The Effects of Postharvest Hot Water and Fungicide Treatments on *Guignardia citricarpa* Growth and the Development of Citrus Black Spot Symptoms on 'Valencia' Orange Fruit

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Abstract. Citrus black spot (CBS), caused by *Guignardia citricarpa*, is a fungal disease that was first described in Australia in the 1890s and has since been discovered in Southwest Florida in 2010. The current study evaluated the effects of hot water treatments on mycelial growth of *G. citricarpa* in vitro and also evaluated postharvest hot-water dips and fungicide treatments on CBS development on 'Valencia' oranges. In vitro exposure to 56 °C for 120 seconds, 59 °C for 60 seconds, or 62 °C for 30 seconds suppressed mycelial growth of all three *G. citricarpa* isolates by >30%. These treatments did not significantly reduce disease incidence or severity of CBS lesion development on whole 'Valencia' oranges from CBS-infected trees when the fruit already had visible CBS symptoms before treatment. On asymptomatic fruit, while the treatments did not significantly reduce the incidence of CBS lesion development, fruit dipped in 56 °C water for 120 seconds significantly reduced disease severity after 2 weeks of storage compared with the control. None of the treatments caused peel scalding or fruit quality deterioration. Postharvest application of azoxystrobin, imazalil, or thiabendazole significantly reduced CBS disease severity on fruit that were asymptomatic at harvest, but did not affect disease incidence. These fungicides were not effective on fruit harvested later in the season (April), possibly because most lesion expression had already occurred before harvest, with little left to develop after harvest. On fruit showing CBS symptoms at harvest, postharvest fungicide treatments did not significantly affect disease incidence or severity after storage. Heating the fungicide solutions did not significantly improve fungicide effectiveness. These results demonstrated that fungicide azoxystrobin, imazalil, or thiabendazole could reduce CBS severity, but not incidence, on orange fruit that are still asymptomatic at harvest.

Citrus black spot is a fungal disease caused by *G. citricarpa* Kiely [anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa]. This disease was first described in Australia in the 1890s and has since been found in the humid subtropical regions of Sub-Saharan Africa, Asia, and South America (Paul et al., 2005). Europe has classified *G. citricarpa* as an A1 quarantine disease (absent from the region) and fruit with CBS are not allowed into the European market (Paul et al., 2005). Almost all commercial citrus cultivars are susceptible to CBS, with sweet oranges, especially mid- to late-harvested cultivars such as Valencia, being the most susceptible. Although CBS does not affect the internal fruit quality, external peel blemishes can render the fruit unmarketable and cause extensive premature fruit drop (Hincapie et al., 2014).

In North America, CBS was first discovered on 'Valencia' oranges in Southwest Florida in Mar. 2010 (Schubert et al., 2012). As of this writing, the disease has been found in three counties: Collier, Hendry, and Polk. The infected groves in these counties have been designated restricted quarantine areas (USDA-APHIS, 2012). However, for the Polk County discovery, no subsequent CBS detections have occurred since the initial find in Nov. 2012. The USDA's pest risk assessment concluded that infected fruit are not a likely vehicle to spread CBS and infected fruit can be shipped to all U.S. states provided grade standards are met and the fruit have been treated according to specific protocols, including washing, brushing, surface disinfesting, imazalil, and/or thiabendazole application and waxing (USDA-APHIS, 2010, 2012). However, previous reports indicated that postharvest thiabendazole or imazalil treatment had no significant inhibition on CBS development (Agostini et al., 2006; Lucon et al., 2010). Infected fruit may develop lesions after packing and shipping that could eventually exceed USDA grade standards, causing economic losses from rejection at destination markets (Canale et al., 2011).

Hot water treatments have been widely evaluated and used to control postharvest decay, reduce physiological disorders, and improve storage quality of a variety of horticultural products (Fallik, 2004). It is an environmentally friendly procedure with increasing acceptability in commercial packinghouses. Ritenour et al. (2003) found that dipping 'Ruby Red' and 'Marsh' grapefruit from Florida in 59 °C water for 10 s resulted in an approximate 90% reduction in stem-end rot (*Lasiodiplodia theobromae*) incidence. Porat et al. (2000a, 2000b) found that a short-duration, hot water brushing (56 °C for 20 s) not only reduced surface microorganism and natural decay on citrus fruit, but also induced defensive mechanisms in fruit against decay organisms. Pavoncello et al. (2001) reported that hot water treatment of 'Star Ruby' at 62 °C for 20 s increased resistance against green mold (*Penicillium digitatum*) as well as induced the accumulation of heat-shock proteins, chitinases, and β-1,3-glucanase in grapefruit peel tissue. Fallik (2004) concluded that the reduction in disease development on fruit treated with hot water was mainly due to the induction of plant disease-resistance compounds as well as the reduction in microorganism population on the fruit surface.

