INCIDENCE OF *XANTHOMONAS CAMPESTRIS* PV *CAROTAE* ON CARROT SEED IRRIGATED BY DRIP OR SPRINKLER IN CENTRAL OREGON, 2004

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Abstract

The proportion of plants from which *Xanthomonas campestris* pv *carotae* was recovered during the season was lower for drip- vs. sprinkler-irrigated fields, for five paired fields in central Oregon. The amount of *Xanthomonas* from seedlots after harvest was less for drip- vs. sprinkler-irrigated fields, 6.5 vs. 7.0 = log (colony forming units/10,000 seeds), respectively. These differences were statistically significant ($P < 0.05$) when the five paired fields were considered valid replications. 2004 was considered a wet season, which may have aggravated *Xanthomonas* to higher levels in drip-irrigated fields than in drier years. Whether the target of $\leq 10^5$ colony forming units/10,000 seeds might be achieved under drip-irrigated fields in drier years remains to be demonstrated. However, because seed from drip-irrigated fields had a higher germination percentage and vigor (see companion report "Drip Irrigation on Commercial Seed Carrots in Central Oregon, 2004" by C. Weber et al.) such seed from drip-irrigated fields should better tolerate hot water treatment for eradication of *Xanthomonas* than seed from sprinkler-irrigated fields.

Introduction

Bacterial blight on carrot plants in carrot seed fields in central Oregon generally is mild and occasional, although disease incidence does flare up in certain fields in some years. More problematic is that levels of *Xanthomonas campestris* pv *carotae* (Xcc) have been high in seedlots harvested from many fields in past years, even in the absence of bacterial blight disease. Care is taken to plant Xcc-free seed and, initially, incidence of Xcc is low, but during the season this incidence increases. Near to harvest, high Xcc populations frequently are present on the foliage of a high proportion of carrot plants even in the absence of symptoms (Du Toit et al. 2005). Currently, most carrot seed fields in central Oregon are irrigated by either sprinkler or furrow irrigation. Little difference in Xcc incidence was noticed for fields irrigated by these two methods in our recent 2-year survey (Du Toit et al. 2005). In central Oregon, seed-to-seed fields that will be furrow or drip irrigated spring-to-fall are frequently sprinkled for seedling emergence in the fall. This practice may tend to promote early establishment of foliar Xcc populations. Spring-to-fall drip irrigation, for both seed-to-seed and steckling-to-seed fields is becoming more popular as research demonstrates its potential for conserving water and promoting high yields and seed quality. Preliminary data gathered in 2002-2003 suggested that Xcc populations were lower in drip-irrigated seed carrot fields where such fields had some physical separation from sprinkler-irrigated seed carrot fields. Our goal in 2003-2004 was to more closely compare bacterial blight incidence in central Oregon seed carrot fields irrigated by drip, furrow, or sprinkler. This study was closely coordinated with and part of the drip-irrigation study discussed in "Drip Irrigation on Commercial Seed Carrots in Central Oregon, 2004."

Commonly in central Oregon, seedlots in sprinkler- or furrow-irrigated fields may be infested with 10^6 to 10^8 colony forming units (CFUs) of Xcc per 10,000 seeds (du Toit et al. 2005), which requires hot water treatment (Pscheidt and Ocamb 2001) to lower the infestation to acceptable levels that would not likely develop into a bacterial problem in commercial fields. In fact, hot water treatment generally eliminates detectable Xcc from seed. In the only study that actually attempted to measure what seed infestation levels resulted in bacterial blight incidence under conducive disease conditions, 10^5 CFUs/10,000 seeds was found to be an upper acceptable level for commercial carrots grown under sprinkler irrigation in the Central Valley of California (Umesh et al. 1998). Hot water treatment is expensive, tricky to manage, and tends to slightly reduce the germination percentage and vigor of the seed. (M. Weber, Central Oregon Seed, Inc., personal communication). The percent germination of seed from drip-irrigated fields tends to be higher than in sprinkler-irrigated fields, so such seedlots may tolerate hot water treatment better. Conversion to drip irrigation for the reason of elevating seed germination percentage and vigor may assist in the *Xanthomonas* issue. However, for seedlots already near germination limitations, hot water treatment can lower germination percentage below acceptable levels. Therefore, in addition to enhanced germination and vigor, we investigated whether seed infestation levels in drip-irrigated fields would be lower than 10^5 CFUs of Xcc/10,000 seed, perhaps eliminating the need for hot water treatment altogether.

