Reduced Xanthomonas on Seed-to-seed Carrot Plants and Harvested Seed Resulting From Early Season Pesticide Applications, 2005-2006

Fred Crowe, Rhonda Simmons, Robert Crocker, Bruce Martens, and Mike Weber

Abstract

A number of antibacterial pesticides were sprayed singly or in combination onto seedling carrots in test plots in a five by five replicated field trial in the fall of 2005 (twice) and early spring of 2006 (once), following artificial point-source infestations of *Xanthomonas campestris* pv *carotae* ("Xanthomonas") in each plot at the two-leaf stage. In late fall 2005, Xanthomonas was recovered at moderate frequency from infested-but-unsprayed plots, but initial post-winter recovery was very low. In infested-but-unsprayed plots, frequency of recovery and net plot Xanthomonas populations on plants increased regularly through 2006, resulting in recovery from all five replications, an average of more than $1x10^7$ colony-forming units (CFU) per g dry carrot foliage by August 2006, and 2.8×10^8 CFU/10,000 seed at harvest in September 2006. In contrast, in plots treated with all antibacterial pesticides, recovery frequency and net Xanthomonas populations on foliage was lower up until harvest, and seed assays were lower than for the untreated check. This suggested that all antibacterial treatments applied to seedlings greatly delayed epidemic spread of Xanthomonas all season long. Nevertheless, seed assays were at least 1×10^5 CFU/10,000 seed for nearly all antibacterial treatments, at or above the level requiring hot water seed treatment. The only exception was the treatment combination of ManKocide® plus Tanos®, for which no Xanthomonas was recovered from plants from any plots in 2005 or 2006, or from harvested seed in 2006. It appeared that early Xanthomonas populations were eradicated by ManKocide plus Tanos, and Xanthomonas did not reestablish later in ManKocide plus Tanos plots. The field trial plot structure (plots were substantially separated by wide alleys and were drip irrigated in 2006 following sprinkler irrigation in fall 2005) allowed season-long product evaluation in spite of abundant spring rain in 2006. A revised working hypothesis of how Xanthomonas develops on seed-to-seed carrots is suggested.

Introduction

Bacterial blight of carrots is incited by *Xanthomonas campestris* pv *carotae* (hereafter simply referred to as "Xanthomonas"). In carrot seed fields in central Oregon and central Washington, bacterial blight disease generally is mild and occasional, even frequently absent, although high disease incidence does flare up in some fields in some years. More problematic is that Xanthomonas is abundant on seed harvested from these regions, even in the absence of bacterial blight disease. Such infested seed may be a source of infection for more damaging disease in commercial plantings.

The following summarizes the results of carrot seed field monitoring over several years in the Pacific Northwest (Du Toit et al. 2005): contamination levels of 10^6-10^8 CFY/10,000 harvested seed are common for commercial seed lots from central Oregon and central Washington. In seed-to-seed production, Xanthomonas incidence on foliage becomes established on a very low proportion of plants soon after planting of seed in fall, increasing progressively in most fields until a high proportion of plants harbor a population before harvest the following year. On individual plants, the

population increases very rapidly, frequently reaching levels of 10^6-10^8 CFY/g dry weight. Seed lots used to plant seed fields almost never test positive for Xanthomonas, indicating that seed lots are either carefully chosen and/or that all or most are hot water treated (see below). Sources for infestation of seed fields typically are local, probably originating in older seed fields. A few fields seem to escape all Xanthomonas infestation each year. Other crops and weeds appear not to harbor this strain of Xanthomonas.

We previously informally reported that as many as 5 percent of over-wintered seed carrot plants harbor internal Xanthomonas populations as high as 10^6-10^{10} CFU/g tissue, distributed in either leaves and stems, or roots, or both. The natural etiology and timing of how such infections establish and develop in the late fall, winter and/or very early spring is undetermined, although we were able to create internally infested carrots post-vernalization by wound or dip inoculating either roots or petioles as carrots were placed into cold storage (F. Crowe and R. Simmons, unpublished except in previous reports to the California Fresh Carrot Research Advisory Board [CFCRAB]). The importance of such internal infection is unclear in the Xanthomonas cycle on seed-to-seed carrots.

