Enhancement of Biocontrol Activity of *Cryptococcus laurentii* by Silicon and the Possible Mechanisms Involved

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ABSTRACT

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Exogenous application of silicon (Si) in the form of sodium metasilicate reduced disease development caused by *Penicillium expansum* and *Monilinia fructicola* in sweet cherry fruit at 20°C. The inhibition of fruit decay was correlated closely with Si concentrations. Silicon at concentrations of 1%, in combination with the biocontrol agent *Cryptococcus laurentii* at 1×10^7 cells per ml, provided synergistic effects against both diseases. Population dynamics of *C. laurentii* were stimulated by Si 48 h after the yeast treatment in the wounds of sweet cherry fruit. Silicon strongly inhibited spore germination and germ tube elongation of *P. expansum* and *M. fructicola* in vitro. Based on results with scanning elec-

tron microscopy, growth of both pathogens was significantly inhibited by Si in the wounds of sweet cherry fruit. Compared with the wounded water control, Si treatment induced a significant increase in the activities of phenylalanine ammonia-lyase, polyphenoloxidase, and peroxidase in sweet cherry fruit but did not increase the levels of lignin. Application of Si activated a cytochemical reaction and caused tissue browning near the site of wounding. Based on our studies, the improvement in biocontrol efficacy of antagonistic yeast when combined with Si may be associated with the increased population density of antagonistic yeast by Si, the direct fungitoxicity property of Si to the pathogens, and the elicitation of biochemical defense responses in fruit.

Additional keywords: blue mold, brown rot, induced resistance, mode of action.

Penicillium expansum Link and *Monilinia fructicola* (G. Wint.) Honey cause serious fruit decay worldwide. Current disease management relies heavily on the use of synthetic chemical fungicides. However, concerns about public health and the development of resistant pathogens have increased the search for alternative methods (21). Heat treatment (24), UV-C light (25), biological control using microbial antagonists (13,33,45), and substances generally regarded as safe (14,17) have potential for control of fruit diseases. Biological control has proven useful for suppression of many plant diseases (12,26,27,40). However, application of biocontrol agents alone often has not provided commercially acceptable control of plant diseases, but in combination with other strategies, the performance margin of biological controls was increased (22,41,43).

Silicon (Si) is a major inorganic constituent of plants where the Si content ranges from 0.1 to 10.0% dry weight (18). Silicon application decreases the severity of plant diseases caused by fungal infections (2,28,30,36). Chérif et al. (9) analyzed the effects of Si on cucumber plants and demonstrated that root decay and wilting caused by *Pythium ultimum* was reduced when cucumber plants were treated with potassium silicate. Efficacy of Si to reduce plant diseases is known especially in rice (11). Seebold et al. (38) reported that the application of Si fertilizers reduced the severity of blast, caused by *Magnaporthe grisea*, in irrigated and upland rice. Silicon is effective in reducing plant diseases of wheat (2), muskmelon (28), peach fruit (4), and pear fruit (39,42). The mechanisms responsible for the protection of plants from fungal diseases by Si are not well understood. Silicon may act by

eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds, and pathogenesis-related proteins in the infected plants (9,19).

Synergistic effects for control of fruit decay were observed when biocontrol agents were combined with selected chemicals such as calcium chloride (43,46), chitosan (15), 2-deoxy-D-glucose (16,21), and salicylic acid (34). Little is known about the efficacy of Si on the activity of biocontrol agents. The objectives of this study were to (i) evaluate the effects of Si, alone or in combination with a yeast biocontrol agent, *Cryptococcus laurentii* (Kufferath) Skinner, on control of the postharvest diseases blue mold and brown rot, caused by *P. expansum* and *M. fructicola*, respectively, in sweet cherry (*Prunus avium* L. cv. Hongdeng) fruit; and (ii) determine the modes of action by which Si enhances the biocontrol efficacy of *C. laurentii*.

