Effects of *Blueberry red ringspot virus* on Yield and Fruit Maturation in Southern Highbush Blueberry

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Abstract

Williford, L. A., Savelle, A. T., and Scherm, H. 2016. Effects of *Blueberry red ringspot virus* on yield and fruit maturation in southern highbush blueberry. *Plant Dis.* 100:171-174.

Blueberry red ringspot virus (BRRV) has become prevalent in southern highbush blueberry in the southeastern United States but information about the yield effects associated with the disease is limited and conflicting. A 3-year study was conducted on mature, container-grown plants of 'Star' and 'Jewel' blueberry that were either systemically infected or not infected with BRRV to determine the effect of the disease on flower bud numbers and fruit yield and on advances or delays in fruit ripening. On Star, flower bud counts were lower for BRRV-positive plants ($P = 0.0137$ in one year and $P = 0.1085$ in another) but no such effect was observed for Jewel. When fruit were harvested over time during the

ripening period in the spring, no consistent yield or berry weight reductions were observed due to BRRV infection for either cultivar. On Star, fruit maturity tended to be slightly advanced in BRRV-positive plants in all years. Specifically, the weight of unripe fruit remaining after the last harvest was consistently lower for BRRV-positive plants than for BRRV-negative plants, suggesting that BRRV infection in Star may lead to a shorter fruit ripening period. No such effect on fruit ripening was observed for Jewel. It is concluded that—for the cultivars examined in this study—BRRV causes a relatively benign infection with no negative yield implications.

With a farm gate value of over \$300 million in 2013 (Anonymous 2015), blueberries have become the most economically important fruit crop in Georgia. More than 6,000 ha of land in the state is now in blueberry production (Anonymous 2013), encompassing both rabbiteye (*Vaccinium virgatum* Aiton) and southern highbush (*V. corymbosum* L. interspecific hybrids) cultivars. Although southern highbush blueberry plants occupy only a small proportion of the acreage, their economic impact is substantial because fruit ripen during a high-price market window early in the season (late April to mid-May), when fresh blueberries are in short supply (Fonsah et al. 2006; Scherm and Krewer 2003). Based on their greater economic value, commercial interest in southern highbush blueberry production continues to increase in Georgia and neighboring states.

Southern highbush blueberry plants, in general, are more susceptible to pests and diseases than the rabbiteye blueberry cultivars traditionally grown in the southeastern United States (Scherm and Krewer 2003). This applies to blueberry viruses as well. Historically, blueberry viruses have been recognized as a common problem in the northern United States, with the Southeast considered essentially virus free. Indeed, a virus survey in 2001 based on enzyme-linked immunosorbent assay testing of suspect southern highbush and rabbiteye plant samples having virus-like symptoms revealed no positive samples in Georgia or North Carolina (Scherm et al. 2008). Since then, however, two viruses have become relatively widespread in Georgia and neighboring states: *Blueberry red ringspot virus* (BRRV) and *Blueberry necrotic ringblotch virus* (Cline et al. 2009; Robinson et al. 2012; Scherm et al. 2008). For BRRV, in particular, a more recent survey in 2008 showed that the virus was present in eight of nine counties surveyed in southern Georgia, on 42.2% (19 of 45) of farms and in 14.9% (25 of 167) of fields sampled, as confirmed by BRRV-specific polymerase chain reaction (PCR) assay (Polashock et al. 2009). This survey further revealed that southern highbush blueberry 'Star', 'Millennia', and 'Emerald' had the highest BRRV prevalence, whereas the disease was not observed in 'Rebel' and 'Windsor'

(Scherm et al. 2008). To date, in Georgia, BRRV has only been observed in southern highbush blueberry plants but not in the more widely grown rabbiteye cultivars. In other states, BRRV has been reported from rabbiteye blueberry, although only sporadically (Polashock et al. 2009; Ramsdell 1995).