It is unclear how much Xanthomonas occurs directly on seed in umbels versus other parts of the plant. Nevertheless, in the harvest combining process Xanthomonas in dust and debris from leaves, stems, and umbels likely becomes deposited onto seed. Carrot seeds have spines that might help collect such dust and debris, but these spines are removed (deburred) during the seed cleaning process. It seems likely that deburring and other manipulations of seed during the cleaning and sizing process removes a substantial amount of harvest dust and small debris that may carry Xanthomonas. Seed quality, percent germination, and Xanthomonas infestation levels are determined after deburring.

By planting out variably infested lots of seed, and measuring the incidence of bacterial blight that resulted in commercial carrot plantings in two different years, a purported economic threshold was established for seed lots planted in central California in the late 1990's (Umesh et al. 1998). It is unlikely that any one such study truly represents a realistic economic threshold risk for all commercial fields in all regions in all years and under all management systems, although it may reasonably represent the risk in central California. Nevertheless, this threshold level of 10^5 CFU Xanthomonas/10,000 seed = 100,000 CFU/10,000 seed, or 10 Xanthomonas per seed, has been accepted as a standard by many in the carrot industry. These assay numbers are typically based on using the highly selective XCS growth medium for carrot Xanthomonas (Williford and Schaad 1984). Seed lots found infested above this level commonly are hot water treated to reduce the infestation level treatment (Strandberg and White 1989). Seed lots infested below this level may be accepted for planting without hot water treatment. Hot water treatment either eradicates Xanthomonas from seed or reduces it below detection levels.

In addition to actual disease, costs to the seed production system result from (a) hot water treatment, (b) reduced seed germination from the effects of hot water treatment, (c) rejection of seed lots if the resultant germination drops too low, and (d) any chemical applications made to seed fields in the attempt to reduce Xanthomonas. Research described here was undertaken towards reducing Xanthomonas levels on carrot seed and reducing the need for hot water treatment.

Currently, the majority of carrot seed fields in central Oregon are irrigated by either sprinkler or furrow irrigation, although the proportion of fields irrigated by drip has increased annually since 2000. Because bacterial populations typically increase and spread in association with free moisture on foliage, especially splashing (but also by equipment movement, etc.), the mode of irrigation continues to be a question with respect to Xanthomonas in carrot fields. Anecdotal observations by growers and seedsmen and our surveys during 2003 and 2004 (Du Toit et al. 2005) failed to note any strong differences in seed lot infestation with respect to mode of irrigation. Nevertheless, more detailed monitoring of drip- versus sprinkler-irrigated fields in 2004 and 2005 (both years with high seasonal rainfall) suggested that seed infestations could be reduced as much as 0.5-1.0 log units under drip irrigation, and it was speculated that such differences might be even more favorable for drip irrigation in years with normally dry seasons. It seems clear that seasonal weather patterns influence Xanthomonas spread, and this may involve timing of rainfall, humidity, etc.

Investigations presented below probed the concept that antibacterial pesticide sprays (e.g., copper materials and some others) might enhance drip and/or furrow irrigation to suppress Xanthomonas development, even though in the past such sprays have not been clearly demonstrated in most sprinkler-irrigated fields to result in lower Xanthomonas in harvested seed lots (F. Crowe, unpublished data). Our working hypothesis has been that Xanthomonas control might be achieved if initial infestation could be eliminated soon after Xanthomonas arrives in newly seeded fields during the first month or so after emergence. It is this period when wind-blown inoculum is most prevalent (DuToit et al. 2005). Once Xanthomonas infestations become established, especially on larger plants where Xanthomonas populations may be very high and where spray coverage is difficult, chemical control would become much more difficult and probably unlikely. Even if fields become infested later (e.g., spring or early summer), fall control might delay Xanthomonas population increase enough that lower seed infestation might result.

Materials and Methods

Five strips of seed-to-seed carrots were established in a trial area suitable for furrow irrigation. Seeding was on 10 August 2005. Carrots were planted in 30-inch rows in long strips through the field. Strips were separated by a 20-ft unplanted alley. Emergence was roughly September 1, after which carrots were removed by tillage from 30-ft cross-alleys between plots along the strips. Thus, plots were 20 ft wide (8 rows) by 20 ft long, separated by alleys either 20 or 30 ft wide. For 65 plots total, plots were arranged in a 5 by 5 replicated randomized block design with 13 treatments per strip replication. Plants were not thinned, and averaged 1 plant per 2 inches.