MATERIALS AND METHODS

Plant material. Sweet cherry (*Prunus avium* cv. Hongdeng) fruit were harvested at commercial maturity and sorted by size and the absence of physical injuries or infections. The firmness of fruit was 13.7 N as determined by a penetrometer (FT-327, UC Fruit Firmness Tester, Milano, Italy), and soluble solids content was 18.7%. Fruit were kept at 0°C and used within 3 days. Prior to use, fruit were disinfested with 2% (vol/vol) sodium hypochlorite for 2 min, washed with tap water, and dried in air.

Yeast antagonist and pathogens. The yeast biocontrol agent, *C. laurentii*, was isolated from the surface of apple fruit following the method of Wilson and Chalutz (44) and identified by CABI Bioscience Identification Services (International Mycological Institute, UK). *C. laurentii* was cultured in 250-ml conical flasks containing 50 ml of nutrient yeast dextrose broth (8 g of nutrient broth, 5 g of yeast extract, and 10 g of dextrose in 1 liter of water) on a rotary shaker at 200 rpm for 48 h at 25°C. Yeast cells were

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centrifuged at $4,000 \times g$ for 10 min, resuspended in sterile distilled water, and adjusted to a concentration of 1×10^7 cells per ml with a hemacytometer.

P. expansum and *M. fructicola* were isolated from infected sweet cherry fruit and maintained on potato dextrose agar (PDA) at 20°C. The spores of both pathogens were removed from the surface of 2-week-old cultures and suspended in 5 ml of sterile distilled water containing 0.05% (vol/vol) Tween 80. The suspensions were filtered through four layers of sterile cheesecloth to remove any adhering mycelia. Spore concentrations of *P. expansum* and *M. fructicola* were determined with a hemacytometer and adjusted to 1×10^4 spores per ml with sterile distilled water.

Effect of concentration of Si on fruit decay. Sweet cherries were wounded (3 mm deep by 3 mm wide) with a sterile nail at the equator of each fruit. Then, 15 μ l of a conidial suspension of *P. expansum* or *M. fructicola*, containing 1×10^4 spores per ml, was placed on each wound. After 12 h, 20 μ l of Si in the form of sodium metasilicate (Sigma Chemical, St. Louis) was added to each wound, with individual fruit being treated with different concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1% [wt/vol]). Treated fruit were put in plastic boxes (200 by 130 by 50 mm) with sterile water to maintain high humidity (about 95%) and stored at 20°C. Disease incidence and lesion diameter were measured after 3 days. There were three replicates of each treatment with 20 fruit per replicate, and the experiment was repeated twice.

Combined effect of Si and *C. laurentii* on fruit decay. In preliminary experiments, *C. laurentii* exhibited persistent protection of fruit from infection by pathogens but did not eradicate pre-existing infections. In contrast, Si suppressed recently established infections but did not protect fruit from infection (data not

shown). In this experiment, *C. laurentii* was applied prior to treatment with Si to suppress diseases of sweet cherry fruit at 20°C. Sweet cherries were wounded as described previously, and 20 μ l of a cell suspension of *C. laurentii* at 1×10^7 cells per ml or sterile distilled water was pipetted into each wound. Within 1 h, each wound was inoculated with 15 μ l of a conidial suspension of *P. expansum* or *M. fructicola* at 1×10^4 spores per ml. After 12 h of incubation, 20 μ l of 1% (wt/vol) sodium metasilicate was added to each wound. Wounds treated with water served as the control. Treated fruit were placed in plastic boxes (200 by 130 by 50 mm) with sterile water to maintain high humidity (about 95%) and stored at 20°C. Disease incidence and lesion diameter were measured after 3, 4, and 5 days. There were three replicates of each treatment with 20 fruit per replicate, and the experiment was repeated twice.

