Vector and transmission investigations for blueberry red ringspot virus in the southeast

Research Report (Final) for SRSFC Grant Code: **2008-09** 1 December 2008

William Cline
Department of Plant Pathology
North Carolina State University
Horticultural Crops Research Station
3800 Castle Hayne Rd.
Castle Hayne, NC 28429
Bill_cline@ncsu.edu

George Kennedy
Department of Entomology
North Carolina State University
Research Annex West
Campus Box 7630
Raleigh, NC USA 27695-7630
George_kennedy@ncsu.edu

Hannah Burrack
Department of Entomology
North Carolina State University
1130 Grinnells Laboratory
Campus Box 7626
Raleigh, NC 27695-7626
Hannah_burrack@ncsu.edu

Objectives:

- We will determine if vector transmission of blueberry red ringspot virus (BRRV) occurs in the southeast, and if so, what vectors may be responsible for the spread of BRRV in North Carolina.
- Once potential vectors are identified, we will determine if transmission can be demonstrated under laboratory or greenhouse conditions.
- We will determine if seed transmission of BRRV occurs.
- We will survey surrounding plants to determine whether BRRV occurs in wild blueberry and other plants

---

Justification:

Blueberry red ringspot caulimovirus (BRRV) has been present in North Carolina since at least the 1960s, but its incidence has been low and the effect on infected plants minimal. The prevalence and economic impact of this virus has, however, recently increased. At least one variety, Ozarkblue, is severely affected by BRRV. In 2007, symptoms of BRRV were noted in highbush blueberry plantings at the NCSU Ideal Tract at Castle Hayne, NC, and the presence of the disease was confirmed by PCR assays. Hundreds of infected bushes were mapped and then removed. BRRV was detected in 3

additional North Carolina counties in summer 2007. The virus has also been detected in southern highbush cultivars in Georgia, and work there is beginning to gauge the extent and nature of the problem. Mapping conducted at the Ideal Tract suggests that the virus spreads by vegetative propagation and, to a lesser extent, by an unknown winged vector. BRRV is known to occur in highbush blueberries and possibly in cranberries, while rabbiteye blueberries at the Ideal Tract remain symptom-free, suggesting that they are either resistant or immune to the disease. While it appears that some highbush blueberries experience less severe effects of BRRV, the susceptibility of newly-released southern highbush cultivars is unknown, and it is these cultivars that are showing symptoms in commercial fields.

Methodologies:

Objective 1: Identification of potential vectors

An insect monitoring program will be initiated at the NCSU Ideal Tract and at previously identified infected commercial blueberry plantings. Monitoring will be targeted to potential vector insects, initially focusing on aphids. Other caulimoviruses are vectored by aphids, and it is possible that BRRV may also follow this pattern. Insects will be collected by hand as well as in a variety of trap types (bucket traps, yellow sticky traps, delta traps, etc.) to ensure that a wide range of species are sampled. Collections will be conducted weekly beginning in April through fall. Other potential vectors include leafhoppers and thrips. A sample of collected insects will be assayed for virus presence using PCR primers developed by Dr. Jim Polashock at Rutgers University.

If virus is detected in insects, those species will then be tracked throughout the year to determine their population dynamics in blueberries.

Objective 2: Determination of vector transmission

If insects carrying BRRV are found in the field, we will conduct virus transmission assays in the laboratory. These experiments will include a range of highbush and rabbiteye varieties, with rabbiteye berries serving as a negative control.

Objective 3: Determination of seed transmission

Blueberry seeds collected from infected commercial and wild blueberries will be grown to determine whether BRRV can be transmitted via seed. Plants will be grown under greenhouse or screenhouse conditions, tested via PCR assays, and held until fall for visual evaluation, when BRRV virus symptoms are more apparent.

Objective 4: Non-crop and wild blueberry surveys

Wild blueberries surrounding areas where BRRV has been detected will be surveyed for disease symptoms and confirmed via PCR assays. The only known hosts of BRRV are in the genus *Vaccinium*, but the disease has been little studied and other hosts may exist. Non-crop hosts exhibiting potential BRRV symptoms will be assayed to determine if the virus is present. Surveys will be conducted in late summer or fall to ensure that disease symptoms are apparent.

Results (progress to date):

Objective 1: Identification of potential vectors

During 2008, we have established insect monitoring locations, begun to quantify the potential vectors present and determine when they are active, and collected several potential vector species which will be tested for BRR virus.

Insect monitoring locations have been established at 3 blueberry plantings in southeastern North Carolina. Monitoring began in mid August 2008 and will continue through August of 2009 to span an entire year. Each location has the following traps placed randomly in approximately 1 acre blocks within larger plantings: 1 Flight intercept trap, 4 AM (yellow sticky traps), 4 blue sticky cards, and 4 branches coated with Tanglefoot® sticky medium. Yellow and blue sticky traps were obtained from Great Lakes IPM (Vestaburg, MI). The 4 sticky branches were included to verify that insects caught on yellow and blue traps were indeed landing and potentially feeding on blueberry plants, rather than simply cuing in on attractive colored traps from a distance.

Traps are changed weekly and all insects are removed and stored for identification. In addition to trapped insects, samples of potential vector species (aphids and whiteflies in 2008) were collected live off plants and preserved for PCR analysis. Live aphids were only observed on 1 trapping date at one location (13 September 2008, Ideal Tract, Castle Hayne Research Station). Whiteflies were observed in August in all trapping locations. Virus assays will be conducted during winter 2009 on preserved samples from summer and fall collection dates.

Yellow sticky traps caught the greatest number and most diverse range of insects of the trap types. Flight intercept traps yielding virtually no potential vector species, and few insects overall, and these traps have been removed from the field. Few aphids were caught in yellow sticky traps, the most likely of the trap types to attract them. This does not mean aphids are not present or not a potential vector species, but they may not occur in great numbers at the times of year for which we currently have data.

