FUNGAL INFECTION AND POSTHARVEST QUALITY OF BLUEBERRY FRUIT IN RELATION TO BERRY FLESH TYPE, HARVEST METHOD, AND POSTHARVEST BIOFUMIGATION

by

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(Under the Direction of Harald Scherm)

ABSTRACT

Postharvest fungal decay is a major concern in blueberry production. Because the risk of infection is increased by fruit bruising, which in turn is increased by machine-harvest, fruit from early-maturing, high-value southern highbush blueberries are not harvested mechanically for the fresh market. This may change fundamentally with the advent of southern highbush genotypes with crisp-textured berries, i.e., fruit with qualitatively firmer flesh and/or skin. In field experiments, machine-harvested crispy fruit had the same or lower natural decay incidence as hand-picked conventional fruit after cold storage. Across cultivar and harvest method treatments, decay incidence was inversely related to fruit firmness. Several plant essential oils were evaluated as postharvest biofumigants to manage fungal decay during cold storage. The plant oil-derived fungicide Sporatec, applied as a biofumigant, reduced decay significantly in most cases. However, biofumigation resulted in significant negative impacts on sensory attributes and no beneficial effects on antioxidant activity of treated berries.

INDEX WORDS: *Alternaria*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Colletotrichum*, postharvest decay, disease management, blueberry, essential oil

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DEDICATION

I would like to dedicate this work to my mother and father for being the source of my inspiration and pride. They have always been there giving me love and support.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Importance and types of blueberries in Georgia. Demand for blueberries has surpassed supply during the past decade, primarily as a result of the widely publicized health benefits of blueberry fruit, which include anti-aging and anti-cancer properties, among others (Beattie et al. 2005; Juranić and Žižak 2005; Brazelton and Strik 2007). Blueberries are now grown worldwide, and increased demand for fresh and processed fruit has resulted in an expansion of planted area as well as higher net returns for growers (Brazelton and Strik 2007). Worldwide, the United States rank first in the production of blueberries, supplying 166,786 t in 2009 with a farm gate value of $507 million (Anonymous 2010). Nationwide, blueberry acreage has increased by 58% in the past 8 years, from 16,341 ha in 2001 to 25,807 ha in 2009 (Anonymous 2002; Anonymous 2010). The state of Georgia ranks second in the nation in total acreage (16%) and fourth to fifth in total production, representing a farm gate value of $102 million at the time of this writing (Anonymous 2010; Boatright and McKissick 2010b) and making blueberry the state's most important fruit crop. There are two species of blueberry grown in Georgia, with the majority of the area (81%) devoted to rabbiteye blueberry (*Vaccinium virgatum* = *V. ashei*) and the remainder planted to southern highbush blueberry (*V. corymbosum* interspecific hybrids) (Boatright and McKissick 2010a). However, despite having lower acreage, one-third of Georgia's 2009 blueberry

farm gate value resulted from sales of southern highbush fruit (Boatright and McKissick 2010a).

Southern highbush blueberry (SHB) cultivars are developed by crossing northern highbush blueberry (*V. corymbosum)* with native southern species such as *V. darrowii* (Lang 1993). In contrast to rabbiteye blueberries, SHB cultivars have a lower chilling requirement (Krewer and NeSmith 2006) and earlier harvest. In Georgia, they are grown successfully in areas with sandy soils naturally high in organic matter, in sandy soils amended with pine bark, or in high-density culture on pine bark beds (Fonsah et al. 2006). There has been a considerable increase in SHB acreage since the mid-1990s, mostly because their fruit ripen earlier than those of rabbiteye blueberries, and earlier fruit satisfies demand for blueberries in a market window which occurs after the late-winter imports from South America and before the earliest northern highbush blueberries are harvested domestically in North Carolina and New Jersey (Fonsah et al. 2006; Scherm and Krewer 2003). Thus, the main advantage of growing SHB cultivars, compared with rabbiteyes, is the considerably higher price received for their early-maturing fruit. Major disadvantages of growing SHB are their greater demand for soil quality and agrichemical inputs, the increased risk of crop loss due to spring freezes associated with their early bloom time, their greater susceptibility to diseases and insect pests, and the increased risk of fruit bruising during harvest due to their generally softer fruit texture.

Mechanical harvest of blueberries. Blueberries can be both hand- and machine-harvested. Hand-picking is labor-intensive (1500 h/ha) (Brown et al. 1996), costs $1.00 to 1.76/kg in southern production areas (Safley et al. 2005), and is mostly

used for fruit intended for the fresh market where high fruit quality is critical (NeSmith et al. 2002). In contrast, machine-harvesting is considerably more labor-efficient (25 h/ha) (Peterson and Brown 1996), costs $0.26 to 0.40/kg (Safley et al. 2005), and is used primarily for late-season harvesting of rabbiteye blueberries when lower fruit prices render hand-harvesting uneconomical (NeSmith et al. 2002). Machine-harvesting, however, is problematic because it can result in bruising and loss of firmness to the fruit, which leads to decreased shelf life and increases the risk of postharvest decay caused by fungal plant pathogens (Mainland et al. 1975; NeSmith et al. 2002). As a result, most machine-harvested fruit is processed (NeSmith et al. 2002), resulting in lower prices than those of blueberries sold fresh ($1.00 vs. 4.34/kg) (Anonymous 2010).

Mechanical blueberry harvesters have been available for nearly half a century. The first harvesters were designed in the early 1960s and have been improved subsequently to reduce fruit and bush damage and to reduce fruit losses (Mainland 1993; Peterson and Brown 1996; Peterson et al. 1997; Takeda et al. 2008). Specifically, a major concern with mechanical harvesting is fruit bruising. This can occur during direct contact between the berries and the harvester's beater rods, when detached berries drop into the harvester's catch pans, when the berries move from the catch pans to the conveyor belt, and when they drop from the conveyor belt into the fruit lugs. The risk of bruising is greater for longer falling distances onto harder surfaces of the harvester (Ballinger et al. 1973). Bruising reduces both external and internal fruit quality by removing the visually appealing fruit surface wax and by reducing fruit firmness (Dale et al. 1994; Mainland et al. 1975; NeSmith et al. 2002). Internal damage results in cellular water leakage and appears as a water-soaked area in the fruit flesh (Labavitch

et al. 1998). This type of damage is the main reason machine-harvesting blueberries for the fresh market in the southeastern United States has traditionally been limited to the firmer, later-maturing, low-value rabbiteye cultivars (Funt et al. 1998; Takeda et al. 2008), whereas the softer, early-maturing, and high-value SHB fruit have almost exclusively been hand-harvested. Indeed, current machine-harvesting capabilities are not yet able to produce high-quality SHB fruit for the fresh market (NeSmith 2009). However, due to shortages in labor and associated increases in labor costs (Fonsah et al. 2004), mechanical harvesting will likely become a necessity for SHB in the near future.

Several drawbacks, in addition to fruit bruising, must be overcome for machine-harvesting to become a viable option for the early fresh fruit market. These include the potential for significant ground losses, excessive green fruit detachment, delayed harvest, ripe fruit remaining in the bush, and mechanical damage to the bush (Ballinger et al. 1973; Mainland et al. 1975; NeSmith et al. 2002; Takeda et al. 2008). Ground loss occurs when the harvester's beater rods detach the fruit but the catch pans fail to collect them, usually because the fruit drop too close to or within the crown of the bush. These losses, which typically amount to 20 to 30%, can be reduced via cultural practices such as pruning to vase shape or trellising (Mainland 1993; Strik and Buller 2002; van Dalfsen and Gaye 1996; Peterson et al. 1997) and also through improvements in harvester technology (Takeda et al. 2008). Green fruit detachment results in reduced overall yield and increased sorting costs (Mainland et al. 1975; Takeda et al. 2008; van Dalfsen and Gaye 1999). Delayed harvest occurs because producers want to reduce the percentage of green berries in the harvested product, which necessitates the first

harvesting by machines to occur 5 to 7 days later than hand-harvesting would generally occur. However, this practice is not desirable in SHB because earlier harvested fruit garner a higher market price. Skipping ripe fruit is also a problem in mechanical harvesting, whereby fruit in the center of the bush may be out of the reach of the harvester's beater rods; this may result in overripe fruit during the next harvest when these fruits are more easily detached, reducing overall fruit quality (Mainland 1993). Again, this type of loss can be reduced by pruning and/or trellising. Finally, mechanical damage to the blueberry bush by the harvester is also problematic because it creates entry wounds for pathogens such as *Botryosphaeria* spp. causing stem blight (Milholland 1972; Wright and Harmon 2010), which can ultimately cause plant death. This damage can be reduced by proper pruning and improved harvester technology (Takeda et al. 2008). Overall, a systems approach integrating cultural and engineering practices is needed to reduce these potential sources of losses associated with machine-harvesting.

Potential for mechanical harvest of novel SHB genotypes. Berry firmness is a key attribute for machine-harvesting blueberry fruit successfully (NeSmith 2009). Current SHB cultivars grown in Georgia, such as the industry standards Star (Lyrene 1998) and Emerald, have lower fruit firmness than rabbiteye cultivars and are therefore not suitable for machine-harvesting. Other examples of SHB cultivars with similar levels of fruit firmness are Scintilla (Lyrene 2008a) and Primadonna (Lyrene 2009a). Recently, the University of Florida blueberry breeding program has developed novel "crisp-textured" SHB cultivars (Padley 2005), such as Bluecrisp (Lyrene 1999) and Sweetcrisp (Lyrene 2009b). These cultivars have firmer berries and better storage shelf life than

conventional SHB cultivars, due primarily to the greater springiness of the outer fruit cuticle (Padley 2005). Other recent Florida cultivar releases, Farthing (Lyrene 2008b) and Meadowlark, possess a semi-crisp berry type that is firmer than that of conventional cultivars but is not as springy as Sweetcrisp or Bluecrisp. The firmer fruit texture of these new cultivars may allow for machine-harvest with reduced bruising, thereby increasing fruit quality and decreasing risk of postharvest fungal decay.

Postharvest decay of blueberries. Several studies have documented postharvest decay of rabbiteye and northern highbush blueberries as an important constraint to blueberry production across the United States (Milholland and Jones 1972; Cappellini et al. 1982; Daykin and Milholland 1984; Makus and Morris 1993; Smith et al. 1996; Schilder et al. 2002). Various fungal pathogens can cause postharvest disease on blueberry fruit, of which *Colletotrichum* spp. (ripe rot), *Alternaria tenuissima* and other *Alternaria* spp. (Alternaria fruit rot), and *Botrytis cinerea* (gray mold) are the most commonly reported. Other fungal genera capable of causing postharvest decay of blueberries are *Aspergillus*, *Aureobasidium*, *Catenophora*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pestalotia*, and *Rhizopus* (Ceponis and Cappellini 1979; Tournas and Katsoudas 2005; Barrau et al. 2006). There are few reports on postharvest fruit decay specifically on SHB, and it is known that SHB cultivars generally are more susceptible than rabbiteye cultivars (Miller et al. 1993; Perkins-Veazie et al. 1994). *Colletotrichum* spp. and *Botrytis* spp. were the main decay-causing organisms on SHB cultivars Gulfcoast and Sharpblue grown in Louisiana (Lang and Tao 1992); *B. cinerea* in Florida (Harmon 2004); and *Colletotrichum*, *Botrytis*, *Alternaria*, and *Monilinia* spp. in Spain (Barrau et al. 2006). Ripe rot caused by *Colletotrichum* spp. is a pre-harvest as

well as postharvest problem and exhibits itself as sunken areas on the fruit surface, supporting orange-colored spore masses (Milholland 1995). *Alternaria* spp. produce greenish-olive mycelia and conidial masses on the fruit surface (Wright et al. 2004). The fungus can also produce mycotoxins in blueberries (Stinson et al. 1980). Gray mold is characterized by gray-colored mycelia and conidial sporulation present on the fruit (Bristow and Milholland 1995). The incidence of postharvest decay may be exacerbated by various factors, including machine-harvesting, storage at higher temperature for long periods, infestation of handling surfaces, wetness of the stem scar, and addition of moisture (Cline 1996; Gillett and Schilder 2009; Mainland et al. 1975).

Postharvest decay management. Several cultural practices and chemical control methods can reduce postharvest decay. For example, postharvest decay commonly starts at the stem scar and is usually higher on stem-less berries than on berries with intact pedicels (Ballinger et al. 1978). However, berries with the stem attached are not marketable. Pre-cooling after harvest is also effective in reducing fungal decay because it eliminates field heat that would otherwise promote fungal growth (Bounous et al. 1997; Ceponis and Cappellini 1979; Ceponis and Cappellini 1983). Other methods to control postharvest disease development include pre-harvest fungicide sprays (Krewer 2010; Milholland and Jones 1972), postharvest chemical dips (Ceponis and Cappellini 1978), and CO_2-enriched storage atmosphere (Ceponis and Cappellini 1983). Pre-harvest fungicide sprays are not reliable in reducing fungal decay, and fungicides such as Captan, Botran, and Benomyl can leave visible residues on the fruit surface (Milholland and Jones 1972). Switch (cyprodinil + fludioxonil) and Pristine (pyraclostrobin + boscalid) are recommended as foliar sprays during bloom, petal fall,

and/or pre-harvest stages to control postharvest diseases but should be coupled with proper postharvest handling and processing to achieve adequate control (Krewer 2010). As postharvest dips, experimentally evaluated fungicides also leave a heavy residue on the fruit surface when they are applied, and sodium hypochlorite washes off the desirable surface bloom (waxy layer), the presence of which is an important quality attribute for fresh market fruit (Ceponis and Cappellini 1978). Although a combination of postharvest cooling and enrichment of storage atmosphere with CO_2 generally provide satisfactory decay control (Ballinger et al. 1973), other means to control blueberry fruit diseases for the fresh market are needed.

Microbial contamination of blueberries. Similar to other types of fresh produce, blueberries are prone to microbial contamination during growing, harvesting, and processing (Tournas and Katsoudas 2005). Indeed, fresh blueberry consumption was linked to an outbreak of listeriosis in Connecticut in 1984 (Ryser 1999). A more recent outbreak was reported in New Zealand where hepatitis A infections were associated with consumption of fresh blueberries, with the source likely being infected food handlers or fecal contamination of groundwater (Calder et al. 2003). Food-borne illnesses caused by *Salmonella* and *E. coli* O157:H7 have been most problematic in fresh produce and were responsible for 50 and 20% of fresh produce-related outbreaks in the United States from 1992 to 2002, respectively (Lynch et al. 2006). More recently, four multistate outbreaks of *Salmonella* infections associated with raw tomato consumption sickened at least 459 people in 21 states during 2005 and 2006 (Anonymous 2007). There have also been outbreaks of *E. coli* O157:H7 in bagged spinach in 2006, and of *Salmonella* on tomato and jalapeno pepper in 2008 (Stuart

2008). While rigid microbial safety standards are currently in place for processed blueberry products, contamination in fresh market berries is also a concern (Popa et al. 2007). The impacts of mechanical harvesting on levels of microbial contaminants are currently unknown. The use of mechanical harvesting could either decrease microbial contamination because there is less handling of fruit by workers, or increase contamination by creating microbial attachment sites via bruising, cracking, and stem-tearing. Thus, it is important to assess microbiological loads on hand- vs. machine-harvested fruit of both conventional and the new crisp-textured SHB cultivars.