Growth of *C. laurentii* in wounds of sweet cherry fruit. Fruit were wounded, and 20 μ l of a cell suspension of *C. laurentii* at 1×10^7 cells per ml was added to each wound. After 12 h, each wound was treated with 20 μ l of 1% (wt/vol) sodium metasilicate or sterile distilled water and stored at 20°C as described previously. Wound tissue was removed at 0, 24, 48, 72, and 96 h after treatment following the method of Janisiewicz et al. (23). The resulting cylinders of excised tissue (10 mm deep by 10 mm wide) from five fruit were placed in a mortar with 10 ml of sterile distilled water and ground with a pestle. Then, 100- μ l serial 10-fold dilutions were plated on nutrient yeast dextrose agar medium. After incubation at 20°C for 72 h, colonies were counted and expressed as $\log_{10}$ CFU per wound. There were three replicates of each treatment, and the experiment was conducted three times.

Inhibition of growth of *P. expansum* and *M. fructicola* in vitro. The effects of Si on spore germination and germ tube elongation of *P. expansum* and *M. fructicola* were assayed in potato dextrose broth (PDB) according to the method of Tian et al. (43). Spore suspensions were prepared as described previously. Aliquots of 100 μ l of both pathogen suspensions were transferred to a glass tube (180 by 16 mm) containing 5 ml of PDB to obtain a final concentration of 5×10^5 spores per ml. The PDB contained various concentrations of sodium metasilicate (0, 0.2, 0.4, 0.6, 0.8, and 1% [wt/vol]). All tubes were placed on a rotary shaker at 100 rpm at 20°C and incubated for 15 h. For each treatment, within each replicate, approximately 200 spores of each pathogen were observed to determine germination and germ tube length. Each treatment was replicated three times, and the experiment was repeated twice.

Scanning electron microscopy. For scanning electron microscopy, fruit were wounded and 15 μ l of a conidial suspension of *P. expansum* or *M. fructicola* at 1×10^5 spores per ml was placed on each wound. Within 1 h, 20 μ l of 1% (wt/vol) sodium metasilicate or sterile distilled water was added to each wound. Treated fruit were incubated at 20°C as described previously. Tissue samples were taken from the wounds 24 and 48 h after inoculation with *M. fructicola* and *P. expansum*, respectively. Samples were held overnight in fixative (4% [vol/vol] formaldehyde and 1% [vol/vol] glutaraldehyde in 0.1 M phosphate buffer, pH 7.2). Residual fixative was removed with repeated phosphate buffer washes. The samples were subsequently dehydrated in a series of ethanol solutions and then critical point dried with liquid CO₂ in a desiccator. The specimens were mounted onto aluminum specimen stubs using conductive silver paint, sputter coated with gold, and observed with a scanning electron microscope (S-800, Hitachi Co., Tokyo) at 30 kV. Samples from five fruit from each treatment were used.

Induced biochemical reactions. For enzyme assay, sweet cherries were wounded, 20 μ l of 1% (wt/vol) sodium metasilicate was placed in each wound, and fruit were incubated at 20°C as described previously. Wounded fruit treated with water and non-wounded fruit served as controls. Samples containing the pericarp

Fig. 1. Relationship of Si concentration with efficacy against *Penicillium expansum* and *Monilinia fructicola* of sweet cherry fruit at 20°C. Fruit were wounded and inoculated with *P. expansum* or *M. fructicola* at 1×10^4 spores per ml. After 12 h, each wound was treated with sterile distilled water or different concentrations of Si. **A**, Disease incidence and **B**, lesion diameter were measured after 3 days. The data were based on three replicates of 20 fruit each, and the test was repeated twice with similar results. Values represent treatment means of pooled data.

and flesh were taken from the wounds of 30 fruit at 0, 6, 12, 24, and 48 h after treatment. There were three replicates of each treatment, and the experiment was repeated twice.

