EFFICACY OF COPPER AND CHLORINE PRODUCTS FOR CONTROL OF *XANTHOMONAS CAMPESTRIS* PV *CAROTAE* ON CARROTS[1]

Fred Crowe and Rhonda B. Simmons

Introduction

In central Oregon, copper products have been used for many years in attempts to suppress early developing populations of the carrot bacterial blight pathogen, *Xanthomonas campestris* pv *carotae* (Xcc), on seed-to-seed carrots. Local applications have been made primarily in the fall and/or early spring. Earlier, we determined that Xcc populations in central Oregon carrot fields were not tolerant to copper ions (Parks and Crowe 2000), but it remains unclear how effective copper treatments have been.

Attempts to gather efficacy data for copper products against carrot bacterial blight in commercial fields in central Oregon have been frustrating, because in most years no bacterial blight develops to make comparisons between treated and untreated plots. Recent studies have shown that bacterial blight symptoms do not commonly form in seed carrot fields, in spite of very high foliar populations (DuToit et al. 2004), which likely would cause symptoms in areas with wetter and hotter weather (Umesh et al. 1998). As a result, we switched to comparing products based on their direct impact on foliar populations of Xcc, but this has not proven to be straightforward testing either.

Preliminary studies during the summer of 2004 showed no differences among Xcc populations on treated or untreated highly infested carrot foliage after application of various copper and other products. The easy interpretation might have been that coppers and other products were ineffective. But foliar assays of carrot tissues involve chopping and extensive washing of tissues in water prior to dilution plating on agar. We previously had determined that internal populations were commonly present in local carrot fields by mid-summer (Crowe and Bafus 2003, Crowe et al. 2004). Further, Xcc was detected in carrot stecklings brought into the Pacific Northwest (PNW) as planting stock for some seed fields (Du Toit et al. 2004). Therefore, we worried that any internal sources of Xcc could be obscuring foliar assays in our copper spray plots, and we might be missing pesticide-induced reduction of Xcc populations on foliage surfaces.

This concern was validated: At mid-summer, additional sampling revealed that 5-10 percent of plants in our field trials areas (within local grower fields) harbored high internal Xcc populations in carrot foliage. *Xanthomonas* from the cut edges of chopped foliage from these plants would be enough to contaminate our assays even if all Xcc present on the foliage surface had been eliminated by surface-applied treatments. Unfortunately, we could not afford to sample individual plants in our spray trials to get around this issue.

Seed used to plant carrots in central Oregon routinely tests negative for Xcc. Such seedlots are presumed free of Xcc or are at very low frequency (Kuan et al. 1985, Umesh et al. 1998). It is highly unlikely that any such bacteria in seedlots could directly account for the proportion of plants from which internal populations are measured in carrot seed fields. Bacteria are deposited from dust generated from harvest of nearby older seed carrot fields onto new seed

[1] These investigations were partially supported by a grant from DuPont de Nemours and Company.

carrots seedlings in central Oregon in the weeks after emergence in the fall (Du Toit et al. 2004). Presumably, internal infections of carrot seed plants result when Xcc finds its way from foliage surfaces into the plants. Dip inoculations of such foliage have resulted in Xcc populations developing in foliage and roots (Crowe and Bafus 2003).

In any case, soon after seedlings emerge the proportion of infested plants is low and foliar populations have not developed to the high levels seen in summer (Du Toit et al. 2004). For copper and other spray evaluations (none of which were systemic in action), we reasoned that there would be less chance that any internal foliar populations would have developed on seedling plants. In fact, we would like to conduct control studies on carrot seedlings to prevent such fall infections, but so few bacteria are naturally present, such studies are impossible in naturally infested plantings. Thus, in the fall of 2004 we artificially and uniformly inoculated Xcc onto seedling carrots to provide a background against which to test coppers and other antibacterial products. We evaluated the impact of antibacterial products applied to the surface of those seedlings before internal populations of Xcc developed on them.

Various copper products are locally sold for control of Xcc on carrots, and several were included in this study. We were particularly interested in ManKocide, which is a combination of the fungicide maneb with the anti-bacterial copper hydroxide. This product has been found more effective than some other anti-bacterial copper products for other plant diseases. In a single previous field trial based on bacterial blight symptoms, we obtained some very limited data that indicated ManKocide might be superior to Kocide (copper hydroxide). More data were required to verify this earlier data in support of a local product pesticide label.