Essential oils as biofumigants for postharvest decay control. Plant essential oils are concentrated, aromatic compounds extracted from a variety of plant species that contain volatile active ingredients with pronounced medicinal, antimicrobial, and antioxidant properties. For example, *p*-cymene is constituent of essential oils from cumin and thyme (Ultee et al. 2000). Linalool is a terpene alcohol naturally found in many plant species belonging to the families *Lamiaceae* (mint), *Lauraceae* (rosewood), and *Rutaceae* (citrus) (Casabianca et al. 1998; Lewinsohn et al. 2001). Several such plant essential oils have been shown to suppress plant pathogens. For example, clove oil, an ingredient of the contact biofungicides Sporan and Sporatec, has documented antifungal properties (Kishore et al. 2007). In addition, such oils have been shown to inhibit pathogenic bacteria such as *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* (Oussalah et al. 2005). Furthermore, a recent study by Wang et al. (2008) showed that volatile compounds derived from carvacrol, anethole, perillaldehyde, *p*-cymene, and linalool were effective in preventing blueberry fruit decay, and three compounds

(carvacrol, anethole and perillaldehyde) were shown to increase antioxidant levels of biofumigated fruit (Wang et al. 2008).

Based on the above considerations, the objectives of my thesis were to compare conventional and crisp-textured SHB genotypes after hand- and machine-harvest with respect to microbial contamination on the fruit surface at harvest and subsequent development of postharvest decay, for both natural and artificial inoculation. In a separate study, the effects of postharvest biofumigation with plant essential oils during cold storage on fungal decay, sensory quality, and antioxidant activity of treated berries were also studied to assess the potential of biofumigation as a postharvest disease management tactic.

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CHAPTER 2

FUNGAL INFECTION AND POSTHARVEST QUALITY OF SOUTHERN HIGHBUSH BLUEBERRY FRUIT IN RELATION TO BERRY FLESH TYPE AND HARVEST METHOD[1]

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Fungal Infection and Postharvest Quality of Southern Highbush Blueberry Fruit in Relation to Berry Flesh Type and Harvest Method

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ABSTRACT

Postharvest decay, incited by various fungal pathogens, is a major concern in most blueberry production areas of the United States. Because the risk of infection is increased by fruit bruising, which in turn is increased by machine-harvest, it has not been possible to mechanically harvest fresh-market fruit from early-maturing, high-valued, but soft-textured southern highbush blueberries (SHB). This could change fundamentally with the recent development of SHB genotypes with crisp-textured berries, i.e., fruit with qualitatively firmer flesh and/or skin. Four replicate row sections of four SHB genotypes having crispy fruit and four with conventional fruit were either hand-picked or machine-harvested at a commercial blueberry farm in northern Florida in April 2009 and May 2010. Harvested fruit were sorted, packed, and placed in cold storage (2°C) for up to 3 weeks. Average counts of total aerobic bacteria, yeasts and molds, coliforms, and *E. coli* on fruit samples from the 0-day cold storage period were below commercial tolerance levels, except for selection FL 01-248 (conventional berry type) in 2010. In both years, natural decay incidence following cold storage was lowest for hand-harvested crispy fruit and highest for machine-harvested conventional fruit. Interestingly,

machine-harvested crispy fruit had the same or lower decay incidence as hand-picked conventional fruit. Across all treatments, natural decay incidence was inversely related to fruit firmness. In separate experiments, samples from the 0-day cold storage period were inoculated at the stem end with *Alternaria alternata*, *Botrytis cinerea*, or *Colletotrichum gloeosporioides*, and fruit decay was assessed after 7 days in the cold room followed by 60 to 72 h at room temperature. In response to artificial inoculation, less disease developed on crispy berries. No significant effect of harvest method was observed, except for *A. alternata* inoculation in 2009, when hand-harvested fruit developed a lower level of disease than machine-harvested fruit. Taken together, results from this study suggest that mechanical harvesting of SHB cultivars with crisp-textured berries is feasible from a postharvest pathology perspective.

Additional keywords: *Vaccinium corymbosum* interspecific hybrid, *Aureobasidium pullulans*, harvest efficiency

INTRODUCTION

Worldwide, the United States rank first in the production of blueberries, supplying 166,786 t in 2009 with a farm gate value of $507 million (Anonymous 2010). Blueberry acreage nationwide has increased by 58% in the past 8 years, from 16,341 ha in 2001 to 25,807 ha in 2009 (Anonymous 2002; Anonymous 2010). Georgia ranks second nationally with 16% of the total cultivated blueberry acreage and fourth to fifth in total blueberry production. With a farm gate value of $102 million (Anonymous 2010; Boatright and McKissick 2010b), blueberry is the state's most important fruit crop.

Currently, >80% of the cultivated acreage is devoted to rabbiteye blueberry (*V. virgatum* = *V. ashei*), with the remainder planted to southern highbush blueberries (*V. corymbosum* interspecific hybrids) (Boatright and McKissick 2010a). In spite of much lower acreage, one-third of the blueberry farm gate value in Georgia in 2009 resulted from sales of southern highbush blueberry fruit (Boatright and McKissick 2010a).

Southern highbush blueberry (SHB) cultivars were developed through interspecific breeding of northern highbush blueberry (*V. corymbosum)* and native southern species such as *V. darrowii* (Lang 1993). These cultivars have low chilling requirements (Krewer and NeSmith 2006) and are grown successfully in areas with sandy soils naturally high in organic matter, in sandy soils enriched with pine bark, or in high-density culture on pine bark beds (Fonsah et al. 2006). There has been a considerable increase in SHB acreage since the mid-1990s because their fruit ripen earlier than those of rabbiteye cultivars, satisfying demand for blueberries in the early-May market window between imports from South America and early-summer domestic fruit production from northern highbush blueberries in North Carolina and New Jersey (Fonsah et al. 2006; Scherm and Krewer 2003).

Blueberries can be both hand- and machine-harvested. Hand-picking is labor-intensive (1500 h/ha) (Brown et al. 1996), costs $1.00 to $1.76/kg in southern production areas (Safley et al. 2005), and is mostly used for fruit intended for the fresh market to achieve high fruit quality (NeSmith et al. 2002). Due to an increasing shortage of farm labor, the cost of hand-harvesting will continue to escalate. Machine-harvesting is highly labor-efficient (25 h/ha) (Peterson and Brown 1996), costs $0.26 to 0.40/kg (Safley et al. 2005) and is usually done late in the season for rabbiteye cultivars when

fruit prices are lower and hand-harvesting becomes uneconomical (NeSmith et al. 2002). Machine-harvesting, however, often results in bruising and loss of firmness to the fruit, which in turn leads to decreased shelf life and increased risk of postharvest decay (Mainland et al. 1975; NeSmith et al. 2002). As a result, much of the machine-harvested fruit goes to the processed market (NeSmith et al. 2002), where it receives a lower price ($1.00/kg) than blueberries sold fresh ($4.34/kg) (Anonymous 2010). Mechanical harvesting systems currently used for the later-maturing and lower-value rabbiteye fruit are generally not capable of delivering SHB fruit of sufficient quality and shelf life for the fresh market (Funt et al. 1998; Takeda et al. 2008). As a result, nearly all SHB cultivars grown in Georgia are hand-harvested, although interest in mechanically harvesting this high-value crop is increasing rapidly (NeSmith 2009).

Berry firmness is a key attribute required for fruit to be machine-harvested successfully (NeSmith 2009). Currently, fruit of SHB cultivars typically grown in Georgia, such as Star (Lyrene 1998) or Emerald, have lower firmness than those of rabbiteye cultivars and are not suitable for machine-harvesting. Other examples of SHB cultivars with similar levels of fruit firmness are Scintilla (Lyrene 2008a) and Primadonna (Lyrene 2009a). Recently, the University of Florida blueberry breeding program has developed novel “crisp-textured” SHB cultivars (Padley 2005), which include Bluecrisp (Lyrene 1999) and Sweetcrisp (Lyrene 2009b). These cultivars have firmer berries and better storage life than conventional SHB cultivars because of the springiness of their skin and the increased force needed to rupture the skin (Padley 2005). Two other recent Florida cultivar releases, Farthing (Lyrene 2008b) and Meadowlark, possess a semi-crisp berry type that is firmer than that of conventional cultivars but does not reach the springiness

of Sweetcrisp or Bluecrisp. The firmer fruit texture of these new cultivars may allow for machine-harvest with reduced bruising, thereby increasing fruit quality and decreasing risk of postharvest fungal decay.

Little is known about the response of SHB cultivars to harvesting method in relation to postharvest disease development; however, several studies have documented postharvest decay on rabbiteye and northern highbush blueberries (Milholland and Jones 1972; Cappellini et al. 1982; Daykin and Milholland 1984; Makus and Morris 1993; Smith et al. 1996; Schilder et al. 2002). Various fungal pathogens attack fruit of these blueberry species, of which *Colletotrichum* spp. (causing ripe rot), *Alternaria tenuissima* and other *Alternaria* spp. (causing Alternaria fruit rot), and *Botrytis cinerea* (causing gray mold) are the most commonly reported. Other fungal genera capable of causing postharvest decay of blueberries are *Aspergillus*, *Aureobasidium*, *Catenophora*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pestalotia*, and *Rhizopus* (Ceponis and Cappellini 1979; Tournas and Katsoudas 2005; Barrau et al. 2006). There are few reports on postharvest fruit decay specifically of SHB cultivars, but it is known that SHB cultivars generally develop higher postharvest decay than rabbiteye cultivars (Miller et al. 1993; Perkins-Veazie et al. 1994). *Colletotrichum* spp. and *Botrytis* spp. were the main decay-causing organisms on SHB cultivars Gulfcoast and Sharpblue grown in Louisiana (Lang and Tao 1992); *B. cinerea* in Florida (Harmon 2004); and *Colletotrichum*, *Botrytis*, *Alternaria*, and *Monilinia* spp. in Spain (Barrau et al. 2006). The incidence of postharvest decay may be exacerbated by various factors, including machine-harvesting, storage at higher temperature for long periods, infestation

of handling surfaces, wetness of the stem scar, and presence of moisture (Mainland et al. 1975; Cline 1996; Gillett and Schilder 2009).

In addition to attack by postharvest pathogens, blueberries are prone to microbial contamination during the growing season as well as during harvesting and processing (Tournas and Katsoudas 2005). While rigid microbial safety standards are currently in place for processed blueberry products, contamination of fresh market berries is also a concern (Popa et al. 2007). The use of mechanical harvesting could either decrease microbial contamination because there is less handling of fruit by workers, or increase the risk of contamination through creation of microbial attachment sites associated with fruit bruising, cracking, and stem-tearing. Hence, it is important to assess microbiological loads on hand- vs. machine-harvested fruit of both conventional and the new crisp-textured SHB cultivars.

Based on the above considerations, the objectives of this study were to compare conventional and crisp-textured SHB genotypes after hand- and machine-harvest in relation to microbial contamination on fruit at harvest and subsequent postharvest decay development. In addition, fungal organisms associated with postharvest decay were identified.

MATERIALS AND METHODS

Study site and genotypes. The study was conducted on a blueberry farm near Waldo, FL, where numerous recent cultivar releases and advanced selections from the University of Florida blueberry breeding program are grown on a commercial scale. The following SHB genotypes having berries with conventional flesh type were included in

the study: Primadonna, Scintilla, Star, and FL 05-486 in 2009; and Scintilla, Star, and FL 01-248 in 2010. Their crisp-textured counterparts were Farthing, Sweetcrisp, FL 98-325, and FL 05-290 in 2009; and Farthing, Sweetcrisp, and Meadowlark in 2010. Plants were between 2 and 5 years old and measured between 1.0 and 2.0 m in height. Plant spacing was 0.75 m within rows and 3.5 m across rows. Crop management followed standard commercial practice (Krewer 2010), which included fungicide applications during bloom and fruit development but not at the pre-harvest stage.

Hand- and machine-harvest. Harvesting experiments were conducted between 24 and 26 April 2009 and 7 and 8 May 2010; the 2010 harvest was unusually late due to below-average winter and spring temperatures. All genotypes were harvested at least once by a commercial hand-harvesting crew prior to the experimental harvest, ensuring that ripe fruit from the different cultivars or selections were at similar maturity levels. Experimental hand- and machine-harvest were conducted in four replicate row sections per genotype, each containing 13 to 50 bushes. The experimental design was a split-plot with genotype as the main-plot and harvest method as the sub-plot.

Fruit were either hand-picked into 3.78-L buckets or harvested by machine. The mechanical harvester used was a self-propelled Korvan 8000 (Oxbo International, Lynden, WA), except for cultivars Star and Farthing in 2010, which were harvested with a tractor-pulled Korvan 930 (Oxbo International). Both harvester models utilize the same fruit detachment mechanism (rotary and oscillating action of beating rods) and differ primarily in their size. The harvesters were operated at a ground speed of 1.1 to 1.6 km/h with the rotor set at 640 to 690/min; the adjustable counter-weights were set according to the width of the bush. Fruit harvested by the mechanical harvester were

collected into standard blueberry lugs (60 cm × 40 cm × 17 cm). Hand-harvested fruit also were transferred from picking buckets into lugs.

Harvested fruit were pre-cooled and stored temporarily (for 1 to 3 days) at 15°C in a mobile field cooler. The same mobile cooler was used to transport the fruit to a packingline at the University of Georgia (UGA) Blueberry Research Farm in Alapaha, GA, where they were sorted and packed into 550-mL (one pint) plastic clamshells. The packingline included a lift belt, an air blower to remove leaves and twigs, a tilted belt to remove green clusters and heavily damaged fruit, an inspection table where fruit were graded manually for size and color, and a filler to feed fruit into the clamshells. Clamshells containing fruit were placed into cold storage (2°C) at the UGA Vidalia Onion Research Lab in Tifton, GA.

Fruit surface contaminants. Microbial load on the fruit surface was assessed on fruit samples after they had been sorted and packed into clamshells but before they were placed into cold storage. A subsample of four genotypes was used in both years, i.e., Primadonna and Scintilla (both with conventional flesh) as well as FL 98-325 and Sweetcrisp (both with crispy flesh) in 2009; and Star and Scinitlla (both conventional) as well as Farthing and Sweetcrisp (both crispy) in 2010. For each genotype and replicate, one 50-g fruit sample (40 to 50 berries) was placed in a sterile 500-mL flask containing 50 mL of sterile phosphate buffer (42.5 mg KH_2PO_4 per L; pH 7.2). The mouth of the flask was wrapped with aluminum foil and Parafilm, and the flask was agitated on a wrist action shaker at medium speed for 15 min. Aliquots of the wash buffer and of 1:20 or 1:100 dilutions were plated in triplicate onto plate count agar (PCA), dichloran rose bengal chloramphenicol (DRBC) agar, and 3M Petrifilms (3M Microbiology, St. Paul,

MN) for enumeration of total aerobic bacteria, yeasts and molds, and *E. coli* and coliforms, respectively. PCA and DRBC agar dishes were incubated at 23 to 25°C, and colonies were counted after 3 and 5 days, respectively. Petrifilms were incubated at 35°C and evaluated after 2 days. Colony-forming units (CFU) per g of fruit were log(CFU+1)-transformed and subjected to split-plot analysis of variance with genotype (or flesh type group) as the main-plot and harvest method as the sub-plot using PROC GLIMMIX in SAS v. 9.2 (SAS Institute, Cary, NC).

