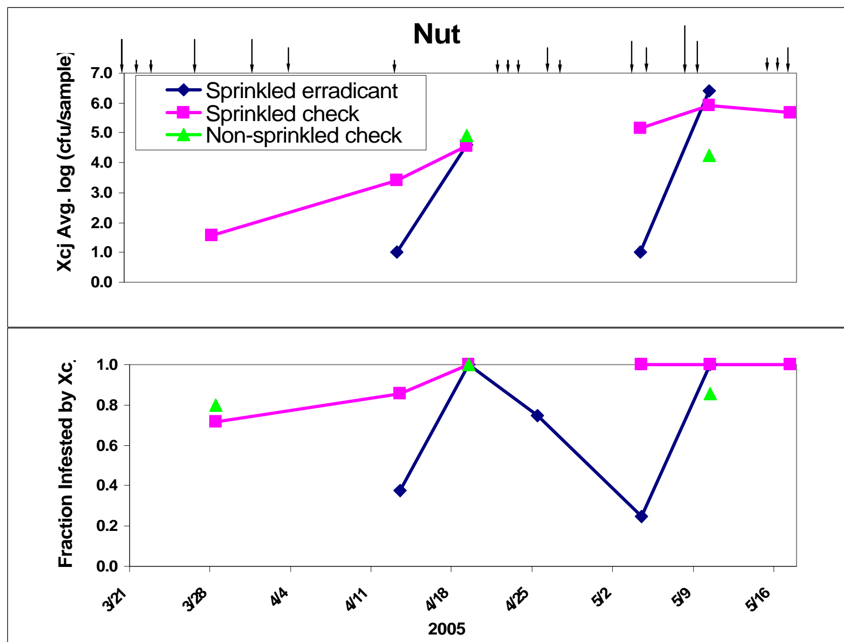

Figure 13. Population size of *Xanthomonas campestris pv. juglandis* on nuts of walnut at various times in 2005. The mean population size of the pathogen on bud scales that harbored detectable pathogen is shown on the top panel while the fraction of bud scales that harbored any pathogen cells are shown in the bottom panel. Vertical arrows refer to natural rainfall event.

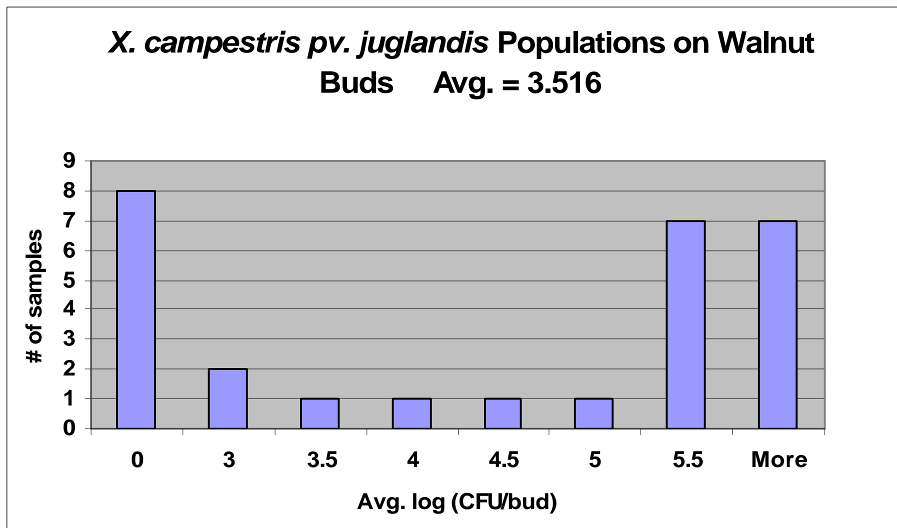

Figure 14. Population sizes of *Xanthomonas campestris pv. juglandis* on individual walnut buds collected in a commercial Chandler walnut orchard in Tehama County in February, 2005.