Materials and Methods

Fields selected for comparisons of Xcc incidence were the same as those included in the companion drip study "Drip Irrigation on Commercial Seed Carrots in Central Oregon, 2004." Materials and methods for the drip study are included there and not in this report. Specific irrigation usage data were not available for every field set and are not shown here. In all cases, each set of comparisons included the same carrot variety and seed source. None of the planted seed was available for testing; however, in previous studies no Xcc was found on any seedlots used in central Oregon (Du Toit et al. 2005). We assume that seed companies direct Xcc -free seed into central Oregon carrot plantings to increase the chance that Xcc will not develop in the seed field. However, inoculum does find its way into central Oregon fields anyway, typically from combine dust from nearby fields (Du Toit et al. 2004), or from from equipment, animals, etc. Further, each field set used in this study included fields that were geographically similar within central Oregon, so soil types, local microclimate, and other factors were similar. Nevertheless, each field was independently farmed and each farmer tended to manage his seed carrot crop similarly, especially for a given variety.

In all, we monitored Xcc populations in seven matched sets of fields during the 2004 season. Samples were collected from each set within a day or two of April 20, July 10, and September 1. Because irrigation systems were installed in the spring, after April 20, the April 20 sampling period was considered a pre-irrigation baseline sample. Typically, irrigation in central Oregon begins in late April or soon after. We had initially intended to collect an additional sample from mid-June. However, heavy spring rains delayed installation of drip systems and eliminated the need for irrigation during the spring of the season, a highly unusual situation in central Oregon that we believe had implications on bacterial incidence, which is discussed later.

Sampling and plant assays were conducted as per our previous survey (Du Toit et al. 2005). At each sampling date, either whole plants or representative parts of plants (foliage, stems, flowers) were collected separately for 30 plants. Hands and equipment were sterilized between each sample, and each sample was collected in a new plastic bag. Samples were kept cool until processed in the lab. If necessary, samples including umbels and stems were chopped prior to washing for 1 hour in phosphate buffer (12.5m M PO_4, pH 7.1), prior to coarse filtering and dilution plating onto XCS agar, a semi-selective medium for Xcc (Williford and Schaad 1984). Petri plates were observed within 1 week and the number of CFUs of Xcc were determined per gram of dry weight in the sample.

Following harvest, cleaned seed samples from each field were assayed by soaking approximately 10,000 seeds overnight at 4^oC in 100 ml of saline (0.85 percent NaCl). Two drops of Tween 20 were then added to each flask, which was placed on a rotary shaker for 5 minutes, diluted and then plated on XCS agar. Seedlots were assayed three times each. Incidence of plant infection was simply the proportion of plants on which Xcc was found, typically expressed as a percentage. Additionally, for plants that tested positive for this pathogen, we expressed the mean of the log(CFU).

Results and Discussion

The proportion of plants from which Xanthomonas was recovered is shown in Figure 1 for five of the seven fields. Two fields were excluded from these figures. In one case, there was no sprinkled field within the set, only a furrow-irrigated field, so this set could not contribute to statistical analyses. Another field set was excluded because the incidence of plants with Xcc already was over 80 percent at the April sampling, an unusually high incidence for which we have no explanation. For this set, too little divergence in incidence between irrigation types was possible because plants remained infested all season. For the five field sets retained in the analysis, each set contained a sprinkler-irrigated field and a drip-irrigated field, so direct comparisons were possible, and each set was considered a replication for statistical purposes. The mean proportion of plants from which Xcc was recovered for these five field sets is shown in Figure 2, including letters showing mean separations for significance level of 5 percent based on analysis of variance. The April 20 sampling date was considered a pre-irrigation baseline sample, because fields had not been handled differently at that time with respect to type of irrigation. Prior to any irrigation, the proportion of plants already harboring Xcc populations in fields to be sprinkled averaged 21 percent in late April, and in drip fields averaged about 15 percent, with a combined average of 19 percent (Fig. 2).

