Blueberry red ringspot virus: Prevalence in Georgia and North Carolina, and yield losses associated with the disease

Final Report (Research Proposal)

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Objectives

The overall aim of this project was to gather basic information on the distribution and damage potential of *Blueberry red ringspot virus* (BRRV), which has emerged as a new threat in Georgia and North Carolina. Specific objectives were to:

1) Conduct field surveys in Georgia and North Carolina production areas to establish the prevalence and geographical distribution of the disease.
2) Determine reductions in yield associated with the disease.

Justification

At least nine species of plant viruses can infect commercial blueberries in North America (Caruso and Ramsdell 1995). Among these, some (such as the scorch and shock viruses) are consistently very damaging, whereas most others are highly variable in the level of crop loss they cause, depending on the cultivar and the production environment. In the long run, however, they are all debilitating in production fields and devastating to propagation because virus-infected nursery stock, in general, can not be sold. In the past, it was assumed that blueberry viruses are mostly a problem of the northern half of the U.S., with production areas in the southeastern states being essentially virus-free. For example, although a few plants with *Blueberry shoestring virus* and *Strawberry latent ringspot virus* were identified in south Georgia in 2000, a subsequent virus survey in 2001 revealed no positives in a set of 55 suspect samples from Georgia and 38 suspect samples from North Carolina. These samples were tested against a battery of 16 plant viruses that might be found on blueberry by a commercial diagnostic lab (Agdia, Elkhart, IN) using ELISA; unfortunately, the Agdia ELISA did not include BRRV.

During the summer and fall of 2007, symptoms resembling infection by BRRV appeared in several commercial fields, propagation nurseries, and research plantings in Georgia and North Carolina. Surprisingly, disease incidence (percentage of affected bushes) and severity (percent affected leaf area) in some of the plantings were very high, especially given the fact that there was little evidence of symptoms in the same fields the previous year. In light of what appears to be a rapidly emerging disease in the region, the objectives of this project were to answer two key questions related to spread and potential impact of the disease: 1) How widespread is red ringspot disease in the commercial blueberry production areas of Georgia and North Carolina? And 2) how significant are the yield losses (if any) associated with the disease?

Methodologies

Objective 1: The BRRV survey in Georgia was coordinated with another regional field survey for blueberry bacterial leaf scorch (*Xylella fastidiosa*) conducted by P. M. Brannen *et al*. (see separate SRSFC progress report). It was carried out between late August and early October 2008 in Appling, Bacon, Brantley, Clinch, Pierce, and Ware counties. In each county, between 4 and 12 farms (sites) were surveyed, each of which consisted of multiple fields (cultivars). In addition, one farm each with multiple fields was scouted in Berrien and Colquitt counties. The entire Georgia survey encompassed a total of 9 counties, 45 farms, 167 fields, and 26 different southern highbush cultivars (Table 1). Plant age ranged from 1st through 12th leaf, with most of the survey focused on younger plantings (≤4th leaf).

In four quadrants within each field the BRRV incidence (percentage of symptomatic plants) was estimated visually using a four-point scale, namely BRRV absent, scattered, present at moderate incidence, or present at high incidence. Visual assessment was based on the presence of typical foliar ringspot symptoms (Fig. 1A) or faint foliar symptoms (mosaic) in conjunction with tell-tale ringspotting on the stem (Fig. 1B). Selected samples assessed as positive or negative in the field were confirmed with a BRRV-specific PCR assay (Glasheen et al. 2002).

In addition to symptoms typical of BRRV (Figs. 1 A and B), a pronounced necrotic ringspotting or blotching on leaves was encountered during the survey (Fig. 1C); similar symptoms had been noticed in previous years, albeit at very low levels. Affected leaves consistently tested negative for BRRV when subjected to the aforementioned PCR assay. In addition, isolation attempts for fungal pathogens gave inconsistent results. However, subsequent isolation of dsRNA by Dr. Robert Martin, USDA-ARS small fruits virologist in Corvallis, OR, strongly suggests presence of another (unknown?) virus(es). This new disease, which has not been fully characterized to date but is distinct from BRRV, will be referred to as “necrotic ringspot” henceforth. Incidence levels were assessed in the field with the same four-point scale as for BRRV, i.e., absent, scattered, moderate, and high.