Natural postharvest disease incidence. Postharvest disease development was assessed on a subsample of six genotypes each year, i.e., Scintilla, Star, and FL 05-486 (all having conventional flesh) as well as Farthing, Sweetcrisp, and FL 98-325 (all having crispy flesh) in 2009; and Scintilla, Star, and FL 01-248 (conventional) as well as Farthing, Sweetcrisp, and Meadowlark (crispy) in 2010. Clamshells were removed from cold storage after 0, 7, 14, and 21 days, at which time firmness was measured on a subsample of 50 berries per genotype and replicate using a FirmTech II instrument (BioWorks Inc., Wamego, KS). Fruit removed from cold storage were maintained at room temperature (23 to 25°C) in clamshells for an additional 4 days, at which time the number of fruit with symptoms or signs of postharvest decay was counted from a total of 50 to 100 fruit per replicate. Percent disease incidence was arcsine-square root-transformed and subjected to split-split-plot analysis of variance with genotype (or flesh type group) as the main-plot, harvest method as the sub-plot, and cold storage time as the sub-sub-plot using PROC GLIMMIX. Furthermore, postharvest disease incidence was regressed against fruit firmness measured after the corresponding cold storage period. Fungal pathogens associated with diseased fruit were identified microscopically

(Barnett and Hunter 1987; Wharton and Schilder 2003) and were reported as the proportion of each pathogen species out of the total number of affected fruit.

Postharvest disease incidence after artificial inoculation. Fruit from the 0-day cold storage period were used in these experiments, using the same genotype and harvest method combinations mentioned for the natural postharvest disease development experiments above. Individual experimental units consisted of 50 fruit per replicate, placed stem end up in two or three Petri dishes (100 mm diameter, 25 mm depth) on Whatman No. 1 filter paper moistened with 1 mL of sterile deionized water. Each fruit was inoculated on the stem end with a 20-µL drop of a spore suspension (1×10^5 conidia per mL) of either *A. alternata* (isolated from blueberry fruit obtained locally), *B. cinerea* (isolated from infected flowers of a *Geranium* sp.), or *C. gloeosporioides* (isolated from blueberry fruit obtained locally). These isolates had been maintained on agar slants at 7°C for long-term storage and had been grown on potato dextrose or V8 juice agar prior to use. An untreated control group consisted of fruit inoculated with 20 µL of sterile distilled water on the stem scar. Inoculated fruit were incubated at 23 to 25°C for 24 h and at 7°C for 7 days. After another 72 h (2009) or 60 h (2010) at 23 to 25°C, each berry was observed under low-power magnification (10 to 63×) for presence of symptoms or fungal signs. Separately for each pathogen, percent disease incidence was arcsine-square root-transformed and subjected to split-plot analysis of variance with genotype (or flesh type group) as the main-plot and harvest method as the sub-plot using PROC GLIMMIX.

RESULTS

Fruit surface contaminants. In both years, average counts of total aerobic bacteria as well as yeasts and molds were below commercial thresholds (for processed blueberry) which are 100,000 CFU/g for aerobic bacteria and 75,000 CFU/g for yeasts and molds (U.S. Highbush Blueberry Council, Folsom, CA, *unpublished*) except for FL 01-248 in 2010 (252,375 CFU/g of aerobic bacteria). In general, microbial counts were higher in 2010 than in 2009 (Fig. 2.1), especially for aerobic bacteria on FL 01-248 and for yeasts on all genotypes. There were no statistically significant effects of flesh type (conventional vs. crispy genotype group) and harvesting method on microbial plate counts (Table 2.1). However, when data were analyzed for individual genotypes (as opposed to genotype groups), counts of aerobic bacteria and yeasts were significantly higher on FL 01-248 in 2010 (P = 0.0236 and 0.0207, respectively).

No colonies of *E. coli* were detected on Petrifilms in either year; however, some coliform colonies were present in one replicate of hand-harvested Primadonna in 2009 (average 7.0 CFU/g) and in machine-harvested replicates of Farthing and Sweetcrisp in 2010 (averages 1.0 and 20.0 CFU/g, respectively).

Natural incidence of postharvest disease. In both years, postharvest disease incidence increased as the duration of the cold-storage period increased (Fig. 2.2), but disease levels were considerably higher in 2010 than in 2009. Machine-harvested conventional genotypes developed the highest disease and hand-harvested crispy genotypes had the lowest disease in both years. Interestingly, machine-harvested crispy fruit performed at least as good or better than hand-harvested conventional fruit in both years (Fig. 2.2). These observations were confirmed by analysis of variance of the data

(Table 2.3). When data were analyzed for individual genotypes, Farthing (a crisp-textured cultivar) performed best and Scintilla (a conventional cultivar) performed worst in 2009 (Table 2.2). In 2010, Farthing and Sweetcrisp (both crispy flesh cultivars) were the best whereas FL 01-248 (a conventional selection) performed worst.

An inverse relationship was observed between postharvest disease incidence and fruit firmness (4 days before disease assessment, when fruit were taken out of cold storage) in both years (Fig. 2.3). In 2009, fruit firmness values >220 g/mm were associated with low disease incidence. However, few fruit samples reached or exceeded this firmness value in 2010 (Fig. 2.3).

Alternaria spp., *Cladosporium* spp., and *Aureobasidium pullulans* were the most common fungi naturally associated with postharvest disease in this study (Table 2.5 and Fig. 2.4); however, there was a higher proportion of *Colletotrichum* spp. in 2010 than in 2009. Other fungi found on diseased fruit were *Pestalotia* spp., *B.cinerea*, and *Penicillium* spp., along with a few others which were not readily identified. Overall, the complex of postharvest decay fungi was similar for the conventional and crispy genotype groups and for hand- and machine-harvested fruit (Fig. 2.4).

Incidence of postharvest disease after artificial inoculation. In both years, fruit of crispy flesh genotypes developed significantly lower disease than those of conventional flesh genotypes following artificial inoculation with *A. alternata*, *B. cinerea*, or *C. gloeosporioides* (Table 2.4 and Fig. 2.5). In 2009, Scintilla (conventional flesh) developed the highest disease (~80 to 90% incidence) as a result of *A. alternata* or *B. cinerea* inoculation. On the other hand, Farthing and Sweetcrisp (both crispy flesh) developed the lowest disease (~40 to 50% incidence) in response to *B. cinerea* and *C.*

gloeosporioides inoculation, respectively (Fig. 2.5). In 2010, Sweetcrisp (crispy flesh) developed lower levels of disease (~70 to 80% incidence) whereas Star (conventional) developed the highest disease (~90 to 100% disease incidence) after artificial inoculations. There was no significant effect of harvest method except for inoculation with *A. alternata* in 2009 (Table 2.4), where hand-harvested fruit developed lower levels of disease than machine-harvested fruit.

DISCUSSION

This study documents that mechanical harvesting of crisp-textured SHB genotypes is feasible from a postharvest pathology perspective. When exposed to natural inoculum, machine-harvested fruit of crispy flesh genotypes developed levels of postharvest disease lower or similar to those of hand-harvested fruit of conventional flesh genotypes. Results of artificial inoculation with *A. alternata*, *B. cinerea*, and *C. gloeosporioides* supported the fact that crispy berries develop less disease than their conventional flesh counterparts, regardless of harvest method. It has been shown previously that fruit of the former genotypes are firmer and more “springy” than those of the latter (Padley 2005), which may explain the lower postharvest disease levels via two different, but related, mechanisms: 1) because of their firmer texture, crispy berries are inherently more resistant to direct penetration by fungal pathogens; and 2) the greater firmness of crispy berries allows lower levels of bruising to occur during harvest, thereby reducing the level of wound-associated infection. Both mechanisms may be in operation according to the results of our comparison between hand- and machine-harvested fruit.

Our study clearly documented a negative association between postharvest disease incidence and fruit firmness across genotypes and harvest methods, indicating that fruit firmness is a good predictor for postharvest decay. This relationship also suggests that the lower level of postharvest disease in crispy flesh genotypes is mostly due to their greater firmness, and not some other form of genetic resistance. Similarly, the higher disease incidence in 2010 compared with 2009 could be due in part to lower values of fruit firmness, possibly in relation to hotter temperatures (reaching up to 35°C during harvest) and/or higher rainfall before harvest in 2010. It is well established that fruit firmness is inversely related to temperature and can vary from year to year for the same cultivar (Ballinger et al. 1973; NeSmith et al. 2002). Also, early ripening is generally associated with higher fruit firmness (Yang et al., 2009), which may be the cause of better firmness levels in 2009 compared with 2010, when harvest was delayed by about 2 weeks due to weather.

Mechanical harvesters for blueberries were first developed in the early 1960s and have been improved since then to produce higher-quality fruit (Mainland 1993; Peterson and Brown 1996; Peterson et al. 1997; Takeda et al. 2008). A major concern with mechanical harvest has been fruit bruising, which occurs as result of direct contact between the berries and the harvester's beater rods, when detached berries drop from the bush into the harvester's catch pans, during passage of the berries from the catch pans to the conveyor belt, and when the berries drop from the conveyor belt into the fruit lugs. Bruising increases with longer falling distance on harder surfaces of the harvester (Ballinger et al. 1973) and reduces both external (by removing the visually appealing fruit surface wax) and internal (by reducing firmness) fruit quality (Dale et al.

1994; Mainland et al. 1975; NeSmith et al. 2002). Internal damage to the fruit results in cellular water leakage and development of water-soaked areas in the flesh (Labavitch et al. 1998). This is the main reason machine-harvesting of blueberries for the fresh market in the southeastern United States has traditionally been limited to the firmer and later-maturing but lower-value rabbiteye blueberries, whereas the softer, early-maturing, high-value SHB fruit have almost exclusively been hand-harvested. Due to shortages in labor and associated increased labor costs (Fonsah et al. 2004), mechanical harvesting will likely become a necessity for SHB in the near future. In this context, the favorable results obtained in our study for fruit firmness and postharvest disease development on machine-harvested crispy flesh SHB are encouraging.

In addition to bruising, however, there are other drawbacks to machine-harvesting that can increase losses and reduce quality and revenue. These include ground losses, excessive green fruit detachment, delayed harvest, ripe fruit remaining in the bush, and bush damage (Ballinger et al. 1973; Mainland et al. 1975; NeSmith et al. 2002; Takeda et al. 2008). Ground loss occurs when the harvester's beater rods detach the fruit but the catch pans fail to collect them, usually because the fruit drop too close to or within the crown of the bush. These losses, which typically amount to 20 to 30%, can be reduced with cultural practices such as pruning to a vase shape or trellising (Mainland 1993; Strik and Buller 2002; van Dalfsen and Gaye 1996; Peterson et al. 1997), as well as via improvements in harvester technology (Takeda et al. 2008). Another drawback of mechanical harvesting is green berry detachment, which results in reduced overall yield and increased sorting costs (Mainland et al. 1975; Takeda et al. 2008; van Dalfsen and Gaye 1999). Machine harvest may need to be delayed by 5 to 7

days compared with hand-harvesting to reduce the percentage of green berries, but this is not desirable in SHB because of rapidly declining fruit prices as the season progresses. Moreover, ripe fruit in the center of the bush are often missed by the mechanical harvester, which results in overripe fruit during the next harvest, thereby reducing overall fruit quality (Mainland 1993). This type of loss can be reduced by pruning and/or trellising. Mechanical damage to the crown of the bush by the harvester creates entry wounds for pathogens such as *Botryosphaeria* spp. causing stem blight (Milholland 1972; Wright and Harmon 2010). However, this damage can be reduced by proper pruning and improved harvester technology (Takeda et al., 2008). Overall, a systems approach integrating cultural and engineering practices is needed to reduce these potential sources of losses associated with machine-harvest.

Although the fruit firmness groups and harvest methods differed in postharvest disease incidence in our study, the pathogen complex responsible was similar across treatments. *Aureobasidium pullulans*, *Cladosporium* spp. (both usually considered secondary pathogens), and *Alternaria* spp. were observed most commonly. Pathogens such as *B. cinerea* and *Colletotrichum* spp. that are common in other blueberry growing areas of the United States (Cappellini et al. 1982; Milholland and Jones 1972; Schilder et al. 2002; Smith et al. 1996; Tournas and Katsoudas 2005) were also observed, albeit in smaller proportions (except for a higher incidence of *Colletotrichum* spp. in 2010). Higher temperature and rainfall before harvest could have increased the incidence of *Colletotrichum* spp. in 2010.

Although this study demonstrated that mechanical harvest of the novel crisp-textured SHB cultivars is feasible from a postharvest pathology perspective, further

research is needed to address potential problems related to in-field losses, postharvest quality, and consumer acceptance of machine-harvested crispy berries. We are currently collaborating with plant breeders, horticulturists, postharvest physiologists, and food scientists to investigate these aspects comprehensively and develop an integrated system for production, harvest, and marketing of mechanically harvested SHB.

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Table 2.1. Results of a split-plot analysis of variance for the effects of fruit flesh type (conventional vs. crispy)[a] and harvest method (hand- vs. machine-harvest) on plate counts of aerobic bacteria, total yeasts, and total molds on southern highbush blueberry fruit in 2009 and 2010.

	2009				2010			
Source	ndf	ddf	*F*	*P*	ndf	ddf	*F*	*P*
Aerobic bacteria								
Flesh type (F)	1	6	4.59	0.076	1	6.16	2.51	0.163
Harvest (H)	1	22	0.090	0.768	1	21.5	0.450	0.510
F × H	1	22	0.160	0.688	1	21.5	0.290	0.597
Total yeast								
Flesh type (F)	1	6	0.030	0.859	1	6	0.130	0.728
Harvest (H)	1	22	0.050	0.822	1	22	4.27	0.051
F × H	1	22	0.010	0.936	1	22	0.000	0.971
Total mold								
Flesh type (F)	1	6	1.21	0.313	1	6	0.040	0.846
Harvest (H)	1	22	0.640	0.431	1	22	2.26	0.147
F × H	1	22	2.46	0.131	1	22	0.570	0.456

[a]Conventional flesh genotypes included Primadonna and Scintilla in 2009 and FL 01-248 and Scintilla in 2010. Crispy flesh genotypes were FL 98-325 and Sweetcrisp in 2009 and Farthing and Sweetcrisp in 2010.

Table 2.2. Natural postharvest disease incidence (%) on fruit of southern highbush blueberry genotypes having conventional or crispy fruit flesh type subjected to hand- or machine-harvest and maintained in cold-storage (2°C) for different periods of time[a].