In addition to copper products, we were interested in the effects of chlorine (actually, sodium hypochlorite, NaOCl) applications to foliage, either applied in irrigation water or sprayed onto foliage with a ground rig sprayer. Chlorine has been used on some crops for control of foliage diseases in the PNW, and is labeled as an irrigation system treatment (Liquichlor 12.5 percent solution). Sodium hypochlorite does not remain active for very long when exposed to organic matter, sunlight, and air. There is a dosage by time effect for killing bacteria: lower doses over longer times are more or less equivalent to higher doses over shorter times. When applied through irrigation systems, application may be for an extended period (hours) at 1-5 ppm, or for shorter periods (minutes) at 15-30 ppm. In central Oregon, higher doses applied through a normal crop spray rig have achieved the same killing effect over (probably) a few seconds to a few minutes at most. Spray coverage of foliage must be excellent when applied in this manner, -compared to application through an irrigation system.

Materials and Methods

Carrot seed that had been assayed free of Xcc was seeded at the Central Oregon Agricultural Research Center (COARC) on August 16, 2004. Plants emerged during late August and were at the 3-4 true leaf stage by the third week of September 2004.

In early September 2004, approximately 20 lbs of infected/infested carrot foliage was collected from old fields and air-dried. Dry foliage was ground in a mill until powdered. Prior to each inoculation, approximately 10 lbs of dried, powdered foliage was soaked for 1 hour in tap water, then filtered through both cheesecloth and fine nylon mesh. Volume was

adjusted to 4 gal. Water samples were assayed by dilution plating directly onto XCS agar medium highly selective for Xcc (Williford and Schaad 1984). Approximately 10^7 colony-forming units (CFU)/ml were found in this filtrate for each date of application.

Within 24 hours of preparation, the filtrate was sprayed over newly planted carrot plots with Xcc recovered from this foliage either before or after applying two rounds of pesticides separated by 2 weeks (see Table 1 for dates of inoculation and pesticide applications).

Copper products used included Copper Count N (active ingredient = copper ammonium complex), Kocide 2000 (active ingredient = copper hydroxide), and ManKocide (active ingredients = a combination of copper hydroxide and the fungicide maneb). Sodium hypochlorite as Liquichlor 12.5 percent was applied at 1 and 2 qt/acre in 50 gal/acre water, equivalent to 1,600 and 3,200 ppm, respectively, by volume. All products used were applied by a backpack compressed-air sprayer in 50 gal/acre water at 30 psi using paired drop nozzles on either side of each carrot seed line.

Two trial areas were located 10 ft apart, one trial area for each date of inoculation and pesticide treatment combination. Plots were 10 ft wide by 20 ft long, with four carrot rows located in each plot. All rows within a plot were inoculated (or not for non-inoculated plots) or treated with coppers or chlorine (or not for untreated plots). Each treatment was replicated three times each, and fully randomized in location within each trial.

As in recent field surveys, foliage from the plots was assayed for Xcc by washing bacteria from the foliage, culturing onto a selective medium (Wiliford and Schaad 1984, Kuan et al. 1985, Umesh et al. 1998, Du Toit et al. 2004), and determining the CFU/g dry carrot tissue in the sample. At each sampling, all the foliage from 30 seedling plants in each plot were collected, composited, and washed for Xcc by standard methods (DuToit et al. 2004). CFUs thus represent a composite of 30 plants/plot at each sampling, and do not represent the population on each plant individually. Other plot information is shown in Table 1.

Results and Discussion

Xanthomonas was not recovered from foliage within a few days after inoculation, but substantial populations had established within 9 days of inoculation.

Data and additional treatment information are shown in Table 1. Data are expressed as the means of the log (CFU) per plot. (Populations on foliage were measured by leaf assay, then transformed to the logarithm of the CFU. Log of 10 = 1, log of 100 = 2, log of 1,000 = 3, ……. log of 1,000,000 = 6, etc.) Because there is no logarithm of 0, we substituted 1 CFU for 0 CFU in the means and statistics, but this made no difference in the outcome because of the very many CFUs found once bacterial populations had established. Means and statistics were calculated on the log data. This is normal and common handling of bacterial data, because populations of bacteria tend to be quite large and variable.

