

Title: Characterization and detection of Blueberry necrotic ring blotch associated virus, a new pathogen threatening the blueberry production in the Southeast

Final report

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Recently, a new problem has been observed on southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids) in several southeastern states. Our current assumption is that blueberry necrotic ring blotch is likely caused by a virus, since double-stranded RNA (dsRNA) has been isolated from symptomatic leaves. Initially observed in 2006, necrotic ring blotch was found in a few locations in southern Georgia. No bacterial or fungal pathogens were observed in symptomatic tissue, and ELISA and PCR tests were negative for known blueberry viruses. The initial distribution of necrotic ring blotch in Georgia was limited to very sporadic reports and observations in 2006 and 2007, but in 2008, it was found in multiple locations throughout the major blueberry production counties; in several locations, premature defoliation occurred on severely affected plants. The disorder was also observed in Florida, Mississippi, South Carolina, and North Carolina, and it has emerged from an initial curiosity to a potential yield-limiting problem among some cultivars. To date, necrotic ring blotch has not been observed in the native, more widely grown rabbiteye (*Vaccinium virgatum*) cultivars, but it is not known if rabbiteye blueberries could be a symptomless host of this virus.

Symptoms of blueberry necrotic ring blotch disorder are shown Fig. 1, initially symptoms of necrotic ring blotch are observed as irregularly shaped circular spots or blotches with green centers on the top and bottoms of leaves. These symptoms are distinct from those caused by BRRV, which produces ringspots that are more reddish in color, have thinner margins, and are less blotchy in appearance and are only present on the upper leaf surfaces. With necrotic ring blotch, spots generally are more prevalent on older tissue (i.e., the lower leaves of plants), but they can inundate a bush from bottom to top. The

outer ring colors can initially vary from dark brown to purplish-black, but they often coalesce to form solid brown to black necrotic spots or blotches which are similar to Septoria leaf spot or other fungal leaf spots.

The cause of the disorder is currently unknown, but presence of dsRNA suggests a virus. In addition, the dsRNA patterns were consistent from at least 8 different samples that were analyzed. Partial sequence has been obtained for three of the dsRNAs and used to develop diagnostic primers for detection by reverse transcription-polymerase chain reaction (RT-PCR). More than 40 individual plants that exhibited necrotic ring blotch symptoms in North Carolina, Georgia and Florida were positive in the RT-PCR assay that was developed. The perfect correlation between the virus and symptoms in this many samples from several states in the southeastern U.S. strongly suggests that the virus that is being characterized is indeed the causal agent of the disease. There are five dsRNA bands and it is possible that there are multiple viruses present. The additional bands have been gel purified and are being subjected to high throughput sequencing to determine if there are multiple viruses, or if the additional dsRNA bands represent subgenomic dsRNAs produced during replication of the virus that we now have partially sequenced.

There is currently no information on how the virus spreads in the field, but transmission can occur fairly quickly plants free of the virus planted adjacent to the infected plants. This rapid movement suggests an aerial vector. Until the vector is identified control measures cannot be developed. Though, it is important to start new plantings with virus-tested planting stock.



Fig. 1. Symptoms of Blueberry necrotic ring blotch symptoms on Star blueberry; Left. Mississippi and, Right. Georgia. Notice green island surrounded by dark reddish brown rings on upper and lower leaf surfaces.

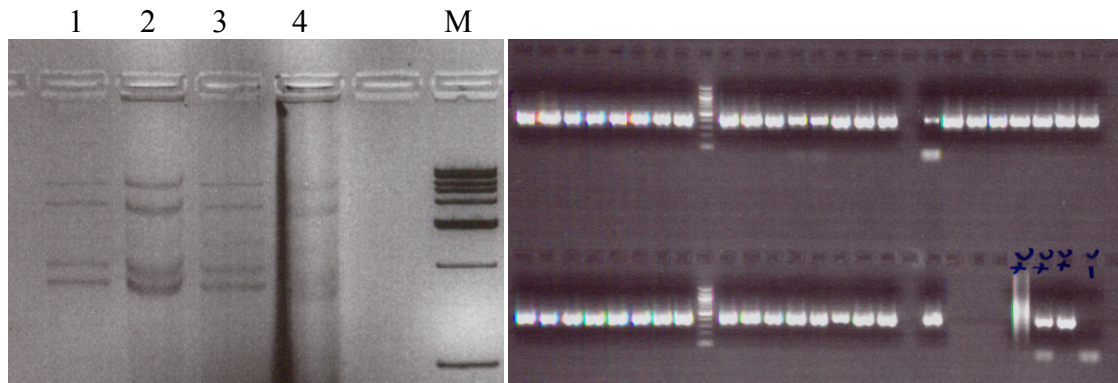


Fig. 2. DsRNA and RT-PCR detection of Blueberry necrotic ringblotch associated virus. Left. DsRNA from four separate samples (1-4) of blueberry exhibiting symptoms of the disease. Right. RT-PCR detection from 41 field samples exhibiting symptoms of the necrotic ringblotch (24 upper row and 17 lower row), right on lower row shows three positive controls and one negative control.