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Effect of postharvest biofumigation on fungal decay, sensory quality, and antioxidant levels of blueberry fruit

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ABSTRACT

Postharvest decay, caused by various fungal pathogens, is an important concern in commercial 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 (rosemary and wintergreen oils) and Sporatec (rosemary, clove, and thyme oils) were evaluated as postharvest biofumigants to manage fungal decay under refrigerated holding conditions. Hand-harvested Tifblue rabbiteye blueberry fruit were inoculated at the stem end with conidial suspensions of *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum acutatum*, or sterile deionized water (check inoculation) and subjected to biofumigation treatments under refrigeration (7 °C) for 1 wk. Sporatec volatiles reduced disease incidence significantly ($P < 0.05$) 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 capacity and individual anthocyanins, and no consistent effects on these antioxidant-related variables were found in 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 biofumigation of blueberries under refrigerated holding conditions appears limited.

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1. Introduction

Worldwide, the United States ranks 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 yr, from 16,341 ha in 2001 to 25,807 ha in 2009 (Anonymous, 2002, 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 potential health benefits such as anti-cancer and anti-aging properties as well as prevention of heart disease, most of which are thought to be related to their high levels of antioxidants (Beattie et al., 2005; Juranić and Žižak, 2005).

In all major blueberry-producing areas of the United States, postharvest decay, associated mostly with fungal infections, is an important production problem. Indeed, numerous 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 most commonly reported. Other fungal genera causing postharvest decay of blueberries are *Aspergillus*, *Aureobasidium*, *Catenophora*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, and *Rhizopus* (Ceponis and Cappellini, 1979; Tournas and Katsoudas, 2005; Barrau et al., 2006).

To manage postharvest decay, pre-harvest fungicide sprays (Milholland and Jones, 1972), postharvest chemical dips (Ceponis and Cappellini, 1978), postharvest cooling (Ballinger et al., 1973;

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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 fruit. Therefore, in addition to cooling and CO_2 storage, other means to control postharvest decay 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 held at a storage temperature of 10 °C (Wang et al., 2008). In the same study, some of these oils also increased the levels of health-promoting antioxidants in treated fruit compared with the untreated check. However, in these pilot experiments 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 (rosemary, clove, and thyme oils) and Sporan (rosemary and wintergreen 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 biofumigants in refrigerated holding conditions to control postharvest decay of blueberry fruit following artificial inoculation with *A. alternata*, *B. cinerea*, and *C. acutatum*; and (2) assess the impact of biofumigation on sensory qualities and antioxidant levels of treated fruit.

2. Materials and methods

2.1. Fruit samples and essential oils

Two independent experimental runs were conducted using fruit of Tifblue rabbiteye blueberry (*Vaccinium virgatum*) hand-harvested from commercial blueberry plantings in northern and southern Georgia, respectively. Both plantings received no pre-harvest fungicide applications. Fruit were transported to the laboratory in an air-conditioned vehicle and 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 plant oils, viz. Sporatec (Brandt Consolidated, Springfield, IL) and Sporan (EcoSmart Technologies, Franklin, TN), were included in the study.

2.2. 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. acutatum* (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 check group consisted of fruit inoculated similarly with 20 μL of sterile water. Inoculated fruit were pre-incubated at 23–25 °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, 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 positioned over the weighing dish. A 1-mL volume of each biofumigant oil (no oil for the check biofumigation) was pipetted into the weighing dish, the lid of the Mason jar was tightened, and the jar was incubated without fruit at 23–25 °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 jars were placed in a dark cold room (7 °C). This temperature was chosen to reflect typical holding conditions in commercial blueberry packinghouses in southern Georgia, where the cold-storage temperatures used commonly in postharvest chains for other fruits (~2 °C) would be uneconomical. After 7 d, fruit were removed, transferred into 550-mL plastic clamshells, and kept at 23–25 °C for 3 d. Infected fruit were counted from each clamshell by observing under low-power magnification (10–63×) 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 check ($P = 0.05$).

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 × 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 d in the cold room (7 °C), fruit were transferred to 550-mL plastic clamshells and pooled from the four replicates to make approximately 0.23 kg of fruit per biofumigant treatment for sensory evaluation.

Sensory analysis was done as described in detail by Smith (2010). Briefly, evaluations were performed by eight trained panelists from the Department of Food Science and Technology, University of Georgia, Athens, under IRB approval no. 2009-10159-0. 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 (least pronounced) to 15 (most pronounced). 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-purchased blueberries. 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 check.

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