In central Oregon, seed-to-seed carrots initially are sprinkler irrigated prior to and after emergence through the fall. Irrigation from spring through fall may be by sprinkler, furrow, or drip. In this trial, irrigation was switched to furrow in the spring of 2006. All fertilization, herbicide, and other practices were as per commercial production for the region and are not detailed here. Only a female carrot line was planted to prevent this field from cross-pollinating with other fields in the region. Carrots in plots were not thinned to 1 plant per 6-12 inches as per most seed-to-seed fields. Initial stands averaged 1 plant per 2 inches. Although frequent plant sampling did thin the plots slightly, plant stands remained at least as thick as commercial fields throughout the season. No bees were installed at pollination, but bees, flies, and other insects were active on flowers. Although seed set did occur, it was lighter than if there had been nearby pollination sources.

We attempted to initiate a mini-epidemic of Xanthomonas in each plot by establishing small, point-source infestations simulating Xanthomonas -infested dust falling onto newly seeded carrot fields. The alley structure discussed above was intended to contain each mini-epidemic within each plot, and prevent plot-to-plot cross-contamination. One outcome of the trial was to evaluate whether this plot and infestation structure had merit for such studies.

Air-dried foliage ground in a Wiley mill and which assayed 10^6 Xanthomonas CFU/g dry tissue was used to infest plots. Prior to infestation, plots were irrigated very lightly on September 12 so that the ground foliage would stick to carrot seedlings. Wind was either very light or absent. At that time 0.5 g of inoculum was sprinkled by hand immediately above plants in the middle 2 rows by 25 cm-long plot area, in each plot. Approximately half of the ground inoculum contacted and stuck to damp foliage. Most remaining particles fell to the ground near the base of seedlings, and some visible smaller dust drifted up to a meter downwind but none noticeably drifted outside the plots. All plots were infested within 15 minutes of starting, and water was still noticed on foliage in the final plot, in spite of sunny conditions. No plots were left noninfested for this 2005-2006 trial.

Treatments were various antibacterial pesticide products sprayed onto foliage. Sprays were made on September 19 at the three- to four-leaf stage of growth (Fig. 1). A repeat application was on October 3 when carrots had approximately five leaves. A third application was made on April 13 at which time only three to six small green leaves were present following winter die back of the fall foliage.

We earlier determined that coverage of carrot foliage was critical to product efficacy (F. Crowe, unpublished data). A commercially designed tractor-mounted spray tank and boom were used in this trial, with nozzle orientation in a triangular fashion over each carrot row. Two nozzles sprayed from the sides of seedling carrots, and one nozzle sprayed from above (Fig. 1). This seemed to be the most optimal commercially used orientation available. All products were applied at 40 gal/acre broad acre, but only the carrot rows were sprayed so that in reality it was 20 gal/acre. For each plot, the tractor-sprayer was started up in the middle of the alley so that it was at application speed by the time any spray was applied. Similarly, the spray was continued at pace until reaching well past each plot. Wide alleys allowed the spray rig to be easily maneuvered among plots. All applications included 9 oz/100-g water Silken (silicone based) spreader sticker, plus an antifoaming agent. Enough product was mixed in the spray tank to allow for spraying all five replications per treatment with a single tankful. The tank and spray rig were purged between each treatment. Spray treatments included the following, with all rates as the amount of commercial product:

1. Untreated
2. Hasachlor 1qt/acre (12.5 percent sodium hypochlorite, Hasa, Inc.)
3. Oxidate 1:100 dilution (peroxyactic acid, Biosafe Corp.)
4. ManKocide® 2.5 lb/acre (mancozeb + copper hydroxide, DuPont/Griffin)
5. Kocide® 2000 1.5 lb/acre (copper hydroxide, DuPont/Griffin)
6. Serenade® 3 lb/acre (*Bacillis subtlis*, AgriaQwest)
7. Hasachlor 1 qt/acre first application, chased 2 days later by BlightBan® A506 150 g, followed by later applications of BlightBan A506 alone (BlightBan = *Psuedomonas flourescens*, Plant Health Tech.)

