JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

REVIEW

pubs.acs.org/JAFC

Control of Postharvest Diseases of Fruit by Heat and Fungicides: Efficacy, Residue Levels, and Residue Persistence. A Review

Mario Schirra,*[,†] Salvatore D'Aquino,[†] Paolo Cabras,[§] and Alberto Angioni[§]

[†]Institute of Sciences of Food Production, National Research Council, Traversa La Crucca 3, Regione Baldinca, 07040 Li Punti, Sassari, Italy

[§]Department of Toxicology, Food and Environmental Unit, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy

ABSTRACT: Extensive research has been done in recent years to reduce the heavy dependence on chemical fungicides to control postharvest diseases and disorders of horticultural crops. Alternative strategies were based on improved cultural practices, biological control, plant-defense promoters, and physical treatments such as UV illumination, radiofrequency treatment, heat therapy, and storage technologies. Among these, postharvest heat treatments such as hot water dips, short hot water rinsing and brushing, and hot air conditioning have reduced rot development and enhanced fruit resistance to chilling injury in sensitive cultivars while retaining fruit quality during cold storage and shelf life. Additive or synergistic increases in effectiveness were observed by integrating heat therapy with various chemical compounds, thus leading to significant reductions in the application of active ingredients to protect produce from decay. This paper highlights the knowledge on this topic with emphasis on heat therapy effects and factors affecting the uptake, persistence, and performance of fungicide residues when they are applied in combination with hot water.

KEYWORDS: cold storage, fungicide residues, heat treatments, postharvest decay

INTRODUCTION

In recent years the agricultural industry has experienced strong market and technological competition among the leading producing countries. Considerable efforts of scientists and producers are devoted to meeting consumer demand for produce with excellent quality traits, with no or minimal pesticide residues. On the other hand, the intensity of the production systems and the low tolerance by the market of fruit diseases, disorders, and insect infestations require a high level of effective pest control, which, despite the important achievements of modern agriculture, is still heavily dependent on synthetic agrochemicals. To reduce the use of pesticides, attention is currently focused on alternative control strategies based on improved cultural practices, biological control, and plant defense promoters. Efforts are also devoted to reducing postharvest losses of horticultural crops by using biological control and physical methods such as ultraviolet illumination, radiofrequency treatment, heat treatments (heat therapy), and storage technologies.[1,2]

Among these, postharvest heat treatments such as hot water treatment, short hot water rinsing and brushing, and hot air treatment provide quarantine security, reduce rot development, enhance fruit resistance to chilling injury in cold-sensitive cultivars, and retain fruit quality during cold storage and shelf life. The effect of heat therapy on horticultural crops has been thoroughly reviewed.[3−8] Despite the beneficial effects of heat treatments, the complete control of decay is rarely accomplished by heat therapy alone, especially when fruit is subjected to cold storage prior to marketing. Thus, heat therapy should be combined with other treatments to enhance its efficiency.

Recently, various effective "reduced risk" fungicides belonging to different chemical classes have been developed to control a wide range of fungal diseases.[9] Some of them have been registered in the United States for postharvest treatments of various horticultural crops, and a number of studies have been done to evaluate the potential of these fungicides to control the main postharvest pathogens of horticultural crops.[10]

The present paper provides an overview of the relevant literature concerning the control of fruit postharvest diseases by heat and fungicide treatments with emphasis on factors influencing the uptake, persistence, and performance of fungicide residues in comparison with standard treatments applied at ambient temperature. The influence of heat and fungicide treatments on ultrastructural changes of epicuticular wax and on fruit tolerance to chilling injury is also discussed.

RESIDUE LEVEL AND PERFORMANCE OF FUNGICIDES APPLIED IN COMBINATION WITH HOT WATER

Various factors affect the deposition of fungicides and their dissipation rate in fruit, including fungicide concentration, treatment mode (spray, drench, or dip), type of mixture (aqueous- or wax-based mixtures), species, cultivar, fruit age, treatment duration, temperature, and pH of the fungicide mixture.[11,12]

Early investigations on peach (*Prunus persica* (L.) Batsch), plum (*Prunus salicina* Lindl.), and nectarine [*P. persica* (L.) Batsch var. *nectarina* (Alt.) Maxim.] have shown that a 1.5 min dip treatment with 2,6-dichloro-4-nitroaniline (DCNA) at 51.5 °C was consistently more effective in controlling postharvest decay than treatments with hot water alone or with DCNA at the ambient temperature of 24 °C.[13] Residues of DCNA capable of providing outstanding decay control in smooth-skinned or glabrous fruit such as nectarine and plum are difficult to attain