	Cold-storage period[b] (days after harvesting)							
	0		7		14		21	
Year/ genotype	Hand	Machine	Hand	Machine	Hand	Machine	Hand	Machine
2009								
Conventional								
Star	2.04 b	5.40 a	5.24 b	17.2 b	6.06 b	23.0 b	7.28 b	21.1 b
Scintilla	7.21 a	3.35 ab	14.6 a	27.5 a	17.5 a	29.1 a	28.6 a	48.5 a
FL 05-486	0.630 b	2.00 ab	5.80 b	10.2 cd	5.98 b	15.8 c	7.73 b	18.6 bc
Crispy								
Farthing	0.533 b	0.473 b	1.80 c	1.76 e	3.05 b	6.73 d	5.72 b	8.31 c
Sweetcrisp	0.358 b	2.87 ab	1.14 c	12.9 bc	5.34 b	11.3 cd	4.49 b	21.4 b
FL 98-325	1.31 b	2.69 ab	4.08 bc	5.60 de	4.94 b	9.05 d	9.69 b	21.1 b
2010								
Conventional								
Star	24.3 b	47.6 a	19.9 c	40.2 b	37.8 ab	64.6 a	—	—
Scintilla	15.8 c	38.1 b	31.7 ab	58.9 a	32.3 bc	62.8 a	—	—
FL 01-248	34.0 a	49.6 a	34.5 a	55.4 a	48.0 a	68.9 a	—	—
Crispy								
Farthing	3.74 d	8.61 d	5.31 d	10.3 d	6.62 d	13.6 c	—	—
Sweetcrisp	4.26 d	12.7 d	5.61 d	17.0 cd	6.90 d	17.7 c	—	—

Meadowlark	24.7 b	28.4 c	21.1 bc	27.9 bc	23.6 c	33.6 b	—	—

[a]Values are means of four replicates. Within each column and year, means followed by the same letter are not significantly different according to Fisher's Protected LSD test (α = 0.05).
[b]Fruit were maintained at room temperature (23 to 25°C) for an additional 4 days following the cold storage period to increase disease pressure.

Table 2.3. Results of a split-split-plot analysis of variance for the effects of fruit flesh type (conventional vs. crispy)[a], harvest method (hand- vs. machine-harvest), and time on natural postharvest disease incidence on southern highbush blueberry fruit in 2009 and 2010.

	2009				2010			
Source	ndf	ddf	*F*	*P*	ndf	ddf	*F*	*P*
Flesh type (F)	1	6	62.75	0.0002	1	6	274.5	<0.0001
Harvest (H)	1	6	139.53	<0.0001	1	6	157.8	<0.0001
F × H	1	6	2.55	0.162	1	6	18.4	0.005
Time (T)[b]	3	164	53.30	<0.0001	2	120	10.4	<0.0001
T × F	3	164	1.53	0.209	2	120	2.83	0.063
T × H	3	164	4.08	0.008	2	120	0.270	0.761
T × F × H	3	164	0.610	0.609	2	120	0.030	0.967

[a]Individual genotypes used in the 2 years are given in Table 2.2.
[b] Disease assessments made after 0, 7, 14, and 21 (2009 only) days in cold storage (2°C) followed by an additional 4 days at room temperature (23 to 25°C) to increase disease pressure.

Table 2.4. Results of a split-plot analysis of variance for the effects of fruit flesh type (conventional vs. crispy)[a] and harvest method (hand- vs. machine-harvest) on postharvest disease incidence following artificial inoculation with *Alternaria alternata*, *Botrytis cinerea*, or *Colletotrichum gloeosporioides* on southern highbush blueberry fruit in 2009 and 2010.

Source	2009				2010			
	ndf	ddf	*F*	*P*	ndf	ddf	*F*	*P*
A. alternata								
Flesh type (F)	1	6	31.4	0.001	1	6	267.9	<0.0001
Harvest (H)	1	38	5.00	0.031	1	38	1.19	0.281
F × H	1	38	0.350	0.557	1	38	1.35	0.253
B. cinerea								
Flesh type (F)	1	6	116.6	<0.0001	1	6	84.9	<0.0001
Harvest (H)	1	38	3.84	0.057	1	38	0.050	0.832
F × H	1	38	0.120	0.728	1	38	1.24	0.273
C. gloeosporioides								
Flesh type (F)	1	6	22.4	0.003	1	6	6.56	0.045
Harvest (H)	1	38	0.470	0.496	1	38	0.180	0.671
F × H	1	38	0.300	0.588	1	38	0.110	0.738

[a]Individual genotypes used in the 2 years are given in Table 2.2 and Fig. 2.5.

Table 2.5. Incidence of different fungal genera causing natural postharvest disease on fruit of southern highbush blueberry genotypes having conventional or crispy fruit flesh type subjected to hand- or machine-harvest and maintained in cold-storage (2°C) for 0, 7, 14, and 21 (2009 only) days in 2009 and 2010.

	Percent postharvest disease-causing organisms[b]					
Year/ harvest method	*Alternaria*	*Botrytis*	*Colletotrichum*	*Cladosporium*	*Aureobasidium*	Others
2009						
Conventional						
Hand	2.15	0.150	0.210	4.21	1.31	1.03
Machine	3.14	0.960	0.340	8.52	2.46	2.01
Crispy						
Hand	1.15	0.08	0.16	1.28	0.44	0.34
Machine	2.53	0.29	0.35	3.98	1.37	0.99
2010						
Conventional						
Hand	10.01	0.500	5.71	12.00	8.61	1.22
Machine	16.37	2.73	7.23	25.47	20.26	3.14
Crispy						
Hand	3.38	0.10	4.11	3.05	2.08	0.097
Machine	7.04	0.32	3.87	6.81	5.34	1.06

[a]Individual genotypes used in the 2 years are given in Table 2.2.
[b] Disease assessments made after 0, 7, 14, and 21 (2009 only) days in cold storage (2°C) followed by an additional 4 days at room temperature (23 to 25°C) to increase disease pressure. Values are percentages across all assessment dates.

Fig. 2.1. Plate counts of total aerobic bacteria, yeasts, and molds from the fruit surface of four southern highbush blueberry genotypes grouped into conventional and crispy fruit flesh types and subjected to hand- or machine-harvest in 2009 (**A**, **B**, and **C**) and 2010 (**D**, **E**, and **F**). Values are means and standard errors of four replicates.

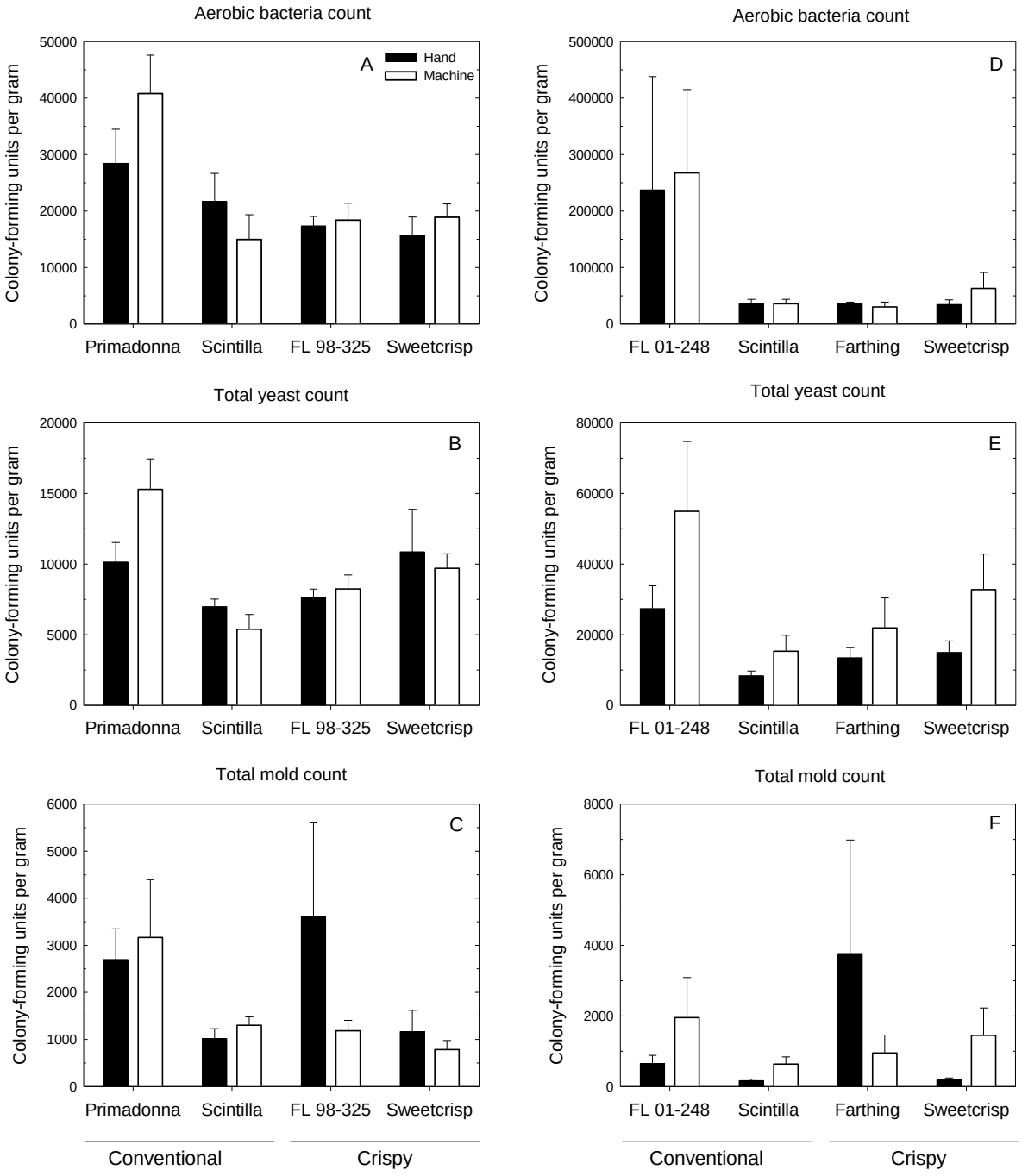
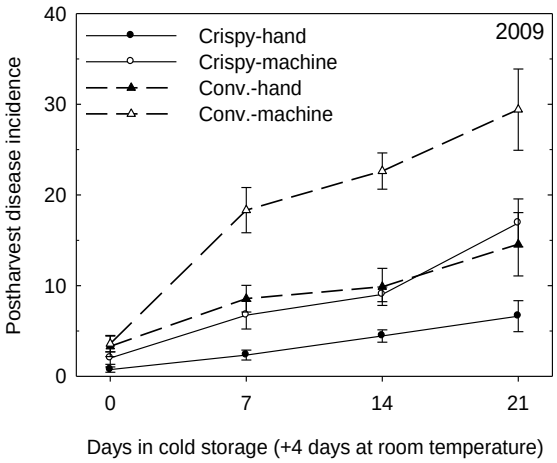

Aerobic bacteria count
A
Hand
Machine
Colony-forming units per gram
50000
40000
30000
20000
10000
0
Primadonna
Scintilla
FL 98-325
Sweetcrisp
Aerobic bacteria count
D
500000
400000
300000
200000
100000
0
FL 01-248
Scintilla
Farthing
Sweetcrisp
Total yeast count
B
20000
15000
10000
5000
0
Total yeast count
E
80000
60000
40000
20000
0
Total mold count
C
6000
5000
4000
3000
2000
1000
0
Total mold count
F
8000
6000
4000
2000
0
Conventional
Crispy

Fig. 2.2. Postharvest disease incidence on fruit of conventional vs. crispy flesh southern highbush blueberry genotypes harvested either by hand or machine and left in cold storage (2°C) for 0, 7, 14, and 21 (2009 only) days. Fruit were kept at room temperature (23 to 25°C) for an additional 4 days after removal from cold storage to increase disease pressure. Individual genotypes used in the 2 years are given in Table 2.2.


2009
Crispy-hand
Crispy-machine
Conv.-hand
Conv.-machine
Postharvest disease incidence
0
10
20
30
40
0
7
14
21
Days in cold storage (+4 days at room temperature)

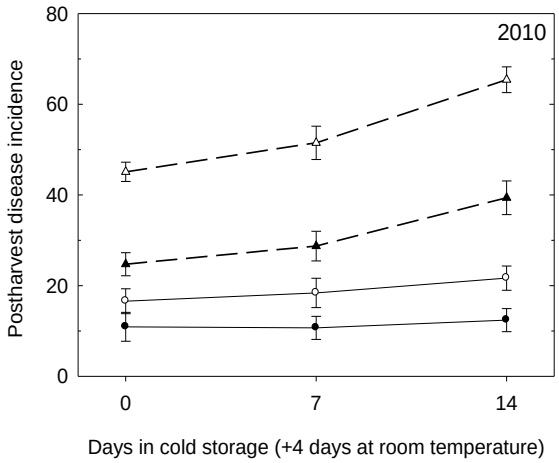
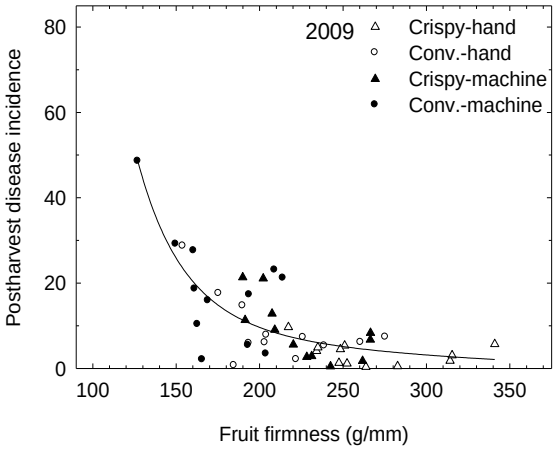

2010
Postharvest disease incidence
0
20
40
60
80
0
7
14
Days in cold storage (+4 days at room temperature)

Fig. 2.3. Relationship between postharvest disease incidence and firmness for fruit of southern highbush blueberry genotypes having conventional or crispy fruit flesh type subjected to hand- or machine-harvest and maintained in cold-storage (2°C) for 0, 7, 14, and 21 (2009 only) days. Same genotype, harvest method, and storage period combinations as shown in Table 2.2.


2009
Crispy-hand
Conv.-hand
Crispy-machine
Conv.-machine
Postharvest disease incidence
Fruit firmness (g/mm)
0
20
40
60
80
100
150
200
250
300
350

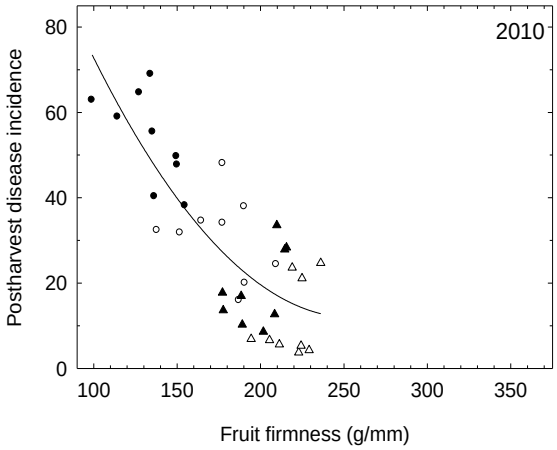

2010
Postharvest disease incidence
Fruit firmness (g/mm)
0
20
40
60
80
100
150
200
250
300
350

Fig. 2.4. Relative proportion of different fungal genera associated with natural postharvest disease on fruit of southern highbush blueberry genotypes having conventional or crispy fruit flesh type subjected to hand- or machine-harvest and maintained in cold-storage (2°C) for 0, 7, 14, and 21 (2009 only) days in 2009 (**A**) and 2010 (**B**). Individual genotypes used in the 2 years are given in Table 2.2.

