Postharvest Biology and Technology 83 (2013) 40–46

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Determination of optimal sulfur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit

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ARTICLE INFO

Article history:
Received 4 July 2012
Accepted 11 March 2013

Keywords:
Vaccinum spp.
Botrytis cinerea
Decay
Postharvest diseases
Sulfur dioxide

ABSTRACT

Highbush blueberries (*Vaccinum* spp.) are a major export fruit crop of Chile which is stored at 0 °C and transported to markets in Asia, Europe, and the USA, using more than 15 d of maritime transportation. Under these conditions, gray mold caused by *Botrytis cinerea* can produce important economic losses. The effectiveness of sulfur dioxide (SO_2) concentration × time treatments on gray mold control was determined in the laboratory and validated prior to refrigerating the fruit, using pallet scale SO_2 fumigation treatment on the following blueberry cultivars: 'Brigitta', 'Legacy', 'Liberty' and 'O'Neal'. In inoculated 'Brigitta' and 'Liberty' blueberries, gray mold prevalence varied from 97.2% to 97.5% in non-treated fruit, and this value was reduced from 7.9% to 6.1% in blueberries that were exposed to a SO_2 concentration × time (*Ct*) product of 400 ($\mu L\,L^{-1}$) h. The relationship between SO_2 *Ct* products and gray mold prevalence under laboratory conditions was best explained by exponential models, which had a determination coefficient (R^2) that ranged from 0.88 to 0.96. The estimated EC_{90} values varied between 245 and 400 ($\mu L\,L^{-1}$) h, and the SO_2 *Ct* between 250 and 350 ($\mu L\,L^{-1}$) h was validated using a pallet scale application treatment to obtain the best control and minimal variation. No visual phytotoxicity symptoms of SO_2 were observed with the *Ct* that was tested in this study. Therefore, SO_2 fumigation was demonstrated to be an effective and practical technology for reducing the risk of blueberry gray mold decay during storage, and further effort should be given to register the use of this product for blueberries in the main Chilean export markets.

1. Introduction

Highbush blueberry (*Vaccinum* spp.), with over 8,400 ha planted and 73,341 t harvested, represents 3.2% of the total fresh fruit that was exported from Chile in 2011. Blueberry export is mainly distributed to the USA (Beaudry et al., 1998), but Europe and Asia are also important markets for blueberries from Chile. However, the deterioration of fruit quality that is associated with fruit decay is the main limitation to blueberry export when the postharvest period extends longer than 30 d. Under this circumstance, gray mold, which is caused by *Botrytis cinerea* Pers. ex Fr. (tel. *Botryotinia fuckeliana* de Bary) Whetzel, is one of the most important deterioration factors that affects blueberries during storage and transport.

B. cinerea is a necrotoph fungus with a wide host range, is widely distributed in nature, and can survive in blueberries and other hosts as mycelium and/or as sclerotia in plant debris (Bristow and Milholland, 1995). In grapes, it is generally accepted that infection by *B. cinerea* during cold storage and transport is initiated during flowering or during the early stages of fruit development and that infection is possible through the fruit pedicel, which develops gray mold symptoms after harvest (Droby and Lichter, 2004; Elmer and Michailides, 2004). Despite the availability of botryticide treatments that are applied periodically from the early pink bud to petal fall stages, *B. cinerea* persist in fruit and cause important postharvest losses.

In this context, sulfur dioxide (SO_2), which is widely used on table grapes to prevent fungal decay during storage (Franck et al., 2005; Zoffoli and Latorre, 2011), appears to be an important technology for reducing *B. cinerea* in blueberry fruit and minimizes gray mold prevalence during storage and transport. In Chile, SO_2 is applied by chamber fumigation as soon as the grape is harvested, and this treatment is followed by SO_2 in-package pad released from sodium metabisulfite ($Na_2S_2O_5$) salt (Franck et al., 2005; Zoffoli and Latorre, 2011). The time of SO_2 fumigation that was required to kill 99% of the conidia of *B. cinerea* varied according to the continuous SO_2 concentration that was used. Furthermore, the effectiveness of the SO_2 treatments depends on the concentration × time product (*Ct*) achieved, *e.g.* 100 ($\mu L\,L^{-1}$) h, has been proposed to be the minimum value to achieve 99% of the control levels after exposure at 0 °C (Smilanick and Henson, 1992). SO_2 treatments have also been suggested for controlling postharvest decay of blueberries (Cantín

