BLUEBERRY RED RINGSPOT VIRUS IN SOUTHERN HIGHBUSH BLUEBERRY: EFFECTS ON FRUIT MATURATION AND YIELD, AND INTERACTIONS WITH BIOTIC AND ABIOTIC SOIL FACTORS

by

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(Under the Direction of Harald Scherm)

ABSTRACT

Blueberry red ringspot virus (BRRV) has become widespread in Georgia, but there is limited quantitative information on yield losses associated with this disease. A 3-year study on two southern highbush cultivars growing in containers outdoors with frequent harvests during the fruit ripening period revealed no effects of BRRV on total fruit yield. On cultivar Star, fruit maturity was slightly advanced in BRRV-positive plants in all years. Interactions between BRRV and Phytophthora root rot were studied in the greenhouse and field, revealing no evidence for synergism between the two diseases in symptom intensity or yield loss. Similar results were obtained in a field study where plants were affected by BRRV and abiotic root damage (presumed herbicide injury). Despite the absence of yield losses, it is important to improve propagation practices to prevent further spread of BRRV and other systemic pathogens that may not be as benign in their yield effects.

INDEX WORDS: Blueberry, *Blueberry red ringspot virus*, crop loss assessment, *Phytophthora cinnamomi*, Phytophthora root rot, *Vaccinium corymbosum*

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DEDICATION

I dedicate this work to my wonderfully unique family and irreplaceable friends. My experiences at the University of Georgia have been momentous. Words of encouragement, prayers, and time away from my own thoughts, were never in want at the most critical times.

I would also like for this dedication to serve as a remembrance of our family members who have departed from this world during my time at the University of Georgia.

"For I know the plans I have for you," declares the Lord, "plans to prosper you and not to harm you, plans to give you hope and a future." - Jeremiah 29:11

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS.....v

CHAPTER

1 INTRODUCTION AND LITERATURE REVIEW.....1

LITERATURE CITED.....10

2 EFFECTS OF *BLUEBERRY RED RINGSPOT VIRUS* ON YIELD AND FRUIT MATURATION IN SOUTHERN HIGHBUGH BLUEBERRY.....17

ABSTRACT.....18

INTRODUCTION.....19

MATERIALS AND METHODS.....21

RESULTS.....22

DISCUSSION.....23

LITERATURE CITED.....26

3 EFFECTS OF CO-INFECTION BY *BLUEBERRY RED RINGSPOT VIRUS* AND *PHYTOPHTHORA CINNAMOMI* ON SYMPTOM INTENSITY, PLANT GROWTH, AND YIELD OF SOUTHERN HIGHBUSH BLUEBERRY.....33

ABSTRACT.....34

INTRODUCTION.....35

MATERIALS AND METHODS.....37

RESULTS.....42

DISCUSSION....44
LITERATURE CITED....47
4 EFFECTS OF *BLUEBERRY RED RINGSPOT VIRUS* AND ABIOTIC ROOT INJURY ON PLANT GROWTH AND YIELD OF SOUTHERN HIGHBUSH BLUEBERRY IN THE FIELD....58
ABSTRACT....59
INTRODUCTION....59
MATERIALS AND METHODS....61
RESULTS....62
DISCUSSION....64
LITERATURE CITED....66
5 DISCUSSION AND CONCLUSIONS....73
LITERATURE CITED....76

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Blueberry production in Georgia. With a farm gate value just under $230 million in 2012 (Wolfe and Stubbs 2013), blueberries have become the top fruit commodity in Georgia. More than 6,000 ha of land in the state are now in blueberry production (Anonymous 2013b). The industry is concentrated in the southeastern part of the state, with Appling, Bacon, Clinch, and Ware Counties accounting for >70% of the total production (Wolfe and Stubbs 2013). The increase in acreage has been driven by several factors, including increased consumer demand associated with the documented health benefits of blueberry fruit (Anonymous 2013a, Wood 2011) and the availability of improved cultivars adapted to the southeastern growing environment.

The majority of the blueberry acreage in Georgia is devoted to production of rabbiteye blueberry (*Vaccinium virgatum*), which is native to the southeastern United States and well adapted to the region's warm and humid climate (Scherm and Krewer 2008). Rabbiteye blueberries are relatively pest-resistant or -tolerant, but they have the disadvantage of late fruit maturity (late May through early July in southeastern Georgia) during a harvest window when fruit prices are past their peak. A smaller percentage of the acreage in Georgia is planted to southern highbush blueberry (SHB), *Vaccinium corymbosum* interspecific hybrids (Scherm and Krewer 2003). It is estimated that SHB currently generate 20 to 30% of Georgia's farm gate value for blueberries (P.M. Brannen, *personal communication).* These early-maturing cultivars allow growers to take advantage of a high-price market window (late April to mid-May) that

occurs after late-winter imports from South America and before northern highbush blueberries are harvested in North Carolina and New Jersey (Fonsah et al. 2006; Scherm and Krewer 2003). However, SHB in general are more disease susceptible than the rabbiteye cultivars traditionally grown in the Southeast (Scherm and Krewer 2003). Indeed, over the years, SHB have suffered from a number of fungus and Oomycete diseases such Septoria leaf spot, anthracnose on leaves and fruit, leaf rust, Botryosphaeria stem blight, and Phytophthora root rot, among others (Scherm et al. 2008b, Smith 2002). In addition, SHB are affected by systemic diseases such as bacterial leaf scorch (caused by *Xylella fastidiosa*) and virus diseases, such as *Blueberry red ringspot virus* (Caruso and Ramsdell 1995). In recent years, these systemic diseases have been particularly problematic from a producer's perspective because there are no effective in-field management tactics. Furthermore, the cost of mitigating infection by systemic diseases can continue into propagation systems, for once a plant is infected with a systemic pathogen such as a virus, it usually remains so for its lifetime (Hull 2009).

Importance of viruses in blueberry. The rapid increase in blueberry production in the Southeast has potentially introduced and spread several virus diseases (Martin et al. 2012). Plant viruses can cause significant economic losses in most agricultural crops, including small fruits such as blueberry, strawberry, and raspberry (Tzanetakis 2010). Plant viruses typically are spread through infected plant material by vegetative propagation, vectors such as arthropods and nematodes, and sometimes pollen. Virus diseases, depending on the virus species and its host range, can vary in symptomatology from asymptomatic to severely symptomatic, with some resulting in plant death, as well as by region and across years (Hull 2009). As a perennial fruit crop, blueberries require intensive preparation, planting, establishment, and maintenance procedures before they reach maturity and full production. For example, a detailed economic

analysis estimated a total establishment cost of $24,700 per ha for the first year in SHB (Fonsah et al. 2007). Blueberry plantings have an estimated productivity of 20 years or longer if they remain healthy.

Vaccinium spp. can harbor viruses belonging to several different groups such as *Sobemovirus, Caulimoviridae*, and *Bromoviridae* (Ramsdell 1979, Glasheen et al. 2002, Martin et al. 2012). Most blueberries are native to and grown in North America, so it is not surprising that most of the blueberry viruses have been reported from this continent. Blueberries have become a prime candidate for problems caused by new and emerging viruses for several reasons: 1) the rapid expansion of the acreage; 2) new plantings being established next to areas where virus-infected neighboring plant hosts, including wild or naturalized *Vaccinium* spp., and vectors may occur; 3) the introduction of new cultivars without information on their susceptibility and reaction to viruses and other plant pathogens; and 4) the lack of grower awareness of diseases that can emerge when propagating non-certified material (Martin et al. 2009, 2012). Ongoing research seeks to address management strategies for plant viruses and understand the effect of existing and novel viruses in blueberry.

Virus diseases have been reported in lowbush, highbush, and rabbiteye blueberries, varying by cultivar and growing region. *Blueberry shoestring virus* (BSSV), one of the earliest reported viruses in blueberry, is both graft- and aphid-transmissible (Varney 1957, Morimoto et al 1985). SHB and rabbiteye blueberry once were thought to be resistant to BSSV, but this assumption was likely due to the fact that the virus had not yet spread into the southern production regions (Acquaah et al. 1995). Economic losses due to BSSV are substantial in Michigan and New Jersey (Converse 1987). Necrotic ringspot disease in blueberry became associated with *Tobacco ringspot virus* (TRSV) and *Tomato ringspot virus* (ToRSV), both of

which are transmitted by the nematode *Xiphinema americanum* (Fuchs 2010). Both TRSV and ToRSV can lead to plant death in northern highbush blueberry (Johnson 1972, Ramsdell 1978). Another relatively common virus in North America is *Blueberry red ringspot virus* (BRRV), whose biology will be discussed in more detail below. *Blueberry scorch virus* (BlSV), reported from New Jersey, Massachusetts, Connecticut, Michigan, Oregon, Washington, and British Columbia (Martin et al. 2006), causes symptoms that depend on virus strain and blueberry species or cultivar - varying from marginal chlorosis to severe blighting of flowers and vegetative growth, as well as dieback (Stretch 1983, Bristow et al. 2000). One of the most serious virus diseases in certain production regions is caused by *Blueberry shock virus* (BlShV). This ilarvirus is present in pollen and can survive in bee hives for up to 2 weeks, playing a role in the transmission of the disease (Martin et al. 2006). Symptoms are similar to those of BlSV showing a 'shock reaction' in the spring where fruit loss is directly related to the extent of flower and foliage blighting. After total or partial blighting, foliage and shoots of infected bushes recover by time of harvest but serve as additional reservoirs of inoculum (Martin et al. 2006).

Historically, blueberry viruses were recognized as a common problem in the northern United States, with the Southeast considered essentially virus-free. Indeed, a virus survey based on ELISA testing of suspect samples in 2001 revealed no positive samples in Georgia or North Carolina (Scherm et al. 2008a). Since then, however, two viruses have become relatively widespread in Georgia and neighboring states, BRRV and *Blueberry necrotic ringblotch virus* (BNRBV) (Scherm et al. 2008a, Cline et al. 2009, Robinson et al. 2012). For BRRV in particular, a more recent survey (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 14.9% (25 of 167) of fields sampled; virus presence was confirmed by BRRV-specific PCR assay (Polashock et al.

2009). This survey further revealed that SHB cultivars Star, Millennia, and Emerald had the highest BRRV prevalence, whereas the disease was not detected in Rebel and Windsor (Scherm et al. 2008a). To date in Georgia, BRRV has only been observed on SHB cultivars, but not in the more widely grown rabbiteye type.

Occurrence and symptoms of BRRV. BRRV was first reported as a virus disease on highbush blueberry in New Jersey in 1954 (Hutchinson and Varney 1954). Symptoms of BRRV include red rings on stems, leaves, and occasionally the fruit. Symptoms are visible as red spots or rings on the green stems in the spring and as red rings with light green centers on the leaves in the summer and fall. These ringspots measure 2 to 3 mm in diameter and can coalesce into blotches over time (Hutchinson and Varney 1954). Often ringspot symptoms develop only on the adaxial leaf surface, but some cultivars infected with BRRV exhibit rings on both sides of the leaves (Martin et al. 2012). 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 not visible on hardwood, which increases the potential of spreading the pathogen when infected cuttings are utilized 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 around the world in the Czech Republic (Přibylová et al. 2010), Japan (Isogai et al. 2009), Korea (Cho et al. 2012), Poland (Kalinowska et al. 2011), and Slovenia (Pleško et al. 2010).

A member of the *Caulimoviridae* family in the *Soymovirus* genus, BRRV is a 42 to 46-nm circular double-stranded DNA virus with a genome of 8.3 kB that is easily detectable by PCR (Glasheen et al. 2002). Both end-point (Polashock et al. 2009) and real-time (J. Polashock,

unpublished) PCR assays currently are available for diagnostic testing. The testing of asymptomatic leaf tissue sometimes can be unreliable; however, testing green bark scrapings from current-season growth has proven successful for detection purposes (Martin et al. 2012).

Effects of virus diseases on yield. Viruses are among the most important plant pathogens contributing to reductions in plant vigor, yield, and market value, even to the point of plant death (Hull 2009). Effects of virus pathogens have been reported in various crop systems, causing a variety of symptoms often dependent on virus strain, plant species and cultivar (Iwaki et al. 1984; Golino et al. 2008), and environmental conditions. Sometimes, co-infection by two viruses can lead to amelioration of disease symptoms, utilized commercially for cross-protection (Fletcher 1978), whereas, more commonly, co-infection between viruses exacerbates symptoms and yield loss through synergistic interactions (Wintermantel 2005; Demski and Jellum 1975; Scott et al. 2001). As such, the interactions between viruses, other biotic and abiotic factors, and plant growth and yield are complex.

In blueberry, some virus diseases are highly damaging whereas others are relatively benign. Some of the most prominent yield losses in blueberry are attributed to infection by BlSV and BlShV, both causing flower and leaf blight in northern highbush blueberry (Caruso and Ramsdell 1995). BlSV symptoms range from no damage to complete necrosis and blighting to plant death after several years of infection (Bristow et al. 2000). Yield assessment of paired northern highbush blueberry plants naturally infected or not infected with BlSV revealed a 70 to 80% reduction in fruit yield when infected for 2 or 3 years. With BlShV, following the first year of severe virus-induced blighting, yield on infected bushes was only a 65% of that on healthy control bushes (Bristow et al. 2002).

