Preventive and curative activity of postharvest potassium silicate treatments to control green and blue molds on orange fruit

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1**Abstract**

2Preventive and curative antifungal activities of postharvest treatments with potassium 3silicate (PSi) against green (GM) and blue (BM) molds were evaluated on oranges (cvs. 4'Valencia' or 'Lanelate') artificially inoculated in rind wounds with *Penicillium digitatum* 5and *P. italicum*, respectively. The most effective PSi concentration, the effect of fungal 6inoculum concentration, and the influence of temporal and spatial factors on antifungal 7activity were assessed in *in vivo* primary screenings. After 6 days of incubation at 20°C, 8significant preventive (treatment before fungal inoculation) and curative (treatment after 9inoculation) activities against GM and BM were observed with PSi at 90 mM (GM and BM 10incidence reductions of 23 and 52%, and 23 and 40%, respectively). In preventive tests, the 11effectiveness of PSi was influenced by inoculum concentration (10^3, 10^4, 10^5, or 10^6 spores 12mL^{-1}), but not by the distance between treatment and inoculation sites (10, 20 or 30 mm). 13PSi applied about 2 h before inoculation showed higher preventive activity than applied 14before 24, 48 or 96 h. In order to determine the best dip treatment conditions, PSi at 90 mM 15was tested at 20 or 50°C for 60 or 150 s in small-scale trials with 'Lanelate' oranges 16artificially inoculated before or after the treatment and incubated for 7 days at 20°C. Dips at 1720°C for 60 s were selected and subsequently applied on inoculated 'Valencia' oranges 18stored at 5°C and 90% RH for up to 6 weeks. Curative postharvest dips effectively reduced 19the incidence and severity of both GM and BM during cold storage, while preventive dips 20significantly reduced the severity but not the incidence. Overall, postharvest PSi treatments 21showed potential as a new tool to be part of non-polluting strategies to control penicillium 22decay of citrus fruit.

23 **Key words** *Penicillium digitatum, Penicillium italicum*, *Citrus sinensis*, silicon, 24 alternative postharvest disease control

Introduction

Green (GM) and blue (BM) molds caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. and *P. italicum* Wehmer, respectively, are major diseases responsible for postharvest losses of citrus fruit worldwide (Palou et al. 2008). In spite of the application of fungicides and the increasing implementation of some alternative control strategies, the high incidence of GM and BM still accounts for important economical problems on stored citrus commodities. Therefore, it is necessary to further develop alternative approaches and novel technologies, including physical, chemical and biological control methods, for a cost-effective integrated control of these molds (Droby et al. 2009; Palou et al. 2008).

Silicon (Si) is considered a functional plant nutrient that plays an important role as a component of cell walls (Laing et al. 2006). In agriculture, the interest of Si is multiple because has shown value as a fertilizer and for pest and disease control. In addition, some positive effects on the reduction of physiological disorders have also been described. In the particular case of citrus fruit, it was found that postharvest Si treatments could alleviate chilling injury, especially on long-term cold-stored lemons (Mathaba et al. 2009). The earliest scientific works on the role of Si in plant disease control were reported in the 1920s and 1930s. Wagner (1940) was the first to report an interaction between Si fertilization and the incidence of cucumber powdery mildew. In Europe, more recent research demonstrated that the addition of potassium silicate (PSi; K_2SiO_3) to nutrient solutions reduced the incidence of powdery mildew and stem lesions caused by the pathogen *Botrytis cinerea* on cucumber plants (O'Neill 1991). While the role of silicified cell walls in protecting plants against pathogens may not be completely discarded, other results suggest that Si acts in the host tissue by affecting the signals between host and pathogen, resulting in a more rapid

and extensive activation of plant defense mechanisms (Chérif et al. 1992a,b, 1994; Samuels et al. 1991). The specific mechanisms responsible for the protection of plants from fungal diseases by Si are not well understood. Si may act by eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds, and pathogenesis-related proteins in infected plants (Chérif et al. 1992a; Epstein 1999). Results by Shen et al. (2010) suggested that reductions in fungal disease after treatment of field plants with low concentrations of PSi were probably not due to fungistatic effects of Si, but rather to other mechanisms such as Si acting as a physical barrier against pathogen penetration or Si-induced defense response in plants.

In contrast to PSi, whose antifungal activity has been practically assessed only as field treatments for herbaceous crops, another Si salt, sodium silicate (SSi), has been tested as postharvest treatments for some fruit or vegetable commodities. Bi et al. (2006) found that SSi at 100 mM was more effective than SSi at 25 or 50 mM to control diseases caused by *Alternaria alternata*, *Fusarium semitectum*, and *Trichothecium roseum* on artificially inoculated Hami melon fruit; SSi at 200 mM was phytotoxic. Furthermore, SSi treatments applied at 100 mM before inoculation with *T. roseum* induced lower decay incidence and severity on melons than treatments applied after fungal inoculation. According to these workers, the protection provided by SSi treatments was correlated with the activation of two families of defense-related enzymes, peroxidase and quitinase. In further research with melons, Guo et al. (2007) reported that SSi and silicon oxide, both applied as postharvest dips, reduced the severity of pink rot, caused by *T. roseum*, on Chinese cantaloupes, with lesion diameters reduced by up to fivefold when compared with the controls. In the same work, the effectiveness of SSi increased at higher concentrations and the growth of the fungus was completely inhibited at 100 mM. Few research results are available on the