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http://dx.doi.org/10.1016/j.postharvbio.2013.03.007

et al., 2012) and other fruit species (Cantín et al., 2011; Cheah et al., 1992; Sivakumar et al., 2010; Smilanick et al., 1995; Williams et al., 2003). The phytotoxicity of SO_2 has limited its use; however, this compound appears to be non-phytotoxic on most blueberry cultivars (Cantín et al., 2012; Rivera et al., 2011). Therefore, the objectives of this study were the following: (i) to demonstrate the effectiveness of SO_2 treatment against gray mold, (ii) to determine the optimum concentration × time product (*Ct*) that is needed to develop an SO_2 fumigation protocol for long transport to markets under normal atmospheres and (iii) to study the effect of SO_2 on fruit quality.

2. Materials and methods

2.1. Fruit materials

These studies were performed using the northern highbush blueberry (*Vaccinum corymbosum*) 'Brigitta' and 'Liberty' fruit cultivars and the southern highbush blueberry (*V. corymbosum* × *V. darrowi*) 'Legacy' and 'O'Neal' fruit cultivars. Blueberry fruit were harvested at commercial maturity (total soluble solids 13.2–15.2%), were uniform in size and lacked any visual injuries or shriveling. The fruit were obtained from orchards that were located between Curacaví (lat. 33°24′ S) and Osorno (lat. 38°46′ S) in Chile. As customary for exported fruit, the pedicels were removed prior to packing in 125 g polyethylene terephthalate (PET) clamshells.

2.1.1. Isolation and inoculation

Small pieces (5 mm in length) of the fruit were selected from the margins of the decay lesions from 'Brigitta' blueberries and placed on potato dextrose agar that was acidified with 0.5 $\mu L\,L^{-1}$ of 92% lactic acid (APDA). Pure cultures of the fungus were obtained by transferring hyphal tips to APDA, incubated at room temperature (20–22 °C) under continuous light for 7 d. Isolates were identified on the basis of colony, conidia and conidiophore morphology. The identification of *B. cinerea* was corroborated molecularly using the internal transcribed spacer region (ITS4 and ITS5) of rDNA (White et al., 1990). The isolate named BCA1 was stored and propagated to use for fruit inoculation.

2.2. Effectiveness and optimum SO_2 concentration–time (Ct) product against gray mold

Under laboratory conditions, 'Brigitta' blueberries were surface disinfected by dipping them for 5 min in 75% ethanol within 8 h after harvest, and fruit were dried out in a flow chambers for 2 h at 20 °C prior to inoculating 30 fruit per replicate. An aliquot (5 μL) of conidial suspension (10^5 conidia mL^{-1}) of *B. cinerea* (isolate BCA1) was deposited on the injury that was left at the insertion of the stem to the berry when removing the fruit pedicels during harvesting.

Fruit were incubated for 4 h at room temperature (20–22 °C) prior to placing them in 30 cm × 30 cm × 11.5 cm polyethylene chambers (Tupperware, Brazil). Fruit were fumigated at 20 ± 1 °C and 92% relative humidity (RH), determined with a Hobo Pro RH/Temperature (Onset Computer Co., MA), using a continuous SO_2 stream (2.5 mL s^{-1}) at final outflow concentrations of 22.5, 50.2 or 101.6 $\mu L\,L^{-1}$, which were reached at 6, 5 and 3 h, respectively, after the beginning of the fumigation process (Fig. 1). The SO_2 concentrations were achieved using a gas mixture valve board with a certificated SO_2 cylinder at a concentration of 500 ± 9.9 $\mu L\,L^{-1}$ (Aga S.A., Santiago, Chile) and a certificated air cylinder (0.3 g L^{-1} CO_2 and 20.3 g L^{-1} O_2) (Indura S.A., Santiago, Chile). The inflow and outflow concentrations were monitored using a SO_2 electrochemical detector gas analyzer (Solus Plus®, ThermoGastech Inc., CA), which had a detection limit between 0.1 and 150 $\mu L\,L^{-1}$ and an accuracy of 0.1 $\mu L\,L^{-1}$.