Compared with BlSV and BlShV, BRRV is thought to have limited impact on plant growth, and many infected blueberry bushes appear to produce a full crop (Martin et al. 2012). However, this conclusion is based largely on anecdotal observations, and only few studies have been made to quantify the effect of BRRV in blueberry. One study in Michigan reported a 25% yield loss in BRRV-infected northern highbush blueberry cultivar Blueray (Gillet 1988) although, this was based on results from 1year and was not repeated. A more recent survey on losses associated with BRRV on SHB in Georgia obtained data both on individual, tagged shoots as well as on whole plants with different levels of BRRV intensity in the field (Scherm et al. 2008a). In the whole-plant study, total fruit yields on bushes of cultivar Star with or without BRRV were not significantly different. However, the infected plants had a significantly higher fruit yield during the first harvest (out of two harvests) than bushes that were asymptomatic, resulting in the tentative conclusion that BRRV may advance fruit ripening slightly (Scherm et al 2008a). 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 fruit number. Data were based on a single fruit harvest, so no harvest data were available over time. As such, quantitative information about the effect of BRRV on yield and fruit ripening in blueberries, including SHB, still is limited and conflicting.

Environmental and biotic interactions. Field observations by blueberry growers and extension agents in southern Georgia have suggested that BRRV symptoms (and presumably associated yield losses) are exacerbated in situations where plants are stressed or co-infected with other plant pathogens, e.g., on sites that are waterlogged and where Phytophthora root rot may be present (Fig. 1.1). In general, it is well established that certain environmental conditions,

such as drought stress (Schoeneweiss 1981), heat stress, or herbicide injury (Griffiths 1981) can greatly affect host predisposition, susceptibility, and symptom severity. Soil conditions in particular are key factors in disease development, especially soil moisture. Locations where the soil remains saturated for extended periods may experience hypoxia and potentially predispose the plant to infection by species of *Fusarium*, *Phytophthora*, and *Pythium* (Calhoun 1973, Bryla et al. 2008). It is possible that a similar predisposition may occur between BRRV and abiotic or biotic soil factors, as suggested in Fig. 1.1, but such an interaction has not been documented experimentally.

Indeed, few studies have evaluated the extent to which viruses and fungus or Oomycete root pathogens co-infect, and their joint impact on symptom development, yield, and survival of plants. In arrowleaf clover (*Trifolium vesiculosum*), for example, the interaction between *Bean yellow mosaic virus* (BYMV) and root rot caused by different species of *Phytophthora* was studied by single and dual inoculations with the causal agents utilizing tolerant, resistant, and susceptible clover species in a greenhouse (Pratt et al. 1982). Foliar dry weight, root volume, and root health ratings were obtained, and dual infection often acted synergistically on these variables (Pratt et al. 1982). Symptoms varied with inoculation timing, with the most severe symptoms occurring after simultaneous inoculation with BYMV and *P. erythroseptica* or *P. megasperma*. In contrast, symptoms were less severe when plants were infected with BYMV subsequent to each *Phytophthora* spp. (Pratt et al. 1982). In light of these observations, similar co-inoculation studies are warranted to quantify interactions between BRRV and *P. cinnamomi*, the common species of *Phytophthora* associated with root rot in southern blueberries (Royle and Hickman 1963).

Project Goals and Objectives. It is clear that quantitative information on the effects of BRRV infection in blueberry is limited and conflicting, with some studies reporting significant yield decreases (albeit based on limited data) whereas others report no effects or even an increase in the first harvest (suggesting accelerated fruit ripening). Thus, multi-year studies in more controlled conditions (e.g., on container-grown plants) and with frequent fruit harvest are needed to better address this question. Furthermore, the potential for interactions between BRRV and abiotic or biotic soil factors, suggested by growers and extension agents, should be addressed. As such, the overall aim of this study was to contribute to a more detailed and comprehensive understanding of the yield effects of BRRV in several economically important cultivars of SHB. Specific objectives are to:

1) quantify the effects of BRRV on fruit maturation and yield of SHB in semi-controlled conditions (on potted plants outdoors);

2) determine the impact of co-infection by BRRV and *Phytophthora cinnamomi* on plant growth and symptom development; and

3) elucidate the effects of BRRV in the presence of abiotic root damage on blueberry plant growth and yield in the field.

Currently, BRRV is not managed actively in established plantings. It is hoped that the present study will provide the data base needed to determine whether or not yield reductions in plants infected with BRRV, alone or in combinations with other biotic or abiotic stressors, are large enough to warrant more active management of the disease in growing conditions in Georgia, e.g., through roguing of infected plants in the field.

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Fig. 1.1. Presumed exacerbation of symptoms of *Blueberry red ringspot virus* (BRRV) in two rows of Star southern highbush blueberry (rows B and C) in the presence of Phytophthora root rot (foreground) compared with BRRV-unaffected plants of FL 89-16 (rows A and D). This observation may suggest interactions between the two diseases. Image courtesy James Jacobs, UGA Cooperative Extension.

CHAPTER 2

EFFECTS OF *BLUEBERRY RED RINGSPOT VIRUS* ON YIELD AND FRUIT MATURATION IN SOUTHERN HIGHBUGH BLUEBERRY[1]

[1]Williford, L.A., Savelle, A.T., and Scherm, H. 2014. To be submitted to *Plant Disease*.

Effects of *blueberry red ringspot virus* on yield and fruit maturation in southern highbush blueberry

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ABSTRACT

Blueberry red ringspot virus (BRRV) has become prevalent on 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 container-grown plants of cultivars Star and Jewel that were either infected or not infected with BRRV to determine the effect of the disease on flower bud set and fruit yield, and on advances or delays in fruit ripening. On Star, flower bud set was reduced on BRRV-positive plants ($P = 0.0137$ in one year and $P = 0.1085$ in another), but no such effect was observed on Jewel. When fruit were harvested six or seven times during the fruit 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 higher for BRRV-negative plants than for BRRV-positive plants, suggesting that BRRV infection on Star may lead to a less protracted 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 benign infection with no negative yield implications.

INTRODUCTION

With a farm gate value just under $230 million in 2012 (Wolfe and Stubbs 2013), blueberries have become the top fruit commodity in Georgia. More than 6,000 ha of land in the state are now in blueberry production (Anonymous 2013). It is estimated that southern highbush blueberries (SHB), *Vaccinium corymbosum* interspecific hybrids, currently generate 20 to 30% of Georgia's farm gate value for blueberries (P.M. Brannen, *personal communication).* Compared with the traditionally grown rabbiteye (*Vaccinium virgatum*) cultivars, SHB fruit mature about 1 month earlier, allowing growers to take advantage of a high-price market window (late April to mid-May) that occurs after late-winter imports from South America and before northern highbush blueberries are harvested in North Carolina and New Jersey (Fonsah et al. 2006; Scherm and Krewer 2003).

SHB in general are less pest-resistant than the rabbiteye cultivars traditionally grown in the Southeast (Scherm and Krewer 2003). This applies to blueberry viruses as well. Historically, blueberry viruses were recognized as a common problem in the northern United States with the Southeast considered essentially virus-free. Indeed, a virus survey based on ELISA testing of suspect plant samples in 2001 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* (BNRBV) (Scherm et al. 2008, Cline et al. 2009, Robinson et al. 2012). For BRRV in particular, a more recent survey (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 14.9% (25 of 167) of fields sampled; virus presence was confirmed by BRRV-specific PCR assay (Polashock et al. 2009). This survey further revealed that SHB cultivars Star, Millennia, and Emerald had the

highest BRRV prevalence, whereas the disease has not been observed in Rebel and Windsor (Scherm et al. 2008). To date in Georgia, BRRV has only been observed on SHB cultivars, but not in the more widely grown rabbiteye cultivars.

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, whereas few studies have been made to quantify the effect of BRRV on yield in blueberry. One study in Michigan reported a 25% crop loss in BRRV-infected northern highbush blueberry cultivar Blueray (Gillet 1988), but this was based on 1-year results and was not repeated. A more recent study on yield losses associated with BRRV on SHB in Georgia obtained data both on individual, tagged shoots as well as on whole plants with different levels of BRRV intensity in the field (Scherm et al. 2008). In the whole-plant study, total fruit yields on bushes of cultivar Star with or without BRRV were not significantly different. However, the infected plants had a significantly higher fruit yield during the first harvest (out of two harvests) than bushes that were asymptomatic, resulting in 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, hence no yield data were available over time. As such, quantitative information about the effects of BRRV on yield and fruit ripening in blueberries, including SHB, is still limited and conflicting. Based on these considerations, the objective of the present study was to generate more accurate and precise data on the yield implications of BRRV on SHB based

on multi-year studies in semi-controlled conditions (on container-grown plants outdoors) with frequent berry harvests to document advances or delays in fruit ripening.

MATERIALS AND METHODS

Plant material and maintenance. The study was carried out from 2012 to 2014 on container-grown plants of SHB cultivars Star and Jewel maintained in 11-liter pots outdoors at the greenhouse complex of the University of Georgia, Athens. The plants had been established 3 years earlier 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 end-point polymerase chain reaction (PCR) from leaf samples as described by Polashock et al. (2009) using primers RRSV3-Forward and RRSV4-Reverse. There were 25 BRRV-positive plants and 22 BRRV-negative plants for Star used across the 3 years. For Jewel, the corresponding numbers were 18 and 23, respectively. Each plant was considered a replicate, and plants were arranged randomly within each cultivar.

Plant maintenance and management including fertilization and summer pruning followed standard practices, with watering and 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.

Data collection and analysis. Flower bud set was determined in 2013 and 2014 by counting all flower buds on each plant 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. 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) included the remaining unripe (green and red) fruit, weighed and counted separately from the ripe blue fruit. Total fruit weights and numbers were determined for each harvest date, and a cumulative total (across all harvests) and an average berry weight was calculated. All data were analyzed, separately by cultivar, using one-way analysis of variance for a completely randomized design (PROC GLM in SAS v. 9.3; SAS Institute, Cary, NC).

RESULTS

Effects of BRRV on flower bud set, yield, and fruit ripening in Star. In the two years where flower bud set was determined, bud counts were numerically higher on BRRV-negative plants than on those positive for the disease (Fig. 2.1A). This effect was statistically significant in 2013 ($P = 0.0137$) and nearly so in 2014 ($P = 0.1085$).

Total fruit yield was numerically higher on BRRV-negative plants in two of the three years (Fig. 2.2A), however, this effect was not statistically significant. Interestingly, the weight of unripe berries remaining after the last harvest was significantly higher for BRRV-negative plants than for BRRV-positive plants (Fig. 2.2A), suggesting that BRRV infection in Star may lead to a less protracted fruit ripening period. Average berry weight was unaffected (Table 2.1), except in 2012 where the berry weight was significantly higher on BRRV-positive plants ($P = 0.0209$).

When fruit yield was examined over the six or seven harvest dates per year (Fig. 2.3), 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 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 time.

Effects of BRRV on flower bud set, yield, and fruit ripening in Jewel. There was no significant difference in flower bud set between BRRV-positive and BRRV-negative plants in either year (Fig. 2.1B). Fruit yield data for Jewel were obtained only in 2012 and 2014 as a late freeze occurred in March 2013, killing most flowers and developing fruit. Total yield was significantly higher on the BRRV-positive plants than on BRRV-negative plants in 2012 (Fig. 2.2B), whereas no significant differences were observed in 2014. There was no significant effect on berry weight on Jewel in either year (Table 2.1).

When fruit yield was examined over time (Fig. 2.4), 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 time.

DISCUSSION

This study was conducted in more controlled conditions than previous trials examining the yield effects of BRRV, with more replications across five cultivar-year combinations and more frequent harvests to capture any effects the disease might have on yield and fruit ripening. Results showed no significant fruit yield reductions associated with BRRV across the five cultivar-year combinations. This supports anecdotal reports of limited yield relevance of BRRV (Martin et al. 2009) but conflicts with a study from Michigan reporting a 25% yield loss in BRRV-infected northern highbush blueberry cultivar Blueray (Gillet 1988). The latter study was not repeated, so it is unclear whether the significant yield loss was unique to Blueray and/or to

the year when the investigation was carried out. The only previous experimental study on BRRV-associated yield losses in SHB (Scherm et al. 2008), conducted also in 1 year only, found no significant effect of BRRV status (positive or negative) on total yield of field-grown Star when whole-plant yields were determined. Combined with our data, it can be concluded that BRRV does not cause significant yield losses in SHB, at least 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 (Table 2.1), a few general trends emerged from the data. For example, flower bud set on Star was consistently higher on BRRV-negative than on BRRV-positive plants, in one year significant at $P = 0.05$ and in the second year at $P = 0.10$ (Fig. 2.1A). However, the 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. 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 no effect of the disease on average berry weight (statistically significant only in 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) affected by BRRV. This was evident not only from the fruit ripening curves in Fig. 3.3, but also from the remaining unripe fruit yield at the last harvest date, which was significantly lower in BRRV-positive than BRRV-negative plants in two years at $P = 0.05$ and in one year at $P = 0.10$ (Table 2.1). This is consistent with a previous 1-year study by Scherm et al. (2008) which showed a significantly higher yield in the first harvest on 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 SHB in Georgia (Scherm et al. 2008), it is fortunate that there are no consistent yield losses associated with the disease. Since BRRV is transmitted only by vegetative propagation (Caruso and Ramsdell 1995), 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. As blueberry acreage is now leveling off 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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Table 2.1. *P*-values of analyses of variance[a] to compare fruit yield variables on container-grown Star and Jewel southern highbush blueberry plants that were either positive or negative for *Blueberry red ringspot virus*.

Cultivar and year	Total yield	Ripe fruit yield	Unripe fruit yield	Berry weight
Star				
2012	0.7081	0.5495	**0.0364**	**0.0209**
2013	0.4524	0.5678	**0.0327**	0.5039
2014	0.1298	0.3466	0.0884	0.1383
Jewel[b]				
2012	**0.0024**	**0.0027**	0.4394	0.4480
2014	0.2882	0.2078	0.6195	0.5501

[a] Corresponding yield data presented in Fig. 2.2.

[b] No yield was obtained for Jewel in 2013 due to freeze damage during bloom and early fruit set.

