Title of Project: Screening Blueberry Seedling Progenies for Pollen Transmission of Blueberry Latent Virus

Progress Report

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Research Proposal

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Objective: To screen blueberry F_1 seedling progenies involving female parents testing negative for Blueberry Latent Virus and male parents testing positive for Blueberry Latent Virus for pollen transmission of the virus.

Justification:

In traditional blueberry production areas of the southeastern US virus diseases have been presumed to have minimum impact until recently. The Southeast is also one of the regions where blueberry production has expanded very rapidly in recent years and virus diseases are now a significant problem in at least parts of the region (Martin, et al. 2011). With the rapid expansion of production in traditional as well as new areas, blueberry plants are being exposed to additional viruses. Symptoms often vary

between cultivars and regions, some viruses may remain latent for years, and diagnosis and detection procedures are often unreliable (Martin, et al. 2012). Complicating the spread of viruses is also the fact that the rapid increase in blueberry plantings has resulted in shortages of planting stocks, leading growers to propagate from field plants that often may harbor asymptomatic virus infections.

Three viruses, blueberry red ringspot virus (BRRV) (Cline, et al. 2009; Polashock, et al. 2009), blueberry necrotic ring blotch virus (BNRBV) (Robinson, et al. 2012) and blueberry latent virus (BBLV) (Martin, et al. 2011) have been identified from cultivated blueberry plantings in the southeastern US in recent years. A fourth, tobacco ringspot virus (TRSV), has the potential to become an emerging problem on blueberry in the region (Martin et al. 2012), especially when plantings are extended to heavier soils where the numerous vectors for TRSV are more abundant.

Blueberry latent virus is one of the most recent viruses to be identified from blueberry (Martin, et al. 2011). As the name implies, BBLV has not yet been shown to cause any obvious symptoms in single infections, however its role, if any, in mixed infections is yet to be determined. BBLV has been shown to be prevalent in all major blueberry production regions in the US. It was found in more than 50% of the samples tested, and is reported likely to be present wherever North American blueberry germplasm is grown (Martin, et al. 2011). Movement of the virus has been suggested to be limited to seed and/or pollen. Transmission studies from three separate crosses involving male and female parents showed that BBLV is very efficiently transmitted by seed. No studies have yet been reported regarding pollen transmission of BBLV. However, when thin sections of BBLV-infected and BBLV-free tissue were observed under scanning transmission electron microscopy, no virions or abnormal structures were observed in pollen or leaf tissue. Therefore the question of pollen transmission of BBLV still remains.

Methodologies:

This proposal was developed to specifically address pollen transmission of BBLV using a series of crosses outlined in Table 1. Four crosses involved crossing BBLV-negative female parents with BBLV-positive male parents, a fifth involved two BBLV-negative parents (BBLV-negative control) and a sixth involved BBLV-positive parents (BBLV-positive control). Crosses were made in a greenhouse at Raleigh, NC, and the seeds were germinated and transplanted into containers in 2012. Recently developed full size leaves were used for testing as recommended by Martin, et al. (2011). The BBLV status of each parent and seedling progeny was determined using the RT-PCR detection protocol of Martin, et al. (2011). Internal control primers for the dehydrogenase ND-2 subunit (NADH) (Tzanetakis, et al. 2007b) were used to verify the reverse transcriptase assay. PCR testing for BBLV was done using primers BBLVdetF and BBLVmidR (Martin, et al. 2011) and One Taq Quick Load 2X Master mix (New England Biolabs). The cDNA used for the positive control was isolated in 2012 from two plants of the cultivar New Hanover (New Hanover A and B). The RT-PCR BBLV sequences from 'New Hanover' were sent for sequencing in November 2013 and it was confirmed to be correct for this virus. A 1% agarose (.5%TBE) gel was run to separate fragments.

In addition to the PIs, other individuals involved in this project included laboratory research specialist Carol George who performed the RT-PCR analyses and undergraduate student assistant Wendy Buchanan who assisted with RT-PCR analyses and who was also responsible for care of the plants.