Received: February 14, 2011
Revised: July 13, 2011
Accepted: July 14, 2011
Published: July 14, 2011

with DCNA application at ambient temperature but may be accomplished by the application of a heated fungicide mixture. In plum and nectarine, treatments with reduced rates (225 mg/L) of DCNA at 51.5 °C produced residues comparable with standard treatments with 900 mg/L DCNA at ambient temperature (3.1 versus 2.7 mg/kg, respectively) and were equally effective in controlling decay.[13]

Sodium *o*-phenylphenate (SOPP) has long been used to control decay in citrus fruit.[14,15] Hayward and Grierson[16] showed that following postharvest treatments with Dowcide A (a commercial product based on SOPP) at 1000 mg/L active ingredient (ai) and 40 °C, *o*-phenylphenol (OPP) residues in 'Pineapple' and 'Valencia' oranges increased with fungicide concentration and length of treatment, but remarkably decreased, from 6.5 to 1 mg/kg, as the pH of the fungicide mixture increased from 10 to 11.5. When the pH of the SOPP solution was 10.5 and the temperature was raised from 43.3 to 48.9 °C, residues increased from 6.5 to 13.5 mg/kg. The pronounced effect of pH on OPP residue deposition was related to SOPP hydrolysis, because a low pH increases its hydrolysis, which increases deposition of its residues, whereas a high pH decreases its hydrolysis and OPP residues in fruit.

Studies on various citrus fruit cultivars[17] showed that a 2 min treatment with a fungicide mixture containing 2% Dowcide A, 1% hexamine, and 0.2% sodium hydroxide in water at the ambient temperature of 22.8 °C resulted in better decay control than treatment at 37.8 °C, although OPP residues in fruit were consistently lower (2.2 versus 5.2 mg/kg). The diminished efficacy of heated SOPP may have occurred as a result of phytotoxic effects to the peel.[16]

Imazalil (IMZ) and thiabendazole (TBZ) are synthetic fungicides employed on a commercial scale to control a wide range of fungal diseases on fruits, vegetables, and ornamentals.[9] Both fungicides are applied to control postharvest decay in citrus fruit.[14,15] IMZ is especially effective in controlling green mold caused by *Penicillium digitatum*, including benzimidazole-resistant-strains, by curative, preventive and antisporulant activities but is less effective than TBZ for stem-end rot control, and both are ineffective against sour rot and brown rot.[14,15] IMZ residues in citrus fruit are affected by treatment mode (spray or dip) and method of application (aqueous- or wax-based mixtures). Indeed, dip applications of IMZ—aqueous mixtures in 'Valencia' orange left significantly higher residues (2.60 mg/kg) than nonrecovery spray treatments (1.13 mg/kg) at equal concentration (1000 mg/L) and treatment time (15 s).[18] In various citrus-producing countries IMZ is usually used as a wax-based mixture due to its beneficial effects against soilage, a cosmetic defect that occurs when healthy fruits within boxes are soiled by conidia from adjacent rotten fruit.[19] However, when IMZ is applied in a water—wax mixture, it is less effective than in an aqueous mixture.[20,21] This is because part of the ai is not available, being immobilized in the wax and because the wax is more viscous than water, the fungicide penetrates less effectively into the wound infection courts that are exploited by *P. digitatum*. Thus, for a wax fungicide mixture to achieve comparable effectiveness to that in a water mixture, the fungicide concentration must be increased. The application of IMZ at 490 mg/L in aqueous solution at 37.8 °C was found to be significantly more effective in decay control than IMZ in a wax mixture at 4200 mg/L sprayed on fruit at ambient temperature.[22] Furthermore, residues of ca. 3.5 mg/kg IMZ deposited by treatments with IMZ in wax mixture reduced the incidence of green mold on lemons, from 94.4% among untreated fruit to 15.1%, whereas an equal residue concentration deposited by treatment with heated aqueous IMZ reduced green mold incidence to <1.0%.