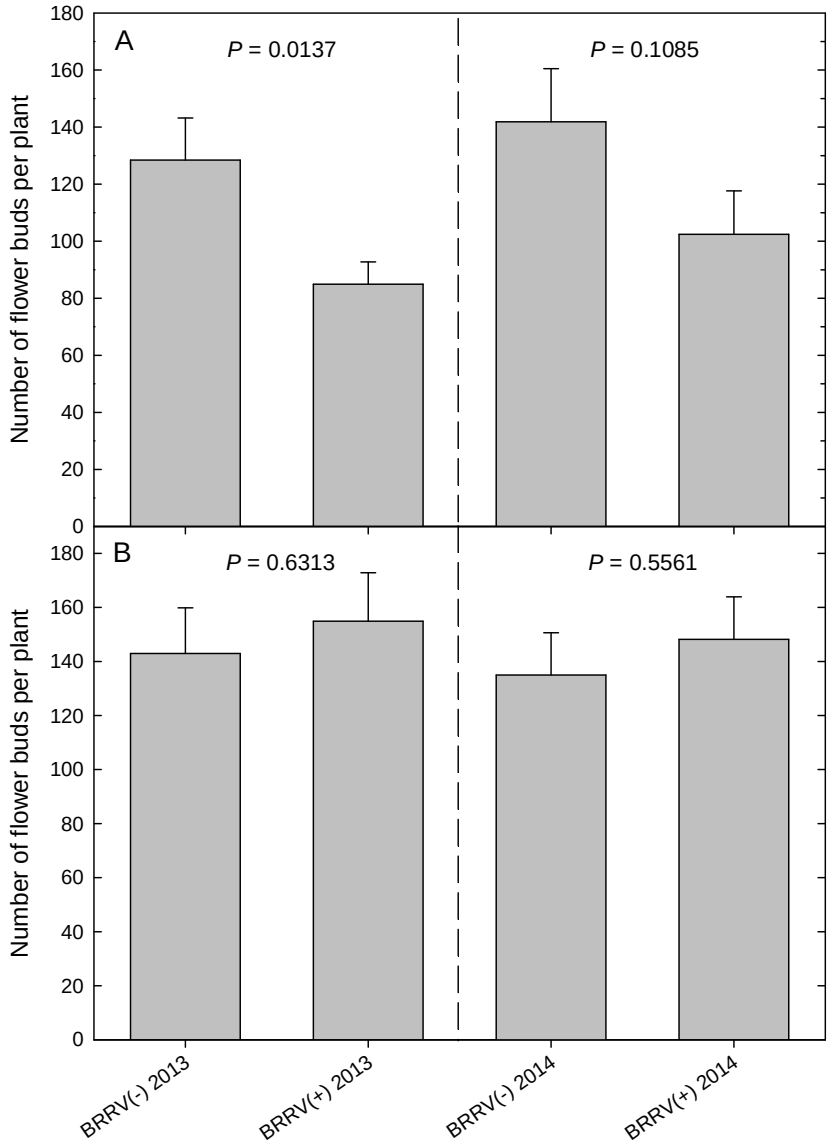

Fig. 2.1. Flower bud set (determined in January or February of 2013 and 2014) on container-grown Star (A) and Jewel (B) southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Values are means and standard errors of 18 to 25 plants (replicates). *P*-values from one-way analysis of variance.

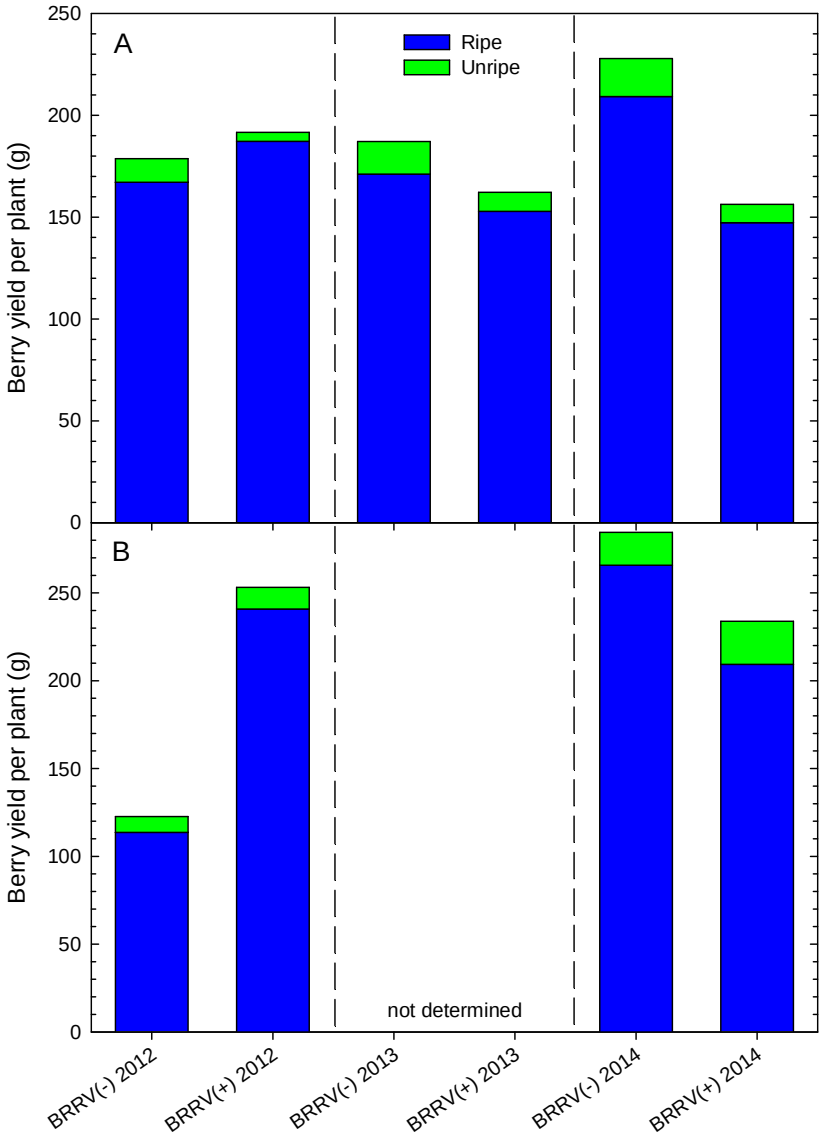

Fig. 2.2. Fruit yield, determined in six or seven successive harvests, on container-grown Star (A) and Jewel (B) southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Values are means of 18 to 25 plants (replicates). Unripe yield corresponds to fruit that still were 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 presented in Table 2.1.

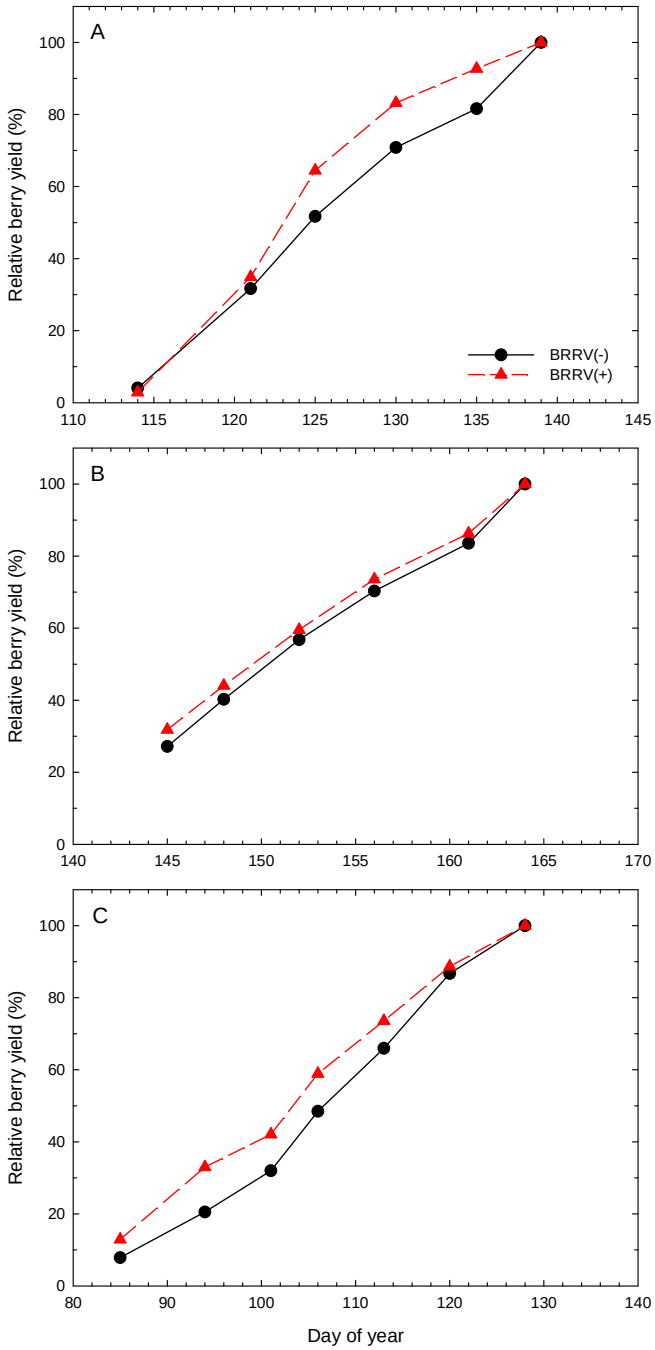

Fig. 2.3. Cumulative fruit yield over time, determined in six or seven successive harvests in 2012 (A), 2013 (B), and 2014 (C), on container-grown Star southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Total yield (Fig. 2.2) was 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 still were immature on the final harvest date.

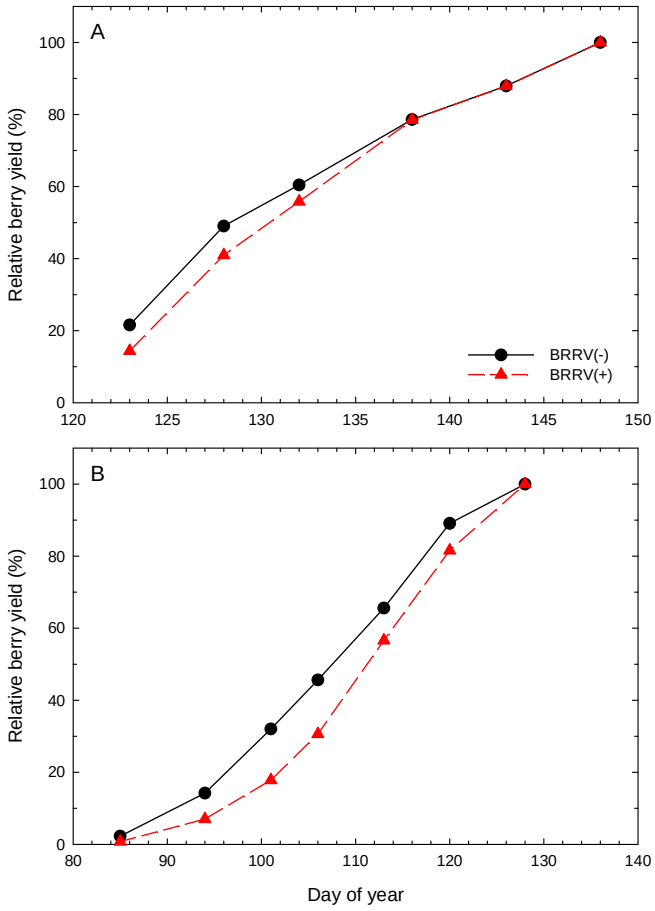

Fig. 2.4. Cumulative fruit yield over time, determined in six or seven successive harvests in 2012 (A) and 2014 (B), on container-grown Jewel southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV). Total yield (Fig. 2.2) was set to 100% 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 still were immature on the final harvest date.

CHAPTER 3

EFFECTS OF CO-INFECTION BY *BLUEBERRY RED RINGSPOT VIRUS* AND *PHYTOPHTHORA CINNAMOMI* ON SYMPTOM INTENSITY, PLANT GROWTH, AND YIELD OF SOUTHERN HIGHBUSH BLUEBERRY[1]

[1]Williford, L.A., Savelle, A.T., Williams-Woodward, J., Jeffers, S.N., and Scherm, H. 2014. To be submitted to *Plant Disease*.

Effects of co-infection by *Blueberry red ringspot virus* and *Phytophthora cinnamomi* on symptom intensity, plant growth, and yield of southern highbush blueberry

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ABSTRACT

Blueberry red ringspot virus (BRRV) is a relatively benign plant virus, causing no reduction in fruit yield of southern highbush blueberry when it is the only pathogen affecting the plant. However, recent field observations have suggested that BRRV symptoms (and presumably associated yield losses) are exacerbated in situations where plants are stressed or co-infected with other plant pathogens, e.g., on sites that are waterlogged and where Phytophthora root rot may occur. Here, we conducted greenhouse trials with Star and Jewel southern highbush blueberry plants that were either positive or negative for BRRV and inoculated or not inoculated with *Phytophthora cinnamomi* when they were 4 or 5 months old. No consistent effects were observed for *P. cinnamomi* infection increasing foliar BRRV symptoms or BRRV-infection increasing foliar Phytophthora symptoms. Shoot and root fresh weights were always significantly ($P < 0.05$) reduced by *P. cinnamomi* infection, whereas the effect of BRRV infection generally was not significant. There generally was no statistically significant interaction in the effects of the two pathogens on yield variables, indicating that BRRV-positive and BRRV-negative plants responded similarly to inoculation with *P. cinnamomi*. When root system discoloration was

assessed quantitatively using *Munsell Soil-Color Charts*, co-infection by BRRV and *P. cinnamomi* always resulted in the same root color contrast rating as infection by *P. cinnamomi* alone, again indicating the lack of an interaction between the two pathogens. In a separate experiment, 2-year-old potted plants that were either BRRV-positive or BRRV-negative were transplanted into a field site harboring *P. cinnamomi*. Flower bud set (over two seasons) and fruit yield (over one season) were not significantly different between the two BRRV groups in the presence of *P. cinnamomi*.

