CSI Black Rot: What DNA-Fingerprinting Technology Has Revealed About This Disease in New York

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Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), is a significant disease of cabbage, and other crucifer crops world-wide. This disease has been a serious problem in New York and is listed as a high priority for the industry. The pathogen can be spread through infected seeds or from plant-to-plant through water droplets, and can spread rapidly in transplant greenhouses and seed beds. In other locations where black rot is prevalent, the disease has been shown to spread from weeds, and debris in the soil. The relative role of weeds and soil debris as a source of the pathogen, compared with infected seed was previously unknown. The overall goal of this project was to gain a better understanding of the potential sources of inoculum of black rot in NY, and the severity of disease from different sources. This information will allow the development of management strategies for control of this disease, and ensure that new varieties are tested against the most aggressive strains of the pathogen.

Since 2004, our program has been using DNA fingerprinting to identify strains of the black rot pathogen, Xcc. We have surveyed black rot pathogen isolates annually from transplants and symptomatic plants in commercial fields using selective media, ELISA, pathogenicity, and DNA fingerprinting. Our studies have shown that while it is possible for the pathogen to over-winter in NY, this has not been the most common source of inoculum. Fingerprinting results have identified new strains of *Xcc* that have NY each year of the study (2004-2013), and the new strains are the predominant strains each season.

To better understand the role of weeds as a source of inoculum, we collected weed samples in the spring, from 5 fields that had severe black rot the previous fall. From each field, 15-20 weed samples were collected, and we attempted to isolate Xcc. The pathogen was isolated (based on molecular data) only from cruciferous weeds, however none of the bacteria isolated produced symptoms on cabbage plants when inoculated in our greenhouse assay. Additionally, the DNA fingerprint patters of the isolates obtained from weeds did not match any of the DNA fingerprint patterns from isolates obtained from cole crops. This means that the isolates we collected from weeds did not come from the severely infected cabbage that were in the same field the previous year. In two or three instances we have isolated Xcc from weeds in fields during a black rot epidemic, with the symptomatic cruciferous weed growing next to the infected cabbage. In these cases, the isolates in the weeds were identical to those in the cabbage plants, based on DNA fingerprinting. It is impossible to know for certain if the pathogen moved from the cabbage to the weed, or from the weed to the cabbage. Our hypothesis is that the Xcc moved from the cabbage to the weed since in each case the isolate had never before been observed in NY, and we identified the identical isolate from cabbage in other geographically separated fields. Thus, while strains of *Xcc* that are pathogenic to cole crops can be harbored and detected in weeds in New York, this is not the predominant source of inoculum.

Based on our fingerprint studies, we now know that new strains of Xcc arrive each year either on seed or on diseased transplants and the pathogen then spreads during transplant production and in the field when appropriate environmental conditions exist. With this information in hand, we

began new studies with the goal of determining the efficacy of available control strategies to prevent the spread of Xcc. We have conducted experiments to determine if applications of copper during transplant production will reduce the spread of the pathogen in the greenhouse and lead to less disease in the field, and have continued the treatments into the field to determine if there was an impact on yield.

Results of three years of study indicate that application of copper during transplant production does reduce the spread of Xcc. Application of Actigard (a plant defense response activator) also reduced spread of Xcc, but some phytotoxicity (cupping of leaves) and smaller head size was observed. Our trial in 2013 compared various formulations of copper-based control products for efficacy against black rot under field conditions. Healthy transplants were planted in a randomized complete block design. Treatments (Table 1) were sprayed on a 7 day schedule until just prior to harvest, and plants were inoculated with Xcc 24 hours after the first spray. Disease incidence and severity were rated four times. No phytotoxicity was noted with any of the treatments. Black rot symptoms were first observed in early July and by the first rating on July 10, 100% of the untreated plants showed black rot symptoms. The wet and cool environmental conditions during the 2013 in June and early July were not optimal for the spread of Xcc, and while the incidence (number of plants with any symptoms of black rot) was high, the severity was fairly low. At the last rating, black rot lesions covered 39% of the inoculated but untreated (no copper) plants. The uninoculated plants with no treatment had a severity rating of only 4% by late July, but 100% of them had symptoms of black rot. All of the treatments on the inoculated plants significantly reduced the severity of the black rot at the final rating (Table 1). Additionally, the Kocide and Cuprofix treatments had significantly less black rot than the Cueva treatment (Table 1), however this was a lower rate of Cueva than had been tested in 2012 when no significant differences were seen between Cueva and Kocide.

Table 1. Results from testing multiple copper-based control products for efficacy against the black rot pathogen Xcc. Numbers are the mean incidence or severity of 4 replicates.

Treatment and rate	**% Incidence**				**% Severity**			
	10 Jul	**15 Jul**	**19 Jul**	**22 Jul**	**10 Jul**	**15 Jul**	**19 Jul**	**22 Jul**
Inoculated control…… . NA	100 a	100 a	100 a	100 a	4.8 a	11.5 a	27 a	39 a
Uninoculated control……..NA	20 c	55 b	80 b	100 a	0.01 c	0.05 c	1.3 c	4.0 c
Kocide 3000 …… . 0.75lb/A	62.5 c	87.5 a	100 a	100 a	0.8 bc	1.0 bc	4.0 c	8.5 c
Champ WG ……0.25lb/A	87.5 ab	95 a	95 ab	100 a	1.9 bc	3.8 bc	6.5 c	12.5 bc
Cueva FL ……0.5gal/100gal	97.5 a	100 a	100 a	100 a	2.5 b	5.8 b	16 b	22.5 b
Cuprofix Ultra 40 Disperss….1.25lb/A	72.5 bc	85 a	98 ab	100 a	1.4 bc	2.7 bc	3.3 c	7.8 c

Means within a column followed by the same letter are not significantly different at *P*=0.05 as determined by Fishers LSD.