Results:

The initial testing for pollen transmission of BBLV virus began with Group A (BBLV positive control). The results from this group of 100 plants is summarized in Table 2. Table 2 includes not only the percent transmission of BBLV but also the maximum and minimum temperatures for each testing date. The testing started on May 5, 2014, with leaves harvested from 17 plants and 7 of the plants produced an amplicon of the correct size and the bands were fairly bright. The maximum and minimum temperatures for May 5 were 75/55^{o}F. This first set of plants showed a 41.2% transmission rate for BBLV. A second set of 24 plants from Group A was tested on May 21, 2014. Only 4 of the 24 plants produced an amplicon of the correct size, but these bands were variable, indicating the overall virus titer for this set of plants was low. One plant did show a bright positive amplicon of the correct size. The maximum and minimum temperatures for this testing date were 87/60^{o}F. The BBLV transmission rate for this second group was 16.6%. A third set of 11 plants from Group A were tested on May 28 and none were found positive for BBLV. The maximum and minimum temperatures for this testing date were 90/64^{o}F. Testing continued through June 26 with Group A. The only date in June resulting in amplicons of the correct size was June 16 with 13% transmission of BBLV, but extremely light bands. The maximum temperatures for June ranged from 89 to 95^{o}F. The total number of seedlings in Group A found to be positive for BBLV was 14, a 14% ratio. This ratio was considerably lower than the expected ratio of near 100% from two parents testing positive for BBLV.

Groups B and C were tested for BBLV during July, 2014. Both these include BBLV-negative female parents and BBLV-positive male parents. Group B included 30 plants and Group C 25 plants. None of the seedlings in Groups B and C tested positive for BBLV, which is possibly accurate if BBLV is not pollen transmitted. The maximum temperatures during the testing periods in July for Groups B and C ranged from 84 to 98^{o}F. Groups E and F remain to be tested for BBLV.

Conclusions:

Many plant viruses are temperature sensitive for viral replication. There is no published information to indicate whether this is the case with BBLV. However in this study it was observed that as the average daytime temperature increased the percent of plants testing positive for BBLV was reduced, and rapidly reached zero, or near zero. The highest percent seedlings testing positive in the positive control cross (Group A) was when the maximum temperature was 73^{o}F. However, even the 41.2% positives at 73^{o}F was far below the near 100% expected from crossing two parents testing positive for BBLV. Therefore, it appears that maximum temperatures under 70^{o}F may be needed for optimum replication of BBLV virions. The role of high night time temperatures in BBLV virus replication is also yet to be determined. As a result virus testing was halted until cooler temperatures predominate in fall and winter. We are presently starting testing again, including retesting all the plants tested earlier in 2014. Results from the most recent tests are not available yet.

Impact Statement:

This is a progress report and the role of temperature in regulating BBLV viral replication needs to be determined more clearly before the impact of this study can be assessed.

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Table 1. Crosses utilized to determine pollen transmission of blueberry latent virus.

Group	Female parent	BBLV status	Male parent	BBLV status
A	NC 2930	positive	New Hanover	positive
B	NC 4301	negative	NC 3104	"
C	NC 5141	"	" "	"
D	NC 4957	"	New Hanover	"
E	NC 5142	"	" "	"
F	NC 5141	"	NC 4957	negative

Table 2. Results from testing Group A for transmission of blueberry latent virus[1].

Testing date	No. of seedlings tested	No. positives	% positives	Max/Min temp.	Notes
5/5/2014	17	7	41.2	73/55°F	strong bands
5/21/2014	24	4	16.6	87/60°F	strong & weak mix
5/28/2014	11	0	0.0	90/64°F	
6/3/2014	7	0	0.0	89/57°F	
6/16/2014	22	3	13.0	95/69°F	extremely light bands
6/24/2014	17	0	0.0	86/65°F	
6/25/2014	2	0	0.0	93/73°F	
Total	100	14	Avg. 14.0		

[1]Group A is a cross of two BBLV-positive parents where virus transmission would be expected to be near 100%.