INTRODUCTION

Viruses have been recognized as a common problem in blueberry production regions in the northern United States for a long time (Caruso and Ramsdell 1995), whereas blueberry production regions in the Southeast traditionally have been considered virus-free. Indeed, a virus survey based on ELISA testing of suspect samples in 2001 revealed no positive samples in Georgia or North Carolina (Scherm et al. 2008a). Since then, however, two viruses have become relatively widespread in Georgia and neighboring states, *Blueberry red ringspot virus* (BRRV) and *Blueberry necrotic ringblotch virus* (BNRBV) (Scherm et al. 2008a, Cline et al. 2009, Robinson et al. 2012). For BRRV in particular, a more recent survey (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 14.9% (25 of 167) of fields sampled; virus presence was confirmed by BRRV-specific PCR assay (Polashock et al. 2009). To date in Georgia, BRRV has only been observed on southern highbush blueberry cultivars (*Vaccinium corymbosum* interspecific hybrids), but not in the native and more widely grown rabbiteye (*Vaccinium virgatum*) cultivars.

BRRV is thought to have limited impact on plant growth, and many affected blueberry plantings appear to produce a full crop (Martin et al. 2012). However, field observations by blueberry growers and extension agents in southern Georgia have suggested that BRRV symptoms (and presumably associated yield losses) are exacerbated in situations where plants are stressed or co-infected with other plant pathogens, e.g., on sites that are waterlogged and where Phytophthora root rot may occur (Fig. 3.1). In general, it is well established that certain environmental conditions, such as drought stress (Schoeneweiss 1981), heat stress, or herbicide injury (Griffiths 1981) can greatly affect host predisposition, susceptibility, and symptom severity. Soil conditions in particular are key factors in disease development, especially soil moisture. Locations where the soil remains saturated for extended periods may experience anaerobic conditions and potentially predispose plants to infection by species of *Fusarium*, *Phytophthora*, and *Pythium* (Calhoun 1973, Bryla et al. 2008). It is possible that a similar predisposition may occur between biotic or biotic soil factors and BRRV, as suggested in Fig. 3.1, but such an interaction has not been documented experimentally.

Few studies have evaluated the extent to which viruses and fungus or Oomycete root pathogens co-infect plants, and their combined impact on symptom development, yield, and survival of plants. In arrowleaf clover (*Trifolium vesiculosum*), the interaction between *Bean yellow mosaic virus* (BYMV) and root rot caused by different species of *Phytophthora* was studied by single and dual greenhouse inoculations with the causal agents on tolerant, resistant, and susceptible clover species (Pratt et al. 1982). Foliar dry weight, root volume, and root health ratings were obtained, and dual infection often acted synergistically on these variables (Pratt et al. 1982). In light of these observations, similar co-inoculation studies are warranted to quantify

interactions between BRRV and *P. cinnamomi*, a typical *Phytophthora* species associated with root rot in southern blueberries (Royle and Hickman 1963).

The potential for interactions between Phytophthora root rot and BRRV, brought to our attention by growers and extension agents, should be addressed experimentally. As such, the overall aim of this study was to determine the impact of co-infection with BRRV and *P. cinnamomi* on blueberry plant growth, symptom development, and fruit yield.

MATERIALS AND METHODS

Plant material and maintenance in the greenhouse. Softwood cuttings (15 to 20 cm long) were taken from mature Star or Jewel southern highbush blueberry plants in June of 2012 and 2013. The mother plants were either BRRV-positive or BRRV-negative as determined previously by testing leaf disks with end-point PCR (Polashock et al. 2009). Cuttings were stored in large plastic bags on ice overnight in a cold room. The next day, a sterile pruner was used to make a 2-cm vertical incision at the base of each cutting to facilitate rooting. The bases of the cuttings were dipped into 2,500 ppm potassium-indole-acetic acid and then stuck into 36-well trays containing milled pine bark. Four trays (144 plants) were prepared for each BRRV treatment (BRRV-positive and BRRV-negative). The trays were arranged on a mist bench in a greenhouse to encourage root development. One week after sticking, a drench of Medallion WP fungicide (50% fludioxonil; Syngenta Crop Protection, Greensboro, NC) was applied. Greenhouse conditions were characterized by a temperature range of 23 to 32°C and a 12 h of photoperiod. Ten weeks after sticking, rooted cuttings were transplanted into 20-cm clay pots containing a 2:1 peat:sand (v:v) mix amended with 8 g/liter of Osmocote (14-14-14 N-P-K)

slow-release fertilizer. These plants subsequently were grown in a greenhouse at 18 to 27°C with a 14 h photoperiod for 4 to 5 months prior to inoculation with *P. cinnamomi*.

***Phytophthora cinnamomi* inoculation.** Two isolates of *P. cinnamomi* (BBRY-1 and BBRY-2) originally isolated from diseased blueberry plants in Georgia were grown in a sterile mixture of 10% V8 broth (100 mL V8 juice, 1.0 g $CaCO_3$, 900 mL distilled water) and horticultural grade vermiculite (1:2 v:v) for 2 weeks. Each flask containing 100 mL of V8-vermiculite mixture was autoclaved twice, on two consecutive days, for 30 min each time (Roiger and Jeffers 1991). After cooling, five agar plugs colonized by an isolate were added to the V8-vermiculite mixture in a flask; each plug was 5 mm in diameter and was cut from a 1-week-old actively growing culture on 15% V8 agar (160 mL V8 juice, 1.5 g $CaCO_3$, 25 g agar, 850 mL distilled water). Flasks were incubated in the dark at room temperature for 2 weeks and shaken every 2 to 3 days to ensure uniform colonization of the medium.

Twenty BRRV-positive plants and 20 BRRV-negative plants were selected based on uniformity and were used in the inoculation experiments with *P. cinnamomi*. The experimental design was a split-plot with two levels of *P. cinnamomi* (inoculated and not inoculated) in the main-plot crossed with two levels of BRRV (positive or negative) in the sub-plot. There were ten plants (replicates) for each treatment combination. One trial was conducted in December 2012 (trial A) with plants of cultivars Star and Jewel and two additional trials in January 2014 (trials B and C) with only Star. For plants inoculated with *P. cinnamomi*, 25 (trial A) or 10 (trials B and C) mL of colonized V8-vermiculite medium was applied to the soil surface around each plant, whereas non-inoculated plants received an equal volume of non-colonized V8-vermiculite medium. The inoculum was covered with 50 mL of 2:1 peat:sand mixture and then lightly watered into the soil.

One week after inoculation with *P. cinnamomi*, plants were subjected to flooding by individually placing each pot into a 12-liter bucket filled with water and submerging it for 48 h so that ~1 cm of water stood above the soil line. In trial A, plants were flooded every 2 weeks for a total of seven flooding periods. The buckets were sanitized with 10% bleach solution between flooding events. In trial B, plants were flooded twice beginning 1 week after inoculation, whereas in trial C, plants were flooded only once 1 week after inoculation.

Foliar disease progression. The first symptoms of Phytophthora root rot (mid-day wilting) began to appear 1 to 2 months after inoculation. Foliar disease assessments for Phytophthora root rot (in all three trials) and BRRV (in trials B and C only) were conducted once or twice per week, depending on disease progress. For BRRV, affected leaves were counted and classified into one of three severity classes (Fig. 3.2): 1 = light green or chlorotic spots, 2 = chlorotic spots with slight red tint or center, and 3 = characteristic red ringspots or blotches. Both the numbers of symptomatic leaves per plant as well as the percentages of leaves assigned to BRRV severity class 3 were analyzed. For Phytophthora root rot, foliar disease progress was assessed as the total number of red, chlorotic, dried, and defoliated leaves per plant at each assessment date. The number of defoliated leaves was obtained cumulatively over time by counting and removing leaves that had dropped onto the soil surface at each assessment date.

BRRV titer in test plants was determined by real-time polymerase chain reaction (PCR) assay (J. Polashock, unpublished). Leaf samples were selected arbitrarily just before conclusion of each experiment (4, 3, and 3 months after inoculation with *P. cinnamomi* in trials A, B, and C, respectively). Plant tissue was frozen in liquid nitrogen and stored in a -20^{o}C freezer prior to processing. Frozen plant material (300 mg) was ground in a Mini-Beadbeater-8 (BioSpec Products, Bartlesville, OK), and total genomic DNA was extracted using the DNeasy Plant Mini

Kit (Quiagen, Valencia, CA). Real-time PCR was conducted with a BRRV Assay including Primer 1: 5'-ACTTGCTGATAATCGCTACCG-3', Primer 2: 5'-GATAATGCTTGCGCTGTATGC-3' and probes 5' 6-FAM, Int ZEN, and 3' Iowa Black (J. Polashock, unpublished). The reaction volume was a total of 20 µL which included 2 µL of genomic DNA from leaf extract, 10 µL of 2X TaqMan Master Mix (LifeTechnologies, Grand Island, NY), 1 µL of 20X BRRV Assay and 7 µL nuclease-free water. Thermocycler run conditions were 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 45 sec. The resulting Ct values were converted into virus copy number per 100 µL based on a standard curve starting with 3,000,000 copies of BRRV DNA diluted 1:10 through 30 copies in100 µL. Ct values below 33 were considered positive.

Root and shoot weights. At the end of each trial, plants were harvested destructively and shoot and root fresh weights were determined. Roots were washed of all soil and blotted dry before being weighed. The bulk root systems were then assessed for discoloration using *Munsell Soil-Color Charts* (Munsell Color 2009) and given a designated hue, value, and chroma based on the most appropriate color chip (Fig. 3.3). Differences in root discoloration relative to the control (BRRV-negative, not inoculated with *P. cinnamomi*) were assessed quantitatively using a contrast rating (adapted from Schoeneberger et al. 2012) where differences were defined as absent, faint, distinct, or prominent. Dry weights were obtained after shoots and roots were held at 65°C for 48 h.

Recovery of *Phytophthora*. *Phytophthora cinnamomi* was re-isolated from the roots of all inoculated replicates. Five 7-mm root pieces from each washed root system were embedded in CMA-PARPH semi-selective medium containing 15 g of Difco cornmeal agar with 1000 mL of distilled water and antimicrobial amendments (400 µL pimaricin, 250 mg ampicillin, 10 mg

rifamycin, 67 mg pentachloronitrobenzene, and 32.5 mg 70% hymexazol) (modified from Ferguson and Jeffers 1999).

Statistical analysis of greenhouse data. Foliar disease intensity (separately for BRRV and Phytophthora root rot), as well as shoot and root weight data, were analyzed with analysis of variance for a split-plot design (PROC GLIMMIX in SAS v.9.3; SAS Institute, Cary, NC). For BRRV, the analysis utilized assessments from 6 weeks after inoculation with *P. cinnamomi*, before foliar BRRV symptoms became masked by those of Phytophthora root rot. For Phytophthora root rot, foliar disease incidence data from the last assessment date prior to conclusion of the study were used in the analysis. Of particular interest in the analysis of variance was the presence of a significant ($P < 0.05$) interaction between the BRRV and *Phytophthora* treatments, indicating synergistic interactions between the two pathogens.

Field study. The study was carried out in a low-lying area in a commercial southern highbush blueberry planting in Ware County, GA (Fig. 3.1) affected by Phytophthora root rot (confirmed by baiting of *P. cinnamomi* from soil samples). Twenty dead plants at the end of two rows of cultivar Star were removed in October 2012 and replaced with ten pairs of 2-year-old potted Star plants that were either BRRV-positive or BRRV-negative, as determined by end-point PCR (Polashock et al. 2009). This design allowed for direct comparison of the plants' performance in the presence of *P. cinnamomi*. Flower bud set was determined by counting the total number of flower buds on each plant in early February of 2013 and 2014. In spring of 2013, developing green fruit were stripped from the test plants to favor plant growth and development. In 2014, fruit yield was determined on 7 May by harvesting all fruit on each bush and counting and recording the weights of ripe (blue) and unripe (green or red) fruit separately. Both flower bud counts and fruit yields were analyzed using paired t-tests.

RESULTS

Effects of co-infection on foliar BRRV incidence and severity in the greenhouse. Foliar BRRV incidence and severity were assessed only in trials B and C. No BRRV symptoms were observed on BRRV-negative plants. On BRRV-positive plants, the number of leaves with BRRV symptoms was not affected by inoculation with *P. cinnamomi* in trial B, but was significantly higher in plants that were not inoculated with *P. cinnamomi* in trial C (Fig. 3.4). When BRRV severity was assessed, there was no significant difference in the relative percentage of leaves in the most severe BRRV class (Fig. 3.2) between plants that were inoculated or not inoculated with *P. cinnamomi* in trial B (Fig. 3.4). However, in trial C, the percentage of leaves in the most severe BRRV class was significantly increased in plants that were co-infected with *P. cinnamomi*.

The real-time PCR assay was capable of detecting BRRV in symptomatic but not in asymptomatic leaves (data not shown). Detailed results will be presented elsewhere.

Effects of co-infection on Phytophthora root rot incidence in the greenhouse. Foliar symptoms associated with Phytophthora root rot following inoculation with *P. cinnamomi* were observed for a total of 4 months in trial A (on Star and Jewel) and 3 months in trials B and C (on Star only) (Fig. 3.5). Final foliar disease incidence was considerably higher in plants inoculated with *P. cinnamomi* than in those not inoculated in all trials (Fig. 3.5). Statistically, the effect of inoculation with *P. cinnamomi* on disease severity was highly significant in all four trial-cultivar combinations ($P < 0.0001$), whereas there was no significant effect of BRRV on Phytophthora root rot symptom severity (Table 3.1). There was no significant statistical interaction between inoculation with *P. cinnamomi* and presence of BRRV (Table 3.1) on development of foliage symptoms associated with Phytophthora root rot, indicating that plants with or without BRRV

reacted similarly to inoculation with *P. cinnamomi* with regard to Phytophthora foliar disease progression.