For phenylalanine ammonia-lyase (PAL) assay, a 3-g tissue sample was homogenized with 30 ml of 50 mM sodium borate buffer (pH 8.8, containing 5 mM β -mercaptoethanol) and 0.5 g of polyvinyl pyrrolidone (PVP) and ground using a polytron tissue grinder (Kinematica CH-6010, Brinkman Co., Kriens-Luzern, Switzerland) at 4°C. The mixture was centrifuged at $15,000 \times g$ for 30 min at 4°C, and the supernatant was collected for enzyme analysis. The PAL activity was assayed following the method of Assis et al. (1) with some modification. One milliliter of enzyme extract was incubated with 2 ml of borate buffer (50 mM, pH 8.8) and 0.5 ml of L-phenylalanine (20 mM) for 60 min at 37°C. The reaction was stopped with 0.1 ml of 6 N HCl. The PAL activity was determined by the production of cinnamate, measured by the absorbance change at 290 nm with a spectrophotometer (UV-160, Shimadzu Corp., Kyoto, Japan). The blank was the crude enzyme preparation mixed with L-phenylalanine with zero time incubation. The protein content of the enzyme extracts was estimated by the method of Bradford (5). Specific enzyme activity was defined as nanomole cinnamic acid per hour per milligram of protein.

Polyphenoloxidase (PPO) and peroxidase (POD) were extracted according to the method of Chen et al. (8). Tissue samples (3 g) of each treatment were homogenized with 30 ml of 0.1 M sodium phosphate buffer (pH 6.4) containing 0.5 g of PVP and ground at 4°C. The homogenate was centrifuged at $15,000 \times g$ for 30 min at 4°C, and the supernatant was used for enzyme assays. The PPO activity was determined by adding 1 ml of enzyme preparation to 2 ml of catechol as a substrate, and the change was measured immediately in absorbance at 398 nm (A_{398}). The activity was expressed as A_{398} per minute per milligram of protein.

The POD activity was determined using guaiacol as substrate (20). The reaction mixture consisted of 2 ml of crude extract, 1 ml of guaiacol, and 1 ml of buffer. The reaction mixture was incubated at 30°C for 30 min before 1 ml of H_2O_2 was added. Absorbance was measured at 460 nm (A_{460}). The activity of POD was defined as A_{460} per minute per milligram of protein.

Lignin analysis. Fruit prepared for the lignin assay were treated as described previously for enzyme measurement. Lignin was detected quantitatively using a lignin thioglycolic acid (LTGA) method (6) with some modification. Samples were taken from the wounds of 30 fruit containing the pericarp and flesh at 0, 6, 12, 24, and 48 h after treatment. Samples (10 g) of each treatment were first extracted in two changes of 30 ml of methanol over 24 h; pellets were collected by centrifugation and transferred to glass beakers for drying (24 h at 60°C). The resulting residue (50 mg) was transferred to glass screw-cap tubes, and then 5 ml of 2 N HCl and 0.5 ml of thioglycolic acid were added. The tubes were sealed and incubated in a water bath at 100°C for 4 h to hydrate the methanol insoluble residue. After cooling, the pellet was collected by centrifugation at $30,000 \times g$ for 10 min at 20°C. The collected pellet was washed once with 5 ml of distilled water. The resulting pellet was solubilized in 5 ml of 0.5 N NaOH, sealed with Parafilm, and agitated gently at 20°C for 24 h to extract the lignin thioglycolate. Samples were centrifuged at $30,000 \times g$ for 10 min at 20°C and the supernatant solutions were transferred to glass centrifuge tubes. Then, 1 ml of concentrated HCl was added to each tube and the tubes were stored at 4°C for 4 h to precipitate the LTGA. The precipitate was collected by centrifugation ($30,000 \times g$, 10 min) and washed once with 0.1 N HCl. The final pellet was dissolved in 10 ml of 0.5 N NaOH, and the insoluble material was removed by centrifugation. The absorbance of the supernatant was measured at 280 nm with a spectro-

Fig. 2. Effect of Si in combination with *Cryptococcus laurentii* on efficacy against **A and B**, *Penicillium expansum* and **C and D**, *Monilinia fructicola* of sweet cherry fruit at 20°C. Fruit were wounded, treated with *C. laurentii* at 1×10^7 cells per ml or sterile distilled water, and inoculated with *P. expansum* or *M. fructicola* at 1×10^4 spores per ml. After 12 h, each wound was treated with Si at 1% (wt/vol). Fruit treated with water served as the control. Disease incidence and lesion diameter were measured after 3, 4, and 5 days. CL = *C. laurentii*. The data were based on three replicates of 20 fruit each, and the test was repeated twice with similar results. Within each day, columns with the same letter are not statistically different according to the least significant difference test ($P < 0.05$).

photometer (UV-160, Shimadzu). Results were expressed as 280 nm absorbance of LTGA in 10 ml of 0.5 N NaOH. There were three replicates of each treatment, and the experiment was repeated twice.