In addition, heated solutions have been reported to enhance fungicide effectiveness for postharvest decay control, allowing lower fungicide concentrations to be used on fruit. For example, D'Aquino et al. (2006) showed that dipping citrus fruit in heated (50 °C) imazalil or pyrimethanil solution required 8-or 16-fold lower fungicide concentration,

respectively, than treatments at 20 °C for control of *P. digitatum* or *Penicillium italicum*. There is currently no report of hot water treatment or heated fungicide treatment to delay CBS lesion development on citrus fruit after harvest.

The objective of this study was to investigate whether hot water or heated fungicide treatments can reduce CBS lesion development on 'Valencia' orange fruit. The effects of heat treatments on mycelial growth of *G. citricarpa* in vitro and the potential changes in fruit quality caused by hot water treatments were also investigated.

Materials and Methods

Effects of heat treatments on mycelial growth of G. citricarpa in vitro. Three isolates of *G. citricarpa* from different locations in Florida were used to evaluate temperature and exposure duration on mycelial growth. The three isolates were designated as GC1 (isolated from Collier County in 2010), CNGC (isolated from Hendry County in 2011), and PPST (isolated from Polk County in 2012). The isolates were taken from lesions of infected 'Valencia' orange fruit and stored on sterile filter papers in sealed bags at –20 °C. For mycelial production, the isolates were transferred to potato dextrose agar and grown at 25 °C with 12 h of light for 14 d. The temperatures and exposure durations evaluated were 53, 56, 59, or 62 °C, for 10, 20, 30, 60, 120, 180, or 300 s. The experimental design consisted of a 3 (isolates) × 4 (temperatures) × 6 (exposure durations) factorial with three replicates (mycelial ball) per treatment combination and the entire experiment was repeated.

The experiment was performed as described by Nevarez et al. (2010) with some modifications. Mycelial plugs (5 mm diameter), from the actively growing area of a 14-d-old colony were transferred into 50-mL bottles containing 15 mL of potato dextrose broth (PDB) and incubated on a shaker (120 rpm) at 25 °C with 12 h of light for 7 d, with one mycelial plug per bottle. After 7 d, a small dark mycelial ball formed in the PDB. Three mycelial balls were chosen randomly, rinsed with sterile water, and placed in petri dishes without lids at 60 °C for 72 h to obtain the original dry weight (DW1). The remaining mycelial balls were used for the heat treatments where one mycelial ball in one flask served as a replicate. Fifty-milliliter flasks with 20 mL of PDB were randomly placed in a hot water bath and two calibrated thermometers were used to measure temperature during the experiment: one in the water bath and the other in the solution within one of the flasks. After the temperature within the flask reached the desired temperature, a mycelial ball was placed in the flask for the desired duration. After treatment, the heated flask was moved to 25 °C water for 1 min and then placed on a shaker (120 rpm) at 25 °C with 12 h of light for mycelial incubation. After 7 d, the mycelial balls were rinsed and dried

as described earlier to obtain the final dry weight (DW2$_{treatment}$). Untreated mycelial balls incubated at 25 °C in PDB for 7 d and then dried served as the control (DW2$_{control}$). Percent growth inhibition was calculated as following:

$$\text{Growth inhibition}(\%)$$
$$= 100 \times (\text{DWC}_{control} - \text{DWC}_{treatment})$$
$$/\text{DWC}_{control}, \text{ where}$$
$$\text{DWC}_{control} = \text{DW2}_{control} - \text{DW1 and}$$
$$\text{DWC}_{treatment} = \text{DW2}_{treatment} - \text{DW1}.$$

Effects of hot water dips on CBS development of naturally infected fruit right after harvest. 'Valencia' oranges were harvested from a CBS-infected grove in Immokalee, FL, on 12 Feb. 2015 and 2 Apr. 2015, with each harvest serving as a separate experiment. The disease incidence in this grove was 100%, with every tree having at least one fruit with CBS lesions, and no preharvest fungicide was used on these trees. The fruit were washed for 1 min on a rotating brush bed with water and then separated into two groups (asymptomatic and symptomatic). Preexisting CBS lesions on symptomatic fruit were marked before administering the treatment to distinguish them from lesions that developed subsequently. Both asymptomatic and symptomatic fruit were dipped in water at 1) 56 °C for 120 s, 2) 59 °C for 60 s, or 3) 62 °C for 30 s. Fruit dipped in 25 °C water for 120 s were used as a wet control and undipped fruit were used as a dry control. The experimental design consisted of a 5 (treatments) × 2 (harvests) factorial with three replicates per treatment combination. Ten asymptomatic fruit and 10 symptomatic fruit dipped together in one tank served as a replicate (Lucon et al., 2010).