Similar results were observed when shoot fresh weight was analyzed at the end of the experiment: highly significant reduction due to *P. cinnamomi* inoculation ($P < 0.0001$), no effect of BRRV presence (with the exception of Star plants in trial A, $P = 0.0436$), and no significant interaction between the two pathogens (Table 3.1 and Fig. 3.6). Root weight data also confirmed the lack of an interaction, except for trial C where the interaction term was significant at $P = 0.0118$ (Table 3.1). In all cases, the main effect of inoculation with *P. cinnamomi* was highly significant whereas the BRRV main effect was significant in two of the four trial-cultivar combinations.

When assessed using *Munsell Soil-Color Charts*, hue was the same for all root systems and determined as 10YR (yellow-red) (Fig. 3.3). Root discoloration always was more pronounced in plants inoculated with *P. cinnamomi* when compared with the BRRV-negative, no-*Phytophthora* control. In all cases, the contrasts in *Phytophthora*-infected plants were quantified as 'distinct' based on the chroma and value numbers (Table 3.2). Infection by BRRV alone only resulted in 'faint' contrasts from the control in all trials. Co-infection by BRRV and *P. cinnamomi* always resulted in the same root color contrast rating as infection by *P. cinnamomi* alone (Table 3.2), indicating the lack of an interaction between the two pathogens.

Effects of co-infection on flower bud set and fruit yield in the field. Two BRRV-positive plants were lost from the trial due to plant death from unknown causes, leaving eight BRRV-positive and ten BRRV-negative plants. Flower bud numbers per plant increased considerably from 2013 to 2014 as the plants became established at the field site, but there were no significant differences between BRRV-positive and BRRV-negative plants in either year (Fig.

3.7). Similarly, there were no significant effects on any of the yield variables evaluated in 2014 with *P*-values of 0.8758, 0.6949, 0.9979, and 0.0858 for total fruit yield, ripe fruit yield, unripe fruit yield, and average berry weight, respectively.

DISCUSSION

Results from this study showed that BRRV and *P. cinnamomi* do not act synergistically in terms of their effect on symptom severity, plant growth, flower bud set, and fruit yield. Out of all the variables examined across three greenhouse trials and one field trial, only one greenhouse trial (trial C) showed a significant interaction between the two pathogens, namely for the effect root fresh weight. This trial also showed the only other effect indicative of a symptom exacerbation due to co-infection, i.e., a significantly greater percentage of BRRV-affected leaves in the most severe symptom class for plants that also had Phytophthora root rot. In all other trials and comparisons, there was no evidence for a synergistic interaction between the two pathogens, thereby contradicting anecdotal field observations that have suggested such an effect.

It is important to note in this context that we based our conclusions primarily on the lack of a statistically significant interaction between the two pathogens in analyses of variance. A significant interaction would indicate that BRRV-positive plants react more strongly to infection by *P. cinnamomi* than do BRRV-negative plants in terms of the response variable of interest. This analysis thus captures synergistic (over-additive) effects. Additive effects, where Phytophthora root rot might reduce yield by a certain percentage regardless of whether plants are BRRV-negative or BRRV-positive, would not result in a statistically significant interaction term in the analysis, although the yield of the double-infected plant would be expected to be lower than that of plants infected with either pathogen alone.

In greenhouse trial C, the total number of BRRV-affected leaves was significantly lower in plants that were co-infected with *P. cinnamomi* (Fig. 3.4). This counterintuitive result could be due to two reasons. First, the onset of foliar symptoms of Phytophthora root rot may have masked some of the symptoms associated with BRRV, especially those in the light symptom class. The low percentage of leaves in BRRV symptom class 1 in Fig. 3.4 for plants infected with *P. cinnamomi* seems to support this idea. Secondly, the significantly reduced shoot growth in plants infected with *P. cinnamomi* would have resulted in a smaller number of leaves per plant, thereby skewing the BRRV incidence data that were based on the number (rather than the proportion) of affected leaves.

After the initial foliar symptoms of Phytophthora root rot appeared in the first greenhouse trial, disease developed rapidly, which may not have allowed for adequate opportunity to observe synergistic interactions between the two diseases. We attempted to address this by lowering the inoculum density of *P. cinnamomi* and reducing the flooding frequency in trials B and C compared with trial A, but the disease still proceeded rapidly on these plants, which were between 7 and 9 months old at the conclusion of the experiments. In theory, mature plants would likely show slower Phytophthora root rot symptom progression, which should increase the likelihood of observing synergistic interactions with BRRV over time. However, this was not observed in the field trial included in this study, where plants were 3.5 years old at the conclusion of the trial.

Numerous papers have reported synergistic interactions after co-infection of plants by different virus species (Wintermantel 2005, Demski and Jellum 1975, Scott et al. 2001), but there are very few studies that investigated interactions between viruses and other soilborne pathogens (Pratt et al. 1982). As shown in Chapter 2, BRRV is a relatively benign virus, causing no

significant yield losses when it is the only pathogen affecting the plant. The present study takes this one step further by demonstrating no significant exacerbation of symptoms or yield loss in plants that are dually infected with BRRV and *P. cinnamomi* - one of the most common soilborne pathogens affecting blueberries in the Southeast.

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Table 3.1. *P*-values of mixed-model analyses of variance for the effects of co-infection by *Blueberry red ringspot virus* (BRRV) and *Phytophthora cinnamomi* (Pc) on foliar disease incidence on and fresh weight of greenhouse-grown southern highbush blueberry plants[a].

Treatment	No. of PRR-affected leaves[b]	Shoot fresh weight	Root fresh weight	Total fresh weight
Trial A - Star				
BRRV main effect	0.2858	**0.0436**	0.1773	**0.0447**
Pc main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.8297	0.9162	0.1170	0.6209
Trial A - Jewel				
BRRV main effect	0.4148	0.3181	0.4538	0.3129
Pc main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.9462	0.1553	0.2985	0.3210
Trial B - Star				
BRRV main effect	0.2899	0.4758	**0.0309**	**0.0435**
Pc main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.4803	0.8526	0.2525	0.4624
Trial C - Star				
BRRV main effect	0.6589	0.8117	**0.0422**	0.2819
Pc main effect	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	0.6407	0.7826	**0.0118**	0.0909

[a] Data presented in Figures 3.5 and 3.6.

[b]Number of leaves per plant showing symptoms of Phytophthora root rot at the conclusion of each trial, 3 to 4 months after inoculation with *P. cinnamomi*.

Table 3.2. Discoloration of roots[a] on greenhouse-grown southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and inoculated (+) or not inoculated (-) with *Phytophthora cinnamomi* (Pc).

Treatment	Δ Value[b]	Δ Chroma[b]	Contrast to BRRV(-) Pc(-)
Trial A - Star			
BRRV (-), Pc(-)	(4.8)	(5.6)	--
BRRV(+), Pc (-)	≤1	≤1	faint
BRRV (-), Pc (+)	≤1	≤2	distinct
BRRV(+), Pc (+)	≤1	≤2	distinct
Trial A - Jewel			
BRRV(-), Pc (-)	(5.1)	(5.3)	--
BRRV(+), Pc (-)	≤1	≤1	faint
BRRV(-), Pc (+)	≤2	≤2	distinct
BRRV(+), Pc (+)	≤2	≤2	distinct
Trial B - Star			
BRRV(-), Pc (-)	(5.9)	(3.6)	--
BRRV(+), Pc (-)	≤1	0	faint
BRRV(-), Pc (+)	≤3	≤1	distinct
BRRV(+), Pc (+)	≤2	≤2	distinct
Trial C - Star			
BRRV(-), Pc (-)	(5.8)	(4.0)	--
BRRV(+), Pc (-)	≤1	0	faint
BRRV(-), Pc (+)	≤3	≤1	distinct
BRRV(+), Pc (+)	≤2	2	distinct

[a] Root discoloration was assessed on bulk root systems using *Munsell Soil-Color Charts* (Munsell Color 2009).

[b]Listed are the differences in means of the Munsell Value and Chroma ratings for each treatment compared with the control (BRRV-negative, *P. cinnamomi*-negative), and the resulting contrast rating (adapted from Schoeneberger et al. 2012).

Fig. 3.1. Presumed exacerbation of symptoms of *Blueberry red ringspot virus* (BRRV) in two rows of Star southern highbush blueberry (rows B and C) in the presence of Phytophthora root rot (foreground) compared with BRRV-unaffected plants of FL 89-16 (rows A and D). This observation may suggest interactions between the two diseases. Image courtesy James Jacobs, UGA Cooperative Extension.

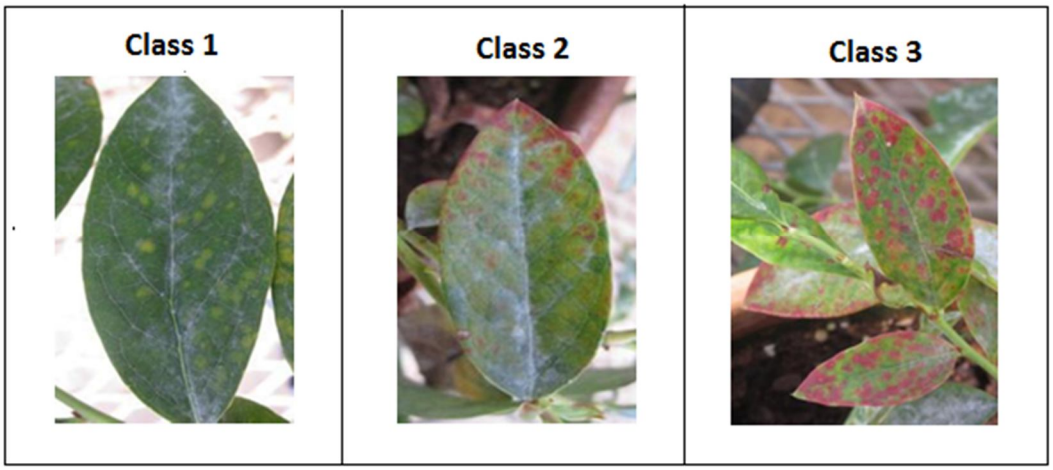

Fig. 3.2. *Blueberry red ringspot virus* severity classification used for foliar disease assessment in the greenhouse. Class 1 = chlorotic spots; class 2 = chlorotic spots with reddish tint or center; class 3 = characteristic red ringspots or blotches.

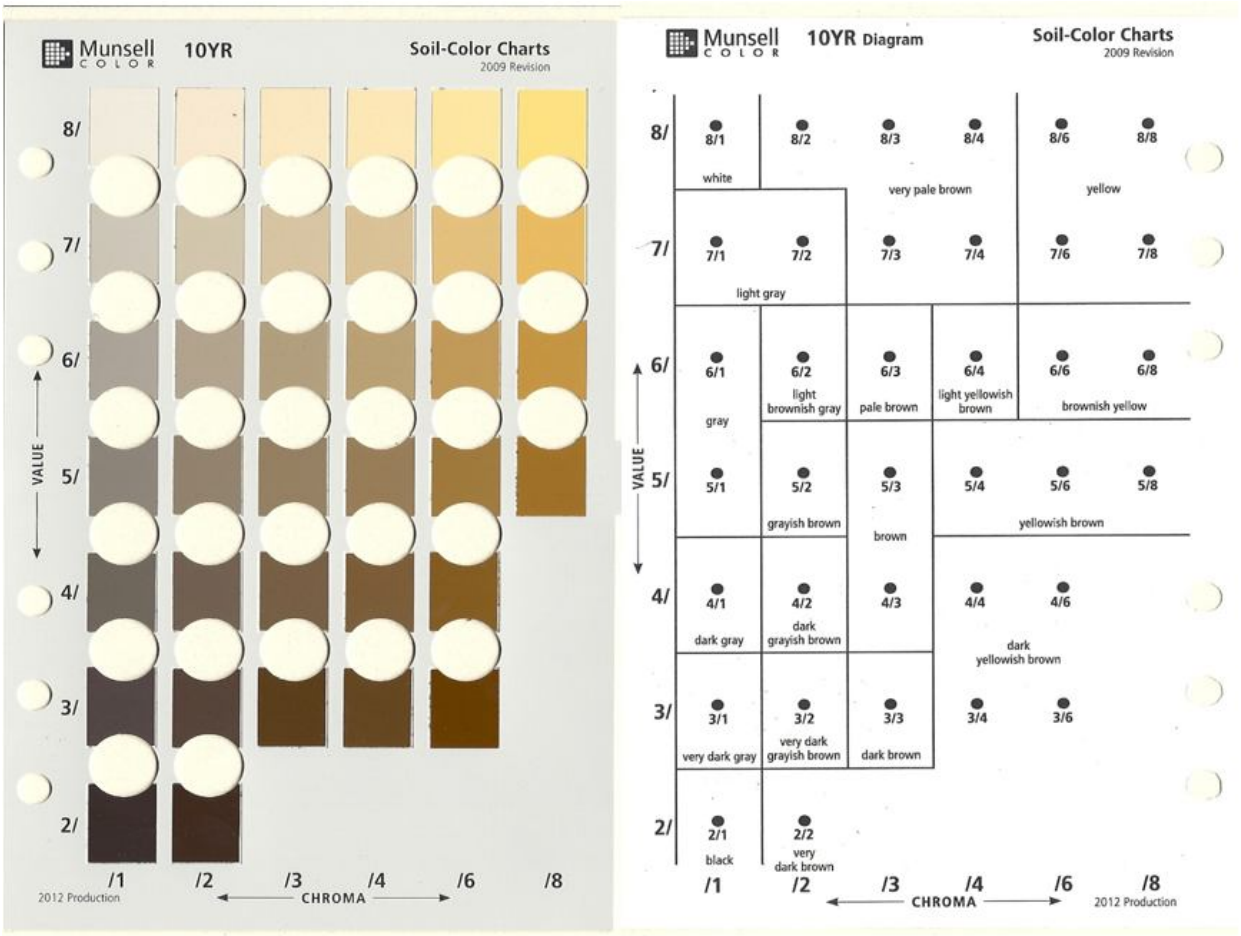

Fig. 3.3. Example of a page from the *Munsell Soil-Color Charts* (Munsell Color 2009) used in this study to assess and compare discoloration of bulk root systems on greenhouse-grown southern highbush blueberry plants that were either positive or negative for *Blueberry red ringspot virus* and inoculated or not inoculated with *Phytophthora cinnamomi*. Color chips of the same hue (wavelength) are arranged on a single page, organized with increasing value (lightness) from bottom to top and chroma (intensity) from left to right.