In North Carolina, survey data was collected by W. O. Cline during routine farm visits from August to October 2008, with most observations in the main commercial production area in the southeastern part of the state (Bladen and surrounding counties) concentrating on younger fields of southern highbush blueberry where problems have been observed; however older fields were also included. Plants were surveyed visually with emphasis on BRRV, necrotic ringspot, and bacterial leaf scorch (*X. fastidiosa*). Visual assessment of disease incidence in each of four quadrants was made on 13 farms ranging from 15 to 400 acres in size for a total of 38 fields (location × cultivar combinations).

Objective 2: Yield loss experiments were conducted in two different BRRV-infected plantings of Star southern highbush blueberry in Appling County, GA. In the first experiment (*whole-plant study*), six plants with and six neighboring plants without symptoms of BRRV were tagged in the fall of 2007. These same plants were revisited during harvest the next spring to determine berry yield. All ripe berries were harvested during the first two hand-harvest dates (2 and 9 May 2008), and the weight of the removed berries as well as the number of berries per 100 g (an indication of berry size) were determined.

The second experiment (*single-shoot experiment*) was carried out in a planting where every plant was infected with BRRV, but symptom severity differed among bushes and among shoots within bushes. In late October 2007, 106 20-cm-long spring shoots were tagged permanently across the planting (one shoot per bush). At the same time, BRRV severity for each whole bush and for each tagged shoot was assessed separately using a healthy-light-medium-severe scale. Furthermore, disease severity for each leaf on each tagged shoot was assessed using the same scale. In late November 2007, the number of flower buds and the number of missing leaves (an indication of defoliation) was counted on each tagged shoot. These shoots were revisited in early March 2008 to determine BRRV severity on the stem (healthy, light = 1-2 rings per stem, medium = 3-4 rings, severe > 4 rings) and to count the number of flower clusters per shoot. Total berry yield per shoot and average berry weight were measured in late April when most fruit were still immature.

Results

Objective 1: In the Georgia survey, BRRV was present in 8 of the 9 counties surveyed, with Colquitt County (where only one farm was scouted) being the lone exception. The prevalence of BRRV at the farm level was 42.2%, i.e., 19 out of 45 farms had the disease in at least one of their fields (Table 1). At the field level, 25 of 167 fields (14.9%) had some BRRV. The two counties with the lowest BRRV prevalence were Clinch and Pierce, where only about 5% of the fields had the disease (Table 1). In most of the symptomatic fields, only few plants were affected, i.e., disease incidence was assessed as "scattered."

Necrotic ringspot disease was more prevalent than BRRV, with 55.6% of all farms and 28.7% of all fields showing symptoms of the disease (Table 1). Thus, field-level prevalence of necrotic ringspot in Georgia was almost twice as high as that of BRRV. In about half of all fields with necrotic ringspot symptoms, the number of infected plants was moderate or high. Thus, necrotic ringspot was not only more common, but also more severe than BRRV in the Georgia survey.

Among the cultivars surveyed in at least 5 fields each, Star, Millennia (but note small sample size, $n = 5$), and Emerald had the highest BRRV prevalence, whereas Rebel and Windsor were BRRV-free (Table 2). For necrotic ringspot, Star, O'Neal, and FL 86-19 (V1) had the highest prevalence (around half of all fields with at least some symptomatic plants), whereas Emerald, Millennia, Rebel, and Windsor were disease-free. Thus, at this time, necrotic ringspot appears to be restricted to fewer cultivars than BRRV (Table 2).

In the North Carolina Survey, BRRV was present on 3 of 13 farms (23.0%) and in 8 of 38 fields (21.1%). Thus, the farm-level prevalence was lower than in Georgia, but the field-level prevalence was higher. BRRV incidence in symptomatic fields ranged from <1% to 100%. Affected cultivars included Legacy, Blue Ridge, Biloxi, Jubilee, Star, and Duke. Previous work in North Carolina (as well as the data shown in Table 2 for Georgia) has confirmed BRRV in O'Neal, but symptoms were not observed in O'Neal in this survey.

Necrotic ringspot was also present on 3 of 13 North Carolina farms (23.0%), but these were different farms than those where BRRV was found. The field-level prevalence was 15.8% (6 of 38 fields), which was lower than in Georgia. Incidence ranged from 25 to 100%, but most commonly all plants in a given monoculture field were infected. In this survey, necrotic ringspot was limited to Star and O'Neal in North Carolina; these two cultivars also showed severe symptoms in Georgia.