8. Oxidate 1:100 first application, chased 2 days later by BlightBan A506 150 g, followed by later applications of BlightBan A506 alone
9. Hasachlor 1 qt/acre plus ManKocide 2.5 lb/acre
10. Oxidate 1:100 plus ManKocide 2.5 lb/acre
11. Kocide 1.5 lb/acre plus Tanos 8oz/acre (famoxadone plus cymoxanil, DuPont)
12. ManKocide 2.5 lb/acre plus Tanos 8 oz/acre
13. ManKocide 2.5 lb/acre plus Serenade 3 lb/acre

Beginning a week after infestation, untreated check plots were sampled weekly until Xanthomonas was detected, after which all plots were sampled on an approximately monthly basis except for mid-winter. Sampling involved collecting foliage from 30 plants per plot, placing foliage into a new plastic bag, and storing each bag refrigerated until plants were assayed within 24 hours of collection. Described in more detail by Du Toit et al. (2005), Williford and Schaad (1984), and Umesh et al. (1998), the assay involved chopping the foliage (except when small), then shaking each sample in phosphate buffer for 1 hour, then dilution plating the water from the shaker onto highly selective XCS agar medium, and calculating the amount of colony-forming units (CFU)/g of dry weight of foliage washed. Thus, foliage from each plot was assayed as a composite of 30 plants, with the plot as the sampled experimental unit. When small, all the foliage from each composited carrot was sampled. As plants became larger and bolted, plants were subsampled to include a representative amount of foliage, petioles, stems, and umbels.

On September 6, umbels were hand clipped from all replications from some but not all treatments. Treatments selected for harvest included the unsprayed check and others that spanned a range of seasonal Xanthomonas assay recoveries. The final irrigation was on July 21, and plants were somewhat dry when umbels were collected. For each sampled plot, 100 umbels representing a typical harvest range (mostly primary and secondary, very few tertiary) were collected and bagged and allowed to further air dry. Several leaves per plant were included with the umbels to provide additional sources of Xanthomonas. Hands and tools were disinfested following collection from each plot. After several additional weeks air drying, seed was hand rubbed from each umbel per plot. Seed was deburred, and then passed through screens by hand, using standard research equipment. This procedure simulated the commercial combine-deburring seed-cleaning process. All tools, equipment, and hands were disinfested between each plot sample at each step of the process. In the end, a fully cleaned seed sample was obtained from each harvested plot, and seeds were assayed as in other studies (Du Toit et al. 2005, Williford and Schaad 1984, Umesh et al. 1998). Plots yielded between 4,000 and 6,000 seeds. Because 10,000 seeds were not obtained from 100 umbels, data were converted to represent CFU/10,000 seeds. Additional research (not shown) suggested that these seed assays resulted in CFU counts 10-20 percent less than those from commercial processes, probably due to reduced amount of foliage and stems in our hand process compared to commercial harvest. Such small differences were unimportant, considering that we are more concerned about treatment differences of tens of thousands, hundreds of thousands or more.

Results and Discussion

For simplicity of presentation in this report, composite plant assays for Xanthomonas (30-plant samples/plot) were rounded off to the nearest order of magnitude for CFU/g dry weight carrot foliage and CFU/10,000 seed:

0 (zero cannot be represented as exponents of 10, nor by a log value)
$1 = 10^0 = \log 0$
$10 = 10^1 = \log 1$
$100 = 10^2 = \log 2$
$1{,}000 = 10^3 = \log 3$
$10{,}000 = 10^4 = \log 4$
$100{,}000 = 10^5 = \log 5$
$1{,}000{,}000 = 10^6 = \log 6$
$10{,}000{,}000 = 10^7 = \log 7$
$100{,}000{,}000 = 10^8 = \log 8$

When rounded off, averages were rounded *after* averaging the nonrounded data.