As indicated earlier, spring rains delayed onset of spring irrigation until about June 1, a highly unusual delay for central Oregon. Theoretically, such rains might splash Xanthomonas within fields irrespective of irrigation type. Further, with delayed irrigation, there were fewer than normal sprinkler-irrigation events that might lessen differences in Xccincidence between sprinkler and drip irrigation. Nevertheless, average Xccplant recovery incidence increased in drip fields from only 15 percent in April to 25 percent around July 10, whereas such incidence increased in sprinkler-irrigated fields from about 20 to 60 percent (Fig. 2). On average, sprinkler-irrigated fields were irrigated five to six times during the June 1 to July 10 period. The three-fold difference in July incidence between drip- vs. sprinkler-irrigated fields suggests that irrigation type had a tremendous influence on plant infestation incidence, even with fewer irrigations contributing than in a normal season. Not shown in the figures are data from two furrow-irrigated fields included in these field sets; the average incidence of Xcc in those fields was intermediate between that found in drip- and sprinkler-irrigated fields.

Year 2004 continued to be unusual with respect to precipitation later in the growing season: very heavy rainfall was recorded in central Oregon late in August, as carrot seed plants were approaching maturity and before the September 1 sampling period. The proportion of plants with Xcc in sprinkler-irrigated fields increased from 60 to 76 percent between July and September, and in drip-irrigated fields the increase was from 25 to 67 percent. We had hoped that incidence would remain low among drip-irrigated fields during this same period, but it seems likely that the heavy August rain promoted rapid spread. Statistically, however, the difference between 76 and 67 percent was still significant ($P < 0.05$)..

Actual population data from plants that assayed positive for Xcc are not shown for any date, but as per our earlier field surveys , populations of 10^4-10^8 CFU/g of dry tissue were common whenever Xccwas present on a plant. As we found in our earlier surveys, once plants become infested, populations tend to become somewhat high even though symptoms do not generally develop. Thus, the main effect of irrigation type is simply the reduction in proportion of plants with Xcc.

Seed assay data for the five field sets are shown in Figure 3, expressed as the mean log (CFU) averages. Seed from sprinkler-irrigated fields averaged log(CFU) or just over 7, whereas seed from drip irrigated fields averaged about 6.5 mean log(CFU). This difference was statistically significant ($P < 0.05$). A log(CFU) rating of 6.5 is higher than our hoped-for log(CFU) target of 5 (i.e., 10^5 CFU/10,000 seed); however, it might be reasonably expected that assays from drip-irrigated fields would be lower in a year with normally low precipitation. We intend to conduct a similar set of comparison evaluations in the 2004-2005 season, we hope with more normal (i.e., less) rainfall and lower Xcc populations in drip-irrigated fields. Perhaps, either by drip irrigation alone or drip irrigation in combination with other field treatments, acceptable seed infestation levels may be achieved. However, elevation of seed germination and vigor via drip irrigation itself can create more hot water tolerant seedlots.

References

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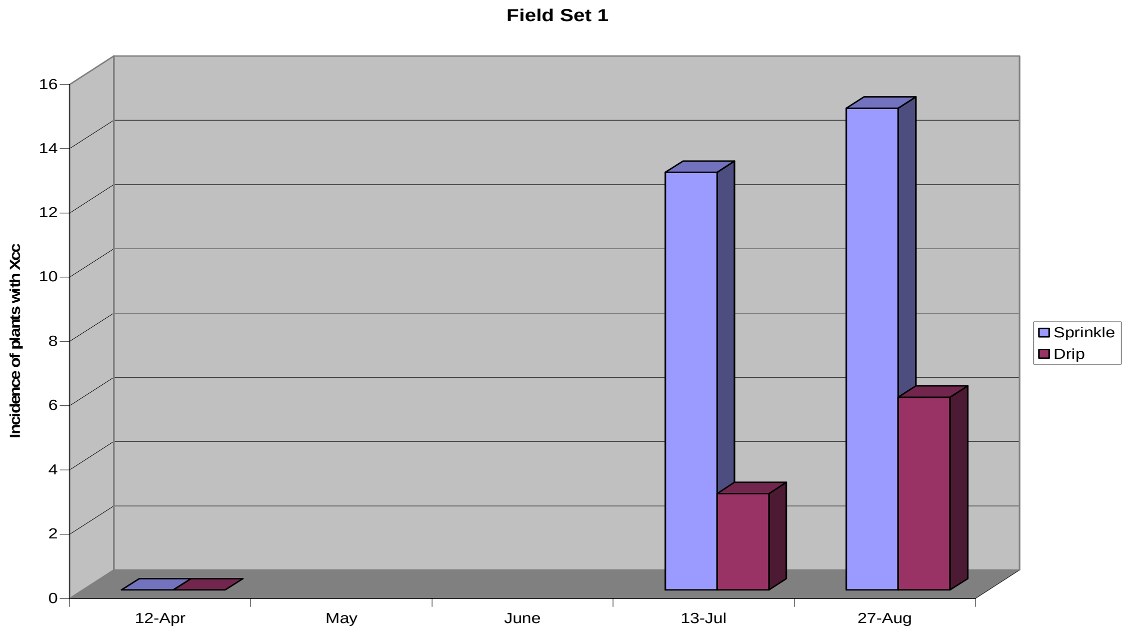
Figure 1. Seasonal incidence of plant infection by *Xanthomonas campestris* pv. *carotae* (Xcc) for different types of irrigation. Each field set included different fields planted with the same variety of carrots and within a common subregion of central Oregon, 2004.