Statistical analysis. Data for antagonist populations (CFU per wound) were transformed to logarithms to improve the homogeneity of variances. The incidence and severity of decay, population dynamics, and growth of pathogens in PDB were analyzed by analysis of variance with SPSS (SPSS Inc., Chicago, IL). When the treatment effects were statistically significantly ($P < 0.05$), the least significant difference test was used for means separation. Data analysis indicated that treatment effects were similar among the experimental repeats. Results presented were pooled across repeated experiments.

RESULTS

Effect of concentration of Si on fruit decay. The effectiveness of Si for controlling blue mold and brown rot in sweet cherry fruit was positively correlated with concentrations of Si (Fig. 1). At 0.6% (wt/vol), Si significantly ($P < 0.05$) reduced decay incidence (Fig. 1A) and lesion diameter (Fig. 1B) of both diseases, while Si at 1% (wt/vol) gave better disease control than 0.6% (wt/vol). After incubation at 20°C for 3 days, fruit treated with Si at 1% (wt/vol) had 63.4 and 86.6% less decay caused by blue mold and brown rot, respectively, than the water controls.

Combining Si with *C. laurentii* for control of fruit decay. Although a single application of Si reduced decay of blue mold and brown rot, better control was achieved when sweet cherry fruit were pretreated with *C. laurentii* (Fig. 2). The combination of Si and *C. laurentii* completely suppressed blue mold (Fig. 2A and B) and brown rot (Fig. 2C and D) after 3 and 4 days of incubation at 20°C. Disease incidence increased and lesion diameter enlarged as storage time increased. After incubation for 5 days, fruit treated with Si combined with *C. laurentii* had 43 and 87% less decay due to blue mold and brown rot, respectively, than the controls. Lesion diameters caused by both diseases also were reduced significantly ($P < 0.05$) by Si combined with *C. laurentii*.

Influence of Si on growth of *C. laurentii* in the wounds of sweet cherry fruit. Population dynamics of *C. laurentii* in the wounds of sweet cherry fruit were significantly influenced ($P < 0.05$) by the application of Si (1% [wt/vol]) at 20°C (Fig. 3). The population density of *C. laurentii* was inhibited by Si during the first 48 h of the incubation period, but after that time, growth of yeast was stimulated. At the end of the study (96 h), the popu-

Fig. 3. Influence of Si on growth of *Cryptococcus laurentii* in the wounds of sweet cherry fruit. Fruit were wounded and treated with *C. laurentii* at 1×10^7 cells per ml. After 12 h, each wound was treated with 1% (wt/vol) Si or sterile distilled water and stored at 20°C. Colonies were expressed as $\log_{10}$ CFU per wound. The data were based on three replicates, and the test was conducted three times with similar results. Bars represent the standard deviation of the treatment mean of pooled data.

lation density of *C. laurentii* in fruit treated with Si was almost 2.4-fold greater than that in the water-treated control.

Effect of Si concentration on growth of *P. expansum* and *M. fructicola* in vitro. Spore germination and germ tube elongation of *P. expansum* and *M. fructicola* pathogens were inhibited significantly ($P < 0.05$) by Si at 0.2% (wt/vol) (Fig. 4). Inhibition was positively correlated with Si concentration. At 0.6% (wt/vol), Si completely inhibited the growth of *P. expansum* and *M. fructicola*. Spore germination was less sensitive to Si than germ tube elongation.

