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

Final 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.

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 remained.

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 and 2013. The screenings were conducted in 2014 (SRSFC project # 2014-11) and 2015. 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 and Martin, 2008) were used to verify the reverse transcriptase assay. PCR testing for BBLV was done using primers BBLVdetF and BBLVmidR (Martin, et al. 2011) in 2014 and early 2015, and One Taq Quick Load 2X Master mix (New England Biolabs). The majority of the screening in 2015 used primers BBLVF5 (TCCCCCTCGAGCTCAGGAAGTT) and BBLVR5 (ATGATCTTCCTCACGCCGGC) produced by Carol George. The cDNA used for the positive control was isolated in 2012 from two plants of 'New Hanover' ('New Hanover' A and B). The RT-PCR BBLV sequences from 'New Hanover' were sent out for sequencing in November, 2013, and it was confirmed to be correct for this virus. A 1% agarose (0.5% TBE) gel was run to separate fragments.

Laboratory research specialist Carol George performed the RT-PCR analyses and undergraduate student Wendy Buchanan assisted with making crosses, RT-PCR analyses and was also responsible for care of the plants.

Results:

The initial testing for pollen transmission of BBLV virus began with Groups A through C in 2014 and these results are summarized in the 2014 Progress Report (SRSFC Project # 2014-11).Since the percent positive seedlings for BBLV appeared to decrease with temperature during 2014, we erroneously proposed that BBLV might be a temperature sensitive virus. Initial results in 2015 followed the 2014 results fairly closely but percent positive appeared to be low even under modest temperatures, so alternative possibilities were explored. The problem was resolved when Carol George produced a new set of primers (BBLVF5 and BBLVR5). The parent plants used in making the crosses were screened again in 2015 using the new primers and the result was identical to 2014 findings (see Table 1).The results from using these primers in 2015 to screen the six progenies is summarized in Table 2. The positive control cross (group A) resulted in 94% seedlings positive for blueberry latent virus, which is near the 100% positive reaction expected. The negative female x positive male crosses (groups B-E) had only modest numbers of seedlings in each progeny, but at least 60% of the seedlings in each cross were determined to be positive and two of these progenies had 80% or more positives. Therefore, it appears clear that blueberry latent virus can be pollen transmitted. With this group of parents it appears that the virus infected the seed and the seedling that developed from it, but did not infect the female parent plant on which the seed developed. This is known as vertical virus transmission (Card et al., 2007). The negative control cross (group F) also had only a modest number of seedlings with none determined to be positive.

Conclusions:

Blueberry latent virus is not temperature sensitive as was proposed in 2014 for this project. All the problems with producing consistent reliable screening results for the virus were resolved by producing and utilizing a new set of primers. The 2015 results demonstrated that blueberry latent virus can be transmitted by pollen as well as seeds. The virus was also determined to be vertically transmitted in these progenies.

Impact Statement:

Although no negative role(s) have been determined for blueberry latent virus infection it is important to be aware that this virus can be both pollen and seed transmitted in case a future problem associated with this virus is identified. It is also significant to know that it is vertically transmitted, since a virus-negative female parent will not be infected if it is pollinated by pollen from an infected plant.

References Cited:

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Table 1. Crosses utilized to determine pollen transmission of blueberry latent virus and reaction of parents to screening for the virus in 2014 and 2015[1].

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

[1]Reaction of parents was identical in both 2014 and 2015.

Table 2. Results from screening six blueberry progenies for pollen transmission of blueberry latent virus.

Group	Cross[1]	Number Tested	Number Positive	Percent Positive
A	NC 2930 (+) x New Hanover (+)	96	90	94
B	NC 4301 (-) x NC 3104 (+)	30	18	60
C	NC 5141 (-) x NC 3104 (+)	25	20	80
D	NC 4957 (-) x New Hanover (+)	14	9	64
E	NC 5142 (-) x New Hanover (+)	24	20	83
F	NC 5141 (-) x NC 4957 (-)	18	0	0

[1]A (+) following a selection number means positive for blueberry latent virus, while a (-) means negative for blueberry latent virus.