Whereas in citrus fruit IMZ is less effective in wax than in water mixtures at equal concentration, in tamarillos [*Cyphomandra betacea* (Cav.) Sendt.] its effectiveness was found to be similar in water or wax.[23] Dip treatments for 10 min in water at 50 °C followed by a 1 min dip in water or wax mixtures containing IMZ at 250, 500, or 750 mg/L at ambient temperature were equally effective in controlling stem-end lesions caused by *Colletotrichum gloeosporioides* (Penz.) Arx and *Colletotrichum acutatum* Simmonds over 8 weeks of storage at 3.5 °C plus 1 week at 20 °C. In addition, water dip treatments at 50 °C followed by IMZ at 750 mg/L, in wax or water mixture at ambient temperature, were equally effective in controlling stem-end lesions and calyx mycelium and necrosis, although IMZ residues in the wax mixture were higher than in the water mixture (1.2 versus 0.5 mg/kg).[23]

IMZ residue levels in lemons following dip treatments were closely correlated with the rate of fungicide applied.[24] After application of IMZ at concentrations ranging from 250 to 1500 mg/L IMZ at 50 °C, its residues were ca. 4.5-fold higher compared to corresponding treatments performed at ambient temperature. Treatment with 250 mg/L IMZ at 50 °C provided the complete control of *Penicillium* spp. in lemons during 13 weeks of storage at 9 °C plus 1 week of shelf life at 20 °C and produced a residue level of approximately 8 mg/kg after shelf life. When treatment was conducted at room temperature, complete decay control was accomplished with 1500 mg/L IMZ, which resulted in a post-shelf-life residue level of approximately 3 mg/kg. Due to the linear relationship between the rate of fungicide employed and ai deposition on fruit, it was estimated that a 50 mg/L IMZ dip at 50 °C should be enough to supply the minimum level of IMZ residue in fruit (ca. 3 mg/kg) to control *Penicillium* decay. Further studies have corroborated this conclusion, as higher levels of IMZ at 50 °C were equally effective in controlling decay.[25]

Studies on 'Marsh' grapefruit reported that a 3 min dip treatment with IMZ at 200 mg/L and 50 °C was significantly more effective in controlling decay during 3 months of storage than treatment with IMZ at 1000 mg/L at ambient temperature and resulted in approximately 2-fold higher residues (3.46 versus 1.80 mg/kg) in fruit.[26] Doubling the IMZ concentration or length of dipping or increasing the fungicide temperature by 5.6 °C increased the residue levels in 'Valencia' oranges and 'Eureka' lemons by approximately 1.5—2 times.[22] Accordingly, IMZ residues in 'Marsh' grapefruit were related to treatment duration.[27] In fact, after treatment with 1200 mg/L IMZ at 20 °C for 0.5 min, the residue level increased from 2.79 mg/kg to levels 47 and 100% higher after treatment for 1.5 and 3.0 min, respectively.[27] Similarly, dipping with 200 mg/L IMZ at 50 °C for 1.5 or 3 min increased the residues from 35.8 to 108% compared to those from 0.5 s dips.

IMZ applied to orange at 20 or 50 °C, alone or in combination with TBZ, resulted in similar IMZ depositions (0.72—0.70 mg/kg at 20 °C and 5.87—5.58 mg/kg at 50 °C), whereas TBZ applied alone produced significantly lower TBZ residues than treatment applied in combination with IMZ (0.62 versus 0.42 mg/kg at 20 °C; 1.55 versus 1.32 mg/kg at 50 °C, respectively). The degradation rate of IMZ was significantly faster in fruit treated with both fungicides versus IMZ applied separately (42 versus 14%). IMZ residues increased with treatment length, doubling when dip time at 50 °C increased from 0.5 to 3 min.

Table 1. Postharvest Treatments with Hot Water in Combination with Fungicides To Control Decay of Horticultural Crops