<u>Objective 2</u>: In the whole-plant study, berry yield during the first hand-harvest was significantly higher in plants that were symptomatic for BRRV in the previous fall than for those that were asymptomatic (Fig. 2). There were no significant differences in yield between the two types of bushes during the second harvest or for both harvests combined (the latter being the result of the first harvest constituting only a relatively small proportion of the total yield). Thus, these results suggest that infection with BRRV may advance fruit ripening somewhat. Infection with BRRV had no effect on average berry weight (Fig. 2).

In the single-shoot study, the number of flower clusters per shoot, the number of berries per shoot, and total berry yield per shoot tended to be higher on healthy shoots than on severely diseased shoots (Fig. 3). In the case of berry number, this reduction was statistically significant. Yield data broken down by harvest date was not available for this study, so we do not know whether BRRV may have increased yield in the first harvest despite decreasing overall yield.

Conclusions

- Both BRRV and necrotic ringspot, a disease of unknown etiology but most likely viral, were found relatively commonly in Georgia and North Carolina (in between 15 and 30% of fields). Prevalence (percentage of symptomatic fields), incidence (percentage of symptomatic plants per field), and symptom severity tended to be higher for necrotic ringspot, although severe symptoms of the disease were restricted to three genotypes (Star, O’Neal, and FL 86-19). In contrast, BRRV occurred across a range of cultivars, and symptom severity was generally lower.
- Similar to what has been reported in the literature from other states (Gillett 1988, Gillett and Ramsdell 1988, Moore and Stretch 1963, Ramsdell 1995), effects of BRRV on berry yield have been inconsistent, indicating the potential for reduced overall yield on severely infected shoots but also a potential for a higher yield in the first hand-harvest, i.e., an advance in ripening.
- For BRRV, additional research is needed to determine to what extent the disease is spread by vectors in the field or whether all spread occurs via infected cuttings. For

example, efforts to backtrack BRRV-positive fields to sources of infected material would provide very useful information. The yield implications of BRRV need to be investigated in more detail by harvesting berries multiple times at a typical grower schedule. With regard to necrotic ringspot, future work is needed to clarify the etiology of the disease, to confirm that severe levels are indeed limited to just a few (but widely planted) cultivars, and to determine effects on berry yield (which may be considerably more pronounced than those of BRRV given the higher symptom severity and observed levels of premature defoliation).

- Results from this study were instrumental in providing preliminary data for a major ($1.7 million) competitive grant to the PIs from the USDA's Specialty Crop Research Initiative. One objective of this new grant is to shed light on the epidemiology and develop management options for emerging systemic diseases of blueberry, which includes bacterial leaf scorch and Botryosphaeria stem blight in addition to the two diseases investigated here. We will utilize the SRSFC's infrastructure for county agent trainings associated with this grant.

Impact Statement

Summary: Field surveys were carried out in Georgia and North Carolina to determine the prevalence and incidence of *Blueberry red ringspot virus* (BRRV) and necrotic ringspot, a disease of unknown etiology but most likely viral, in the main blueberry production regions in the two states. In addition, field studies were conducted to quantify yield losses associated with infection by BRRV.

Situation: At least nine different plant viruses can infect blueberries in North America. Among these, some (such as the scorch and shock viruses) are consistently very damaging, whereas others are highly variable in the level of crop loss they cause, depending on the cultivar and the production environment. In the long run, however, they are all debilitating in production fields and devastating to propagation because virus-infected nursery stock, in general, can not be sold. In the past, it was assumed that blueberry viruses are mostly a problem of the northern half of the U.S., with the southeastern states being essentially virus-free. However, during the summer and fall of 2007, symptoms resembling infection by BRRV appeared in several commercial fields, propagation nurseries, and research plantings in Georgia and North Carolina. Quantitative information on the geographical distribution of the disease in blueberry production areas in the two states and on the yield losses associated with BRRV is lacking.

Response: In collaboration with county extension agents, 167 and 38 fields planted to a range of southern highbush blueberry cultivars were scouted during fall 2008 for prevalence and incidence of BRRV in Georgia and North Carolina, respectively. At the same time, symptoms of necrotic ringspot, a novel disease of unknown etiology but most likely viral, were noticed, and prevalence and incidence of this disease was assessed as well. Berry yield reductions in relation to BRRV severity were quantified in whole-plant and single-shoot studies.