Typical symptoms of BRRV include red rings on stems and leaves that measure 2 to 3 mm in diameter and can coalesce into blotches over time. Occasionally, red rings will appear on the unripe green fruit but such symptoms generally are not evident on the ripe fruit. Symptoms are variable on softwood and less distinguishable on hardwood, which increases the potential of spreading the pathogen when infected cuttings are used for vegetative propagation. Vegetative propagation is currently considered the only means of BRRV transmission, with no other vector confirmed (Cline et al. 2009). Nevertheless, the spread of BRRV has continued, with reports in several of the United States' largest blueberry-producing states (Polashock et al. 2009), as well as in Europe (Kalinowska et al. 2011; Pleško et al. 2010; Přibylková et al. 2010) and Asia (Cho et al. 2012; Isogai et al. 2009).

BRRV is thought to have limited impact on plant growth, and many infected blueberry plantings appear to produce a full crop (Martin et al. 2012). However, this conclusion is based largely on anecdotal observations, with few studies having been made to quantify the effect of BRRV on berry yield. One study in Michigan reported a 25% crop loss in BRRV-infected northern highbush blueberry 'Blueray' (Gillett 1988) but this was based on a single year of observation. A preliminary 1-year study on yield losses associated with BRRV in southern highbush blueberry in Georgia obtained data on both individual, tagged shoots as well as on whole plants, with different levels of BRRV symptom intensity in the field (Scherm et al. 2008). In the whole-plant study, total fruit yields on bushes of Star with or without the virus (confirmed by BRRV-specific PCR) were not significantly different. However, the infected plants had a significantly higher fruit yield during the first of two harvests than bushes that were uninfected and asymptomatic, leading to the tentative conclusion that BRRV may advance fruit ripening slightly (Scherm et al. 2008). In the single-shoot experiment, yield variables (flower clusters per shoot, number of berries per shoot, and fruit yield per shoot) were reduced on severely diseased shoots compared with asymptomatic shoots. However, the reduction was statistically significant only in the case of berry number. Data were based on a single fruit harvest in a single year, and no yield data were available over time. As such,

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Accepted for publication 15 June 2015.

quantitative information about the effects of BRRV on yield and fruit ripening in blueberry is still limited and conflicting. Hence, the overall objective of the present study was to generate more accurate and precise data on the yield implications of BRRV in southern highbush blueberry. Specifically, we conducted multiyear studies in semicontrolled conditions (on container-grown plants outdoors) with frequent berry harvests to document not only variations in fruit yield but also advances or delays in fruit ripening.

Materials and Methods

Plant material and maintenance. The study was carried out from 2012 to 2014 with container-grown plants of southern highbush blueberry Star and 'Jewel' maintained in 11-liter pots outdoors at the University of Georgia Plant Pathology greenhouse complex. The plants had been established in 2009 from softwood cuttings taken from mother plants that were either positive or negative for BRRV. The BRRV status of the test plants was confirmed in June 2013 by PCR from leaf samples, as described by Polashock et al. (2009), using primers RRSV3 and RRSV4. All positive plants also showed foliar symptoms of the disease during those times of the year when symptoms are usually apparent. For Star, 25 BRRV-positive plants and 22 BRRV-negative plants were used across the 3 years. For Jewel, the corresponding numbers were 18 and 23, respectively. Each plant was considered a replicate, and replicates were arranged randomly within each cultivar.

Plant maintenance and management including watering and fertilization followed standard practices, with weed control performed manually as needed. Plants were maintained outdoors throughout the year, except in the spring of 2014, when they were moved temporarily into a greenhouse during bloom and early fruit development

for freeze protection. After harvest each year, summer pruning was conducted such that plants had similar canopy volumes across cultivars and BRRV status.