Scanning electron microscopy observation. Inhibition of the growth of *P. expansum* and *M. fructicola* in the wounds of sweet cherry fruit by Si at 1% (wt/vol) was investigated by scanning electron microscopy (Fig. 5). In Si-treated wounds, restricted mycelial growth was observed at 48 h for *P. expansum* (Fig. 5B) and at 24 h (Fig. 5D) for *M. fructicola*, whereas extensive mycelial ramification was evident in water-treated wound sites (Fig. 5A and C). Abnormal germ tubes, distorted mycelium, and swollen conidia of *P. expansum* were observed in wounds treated with Si (Fig. 5B). In contrast, normal spores and mycelial shapes were evident in the water-treated control fruit (Fig. 5A).

Induction of biochemical defense responses by Si treatment. Application of Si induced the accumulation of PAL, PPO, and POD in sweet cherry fruit (Fig. 6). Both wounding and Si treatment increased the activity of PAL, but the level in Si-treated fruit was higher than that in wounded control fruit (Fig. 6A). The PAL activity increased gradually and reached its highest value at 24 h in Si-treated fruit. The level was almost twofold greater than that in nonwounded control fruit at that time and was also significantly higher ($P < 0.05$) than that in wounded control fruit. Non-

Fig. 4. Effect of Si on A, spore germination and B, germ tube elongation of *Penicillium expansum* and *Monilinia fructicola* in vitro. Pathogen suspensions were incubated in potato dextrose broth containing different concentrations of Si. Spore germination and germ tube elongation were observed after 15 h incubation on a rotary shaker at 100 rpm at 20°C. Approximately 200 spores of each pathogen were measured for germination rate and germ tube length per treatment within each replicate. Each treatment was replicated three times, and the experiment was repeated twice.

wounded control fruit had a constant low level of PAL activity throughout the experiment.

Activity of PPO was stimulated by wounding. Adding Si to the wounds increased the accumulation of PPO in wounded fruit. In sweet cherry fruit treated with Si, PPO increased quickly and reached the maximum level 12 h after treatment (Fig. 6B).

Application of Si resulted in a gradual increase of POD activity in sweet cherry fruit. The change in POD activity in wounded control fruit was similar to that in fruit treated with Si. However, the levels of POD activity were increased further by treatment with Si. Throughout the experiment, nonwounded control fruit maintained low levels of POD activity (Fig. 6C).

Browning of epidermal cells at the site of wounding was observed after Si treatment, whereas tissue browning was not evident in wounded control fruit.

Quantitative determination of lignin induced by Si. Both wounding and Si induced the biosynthesis of lignin in sweet cherry fruit (Fig. 7). Lignin maintained high levels throughout the experiment in the wounded control and Si-treated fruit. The content of lignin in Si-treated fruit increased gradually and reached a maximum after 48 h. Lignin accumulation in Si-treated fruit was not significantly ($P < 0.05$) greater than that in wounded control fruit during all stages of the 48-h incubation period.

DISCUSSION

Silicon was effective in suppressing blue mold and brown rot caused by *P. expansum* and *M. fructicola*, respectively, in sweet cherry fruit, and efficacy was influenced by the concentration of

Si. This result was consistent with the observation of Menzies et al. (29), who found that disease caused by *Sphaerotheca fuliginea* in cucumber plants was reduced significantly with increasing concentration of Si in nutrient solutions. Application of Si significantly improved the biocontrol ability of *C. laurentii* against blue mold and brown rot in sweet cherry fruit. Silicon, combined with *C. laurentii*, completely suppressed both diseases after 3 or 4 days of incubation at 20°C. Combinations of biocontrol agents with safe chemicals, such as calcium chloride (43,46), chitosan (15), 2-deoxy-D-glucose (16,21), and salicylic acid (34), are effective methods to enhance biological control activity. To our knowledge, this is the first report of a beneficial effect derived from the combination of Si and a biocontrol agent.