activity of SSi against citrus postharvest pathogens. Liu et al. (2010) found that the plasma membrane of Si-treated spores of *P. digitatum* was damaged, which suggested that Si played a crucial role as antifungal agent. This author showed that treatments with SSi significantly controlled GM caused by *P. digitatum* on citrus fruit. In addition, Youssef et al. (2012) recently reported that both preharvest and postharvest treatments with SSi were effective against *P. digitatum* or *P. italicum* on oranges and mandarins. Furthermore, we observed very recently that postharvest treatments with SSi significantly reduced penicillium molds on oranges artificially inoculated with *P. digitatum* or *P. italicum* about 24 h after treatment. However, these treatments left visible salt residues and were potentially phytotoxic to the fruit peel (Moscoso-Ramírez and Palou 2013).

Before the promising activity of Si, applied as SSi, against some important postharvest diseases and the lack of information on the performance of PSi, this research focused on the evaluation of the antifungal properties of postharvest treatments with PSi against GM and BM. Particularly, the objectives of this study were to: i) determine in *in vivo* primary screenings the range of effective PSi concentrations for optimization of both preventive and curative activities of PSi against GM and BM on orange fruit, ii) determine the influence of temporal and spatial factors on the preventive activity of PSi against *P. digitatum*, iii) optimize the conditions for postharvest dip treatments with PSi, and iv) evaluate the effectiveness of postharvest PSi treatments during prolonged cold storage of oranges.

Material and methods

Fruit

The experiments were carried out with ‘Valencia’ and ‘Lanelate’ oranges (*Citrus sinensis* (L.) Osbeck) collected from commercial orchards in the Valencia area (Spain). The fruit

were used the same day or stored up to 1 week at 5°C and 90% relative humidity (RH) before use. Before each experiment, fruit were selected, randomized, washed with fresh water and allowed to air dry at room temperature.

Fungal inoculum

P. digitatum and *P. italicum* , isolates NAV-7 and MAV-1, respectively, from the fungal culture collection of the Laboratori de Patologia, Centre de Tecnologia Postcollita (CTP), Institut Valencià d´Investigacions Agràries (IVIA), were cultured on potato dextrose agar (PDA, Sigma-Aldrich Chemical Co., St. Louis, MA, USA) plates at 25ºC. Conidia of each fungus from 7- to 14-days-old were taken from the agar surface with a sterile glass rod and transferred to a sterile aqueous solution of 0.05% Tween® 80 (Panreac, S.A.U., Barcelona, Spain). Conidial suspensions were filtered through two layers of cheesecloth to separate hyphal fragments and, depending on the experiment, adjusted to 10^3, 10^4, 10^5 or 10^6 spores ml^{-1} using a haemocytometer.

Determination of potassium silicate concentration

In vivo primary screenings were used to establish the most appropriate concentration for both preventive and curative activity of PSi against GM and BM. For preventive activity, 30 µl of solution of PSi (K_2SiO_3; syns.: potassium salt of silicic acid, potassium metasilicate; Sil-MATRIX®, 29.1% a.i. PSi, PQ Corporation, Valley Forge, Pennsylvania, USA) at 0.9, 9 or 90 mM a.i. were placed, using a micropipette, in a 1 mm wide, 2 mm long, 1 mm diameter wound made with a stainless steel rod on the equatorial region of ‘Valencia’ oranges. About 24 h later, 30 µl of conidial suspension at 10^5 spores ml^{-1} of *P.*

digitatum or *P. italicum* were placed, using a micropipette, in a new identical wound adjacent to the first one (about 1-2 mm of separation between wounds). No good decay rate had been obtained in control fruit in previous experiments when the fungal inoculation had been performed in the same wound inflicted 24 h before, probably due to wound lignification during this period of time. To evaluate the curative activity, 30 µl of PSi solution at 0.9, 9, 30, 90 or 150 mM were placed, using a micropipette, in the same inoculation rind wound about 24 h after the inoculation of the pathogen. Control fruit were treated with 30 µl of sterile distilled water. In all cases, 4 replicates of 5 oranges each were used for each treatment. Treated fruit were stored at 20ºC and 90% RH for 6 days, at which time disease incidence (% of infected fruit) and severity (lesion diameter) were evaluated.

Influence of inoculum concentration

To evaluate the influence of inoculum concentration of *P. digitatum* on the preventive or curative activity of PSi, concentrations of 10^3, 10^4, 10^5 and 10^6 spores ml^{-1} of *P. digitatum* were prepared following the procedure described above. Thirty µl of PSi solution at 90 mM (13.9 g l^{-1}) were placed, using a micropipette, in a 1 mm wide, 2 mm long, 1 mm diameter wound made with a stainless steel rod on the equatorial region of 'Lanelate' oranges. For preventive and curative activity, 30 µl of conidial suspension of *P. digitatum* at the desired inoculum concentration were placed, using a micropipette, in the same peel wound about 2 h after and before, respectively. Control fruit were treated with 30 µl of sterile distilled water. Each treatment was applied to 4 replicates of 5 oranges each. Treated fruit were stored at 20ºC and 90% RH for 6 days, at which time disease incidence and severity were recorded. The experiment was repeated once.