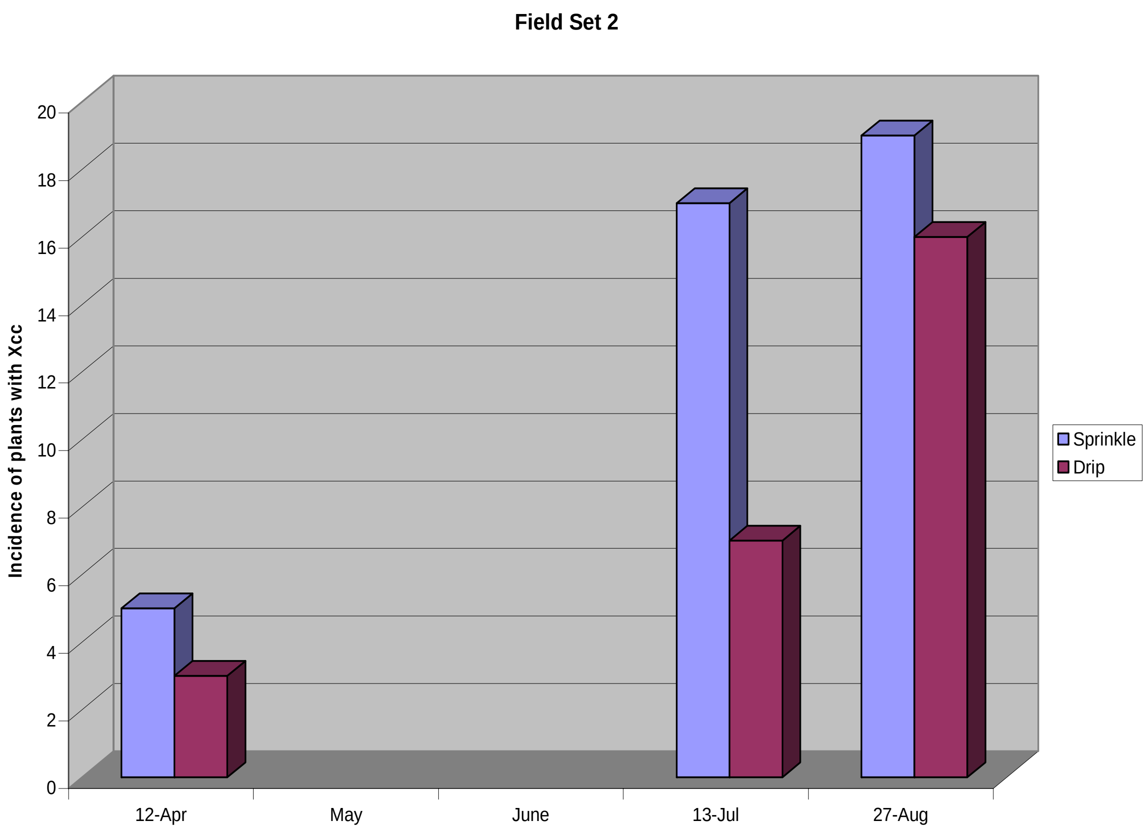
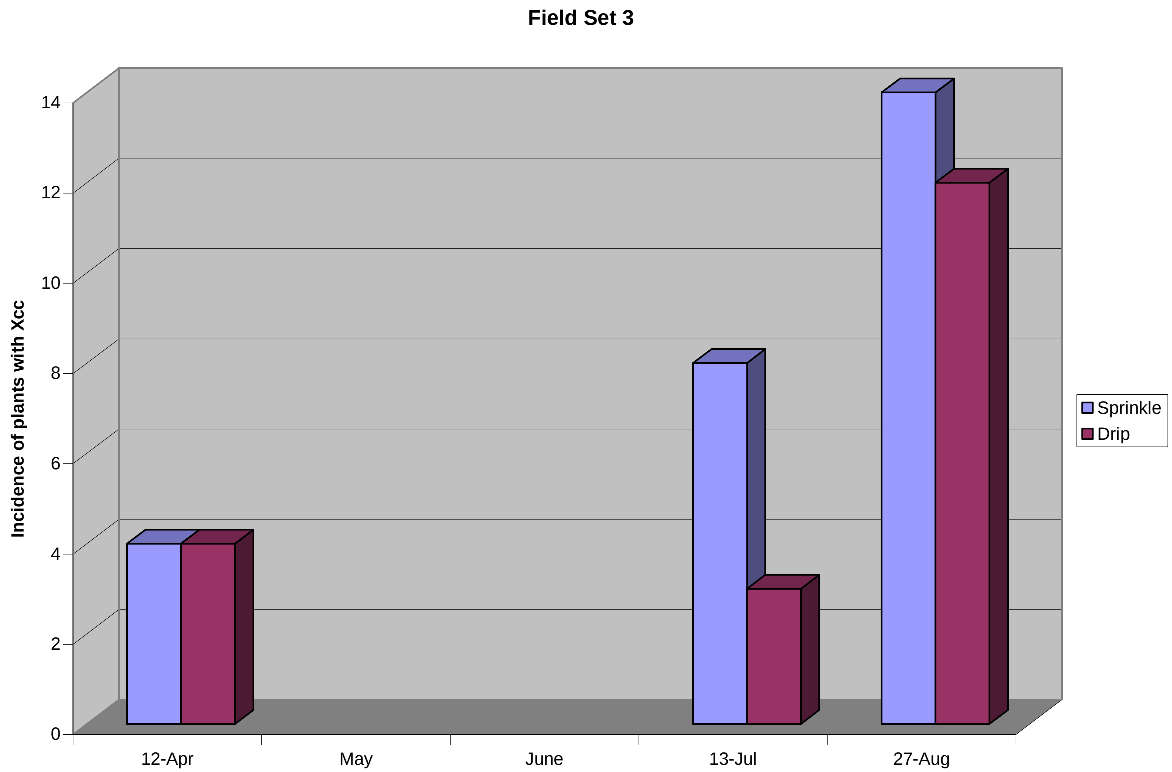