The mechanisms of Si enhancement of the biocontrol efficacy of *C. laurentii* appear to be complex. This may be associated with the effects of Si on the biocontrol agent, the pathogen, and sweet cherry fruit. In this study, the growth of *C. laurentii* in sweet

Fig. 5. Scanning electron micrographs of **A and B**, *Penicillium expansum* and **C and D**, *Monilinia fructicola* in the wounds of sweet cherry fruit. Fruit were wounded and inoculated with a conidial suspension of *P. expansum* or *M. fructicola* at 1×10^5 spores per ml. Within 1 h, each wound was treated with **B and D**, 1% (wt/vol) Si or **A and C**, sterile distilled water and stored at 20°C. Samples were removed from the wounds 24 and 48 h after *M. fructicola* and *P. expansum* inoculations, respectively. **A and B**, Bar = 15 μ m; and **C and D**, Bar = 30 μ m. agt = abnormal germ tube; dt = distorted mycelium; and sc = swollen conidia. Samples from five fruit of each treatment were examined.

Fig. 6. Induction of **A**, phenylalanine ammonia-lyase (PAL), **B**, polyphenol-oxidase (PPO), and **C**, peroxidase (POD) activities by Si in sweet cherry fruit. Fruit were wounded, treated with 1% (wt/vol) Si, and stored at 20°C. Fruit wounded and treated with water or nonwounded fruit served as controls. Each treatment contained three replicates, and the experiment was repeated twice with similar results. Bars represent the standard deviation of the treatment mean of pooled data.

Fig. 7. Induction of lignin biosynthesis by Si in sweet cherry fruit. Fruit were wounded, treated with 1% (wt/vol) Si, and stored at 20°C. Fruit wounded and treated with water or nonwounded fruit served as controls. Results were expressed as 280 nm absorbance of lignin thioglycolic acid in 10 ml of 0.5 N NaOH. Each treatment contained three replicates, and the experiment was repeated twice with similar results. Bars represent the standard deviation of the treatment mean of pooled data.

cherry fruit was stimulated by Si 48 h after incubation. Yeast antagonists often exert biocontrol activity through competition for nutrients and space (35). To control pathogens effectively, biocontrol agents must multiply more quickly than pathogens. Conversely, Si directly inhibited spore germination and germ tube elongation of *P. expansum* and *M. fructicola* in vitro in our study. Growth of both pathogens was inhibited completely when the concentration of Si was 0.6% (wt/vol). This result is similar to the observations of Biggs et al. (4), who found that calcium silicate could inhibit the growth of *M. fructicola* in vitro. Results of scanning electron microscopy observation of *P. expansum* and *M. fructicola* in the wounds of sweet cherry fruit confirmed that growth of the two pathogens was inhibited by Si treatment.

Plants defend themselves from pathogen infection through a wide variety of mechanisms (3). Resistance can be induced by abiotic and biotic inducers (37). Exogenous factors such as salicylic acid (31), β -aminobutyric acid (10), and benzothiadiazole (3) can lead to enhanced plant protection against various pathogens. In our study, Si induced biochemical defense reactions in sweet cherry fruit. Activities of PAL, PPO, and POD in fruit treated with Si were increased significantly compared with those in wounded or nonwounded control fruit. These three enzymes may combine to account for increased disease resistance. PAL is the first enzyme of the phenylpropanoid pathway and is involved in the biosyntheses of phenolics, phytoalexins, and lignins (32). The resulting phenolics could be oxidized by the action of PPO and POD to produce slightly colored compounds (i.e., quinines) (7). Quinines can polymerize to produce colored compounds typically seen in browning processes, which are highly toxic to invading microorganisms (7). Tissue browning of sweet cherry fruit around the site of wounding was observed after Si treatment. Lignin is thought to play a role in barricading the pathogen from invading the plant through physical exclusion (6). However, lignin content in Si-treated fruit was not significantly increased compared with that in wounded control fruit. It is likely that lignin deposition does not play as significant a role as accumulation of toxic materials in the defense response activated by Si in sweet cherry fruit.

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