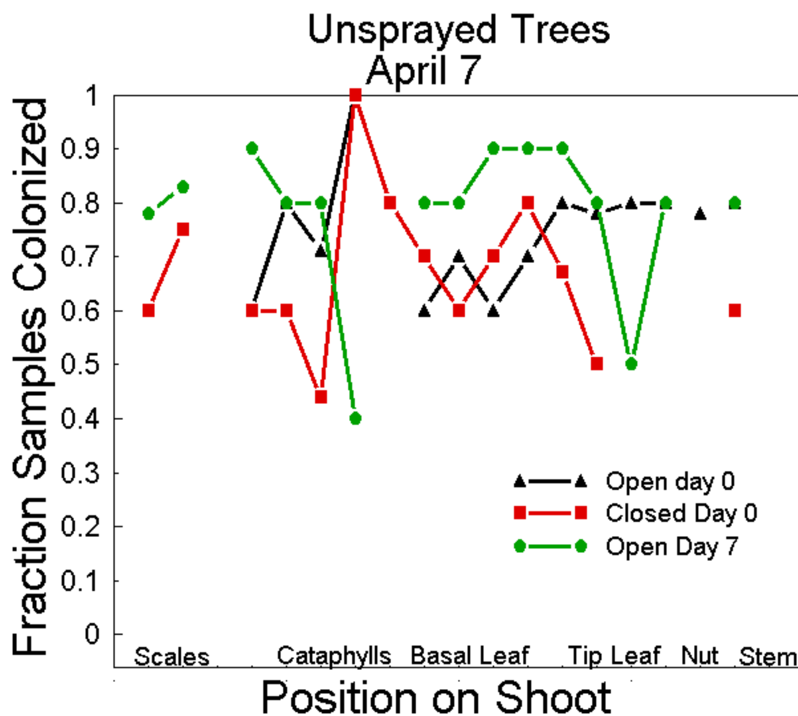

Figure 15. Fraction of samples of various tissues (shown on abscissa) on developing shoots of unsprayed Chandler walnut that had detectable *X.c. juglandis* that developed from buds that opened on March 25 (triangles), were closed on March 25 (squares) or opened on March 31 when assayed by dilution plating of tissue macerates on April 7.

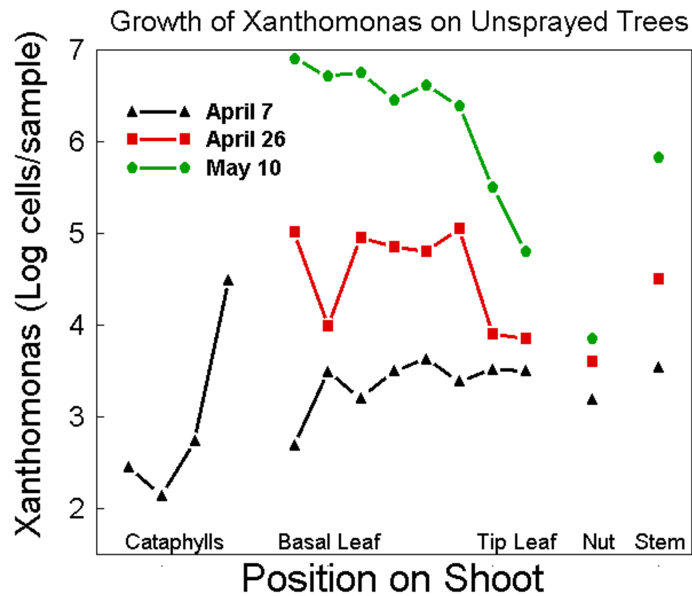

Figure 16. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) of unsprayed Chandler walnut trees that were assayed by dilution plating of tissue macerates on April 7 (triangles), April 26 (squares) or May 10 (circles).

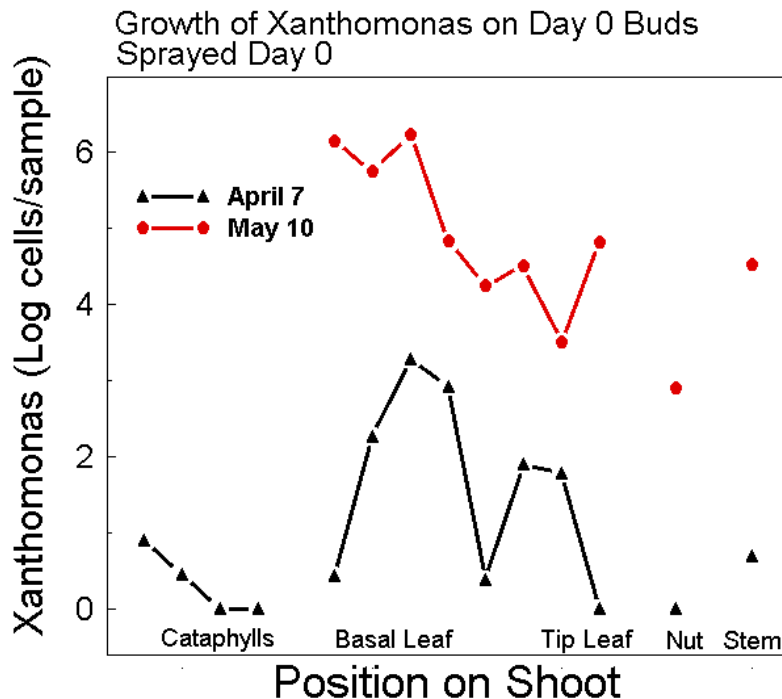

Figure 17. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 25 on Chandler walnut trees that were sprayed on March 25 when assayed by dilution plating of tissue macerates on April 7 (triangles), and May 10 (circles).