Assessment of temporal and spatial characteristics of preventive activity

An experiment with 'Valencia' oranges was conducted in order to temporally characterize the preventive activity of PSi. Thirty µl of PSi aqueous solution at 90 mM were placed, using a micropipette, in a 1 mm wide, 2 mm long, 1 mm diameter wound made with a stainless steel rod in the equator of the fruit. Subsequently, 30 µl of a 10^5 spores ml^{-1} *P. digitatum* conidial suspension were pipetted about 2, 24, 48 or 96 h after treatment in a new adjacent wound (about 2 mm of separation between wounds). Each treatment was applied to 4 replicates of 5 oranges each. Treated fruit were stored at 20ºC and 90% RH for 6 days, at which time disease incidence and severity were evaluated. The experiment was repeated once.

Another experiment was designed to evaluate the spatial influence on the preventive activity of PSi. Fifty µl of PSi aqueous solution at 90 mM were pipetted in a wound made with a stainless steel rod in the fruit equatorial region as described above. About 2 h after treatment, 30 µl of a 10^4 spores ml^{-1} *P. digitatum* conidial suspension were placed in a new rind wound inflicted at a distance of 10, 20 or 30 mm from the initial treatment wound. Control fruit were treated with sterile distilled water and then inoculated in the same rind wound. Four replicates of 5 oranges each were used per treatment. Treated fruit were stored at 20ºC and 90% RH for 6 days, at which time disease incidence and severity were recorded. The experiment was repeated once.

Assessment of optimal dip treatment conditions

Small-scale trials were conducted using 'Lanelate' oranges to establish the best dip treatment conditions to resemble potential commercial applications in citrus packinghouses. Fungal inoculation in one peel wound per fruit at a concentration of 10^5 spores ml^{-1} of *P.*

digitatum or *P. italicum* was carried out following the procedure previously described. Dips were performed in stainless steel buckets containing 10 L of 90 mM aqueous solution of PSi. This concentration of PSi was selected according to previous results obtained in the *in vivo* primary screenings. When needed, PSi solutions were heated by placing the buckets in a 250-L stainless steel water tank fitted with two electrical resistances (4.5 kW each), a thermostat, and an automatic water-recirculating system. Fruit were placed into 18 L multi-perforated wall stainless steel containers, exactly fitting in the above-mentioned buckets, and completely immersed in the treatment solution for 60 or 150 s at 20 or 50°C. To assess preventive activity, dips were performed about 2 h before fungal inoculation and control fruit were dipped for 60 s in water alone at both 20 and 50°C. To assess curative activity, dips were performed about 24 h after fungal inoculation and control fruit were dipped in water alone at 20°C for 60 s. After treatment, fruit were not rinsed with tap water. Forty fruit per treatment (4 replicates of 10 fruit each) were arranged in plastic cavity sockets on cardboard trays. Treated fruit were incubated at 20°C and 90% RH. Disease incidence and severity were assessed after 7 days of incubation. Potential fruit phytotoxicity caused by PSi or heat was visually assessed after 3 days at 20°C. For this purpose, fruit were classified into one of four categories, depending on peel appearance: 0 = no peel damage; 1 = slight brownish blemishes present (<10% fruit surface); 2 = moderate brownish blemishes present (10% <fruit surface<25%) and 3 = severe peel injury (>25% fruit surface). A ponderate peel pitting index (0–3 scale) was calculated for each treatment. These trials were performed twice.

Effectiveness on long-term cold-stored fruit

Both preventive and curative activities of postharvest PSi dips against GM and BM were evaluated on 'Valencia' oranges subjected to long-term cold storage. Stainless steel buckets containing 10 L of 90 mM PSi aqueous solution were used to dip the fruit at 20ºC for 60 s. Fruit were inoculated and treated or vice versa as described before and stored up to 6 weeks at 5ºC and 90% RH in a cold room in the IVIA CTP facilities. Control fruit were dipped in water alone at 20°C for 60 s. Forty fruit per treatment (4 replicates of 10 fruit each) were used. Disease incidence and severity were assessed after 2, 4, and 6 weeks at 5ºC.

Statistical analysis

Data were analyzed by analyses of variance (ANOVA) with Statgraphics software (Statgraphics Plus, v. 5.1; Manugistics Inc., Rockville, Maryland, USA). Data on disease incidence were transformed to the arcsine of the square root of the proportion of infected fruit to assure the homogeneity of variances. In some cases, reductions with respect to the control treatments were calculated as percentages. Statistical significance was judged at the level $P = 0.05$. Unless otherwise stated, results are means from two repeated experiments. When appropriated, the Fisher's Protected Least Significant Difference (LSD) test was applied to separate means. Shown values are non-transformed means.

Results

Potassium silicate concentration

Among the concentrations of PSi evaluated in this set of *in vivo* experiments to prevent the molds, the concentration of 90 mM significantly inhibited the development of GM and BM on 'Valencia' oranges and was clearly superior to the concentrations of 0.9 and 9 mM (Fig.

11). The incidence of GM and BM was reduced by 23 and 52%, respectively, by PSi at 90 2mM after 6 days of incubation at 20°C. Furthermore, PSi at 90 mM effectively reduced the 3severity of GM and BM by 74 and 67%, respectively (Fig. 1).