Included were a set of plots that were not inoculated with Xcc, nor sprayed with pesticide. This proved interesting, because as Table 1 shows, Xcc appeared in these non-inoculated plots as time progressed. This most likely represents the natural ability of Xcc to spread under sprinkler irrigation over plot-to-plot distances used in these trials, which was about 10 ft.

There also may have been some slight drift from our spray-on inoculation. Population levels in non-inoculated plots remained lower than for other treatments, even those that were treated with coppers or chlorine.

We also randomly sampled plants from all plots and tested for internal populations of Xcc, as described previously (Crowe and Bafus 2003, Crowe et al. 2004). Briefly, roots were surface sterilized, peeled, and pureed, and the filtrate filtered before dilution plating of filtrate; a modified procedure was used for stems and leaves. We found no such internal population during the fall or early winter (data not shown), in contrast to when we sampled during mid-summer. Thus, we do not believe any internal populations compromised the data shown in Table 1. This was an important assessment, as we feel confident that all our recoveries were from foliar populations only. These plots were resampled during January 2005. In January, internal populations of Xcc were detected in 15 percent of the plants, although the CFUs/plant was low (approx. 10^3-10^4 CFU/g dry wt). Apparently, internal populations may develop during the winter.

Early inoculated trial

Mean log(CFU) for copper and chlorine treatments are shown in Table 1. All data from this trial were complicated by variability in the data, and no statistical differences ($P \leq 0.05$) were found among treatment means. Without strong statistical differences present, only trends can be discussed. When a uniform background of Xcc was established before any pesticides were applied, evidence for early suppression (in the first few days after either application) of Xcc was limited at best by the copper products, but probably for both chlorine rates. Considering untransformed numbers, CFU data for chlorine applications were 10-1,000 times less than the mean CFU for untreated but inoculated plots. However, 8 days after the final spray application, mean log(CFU) for all copper products was around 10-fold lower than the mean log(CFU) of the untreated but inoculated plots.

As a modified anlaysis, all copper data were combined within a single “copper” treatment of nine replications, and all chorine data were combined within a single “chlorine” treatment of six replications. Analysis of variance was rerun, but no statistical separation emerged in this approach, either. In Figure 1, mean log(CFU) for all copper products are averaged together, as are the two chlorine treatment rates.

Late-inoculated trial

Data from this trial were also complicated by variability, but some statistical separation was found, at least between non-inoculated and other treatments. Means are shown in Table 1. Inoculation with Xcc occurred *after* the first spray application and *before* the second spray application of pesticides. Bacterial populations were not well established and detectable until about the date of the second spray application. On that date, untransformed CFU estimates for Xcc were 10-1,000 times lower for all pesticide treatments compared to the untreated but inoculated plots. Eight days after the second application, mean log(CFU) for all pesticides were about 10-fold lower than the mean log(CFU) for the untreated but inoculated plots. For October 12 and 20, there was statistical mean separation ($P \leq 08$) between non-inoculated and all other treatments, but individual treatments could not be separated from the inoculated control treatment.

As a modified analysis, all copper data were combined into a single "copper" treatment of nine replications, and both chlorine treatments were combined into a single "chlorine" treatment, and analysis of variance was again performed. Data are shown in Figure 2. By this approach, on both October 12 and 20, "copper" and "chlorine" treatments were statistically distinguished from the inoculated treatment at the $P < 0.05$ level (5 percent), even though we could not statistically distinguish any differences among specific copper or chlorine treatments. The LSD 5 percent value for October 12 was 3.2 log units and 1.3 log units, respectively, but these LSDs are not shown on the figure. For the late trial, therefore, copper products as a group did provide statistically significant suppression of Xcc populations, as did chlorine.