Figure 1. cont.



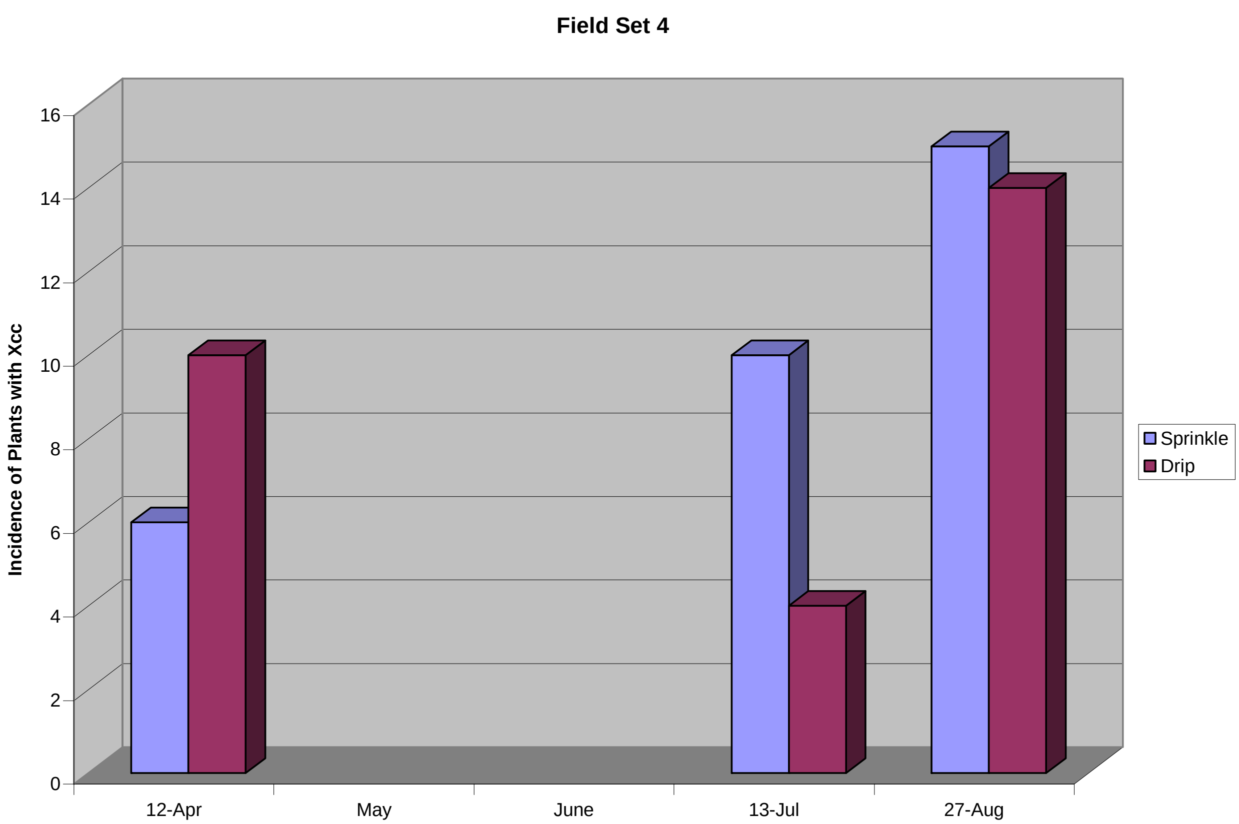