Fruit were dipped into water within stainless steel tanks holding 40 L of rapidly stirred water that could be heated as necessary as described by Ritenour et al. (2003). Water temperature varied by ±0.5 °C. After treatment, the fruit were air-dried and incubated at 12 °C with 90% relative humidity (RH) and exposed to 5–10 ppm ethylene and continuous light to speed lesion appearance (Baldassari et al., 2007; Brodrick and Rabie, 1970; Er et al., 2013). The number of new lesions on each fruit (disease severity) and percent of fruit that developing new lesions (disease incidence) were determined after 2 weeks.

Effects of hot water dips on fruit quality after storage. 'Valencia' orange fruit of uniform size and color were obtained from a nonCBS-infected grove in Ft. Pierce, FL, on 11 Mar. 2015 and 30 Apr. 2015, with each harvest serving as a separate experiment. Fruit were treated as above and stored at 4 °C with 90% RH. Peel scalding, weight loss, peel color, peel puncture resistance (PPR), total soluble solids (TSS), and titratable acid (TA) were measured every 4 weeks. The experimental design consisted of

a 5 (treatments) × 2 (harvests) factorial with three replicates per treatment combination. Thirty fruit dipped together in one tank served as a replicate.

Fruit quality evaluation was performed as described by Ritenour et al. (2005) with some modifications. The peel scalding index was estimated visually as the percentage of peel injury area compared with the total surface, where: 0 = no peel injury; 1 = less than 25% peel injury; 2 = about 25% to 50% peel injury; 3 = about 50% to 75% peel injury; and 4 = more than 75% peel injury. Peel color was measured at three evenly spaced locations around the fruit equator using a Minolta Chroma Meter (model CR-300; Minolta Camera Corp., Ramsey, NJ). Color was reported as a*:b* ratio where a* measures green (negative) to red (positive) and b* measures blue (negative) to yellow (positive). As fruit peel color turns from green to yellow orange, the a*:b* value increases. PPR was determined by puncturing each fruit at three evenly spaced locations around the fruit equator using a texture analyzer (model TA-XT2; Stable Micro Systems, Godalming, England) with a 2-mm-diameter, flat-tipped, cylindrical probe attached to a texture analyzer. After making contact with the fruit surface, the probe was set to travel at a speed of 8 mm·s⁻¹ and the maximum force exerted to puncture the peel was recorded. Juice TSS (°Brix) was measured using a temperature-compensating refractometer (Spectronic Instruments, Rochester, NY). Juice TA (% citric acid) was measured by titrating juice to pH 8.3 with sodium hydroxide (NaOH) using an automatic titrimeter (model DL12; Mettler, Highstown, NJ).

Effects of heated fungicide dips on CBS development of naturally infected fruit right after harvest. Fungicides used in this experiment were formulated products of azoxystrobin, fludioxonil, imazalil, thiabendazole, pyrimethanil, and phosphorous acid. All the fungicides were used at commercially recommended concentrations, based on the active ingredient (Table 1). 'Valencia' oranges were harvested from a CBS-infected grove in Immokalee, FL, on 25 Feb. 2014, 2 Apr. 2014, and 12 Feb. 2015, with each harvest serving as a separate experiment. The disease incidence in this grove was 100%, with every tree having at least one fruit with CBS lesions, and no preharvest fungicide was used on these trees. Fruit were separated into two groups (asymptomatic and symptomatic), and pre-existing CBS lesions on symptomatic fruit were marked before administering the treatment to distinguish them from lesions that developed subsequently. Both asymptomatic and symptomatic fruit were dipped into one of the above fungicides for 30 s. Solutions were at 25 or 50 °C in the first experiment, and 25 or 56 °C in the second and third experiments. Fruit dipped in 25, 50, or 56 °C water for 30 s were used as a control. The experimental design consisted of a 7 (fungicides) × 2 (solution temperatures) × 2 (harvests) factorial with three replicates per treatment combination. Ten asymptomatic

Table 1. Common names of each fungicide tested, their corresponding trade name, percentage of active ingredient manufacturer, and postharvest application concentration.