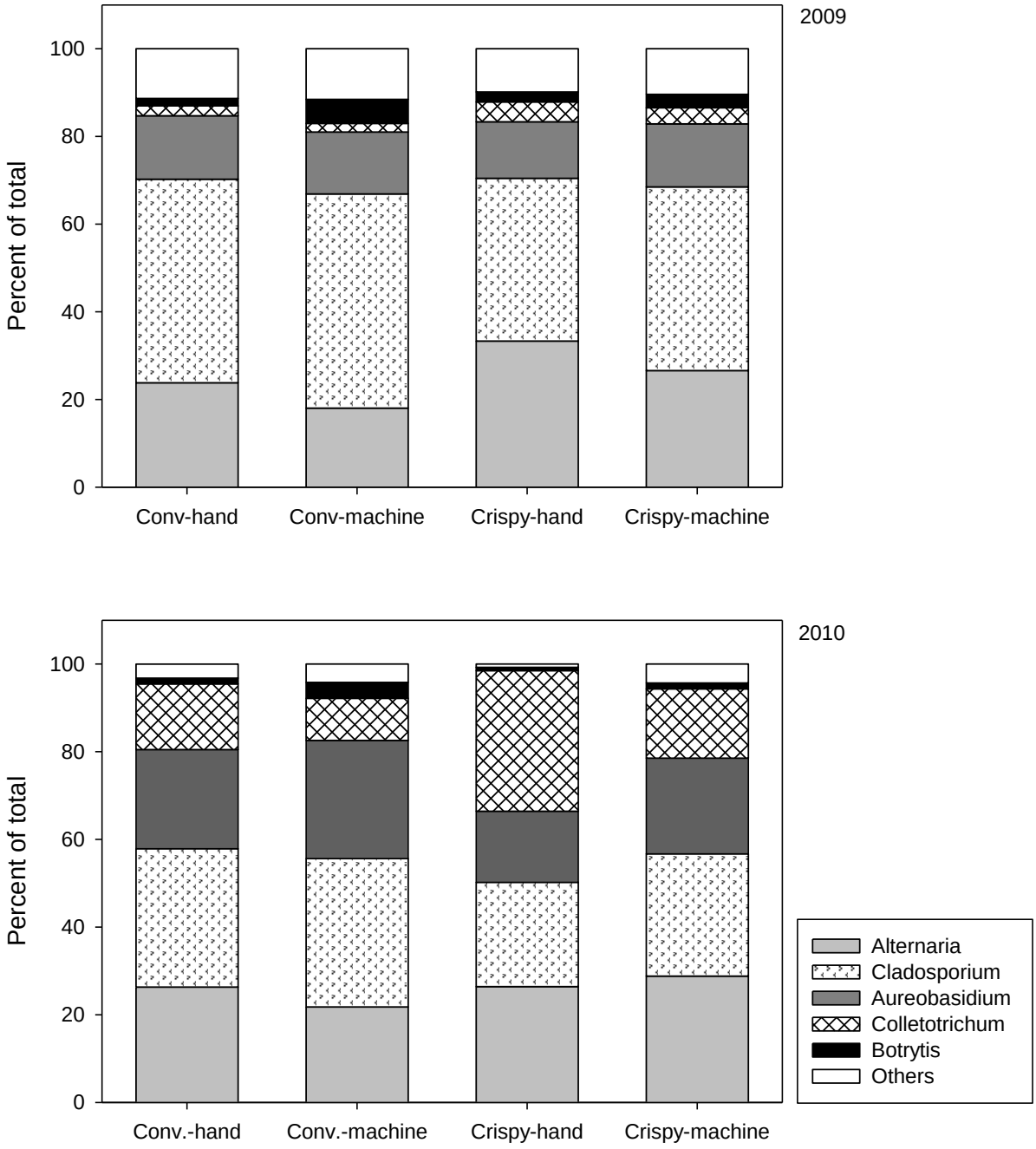
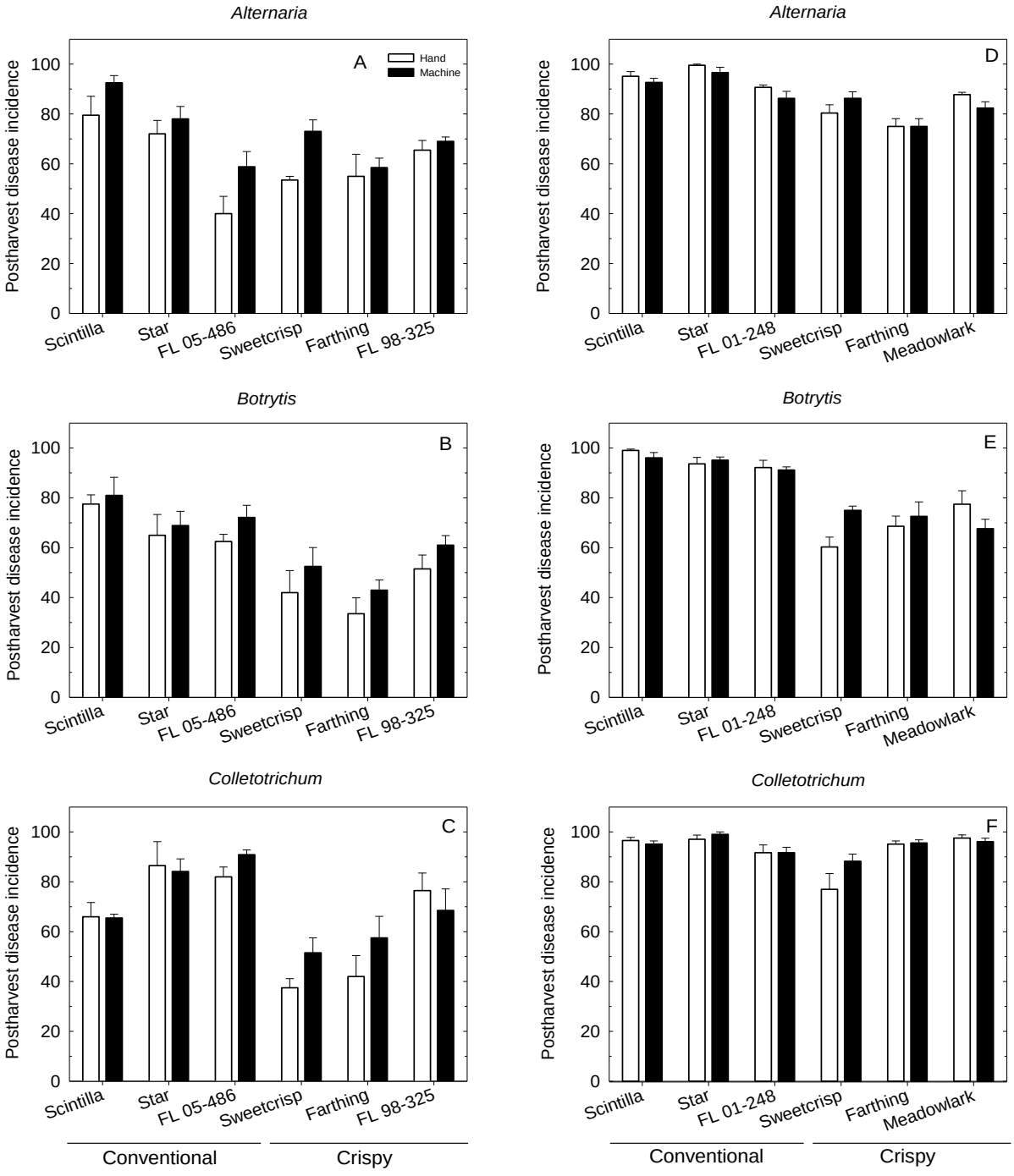

2009
Percent of total
100
80
60
40
20
0
Conv-hand
Conv-machine
Crispy-hand
Crispy-machine
2010
Percent of total
100
80
60
40
20
0
Conv.-hand
Conv.-machine
Crispy-hand
Crispy-machine
Alternaria
Cladosporium
Aureobasidium
Colletotrichum
Botrytis
Others

Fig. 2.5. Percent fruit disease incidence following artificial inoculation with *Alternaria alternata*, *Botrytis cinerea*, or *Colletotrichum gloeosporioides* on six southern highbush blueberry genotypes grouped into conventional and crispy fruit flesh types and subjected to hand- or machine-harvest in 2009 (**A**, **B**, **C**) and 2010 (**D**, **E**, **F**). Values are means and standard errors of four replicates.


Alternaria
A
Hand
Machine
Postharvest disease incidence
0
20
40
60
80
100
Scintilla
Star
FL 05-486
Sweetcrisp
Farthing
FL 98-325
Alternaria
D
Scintilla
Star
FL 01-248
Sweetcrisp
Farthing
Meadowlark
Botrytis
B
Botrytis
E
Colletotrichum
C
Colletotrichum
F
Conventional
Crispy

CHAPTER 3

EFFECT OF POSTHARVEST BIOFUMIGATION WITH PLANT ESSENTIAL OILS ON FUNGAL DECAY, SENSORY QUALITY, AND ANTIOXIDANT ACTIVITY OF BLUEBERRY FRUIT[1]

[1]Mehra, L.K., MacLean, D.D., Shewfelt, R.L., Smith, K.C., Kays, S.J., and Scherm, H. 2010. To be submitted to *Crop Protection*.

Effect of Postharvest Biofumigation on Fungal Decay, Sensory Quality, and Antioxidant Activity of Blueberry Fruit

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ABSTRACT

Postharvest fungal decay, caused by various fungal pathogens, is a significant concern in blueberry production, but current options for managing postharvest diseases are limited for this crop. Four plant essential oils (cinnamon oil, linalool, *p*-cymene, and peppermint leaf oil) and the plant oil-derived biofungicides Sporan and Sporatec were evaluated as postharvest biofumigants to manage fungal decay during cold storage. Hand-harvested Tifblue rabbiteye blueberry fruit were inoculated at the stem end with *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, or sterile deionized water (control inoculation) and subjected to biofumigation treatments in cold storage (7°C) for 1 week. Sporatec volatiles reduced disease incidence significantly in most cases, whereas other treatments had no consistent effect on postharvest decay. Sensory analysis of uninoculated, biofumigated berries was performed utilizing a trained sensory panel, and biofumigation was found to have significant negative impacts on

several sensory attributes such as sourness, astringency, juiciness, bitterness, and blueberry-like flavor. Biofumigated fruit were also analyzed for antioxidant levels, for total oxyradical scavenging capacity, and for total phenolics and anthocyanins. There were no consistent effects on any of the antioxidant-related variables of treated berries. Because of limited efficacy in reducing postharvest decay, negative impacts on sensory qualities, and failure to increase antioxidant levels, the potential for postharvest cold-storage biofumigation in harvested blueberries is limited.

Keywords: *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, biofumigation, essential oils, disease management, blueberry, *Vaccinium virgatum*

1. Introduction

Worldwide, the United States rank first in the production of blueberries, supplying 166,786 t in 2009 with a farm gate value of $507 million (Anonymous, 2010). Blueberry area nationwide has increased by 58% in the past 8 years, from 16,341 ha in 2001 to 25,807 ha in 2009 (Anonymous, 2002; Anonymous, 2010). Georgia ranks second nationally with 16% of the total cultivated blueberry area and fourth to fifth in total blueberry production (Anonymous, 2010). With a farm gate value of $102 million, blueberry is the state's most important fruit crop (Boatright and McKissick, 2010). Blueberries are appreciated by consumers owing to their well-documented health benefits such as anti-cancer and anti-aging properties as well as prevention of heart disease, all of which are related to their high level of antioxidants (Beattie et al., 2005; Juranić and Žižak, 2005). Like most other fresh fruits, however, blueberries have a short

shelf life and are perishable, which may be associated with their high sugar content and low pH levels.

In all major blueberry-producing areas of the United States, postharvest decay, associated mostly with fungal infections, is an important production problem. Indeed, several studies have documented postharvest decay of rabbiteye, northern highbush, and southern highbush blueberries (Milholland and Jones, 1972; Cappellini et al., 1982; Daykin and Milholland, 1984; Miller et al., 1993; Perkins-Veazie et al., 1994; Smith et al., 1996; Schilder et al., 2002; Barrau et al., 2006). Various fungal pathogens can attack blueberry fruit, of which *Colletotrichum* spp. (causing ripe rot), *Alternaria tenuissima* and other *Alternaria* spp. (causing Alternaria fruit rot), and *Botrytis cinerea* (causing gray mold) are the most commonly reported. Other fungal genera capable of causing postharvest decay of blueberries are *Aspergillus*, *Aureobasidium*, *Catenophora*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pestalotia*, and *Rhizopus* (Ceponis and Cappellini, 1979; Tournas and Katsoudas, 2005; Barrau et al., 2006).

To control postharvest decay, pre-harvest fungicide sprays (Milholland and Jones, 1972), postharvest chemical dips (Ceponis and Cappellini, 1978), postharvest cooling (Ballinger et al., 1973; Ceponis and Cappellini, 1979), and CO_2-enriched atmospheres (Ceponis and Cappellini, 1983) have been evaluated. Cooling and storage atmosphere enrichment with CO_2 generally provide the most effective control. Pre-harvest fungicide sprays are not always effective since contamination with pathogen propagules may occur during harvesting and processing. Postharvest chemical dips cannot be applied because such treatments would wash off the fruit surface bloom (waxy layer), an important quality characteristic for fresh market fruit. Therefore, in

addition to cooling and CO_2 storage, other means to control fruit diseases of blueberries for the fresh market are needed.

Several natural plant volatiles have antimicrobial properties (Gardini et al., 2001; Utama et al., 2002; Oussalah et al., 2005; Almenar et al., 2007). Examples of volatile-producing essential oils are cinnamon, clove, peppermint, and thyme oils, all of which have well-documented antimicrobial properties (Wilson et al., 1997; Hammer et al., 1999; Guynot et al., 2003; Kishore et al., 2007; Ayala-Zavala et al., 2008). In a recent laboratory-scale pilot study, anethole, carvacrol, linalool, perillaldehyde, and *p*-cymene inhibited postharvest decay of northern highbush blueberries (Wang et al., 2008). In the same study, some of these oils also increased the levels of health-promoting antioxidants of treated fruits compared with the untreated control. However, these pilot experiments were conducted at a relatively high temperature (10°C instead of a more typical cold-storage temperature), the decay-causing organisms were not identified, controlled artificial inoculations were not investigated, and the effect of biofumigation on sensory attributes of treated fruit was not assessed. Furthermore, several recently labeled, commercial plant oil-based fungicides, such as Sporatec (Brandt Consolidated, Springfield, IL), and Sporan (EcoSmart Technologies, Franklin, TN), both of which are blends of rosemary, clove, and thyme oils, also may have biofumigant properties and should be evaluated for their potential to control postharvest decay of blueberry.