4In the curative tests, the most effective treatment was also PSi at a concentration of 90 mM, 5which significantly reduced the incidence of GM and BM on 'Valencia' oranges by 23 and 640%, respectively, after 6 days of incubation at 20°C (Fig. 1). Likewise, the severity of GM 7and BM was effectively reduced by 57 and 66%, respectively, by treatment at 90 mM. 8Conversely, PSi treatments at 0.9, 9, 30 and 150 mM did not significantly reduce or 9reduced very slightly the incidence and severity of both molds (Fig. 1).

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11Influence of inoculum concentration

12Regardless the inoculum concentration of *P. digitatum*, the preventive activity of PSi at 90 13mM on 'Lanelate' orange fruit was clearly significant. On PSi-treated fruit GM incidence 14was reduced by 90, 66, 65, and 30% at concentrations of *P. digitatum* of 10^3, 10^4, 10^5, and 15$10^6$ spores ml^{-1}, respectively, while it was 50, 74, 100, and 100% on control fruit (Fig. 2). 16Similarly, GM lesion diameters were significantly reduced from 33, 51, 62 and 98 mm on 17control fruit to lesions of 4, 13, 28 and 52 mm on PSi-treated fruit when the oranges had 18been inoculated with concentrations of 10^3, 10^4, 10^5 and 10^6 spores ml^{-1} of *P. digitatum*, 19respectively (Fig. 2).

20In the curative tests, the effectiveness of PSi treatments at 90 mM to reduce GM incidence 21was lower and there were only significant differences with the control treatment when an 22inoculum concentration of *P. digitatum* of 10^4 spores ml^{-1} was used (GM incidence 23reduction of about 30%; Fig. 2). Likewise, GM severity was not significantly reduced by 24PSi treatment regardless of the inoculum concentration (Fig. 2).

In general, both GM incidence and severity significantly and expectedly increased on both control and PSi-treated fruit as the concentration of *P. digitatum* used for inoculation increased. The main exception was GM incidence on control fruit used on curative trials, which already reached 100% with an inoculum concentration of 10^4 spores ml^{-1} (Fig. 2).

Influence of temporal and spatial characteristics on preventive activity

The preventive activity of PSi at 90 mM against GM on 'Valencia' oranges incubated at 20°C for 6 days was significantly different only when the fruit were inoculated with *P. digitatum* about 2 h after the treatment. GM incidence and severity were 35, 95, 95 and 100% and 11, 69, 68 and 93 mm, respectively, when the time interval between treatment and inoculation were of 2, 24, 48 and 96 h (Table 1).

On the other hand, the performance of PSi at 90 mM against GM on 'Valencia' oranges was not affected by the distance between the treatment and inoculation sites (10, 20 or 30 mm). None of the PSi preventive treatments at these distances significantly reduced green mold in comparison with water-treated control fruit (Table 1).

Dip treatment conditions

A concentration of aqueous solution of 90 mM PSi was selected as the most effective in the previous *in vivo* primary screenings. Thus, this concentration was used in this subsequent set of trials. Dips of PSi at 90 mM at 20 or 50°C for 60 or 150 s significantly prevented the incidence of GM and BM on 'Lanelate' oranges inoculated about 2 h after treatment and incubated for 7 days at 20°C. On fruit dipped for 60 or 150 s at 20 or 50°C, PSi at 90 mM significantly reduced GM incidence by 37 and 27% and 50 and 55%, respectively, with

respect to water-treated control treatments (GM incidence of 100%; Fig. 3). On fruit dipped for 60 or 150 s at 20 or 50°C, PSi treatment significantly reduced BM incidence by 18 and 37% and 40 and 28%, respectively, with respect to the controls (Fig. 3). Likewise, the severity of both molds was similarly reduced by PSi at 90 mM on these preventive tests. GM and BM lesion diameters were reduced from 105 and 46 mm on control fruit to 50-70 and 15-20mm, respectively, on PSi-treated oranges (Fig. 3). Thus, mold development on PSi-treated oranges was not significantly affected by immersion time.

In tests to assess the curative activity, dips with PSi at 90 mM applied at 20 or 50°C for 60 or 150 s significantly reduced the incidence of GM and BM on 'Lanelate' oranges inoculated 24 h before treatment and incubated for 7 days at 20°C. On fruit dipped for 60 or 150 s at 20 or 50°C, PSi at 90 mM reduced the incidence of GM by 35 and 38% and 40 and 62%, respectively. There was, therefore, a significant effect of immersion time, but only when the dips were performed at 50ºC (Fig. 4). This pattern was also observed for the incidence of BM, with reductions of 42 and 47%, and 35 and 57%, respectively, on fruit dipped for 60 or 150 s at 20 or 50°C (Fig.4). Likewise, the severity of both molds also followed very similar patterns, and significant differences between immersion times were only observed for GM on oranges dipped at 50ºC (Fig. 4).

As a conclusion of both preventive and curative tests, PSi dips at 20°C for 60 s were selected for use in subsequent experiments.