Generic name	Trade name	Active ingredient (%)	Company, city, state	Concn applied (ppm)
Azoxystrobin	Abound flowable	22.9	Syngenta Crop Production Inc., Greensboro, NC	1,200
Fludioxonil	Scholar	50	Syngenta Crop Production Inc., Greensboro, NC	1,200
Imazalil	Freshgard 700	44.6	FMC FoodTech, Lakeland, FL	1,000
Pyrimethanil	Penbotec 400	37.14	DECCO Cerexagri Inc., Monrovia, CA	1,000
Phosphorous acid	Kphos	55.5	Pace International, Wapato, WA	10,000
Thiabendozale	Freshgard 598	98.5	FMC FoodTech, Lakeland, FL	1,000

fruit and 10 symptomatic fruit dipped together in one tank served as a replicate (Lucon et al., 2010).

Fruit were dipped into the fungicide solutions within stainless steel tanks holding 40 L of a rapidly stirred solution as described above. Water temperature varied by ±0.5 °C. After treatment, the fruit were air dried and incubated at 25 °C with 90% RH and exposed to 3 ppm ethylene and continuous light to speed lesion appearance (Baldassari et al., 2007; Brodrick and Rabie, 1970; Er et al., 2013). The number of new lesions on each fruit (disease severity) and percent of fruit developing new lesions (disease incidence) were evaluated after 2 weeks.

Statistical analysis. The effects of pathogen isolate, temperature, exposure duration, and their interaction on mycelial growth were analyzed by three-way analysis of variance (ANOVA) using the statistical software of SAS 9.4 for Windows (SAS Institute Inc., Cary, NC). The effects of hot water treatment, harvest, and their interaction on CBS development were analyzed by two-way ANOVA. Treatment means were separated using least significant difference (LSD) at α = 0.05. The effects of hot water treatment, harvest, and their interaction on fruit storage quality were analyzed by two-way ANOVA. Treatment means were separated using LSD at α = 0.05. The effects of fungicide, solution temperature, harvest, and their interaction on CBS development were analyzed by three-way ANOVA. Treatment means were separated using LSD at α = 0.05.

Results and Discussion

Effects of heat treatments on mycelial growth of G. citricarpa. There was no significant difference in response of the three *G. citricarpa* isolates to the heat treatment, so the data were pooled. As expected, inactivation of mycelial growth was dependent on both temperature and duration of exposure (Fig. 1), with a significant interaction ($P < 0.001$). Growth inhibition of 0% indicated the mycelial ball grew after treatment, but to the same extent as the control. Negative growth inhibition indicated the mycelial balls grew larger than the control after treatment. Growth inhibition of 100% indicated no growth, whereas values greater than 100% indicated mycelial ball shrinkage compared with pretreatment size.

In agreement with Nevarez et al. (2010) who found less intense shock conditions increased mycelial growth of *Penicillium glabrum* compared with the control, the
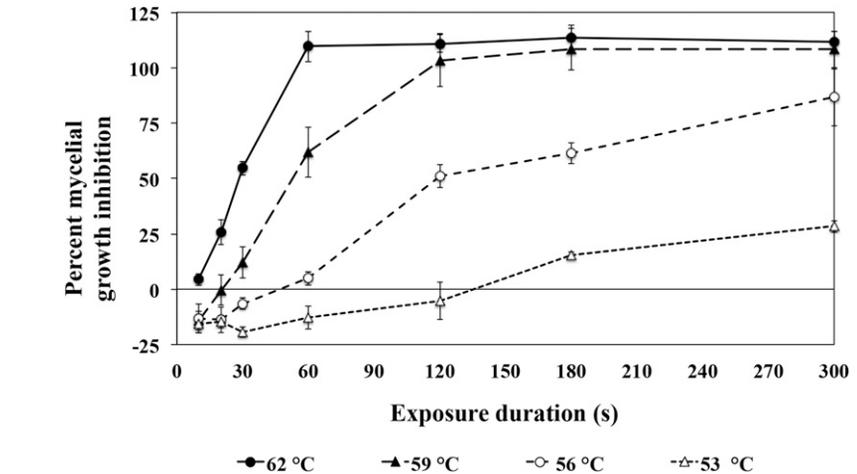

Fig. 1. Effects of heat treatments on mycelial growth of *Guignardia citricarpa*. Mycelial balls were subjected to different temperature × exposure duration and incubated on a shaker at 25 °C with 12 h of light for 7 d. Each value is the mean of three isolates and the vertical bar indicates standard error.