Based on the above considerations, the specific objectives of this study were to 1) evaluate the effect of selected essential oils as cold-storage biofumigants to control postharvest decay of blueberry fruit following artificial inoculation with *A. alternata*, *B.*

cinerea, and *C. gloeosporioides*; and 2) assess the impact of biofumigation on sensory qualities and antioxidant capacity of treated fruit.

2. Materials and methods

2.1. Fruit samples and essential oils

Two independent experimental runs were conducted using Tifblue rabbiteye blueberry (*Vaccinium virgatum*) fruit hand-harvested from commercial blueberry plantings in northern and southern Georgia, respectively. Both plantings had received no pre-harvest fungicide applications. Fruit were sorted manually for uniform maturity and absence of blemishes. Essential oils used for biofumigation treatments were cinnamon leaf oil, peppermint oil, linalool, and *p*-cymene (all obtained from Sigma Aldrich, St. Louis, MO). Furthermore, two commercial biofungicides containing rosemary oil, clove oil, and thyme oil, viz. Sporatec and Sporan, were included in the study.

2.2. Fruit inoculation and pre-incubation

Experimental units (replicates) consisted of 50 fruit, placed calyx side down in three Petri dishes (100 mm diameter, 25 mm depth) on Whatman No. 1 filter paper moistened with 1 mL of sterile deionized water. Each fruit was inoculated on the stem end with a 20-µL drop of a spore suspension (1×10^5 conidia per mL) of either *A. alternata* (isolated from blueberry fruit obtained locally), *B. cinerea* (isolated from infected flowers of a *Geranium* sp.), or *C. gloeosporioides* (isolated from blueberry fruit obtained locally). These isolates had been maintained on agar slants at 7°C for long-term storage and had been grown on potato dextrose agar prior to use. An untreated

control group consisted of fruit inoculated similarly with 20 µL of sterile water. Inoculated fruit were pre-incubated at 23 to 25°C for 24 h prior to application of biofumigation treatments. There were four replicates of each inoculation treatment.

2.3. Biofumigation and disease assessment

Sterile 473-mL wide-mouth Mason glass jars (Ball Corporation, Broomfield, CO) were used as biofumigation chambers. Aluminum weighing dishes (43 mm diameter × 13 mm depth) were placed inside each jar, and wire mesh was placed over the weighing dish. A 1-mL volume of each biofumigant oil (no oil as control biofumigation) was pipetted into the weighing dish, the lid of the Mason jar was tightened, and the jar was incubated without fruit at 23 to 25°C. After 24 h, the lid was opened to introduce inoculated fruit (one 50-fruit sample per jar), the lid was closed to seal the jar, and the jar was placed in a cold room (7°C). After 7 days, fruit were removed and transferred into 550-mL plastic clamshells and kept at 23 to 25°C for 3 days. Infected fruit were counted from each clamshell by observing under low-power magnification (10 to 63×) for presence of symptoms or fungal signs, and percent disease incidence was calculated.

The experiment was conducted in a split-plot design with the four pathogen treatments as the main-plot and the seven biofumigation treatments as the sub-plot. Separately for each pathogen, percent disease incidence (arcsine-square root-transformed) was subjected to one-way analysis of variance with biofumigation treatment as a fixed effect using PROC GLM in SAS v. 9.2 (SAS Institute, Cary, NC).

Dunnett's test was applied to compare means of biofumigation treatments with that of the control ($\alpha = 0.05$).

2.4. Sensory analysis of biofumigated fruit

To determine the sensory quality of blueberries after biofumigation, a separate experiment was set up without pathogen inoculations. Sixty uninoculated fruit were added to each of 28 biofumigation chambers (7 biofumigation treatments $\times$ 4 replicates). Fruit were biofumigated with 1 mL of each essential oil placed in an aluminum weighing dish inside the Mason jar, as described previously. After 7 days in the cold room (7°C), fruit were transferred to 550-mL plastic clamshells and pooled from the four replicates to make approximately 230 g of fruit per biofumigant treatment for sensory evaluation.

Sensory analysis was done as described by Smith (2010). Briefly, evaluations were performed by eight trained panelists from the Department of Food Science and Technology, University of Georgia, Athens, GA. Standards of pre-determined descriptors were given to the panelists to compare treatments. The descriptors were sweetness, sourness, bitterness, astringency, blueberry-like flavor, firmness, crispness, color, and juiciness. These descriptors were ranked by panelists on a scale of 0 to 15. An example of the quantitative sensory evaluation sheet is given in Appendix A, and definitions and references for all the descriptors are included in Appendix B (Smith, 2010). The scale for blueberry-like flavor was developed by the panel, and a value of 7 was considered the standard based on the flavor of store-bought blueberries (Smith, 2010). Ratings given by panelists were subjected to analysis of variance using PROC GLIMMIX (SAS v. 9.2; SAS Institute, Cary, NC), with biofumigation treatment as a fixed

effect and panelists as blocks (random effect). Dunnet's test was applied to compare means of treatments with the corresponding control.

2.5. Antioxidant analysis of biofumigated fruit

Antioxidant analysis of treated fruit was done by performing the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, determining total oxyradical scavenging capacity (TOSC), quantifying total phenolics using the Folin-Ciocalteau (FC) method, and identifying and quantifying individual anthocyanins. A 20-g subsample of the fruit from each replicate of the sensory analysis experiment was flash-frozen with liquid nitrogen and stored at −80°C. For sample extraction, fruit were transferred to −20°C, and a 10-g sample was blended for 1 min with 30 mL of methanol:water:88% formic acid (60:37:3 v/v/v) extraction buffer. After blending, a 20-g sample of the slurry mixture was centrifuged (Beckman Coulter Allegra 25R Centrifuge with TA-14-50 rotor; Brea, CA) at 4,000 *g* for 10 min at 8°C in a 50-mL Falcon tube. Two milliliters of the supernatant were transferred to a microcentrifuge tube for DPPH, TOSC, and total phenolics assays. Another 2-mL aliquot was centrifuged at 14,000 *g* for 15 min at 8°C in the same centrifuge equipped with TA-15-1.5 rotor for subsequent anthocyanin quantification.

2.5.1. DPPH assay to determine antioxidant activity

Extract sample was diluted 1:160 by mixing 5 µL of sample with 795 µL of methanol in disposable glass test tubes. A 200-µL volume of freshly prepared 0.5-mM DPPH was added to initiate the reaction. Samples were incubated in the dark at 25°C for 20 min. Following incubation, absorbance was measured with a spectrophotometer

(Beckman Coulter DU 730) at 517 nm. Methanol (1 mL) was used to blank the spectrophotometer, and a mixture 800 µL methanol + 200 µL of 0.5-mM DPPH was used as a control. Percent inhibition of the DPPH molecule by antioxidants present in the sample was calculated and subjected to analysis of variance with biofumigation treatment as a fixed effect using PROC GLM.

2.5.2. Total antioxidant capacity using the TOSC assay

Total antioxidant capacity was determined using the modified total oxyradical scavenging capacity (TOSC) assay as described by MacLean et al. (2003) with the following modifications. Extract sample was prepared as above, with a 2-mL aliquot concentrated to near dryness using a rotor evaporator (55°C) to remove any interfering methanol, and resuspended in 100-mM phosphate buffer (pH 7.4) to make the total weight up to 1 g. The sample was then diluted to a final ratio of 1:100 with 100-mM phosphate buffer (pH 7.4) prior to analysis. To 700 µL of phosphate buffer, 100 µL of 2-mM KMBA (α-keto-γ-methiolbutyric acid) substrate and 100 µL of diluted sample were added in a disposable glass test tube. A 100-µL volume of 20-mM 2,2'-azobis(2-amidinopropane) radical was added to initiate the reaction, and the glass tube was capped immediately with a rubber septum and incubated for 90 min in a water bath at 39°C. For determination of ethylene content, a 1-mL headspace sample was withdrawn using a gas-tight 1-mL Hamilton syringe, and injected into an Agilent 7890A GC equipped with a capillary electronic pressure control direct-injection port (50°C) running in split mode (20:1), an HP-Plot-Q column (15 m × 0.32 mm × 20 µm; 40°C), and a Flame Ionization Detector (FID) with electronic pressure control (225°C). Helium was

used as the carrier gas at a constant linear flow rate of 2.66 mL/min. Data collection was at a sample rate of 50 Hz, all controlled by Chemstation software (rev. B.03.01; Agilent, Foster City, CA). Ethylene content was determined by comparison of retention time and peak area to a certified standard (Airgas South, Atlanta, GA). Results are expressed in µmol of Trolox equivalents/kg after using a trolox-ethylene standard curve (r^2 = 0.9923; P < 0.0001). Data were subjected to analysis of variance with Dunnett's test to compare the control with the other biofumigation treatments using PROC GLM in SAS v. 9.2.

2.5.3. Total phenolics assay

Extract sample was diluted 1:5 by mixing 30 µL of sample with 120 µL of deionized water, and the Folin-Ciocalteau (FC) reagent was diluted 1:10 with deionized water. A 750-µL volume of diluted FC reagent and 150 µL of diluted sample was added to a disposable glass test tube. To initiate the reaction, 600 µL of 7.5% sodium carbonate solution was added and the mixture was incubated at 21°C for 90 min in the dark. Following incubation, absorbance was measured with a spectrophotometer (Beckman Coulter DU 730) at 760 nm. The spectrophotometer was blanked with 1.5 mL of deionized water before measuring the absorbance of samples. Total phenolics content was calculated as gallic acid equivalent (GAE) from a gallic acid standard curve and was reported as GAE mg/100g of fruit. To prepare the standard curve,150 µL of a dilution series (1:10, 1:20, 1:33.3, 1:50, 1:100) in triplicate of freshly prepared gallic acid (100 mg in 20 mL of deionized water) were added in place of sample. Total phenolics

content was subjected to analysis of variance using PROC GLM, with biofumigation treatment as a fixed effect.

2.5.4. Quantification of individual anthocyanins

A 1-mL extract sample was transferred to an amber HPLC vial (1.8 mL) fitted with a polytetrafluoroethylene screw cap. Anthocyanins were separated and identified using an Agilent 1200 series HPLC system equipped with an inline continuous vacuum solvent degasser, binary pump, temperature-controlled autosampler, column compartments, and a photodiode-array detector (PDA), all controlled by Chemstation (rev. B.03.01) software. Solvents used were 5% formic acid and acetonitrile (ACN) at a flow rate of 1.0 mL/min. The gradient expressed as percent ACN was; 0 to 2 min, 7%; 15 min, 15%; 18 min, 30%; 20 to 24 min, 100%; and 24 to 28 min, 7%. The autosampler compartment was maintained at 4ºC. The injection volume for both samples and standards was 10 µL at a draw speed of 100 µL/min. The anthocyanin compounds were retained using an Agilent Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm × 5 µm) protected by a guard column (12.5 mm × 4.6 mm × 5 µm) of the same phase, all held at 30ºC within the column compartment. Eluted compounds were detected using the PDA equipped with a semi-micro flow cell with a full spectral scan set from 190 to 650 nm (2 nm steps), and monitored at 525 nm for the detection of anthocyanins with a bandwidth of 4 nm.

Fractions for each peak were collected and concentrated to near dryness using a rotor evaporator. A Bruker Autoflex MALDI-TOF/MS (Bruker Daltonics, Billerica, MA) was used to produce ions (*m/z*) which were compared against libraries and published

literature (Wu and Prior, 2005) to identify peaks of interest. Authentic standards for the majority of the peaks are not available; thus, all peaks are expressed in cyanidin 3-*O*-galactoside (ideain chloride, Indofine Chemical Company, Hillsborough, NJ) equivalents. A standard curve based on concentrations ranging from 2 to 500 µg/g was used to quantify the compounds (r^2 = 0.999; $P \leq 0.0001$). Cyanidin 3-*O*-galactoside equivalents (µg/g) were subjected to analysis of variance with Dunnett's test to compare the control with biofumigation treatments (fixed effects) using PROC GLM in SAS v. 9.2.

2.6. Volatile concentrations in biofumigation chambers

A separate experiment was conducted to determine volatile concentrations in biofumigation chambers over time. Holes were drilled in the lid of Mason jars and a rubber septum was inserted. Biofumigants were placed in aluminum dishes inside Mason jars, as described for the above experiments (except that no fruit were added), and jars were placed at 23 to 25°C for 24 h, after which they were transferred to 7°C. After 0, 1, 2 or 3, 4 or 5, 6, and 7 days, a 1-mL headspace sample was drawn with a 1-mL Agilent glass syringe through the septum and injected into an HP 5890 Series II (Hewlett-Packard, San Fernando, CA) gas chromatograph (GC) equipped with an Agilent DB5 (30 m length × 0.32 mm diameter × 0.25 µm film thickness) fused silica capillary column with a flame ionization detector (FID) at 280°C. Day 0 refers to the headspace sampling before the jars were transferred from 25^{o}C to 7^{o}C. The injection port temperature was 225°C, and helium was used as carrier gas at a flow rate of 1.7 mL/min. The sample was injected in split mode at 49°C with a purge time of 0.5 min. The column temperature at the beginning was 49°C, increased to 135°C at a rate of

10°C/min and held for 0.5 min, increased to 280°C at a rate of 30°C/min and held for 5 min. HP 3396 Series III software was used to integrate the signal output and calculate the area under each peak. Standard curves for each compound were made by running different dilutions of pure compounds in redistilled hexane (95% n-hexane, J.T. Baker, Phillipsburg, NJ) in triplicate. These standard curves were used to calculate the concentrations of compounds in biofumigation chambers from their respective peak areas (Table 3.1).

Peak identification was done by injecting 1 mL of headspace gas sample from the biofumigation jar into an Agilent 6890 GC/MS equipped with Agilent DB5-MS column (30 m length × 250 µm diameter × 0.25 µm film thickness). Initial oven temperature was 49°C with a purge time of 0.5 min, and was increased to 280°C at a rate of 10°C/min held for 5 min. Sample was injected in split mode with inlet port at 225°C, pressure 47 kPa, split ratio 5:1, and helium as a carrier gas with a flow rate of 7.9 mL/min. The volatiles were identified using their relative abundance in NIST 2 and Wiley 7 libraries.

3. Results

Postharvest disease incidence following artificial inoculation was always highest in the control (no biofumigation) and in *p*-cymene-treated fruit (Fig. 3.1). Overall, there was no consistent effect of biofumigation treatments on disease incidence, except for Sporatec which resulted in significant disease reduction compared with the untreated control in five out of eight cases. Sporan and linalool also decreased disease, but the effect was significant only in three out of eight cases. In general, postharvest disease

incidence and significance level of treatment effects was higher in the second run of the experiment than in the first run (Fig. 3.1).

Postharvest biofumigation had negative effects on sensory quality of treated fruit (Tables 3.2 and 3.3). Blueberry-like flavor and juiciness were decreased significantly by all biofumigation treatments as compared to the non-biofumigated control in the first experimental run (Table 3.2). Linalool, *p*-cymene, and peppermint oil also resulted in significant increases of sourness in the first run. Astringency was also significantly increased in *p*-cymene, peppermint oil, and Sporan treatments (Table 3.2). Also in the second run, there was a significant reduction in the rating of blueberry-like flavor in all treatments (Table 3.3).