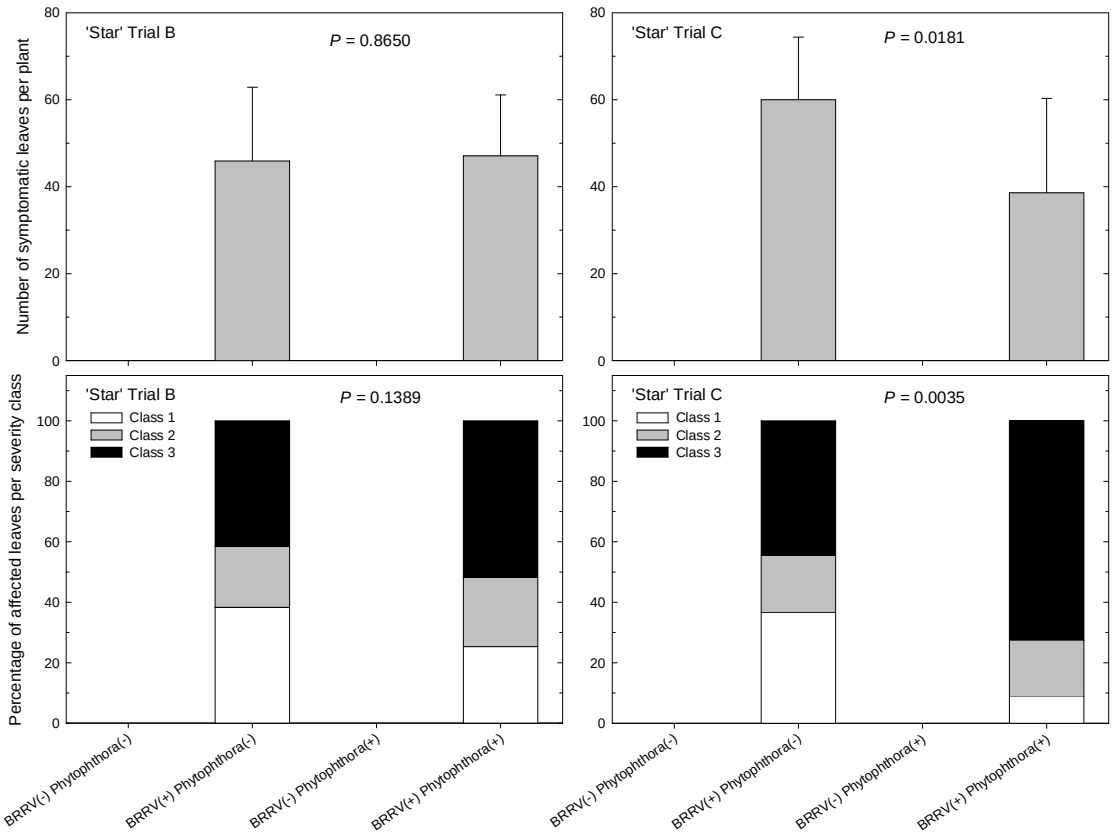

Fig. 3.4. Incidence (top row) and severity (bottom row) of foliar symptoms associated with *Blueberry red ringspott virus* (BRRV) in greenhouse-grown southern highbush blueberry plants that were either positive (+) or negative (-) for BRRV and inoculated (+) or not inoculated (-) with *Phytophthora cinnamomi*. Values are means of 10 plants (replicates) per treatment combination. Standard errors are shown for incidence only. *P*-values based on t-tests to compare BRRV-positive plants that were inoculated or not inoculated with *P. cinnamomi*.

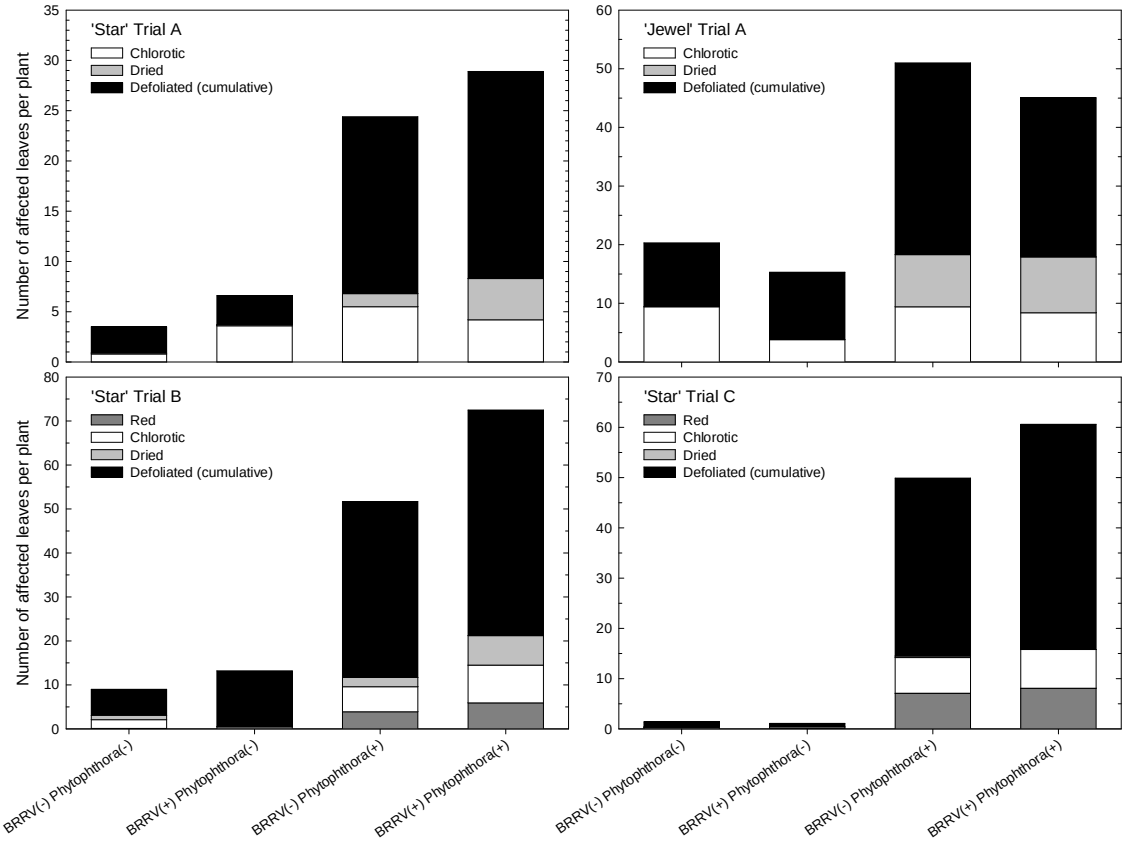

Fig. 3.5. Incidence of foliar symptoms associated with Phytophthora root rot in greenhouse-grown southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and inoculated (+) or not inoculated (-) with *Phytophthora cinnamomi*. Values are means of 10 plants (replicates) per treatment combination. *P*-values reported in Table 3.1.

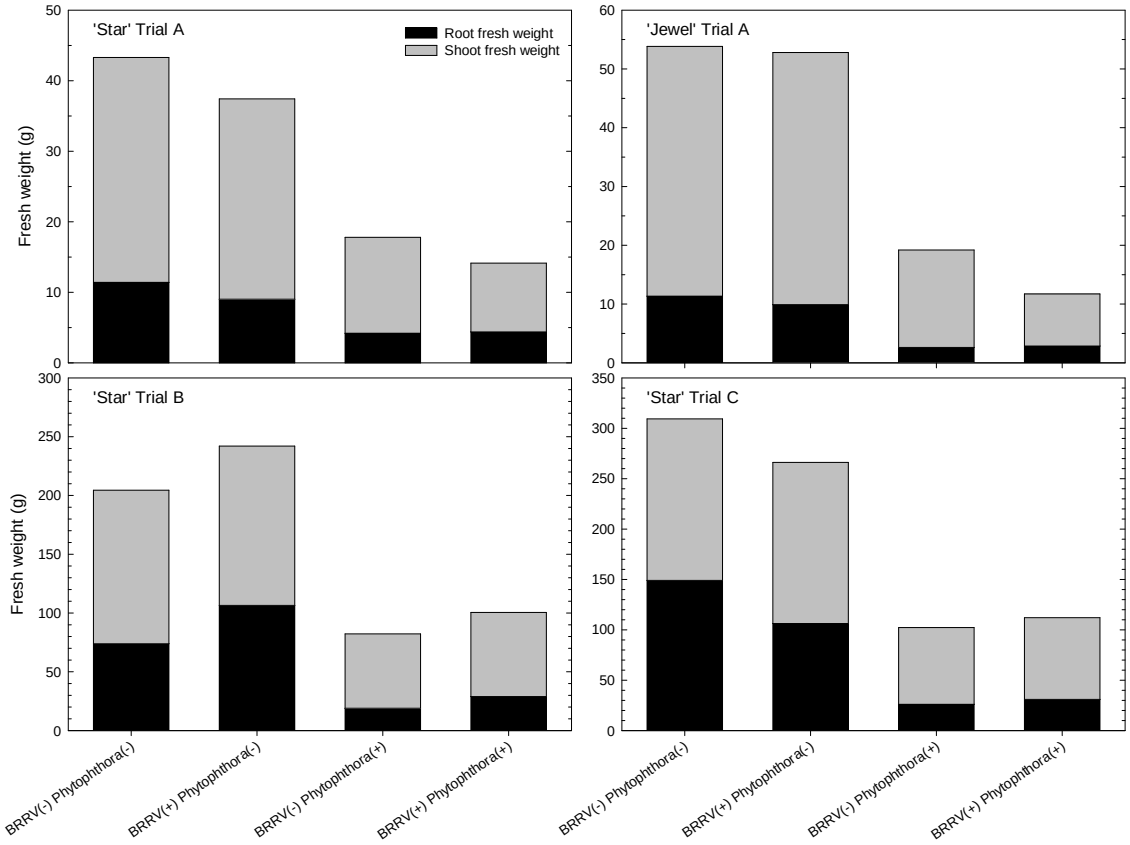

Fig. 3.6. Root and shoot fresh weights of greenhouse-grown southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and inoculated (+) or not inoculated (-) with *Phytophthora cinnamomi*. Values are means of 10 plants (replicates) per treatment combination. *P*-values reported in Table 3.1.

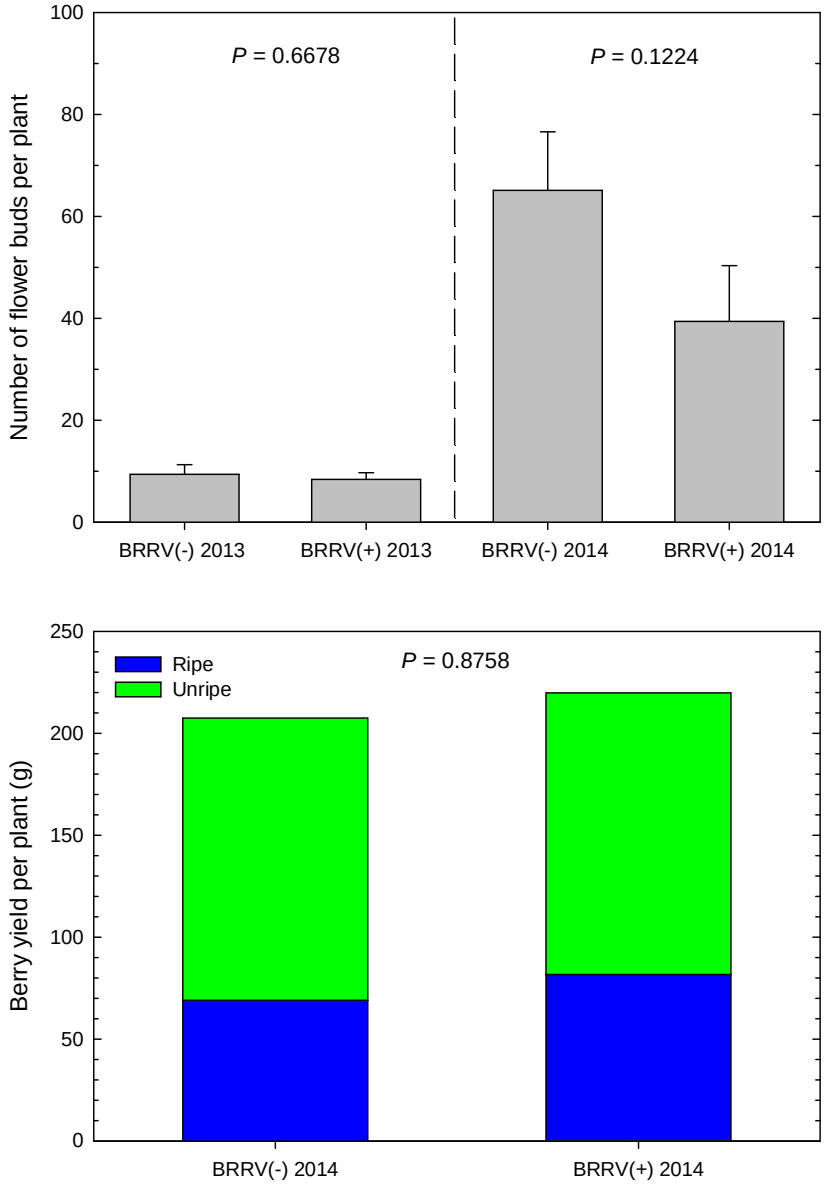

Fig. 3.7. Flower bud set (top) and total fruit yield (bottom) on Star southern highbush blueberry plants that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and transplanted into a field site where *Phytophthora cinnamomi* was present in the soil. No yield was obtained in 2013 because plants were stripped of all fruit to encourage plant establishment and root development. Values are means of 8 to 10 plants (paired replicates), and *P*-values are based on paired *t*-tests.

