Title of Project: Effects of co-infection with *Blueberry red ringspot virus* and Phytophthora root rot on symptom severity, plant vigor, and yield in southern highbush blueberry

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Principal Investigator
Harald Scherm
Professor & Assistant Dean for Research
Department of Plant Pathology
120 Carlton St.
Athens, GA 30602
scherm@uga.edu

Collaborator
Laura Williford
Graduate Research Assistant
Department of Plant Pathology
120 Carlton St.
Athens, GA 30602
law89@uga.edu

Objectives

Determine the effects of simultaneous infection with *Blueberry red ringspot virus* and Phytophthora root rot on symptom severity, plant vigor, and yield in southern highbush blueberry in greenhouse and field experiments, compared with plants infected with one of the two diseases alone.

Justification

Blueberry red ringspot disease, caused by *Blueberry red ringspot virus* (BRRV), and Phytophthora root rot (PRR), caused by the oomycete *Phytopthora cinnamomi*, occur commonly in blueberry plantings in Georgia and neighboring states (Scherm *et al*. 2001, 2008). However, the two diseases differ in many aspects, such as in-field distribution (BRRV often occurs uniformly throughout the field due to spread by vegetative propagation, whereas PRR occurs localized in poorly drained areas) or severity of plant damage (BRRV is thought to have minimal impacts on plant vigor and yield [Gillett 1988], whereas PRR can kill plants). Apart from ensuring clean nursery stock, there are currently no in-field management practices applied against BRRV. In contrast, multiple management tactics are applied against PRR, including high bedding, use of less susceptible cultivars, and fungicide applications (Milholland 1995).

Recently, growers and county extension agents reported substantial exacerbation of BRRV symptoms in areas where PRR is also present (e.g., Fig. 1), suggesting that damage due to the two diseases occurring in combination is synergistic in terms of their negative impact on plant growth and productivity. If this hypothesis is correct, there is an increased urgency toward eliminating BRRV from southeastern blueberry plantings, e.g., by roguing. The quantitative data generated in this study will provide the answer to this important question.

Methodologies

Greenhouse experiments

Softwood cuttings were obtained from confirmed BRRV-infected and unaffected 'Star' and 'Jewel' southern highbush blueberry plants in late spring and early fall of 2012 (two separate experiments). The cuttings were rooted under mist for ~10 weeks. Rooted cuttings of uniform growth were subsequently transplanted into 20-cm clay pots containing a 2:1 peat:sand (v:v) mix and grown for 5 months at 18 to 27^{o}C with 14 h of light per day. Leaf disk samples were taken to confirm BRRV presence or absence in these plants by PCR (Polashock *et al*. 2009) prior to inoculation with *P. cinnamomi*. For each cultivar, the experimental design was a split-plot with two levels of PRR (inoculated or non-inoculated) in the main-plot crossed with two levels of BRRV (presence or absence) in the sub-plot. There were ten plants (replicates) for each treatment combination.

Phytophthora cinnamoni (mixture of isolates BBRY-1 and BBRY-2 obtained from Dr. Steven Jeffers, Clemson Univ.) was grown in a sterile V8-vermiculite (1:2 v:v) medium for 2 weeks. Flasks containing the medium were shaken every 2 to 3 days to ensure uniform colonization. Twenty-five milliliters of colonized medium were applied onto the soil surface of PRR-inoculated plants, whereas non-inoculated plants received an equal volume of V8-vermiculite medium that had not been inoculated with *P. cinnamomi*. The inoculum was lightly watered into the soil after covering it with 50 ml of a 2:1 peat:sand mixture. One week after PRR inoculation and subsequently every 2 weeks, plants were subjected to flooding by placing each pot individually into a 12-liter bucket and submerging it for 48 h so that ~1 cm of water stood above the soil line. The buckets were sanitized with bleach between flooding events.