Impact: BRRV and necrotic ringspot were found relatively commonly in both states (in between 15 and 30% of fields). Prevalence (percentage of symptomatic fields), incidence (percentage of symptomatic plants per field), and symptom severity tended to be higher for necrotic ringspot, although severe symptoms of the disease were restricted to three genotypes (Star, O'Neal, and FL 86-19). Molecular work by a collaborator at USDA-ARS in Oregon strongly suggests that the cause of necrotic rings pot is viral, based on the presence of dsRNA in symptomatic leaves. Effects of BRRV on berry yield were inconsistent, with one trial indicating the potential for reduced overall yield on severely infected shoots and another a potential for a higher yield in the first hand-harvest, i.e., an advance in ripening. Results from this study were instrumental in providing preliminary data for a major ($1.7 million) competitive grant to the PIs from the USDA's Specialty Crop Research Initiative. One objective of this grant is to shed light on the epidemiology and develop management options for emerging systemic diseases of blueberry, which includes bacterial leaf scorch and Botryosphaeria stem blight in addition to the two diseases investigated here.

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Table 1. Prevalence of *Blueberry red ringspot virus* (BRRV) and necrotic ringspot disease in different counties in Georgia, based on a survey conducted in fall of 2008.

	Farm-level prevalence[a]			Field-level prevalence[b]		
County	No. of farms	% farms with BRRV	% farms with nec. ringspot	No. of fields	% fields with BRRV	% fields with nec. ringspot
Appling	12	50.0	50.0	37	18.9	27.0
Bacon	4	50.0	25.0	17	17.7	23.5
Berrien	1	…[c]	…	5	…	…
Brantley	7	57.1	57.1	34	23.5	25.5
Clinch	6	16.7	33.3	19	5.3	21.1
Colquitt	1	…	…	7	…	…
Pierce	8	12.5	75.0	22	4.6	45.5
Ware	6	66.7	66.7	26	15.4	19.2
TOTAL	45	42.2	55.6	167	14.9	28.7

[a] Percentage of farms where BRRV or necrotic ringspot was present in at least one field.
[b] Percentage of fields where BRRV or necrotic ringspot was present. Each farm consisted of multiple fields (usually different cultivars).
[c] No percentages calculated for counties where only one farm was surveyed.

Table 2. Prevalence of *Blueberry red ringspot virus* (BRRV) and necrotic ringspot disease on different southern highbush cultivars in Georgia, based on a survey conducted in fall of 2008.

		BRRV prevalence[a] (%)		Necrotic ringspot prevalence[a] (%)	
Cultivar[b]	No. of fields	All incidence classes[c]	Moderate or severe incidence	All incidence classes	Moderate or severe incidence
Emerald	30	16.7	3.3	0	0
FL 86-19 (V1)	22	4.6	0	45.5	18.2
Millennia	5	20.0	10.0	0	0
O’Neal	11	9.1	0	45.5	27.9
Rebel	7	0	0	0	0
Star	55	23.6	9.3	58.2	25.0
Windsor	6	0	0	0	0

[a] Percentage of fields where BRRV or necrotic ringspot was present.
[b] Only cultivars with at least five data points (separate fields) are shown.
[c] Includes scattered, moderate, and severe incidence levels per field.