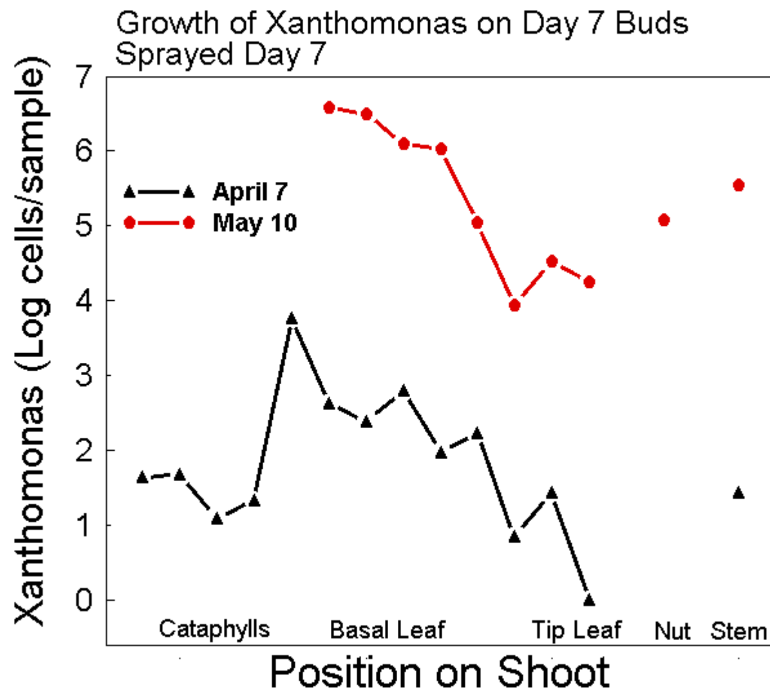

Figure 18. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 31 on Chandler walnut trees that were sprayed on March 31 when assayed by dilution plating of tissue macerates on April 7 (triangles), and May 10 (circles).

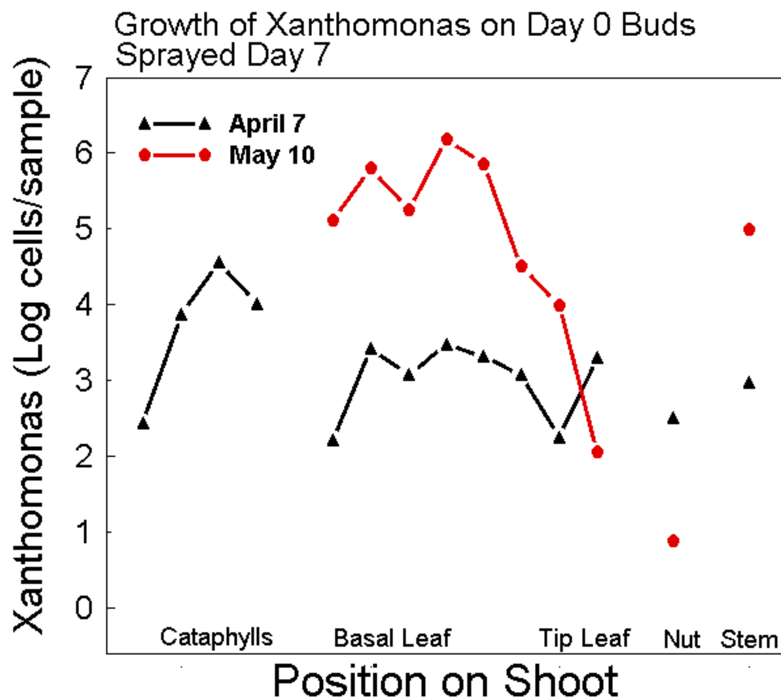

Figure 19. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 25 on Chandler walnut trees that were sprayed on March 31 when assayed by dilution plating of tissue macerates on April 7 (triangles), and May 10 (circles).

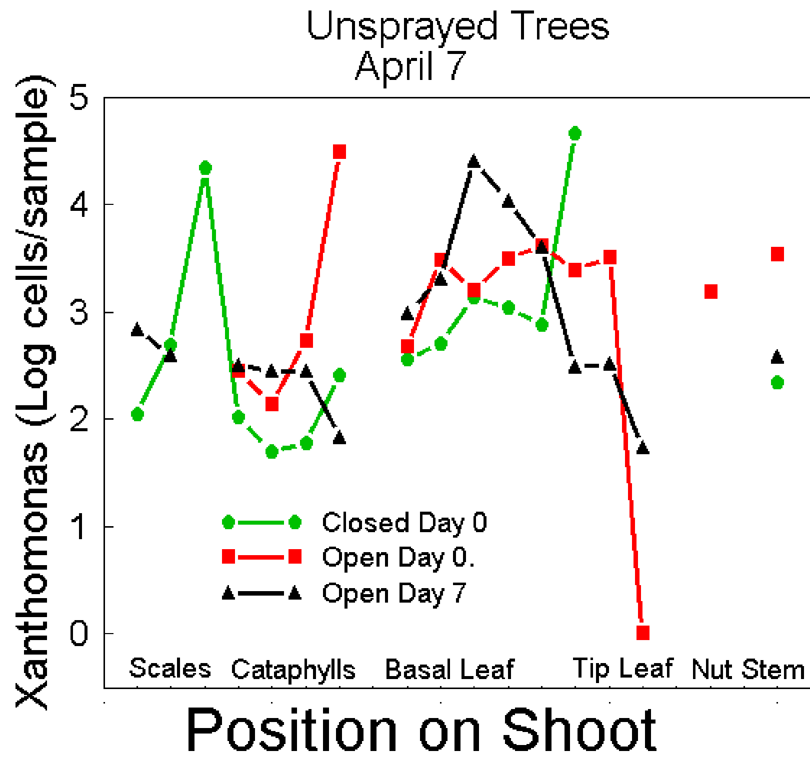

Figure 20. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that were closed on March 25 (circles), opened on March 25 (squares), or opened March 31 (triangles) on unsprayed Chandler walnut trees when assayed by dilution plating of tissue macerates on April 7.