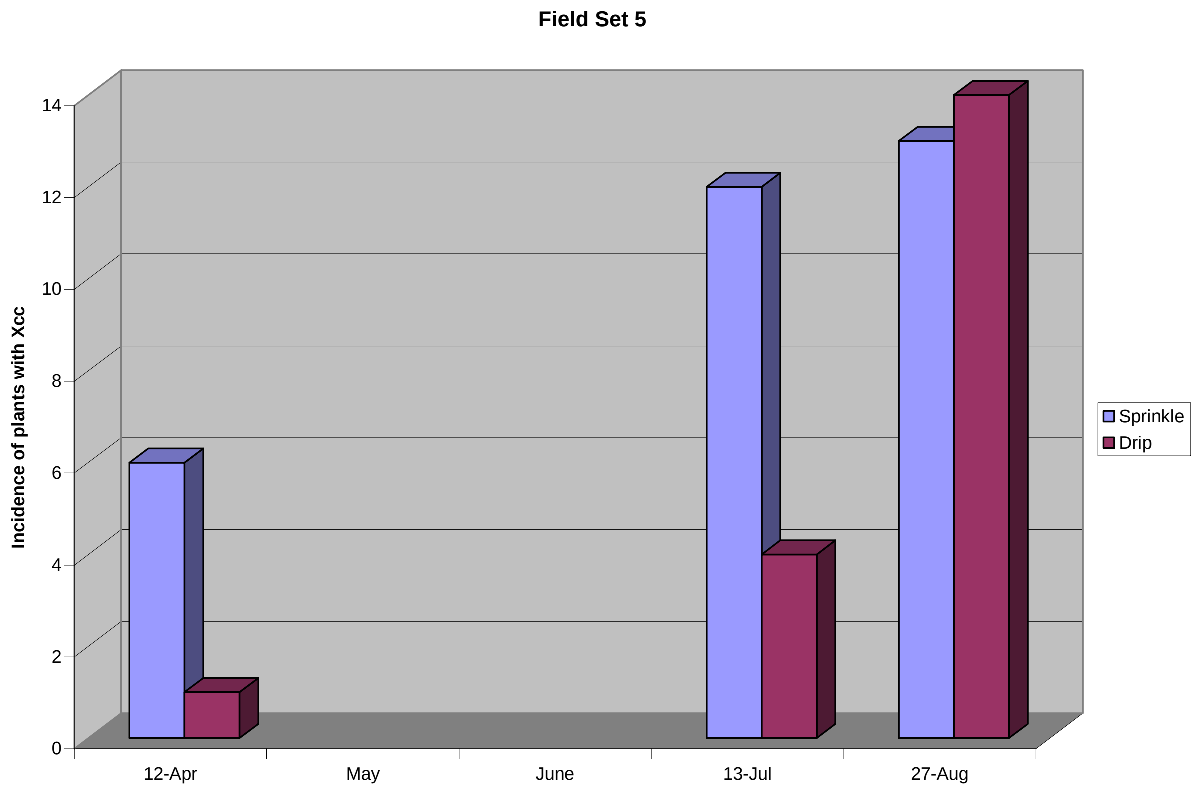