Data collection and analysis. Flower bud counts on each plant were made in 2013 and 2014 in late winter (January or February). Fruit yield was obtained by hand-harvesting mature, blue fruit once or twice a week beginning in April (2012), May (2013), or March (2014) for a total of six to seven harvests per season. The onset of fruit maturity across the 3 years varied based on weather conditions during the preceding winter and early spring. The final harvest (which occurred in May or June each year and was determined based on having harvested at least 90% of the blue fruit per plant) included the remaining unripe (green and red) fruit, weighed and counted separately from the ripe blue fruit. Total fruit weight and number were determined for each harvest date, and a cumulative total and an average berry weight was calculated. All data were analyzed separately by cultivar using one-way analysis of variance for a completely

Fig. 1. Flower bud number (determined in January or February of 2013 and 2014) on container-grown **A**, 'Star' and **B**, 'Jewel' southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Values are means and standard errors of 18 to 25 plants (replicates). *P* values from one-way analysis of variance.

Fig. 2. Total fruit yield, determined in six or seven successive harvests, on container-grown **A**, 'Star' and **B**, 'Jewel' southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Values are means of 18 to 25 plants (replicates). Unripe yield corresponds to fruit that were still immature on the final harvest date. No yield was obtained for Jewel in 2013 due to freeze damage during bloom and early fruit set. Corresponding *P* values from one-way analysis of variance comparing BRRV(+) and BRRV(-) plants are shown below each graph.

randomized design (PROC GLM in SAS v. 9.3; SAS Institute, Cary, NC).

Results

Effects of BRRV on flower bud number, yield, and fruit ripening in Star. In the 2 years where flower bud number was determined, bud counts on Star were statistically lower in 2013 ($P = 0.0137$) and numerically lower in 2014 ($P = 0.1085$) for BRRV-positive plants than for those negative for the disease (Fig. 1A). Total fruit yield was also lower for BRRV-positive plants in 2 of the 3 years (Fig. 2A) but this effect was not statistically significant. Interestingly, the weight of unripe berries remaining after the last harvest was significantly lower for BRRV-positive plants than for BRRV-negative plants (Fig. 2A), suggesting that BRRV infection in Star may lead to a shorter fruit ripening period. Average berry weight was unaffected

(data not shown), except in 2012, where it was significantly higher on BRRV-positive plants ($P = 0.0209$).

To better compare fruit maturity progression over time between BRRV-positive and -negative plants, cumulative yield was expressed on a relative scale, where total yield was set to 100% for each treatment (Fig. 3). When this was done for Star, there was a tendency for fruit on BRRV-positive plants to mature slightly earlier than that on the BRRV-negative plants. For example, the first and second harvests combined in 2014 included 35.0% of the total ripe fruit weight on BRRV-positive plants, compared with only 22.4% on BRRV-negative plants at the same dates.

Effects of BRRV on flower bud number, yield, and fruit ripening in Jewel. There was no significant difference in flower bud numbers between BRRV-positive and BRRV-negative plants of Jewel in either year (Fig. 1B). Fruit yield data for Jewel were obtained only in 2012 and 2014 because a late freeze occurred in March 2013, killing most flowers and developing fruit. Total yield was significantly higher for the BRRV-positive plants than for BRRV-negative plants in 2012 (Fig. 2B), whereas no significant differences were observed in 2014. There was no significant effect on berry weight for Jewel in either year ($P > 0.44$).

When fruit yield was examined over time (Fig. 4), there was a tendency for fruit on BRRV-positive Jewel plants to mature more slowly than on BRRV-negative plants. For example, the first and second harvests combined in 2014 included 7.0% of the total ripe fruit weight on BRRV-positive plants, compared with 14.2% on BRRV-negative plants at the same dates.

Fig. 3. Cumulative fruit yield over time, determined in six or seven successive harvests in **A**, 2012; **B**, 2013; and **C**, 2014 on container-grown 'Star' southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Total yield was set to 100% to allow for better comparison of positive and negative plants. Values are means of 22 to 25 plants (replicates). The last data point includes berries that were still immature on the final harvest date.