crop	treatment[a]	decay/pathogen	ref
apple (*Malus pumila* Mill.)	benomyl or TBZ, 500 mg/L (54.4 °C/4 min dip)	blue mold/*Penicillium expansum*	35
	carbendazim, 200 mg/L (50 °C/4 min dip)	brown rot/*Monilia laxa* and *M. fructigena*	36
cactus pear [*Opuntia ficus-indica* (L.) Mill.]	TBZ, 1000 mg/L (55 °C/5 min dip)	various fungi	37
citrus fruit			
oranges [*Citrus sinensis* (L.) Osbek]	SOPP, 500−1000 mg/L (25−45 °C/3 min dip)	green mold/*Penicillium digitatum* and blue mold/*Penicillium italicum*	38
	TBZ, 1500 mg/L; Kilol DF-100, 200 mg/L (52 °C/3 min)	unspecified	39
	IMZ, 400 mg/L (55 °C/20 s drench); IMZ, 1000 mg/L (room temp.)	green mold and blue mold	40
	water- or wax-TBZ, 2000 mg/L (52 °C/3 min dip)	*Penicillium* spp.	41
grapefruit (*Citrus paradisi* Macf.)	TBZ or IMZ, 1000 mg/L (53 °C/2 min dip)	stem-end rot and *Penicillium* rot	42
	TBZ or IMZ, 1500 mg/L (50 °C/3 min dip)	green mold and blue mold	43
grapefruit type (*C. grandis* × *C. paradisi*)	TBZ or IMZ, 1500 mg/L (50 °C/2 min dip)	green mold, blue mold, and gray mold/*Botrytis cinerea*	44
lemons [*Citrus limon* (L.) Burm]	IMZ, 1000 mg/L (52 °C/3 min dip)	unspecified	45
satsumas (*Citrus unshiu* Marc.)	IMZ, 200 mg/L (54 °C/3 min dip)	green mold and *Alternaria* rot/*Alternaria* spp.	46
guava [*Psidium guajava* (L.)]	benomyl, 500−2000 mg/L (48−50 °C/2 min dips); guazatine, 500 mg/L, (50 °C/5 min dip)	anthracnose/*Colletotrichum gloeosporioides* and fruit canker/*Pestalotia psidii*	47
litchi (*Litchi chinensis* Sonn.)	benomyl, 500 mg/L (52 °C/2 min dip) + packaging	various fungi	48
	benomyl, 1000 mg/L (48 °C/1−18 min, 50 °C/1−8 min, 52 °C/ 0.5−4 min dips)	unspecified	49
mango [*Mangifera indica* (L.)]	TBZ or benomyl, 1000 mg/L (54.5 °C/5 min dip)	anthracnose	50
	benomyl, 1000 mg/L (52 °C/1−3 min dip)	anthracnose and diplodia stem end rot	51
	IMZ, 1000 mg/L (53 °C/3 min)	anthracnose	52
	benomyl, 1000 mg/L (52 °C/5 min)	stem end rot/ *Lasiodiplodia theobromae*	53
	prochloraz, 225 mg/L (48−64 °C/ 15−20 s brushing)	*Alternaria* rot/*Alternaria alternate*	54
	benomyl, 850 mg/L (52−55 °C/ 10 min dip)	anthracnose	55
	hot air (core temp 46.5 °C/10 min, relative humidity >95%) + benomyl, 500 mg/L (52 °C/5 min dip) or prochloraz, 250 mL/L (28 °C/30 s dip)	anthracnose	56
	IMZ, 500 mg/L (53 °C/3 min dip)	anthracnose and stem-end rot	57
	bavistin, 1000 mg/L or captan, 2000 mg/L (52 °C/10 min) + packaging	anthracnose and stem-end rot	58
	prochloraz, 250 mg/L or carbendazim, 1000 mg/L (55 °C/5 min dip)	unspecified	59

dx.doi.org/10.1021/jf201899t |*J. Agric. Food Chem.* 2011, 59, 8531–8542

Table 1. Continued

crop	treatment[a]	decay/pathogen	ref
muskmelon [*Cucumis melo* (L.) var. *cantalupensis*]	SDDC, 4000 mg/L; benomyl, 1000 mg/L; IMZ, 1000 mg/L (57 °C/0.5 min dip)	*Fusarium* rot/*Fusarium roseum* and phomopsis rot/*Diaporthe melonis*	60
	SDDC, 4000 mg/L or SOPP, 2500 mg/L (57 °C/0.5 min dip)	*Fusarium* rot and phomopsis rot	61
	IMZ, 1000 mg/L (60 °C/3 min dip) + polyethylene wrapping	unspecified	62
stone fruits			
peaches [*Prunus persica* (L.) Batsch.]	DCNA or benomyl, 100 mg/L (46 or 52 °C/4 min dip)	brown rot and rhizopus rot/*Rhizopus stolonifer*	63
nectarines [*Prunus persica* var. nectarine (Ait.)]	DCNA, 225 mg/L or benomyl 150 mg/L, (52 °C/0.5 min dip)	brown rot and rhizopus rot	64
peaches and nectarines	DCNA, 450 mg/L or benomyl, 100 mg/L (52 °C/10 s).	brown rot and rhizopus rot	65
	benomyl, 100 mg/L, (46 °C/2.5 min)	*Penicillium* rot/*Penicillium* spp., *Alternaria* rot, and gray mold	66
	benomyl, 100 mg/L (46 °C/2.5 min dip)	brown rot	67
sweet cherries [*Prunus avium* (L.)], peaches and nectarines	various fungicides, ~300 mg/L (1/4 of the label rates, 51.5 °C/1.5−2 min dip) or at 1232 mg/L at 15.5 °C	brown rot and *Alternaria* rot	68
taro [*Colocasia esculenta* (L.) Schott]	benomyl, 200 mg/L (50 °C/5 min dip)	unspecified	69
red tamarillos [*Cyphomandra betacea* (Cav.) Sendtner]	hot water (50 °C/8 min dip) followed by 250 mg/L unheated IMZ/1 min dip	*Colletotrichum gloeosporioides*, *C. acutatum*, *Phomopsis* spp., and *Phoma exigua*	70