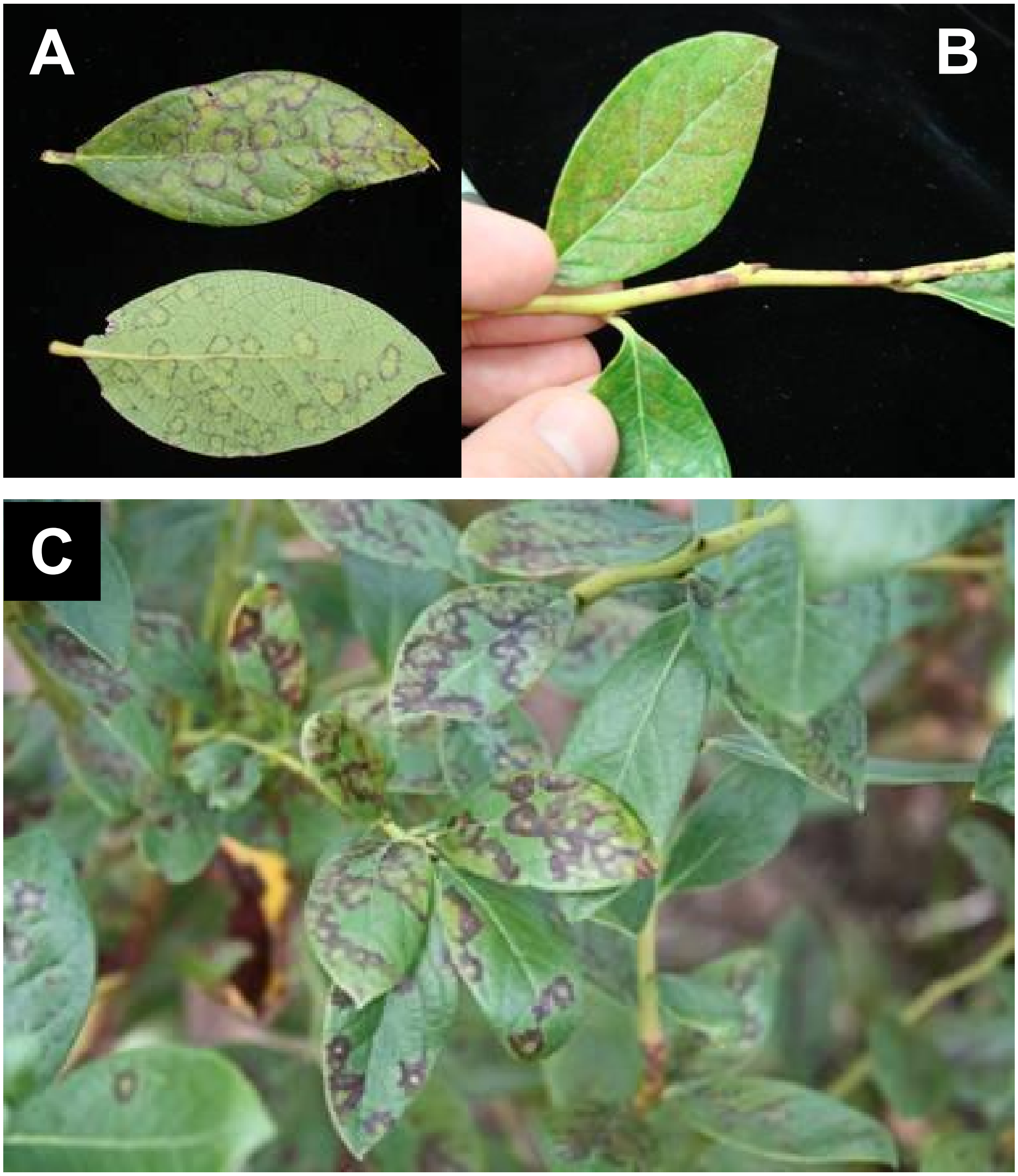

Fig. 1. Typical symptoms on southern highbush blueberry of *Blueberry red ringspot virus* on leaves (A) and shoots (B), and foliar symptoms of necrotic ringspot (C), a novel disease of unknown etiology. Image in C courtesy of P.F. Harmon, Univ. of Florida.