Fig. 4. Cumulative fruit yield over time, determined in six or seven successive harvests in **A**, 2012 and **B**, 2014 on container-grown 'Jewel' southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Total yield was set to 100% to allow for better comparison of positive and negative plants. Values are means of 18 to 23 plants (replicates). The last data point includes fruit that were still immature on the final harvest date.

Discussion

Results of this study showed no significant fruit yield reductions associated with BRRV across five cultivar-year combinations. The study was conducted in more controlled conditions than previous trials examining the yield effects of BRRV, with a greater number of replications and more frequent harvests to capture effects of the disease on yield and fruit ripening. Our results support anecdotal reports of limited yield effects of BRRV (Martin et al. 2009) but conflict with a study from Michigan reporting a 25% yield loss in BRRV-infected northern highbush blueberry Bluejay (Gillett 1988). The latter study was not repeated; therefore, it is unclear whether the significant yield loss was unique to Bluejay or to the year when the investigation was carried out. The only previous experimental study on BRRV-associated yield effects in southern highbush blueberry (Scherin et al. 2008), also conducted in a single year only, found no significant effect of BRRV status (positive or negative) on whole-plant yield of field-grown Star plants. Combined with our data, it can be concluded that BRRV does not cause significant yield losses in southern highbush blueberry on the cultivars examined to date.

Although most of the comparisons of yield variables between BRRV-positive and BRRV-negative plants were statistically not significant (Fig. 2), a few general trends emerged from the data. For example, flower bud counts on Star were consistently lower for BRRV-positive than for BRRV-negative plants, significant in one year at $P = 0.05$ and in the second year at $P = 0.10$ (Fig. 1A). However, greater flower bud numbers on uninfected plants did not translate into higher fruit yields, possibly due to compensatory effects whereby not all flower buds on a given shoot produce flowers, or there are fewer florets produced per flower bud on shoots with more buds. Indeed, it has been documented previously that yield component relationships in *V. corymbosum* are complex, and that flower buds per cane explain only about one-third of the annual variation in fruit yield, with other significant components being cane number per plant and flowers per bud (Pritts and Hancock 1985). Therefore, the effects of BRRV infection on flower phenology, not assessed in this study, would be worthy of further research. Our study did show that there was little impact of the disease on average berry weight, which was statistically significant in only one of five cultivar-year combinations.

Another general pattern observed in this study was a trend for slightly earlier (or less protracted) fruit maturity in Star but not Jewel plants affected by BRRV. This was evident not only from the fruit ripening curves in Figure 3 but also from the yield of the remaining unripe fruit at the last harvest date, which was significantly lower in BRRV-positive than BRRV-negative plants in 2 years at $P = 0.05$ and in 1 year at $P = 0.10$ (Fig. 2). This is consistent with a previous 1-year study by Scherin et al. (2008), which showed a significantly higher yield in the first harvest for BRRV-infected Star plants compared with their asymptomatic counterparts, although total yields (across two harvests) were not significantly different. Understanding the economic relevance of this slight advance in berry maturation and its underlying physiological mechanisms requires further study.

Given the relatively widespread distribution of BRRV in southern highbush blueberry in Georgia (Scherin et al. 2008), it is fortunate that there are no consistent yield losses associated with the disease on the cultivars examined to date. Based on currently available information, BRRV is transmitted only by vegetative propagation (Ramsdell

1995); hence, no further spread is expected in established plantings. As such, no additional practices to manage the disease in the field, such as by roguing, are warranted at this time. It is likely that BRRV was introduced and spread during the 1990s and early 2000s when the blueberry industry in Georgia underwent a rapid acreage expansion, prompting many growers to produce their own nursery stock with relatively limited quality control. Because blueberry acreage expansion is now slowing down in the state, it is important to improve propagation practices to prevent further spread of BRRV as well as other systemic blueberry pathogens that may not be as benign in terms of their yield implications.

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