Effectiveness on long-term cold-stored fruit

After 2 weeks of storage at 5°C, the incidence of both GM and BM on 'Valencia' oranges artificially inoculated about 2 h after treatment (preventive activity) was totally prevented by the application of PSi at 90 mM as 60 s dips at 20°C. At this time, however, the

incidence of GM and BM on control fruit was only of 23 and 20%, respectively. After 4 and 6 weeks of cold storage, PSi at 90 mM showed no protective effect, since the incidence of GM and BM was not significantly different from that on control fruit (Fig. 5). In general, PSi treatments significantly reduced the severity of GM and BM throughout the entire storage period of 6 weeks at 5°C. GM lesion diameters after 4 and 6 weeks on control and PSi-treated oranges were 93 and 48 mm, and 200 and 159 mm, respectively. In the case of BM, these diameters were 48 and 35 mm, and 100 and 84 mm, respectively (Fig. 5).

In the curative tests, the incidence of both GM and BM on 'Valencia' oranges artificially inoculated 24 h before treatment, and stored up to 6 weeks at 5°C and 90% RH were significantly reduced by the application of PSi at 90 mM as 60 s dips at 20°C. At the end of the cold storage period, the incidence of both GM and BM was significantly reduced by up to 45% with respect to the control fruit (GM and BM incidence of 100%) (Fig. 6). The treatment with PSi at 90 mM also effectively reduced the severity of GM and BM during cold storage at 5°C. GM and BM lesion diameters at the end of the 6-week cold storage period on control and PSi-treated oranges were 200 and 98 mm, and 100 and 48 mm, respectively (Fig. 6).

Discussion

This study reports that postharvest treatments with Si in the form of PSi aqueous solutions applied to 'Valencia' or 'Lanelate' oranges both before (preventive activity) and after (curative activity) artificial fungal inoculation, showed significant antifungal activity against citrus GM and BM. Results from *in vivo* primary screenings showed that PSi treatments inhibited mold development in a concentration-dependent manner, and they were more effective at a concentration of 90 mM. Therefore, PSi acted here probably more

as a plant growth regulator, which are typically more effective when applied at an optimal concentration, than as a conventional fungicide, whose efficacy gradually increases as the application dose increases. This hypothesis was supported by the fact that in the screenings for curative action, disease reduction was lower after PSi application at 150 than at 90 mM (Fig. 1). Results from these tests showed that the effectiveness of PSi at 90 mM to prevent GM or BM on 'Valencia' orange fruit (incidence reduction of 23 and 52%, respectively) was slightly inferior or similar to that reported after sodium silicate treatments on citrus (Liu et al. 2010), and it was also similar to that observed on jujube fruit (Tian et al. 2005). Further, it was found in previous work in our laboratory that preventive treatments with SSi at 1000 mM reduced GM and BM on artificially inoculated oranges by 70-90%, but this treatment caused injuries in the fruit peel (Moscoso-Ramírez and Palou 2013). It can be therefore concluded that, from a practical point of view, postharvest PSi treatments are superior to SSi treatments for the purpose of postharvest disease reduction. In addition, PSi treatments not exceeding 1% (wt/vol) were exempted in the USA from the requirement of a tolerance for residues in or on all food commodities when applied or used as a fungicide, insecticide or miticide (US EPA 2006). Moreover, aqueous PSi was also included in the list of synthetic substances allowed for use in organic crop production. The only requirement is that the silica used for the manufacture of PSi must be sourced from naturally occurring sand (USDA AMS NOP 2010).

It has been proposed that the mode of action of Si treatments is through a direct effect on the pathogen. For instance, Liu et al. (2010) reported damage on the plasma membrane of Si-treated *P. digitatum* spores, leading to higher leakage of proteins and sugars. However, other research works contradicted this hypothesis and attributed the antifungal action of Si to indirect effects to the fruit host. Hereto, Si can play an important role on the formation of

physical and mechanical barriers to the penetration of pathogens at the cell wall level (Buonario et al. 2009; Datnoff et al. 2001). Si treatments may also act by eliciting biochemical defense reactions, including the accumulation of lignin, phenolic compounds and pathogenesis-related (PR) proteins in infected plants (Epstein 1999). According to our results, we can hypothesize that in the case of citrus penicillium molds, the mode of action of postharvest PSi treatments for disease control might be a combination of both direct effects on the pathogen *P. digitatum* or *P. italicum* and indirect effects on the fruit host. Curative tests were performed with oranges artificially inoculated in rind wounds with a conidial suspension about 24 h before the treatment. This is the usual procedure to simulate the most common natural infections on laboratory assays to test the efficacy of postharvest fungicides against citrus GM and BM (Eckert and Brown 1986). Since the spore suspensions were freshly prepared the same inoculation day and the inoculated fruit were kept at a constant temperature of 20ºC during the 24-h period between inoculation and treatment, the effect of the treatment was mostly on recently germinated conidia (on germ tubes or young hyphae), although a variable proportion could still be ungerminated conidia. On the other hand, preventive tests were carried out with oranges wounded, treated and inoculated about 2 or 24 h after treatment, depending on the experiment. It seems that PSi treatment effectively conferred by some mechanism some degree of resistance to the fruit peel, since significant disease reductions were observed on both primary screenings and dip trials with a PSi concentration of 90 mM.