Figure 1. cont.



Field Set 5
Incidence of plants with Xcc
0
2
4
6
8
10
12
14
12-Apr
May
June
13-Jul
27-Aug
Sprinkle
Drip

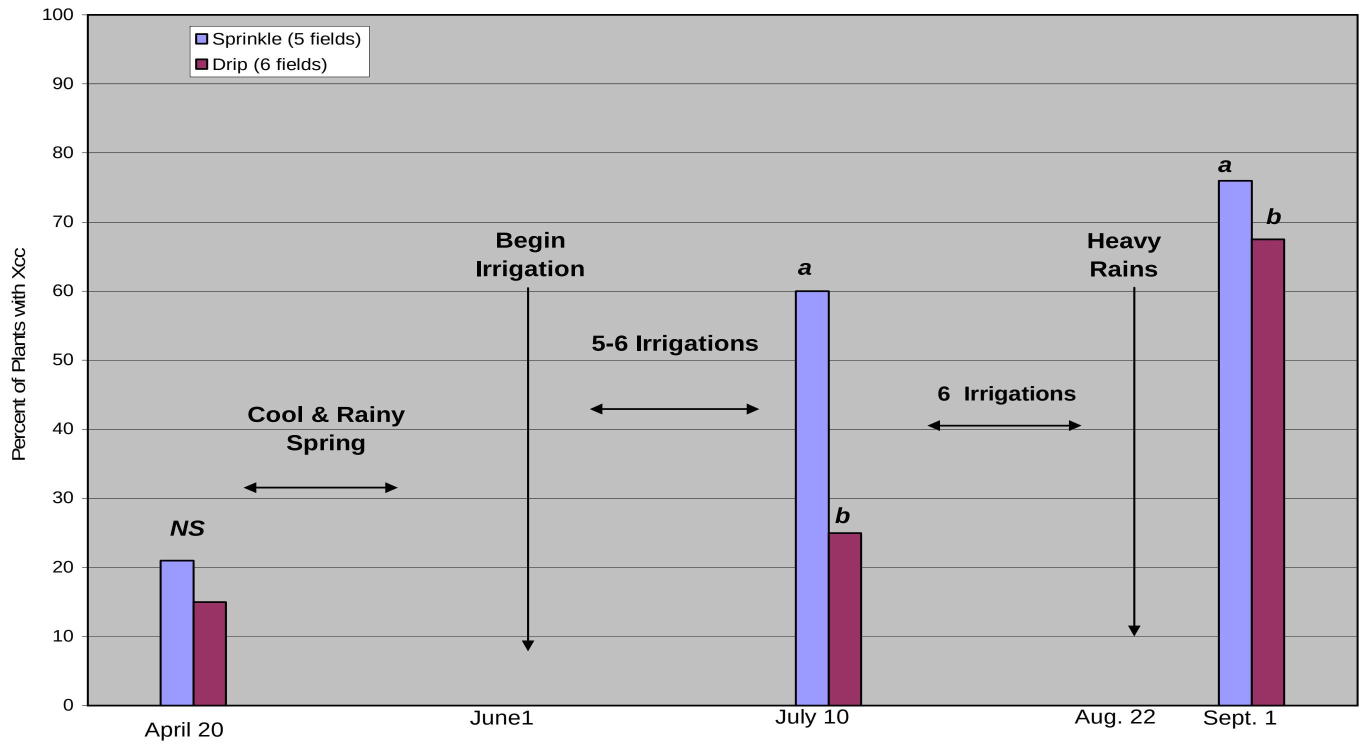


Figure. 2. Proportion means of carrot plants infected with *Xanthomonas campestris* pv. *carotaec* (Xcc), central Oregon irrigation survey, 2004. Means labeled with different letters are statistically significant ($P < 0.05$).