[a] SDDC, sodiun dimethyldithiocarbamate; SOPP, sodium orthophenylphenate; IMZ, imazalil; TBZ, thiabendazole; DCNA, 2,6-dichloro-4-nitroaniline.

In contrast, TBZ residues were not affected by dip length, but they increased when fungicide rate and treatment temperature increased.[27]

TBZ residues in citrus fruit were reported to be related to pH mixtures, increasing from 0.5 to 1.4 mg/kg when the pH was increased from 7.9 to 10.8,[28] and to increased fungicide concentration and temperature, but were not influenced by treatment time.[27] When TBZ was applied at 50 °C at 100−600 mg/L ai, its residues in lemons were closely correlated with the amount of fungicide applied.[29] TBZ applied at 1200 mg/L and 20 °C produced 3.20 mg/kg residue in fruit, the same level that would have accumulated after treatment with TBZ at ca. 150 mg/L ai and 50 °C (calculated value), that is, 8-fold lower than at ambient temperature.

A 3 min TBZ dip treatment on cactus pear [*Opuntia ficus-indica* (L.) Miller] at 150 mg/L and 52 °C produced lower residue level (1.09 mg/kg) than standard treatment with TBZ at 1000 mg/L at ambient temperature (2.82 mg/kg) but was considerably more effective in controlling decay (approximately 89 versus 64% decay control in comparison to untreated fruit) after 6 weeks of storage at 6 °C plus an additional week of simulated shelf life at 20 °C.[30]

Besides heat, the coapplication of sodium bicarbonate (SBC) significantly increased the efficacy of IMZ[31] and TBZ[32] to control green mold, even when fruits were inoculated with an IMZ-/TBZ-resistant isolate of *P. digitatum*. Similar results have been reported when heated mixtures of TBZ or IMZ were applied in combination with potassium sorbate.[33] IMZ and TBZ residues in fruit were affected by heat but not by the presence of SBC[31,32] or potassium sorbate.[33] Although the residues levels and the degradation rate of TBZ in oranges were unaffected by the coapplication of SBC, the rates of TBZ uptake into cuticular wax significantly increased in the presence of SBC or when TBZ was applied at 50 °C.[34] Both SBC and heat affected green mold decay and similarly enhanced TBZ uptake by cuticular wax, thus increasing the efficiency of TBZ.[34]

Many other studies have been performed on a wide range of diseases and horticultural crops to investigate the combined effects of hot water and fungicides in controlling postharvest decay, although data on residue levels in fruit as a result of heated fungicide application were not reported[35−70] (Table 1). In most cases, treatments with heated fungicides were considerably more effective than fungicides applied at ambient temperature, whereas only occasionally no synergistic or additive effects of heat and fungicide treatments in controlling diseases were recorded.[59,60] The phytotoxic effects recognized on various fruit species subjected to heated fungicides[35,38,39,43,44,48,60] may be ascribed to excessive temperature, prolonged exposure of fruit to heat, or excessive accumulation of residues in fruit.

An important aspect of current fungicide research concerns the inclusion of fungicides into complexes to develop controlled-release (CR) formulations, using polymeric materials as carriers, in an attempt to enhance pesticide performance, increase safety, and extend the shelf life of the active ingredient.[71,72] CR formulations differ from conventional pesticides as the active agent is released gradually from the polymeric materials, resulting in a more constant level of fungicide.[71,72]

Lezcano et al.[73] have demonstrated that the solubility of certain benzimidazole-type fungicides (thiabendazole, carbendazim, and fuberidazole), which are widely employed in pre- or postharvest treatments of horticultural crops,

notably increases after complexation with cyclomaltoheptanose (β-cyclodextrin, βCD).