Perhaps the most notable finding to date is the high fall trap captures of the candy-striped leafhopper (*Graphocephala coccinae*). The relatively high populations of this insect are interesting because *G. coccinae* is a potential vector of *Xylella fastidiosa*, the causative agent of bacterial leaf scorch in blueberry. *X. fastidiosa* has been isolated from *G. coccinae* (Pooler et al. 1997), although this is not definitive evidence of vector capability. *G. coccinae* trap captures began during late October, and at one of the trapping locations, 78 *G. coccinae* were caught in a single week (31 October through 6 November 2008).

Objective 2: Determination of vector transmission

We were unable to complete this objective in a single year, therefore vector transmission studies will be conducted in future years. These studies will be based on assays to be conducted this winter on insects collected to date. If insects carrying BRRV are discovered, we will conduct virus transmission assays in the laboratory. These experiments will include a range of highbush and rabbiteye varieties, with rabbiteye berries serving as a negative control.

Objective 3: Determination of seed transmission

Ripe berries were collected from12 visibly infected bushes (individual clones, i.e., seedlings or selections) and three visibly non-infected cultivars at the NCSU Horticultural Crops Research Station in Castle Hayne during July 2007. Seeds were extracted 23-27 July and stored at 40 ^{o}F. In November, seeds were treated with 500 ppm gibberellic acid for 24 hr, then sown in pots containing a 1:1:1 mix of sand:peat:pine bark. Pots were sealed in clear plastic bags and held on a lab bench at room temperature (68-74 ^{o}F) under a continuous mix of fluorescent and incandescent light. Seeds germinated in stages during December 2007 and were transplanted to 1.5-inch square pots (plug trays) in stages during February and March. In late June, seedlings were transplanted to 3-inch pots and moved to an outdoor shade house. Approximately 80 to 100 seedlings 3-4 inches tall were obtained by this process. Seedlings will be grown to adequate size for leaf and stem tissue sampling in 2009, at which time samples will be tested via PCR assay for the presence of BRRV. Plants that test positive for BRRV will be held until symptomatic then re-tested.

Objective 4: Non-crop and wild blueberry surveys

This objective was changed to a survey of commercial fields, due to (1) the greater immediate benefit to growers in avoidance of infected propagation stock, and (2) concern over other possible diseases in commercial fields, such as bacterial scorch. Results are presented here and as part of another 2008 SRSFC report (See project 2008-04 *Blueberry red ringspot virus: prevalence in Georgia and North Carolina and yield losses associated with the disease* Scherm, Brannen, Cline). Survey data in North Carolina was collected statewide during routine farm visits from August to October 2008. Most observations were made in the main commercial production area of southeastern NC (Bladen and surrounding counties). We concentrated on younger fields of southern highbush blueberry where problems have been observed; however older fields/cultivars were also included. Plants were surveyed visually with emphasis on observing symptoms of *Blueberry red ringspot virus* (BRRV), but also Blueberry bacterial leaf scorch (caused by the bacterium *Xylella fastidiosa*), and 'Funky spot', a viral-appearing blueberry necrotic ringspot disease of unknown cause. Visual evaluations of percent disease in each of four random quadrants was recorded at each site for each cultivar, on 13 commercial farms ranging from 15 to 400 acres in size for a total of 38 location × cultivar observations. Bacterial leaf scorch symptoms were not observed at any site, and those plants tested for *Xylella* were negative. BRRV was present in 8 of 38 cultivar × site observations, but was not widespread; only 3 of 13 farms were affected. Incidence in infected areas ranged from <1% to 100%. Affected cultivars included Legacy, Blue Ridge, Biloxi, Jubilee, Star

and Duke. Previous work has confirmed the virus in cv O'Neal, but the virus was not observed in O'Neal in this survey. "Funky spot" was present in 6 of 38 location × cultivar observations, but again only on 3 of 13 farms. These were 3 totally different farms from the 3 with BRRV. Incidence of Funky spot ranged from 25 to 100%, but most commonly all plants in a given monoculture field were infected. Funky spot was strongly correlated to certain monoculture fields, while adjacent fields of different cultivars (on the same farm) had no symptoms. In this survey, Funky spot was limited to 'Star' and 'O'Neal' in NC. Older highbush cultivars, and rabbiteye blueberries, when surveyed, were not symptomatic. Rabbiteye blueberries were not specifically targeted in the survey, but were examined on several occasions throughout the year. These diseases (BRRV, Bacterial leaf scorch, Funky spot) were not observed on rabbiteye blueberries at any location.

Impact Statement:

This project has narrowed the range of potential vectors of BRRV in blueberry and will further do so as trapping continues and PCR analysis of samples commences. We have also identified other potentially interesting insect species, particularly potential vectors of other diseases, present in southeastern blueberry plantings. Our surveys have begun to reveal the incidence and severity of BRRV and other emerging diseases in NC.

Conclusions:

Insect trapping has developed new information on potential vectors, but conclusions cannot yet be drawn. The finding of previously unreported insects, and their potential as vectors, is significant. Our survey to determine the incidence and severity of three emerging blueberry diseases in NC seem to indicate plant-borne infections that are transmitted vegetatively, perhaps via a few, but not all, sources of cuttings (nurseries or growers), for certain newer southern highbush cultivars. Older highbush cultivars and rabbiteye blueberries, where surveyed, were not symptomatic.

Citations:

Cline, W. O. 2008. Scouting your fields for Blueberry Red Ringspot Virus. www.smallfruits.org/Blueberries/pestinformation/2008/BRRVscoutingguide26feb08.pdf