Seasonal composite plant assay data are shown in Table 1 for all plots and all treatments at every date of sampling. Rounded averages and statistics are shown only for the last time of sampling, 9 August 2005. We did not sample all plots in the trial until we could first detect Xanthomonas in untreated plots. Xanthomonas first was detected on 27 October 2005 in untreated check plots. Because it takes a week or so for Xanthomonas to grow and be observed on the selective medium, the entire trial area was not sampled until 28 November 2005. There appeared to be a decline in detection beginning late November 2005, and only a limited frequency of Xanthomonas was detected in any plots on 13 April 2006, including none in untreated check plots on that date. Such an over-winter decline in detection was not apparent in our previous surveys (DuToit et al. 2005), and we are uncertain whether this was atypical or normal.

Beginning in the spring of 2006, detection frequency increased regularly in untreated check and most treated plots through the end of the season. Xanthomonas was detected in all five replications of the check plots by 9 August 2006. All pesticide-treated plots had a lower frequency of detection, but in general the frequency increased in many of these treatments, although it remained low in several. Most interesting, no Xanthomonas was detected season-long in plots treated with the combination of ManKocide plus Tanos.

Detection was erratic among replications within treatments. Detection of Xanthomonas in a plot in 1 month frequently was followed by failure to detect it in some or all subsequent months. Based on earlier surveys (DuToit et al. 2005), we knew that this erratic recovery was due to sampling methods and the probability of finding an infested plant among many noninfested plants. Most likely, the true frequency of plant infestation was in the 2-5 percent range when detection was erratic: a 30-plant sample might contain an infested plant or it might not. To reaffirm this likelihood, in July 2006 individual plants were assayed separately for several 30-plant samples and indeed we detected a single plant as positive in one sample, two in another, and none in a third. (The population measured on those individual plants was very high, 10^6-10^8 CFU/g dry weight of foliage, so the 30-plant average also was high: divide 10 million by 30 and the resulting number still is very large!) As the true frequency of plant infestation increased, so did our frequency of detection later in the season. Based on the earlier surveys, the frequency of plant infestation in the untreated checks by August 2005 probably was around 60-80 percent, so our likelihood of detection in a 30-plant sample was certain. We were somewhat encouraged that so many pesticide products and product combinations reduced the frequency of detection to much lower than the untreated checks.

In August, we decided that seed harvest samples needed to be taken, but because of the time and expense, not all plots could be included in harvest. Treatments were ranked in the following categories according to the number of times Xanthomonas was detected in plots during the period April through August:

A.	High Frequency:	Untreated check (58 percent).
B.	Moderately High:	Oxidate (33 percent), BlightBan after Chlorine (23 percent), Kocide 27 percent)
C.	Moderate:	ManKocide (13 percent), Chlorine plus ManKocide (20 percent), ManKocide plus Serenade (17 percent), Chlorine (17 percent), Kocide plus Tanos (13 percent)
D.	Low:	Serenade (7 percent), Oxidate plus ManKocide (10 percent)
E.	None:	ManKocide plus Tanos (0 percent)

We decided to not include any treatment from the "Moderately High" category because the seed assay would almost certainly be very high. Two treatments were included from the "Moderate" category (ManKocide and Chlorine). Serenade was included from the "Low" category, and only ManKocide was in the "None" category.

The range of treatments included in harvest resulted in a range of recovery of Xanthomonas from seed lots. CFU/10,000 seed is shown in Table 2 for seed harvested in September 2006. Seed from untreated check plots was uniformly highly infested, as expected based on the "Very High" rating from seasonal foliage: Xanthomonas was recovered from seed from all five replications, and the average CFU/10,000 seed was 2.80×10^8, or log[CFU] = 8.5.

Recovery of Xanthomonas from seed was lower from all plots originally treated with antibacterial pesticides. For treatments rated as "Moderate" (Chlorine and ManKocide) or "Low" (Serenade) based on seasonal Xanthomonas recovery from plants, no Xanthomonas was recovered from seed in several replications. Seed from Chlorine-treated, ManKocide-treated, and Serenade-treated plots averaged 3.86×10^7 CFU/10,000 seed (log [CFU] = 7.6); 5.80×10^4 CFU/10,000 seed (log [CFU] = 4.6), and 7.00×10^6 CFU/10,000 seed (log [CFU] = 6.8), respectively. Among these treatments, only ManKocide-treated seed was below the 10^5 or log unit 5 threshold, and this just barely.