In any case, both preventive and curative effects were, as expected, dependent on the concentration of pathogenic inoculum. Early research showed that this factor clearly influenced the rate of successful infections when citrus peel wounds are inoculated with *P. digitatum* or *P. italicum* (Eckert and Eaks 1989). This was confirmed in this work, as

disease incidence and severity on control fruit consistently increased as inoculum density increased (Fig. 2). Conversely, the protective action of PSi consistently decreased as the inoculum load increased (GM incidence reductions after 6 days at 20ºC of 90, 70 and 30% on oranges inoculated with 10^3, 10^5 and 10^6 spores ml^{-1} of *P. digitatum*). This effect on the incidence of GM was not as pronounced in the tests to assess the influence on the curative activity of PSi, probably because control fruit was already 100% decayed with a concentration of 10^4 spores ml^{-1} of *P. digitatum*. In contrast, the effect of inoculum density in these tests was clear for GM severity, which steadily increased on both control and PSi-treated oranges as the inoculum load increased.

The period of time between treatment and inoculation (temporal factor) affected the preventive performance of PSi only when it was of about 2 h. In this case, GM incidence and severity were significantly lower than with the rest of time intervals (Table 1). We assume that after 2 h, a large amount of active PSi residues might be present into the treated rind wound and they might adversely affect the viability of ungerminated spores that had just been inoculated into the adjacent wound. In contrast, time intervals of 24, 48 and 96 h between treatment and inoculation were too long to maintain equivalent proportions of active residues in the peel tissue. It appears, in addition, that during these time periods the treatment did not induce any defense or protection mechanisms against *P. digitatum* important enough to significantly reduce disease on inoculated fruit. The importance of the timing of defense responses in phenomena related to acquired or induced plant resistance to pathogens is critical, as it has been suggested by some researchers (Vallad and Goodman 2004). Our data also showed that PSi locally applied to a peel wound after harvest had no systemic activity and GM incidence and severity were not reduced on inoculated wounds located at 10, 20, or 30 mm from the treatment site. Therefore, the preventive action of

postharvest PSi treatments would be a type of local acquired resistance (LAR), since resistance is only manifested in the same plant tissues that receive the resistance induction treatments. In contrast, most of preharvest or field applications with chemical resistance inducers typically induce systemic acquired resistance (SAR) or induced systemic resistance (ISR), in which pathogen resistance is produced in plant organs other than those that had been directly treated (Edreva 2004; Vallad and Goodman 2004). The production of SAR or IRS is favored by the high metabolic activity of the plant growing in the field. In general, resistance to plant pathogens are more easily induced in vegetative parts of the plant than in reproductive portions, such as the peel tissues of citrus fruit (Porat et al. 2003). In this study, variable results were obtained when postharvest PSi treatments at 90 mM were applied with micropipette into wounds in *in vivo* primary screenings or as aqueous dips in laboratory trials. With the exception of BM incidence in preventive tests, disease incidence was generally higher on dip-treated oranges than on fruit treated with a solution drop. This could be explained by different penetration capability of the product into peel wounds and different length of contact between the product and the treated fruit.

In general, dip times and temperatures did not consistently influence the effectiveness of PSi dips. Therefore, dips at room temperature (20°C) for 60 s were selected and applied on subsequent trials with long-term cold stored oranges. This is a result that might facilitate the commercial adoption of postharvest PSi treatments in citrus packinghouses, since implementation and application costs of non-heated solutions would be considerably lower than that of solutions heated to temperatures of 40-50ºC. In our work, the curative effectiveness of these PSi dips to reduce the incidence of GM and BM was similar to that obtained by Bi et al. (2006) using SSi treatments to control melon pink rot caused by *T. roseum*. GM and BM severity on oranges was reduced by up to two-fold by PSi dips at

room temperature for 60 s in either preventive or curative tests. Although these reductions were significant, they were lower than those obtained by Guo et al. (2007) after dipping Chinese cantaloupes in SSi solutions to control pink rot (severity reduction of about five-fold).

In preventive tests with long-term cold-stored fruit at 5°C, we found that PSi at 90 mM applied as 60 s dips at 20°C did not significantly reduce the incidence of GM and BM after 4 or 6 weeks of storage, but it was effective to reduce the severity of both molds. In contrast, in curative tests, this same treatment effectively reduced both incidence and severity of GM and BM with respect to control fruit (Figs. 5, 6). Therefore, before the overall performance of cold-stored oranges treated with PSi, this treatment could be recommended for long-term storage of citrus fruit at commercial refrigeration temperatures. The primary findings of this research work were that postharvest PSi treatments showed significant preventive and curative antifungal activity against citrus penicillium molds. Considering that Si is the second most abundant atom in the earth's crust and it is readily available, the cost of Si treatments are relatively inexpensive and, in any case, will be lower than that of other new alternative strategies for citrus postharvest disease control. Although large-scale semicommercial trials are needed for efficacy assessment before commercial implementation, Si treatments show great potential as part of non-polluting integrated disease management programs. According to Laing et al. (2006), the application of Si in crops provides a viable component of integrated management of insect pests and diseases because it leaves no insecticide residues in food or the environment, and it can be easily integrated with other pest management practices, including biological control.

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References

Bi, Y., Tian, S. P., Guo, Y. R., & Ge, Y.H. (2006). Sodium silicate reduces postharvest decay on Hami melons: Induced resistance and fungistatic effects. *Plant Disease*, *90*, 279-283.

Buonario, R., Iriti, M., & Romanazzi, G. (2009). Induced resistance to plant diseases caused by oomycetes and fungi. *Petria*, *19*, 130-148.