There were no consistent effects of biofumigation treatment on antioxidant levels of treated fruit, except for reduced total phenolics content in *p*-cymene-treated fruit in the second run (Fig. 3.2). Postharvest biofumigation had some negative effects on the anthocyanin content of fruit (Tables 3.4 and 3.5). In the first experimental run, *p*-cymene treatment resulted in a significant decrease in the content of delphinidin-arabinoside, whereas in the second run *p*-cymene treatment resulted in a significant decrease of 10 anthocyanin compounds from a total of 12 assayed (Table 3.5). Cinnamon oil also decreased the contents of three anthocyanin compounds (cyanidins) significantly.

Concentrations of the volatiles were relatively constant during the 7-day cold-storage period, after an initial drop in concentrations after biofumigation chambers were transferred from room temperature to cold storage (Fig. 3.3). Actual volatile concentrations during cold storage ranged from a low of ~0.1 µL/L for linalool to a high of ~15 µL/L for Sporan.

4. Discussion

Out of the six plant essential oils evaluated in this study, only one (Sporatec) resulted in significant and relatively consistent postharvest disease suppression. However, even for Sporatec the final disease incidence after artificial inoculation was still relatively high. This is in contrast to results reported previously by Wang et al. (2008), who demonstrated reduced natural decay of northern highbush blueberry fruit following biofumigation with *p*-cymene and linalool. These contrasting results are likely due to differences in experimental methodology. In Wang et al. (2008) fruit were incubated at 10°C during biofumigation and evaluated 4 weeks after the onset of treatment, whereas we evaluated fruit after 1 week of biofumigation at 7°C followed by 3 days outside the biofumigation chamber at room temperature (the latter period mimicking unrefrigerated storage in the consumer's home). Thus, the combination of artificial inoculation, lower biofumigation treatment temperature, and the 3-day post-treatment period at room temperature could explain the reduced treatment efficacy in our study compared with that of Wang et al. (2008). The volatile concentrations measured in biofumigation chambers in our study were relatively low (ranging from ~0.1 to 15 µL/L), which was clearly due to low volatility of the essential oils at low temperature, as indicated by the considerable drop in headspace concentrations when Mason jars were moved from room temperature to 7°C. Unfortunately, it is not feasible to store blueberries commercially at higher temperatures where the volatility of the biofumigation products would be greater.

Even if blueberries could be stored at a higher temperature where volatility of the biofumigants would be increased, negative impacts on the sensory quality of treated fruit would likely result, as documented in our study for fruit biofumigated at 7°C. Indeed, biofumigation treatment left a strong flavor on the fruit which detracted from blueberry-like flavor and impacted other desirable characteristics such as astringency, bitterness, and juiciness negatively. To our knowledge this is the first study reporting the sensory implication of using volatiles produced from essential oils on blueberries.

The essential oils used in this study did not improve the antioxidant levels in treated fruit. On the contrary, *p*-cymene-treated fruit resulted in decreased antioxidant activity in the most of the tests performed. Thus, the finding of increased antioxidant levels following biofumigation reported previously for northern highbush blueberry (Wang et al., 2008) could not be confirmed in our study. Again, differences in methodology (storage temperature and duration, presence or absence of a post-fumigation exposure period at room temperature) are likely responsible for these discrepancies.

In summary, the potential for postharvest cold-storage biofumigation in harvested blueberries is limited. We arrive at this conclusions based on the limited efficacy of biofumigation treatments in reducing postharvest decay, negative impacts on sensory attributes, and failure to increase antioxidant levels. The search for new approaches to supplement the currently limited arsenal of postharvest decay management strategies in harvested blueberries continues.

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Wu, X., and Prior, R.L., 2005. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: Fruits and berries. J. Agric. Food Chem. 53, 2589–2599.

Table 3.1. Standard curves used to calculate volatile concentrations of plant essential oils in biofumigation chambers over time.

Essential oil	Standard curve[a]	r^2	*P*	*n*
Cinnamon oil	$y = 18{,}064{,}644.0\ x$	0.998	<0.0001	5
Linalool	$y = 839{,}932{,}063.7\ x$	0.942	0.0023	4
p-Cymene	$y = 1{,}988{,}154{,}509.9\ x$	0.998	<0.0001	5
Peppermint oil	$y = 103{,}018{,}610.2\ x$	0.998	<0.0001	5
Sporan fungicide	$y = 50{,}748{,}712.9\ x$	0.993	<0.0001	5
Sporatec fungicide	$y = 55{,}909{,}939.6\ x$	0.972	<0.0001	5

[a]y = peak area, x = amount of essential oil injected (µL). Regression equation for standard curve was fitted without an intercept.

Table 3.2. Results of sensory analysis of Tifblue rabbiteye blueberry fruit after cold-storage biofumigation with different essential oils in the first run of the experiment[a].

Treatment	Sweet	Sour	Bitter	Astringent	Flavor	Crisp	Firm	Juiciness	Color
Control	5.35	1.05	1.49	0.988	7.31	2.96	3.60	6.92	11.0
Cinnamon oil	5.18	1.98	1.52	1.69	4.76 *	2.09	2.80	4.72 *	10.8
Linalool	3.92	4.20 *	5.95 *	2.01	2.18 *	1.76	2.40	4.56 *	10.6
p-Cymene	3.66	3.39 *	3.00	3.85 *	3.02 *	2.34	2.60	3.78 *	10.0
Peppermint oil	3.65	2.98 *	3.58 *	2.54 *	3.31 *	1.40	2.00	4.45 *	10.4
Sporan fungicide	4.10	1.60	2.30	2.55 *	3.15 *	2.20	3.00	4.91 *	11.0
Sporatec fungicide	3.41	2.50	2.95	2.12	2.32 *	1.64	1.98	4.81 *	10.8
P-value	0.0571	0.0091	0.0003	0.0231	<0.0001	0.2351	0.0615	0.0389	0.1773

[a]Sensory descriptors were ranked on a scale of 0 to 15 by eight trained panelists. Values in the same column without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test ($\alpha = 0.05$).

Table 3.3. Results of sensory analysis of Tifblue rabbiteye blueberry fruit after cold-storage biofumigation with different essential oils in second run of the experiment[a].

Treatment	Sweet	Sour	Bitter	Astringent	Flavor	Crisp	Firm	Juiciness	Color
Control	3.34	3.45	1.86	2.10	7.10	3.00	3.19	5.49	8.43
Cinnamon oil	3.96	3.50	2.05	3.24	5.03 *	2.70	3.10	5.26	9.06
Linalool	2.50	4.48	4.13	3.34	1.96 *	1.94	1.91	5.08	8.76
p-Cymene	3.05	3.14	2.44	2.99	3.06 *	2.83	3.00	3.79 *	8.30
Peppermint oil	2.25	3.16	2.79	3.71	1.70 *	2.55	2.38	3.64 *	9.16
Sporan fungicide	3.30	4.06	2.80	3.06	3.40 *	2.06	2.36	4.98	9.11
Sporatec fungicide	3.23	4.79	2.38	2.54	2.41 *	1.99	2.53	4.23 *	8.51
P-value	0.2548	0.5321	0.2924	0.6612	<0.0001	0.3265	0.1980	0.0015	0.8165

[a]Sensory descriptors were ranked on a scale of 0 to 15 by eight trained panelists. Values in the same column without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test (α = 0.05).

Table 3.4. Anthocyanin content expressed as cyanidin-3-*O*-galactoside equivalent (µg/g) in Tifblue rabbiteye blueberry fruit after cold-storage biofumigation with different essential oils in the first run of the experiment[a].

Treatment	Cya-arab	Cya-gal	Cya-glu	Del-arab	Del-gal	Mal-gal	Mal-glu	Peo-gal	Peo-glu	Pet-arab	Pet-gal	Pet-glu
Control	46.5	90.9	38.5	67.4	110.9	238.2	115.5	60.7	137.9	40.2	73.6	46.3
Cinnamon oil	44.9	85.4	37.1	68.4	112.1	239.7	121.0	56.6	140.8	40.8	74.5	47.6
Linalool	41.2	79.4	34.9	64.3	108.0	233.0	110.5	52.8	134.4	38.0	71.8	45.8
p-Cymene	40.2	76.1	32.6	47.4 *	77.6	177.9	86.3	47.4	105.1	30.6	56.1	36.2
Peppermint oil	42.8	85.2	36.1	65.3	112.5	211.7	101.6	50.3	119.3	37.8	72.1	43.4
Sporan fungicide	42.7	83.7	35.5	58.3	97.3	191.9	95.4	50.4	112.1	33.7	62.3	40.0
Sporatec fungicide	45.1	87.6	36.9	66.2	109.5	224.2	111.4	56.0	130.7	39.0	72.4	45.5
MSD[b]	13.9	31.2	11.3	17.8	34.1	70.1	29.3	15.9	38.4	9.75	21.9	12.5

[a]Means of four replicates. Values in the same column without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test ($\alpha = 0.05$).Cya: cyanidin, Del: delphinidin, Mal: malvidin, Peo: peonidin, Pet: petunidin, arab: arabinoside, gal: galactoside, glu: glucoside.

[b]Minimum significant difference.

Table 3.5. Anthocyanin content expressed as cyanidin-3-*O*-galactoside equivalent (µg/g) in Tifblue rabbiteye blueberry fruit after cold-storage biofumigation with different essential oils in the second run of the experiment[a].

Treatment	Cya-arab	Cya-gal	Cya-glu	Del-arab	Del-gal	Mal-gal	Mal-glu	Peo-gal	Peo-glu	Pet-arab	Pet-gal	Pet-glu
Control	64.7	149.1	59.6	78.2	142.3	257.3	130.0	67.3	121.2	41.0	80.7	52.0
Cinnamon oil	51.5 *	112.9 *	45.5 *	68.0	118.3	252.2	119.5	63.9	142.6	37.2	70.5	43.8
Linalool	61.9	132.6	54.6	74.5	130.2	271.5	129.0	69.8	157.4	40.8	79.2	51.1
p-Cymene	48.2 *	104.3 *	45.6 *	48.5 *	85.8 *	219.0	106.1	52.5 *	133.1	32.3 *	63.0 *	42.9
Peppermint oil	56.2	122.8	52.3	69.1	123.1	235.6	119.8	63.1	107.1	36.8	71.9	48.6
Sporan fungicide	57.5	126.0	52.5	65.6	117.0	243.0	114.4	62.1	143.3	36.5	72.0	47.5
Sporatec fungicide	57.1	126.6	52.3	71.6	125.5	250.3	120.1	63.2	148.8	38.9	74.7	48.9
MSD[b]	11.7	32.7	13.1	14.2	28.8	55.3	27.1	11.3	78.8	6.93	14.2	10.0

[a]Means of four replicates. Values in the same column without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test (α = 0.05).Cya: cyanidin, Del: delphinidin, Mal: malvidin, Peo: peonidin, Pet: petunidin, arab: arabinoside, gal: galactoside, glu: glucoside.

[b]Minimum significant difference.

Fig. 3.1. Postharvest disease incidence on Tifblue rabbiteye blueberry following artificial inoculation with sterile water (control), *Alternaria alternata*, *Botrytis cinerea*, or *Colletotrichum gloeosporioides,* followed by cold-storage biofumigation with different essential oils in the first (**A**, **B**, **C** and **D**) and second (**E**, **F**, **G** and **H**) experimental run. Values are means and standard errors of four replicates. Treatments without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test (α = 0.05).

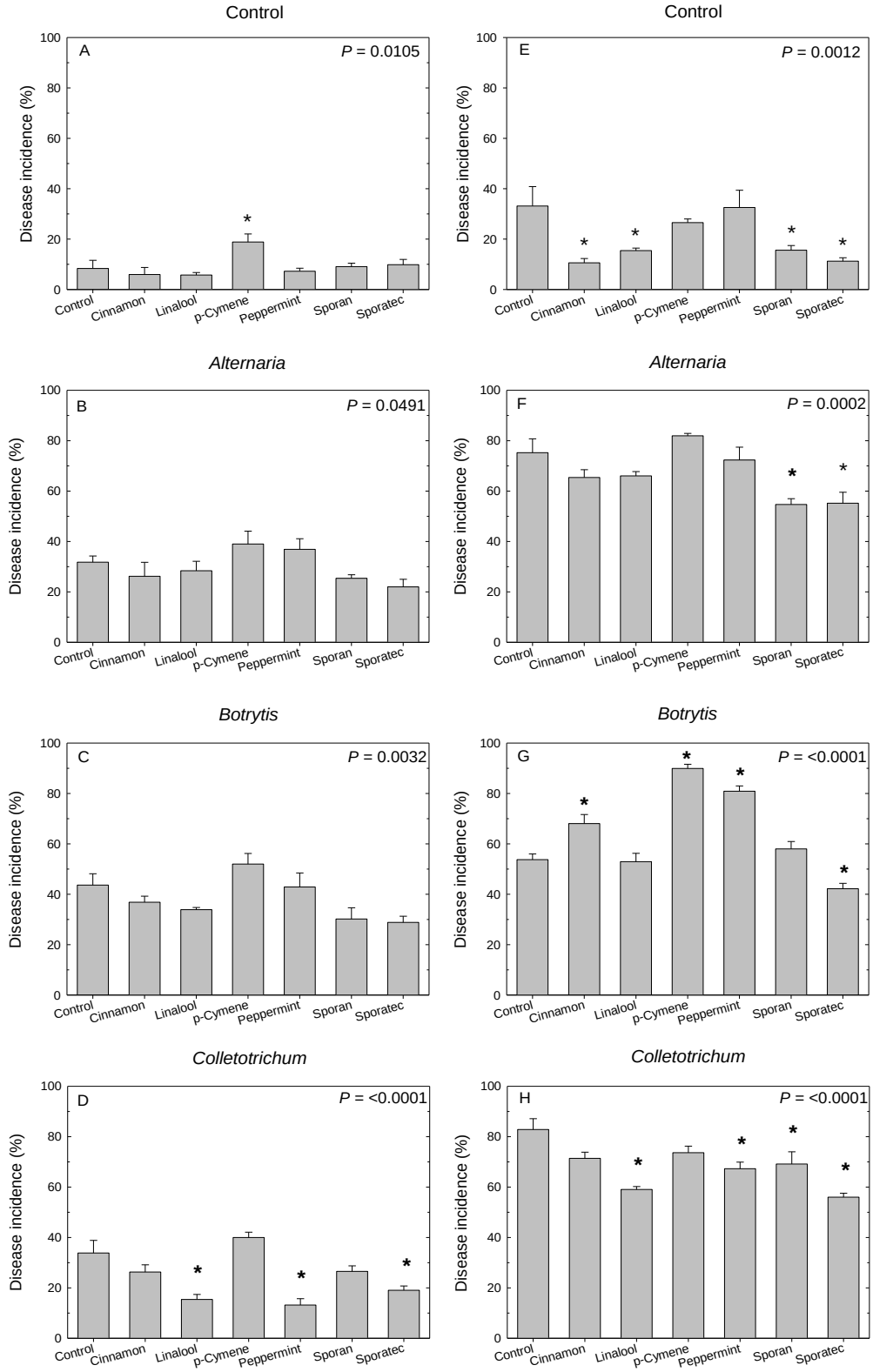

Control
A
P = 0.0105
Control
E
P = 0.0012
Alternaria
B
P = 0.0491
Alternaria
F
P = 0.0002
Botrytis
C
P = 0.0032
Botrytis
G
P = <0.0001
Colletotrichum
D
P = <0.0001
Colletotrichum
H
P = <0.0001
Disease incidence (%)
0
20
40
60
80
100
Control
Cinnamon
Linalool
p-Cymene
Peppermint
Sporan
Sporatec

Fig. 3.2. Total oxyradical scavenging capacity (TOSC) (first and second run, **A** and **D**, respectively), percent inhibition of the DPPH molecule by antioxidants present in the fruit (first and second run, **B** and **E**, respectively), and total phenolics content (first and second run, **C** and **F**, respectively) in Tifblue rabbiteye blueberry fruit after cold-storage biofumigation with different essential oils. Values are means and standard errors of four replicates. Treatments without an asterisk (*) are not significantly different from the untreated control according to Dunnett's test (α = 0.05).