Interestingly, just as no Xanthomonas was recovered from foliage in plots treated with ManKocide plus Tanos ("None" rating), neither was Xanthomonas recovered from seed from those plots. This strongly suggests that no Xanthomonas survived the initial fall and early spring sprays with this product, *and* that no Xanthomonas reestablished within these plots after spraying.

As per the discussion on erratic recovery and sampling frequency above, Xanthomonas was detected on seed from some plots that had not tested positive on plants earlier, and vice versa (i.e., Xanthomonas was not detected from seed of some plots where plants earlier had tested positive). Once again, this simply indicates that in the plots earlier treated with antibacterial pesticides, only a few plants or umbels might be infested. In spite of this, a very high infestation on even a few plants or umbels may result in high populations in a composited sample. We conclude that Xanthomonas frequency must be kept *very* low in a plot or field to keep seed lots from having composited populations that must be hot water treated.

All the products used in this trial are nonpersistent and none would be expected to sustain control for more than a short period of time or to protect new foliage. Any effects from them must have been from eliminating early infestations and either eliminating or reducing them, thus greatly delaying epidemic buildup of Xanthomonas. We were very pleased that no apparent plot-to-plot cross-contamination was detected in this trial, which suggests the trial structure was fairly robust in design. For example, in a number of locations, the ManKocide plus Tanos plots were adjacent to untreated checks.

Not mentioned earlier was the fact that the spring of 2006 was a wet spring, with abundant opportunities for rain splashing Xanthomonas from plot to plot. Because no plot-to-plot movement was detected, this may suggest a more limited movement by Xanthomonas in this manner than previously thought, at least during cooler spring weather and/or when plants are shorter. The risk from spread by equipment and people in our trial probably was comparable to that risk of movement in commercial fields. On the other hand, because our plots were rather small (20 ft by 20 ft), there were larger "edge" effects than occur in commercial fields, which may have influenced humidity. Nevertheless, because the infested-but-untreated plots developed high levels of Xanthomonas, we suspect relatively comparable activity of Xanthomonas occurred in those plots compared to commercial fields. Thus, the more limited occurrence of Xanthomonas in treated plots is probably mostly explained by the antibacterial treatments alone.

We were very surprised not to get rapid spread of Xanthomonas within plots where Xanthomonas could be recovered. There was abundant opportunity for it to spread via plant-to-plant contact, rainfall plus wind, and mechanical transfer during sampling. Infested plants are very highly populated, so there should be plenty of inoculum. Clearly, in 2005-2006, most nonpersistent antibacterial products applied early in the season did not fully eradicate Xanthomonas, but they did delay epidemic development until late in the season. Based on these and past data, we present a revised working hypothesis of how Xanthomonas develops in carrot seed fields: Xanthomonas becomes deposited onto a limited amount of foliage in some manner (wind, equipment, animals, seed, etc…) during the fall. Surface populations develop on some leaves, and Xanthomonas may spread somewhat during the fall. On a limited number of plants (5 percent or so), an internal population of Xanthomonas may develop in late fall or winter. Most or all Xanthomonas located on plant surfaces die off during the winter. Fall antibacterial sprays may reduce or even eradicate Xanthomonas on carrot foliage before the transition to internal infestation, or somehow reduce or prevent internal infestations. By the time carrot plants resume regrowth in spring, all or nearly all Xanthomonas bacteria in the field are harbored in a small proportion of internally infested plants. These bacteria are not immediately able to move epidemically from plant to plant until undetermined events expose them (e.g., wounding, insect feeding, guttation, etc). Nevertheless, as the spring and summer develops, these undetermined events occur and some internally located Xanthomonas do begin to spread among plants by typical means such as rain, sprinkler irrigation, plant-to-plant contact, animals, etc… Eventually, in unsprayed fields, very high epiphytic populations of Xanthomonas are present on many plants.