CHAPTER 4

EFFECTS OF *BLUEBERRY RED RINGSPOT VIRUS* AND ABIOTIC ROOT INJURY ON PLANT GROWTH AND YIELD OF SOUTHERN HIGHBUSH BLUEBERRY IN THE FIELD[1]

[1]Williford, L.A., Savelle, A.T., and Scherm, H. 2014. To be submitted to *Plant Health Progress*.

Effects of *Blueberry red ringspot virus* and abiotic root injury on plant growth and yield of southern highbush blueberry in the field

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ABSTRACT

Blueberry red ringspot virus (BRRV) occurs commonly in blueberry plantings in Georgia, but there is no information about interactions of the virus with other factors affecting plant health and yield. Here, we studied the interactive effects of BRRV and abiotic root damage on southern highbush blueberry. Field plants were either stunted due to root damage (presumed herbicide injury) or not stunted, and infected or not infected with BRRV as determined by symptoms and BRRV-specific PCR. Flower bud set and fruit yields were highest in BRRV-negative plants that were not stunted and lowest in stunted, BRRV-positive plants. BRRV alone reduced flower bud set but not fruit yields, whereas root damage alone reduced yields significantly. The combined effects of BRRV infection and stunting were additive but not synergistic with regard to yield reduction.

INTRODUCTION

The size of the blueberry industry in the southeastern United States has grown considerably during the past two decades, making the region one of the largest blueberry producers in the country. With the expansion of the industry has come an increase in the importance of fungal, bacterial, and viral diseases. Among blueberry viruses, *Blueberry red*

ringspot virus (BRRV) is most prevalent in in Georgia and other southeastern states. Indeed, a recent survey (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 14.9% (25 of 167) of fields sampled (Scherm et al. 2008a); virus presence was confirmed by BRRV-specific PCR assay (Polashock et al. 2009). To date in Georgia, BRRV has only been observed on southern highbush blueberry cultivars (*Vaccinium corymbosum* interspecific hybrids), but not on the native and more widely grown rabbiteye (*Vaccinium virgatum*) cultivars.

BRRV is thought to have limited impact on plant growth, and many infected blueberry bushes appear to produce a full crop (Martin et al. 2012), an assumption confirmed in Chapter 2. However, recent field observations by blueberry growers and extension agents have suggested that BRRV symptoms (and presumably associated yield losses) are exacerbated in situations where plants are stressed or co-infected with other plant pathogens, e.g., on sites that are waterlogged. In general, it is well established that certain environmental factors, such as drought stress (Schoeneweiss 1981), heat stress, or herbicide injury (Altman and Campbell 1977, Altman and Rovira 1989, Griffiths 1981) can greatly affect host predisposition, susceptibility, and symptom severity of plant diseases. Soil conditions in particular are key factors in disease development. In Chapter 3, we examined the interactive effects of BRRV and Phytophthora root rot on plant growth and disease intensity in greenhouse conditions. Here we determine the potential for interactive effects in BRRV-positive and BRRV-negative plants subjected to abiotic root injury, presumably caused by herbicide injury, in the field.

MATERIALS AND METHODS

Field site. The study was carried out in a southern highbush blueberry planting at the Bacon County Blueberry Research and Demonstration Farm near Alma, GA, in 2013 and 2014. The site consists of a 2288-plant block of mature Star plants in which symptoms of BRRV and plant stunting (characterized by reduced plant height and more compact growth habit) 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, canopy volume (width × depth × height) was recorded and leaf samples were collected for confirmation of BRRV by end-point PCR (Polashock et al. 2009). The average volume of stunted plants was 0.95 m^3, whereas that of non-stunted plants was 1.8 m^3.

Plant stunting was initially thought to be caused by Phytophthora root rot, but soil samples collected in May and October of 2013 and baited with *Camellia* leaf disks (Ferguson and Jeffers 1999) did not reveal any species of *Phytophthora*. Soil samples were also assayed for plant-parasitic nematodes, and counts revealed no differences in spiral, ring, stunt, or lance nematode densities per 100 cm^3 of soil. When entire root systems of stunted and control plants were excavated, the roots 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, with substantially reduced lateral expansion evident in some cases (Fig. 4.1), especially toward the row middles. Furthermore, malformation in the crown area was observed occasionally. When recent herbicide use at the site was

reviewed, a herbicide mixture of Chateau WDG (51% flumioxazin; Valent, Walnut Creek, CA), Simazine 4L (42.1% simazine; Drexel, Memphis, TN), and Surflan AS (40.4% oryzalin: United Phosphorus, Trenton, NJ) was found to have been applied with high-volume spray nozzles. Among these herbicides, root suppression and stunting has been associated especially with surflan (Nelson et al. 1983). As such, the stunting observed in this planting was attributed to abiotic herbicide injury on shallow roots or crowns.

Data collection and analysis. In February 2013 and 2014, ten shoots (15 to 20 cm long) per plant formed in the previous year were selected randomly and tagged on each of the 40 plants, and all flower buds were counted on these shoots. In 2013, three weekly fruit harvests were conducted on the test plants between 8 and 21 May, and separately on each of the previously tagged shoots on each plant. The first two harvests included only ripe, blue fruit, whereas the final harvest included all remaining fruit, weighed and counted separately for ripe and unripe (red or green) fruit. In 2014, three harvests were conducted similarly between 7 and 23 May.

Flower bud numbers and fruit yields were analyzed by two-way analysis of variance (PROC GLIMMIX in SAS v. 9.3; SAS Institute, Cary, NC) with BRRV and stunting (both recorded as presence or absence) as fixed effects and replication (block) as a random effect. Of particular interest was the presence of a significant ($P < 0.05$) interaction between BRRV and stunting, indicating synergistic interactions between the two factors.

RESULTS

Effects on flower bud set and fruit yield. In general, flower bud numbers were highest on BRRV-negative plants that were not stunted and lowest on BRRV-positive, stunted plants

(Fig. 4.2). In 2013, the effect of BRRV presence and stunting both were statistically significant ($P < 0.0001$), but their interaction was not (Table 4.1). Thus, BRRV and stunting had an additive effect rather than a synergistic effect on flower bud set. In 2014, the BRRV main effect remained statistically significant, but stunting was not statistically significant, nor was there an interaction between the two factors (Table 4.1).

In whole-plant harvests over 2 years, fruit yields (Fig. 4.3) were highest on BRRV-negative plants that were not stunted, followed by those of BRRV-positive plants that were also not stunted. In both years, the stunting main effect was highly significant in reducing fruit yield, whereas the BRRV main effect was not (Table 4.1). There was also no significant interaction between BRRV and stunting relative to these yield variables, indicating that stunted and non-stunted plants responded similarly to BRRV infection. The only yield-related variable (other than flower bud set) affected by BRRV was the weight of unripe fruit remaining at the last harvest (Table 4.1), which was significantly lower for BRRV-positive plants than for BRRV-negative plants (Fig. 4.3). Thus, with total yield remaining unchanged and the yield of unripe fruit at the last harvest reduced, BRRV compressed the period of fruit ripening. Indeed, when ripe fruit yield was examined over the three harvest dates per year (Fig. 4.4), there was a tendency each year of fruit from BRRV-positive plants to ripen slightly earlier than that from the BRRV-negative plants. For example, the first harvest in 2013 included 59.7% of the total ripe fruit weight on BRRV-positive, non-stunted plants, which was greater than the corresponding value of 52.3% on BRRV-negative, non-stunted plants. Average berry weight was unaffected by BRRV, but reduced significantly by stunting (Table 4.1).

DISCUSSION

Circumstantial evidence suggested that root damage and associated plant stunting at the test site was caused by aggressive use of a herbicide cocktail over multiple years. All of the herbicide active ingredients used at the site have been linked to potential phytotoxicity in perennial crops (Bellinder et al. 2010, Wooten et al. 2003, Ryan et al. 1981, Nelson et al. 1983), although most cause aboveground rather than belowground symptoms. However, oryzalin, the active ingredient in Surflan, has been associated with root suppression and stunting (Nelson et al. 1983), similar to the symptoms observed in the present study. Blueberries are shallow-rooted, rendering them particularly susceptible to herbicide injury. The scattered nature of the stunting symptoms observed at the test site could have been due to variations in the depth of the bark mulch layer above the roots or other soil-related variations, although this was not explored in detail in the present study.

Regardless of the ultimate cause of the root damage and associated stunting, stunting always caused significant reductions in yield-related variables, with the only exception being flower bud set in 2014 (Table 4.1). As such, the abiotic root damage observed in this planting had important yield implications. Therefore, it is critical to further investigate the cause(s) of the root damage and develop means for its mitigation. For example, new bark mulch should be applied to the planting to improve root coverage and encourage root and plant growth, and a more careful weed management strategy should be employed.

In contrast, BRRV caused no significant reductions in fruit yield, although flower bud set was reduced in both years. Thus, similar to Chapters 2 and 3, there was no significant direct effect of BRRV on fruit yield. Also, similar to these previous Chapters, the yield of unripe fruit at the last harvest was decreased, supporting our previous conclusion that fruit ripening is less

protracted in BRRV-affected Star. With the exception of unripe fruit yield in 2013, there were no significant interactions between BRRV and abiotic root damage, showing that the two factors do not act synergistically. This confirms the results of Chapter 3 where we observed no synergistic interactions between BRRV and biotic root damage (caused by Phytophthora root rot).

Given the relatively widespread distribution of BRRV in southern highbush blueberry in Georgia (Scherm et al. 2008), it is fortunate that there are no consistent yield losses associated with the disease, neither by the virus alone (Chapter 2), nor when it co-occurs with biotic (Chapter 3) or abiotic (Chapter 4) root damage. Since BRRV is transmitted only by vegetative propagation (Caruso and Ramsdell 1995), 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. As blueberry acreage is now leveling off 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 their yield implications.

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Table 4.1. *P*-values from analyses of variance to compare flower bud numbers and fruit yield on Star southern highbush blueberry plants in the field that were either positive or negative for *Blueberry red ringspot virus* (BRRV) and affected or not affected by stunting associated with abiotic root damage in 2013 and 2014.

Effect	Bud number	Total yield	Ripe fruit yield	Unripe fruit yield	Berry weight
2013					
BRRV main effect	**<0.0001**	0.0550	0.1833	**<0.0001**	0.4977
Stunting main effect	**<0.0001**	**0.0004**	**0.0007**	**<0.0001**	**0.0020**
Interaction	0.0572	0.9030	0.5061	**0.0056**	0.4349
2014					
BRRV main effect	**<0.0001**	0.1146	0.1377	**0.0021**	0.5354
Stunting main effect	0.9439	**0.0005**	**0.0006**	**0.0244**	**0.0010**
Interaction	0.3352	0.7989	0.7696	0.3328	0.9943

[a] Flower bud numbers determined on ten arbitrarily selected shoots per plant in late winter of each year. All other yield variables determined based on whole-plant harvests over time. Unripe fruit yield refers to berries that were immature (red or green) on the final harvest date. Berry weight determined based on 50 fruit per replicate.

Fig. 4.1. Examples of root systems of Star southern highbush blueberry plants in the field trial to determine interactions between *Blueberry red ringspot virus* and abiotic root damage. Root systems from unaffected plants (left) and those from stunted plants (center and right). Stunted plants had a smaller root system that expanded within-row but not across-row (center). Crown malformation was observed occasionally (right).