A high and uniform background level of Xcc developed on the carrots in our plots, a result of inoculation with Xcc associated with naturally infested carrot foliage. Most likely, because we did not immediately recover XCC following inoculation, the bacterial pathogen populations had to re-establish on the inoculated foliage. In our field survey of commercial fields (Du Toit et al. 2004), Xcc was found on only about 5 percent of plants in fall in both Oregon and Washington. Our infestation was undoubtedly 100 percent, although we did not sample plants individually to verify this. Clearly, this is a higher and more broadly based population than would be expected to develop naturally on seedling carrots in the fall, but it is comparable to a mid-summer population. It is a valid criticism to question whether a lower population (similar to what naturally may find its way onto carrot seedlings) might have been more easily controlled than a very high population. Consider, however, that commercial applications of copper products in fall have not prevented XCC populations from establishing in most local commercial fields.

While it is tempting to look for differences among products, all three products containing copper worked approximately equally well in these experiments. With only three replications, it was difficult to determine whether one product or another was favored; statistically significant differences were found only in the late trial and only between the non-inoculated check vs all other treatments. Nevertheless, there was consistently lower recovery in copper- and chlorine-treated plots than in inoculated plots, although pesticide-treated plots typically had higher recovery than non-inoculated plots. When all copper and chlorine data were combined into generic "copper" and "chlorine" treatments, statistically significant reductions of Xcc populations were found in the late trial, where treatment began before Xcc inoculation.

Considering trends, copper materials worked better when applied before Xcc populations were established. This supports the concept that, at least in central Oregon, where inoculum arrives early, seedling carrots should be treated early in the fall. All the coppers provided some control of Xcc for at least 8 days after treatment. Because these are not systemic materials, the protection that copper products provided will not extend to new untreated foliage. With the high and widespread populations in these experiments, copper products probably would not keep Xcc from spreading to new foliage. They may work better on lightly infested seedling carrots, or to protect against initial infestation by small amounts of Xcc arriving on new plantings. Application of a copper product could be valuable immediately following wounding events (hail, excessive wind that blows sand, trimming activities, etc.). Still, the questions remain: are applications of copper products worth their

cost of application, and can they be applied in a way that will reduce XCC through the season?

These limited data suggest that coppers might be useful if started before the weather was conducive for disease development, before Xcc infestations are well-established, and if applied repeatedly during conducive weather conditions, although more work is required on the efficacy of repeated applications and application timing. Even though copper reduced the high populations found under our experimental inoculations, the resultant populations still were moderately high. This supports common experience that copper products probably cannot be fully depended upon to provide reliable control of bacterial blight if application is started later in the season and if weather conditions are conducive for disease.

We could not determine differences among copper products in these trials. Perhaps with more replications, such differences might have been found. There are, however, differences in cost, ability to tank mix, etc., that could influence choice of materials. Whereas ManKocide did not prove superior to Kocide or Copper Count N in these trials, the combined copper + fungicide product ManKocide may offer some advantage over copper alone if some additional control of fungal diseases is achieved.

The data reported here seem to confirm that chlorine provides a rapid and extensive kill of bacteria on foliage contacted, but Xcc populations quickly rebound. In other field studies, we recovered *no* Xcc on foliage for 1-2 days after application of chlorine, but within a week we found that the population had re-established near to or just under the pretreatment population (F. Crowe, unpublished data). In the trials reported here, the XCC populations were 10- to 100-fold or so below the pretreatment population about a week later. If chlorine could be repeatedly applied through the season, or applied soon before a likely infection period, then such applications could be very useful. Frequent repetition would be easier and cheaper if chlorine was chemigated rather than sprayed by ground rig, however.

References

Crowe, F., and R. Bafus. 2003. Evaluation of the potential for systemic infection of carrot seed by *Xanthomonas campestris* pv. *carotae*. Pages 1-3 *in* 2002 Central Oregon Agricultural Research Center Annual Reports, Special Report 1046, Agricultural Research Station, Oregon State University, Corvallis.

Crowe, F., R. Simmons, R. Davis, A. Mahaffee, W., and V. Stockwell. 2004. Evaluation of the potential for systemic infection of carrot seed crops by *Xanthomonas campestris* pv. *carotae*. Pages 1-8 *in* 2003 Central Oregon Agricultural Research Center Annual Reports, Special Report 1053, Agricultural Research Station, Oregon State University, Corvallis.