Additional support of this working hypothesis includes the following: within the species and strains of the Xanthomonas group of bacteria, few tolerate cold very well, so our finding that the carrot Xanthomonas population declined precipitously over the winter of 2005-2006 seems reasonable– winters in central Oregon commonly drop well below freezing, and exposed carrot foliage typically

is killed. In central Oregon, we associate the worst Xanthomonas with earlier spring events such as hail and sudden late season frosts–perhaps these are opening up wounds that allow early and rapid epidemic spread.

Our plant sampling and assay methods for measuring Xanthomonas in/on plants usually involves some or even much tissue disruption, so it does not discern the location of Xanthomonas in or on foliage, stems, petioles and umbels. To determine that internal populations existed, in our earlier work we devised special procedures to make this distinction; this was easy with carrot storage roots, stems and petioles, but less clear with leaves.

A very similar trial was initiated in the fall of 2006 that will be carried through 2007. This new trial will be sprinkler irrigated beginning April 2007. An additional noninfested, nonsprayed treatment is included that was absent from 2005-2006. It will be interesting to see if Xanthomonas populations develop, if cross-contamination among plots occurs, whether the trial structure and infestation methods used in 2005-2006 under furrow irrigation hold up season long in 2006-2007 under sprinkler irrigation, and whether any products seem to work as well as ManKocide plus Tanos did in 2005-2006. We will try some additional sampling and procedures to further refine or reject our working hypothesis.

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Table 1. Per plot Xanthomonas recovery from carrot foliage from October 2005 to August 2006. (Colony-forming units [CFU] per gram dry weight carrot foliage composited from 30 plants/ plot.)

Treatment	Plot no.	10/27	11/8	11/28	4/13	5/17	6/14	7/18	9/9
Check	7	0	**10^6**	**10^6**	0	**10^5**	**10^7**	**10^7**	**10^7**
	15	**10^2**	**10^4**	**10^2**	0	0	**10^4**	**10^7**	**10^5**
	29	**10^3**	**10^6**	0	0	0	0	**10^5**	**10^7**
	44	0	**10^6**	0	0	0	0	**10^5**	**10^7**
	64	**10^2**	**10^4**	**10^3**	0	0	0	0.0	**10^8**
									$X=10^7$ a
Chlorine	2	NS*	NS	0	0	**10^5**	0	0	0
	16	NS	NS	0	0	0	0	0	**10^4**
	35	NS	NS	0	0	**10^6**	0	0	**10^6**
	41	NS	NS	0	0	0	0	0	0
	59	NS	NS	0	0	0	0	**10^6**	0
									$X=10^5$ c
Oxidate	3	NS	NS	0	0	0	0	0	**10^6**
	20	NS	NS	**10^2**	0	0	**10^5**	0	**10^7**
	38	NS	NS	0	**10^6**	**10^4**	0	0	**10^8**
	47	NS	NS	0	0	0	0	0	**10^6**
	54	NS	NS	0	0	**10^7**	0	**10^6**	0
									$X=10^7$ ab
Mankocide	10	NS	NS	0	0	0	0	0	0
	25	NS	NS	0	**10^5**	0	0	0	0
	39	NS	NS	0	0	0	**10^5**	0	**10^6**
	45	NS	NS	0	0	0	0	0	0
	62	NS	NS	0	0	0	0	0	**10^3**
									$X=10^5$ c
Kocide	11	NS	NS	0	0	0	0	0	**10^7**
	22	NS	NS	0	0	0	**10^5**	**10^6**	0
	37	NS	NS	0	0	0	0	**10^3**	**10^7**
	42	NS	NS	0	0	0	0	0	0
	56	NS	NS	**10^2**	0	**10^7**	0	**10^5**	0
									$X=10^6$ bc
Serenade	13	NS	NS	0	0	0	0	0	0
	18	NS	NS	0	0	0	0	0	**10^5**
	30	NS	NS	0	0	0	0	0	0
	50	NS	NS	0	**10^8**	0	0	0	0
	53	NS	NS	0	0	0	0	0	0
									$X=10^4$ c

Table 1. Continued…..