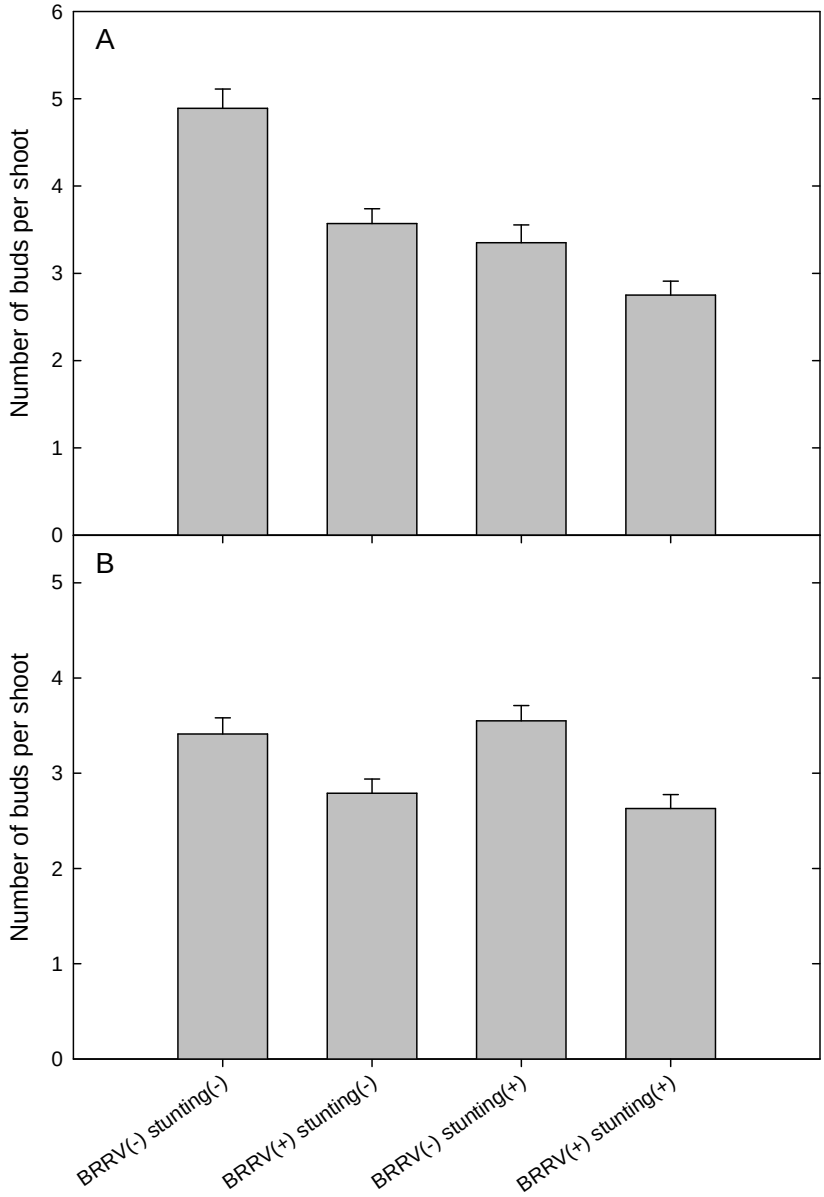

Fig. 4.2. Flower bud set, determined in February of 2013 (A) and 2014 (B) on Star southern highbush blueberry plants in the field that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and affected (+) or not affected (-) by stunting associated with abiotic root damage. Values are means and standard errors of 9 or 10 plants (replicates) per treatment combination, each with 10 shoots assessed per plant. Statistical analysis presented in Table 4.1.

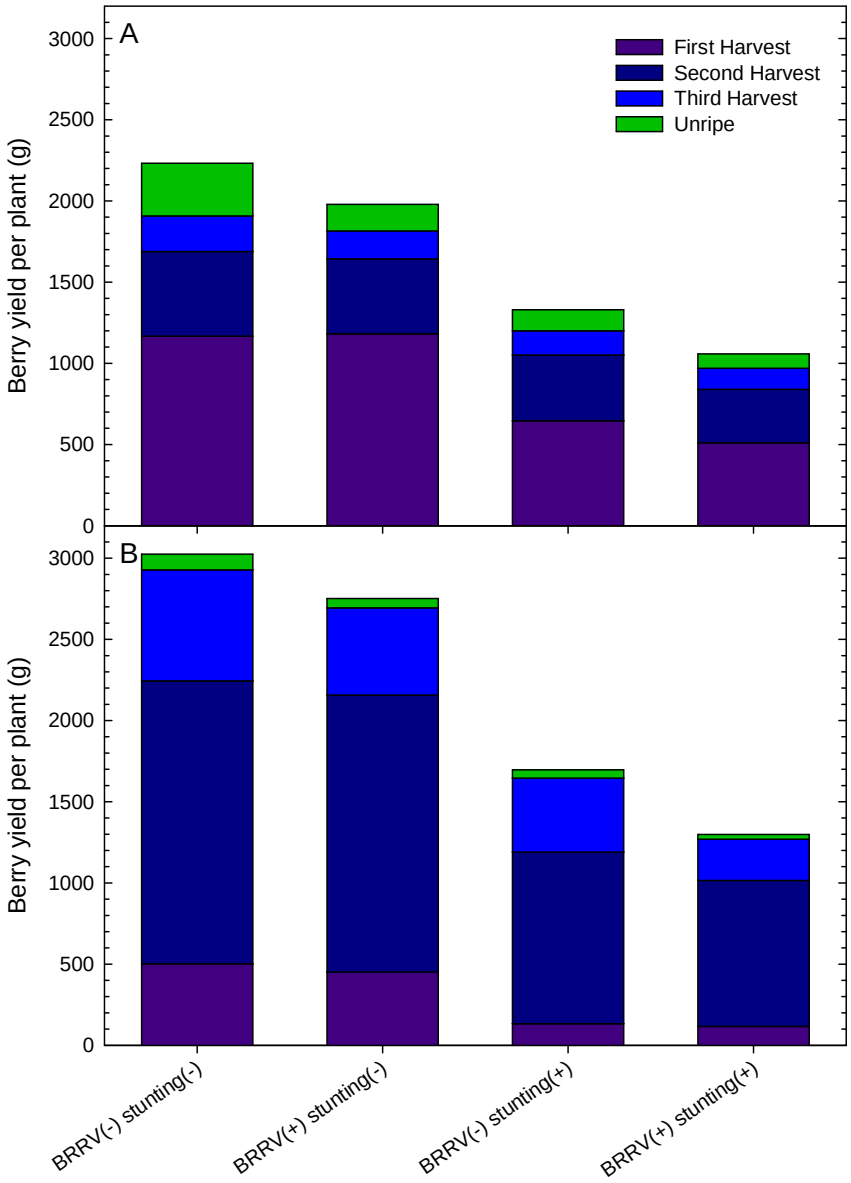

Fig. 4.3. Fruit yield, determined in three successive harvests in 2013 (A) and 2014 (B) on Star southern highbush blueberry plants in the field that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and affected (+) or not affected (-) by stunting associated with abiotic root damage. Values are means of 9 or 10 plants (replicates) per treatment combination. Unripe yield corresponds to berries that were still immature (red or green) on the final harvest date. Statistical analysis presented in Table 4.1.

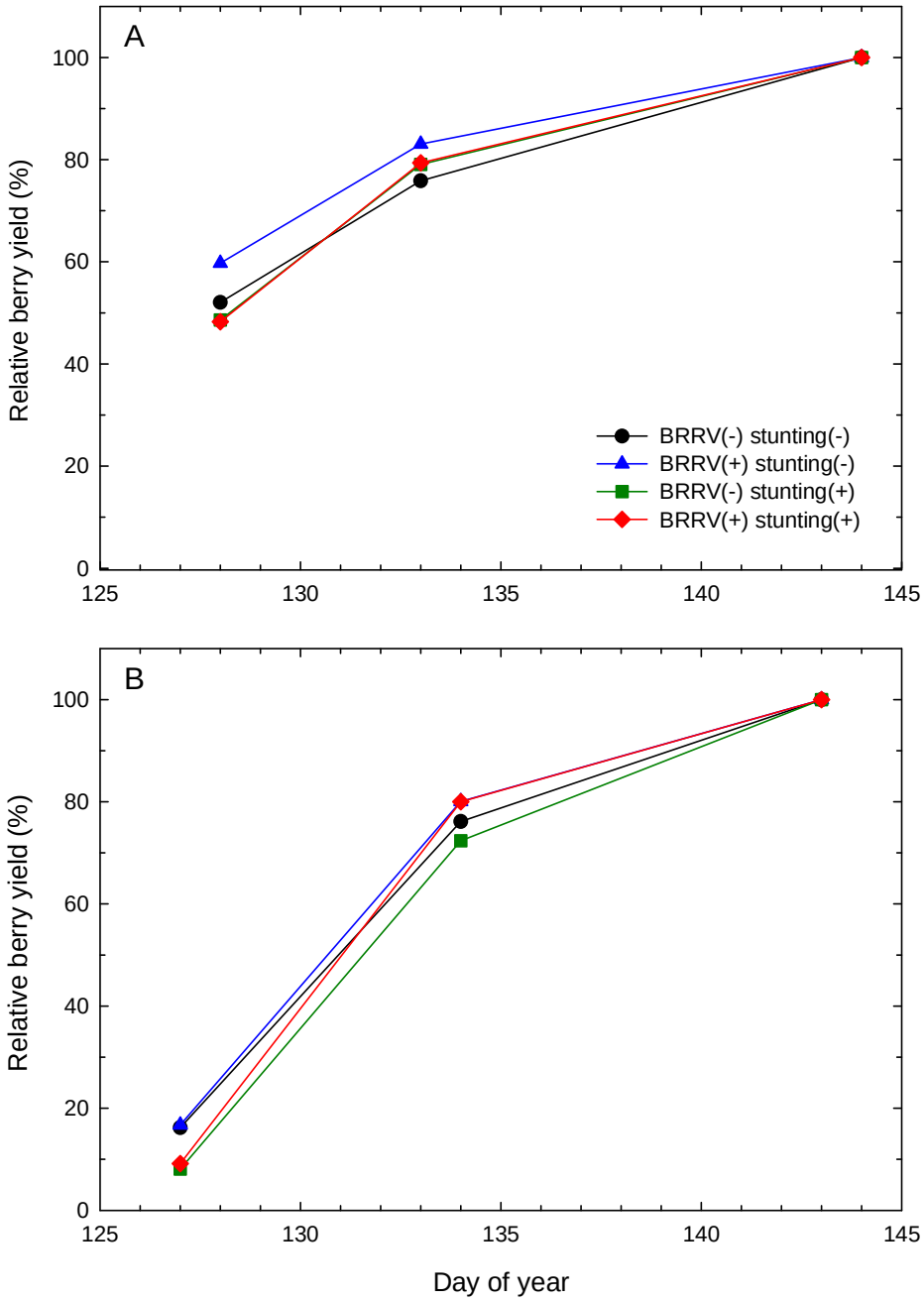

Fig. 4.4. Cumulative fruit yield, determined in three successive harvests in 2013 (A) and 2014 (B) on Star southern highbush blueberry plants in the field that were either positive (+) or negative (-) for *Blueberry red ringspot virus* (BRRV) and affected (+) or not affected (-) by stunting associated with abiotic root damage. Total yield (Fig. 4.3) was set to 100% to allow for better comparison of positive and negative plants. Values are means of 9 or 10 plants (replicates) per treatment combination.

CHAPTER 5

DISCUSSION AND CONCLUSION

Research in this thesis addressed the lack of quantitative effects on fruit yield and berry maturation associated with infection by *Blueberry red ringspot virus* (BRRV), a widely distributed pathogen in southern highbush blueberry (SHB) plantings in the southeastern United States. In Chapter 2, a 3-year study in controlled conditions (on container-grown plants outdoors) and with frequent fruit harvest was conducted to obtain more precise information about yield losses associated with the disease and its impact on fruit ripening. In Chapters 3 and 4, the potential for interactions between BRRV and biotic (*Phytophthora cinnamomi*) and abiotic (presumed herbicide damage) soil factors was addressed. The overall aim of this study was to contribute to a more detailed and more comprehensive understanding of the yield implications of BRRV on SHB. Results consistently showed that BRRV is a benign virus, causing no consistent yield losses either alone or on combination with biotic or abiotic root damage

Multiple harvests over time were conducted in the experiments in Chapter 2 to fill critical knowledge gaps about the direct effect of BRRV infection on fruit maturation and yield. Results revealed no effects of BRRV on total fruit yield on two SHB cultivars. This supports anecdotal reports of limited yield impact from infection by BRRV (Martin et al. 2009) but conflicts with a study from Michigan reporting a 25% crop loss in BRRV-infected northern highbush blueberry plants (cultivar Blueray) (Gillet 1988). The latter study was not repeated or conducted with other cultivars, so it is unclear whether the significant yield loss was unique to Blueray plants or to the year when the investigation was carried out. The only other previous experimental study on

BRRV-associated yield losses in SHB (Scherm et al. 2008) also was conducted in only one year and found no significant effect of BRRV status (positive or negative) on total yield of field-grown Star pants when whole-plant yields were determined. Combined with our data, it can be concluded that BRRV does not cause significant yield losses in SHB, at least on the cultivars examined to date.

On cultivar Star, berry maturity was slightly advanced on BRRV-positive plants in all years, whereas no such effect was observed on the cultivar Jewel. Evidence for less protracted fruit ripening on BRRV-infected Star also was obtained in the experiments in Chapters 3 and 4. Understanding the economic relevance of this slight advance in fruit maturation, and its underlying physiological mechanisms, will require further study.

In Chapter 3, potential interactions between BRRV and *P. cinnamomi*, brought to our attention by growers and extension agents based on field observations, were examined in greenhouse and field conditions. Results demonstrated that BRRV and *P. cinnamomi* do not act synergistically to affect symptom severity, plant growth, flower bud set, or fruit yield. Similar conclusions were obtained in Chapter 4, where interactions between BRRV infection and presence or absence of abiotic root damage, presumed to be caused by herbicide injury based on symptoms and herbicide use history, were examined in the field. In contrast to BRRV, both Phytophthora root rot and abiotic root damage alone caused significant yield losses and plant damage. Therefore, it is critical to further investigate the cause(s) of the abiotic root damage and develop means for its mitigation. For example, assuming these symptoms were indeed caused by herbicide injury, new bark mulch should be applied to the plants to improve root coverage and encourage root and plant growth, and a more careful weed management strategy should be employed.

Given the relatively widespread distribution of BRRV in Georgia (Scherm et al. 2008), it is fortunate that there are no consistent yield losses associated with the disease, neither by the virus alone, nor when it co-occurs with biotic or abiotic root damage. Since BRRV is transmitted only by vegetative propagation (Caruso and Ramsdell 1995), no further spread is expected in established plantings. As such, no additional practices to manage the disease in the field, such as 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. As blueberry acreage is now levelling off 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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