Du Toit, L., F.J. Crowe, M.L. Derie, R. Bafus, and G.Q. Pelter. 2004. Bacterial blight of carrot seed crops: Identification of sources of inoculum. Pages 13-19 *in* 2003 Central Oregon Agricultural Research Center Annual Reports, Special Report 1053, Agricultural Research Station, Oregon State University, Corvallis.

Kuan, T.-L., G.V. Minsavage, and R.L. Gabrielson. 1985. Detection of *Xanthomonas campestris* pv *carotae* in carrot seed. Plant Dis. 69:758-760.

Parks, R., and F. Crowe. 1999. Sensitivity of *Xanthomonas campestris* pv *carotae* to copper pesticides in central Oregon carrot seed fields. Final Report to the Oregon State University Integrated Plant Protection Center, 4 pages.

Umesh, K.C., R.M. Davis, and R.L. Gilbertson. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv *carotae*. Plant Dis. 82:1271-1275.

Williford, R.E., and N.W. Schaad. 1984. Agar medium for selective isolation of *Xanthomonas campestris* pv *carotae* from carrot seeds. Phytopathology 74:1142.

Table 1. Means of log-transformed CFU/g dry wt of carrot foliage for *Xanthomonas campestris* pv *carotae* recovered from seedling carrot foliage that had been sprayed with copper and chorine products either after artificial inoculation in the field with the bacterial pathogen, or both before and after such inoculation[a].

	Treatments and rates (product/acre applied in 50 gal/acre water with drop nozzles)							
Date sampled	Copper Count N 3 pt	Kocide 2000 1.5 lb	ManKocide 2.5 lb	Chlorine 12.5% 1 qt	Chlorine12.5% 2 qt	Inoculated but no trt	Not inoculated	Stat prob.Y
EARLY-INOCULATED TRIAL [Xanthomonas inoculated on Sept 21, before either of two pesticide spray applications Sept 24 and Oct 8]								
Pre-inoculation (Sept 9)	0[z]	0	0	0	0	0	0	NA
Post-inoc/pre-trt (Sept 23)	2.3	0	0	0	0	0	0	NS
After 1st spray trt (Oct 1)	6.7	6.1	5.8	3.1	5.5	6.8	4.2	NS
After 2nd spray trt (Oct 12)	6.5	6.4	3.7	4.8	1.7	6.8	4.2	NS
8 days after 2nd trt (Oct 20)	6.8	6.7	6.0	3.5	6.5	7.3	4.9	NS
LATE-INOCULATED TRIAL [Xanthomonas inoculated on Sept 27, after first spray application Sept 24 and before second spray on Oct 8]								
Pre-inoculation (Sept 9)	0	0	0	0	0	0	0	0
Pre- 1st trt (Sept 23)	0	0	0	0	0	0	0	0
Post-inoculation (Oct 1)	1.6	0	2.4	1.6	2.1	2.4	0	NA
After 2nd spray trt (Oct 12)	3.2	3.5	4.6	2.6	2.0	5.5	0	$P = 0.08$
8 days after 2nd trt (Oct 20)	6.8	6.9	7.0	6.5	6.8	8.0	1.8	$P < 0.001$

[a]. Trial was planted on August 16. First inoculation on September 21 was at the 3- to 4-true-leaf stage of development. Last spray treatment October 8 was at the 7-leaf stage of development. Inoculum was prepared by drying carrot foliage from older, infested fields, then grinding. Ground foliage was washed and filtered prior to spraying filtrate over foliage of plots. Inoculum concentration was 4×10^6 and 2×10^6 CFU/ml filtrate at time of inoculation, sprayed to runoff at 50 gal/acre.

[b]. Analysis of variance was conducted on log-transformed CFU/g dry wt foliage. NA = not applicable; NS = significance levels were much higher than 10 percent. Probabilities of less than 0.01 (10 percent) suggest significant differences exist among some means. There were three replications per treatment per trial, with plots arranged in a randomized block pattern for each trial.