Foliar disease progression was assessed periodically as the cumulative number of dried and defoliated leaves per plant (Fig. 2). At the end of the experiment (4 and 1.5 months after inoculation with *P. cinnamomi* in trials 1 and 2, respectively), shoot and root fresh weight was determined for each plant. In addition, root symptom severity was assessed using a contrast rating (faint, distinct, or prominent) relative to the BRRV-negative, PRR non-inoculated control based on color and hue using *Munsell Soil Color Charts* (Anonymous 2000). *Phytophthora cinnamomi* was re-isolated from the roots of all inoculated replicates on PARP-H medium (Mitchell and Kannwischer-Mitchell 1992). Foliar disease severity, as well as shoot and root weight data, were analyzed with analysis of variance for a split-plot design (PROC GLIMMIX in SAS v.0.3; SAS Institute, Cary, NC).

Field experiments

Ware County trial – This experiment was initiated in the field depicted in Fig. 1. Plants at the end of rows of 'Star' that had died due to PRR were removed in October 2012, and replaced with 2-year-old 'Star' plants that were either confirmed infected with BRRV or unaffected by the virus. Along rows, replants were arranged in ten pairs of BRRV-positive and negative plants, allowing for direct comparison of the plants' performance in the presence of PRR. In spring of 2013, developing fruit were stripped from these plants to favor plant establishment and vegetative growth. Plants will be monitored over the next 2 years for symptom development (chlorosis, BRRV severity), plant growth (width × depth × height), mortality, flower bud set, and berry yield. At the end of the experiment, plants will be uprooted and *P. cinnamomi* isolations conducted and root weights determined. Data will be analyzed using paired t-tests.

Bacon County trial – This site consists of a 2288-plant block of mature Star plants in which BRRV and stunting (initially thought to be due to PRR) occur in a scattered pattern throughout the field. In September 2012, ten groups of four plants each were selected and marked, whereby each group contained one plant each of the following: 1) not stunted (height >115 cm), no BRRV symptoms; 2) not stunted, BRRV symptoms; 3) stunted (≤115 cm), no BRRV symptoms; and 4) stunted, BRRV symptoms. At the same time, bush size (width × depth × height) was recorded and leaf samples were collected for confirmation of BRRV by PCR. In February 2013, ten shoots formed in the previous year were tagged on each of the 40 plants, and all flower buds were counted on these shoots. Between 8 and 21 May 2013, three weekly harvests of mature fruit were conducted on the test plants, and separately on each of the previously tagged shoots on each plant. Flower bud numbers per shoot, as well as total yields per plant, were analyzed by two-way analysis of variance (PROC GLIMMIX) with BRRV and stunting (both recorded as presence or absence) as fixed effects and replication (block) as a random effect.

In May and October 2013, soil samples were obtained from the base of each of the 40 tagged plants to determine the cause(s) of stunting. A soil probe was used to collect six 15-cm cores per plant which were subsequently pooled and baited with *Camellia* leaf disks in covered plastic containers in the lab.

Leaf pieces were embedded into PARP-H agar at 1-, 2- and 3-day intervals and inspected for presence of *Phytophthora* spp.

Results

Greenhouse experiments

Symptoms of PRR following inoculation with *P. cinnamomi* developed much more quickly in trial 2 than in trial 1, presumably because the plants in trial 2 grew faster and were more tender than those in trial 1. As a consequence, fewer biweekly floodings were needed in trial 2 (three) than in trial 1 (seven), and foliar disease severity (number of dried and defoliated leaves) was considerably higher in trial 2 (Table 1). In each of the two trials, foliar disease severity was increased significantly by PRR inoculation, but not by presence of BRRV (Tables 1 and 2). There was no significant statistical interaction between presence of BRRV and PRR (Table 2), indicating that plants with or without BRRV reacted similarly to inoculation with *P. cinnamomi* with regard to foliar disease progression. Similar results were observed when plant fresh weight was analyzed at the end of the experiment: significant reduction due to PRR inoculation, no effect of BRRV presence (with the exception of 'Star' in trial 1, marginal significance for shoot and total fresh weight), and no significant interaction between the two diseases (Table 2).

When assessed using *Munsell Soil Color Charts*, root discoloration was always more pronounced in plants infected with BRRV or PRR than in the no-BRRV, no-*Phytophthora* control, although in most cases the contrasts were quantified as faint based on the chroma and hue figures (Table 3). Co-infection with BRRV and PRR resulted in a distinct difference in root coloration from the control in two of four cultivar-trial combinations.