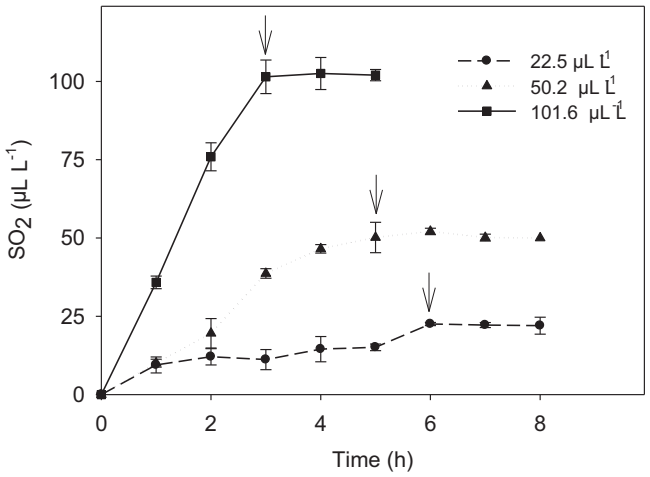

Fig. 1. The dynamics of sulfur dioxide (SO_2) concentration, which was applied into a 10.4 L gasification chamber at 20 ± 1 °C and 92% relative humidity that contained 110 blueberry fruits. The SO_2 concentration was stabilized at 101.6, 50.2 and 22.5 $\mu L\,L^{-1}$ (arrows). The concentration time started to count at time = 0 h. Bar = standard deviation of the means of four replications.

The fruit were exposed to SO_2 concentrations of 22.5, 50.2 or 101.6 $\mu L\,L^{-1}$ for 19.5, 10.0 and 5.5 h, respectively, to attain a SO_2 *Ct* product of 50, 100, 300 and 400 ($\mu L\,L^{-1}$) h for each stream concentration. During SO_2 fumigation, the *Ct* product was monitored using passive dosimeter tubes (5DH, Gastec, Japan) that were graduated from 0 to 600 ($\mu L\,L^{-1}$) h. After SO_2 fumigation, blueberry fruit were distributed into humid chambers (100% RH) at 0 ± 0.5 °C for 15 d. and 3 d. at 20 ± 1 °C prior to determination of gray mold prevalence (% infected fruit divided by 120 fruit) and severity (% lesion length divided by the fruit diameter). An equal number of fruit were inoculated but not fumigated with SO_2 as controls. This experiment was repeated using the blueberry cv., 'Liberty'.

2.3. Effect of SO_2 treatment in fruit firmness

Non-inoculated blueberry fruit cv. 'Brigitta' (125 g, approximately 80 fruit) were exposed to the above-mentioned SO_2 treatments, were stored in PET clamshells and placed in polyethylene bags (20 μm thickness) at 0 ± 0.5 °C for 45 d. The firmness of 20 healthy fruit, as replicates, was determined using a texture analyzer (TA.XT Plus, Stable Micro Systems, Surrey, UK) that was fitted with a 75 mm flat probe, which worked at a speed of 0.2 mm s^{-1} and to a depth of 3 mm. The maximum force that was developed during the test was recorded, and this experiment was repeated under the same experimental conditions using the 'Liberty' blueberry.

2.4. Validation of the effectiveness of SO_2 fumigation treatment

To validate the SO_2 treatment that was obtained under laboratory conditions, the naturally infected 'Brigitta', 'Legacy', 'Liberty' and 'O'Neal' blueberries were obtained from the main commercial planting area of blueberries in Chile. For each cultivar, 12 PET clamshells (125 g of fruit) were placed inside 24-cm × 30-cm × 8.6-cm carton boxes, and 375 boxes (approximately 560 kg fruit) were distributed on wooden pallets (1.2 m × 1.0 m × 1.8 m). Each pallet was covered with a 1.2 m × 1.0 m × 2.3 m tent, and SO_2 was applied using a commercial portable SO_2 injector (Minigas, Proquivi S.A., Santiago, Chile) that was integrated with a SO_2 dosimeter, which was provided with a 90 cm perforated spear that allows the injection of SO_2 gas at a constant flow varying the time thought an integrated microprocessor. The treatment consisted of 20 g (=7.2 L) of SO_2 per pallet for 20 min at 20–22 °C, injected in 35 positions in