Blueberry necrotic ring blotch disorder: A new disease of southern highbush blueberries.

Final report (Research Proposal)

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Objectives: 1) Determine how blueberry necrotic ring blotch disorder (BNRBD) spreads within the plant, 2) conduct an epidemiological study of BNRBD spread in the field, and 3) define the effect BNRBD has on photosynthesis.

Justification: The blueberry industry in Georgia (valued at $102.5 million according to the 2009 Georgia Farm Gate Value Report) and other southeastern states has experienced considerable growth during the past 30 years due to improved cultivars and marketing opportunities (1, 4). This expansion is likely to continue in the near future, since consumer demand has increased due to the widely publicized health benefits of blueberries (2, 3).

Recently, a new disease was observed on southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids). Initially observed in 2006, plants with symptoms reminiscent of a pathogen-induced disease were found in multiple locations in southeastern Georgia. The disease also has been observed in Florida, Mississippi, South Carolina, and North Carolina. Our current assumption is that a virus that has not previously been identified in blueberries causes BNRBD. Double-stranded RNA (dsRNA) has been isolated from symptomatic leaves from multiple sites in Georgia, Florida and North Carolina. The presence of dsRNA is an initial indicator of a virus infection and is routinely used to identify viruses in plants, such as blueberries, that are recalcitrant to standard virus

isolation procedures. To date, BNRBD has not been observed in the native, more widely grown rabbiteye (Vaccinium virgatum) cultivars. Provisionally, the new virus has been designated blueberry necrotic ring blotch associated virus (BNRBaV) (I. E. Tzanetakis and R. R. Martin, unpublished data).

Leaves of susceptible cultivars develop irregular red or brown spots that may or may not have green centers, depending on the cultivar. Eventually the spots may coalesce to cover the entire leaf. The impact that these symptoms have on crop yield is also unknown. With BNRBD, spots are more prevalent on older tissue (i.e., the lower leaves of plants), but they can inundate a bush from bottom to top and in some cultivars cause defoliation of the plant. BNRBD is more apparent after harvest in the summer, but it is not clear when symptoms first appear.

There is currently no information about the epidemiology of the disorder, including its means of transmission or spread, potential involvement of vectors, and the role of alternate hosts or other sources of inoculum. However, if viral, BNRBD is likely transmitted by an insect vector. Other means of spread could include nematodes or root grafting, but these are less likely since the disease spreads extremely rapidly, and viral diseases transmitted by nematodes and root grafting are typically slower processes. No control measures for BNRBD are currently known.

This grant addresses both the simple epidemiological and plant impact information for this new disorder of blueberries. Determining the development and spread of the disease *in planta* during the growing season will be important for understanding the dynamics and possible mechanism by which the disease progresses as well as providing descriptive data on relative symptom development. Field spread studies will be particularly helpful in determining potential means of plant to plant spread, i.e. pollen, insect vectors, etc. Photosynthesis measurements will allow us to determine whether BNRBD interferes substantively with energy production in the plant, which would also relate to total plant health and potential yield reduction.

Methodologies: To determine how BNRBD spreads within a blueberry plant. In order to determine in-plant spread, an architectural study of multiple infected plants was conducted (10 plants showing initial stages of symptom development per field site; three to five field locations). This was conducted in two ways. The first technique utilized a grid system in which different parts of the plant were given a rating of disease severity. These measurements were taken over time, providing a general description of where the disorder first appeared within plants and severity within the plant, as well as its spread in a plant throughout the spring and summer. Only one side of the bush was observed. The second technique in architectural study involved more detail. In the second technique, individual stems with limited symptomatic leaves were tagged after the spring growth flush as symptoms were starting to appear, the location and number of spots were recorded for each leaf on the stem, and the progress of symptoms were recorded over time for the leaves on each stem. The number of spots per leaf over time wererecorded. To conduct an epidemiological study of BNRBD spread in the

field. To better define and understand the dynamics of disease spread, a visual determination of disease development (% symptomatic plants) were taken from 6 sites throughout the growing season. For each site, symptomatic plant coordinates were recorded to develop a map of the in-field epidemic development. Maps will serve as a baseline for new epidemic observations in the future and will give insights into the mechanism by which the disease spreads. To define the effect BNRBD has on photosynthesis. The study will be carried out at 3-5 sites on 1-2 susceptible cultivars. Test plants at all sites will have been sprayed with fungicide to control and minimize fungal leaf spots. The net CO_2 assimilation rate and leaf conductance will be measured with a portable photosynthetic monitoring system (CIRAS-2; PP systems, Amesbury, MA) on mature leaves as described by Roloff et al. (5). After measurements, analyzed leaves will be detached and taken to the laboratory to determine disease severity (% of blotch covered leaf area) as determined by computer analysis. Net CO_2 assimilation rate and leaf conductance will be plotted against disease severity and each other and analyzed according to Roloff et al (5).

Results: During the growing season of 2011, an epidemiological study was conducted on BNRBD on southern highbush blueberries, specifically the highly susceptible cultivar 'Star' at three independent sites. An analysis of in-field spread of the disease indicated that at two sites bordered by woods the disease pressure was heaviest near the woods and progressively became lighter further from the woods At the third site the highest disease pressure was initially observed near two opposite ends of the field, although the plot did not have woods in the immediate vicinity.

When spread within the plant was analyzed the diseased developed first on older leaf tissue and progressed out to newer leaf tissue. This was also reflected on individual canes, which showed the development of disease on older leaves first with the slow progression to newer leaves.

A diagnostic test for the putative causative agent of BNRBD was developed using polymerase chain reaction primer (PCR). In all cases analyzed, positive PCR reactions for the putative pathogen were correlated with the presence of disease symptoms.

Because of equipment malfunctions, the BNRBD effect on photosynthesis was not determined. This analysis will be performed and data analyzed during the growing season of 2012.

Conclusions: BNRBD was widespread in GA in 2011 and is quickly becoming a major disease with the potential to significantly impact production. The results from the study presented here represent the first epidemiological studies performed to better understand disease development and progress in the field. This initial work suggests that the disease is moving into fields from the perimeter and progressing in a gradient fashion through the growing season. The initial assumption is that an insect vector, yet unknown, is transmitting the disease. This conclusion is especially apparent from the sites that were bordered by woods. The appearance of the disease in older leaf tissue and its progression

into newer leaf tissue as the growing season developed suggests that pathogen transmission occurs early in the growing season.

Impact: Reports from other states in the southeastern US, as well as the widespread appearance of the disease in Georgia suggested that BNRBD is an emerging disease that may have a long-term impact of blueberry production. While the pathogen is thought to be a virus, there is presently no definitive proof. Nor is there any information on the vector or the transmission efficiency of the vector. The continued study of the disease will help confirm the causative agent and its source of transmission. Understanding both aspects of the disease etiology are important for developing future management strategies for controlling the disease

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