

Investigation of *Xanthomonas euvesicatoria* on Seed Germination and Seed to Seedling Transmission in Tomato

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Abstract : Infested tomato seeds were used to investigate the influence of *Xanthomonas euvesicatoria* on germination and seed to seedling transmission in a controlled environment and greenhouse assays in an effort to develop effective seed treatments and characterize seed borne transmission of bacterial leaf spot of tomato. Bacterial leaf spot of tomato, caused by four distinct *Xanthomonas* species, *X. euvesicatoria*, *X. gardneri*, *X. perforans*, and *X. vesicatoria*, is a serious disease worldwide. In the United States, disease prevention is expensive for commercial growers in warm, humid regions of the country, and crop losses can be devastating. In this study, four different infested tomato seed lots were extracted from tomato fruits infected with bacterial leaf spot from a field in New York State in 2017 that had been inoculated with *X. euvesicatoria*. In addition, vacuum infiltration at 61 kilopascals for 1, 5, 10, and 15 minutes and seed soaking for 5, 10, 15, and 30 minutes with different bacterial concentrations were used to artificially infest seed in the laboratory. For controlled environment assays, infested tomato seeds from the field and laboratory were placed on a moistened blue blotter in square plastic boxes (10 cm x 10 cm) and incubated at 20/30 °C with an 8/16 hour light cycle, respectively. Infested tomato seeds from the field and laboratory were also planted in small plastic trays in soil (peat-lite medium) and placed in the greenhouse with 24/18 °C day and night temperatures, respectively, with a 14-hour photoperiod. Seed germination was assessed after eight days in the laboratory and 14 days in the greenhouse. Polymerase chain reaction (PCR) using the *hrpB7* primers (RST65 [5'-GTCGTCGTTACGGCAAGGTGGTG-3'] and RST69 [5'-TCGCCCAGCGTCATCAGGCCATC-3']) was performed to confirm presence or absence of the bacterial pathogen in seed lots collected from the field and in germinating seedlings in all experiments. For infested seed lots from the field, germination was lowest (84%) in the seed lot with the highest level of bacterial infestation (55%) and ranged from 84-98%. No adverse effect on germination was observed from artificially infested seeds for any bacterial concentration and method of infiltration when compared to a non-infested control. Germination in laboratory assays for artificially infested seeds ranged from 82-100%. In controlled environment assays, 2.5 % were PCR positive for the pathogen, and in the greenhouse assays, no infected seedlings were detected. From these experiments, *X. euvesicatoria* does not appear to adversely influence germination. The lowest rate of germination from field collected seed may be due to contamination with multiple pathogens and saprophytic organisms as no effect of artificial bacterial seed infestation in the laboratory on germination was observed. No evidence of systemic movement from seed to seedling was observed in the greenhouse assays; however, in the controlled environment assays, some seedlings were PCR positive. Additional experiments are underway with green fluorescent protein-expressing isolates to further characterize seed to seedling transmission of the bacterial leaf spot pathogen in tomato.

Keywords : bacterial leaf spot, seed germination, tomato, *Xanthomonas euvesicatoria*

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