current study also found that less intense treatments (53 °C for less than 60 s, 56 °C for less than 30 s, and 59 °C for less than 20 s) promoted mycelia growth. That may be related to stimulated metabolism during the heat treatment and/or the possible induction of fungal defense mechanisms that promoted growth during incubation. Progressively increased temperature and duration above those levels resulted in progressively greater mycelial growth inhibition. Instead of growing, the mycelial mass decreased by up to 34% after exposure to the most severe heat treatments (62 °C for more than 60 s or 59 °C for more than 120 s). Nevarez et al. (2010) explained this phenomenon as fungal lysis that occurred in response to a wide range of drastic environmental stresses such as heat, pH, or chemical treatments. Exposure to 56 °C water for 120 s, 59 °C water for 60 s, and 62 °C water for 30 s reduced mycelial growth by 51%, 62%, and 55%, respectively, compared with control ($P < 0.0001$ respectively; Fig. 1). The results were similar when the experiment was repeated. These treatments were used for a subsequent experiment on whole fruit because they were considered the most severe treatments the fruit might withstand without scalding (John-Karuppiah et al., 2004)

Effects of hot water dips on CBS development of naturally infected fruit right after harvest. Results for asymptomatic fruit showed no significant harvest and treatment interaction. However, the hot water treatments significantly reduced the number of lesions developed on each fruit (disease severity; $P = 0.0498$), but not the proportion of fruit that developed lesions (disease incidence). Harvest date did not significantly affect CBS development.

Fruit harvested later in the season (Apr. 2015) tended to develop fewer lesions than fruit harvested earlier in the season (Feb. 2015) during storage, but the difference was not significant and it did not significantly influence the efficiency of hot water treatments on CBS development (data not shown). In general, it became more difficult to find fruit without lesions on the tree as the season processed, possibly because most latent infections had already developed into visible symptoms. When results from the two harvests were pooled, asymptomatic fruit dipped in 56 °C water for 120 s developed 35% fewer lesions (disease severity; $P = 0.009$) after 14-d incubation compared with the control (Fig. 2A). Disease severity on the fruit exposed to the other two hot water treatments did not differ significantly than that on the control fruit.

When fruit were symptomatic at harvest, fruit developed more lesions than asymptomatic fruit during subsequent incubation (data not shown). However, there was no significant treatment or harvest effect, nor their interaction. The results show that the hot water treatments were more effective on asymptomatic 'Valencia' oranges than on symptomatic fruit. That might be because a higher level of infection is present in the peel, which overcomes the hot water effects.

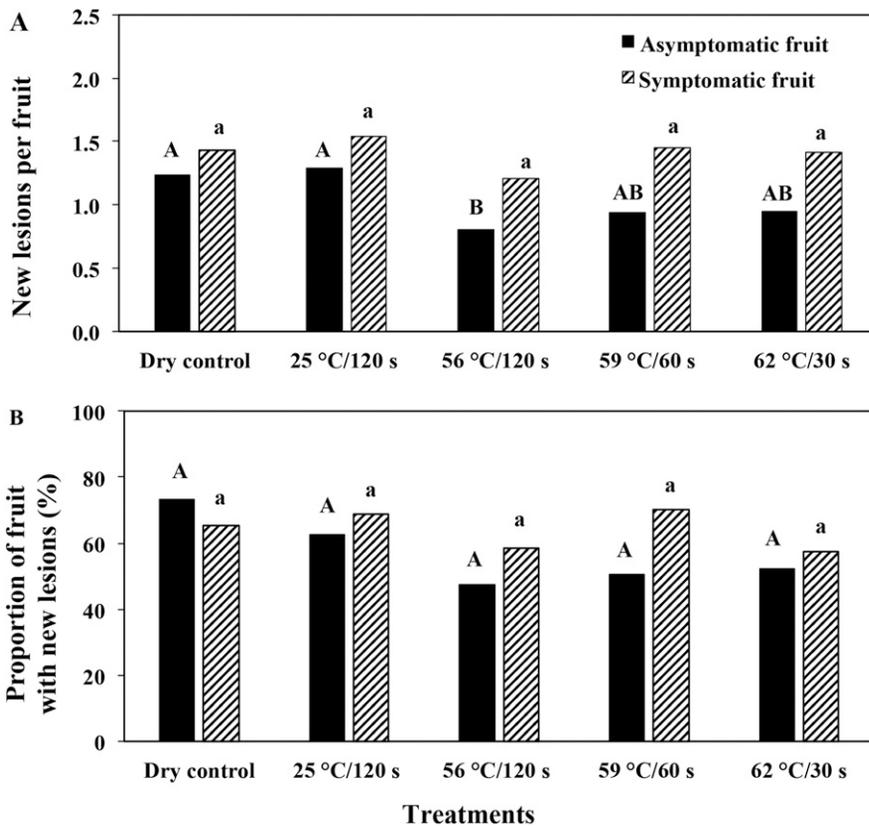Exposure to hot water has been reported to be a safe, nonchemical method to reduce