[c]. All units are log units: 1, 2, 3, 4, 5, 6, 7, and 8 represent 10, 100, 1,000, 10,000, 100,000, 1,000,000, 10,000,000, and 100,000,000 CFU, respectively. Zeros are log units that technically represent 1 CFU, but because 1 CFU was substituted for 0 CFU to allow log transformation, these do in fact represent true zero amounts in the original data.

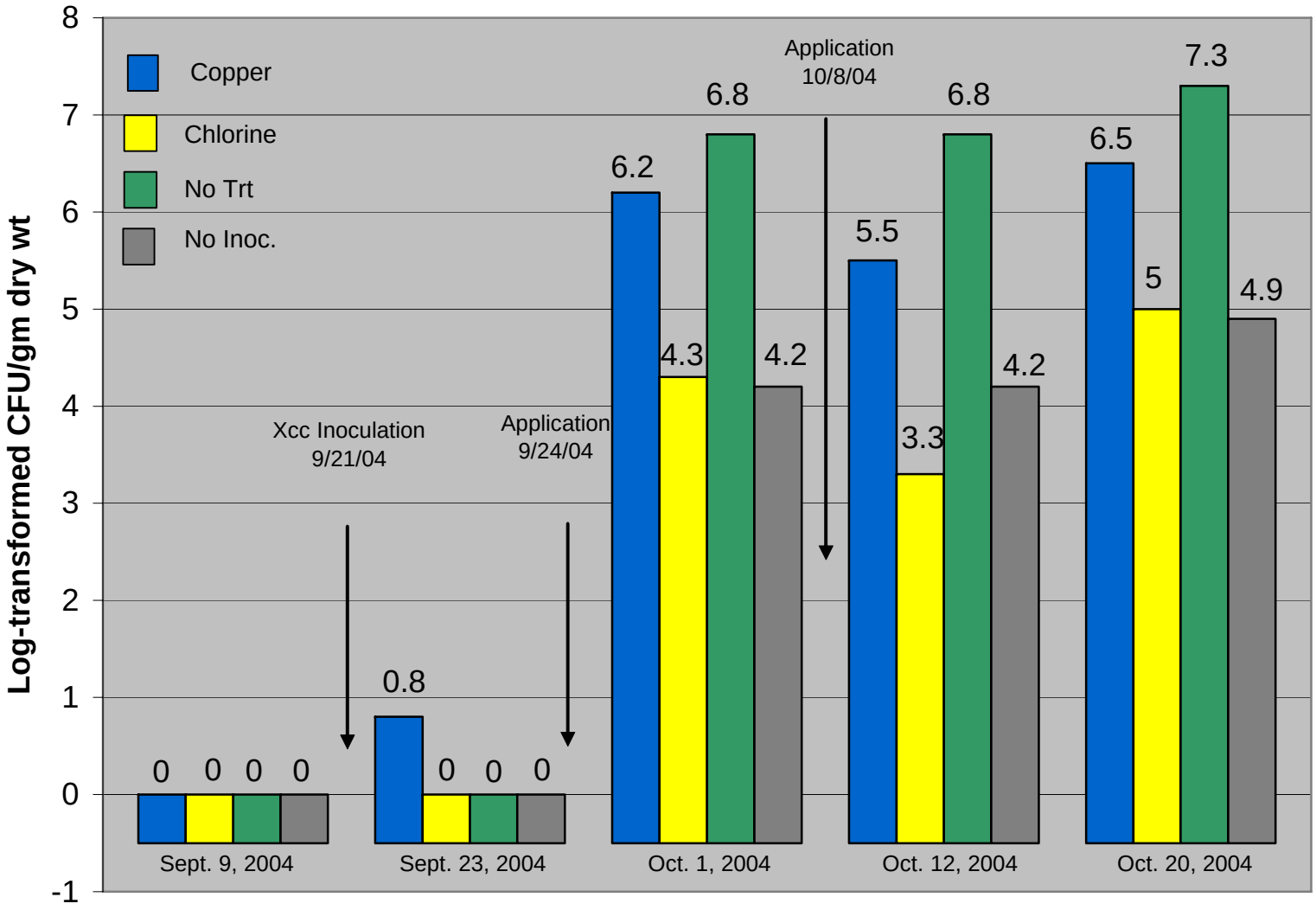

Figure1. Early trial: carrot seedlings inoculated with *Xanthomonas campestris* pv *carotae* before two treatments with copper or chlorine.