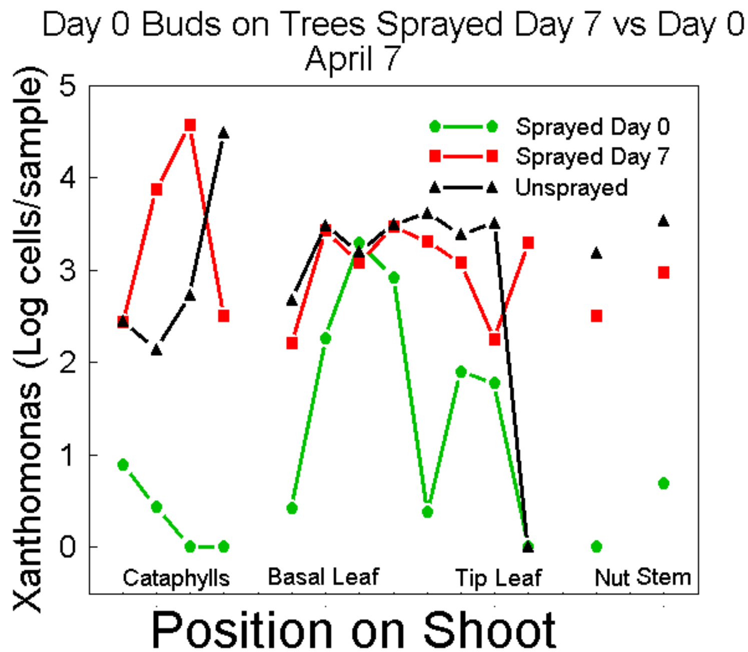

Figure 21. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 25 on Chandler walnut trees that were sprayed on March 25 (circles), March 31 (squares) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on April 7.

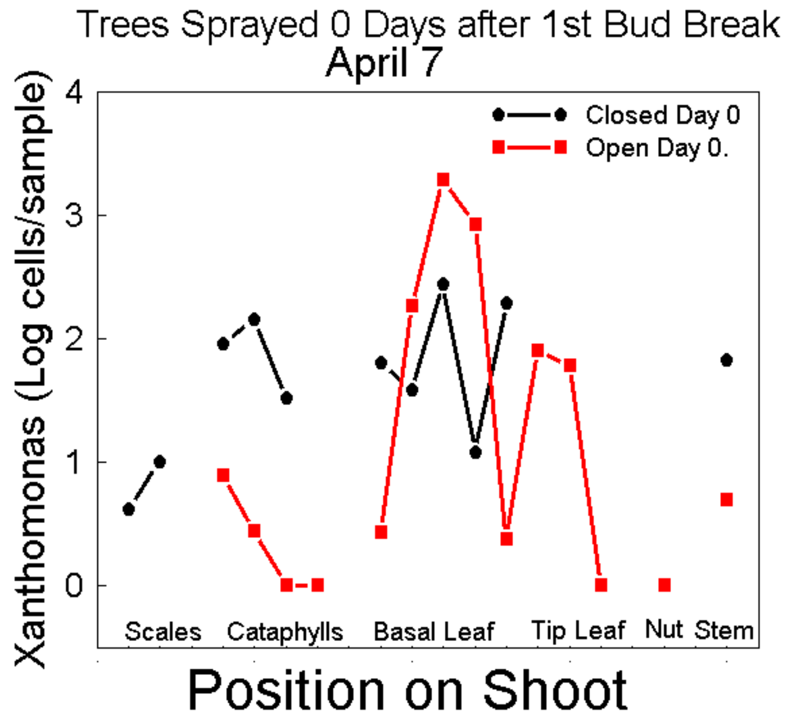

Figure 22. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that were closed on March 25 (circles), or opened on March 25 (squares) on Chandler walnut trees sprayed on March 25 when assayed by dilution plating of tissue macerates on April 7.

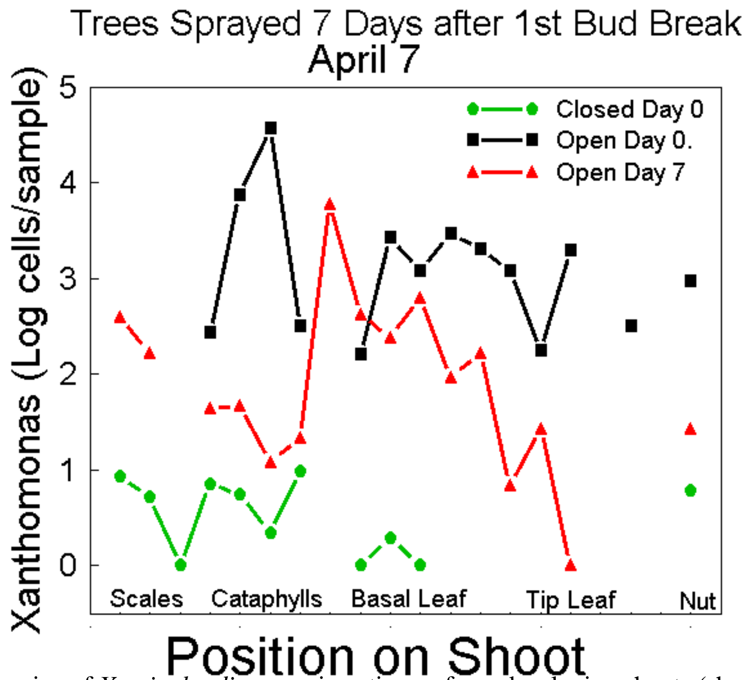

Figure 23. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that were closed on March 25 (circles), opened on March 25 (squares), or opened March 31 on Chandler walnut trees sprayed on March 31 when assayed by dilution plating of tissue macerates on April 7.