Fig. 2. Effects of hot water dips on (**A**) citrus black spot (CBS) disease severity and (**B**) disease incidence on 'Valencia' orange fruit. 'Valencia' orange fruit from a CBS-infected grove were treated with different temperature × exposure duration combination and then incubated at 12 °C with 90% relative humidity, 5–10 ppm ethylene and continuous light to speed lesion appearance. Each value represents the mean of two harvests (Feb. 2015 and Apr. 2015). Means with different letters within fruit disease precondition (asymptomatic or symptomatic) are significantly different based on least significant difference ($P < 0.05$).

postharvest citrus fruit diseases caused by *Penicillium*, *Alternaria*, and *Lasiodiplodia* (Ritenour et al., 2003; Zhou et al., 2014). Hot water can reduce microorganism populations on commodity surfaces or latent pathogens in the outer cell layers and may also induce plant disease resistance against various pathogens. For example, Pavoncello et al. (2001) found exposing fruit to 62 °C water for 20 s induced the accumulation of chitinase and β-1,3-glucanases proteins in the flavedo and inhibited green mold incidence on grapefruit. Yun et al. (2013) explored disease-resistance mechanisms induced by heat in 'Kamei' satsuma mandarin fruit and found a significant accumulation of metabolites, such as tetradecanoic acid and oleic acid in fruit pericarp, which might play an important role in plant disease resistance. It is possible that induced defense compounds in heat-treated fruit of the current study are aiding in reducing CBS lesion development, but defense compounds were not measured. In the current study, a dip in 56 °C water for 120 s produced the greatest efficiency against CBS development. *G. citricarpa* only infected fruit flavedo without affecting fruit internal quality (Hincapie et al., 2014). Therefore, 120 s might be enough time for heat to penetrate into the fruit flavedo,

killing the pathogen or inducing fruit disease-resistance mechanisms.

Effects of hot water dips on fruit quality after storage. Hot water treatments did not significantly or consistently influence fruit weight loss, peel color, PPR, internal TSS, TA, or TSS:TA ratio during storage. Although there were a few values that were significantly different after 4 or 8 weeks of storage, there was no consistency between storage dates or when the experiment was repeated (data not shown).

John-Karuppiah et al. (2004) reported that 20% of the 'Valencia' oranges dipped in 60 °C water for 30 s developed peel scalding. Ritenour et al. (2003) also reported that 'Ruby Red' and 'Marsh' grapefruit developed peel scalding after hot water dips of 56 °C for 120 s, 59 °C for 20 s, or 62 °C for 10 s. However, in the current experiment, none of the hot water treatments caused peel scalding on 'Valencia' orange (data not shown). Difference in scalding susceptibility can be due to many factors such as cultivar, harvest season, and postharvest treatment. Ritenour et al. (2003) found that early-season 'Ruby Red' grapefruit (October) were more sensitive to hot water dips than late-season grapefruit (May), and pretreatment storage conditions also influenced fruit tolerance to hot water dips. When fruit were

stored for 3 d at 10 °C, they withstood higher temperatures than fruit just harvested. Therefore, there are many factors influencing fruit tolerance to hot water dips. Although there was no peel injury observed in the current study, the temperature and exposure duration is still a risk for commercial application.

Effects of heated fungicide dips on CBS development of naturally infected fruit right after harvest. For asymptomatic fruit, there was a significant fungicide–harvest interaction for the number of lesions that developed on each fruit (disease severity; $P = 0.0083$). Fungicide significant reduced disease severity ($P < 0.0001$). Harvest date significantly affected CBS development after harvest, where late-season fruit had lower disease severity and disease incidence ($P < 0.0001$). Heating the fungicide solutions to 50 or 56 °C did not affect fungicidal performance against CBS development.

As the season progressed, latent infections apparently developed into visible symptoms so that fewer lesions developed after harvest on fruit. Late-season fruit (Apr. 2014) had less disease severity and disease incidence after incubation compared with fruit harvested just 2 month earlier (Feb. 2014) (data not shown). As such, harvest date significantly influenced the efficiency of fungicides against CBS lesion development ($P = 0.0083$) so that in the second harvest fungicides no longer significantly affected either disease severity or disease incidence (data not shown).