Accordingly, Schirra et al.[74] showed that the water solubility of IMZ increased after complexation with β-cyclodextrin. Residue level, persistence, and efficacy of βCD−IMZ postharvest treatments in the *Penicillium* decay control of citrus fruit were investigated at 20 and 50 °C in comparison with an IMZ-based commercially available fungicide.[74] When the βCD−IMZ inclusion complex or the commercially available IMZ fungicide was applied to grapefruits at equal rates (250 mg/L) and dip temperatures (20 or 50 °C), IMZ residues in fruit were similar (1.27−1.32 mg/kg at 20 °C and 5.31−5.35 mg/kg at 50 °C). Accordingly, no significant differences were recorded in oranges and lemons after treatments with βCD−IMZ or IMZ at 250 mg/L and 20 °C (2.41−1.06 mg/kg in oranges and 2.04−1.59 mg/kg in lemons). By contrast, when treatments with βCD−IMZ were performed at 50 °C, residues in oranges and lemons were significantly lower and higher, respectively, in comparison with IMZ treatments alone. The degradation rate of ai in grapefruit was not affected by the formulation type or dip temperature, whereas in oranges and lemons IMZ residues were more persistent when the fungicides were applied at 50 °C.[74] Results of this study indicate that the βCD−IMZ complex can be used without loss in performance compared to the conventional fungicide. In addition, use of the βCD−IMZ complex is facilitated because β-cyclodextrin is nontoxic when used in oral or topical formulations and, hence, the βCD−IMZ inclusion complex reduces IMZ exposure to personnel that handle it.

A great deal of attention has been paid in recent years to naturally occurring compounds both as a source of antimicrobials and as starting points for chemical synthesis of active agents. Knowledge of the physicochemical characteristics of certain compounds has prompted the synthesis of broad-spectrum, "natural mimetic" fungicides.[75−77] Among them, azoxystrobin (AZX), trifloxystrobin (TFX), fludoxonil (FLU), pyrimethanil (PYR), and cyprodinil (CYP) are broad-spectrum fungicides with different modes of action, developed in the mid-1990s to control fungal diseases of fruits, vegetables, and ornamental crops.[9,98] All are classified as reduced-risk fungicides by the U.S. Environmental Protection Agency.[10] The potential of these fungicides to control postharvest decay has been investigated on a wide range of diseases and horticultural crops,[10,79−82] and recently AZX, FLU, and PYR were registered in the United States for postharvest treatment of various fruit species.[80,81] In addition, much postharvest research into these fungicides has been devoted to finding the best conditions in terms of ai concentration, treatment time, and temperature so as to enhance fungicide efficacy, reduce the fungicide rate and residues, and minimize their environmental impact.[33,83−85]

AZX and TFX are "natural-like" fungicides belonging to the class of the strobilurin group.[86,87] Their site of action is the fungal mitochondrion, where they bind to the cytochrome bc1 complex, thereby preventing electron transfer and energy production via oxidative phosphorilation. Both AZX and TFX have very favorable eco-toxicological profiles, dissipating rapidly from soils and groundwater and being risk-free to nontarget organisms under field conditions at recommended application rates.[86] The use of AZX is recommended prior to infection or in the early stages of disease development to maximize its potent inhibitory action on spore germination and zoospore motility.[86] The effectiveness of AZX and TFX against various field diseases was established on many horticultural crops.[88−91]

Preharvest AZX applications significantly controlled *Alternaria alternata* in citrus fruit[92] and were effective as a preventive spray against citrus scab (*Elsinoe faucettii*) in potted seedlings of rough lemon (*Citrus jambhiri* Lush) and against melanose caused by *Diaporthe citri* in grapefruit seedlings.[93]