Field Experiments

Ware County trial – In spring of 2013, flowers were stripped from these plants to favor plant establishment and vegetative growth. As such, no yield or disease data have been collected thus far. The first set of flower bud counts and yield data will be gathered in January and April of 2014, respectively. Plants will be monitored over the next 2 years for symptom development, plant growth, mortality, flower bud set, and berry yield. At the end of the experiment, plants will be uprooted and *P. cinnamomi* isolations conducted and root weights determined.

Bacon County trial – Baiting from soil samples collected in May and October 2013 did not yield any *Phytophthora* isolates, nor were there any significant differences in nematode numbers among the stunted plants compared with those that were not stunted (data not shown). Inspection of the root systems of stunted plants did not reveal any necrosis or discoloration compared with their non-stunted counterparts, nor was there any evidence of pathogen infection or arthropod infestation at the crowns. Although main roots and fine roots were well developed, the overall root volume was considerably smaller (Fig. 3), with substantially reduced lateral expansion evident in some cases. Furthermore, malformation in the crown area was observed occasionally (Fig. 3). We hypothesize that stunting in this planting was likely due to abiotic causes, such as soil compaction or herbicide injury to shallow roots or crowns.

Flower bud counts in February 2013 were highest (4.9/shoot) on BRRV-negative plants that were not stunted and lowest (2.8/shoot) on BRRV-positive, stunted plants. The effect of BRRV presence and stunting both were statistically significant ($P < 0.0001$), as was their interaction, albeit marginally ($P = 0.0464$). Thus, BRRV-infected plants were affected more strongly by stunting than uninfected plants with regard to flower bud set. Berry yields (Fig. 4) were highest in BRRV-negative plants that were not stunted, followed by those of BRRV-positive plants that were also not stunted. The effect of stunting on

yield was highly significant ($P < 0.0001$), whereas that of BRRV infection was only marginally so ($P = 0.0402$). In contrast to the flower bud data, there was no significant interaction between BRRV infection and presence or absence of stunting on yield ($P = 0.9899$). The relative proportion of berry yields at the three harvest dates, as well as that of the unripe berries remaining at the last harvest, were similar across the four treatments (Fig. 4), indicating that treatments did not affect the fruit maturation process.

This trial will be monitored for another year for chlorosis, BRRV severity, plant growth, flower bud set, and yield. In addition, the titer of BRRV in the test plants will be determined by real-time quantitative PCR to relate BRRV titer to visual disease severity in the presence or absence of stunting.

Conclusions

Although additional greenhouse and field experiments are being conducted, preliminary data obtained on two cultivars to date do not support the hypothesis of more than additive disease severity and berry yield loss when both BRRV and PRR are present on southern highbush blueberry. Thus, the two diseases appear to operate independently in affecting their host. Disease severity or yield reduction effects due to PRR (or abiotic stunting) were always greater than those associated with BRRV. Our results thus confirm previous observations of limited yield relevance of BRRV infections.

Literature Cited

Anonymous. 2000. Munsell Soil Color Charts, Revised Washable Edition. Munsell Color Co., Gretag Macbeth, New Windsor, NY.

Gillett, J. M. 1988. Physical and Chemical Properties of Blueberry Red Ringspot Virus. M.S. thesis, Michigan State Univ., East Lansing.

Milholland, R. D. 1995. Phytophthora root rot. Pages 7-8 in: Compendium of Blueberry and Cranberry Diseases. F. L. Caruso and D. C. Ramsdell, eds. American Phytopathological Society, St. Paul, MN.

Mitchell, D. J., and Kannwischer-Mitchell, M. E. 1992. Phytophthora. Pages 31-38 in: Methods for Research on Soilborne Pathogenic Fungi. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. American Phytopathological Society, St. Paul, MN.

Polashock, J. J., Ehlenfeldt, M. K., and Crouch, J. A. 2009. Molecular detection and discrimination of *Blueberry red ringspot virus* strains causing disease in cultivated blueberry and cranberry. Plant Dis. 93:727-733.

Scherm, H., Brannen, P. M., and Cline, W. O. 2008. *Blueberry red ringspot virus*: Prevalence in Georgia and North Carolina, and yield losses associated with the disease. Final report 2008-04, Southern Region Small Fruit Consortium, North Carolina State Univ., Raleigh.