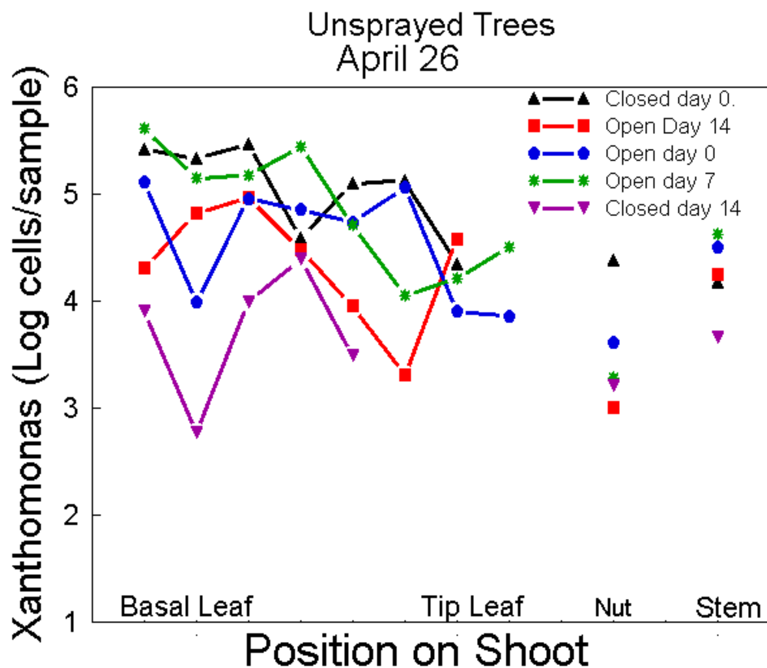

Figure 24. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) of unsprayed Chandler walnut trees that developed from buds that were closed on March 25 (triangles), opened on March 25 (circles), opened on March 31 (stars), opened on April 7 squares) or were closed on April 7 (inverted triangles) when assayed by dilution plating of tissue macerates on April 26.

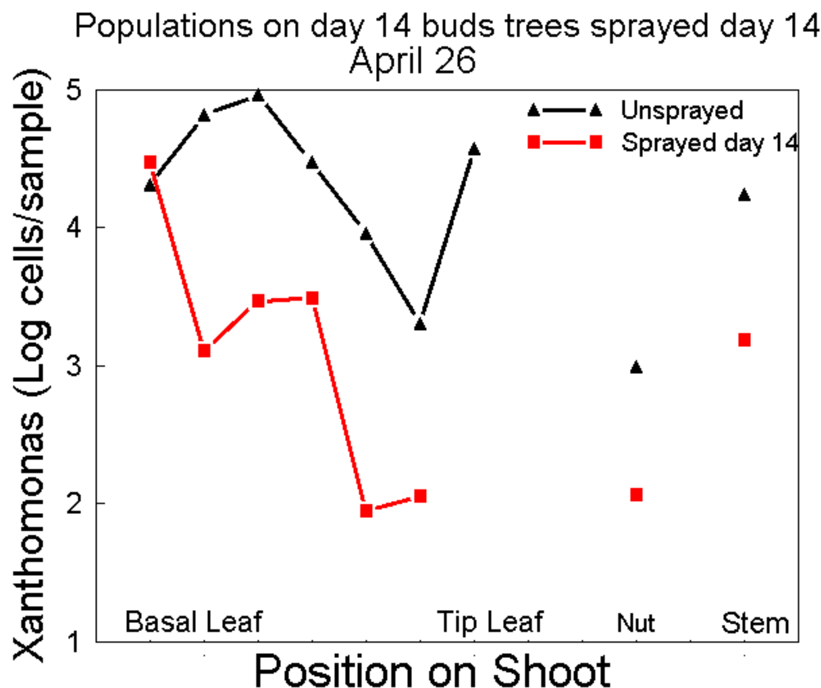

Figure 25. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on April 7 on Chandler walnut trees that were sprayed on April 7 (squares) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on April 26.