Treatment	Plot no.	10/27	11/8	11/28	4/13	5/17	6/14	7/18	9/9
Blightblan (after Chlorine)	8	NS	NS	0	**10^7**	0	0	0	**10^5**
	24	NS	NS	0	0	0	**10^5**	0	0
	32	NS	NS	0	0	0	0	**10^5**	**10^7**
	52	NS	NS	0	0	0	0	0	**10^5**
	58	NS	NS	0	0	0	0	**10^6**	0
									X=10^6 bc
Blightblan (after Oxidate)	9	NS	NS	0	0	0	0	0	0
	14	NS	NS	0	0	0	0	0	0
	27	NS	NS	0	0	0	0	0	**10^7**
	48	NS	NS	0	0	0	0	0	**10^7**
	61	NS	NS	0	0	0	**10^7**	**10^7**	**10^7**
									X=10^6 abc
Chlorine + Mankocide	1	NS	NS	0	0	0	0	0	**10^6**
	23	NS	NS	0	0	0	0	0	**10^7**
	28	NS	NS	0	0	0	0	0	0
	46	NS	NS	0	0	**10^4**	0	**10^6**	0
	57	NS	NS	0	0	**10^4**	0	**10^2**	0
									X=10^6 bc
Oxidate + Mankocide	5	NS	NS	0	0	0	0	0	0
	21	NS	NS	0	0	0	0	0	0
	34	NS	NS	0	0	0	0	0	0
	43	NS	NS	0	0	0	0	**10^6**	**10^5**
	55	NS	NS	0	0	0	0	0.0	**10^5**
									X=10^5 c
Kocide + Tanos	4	NS	NS	0	0	0	0	0	**10^6**
	19	NS	NS	0	0	0	0	0	**10^7**
	36	NS	NS	0	0	0	0	0	0
	49	NS	NS	**10^6**	0	0	0	0	**10^5**
	65	NS	NS	0	0	0	0	0	0
									X=10^6 bc
Mankocide + Tanos	6	NS	NS	0	0	0	0	0	0
	26	NS	NS	0	0	0	0	0	0
	33	NS	NS	0	0	0	0	0	0
	51	NS	NS	0	0	0	0	0	0
	63	NS	NS	0	0	0	0	0	0
									X=0 c
Mankocide + Serenade	12	NS	NS	0	0	0	0	0	0
	17	NS	NS	**10^6**	0	0	0	0	**10^6**
	31	NS	NS	0	0	0	0	0	0
	40	NS	NS	0	0	0	**10^6**	0	**10^4**
	60	NS	NS	0	0	0	0	**10^6**	0
									X=10^5 c

Analysis of variance was performed on log(X+1) transformed data, not raw data. Log(X+1) transformed means determined different (P > 5 percent) are designated by different letter

Table 2. Xanthomonas seed assays from all plots from selected treatments. Seed was aggregated from 100 umbels per plot. Assay data represent colony forming units (CFU) per 10,000 seed.

Treatment	Plot no.	CFU	Log(CFU)
Check	7	**$9.6x10^6$**	**7.0**
	15	**$3.8x10^8$**	**8.6**
	29	**$9.6x10^8$**	**9.0**
	44	**$6.7x10^7$**	**7.8**
	64	**$9.6x10^5$**	**6.0**
mean		**$2.8x10^8$**	**8.5 a**
Chlorine	2	0	***
	16	**$9.6x10^7$**	**8.0**
	35	0	***
	41	**$9.6x10^7$**	**8.0**
	59	**$9.6x10^5$**	**6.0**
mean		**$3.86x10^7$**	**7.6 ab**
ManKocide	10	**$2.9x10^5$**	**5.5**
	25	0	***
	39	0	***
	45	0	***
	62	0	***
mean		**$5.80x10^4$**	**4.8 bc**
Serenade	13	0	***
	18	0	***
	30	0	***
	50	**$1.6x10^7$**	**7.2**
	53	**$1.9x10^7$**	**7.3**
mean		**$7.0x10^6$**	**6.8 bc**
ManKocide & Tanos	6	0	***
	26	0	***
	33	0	***
	51	0	***
	63	0	***
mean		**0**	***** c**

*** Zero has no logarithm.

Analysis of variance was performed on log(X+1) transformed data, not raw data.

Log(X+1) transformed means determined different (P > 5 percent) are designated by different letters.

Figure1. Note nozzle orientation of seeding carrots being sprayed; this ensured total coverage of all seeding carrot surfaces.