Scherm, H., NeSmith, D. S., Horton, D. L., and Krewer, G. 2001. A survey of horticultural and pest management practices of the Georgia blueberry industry. Small Fruits Review 1(4):17-28.

Impact Statement

Summary

Field observations by blueberry growers and extension agents suggested exacerbated symptom severity and yield loss when both *Blueberry red ringspot virus* (BRRV) and Phytophthora root rot (PRR) are present simultaneously. Previously, losses due to BRRV have been considered minimal, hence it is critical to quantify the effects of co-infection between BRRV and PRR.

Situation

Blueberry red ringspot virus (BRRV) and Phytophthora root rot (PRR) occur commonly in blueberry plantings in Georgia and neighboring states. Whereas BRRV is thought to have minimal impacts on plant vigor and yield, PRR can kill affected plants. Apart from ensuring clean nursery stock, there are currently no management practices applied against BRRV, i.e., infected plants are not rogued. In contrast, multiple management tactics are applied against PRR, including high bedding, use of less susceptible cultivars, and fungicide applications.

Recently, blueberry growers and county extension agents reported substantial exacerbation of BRRV symptoms in areas where PRR is also present, suggesting that damage due to the two diseases occurring in combination is synergistic in terms of their negative impact on plant growth and productivity. If this hypothesis is correct, there is an increased urgency for eliminating BRRV from southeastern blueberry plantings.

Response

CAES scientists and graduate students are conducting a range of experiments to determine the effects of co-infection with BRRV and PRR on symptom severity, plant growth, and yield of southern highbush blueberry cultivars 'Star' and 'Jewel'. Studies are carried out with controlled inoculations on potted plants in the greenhouse, and with natural infection (confirmed by pathogen assays) on field-grown plants. Statistical analysis thus far has produced limited evidence for sig nificant interactions between BRRV and PRR infection relative to symptom severity and yield.

Impact

Based on experimental data obtained thus far, the two diseases appear to operate independently in affecting their plant host, with no convincing evidence of synergistic interactions. Disease severity or yield reduction effects due to PRR (or abiotic stunting) were always greater than those associated with BRRV. Our results thus confirm previous observations of limited yield relevance of BRRV infections. Hence, current recommendations for managing BRRV do not need to be changed in the presence of PRR, or vice versa.

Citation(s) for any publications arising from the project

None to date.

Tables

Table 1. Effects of co-infection with *Blueberry red ringspot virus* (BRRV) and *Phytophthora cinnamomi* (Phytophthora root rot, PRR) on final foliar disease severity in two greenhouse trials.

Treatment	Number of dried and defoliated leaves per plant	
	Trial 1	Trial 2
'Star'		
No BRRV, no PRR	2.7	0
No BRRV, PRR	18.9	137.2
BRRV, no PRR	2.9	0.3
BRRV, PRR	24.7	131.5
'Jewel'		
No BRRV, no PRR	10.9	1.2
No BRRV, PRR	41.6	179.3
BRRV, no PRR	11.5	0
BRRV, PRR	36.7	146.3

Values are means of 10 plants per treatment. Statistical analysis in Table 2.

Table 2. *P*-values of a mixed-model analysis of variance of the effects of infection with *Blueberry red ringspot virus* (BRRV) and *Phytophthora cinnamomi* (Phytophthora root rot, PRR) on foliar disease severity and plant fresh weight in two greenhouse trials.

Effect	Number of affected leaves	Shoot fresh weight	Root fresh weight	Total fresh weight
Trial 1 – 'Star'				
BRRV main effect	0.3557	**0.0436**	0.1773	**0.0447**
PRR main effect	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.3879	0.9162	0.1170	0.6209
Trial 1 – 'Jewel'				
BRRV main effect	0.7540	0.3181	0.4538	0.3129
PRR main effect	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.6885	0.1553	0.2985	0.3210
Trial 2 – 'Star'				
BRRV main effect	0.8743	0.6543	0.6586	0.6383
PRR main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.8604	0.9221	0.9058	0.9655
Trial 2 – 'Jewel'				
BRRV main effect	0.2400	0.4501	0.2762	0.3683
PRR main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.2733	0.0339	0.6389	0.0611

Table 3. Effects of co-infection with *Blueberry red ringspot virus* (BRRV) and *Phytophthora cinnamomi* on root discoloration, assed using Munsell color charts, in two greenhouse trials.