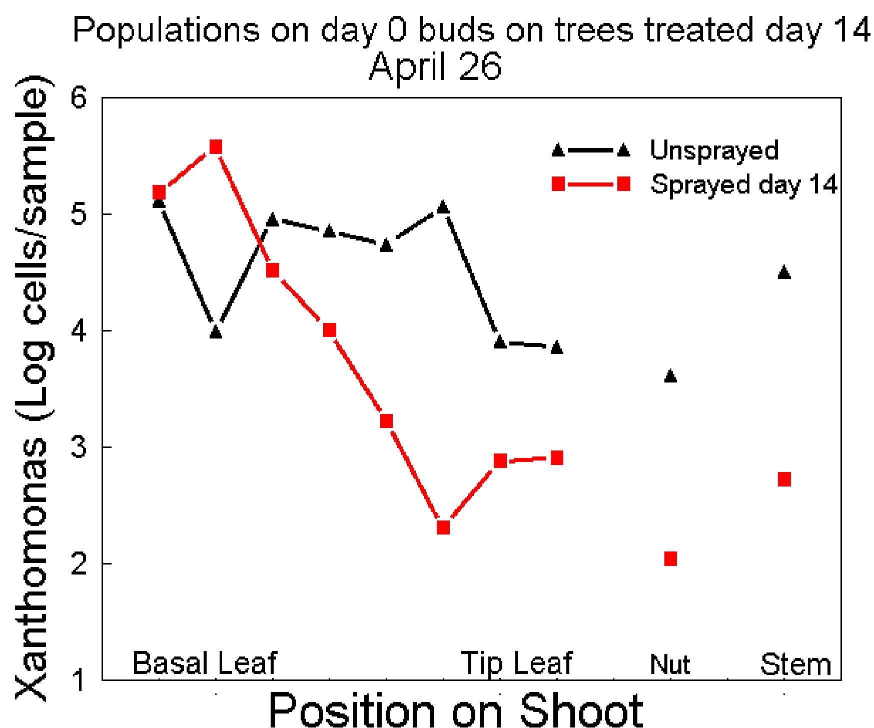

Figure 26. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 25 on Chandler walnut trees that were sprayed on April 7 (squares) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on April 26.

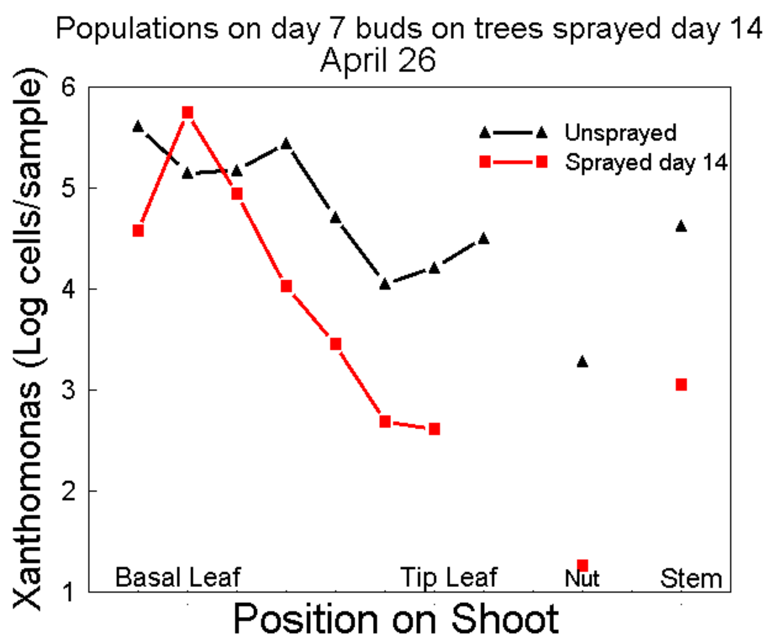

Figure 27. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 31 on Chandler walnut trees that were sprayed on April 7 (squares) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on April 26.

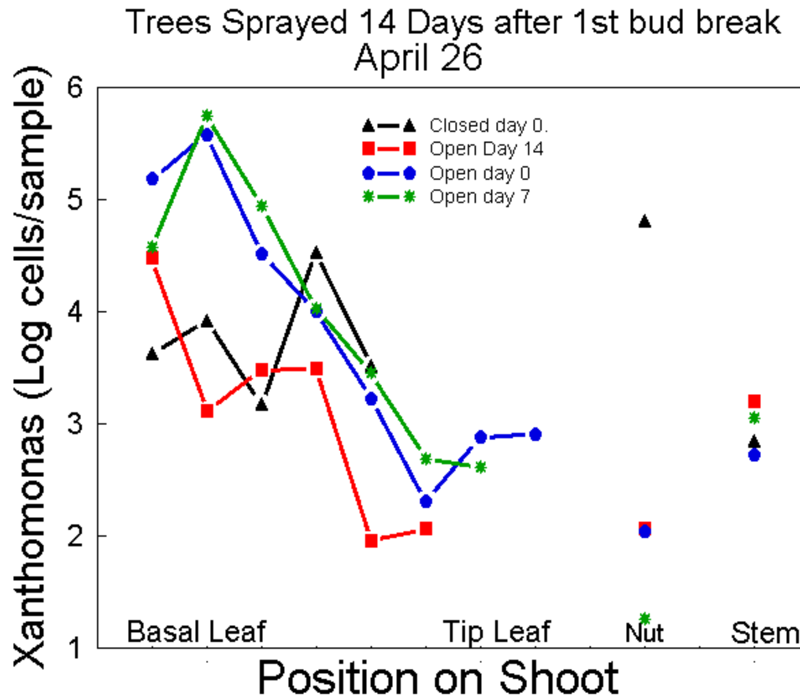

Figure 28. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that were closed on March 25 (triangles), opened on March 25 (circles), opened March 31 (stars) or opened April 7(squares) on Chandler walnut trees that were sprayed on April 7 when assayed by dilution plating of tissue macerates on April 26.