In early season, because there was no significant effect of heated solution to the fungicide performance, the results from experiments at two solution temperatures were pooled. These experiments tested all six fungicides and found that fludioxonil, pyrimethanil, and phosphorous acid did not significantly affect lesion development on asymptomatic fruit. However, azoxystrobin, imazalil, or thiabendazole significantly inhibited disease severity on asymptomatic fruit by 52%, 52%, or 66%, respectively ($P = 0.002$, 0.002, or 0.001, respectively), compared with the control (Fig. 3A). When the experiment was repeated the following year (Feb. 2015) only for azoxystrobin, imazalil, and thiabendazole and the data combined with the first two harvests, all three fungicides still demonstrated significant reduction in CBS disease severity on asymptomatic fruit compared with the control ($P = 0.002$, 0.006, and 0.001, respectively; Fig. 4).

Both imazalil and thiabendazole are systemic fungicides used in commercial packinghouses to control citrus decay (Smilanick et al., 1997; Zhang, 2007). Azoxystrobin is a strobilurin fungicide that inhibits fungal mitochondrial respiration. Miles et al. (2004) reported that preharvest application of strobilurin fungicides (azoxystrobin, trifloxystrobin, and pyraclostrobin) significantly reduced the severity and incidence of black spot on citrus fruit. Therefore, respiration inhibitory fungicides may play an important role in CBS control. The current study is the first to evaluate the effects of these fungicides after
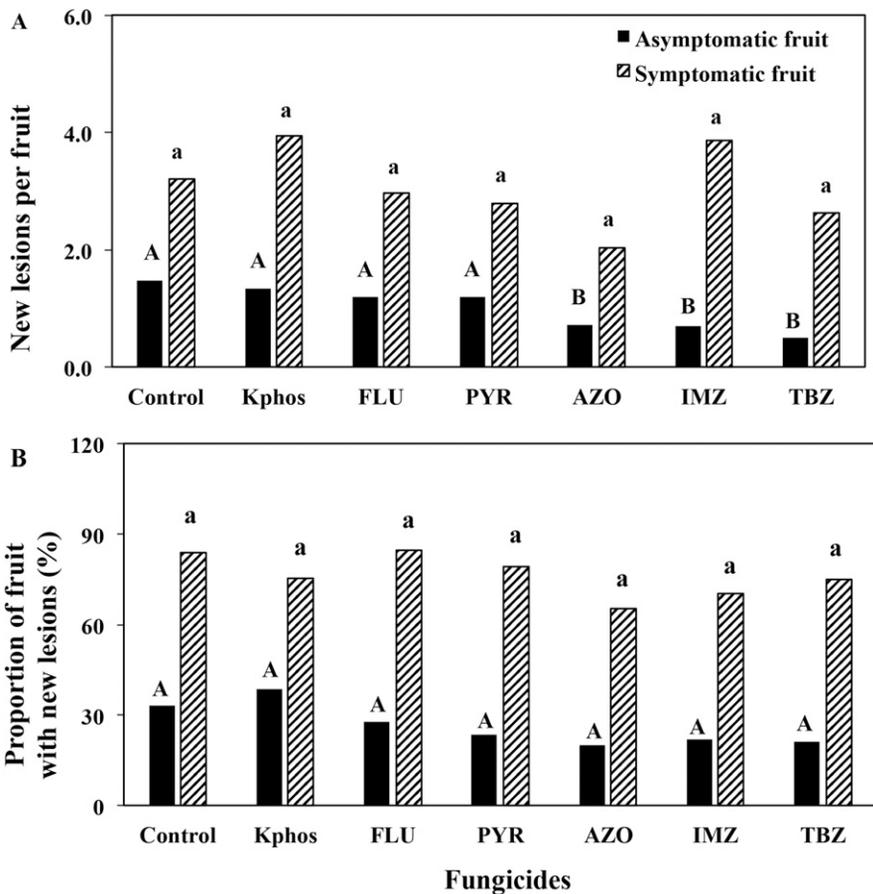
Fig. 3. Effects of 30 s dips in different fungicide solutions on (**A**) citrus black spot (CBS) disease severity and (**B**) disease incidence on 'Valencia' orange fruit harvested in Feb. 2014. 'Valencia' orange fruit from CBS-infected grove were treated with different fungicide × temperature combination and then incubated at 25 °C with 90% relative humidity, 3 ppm ethylene and continuous light to speed lesion appearance. Each value represents the mean of experiments tested at two temperatures. Means with different letters within fruit disease precondition (asymptomatic or symptomatic) are significantly different based on least significant difference ($P < 0.05$). Kphos, phosphorous acid; FLU, fludioxonil; PYR, pyrimethanil; AZO, azoxystrobin; IMZ, imazalil; and TBZ, thiabendazole.
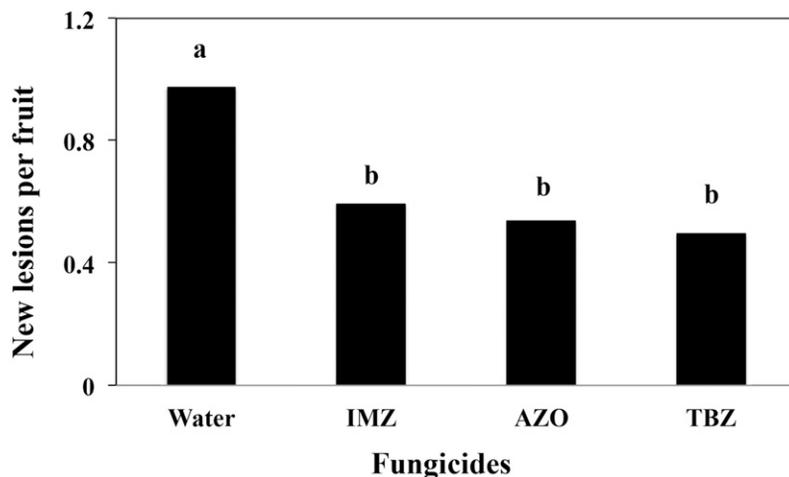