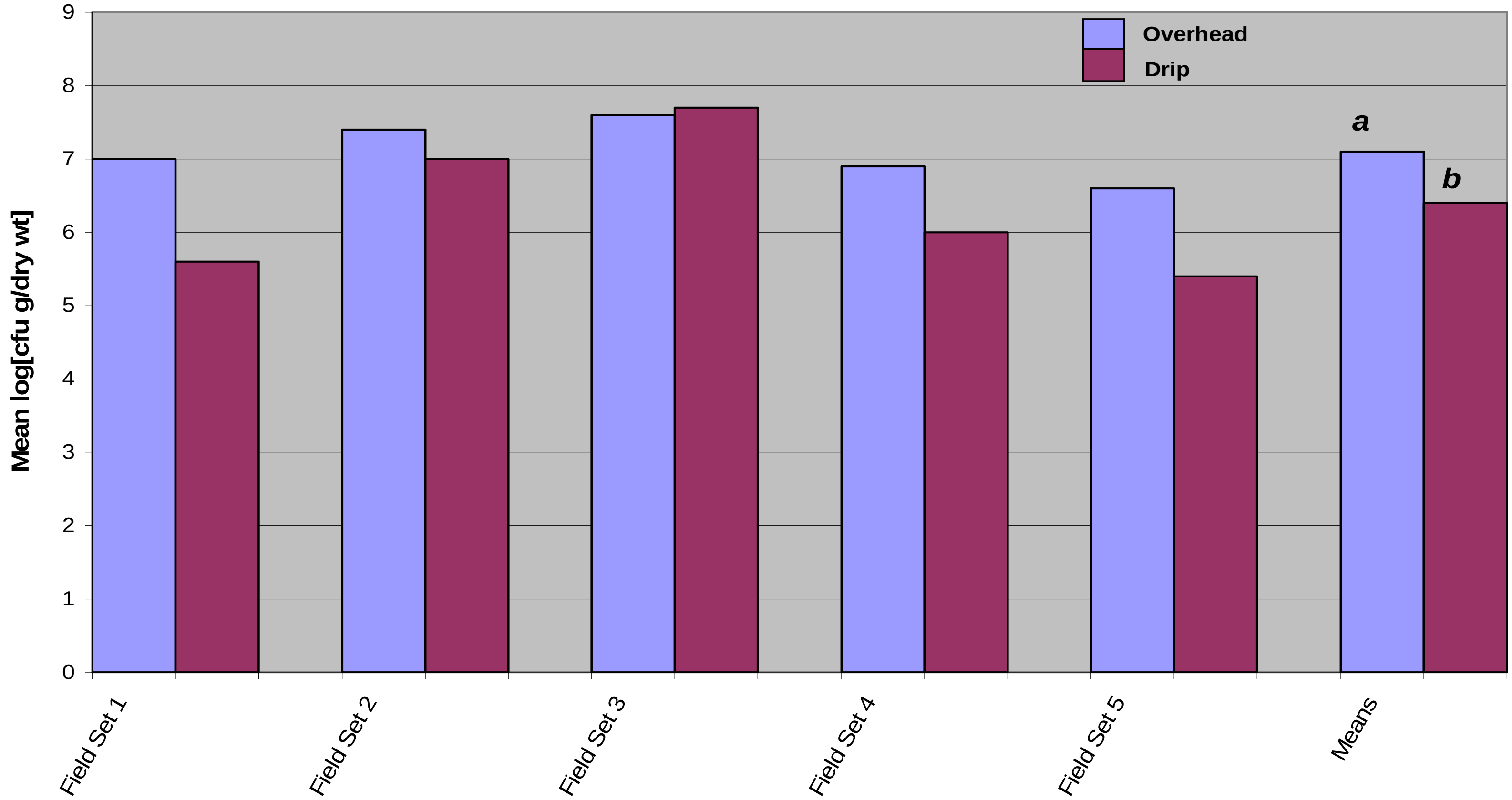


Figure. 3. Detection of *Xanthomonas campestris* pv. *carotae* population on harvested seed. Means with different letters are statistically significant ($P < 0.05$).