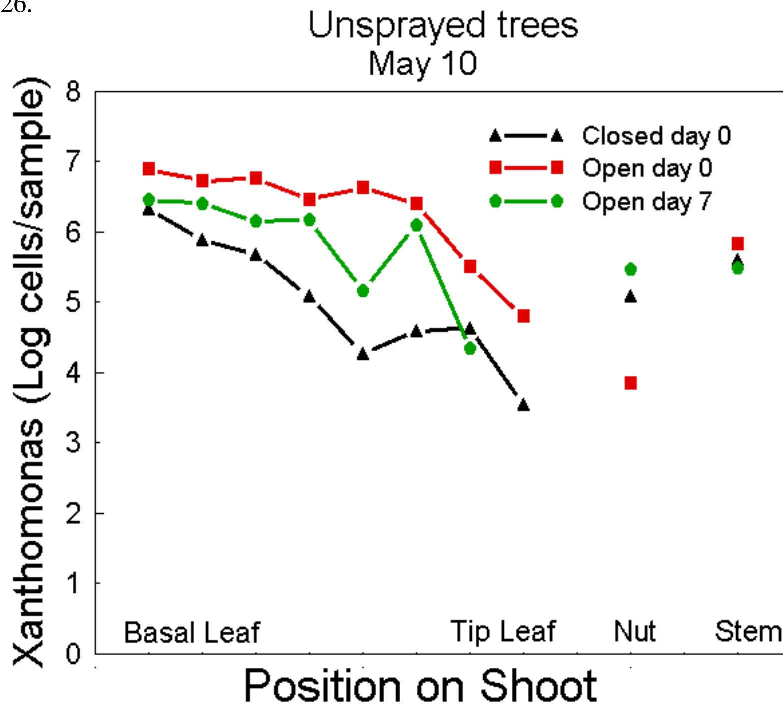

Figure 29. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) of unsprayed Chandler walnut trees that developed from buds that were closed on March 25 (triangles), opened on March 25 (squares), or opened on March 31 (circles) when assayed by dilution plating of tissue macerates on May 10.

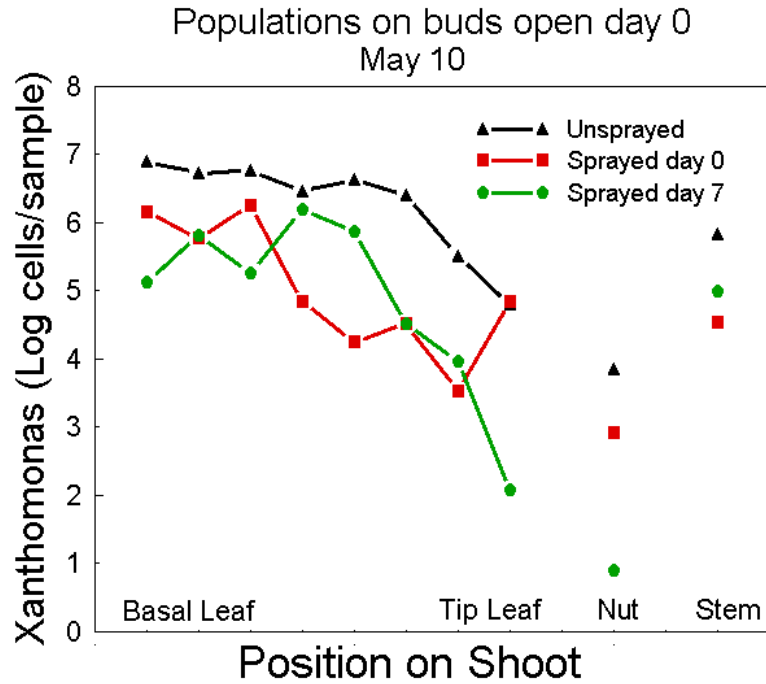

Figure 30. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 25 on Chandler walnut trees that were sprayed on March 25 (squares), sprayed on March 31 (circles) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on May 10.