Chérif, M., Asselin, A., & Bélanger, R. R. (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Phytium* spp. *Phytopathology*, *84*, 236-242.

Chérif, M., Benhamou, N., Menzies, J. G., & Bélanger, R. R. (1992a). Silicon induced resistance in cucumber plants against *Phytium ultimum*. *Physiological and Molecular Plant Pathology*, *41*, 411-425.

Chérif, M., Menzies, J. G. Benhamou, N., & Bélanger, R. R. (1992b). Studies of silicon distribution in wounded and *Phytium ultimum* infected cucumbers plants. *Physiological and Molecular Plant Pathology*, *41*, 371-385.

Datnoff, L. E., Seebold, K. W., & Correa, V. F. J. (2001). The use of silicon for integrated disease management: reducing fungicide applications and enhancing host plant resistance. In L. E. Datnoff, G. H. Snyder, G. H. Korndorfer (Eds.), *Silicon in Agriculture* (pp. 171-183). The Netherlands: Elsevier Science.

Droby, S., Wisniewski, M., Macarisin, D., & Wilson, C. L. (2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm? *Postharvest Biology and Technology, 52,* 137-145.

Eckert, J. W., & Brown, G. E. (1986). Evaluation of postharvest treatments for citrus fruits. In K. D. Hickey (Ed.), *Methods for Evaluating Pesticides for Control of Plant Pathogens* (pp. 92-97). St. Paul, MN, USA: American Phytopathological Society Press.

Eckert, J. W., & Eaks, I. L. (1989). Postharvest disorders and diseases of citrus fruits. In W. Reuter, E. C. Calavan, G. E. Carman (Eds.), *The Citrus Industry* (Vol. 5, pp.179-260). Berkeley, CA, USA: University of California Press.

Edreva, A. (2004). A novel strategy for plant protection: Induced resistance. *Journal of Cell and Molecular Biology, 3*, 61-69.

Epstein, E. (1999). Silicon. *Annual Review of Plant Phisiology and Plant Molecular Biology*, *50*, 641-664.

Guo, Y., Liu, L., Zhao, J., & Bi, Y. (2007). Use of silicon oxide and sodium silicate for controlling *Trichothecium roseum* postharvest rot in Chinese cantaloupe (*Cucumis melo* L.). *International Journal of Food Science and Technology*, *42*, 1012-1018.

Laing, M. D., Gatarayiha, M. C., & Adandonon, A. (2006). Silicon use for pest control in agriculture: A review. *Proceedings of the South African Sugar Technologists' Association*, *80*, 278-286.

Liu, J., Zong, Y., Qin, G., Li, B., & Tian, S. (2010). Plasma membrane damage contributes to antifungal activity of silicon against *Penicillium digitatum*. *Current Microbiology*, *61*, 274-279.

Mathaba N., Tesfay, S., Bower, J. P., & Bertling, I. (2009). The potential of postharvest silicon dips to mitigate chilling injury in citrus fruit with special emphasis on lemon (c.v Eureka). In *Proceedings of the 1st Annual Research Day, Silicon in Agriculture*. Durban, South Africa: South African Sugar Association.

Moscoso-Ramírez, P. A., & Palou, L. (2013). Evaluation of postharvest treatments with chemical resistance inducers to control green and blue molds on orange fruit. *Postharvest Biology and Technology, 85*, 132-135.

O'Neill, T. M. (1991). Investigation of glasshouse structure, growing medium and silicon nutrition as factors affecting disease incidence in cucumber crops. *Medelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, 56,* 359-367.

Palou, L., Smilanick, J. L., & Droby, S. (2008). Alternatives to conventional fungicides for the control of citrus postharvest green and blue molds. *Stewart Postharvest Review, 2:2*, 1-16.

Porat, R., Vinokur, V., weiss, B., Cohen, L., Daus, A., Goldshmidt, E. E., & Droby, S. (2003). Induction of resistance to *Penicillium digitatum* in grapefruit by β-aminobutyric acid. *European Journal of Plant Pathology*, *109*, 901-907.

Samuels, A. L., Glass, A. D. M., Ehret, D. L., & Menzies, J. G. (1991). Mobility and deposition of silicon in cucumber plants. *Plant Cell & Environment*, *14*, 485-492.

1

1Shen, G. H., Xue, Q. H., Tang, M., Chen, Q., Wang, L. N., Duan, C. M., Xue, L., & Zhao,
2 J. (2010). Inhibitory effects of potassium silicate on five soil-borne phytopathogenic
3 fungi in vitro. *Journal of Plant Diseases and Protection, 117*, 180-184.

4Tian, S., Qin, G., & Xu, Y. (2005). Synergistic effects of combining biocontrol agents with
5 silicon against postharvest diseases of jujube fruit. *Journal of Food Protection*, *68*, 544-
6 550.

7USDA AMS NOP. United States Department of Agriculture, Agricultural Marketing
8 Service, National Organic Program. (2010). 7 CFR 205.601. Synthetic substances
9 allowed for use in organic crop production. Final rule. *Federal Register, 75*(238),
10 77521-77524.

11US EPA. United States Environmental Protection Agency. (2006). 40 CFR 180.1268.
12 Potassium silicate; exemption from the requirement of a tolerance. Final rule. *Federal*
13 *Register, 71*(114), 34267-34272.

14Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced
15 systemic resistance in conventional agriculture. *Crop Science*, *44*, 1920-1934.

16Wagner, F. (1940). Die bedeutung der kieselsaüre für das wachstun einiger kulturpflanzen
17 ihren nährstoffhaushalt und ihre anfälligkeit gegen echte mehltaupilze.
18 *Phytopathologische Zeitschrift, 12*, 427-479.

19Youssef, K., Ligorio, A., Sanzani, S. M., Nigro, F., & Ippolito, A. (2012). Control of
20 storage diseases of citrus by pre- and postharvest application of salts. *Postharvest*
21 *Biology and Technology*, *72*, 57-63.

Table 1 Influence of temporal and spatial factors on the preventive activity of potassium silicate (PSi) against citrus green mold on 'Valencia' oranges artificially inoculated with *Penicillium digitatum* after treatment

	Green mold	
	Incidence	Severity
Time between PSi treatment and fungal inoculation (h)	(%)	(mm)
2	35 b	11 b
24	95 a	69 a
48	95 a	68 a
96	100 a	93 a
Distance between PSi treatment application site and fungal inoculation site (mm)		
Control (water)	85 a	79 a
10	95 a	80 a
20	90 a	79 a
30	90 a	77 a

Fruit were treated in a rind wound with 30 µl of PSi at 90 mM, inoculated with *P. digitatum* and incubated at 20°C and 90% RH for 6 days. Means followed by different letters are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Results are means from two experiments. Green mold incidence was arcsine transformed. Non-transformed means are shown.

FIGURES (Created with SigmaPlot)

Fig. 1. Preventive and curative activity of potassium silicate (PSi) at different concentrations against green (GM) and blue (BM) molds in in vivo primary screenings with ‘Valencia’ oranges. In preventive tests, oranges were wounded, treated with 30 µl of PSi solution at different concentrations and artificially inoculated 24 h later with *Penicillium digitatum* or *P. italicum*. In curative tests, fungal inoculation was performed 24 h before the application of PSi. Treated fruit were incubated for 6 days at 20°C and 90% RH. Disease incidence and severity reductions were determined with respect to control fruit treated with sterile water. For each mold, columns followed by different letters are significantly different according to Fisher’s protected LSD test ($P \leq 0.05$) applied after an ANOVA. Disease incidence was arcsine transformed. Non-transformed means are shown.

Fig. 2. Influence of inoculum concentration on the preventive and curative activity of postharvest treatments with 90 mM potassium silicate (PSi) against green mold on ‘Lanelate’ oranges. In preventive tests, oranges were wounded, treated with 30 µl of PSi solution at 90 mM and artificially inoculated about 2 h later with *Penicillium digitatum*. In curative tests, fungal inoculation was performed about 2 h before the application of PSi. Control fruit were treated with sterile distilled water. Treated fruit were incubated for 6 days at 20°C and 90% RH. For each inoculum concentration and treatment, columns with different lowercase and capital letters, respectively, are significantly different according to Fisher’s protected LSD test ($P \leq 0.05$) applied after an ANOVA. Results are means from

two experiments. Disease incidence was arcsine transformed. Non-transformed means are shown.

Fig. 3. Preventive activity of dips with 90 mM potassium silicate (PSi) against green (GM) and blue (BM) molds on artificially wounded 'Lanelate' oranges treated for 60 or 150 s at 20 or 50°C, inoculated about 2 h later with *Penicillium digitatum* or *P. italicum*, and incubated for 7 days at 20°C and 90% RH. Control fruit were dipped in water at 20 or 50°C for 60 s. For each mold and dip temperature and for each mold and PSi dip time, columns with different lowercase and capital letters, respectively, are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Results are means from two experiments. Disease incidence was arcsine transformed. Non-transformed means are shown.

Fig. 4. Curative activity of dips with 90 mM potassium silicate (PSi) against green (GM) and blue (BM) molds on 'Lanelate' oranges artificially inoculated with *Penicillium digitatum* or *P. italicum*, dipped for 60 or 150 s at 20 or 50°C, about 24 h later and incubated for 7 days at 20°C and 90% RH. Control fruit were treated with water at 20ºC for 60 s. For each mold and for each mold and PSi dip time, columns with different lowercase and capital letters, respectively, are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Results are means from two experiments. Disease incidence was arcsine transformed. Non-transformed means are shown.

Fig. 5. Preventive activity of potassium silicate (PSi) dips at 90 mM against green (GM) and blue (BM) molds on wounded 'Valencia' oranges dipped for 60 s at 20°C, artificially inoculated with *Penicillium digitatum* or *P. italicum* about 2 h later, and cold-stored at 5°C and 90% RH for 6 weeks. For each mold and evaluation date, means with different letters are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Disease incidence was arcsine transformed. Non-transformed means are shown.

Fig. 6. Curative activity of potassium silicate (PSi) dips at 90 mM against green (GM) and blue (BM) molds on 'Valencia' oranges artificially inoculated with *Penicillium digitatum* or *P. italicum* in rind wounds, dipped 24 h later for 60 s at 20°C, and cold stored at 5°C and 90% RH for 6 weeks. For each mold and evaluation date, means with different letters are significantly different according to Fisher's protected LSD test ($P \leq 0.05$) applied after an ANOVA. Disease incidence was arcsine transformed. Non-transformed means are shown.