'Star'

	Virus	*Phytophthora*	Δ Chroma	Δ Value	Contrast
Trial 1	No BRRV	-	(4.9)	(5.6)	--
		+	≤ 2	2	**Distinct**
	BRRV	-	1	≤ 1	Faint
		+	≤ 2	2	**Distinct**
Trial 2	No BRRV	-	(5.1)	(3.8)	--
		+	1	≤ 1	Faint
	BRRV	-	1	≤ 1	Faint
		+	≤ 2	≤ 1	Faint

'Jewel'

	Virus	*Phytophthora*	Δ Chroma	Δ Value	Contrast
Trial 1	No BRRV	-	(5.1)	(5.3)	--
		+	≤ 2	≤ 1	Faint
	BRRV	-	1	≤1	Faint
		+	≤ 2	2	**Distinct**
Trial 2	No BRRV	-	(5.0)	(4.0)	--
		+	≤ 2	≤ 1	Faint
	BRRV	-	1	≤ 1	Faint
		+	1	≤ 1	Faint

Contrast calculated quantitatively based on value (lightness or darkness) and chroma (saturation or intensity) relative to the no-BRRV, no-*Phytophthora* control.

Figures

Fig. 1. Presumed exacerbation of symptoms in two BRRV-infected rows of 'Star' southern highbush blueberry (rows B and C) in the presence of Phytophthora root rot (foreground) compared with BRRV-unaffected 'FL 89-16' (rows A and D). This observation may suggest synergism between the two diseases. Image courtesy James Jacobs, UGA Coop. Extension.

Fig. 2. Greenhouse-grown 'Jewel' plants inoculated with *P. cinnamomi* (front) compared with non-inoculated plants (background) in trial 2. Image taken ~ 6 weeks after inoculation. Infected plants exhibit severe wilting and drying of leaves, typical of Phytophthora root rot.

Fig. 3. Root system of unaffected 'Star' southern highbush blueberry plants (left) and those of stunted plants (center and right) in the Bacon County field trial. Stunted plants had a smaller, often one-dimensional root system (center), and crown malformation was observed occasionally (right).

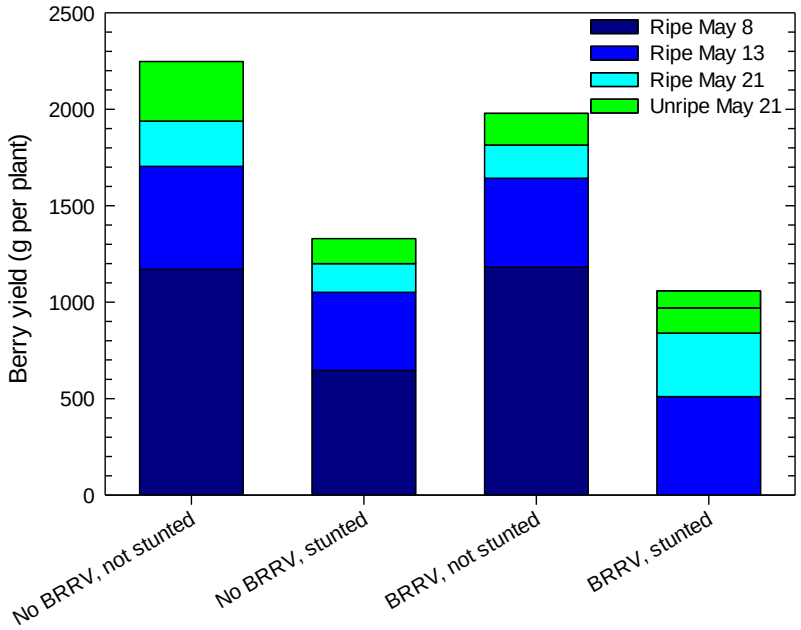

Fig. 4. Interactive effects of *Blueberry red ringspot virus* (BRRV) and abiotic stunting on berry yield of 'Star' southern highbush blueberry plants (*n* = 10) during three harvest periods in the Bacon County field trial in 2013.