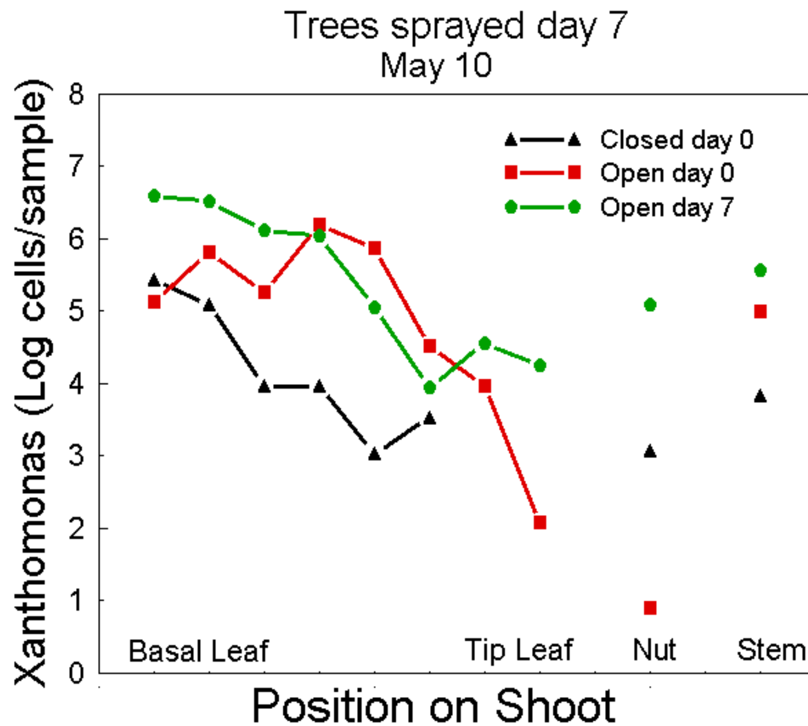

Figure 31. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that were closed on March 25 (triangles), opened on March 25 (squares), or opened on March 31 (circles) on Chandler walnut trees that were sprayed on March 31 when assayed by dilution plating of tissue macerates on May 10.

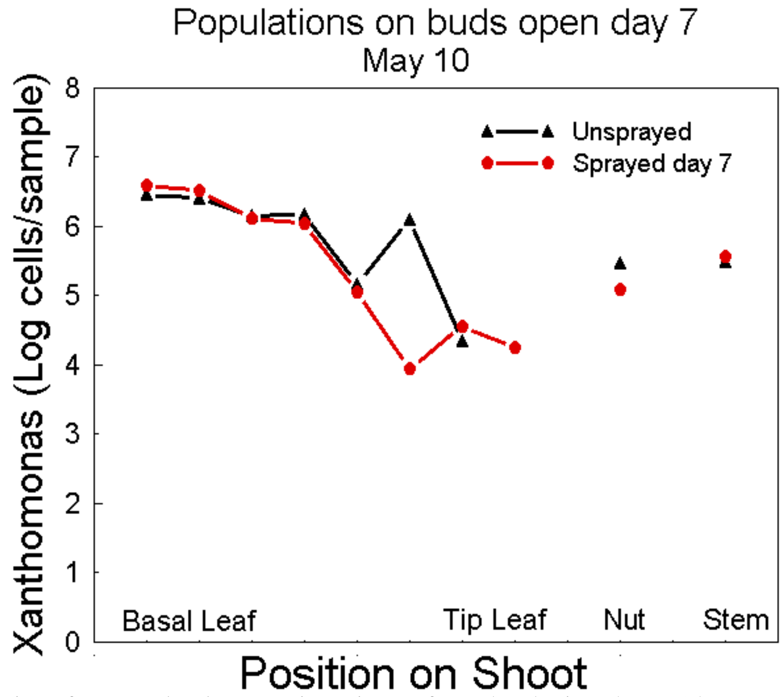

Figure 32. Population size of *X.c. juglandis* on various tissues from developing shoots (shown on abscissa) developing from buds that opened on March 31 on Chandler walnut trees that were sprayed on March 31 (circles) or left unsprayed (triangles) when assayed by dilution plating of tissue macerates on May 10.

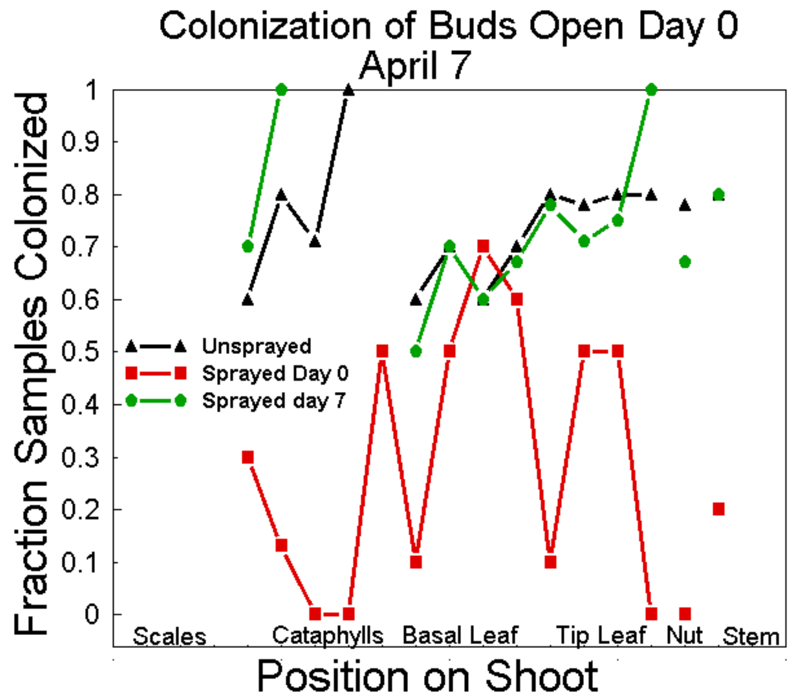

Figure 33. Fraction of samples of various tissues (shown on abscissa) on developing shoots of Chandler walnut that was sprayed on March 25 (squares), sprayed on March 31 (circles) or left unsprayed (triangles) that had detectable *X.c. juglandis* that developed from buds that opened on March 25 when assayed by dilution plating of tissue macerates on April 7.