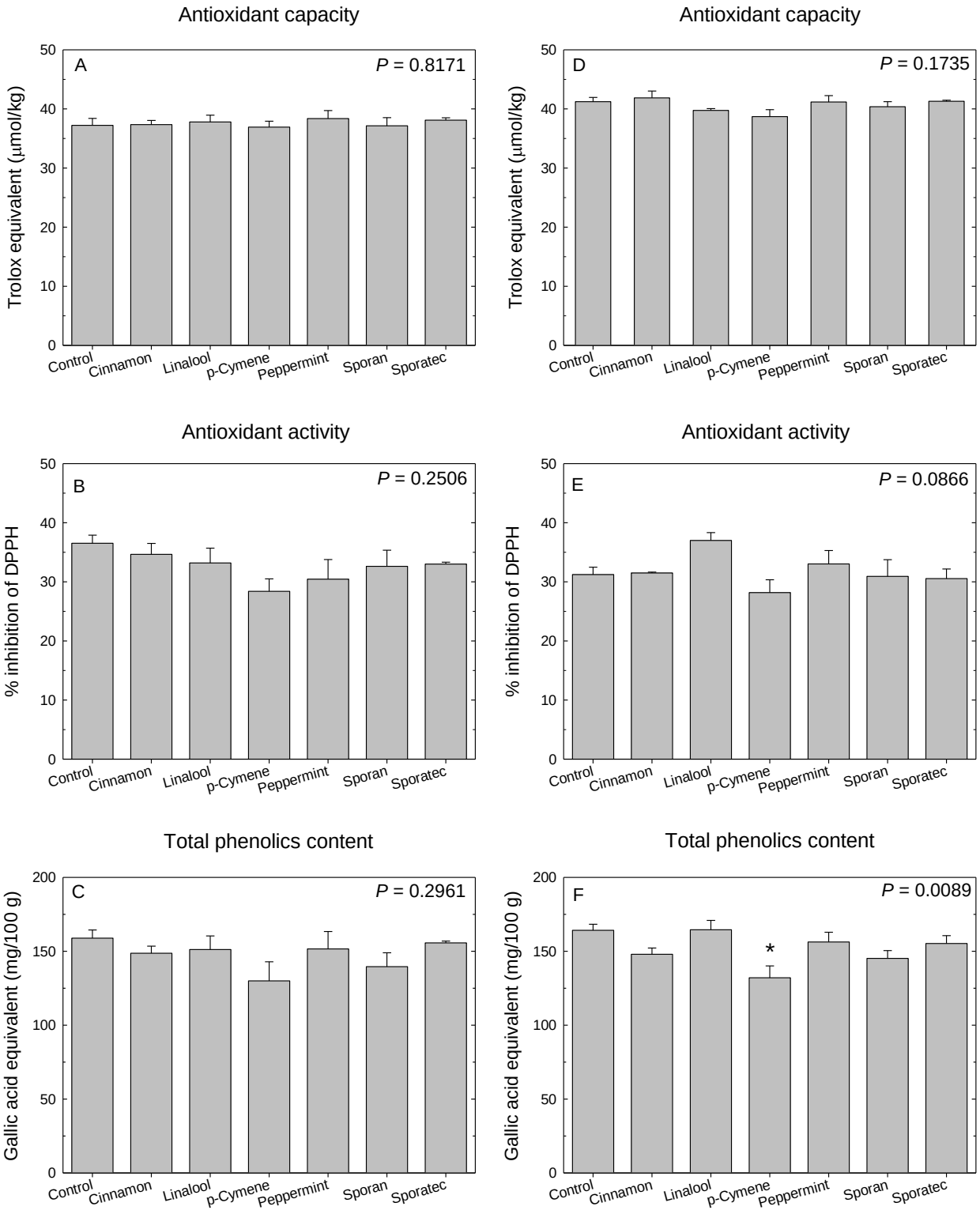
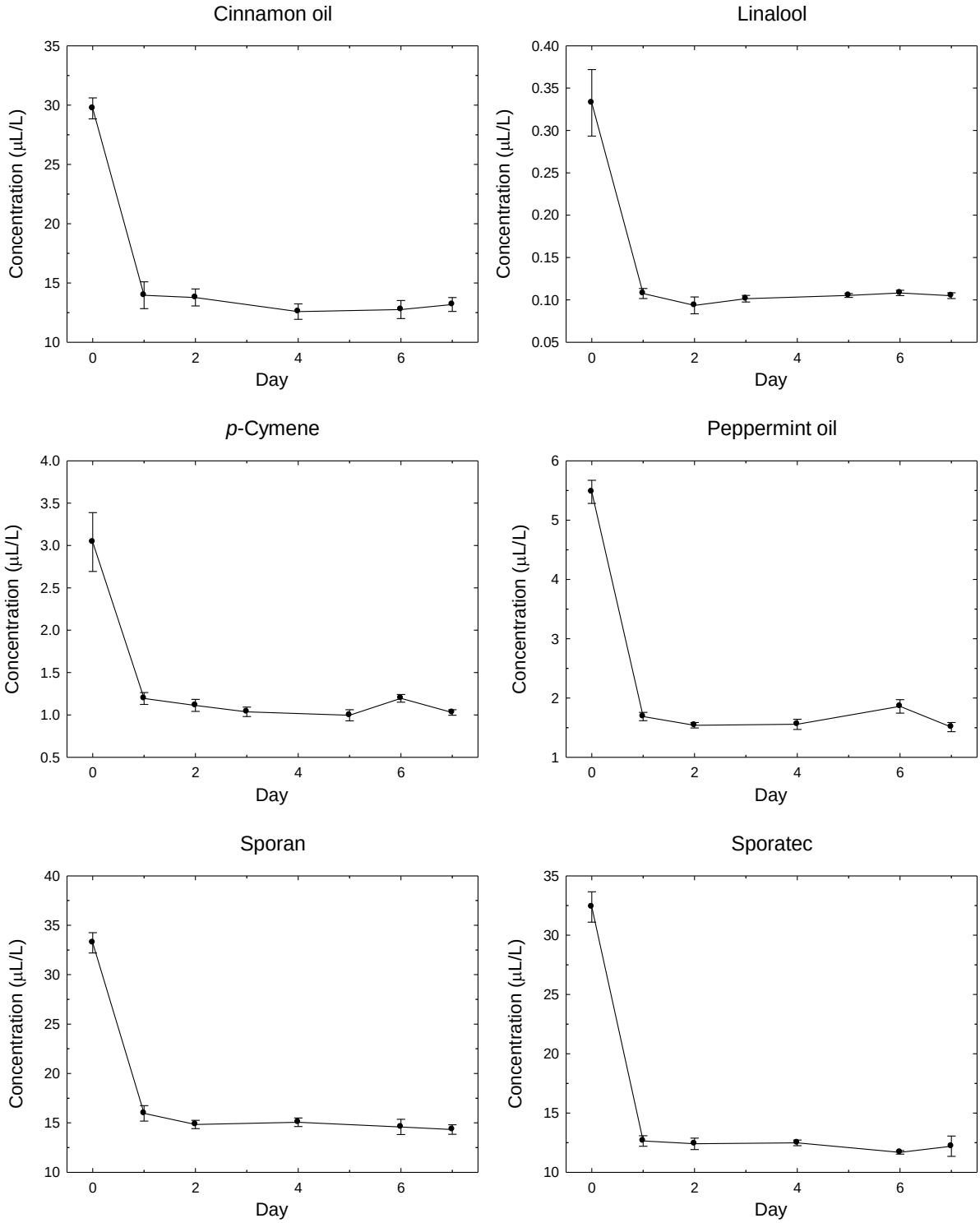

Antioxidant capacity
A
P = 0.8171
Trolox equivalent (μmol/kg)
Antioxidant capacity
D
P = 0.1735
Trolox equivalent (μmol/kg)
Antioxidant activity
B
P = 0.2506
% inhibition of DPPH
Antioxidant activity
E
P = 0.0866
% inhibition of DPPH
Total phenolics content
C
P = 0.2961
Gallic acid equivalent (mg/100 g)
Total phenolics content
F
P = 0.0089
*
Gallic acid equivalent (mg/100 g)
Control
Cinnamon
Linalool
p-Cymene
Peppermint
Sporan
Sporatec

Fig. 3.3. Concentration of volatiles from essential oils in the headspace of biofumigation chambers over time. Day 0 corresponds to the assessment after 24 h of incubation at room temperature (23 to 25°C), just before transfer to cold storage (7°C). All subsequent measurements are after exposure in cold storage.


Cinnamon oil
Concentration (µL/L)
Day
Linalool
Concentration (µL/L)
Day
p-Cymene
Concentration (µL/L)
Day
Peppermint oil
Concentration (µL/L)
Day
Sporan
Concentration (µL/L)
Day
Sporatec
Concentration (µL/L)
Day

CHAPTER 4

CONCLUSIONS

The research reported in this thesis covered two different but related aspects of the postharvest pathology of blueberry fruit. In the first study, the potential for machine-harvesting southern highbush blueberry (SHB) genotypes having the novel crispy flesh berry type was evaluated with respect to postharvest fungal decay and microbial contamination of fruit. Results from this study documented that mechanical harvesting of crisp-textured SHB genotypes is feasible from a postharvest pathology perspective. Most importantly, machine-harvested fruit of crispy flesh genotypes developed levels of natural postharvest disease lower or similar to those of hand-harvested fruit of conventional genotypes. Results of artificial inoculations with *Alternaria alternata*, *Botrytis cinerea*, and *Colletotrichum gloeosporioides* supported the fact that crispy berries develop less disease than their conventional counterparts, regardless of harvest method. Our study further documented a negative association between postharvest disease incidence and fruit firmness across genotypes and harvest methods, indicating that fruit firmness is a good predictor for postharvest decay risk. This relationship also suggests that the lower level of postharvest disease in crispy flesh genotypes is mostly due to their greater firmness, and not some other form of genetic resistance.

Although the two fruit firmness groups and harvest methods differed in postharvest disease incidence in our study, the pathogen complex responsible was similar across treatments. *Aureobasidium pullulans*, *Cladosporium* spp. (both usually

considered secondary pathogens) and *Alternaria* spp. were observed most commonly. Pathogens such as *B. cinerea* and *Colletotrichum* spp. that are common in other blueberry growing areas of the United States were also observed, albeit in smaller proportions (except for a higher incidence of *Colletotrichum* spp. in 2010).

Although this study demonstrated that mechanical harvest of the novel crisp-textured SHB cultivars is feasible from a postharvest pathology perspective, further research is needed to address potential problems related to in-field losses, postharvest quality, and consumer acceptance of machine-harvested crispy berries. We are currently collaborating with plant breeders, horticulturists, postharvest physiologists, and food scientists to investigate these aspects comprehensively and develop an integrated system for production, harvest, and marketing of mechanically harvested SHB.

In the second study that formed part of this thesis, the potential of plant essential oils as cold-storage biofumigants was evaluated with respect to postharvest disease control, sensory quality, and antioxidant content of treated blueberry fruit. Out of the six essential oils evaluated, only one (the plant oil-derived biofungicide Sporatec) resulted in significant and relatively consistent disease suppression. However, even for Sporatec the final disease incidence after artificial inoculation was still relatively high. Furthermore, biofumigation had a negative impact on the sensory quality of treated berries, leaving a strong flavor which detracted from blueberry-like flavor and changing other desirable characteristics such as sweetness and juiciness negatively. Finally, the finding of increased antioxidant levels following biofumigation reported previously for northern highbush blueberry fruit (Wang et al. 2008) could not be confirmed in our study, where antioxidant activity, total phenolics levels, and the types and

concentrations of anthocyanins remained largely unchanged. Taken together, the results of this study suggest that the potential for postharvest cold-storage biofumigation in harvested blueberries is limited. The search for new approaches to supplement the currently limited arsenal of postharvest decay management strategies in harvested blueberries continues.

LITERATURE CITED

Wang, C.Y., Wang, S.Y., and Chen, C. 2008. Increasing antioxidant activity and reducing decay of blueberries by essential oils. J. Agric. Food Chem. 56:3587–3592.

APPENDICES

Appendix A. Illustration of quantitative descriptive ballot used for sensory evaluation of biofumigated blueberries[a].

Panelist _____ Sample_____

Instructions: Please cleanse the palate with water and cracker between each sample. Taste the blueberry and mark the line for intensity of each characteristic below. The middle line is standard.

Sweetness

| | |

slight intense

Please evaluate the blueberry texture and mark the line that best describes the product from each characteristic below.

Crispness

| | |

soggy very crisp

Please evaluate the blueberry appearance and mark the line at the point that best describes the product from the characteristic below.

Color intensity

|

light dark

[a]Modified from Smith, K.C. 2010. The Sensory Evaluation and the Effect of Radio Frequency Application on Southern Higbush Blueberries. M.S. thesis. Dept. of Food Science and Technology, Univ. of Georgia, Athens.

Appendix B. Definitions and references of descriptors used for the sensory evaluation of biofumigated blueberries[a].

Descriptor	Definition	Reference
Sweetness	The taste stimulated by sucrose, glucose, or fructose	Cane sugar (Wal-Mart, Bentonville, AR) Intensity = 5
Sourness	The taste stimulated by acids such as citric, malic, and phosphoric acid	Citric acid (Sciencelab.com, Houston, TX) Intensity = 2
Bitterness	The taste stimulated by substances such as caffeine and hops	Caffeine (Sciencelab.com, Houston, TX) Intensity = 2
Blueberry-like flavor	The expected flavor associated with consuming a blueberry	NA
Astringency	The shrinking or puckering of the tongue surface caused by substances such as tannins or alum	Alum (McCormick and Co., Hunt Valley, MD) Intensity = 2
Crispness	The force and sound (pitch) with which a sample breaks and fractures on the first and second chewing	Club cracker Intensity = 5
Firmness	The force required to fracture the sample between molars	Queen-size olives Intensity = 6
Juiciness	The quantity of juice released by the sample when chewed up to five times	Cucumber Intensity = 8
Blue color	The blue color intensity from light to very dark	Light = 1; light medium = 4; medium = 7.5; medium dark = 11; dark = 14

[a]Modified from Smith, K.C. 2010. The Sensory Evaluation and the Effect of Radio Frequency Application on Southern Highbush Blueberries. M.S. thesis. Dept. of Food Science and Technology, Univ. of Georgia, Athens.