Fig. 4. Effects of 30 s dips in azoxystrobin, imazalil, or thiabendazole solution on citrus black spot (CBS) disease severity on asymptomatic 'Valencia' orange fruit. 'Valencia' orange fruit from CBS-infected grove were treated with different fungicide × temperature combination and then incubated after treatment at 25 °C with 90% relative humidity, 3 ppm ethylene and continuous light to speed lesion appearance. Each value is the mean of three harvests and experiments tested at two temperatures. Means with different letters are significantly different based on least significant difference ($P < 0.05$). AZO, azoxystrobin; IMZ, imazalil; and TBZ, thiabendazole.

harvest on CBS disease severity on asymptomatic fruit, finding that they significantly reduced the number of new lesions developing on each fruit.

Fruit that were symptomatic at harvest developed more CBS lesions during subsequent incubation than did asymptomatic fruit (data not shown). That may be related to high fruit infection on the tree. No interaction between fungicide, temperature, and harvest were found on symptomatic fruit. Fungicide and temperature did not affect CBS lesion development. Only harvest date influenced CBS development ($P < 0.0001$), with late-season fruit developing fewer lesions after harvest, likely because more infections had formed lesions (data not shown). Although azoxystrobin, imazalil, or thiabendazole significantly inhibited disease severity on asymptomatic fruit early in the season, no fungicides significantly reduced disease severity or disease incidence on symptomatic fruit (Fig. 3A and B). This is in agreement with the reports of Lucon et al. (2010) and Rappussi et al. (2011). Korf et al. (2001) also reported that the commercial postharvest chemicals guazatine, imazalil sulphate, and 2,4-D sodium salt could reduce viability of *P. citricarpa* on CBS-infected fruit, but did not eliminate the pathogen completely. The current study showed that postharvest treatments had better effects on fruit that were asymptomatic at harvest. Thus, effective preharvest control is important for maximizing overall reductions of CBS on 'Valencia' oranges (Agostini et al., 2006).

These results indicated that a postharvest dip in 56 °C water for 120 s reduced CBS lesion development on asymptomatic 'Valencia' orange fruit at harvest without negatively impacting fruit quality. However, the temperature/duration is close to values that can cause peel scalding of 'Valencia' orange and grapefruit. Thus, because fruit sensitivity to heat injury can vary with cultivar, season, or postharvest treatment, these treatments cannot be currently recommended unless other mitigation treatments reduce the possibility of injury. Such treatments could include immediate cooling after heat treatment or wax application, both of which have been shown to reduce fruit scalding after heat treatment (John-Karuppiah et al., 2004; Ritenour et al., 2003; Shellie and Mangan, 2002). In addition, postharvest applications of azoxystrobin, imazalil, or thiabendazole at commercially recommended concentrations significantly reduced CBS lesion development on 'Valencia' orange fruit that were asymptomatic at harvest. These effects were more pronounced when fruit were harvested earlier in the season, when fewer lesions were visible on the fruit. The use of heated solutions did not improve fungicidal performance against CBS.

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