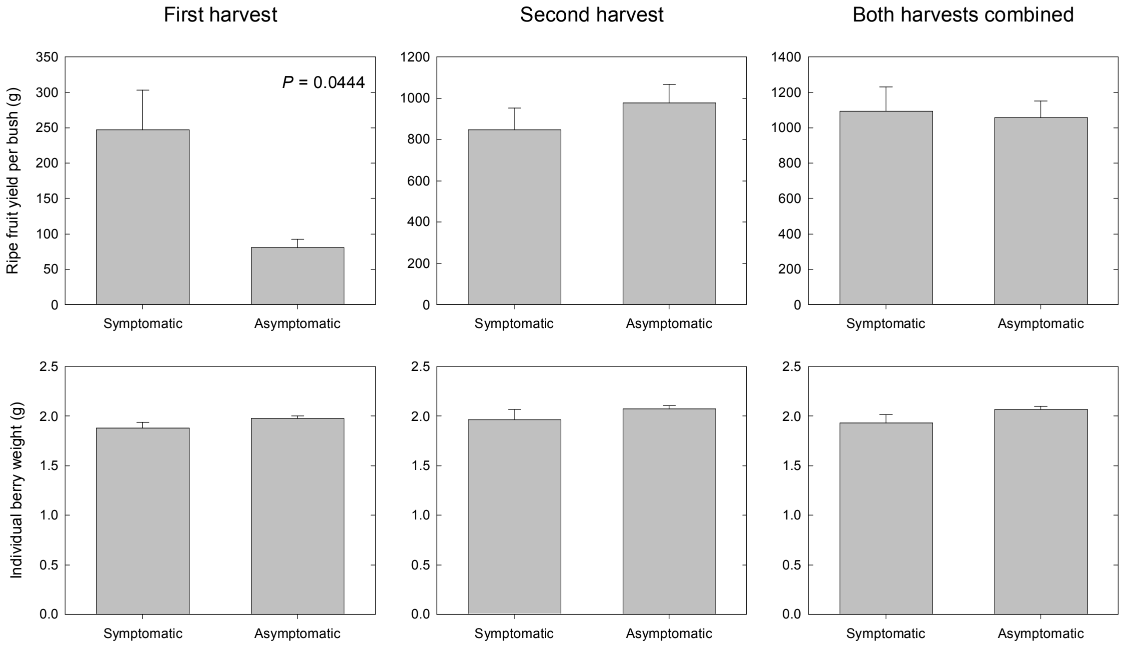

Fig. 2. Effect of presence (symptomatic) or absence (asymptomatic) of *Blueberry red ringspot virus* on berry yield of southern highbush cultivar Star in the whole-plant yield loss study. Values are means and standard errors of six bushes per treatment.

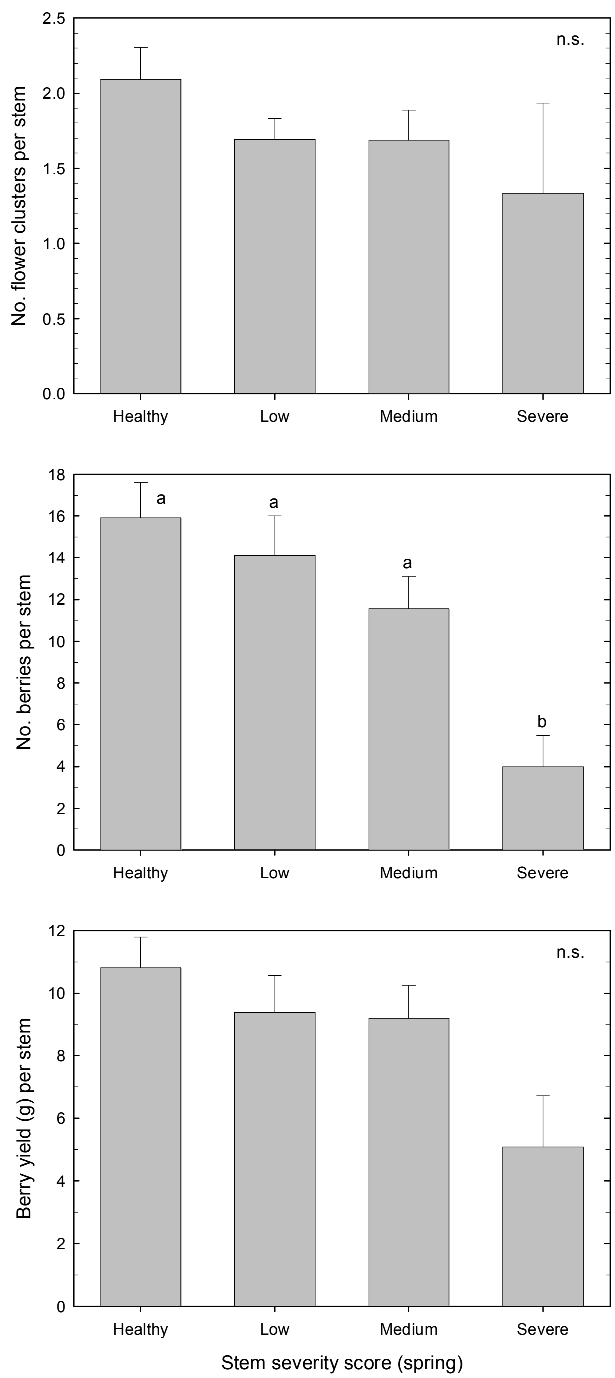

Fig. 3. Effect of severity of *Blueberry red ringspot virus* on berry yield of southern highbush cultivar Star in the single-shoot yield loss study. Values are means and standard errors of a total of 106 shoots across the four shoot severity classes. n.s. = not significant.