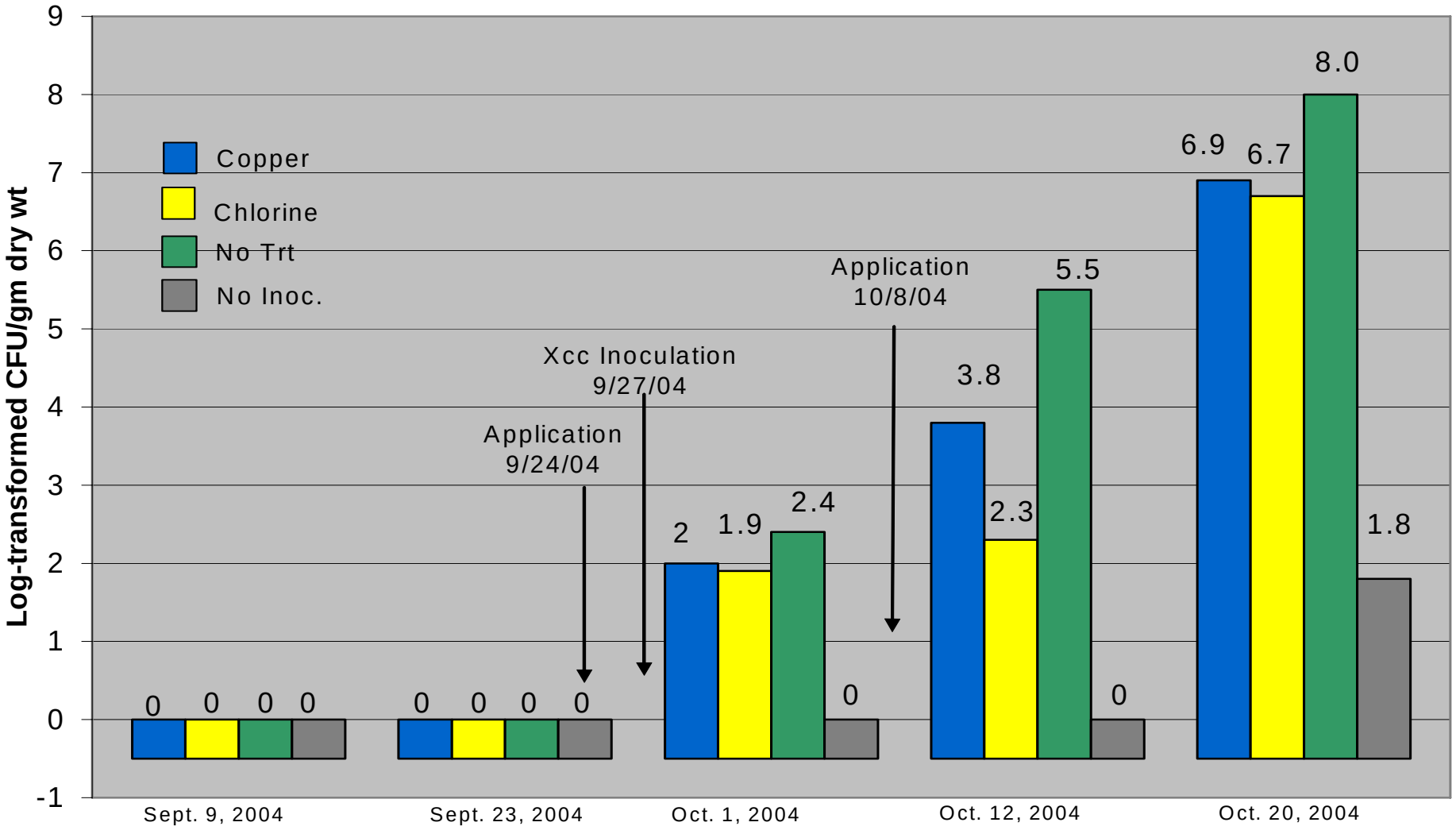

Figure 2. Late trial: carrot seedlings treated with copper or chlorine before and after inoculation with Xanthomonas campestris pv carotae.