When AZX was applied as a postharvest dip for 3 min at 50 °C, reduced doses of ai (50−100 mg/L) were enough to achieve the complete or almost complete control of decay in 'Star Ruby' grapefruit during simulated cold quarantine treatment for 3 weeks at 2 °C, subsequent standard storage for 5 weeks at 8 °C, and an additional 2 weeks of simulated shelf life at 20 °C.[94] AZX applied at 50−100 mg/L at ambient temperature was less effective than heated AZX, reducing the percentage of rotten fruit to 4.7−6% compared to the control with 16.4% decay. Following treatments with AZX at 25−100 mg/L at 20 or 50 °C, residue levels in grapefruit were positively correlated with the amount of fungicide applied. When equal fungicide doses were applied, treatments at 50 °C produced 63−84% higher residues than those at 20 °C. AZX residues in grapefruit remained unchanged during both cold storage and shelf life. The prolonged stability of AZX residues during cold storage and in darkness was confirmed by further studies on oranges,[95] apples,[96] and peppers (*Capsicum annuum* L.).[97] By contrast, a relatively rapid decline of AZX residues was observed in various crops over the preharvest period.[97,98]

Although AZX was highly effective at reduced rates in controlling natural decay of *Penicillium* spp.,[94] remarkably higher doses of ai (600 mg/L at 20 °C or 300 mg/L at 50 °C) were able to achieve only a modest measure of decay control when AZX was applied on oranges artificially inoculated with *P. digitatum*.[95] The relatively moderate decay control of AZX may be ascribed to a poor systemic property that makes the ai incapable of reaching the active infection sites.[80] AZX applied to oranges at 600 mg/L and 20 °C or at 300 mg/L and 50 °C for 30−60 s produced similar AZX residues in fruit (0.45−0.48 mg/kg at 20 °C and 0.53−0.57 mg/kg at 50 °C) and equally reduced green mold decay.[95]

Unlike AZX, TFX was highly effective in controlling decay caused by green and blue mold in fruit artificially inoculated with *P. digitatum* or *P. italicum*, when applied at 100 mg/L and 50 °C.[99] Following a 3 min dip treatment with TFX at 100 mg/L, residues of TFX in Tarocco orange were significantly affected by dip temperature, doubling when the fungicide mixture containing 100 mg/L TFX was heated from 20 to 50 °C. When the treatment was performed at 20 °C, TFX residues doubled (from 0.15 to 0.30 mg/kg) when the dip time was increased from 0.5 to 3 min, whereas TFX residues were unaffected by dip time when treatments were performed at 50 °C.[99]

PYR is a fungicide belonging to the anilinopyrimidine class that includes cyprodinil and mepanipyrin.[9] PYR inhibits mycelial elongation and the secretion of certain enzymes involved in plant cell wall degradation.[100−102] PYR has little effect on spore germination but effectively inhibits germ tube elongation. The compound is effective against diseases caused by *Botrytis cinerea* and *Monilinia* spp., *Venturia inaequalis*, *P. expansum*, and other pathogens.[75,100−103]

PYR has been shown to be an effective fungicide for postharvest management of citrus green mold, especially to control isolates of *P. digitatum* resistant to the fungicides commonly used in packinghouses such as IMZ, TBZ, and SOPP.[83] Postharvest dip treatment for 30 s or drenching citrus fruit at room temperature in a water mixture containing PYR at 500 mg/L

was more effective in controlling green mold (>90% control) and sporulation than PYR application in wax over rotating brushes at 1000 or 2000 mg/L (about 65% control). Postharvest dip treatments of oranges in water mixtures of PYR (300 mg/L for 30 s) at ambient temperature and of lemons (500 mg/L for 60 s) significantly controlled green mold and resulted in ca. 1−2 mg/kg residues.[83]

PYR residues in oranges and lemons were greatly dependent on treatment temperature, approximately doubling for each 5 °C increase in the mixture above 30 °C. The addition of sodium bicarbonate[83] or potassium sorbate[33] improved the performance of PYR, whereas the influence of heat was irregular and small, although PYR residues in fruit were greatly increased by heat.[83] A moderate effect of heat on PYR efficacy was found by Schirra et al.[95] The lack or modest effect of heat on PYR efficacy may be ascribed to the relatively short treatment durations (30−60 s) employed by Smilanick et al.[83] and Schirra et al.,[95] which were not long enough to achieve the beneficial effects of heat.[34,104] Indeed, when longer times were used (180 s), the efficacy of PYR increased dramatically as treatments with PYR at 100 mg/L at 50 °C produced more residues (3.98 mg/kg) than treatment with PYR at 400 mg/L and 20 °C (0.84 mg/kg), being equally or more effective in controlling decay.[105] Residue levels of PYR in oranges were significantly correlated to fungicide dosage and dip temperature. When equal amounts of the fungicide were used, treatment at 50 °C produced ca. 13−19 times more residues than that at 20 °C.[105]

The broad-spectrum activity of CYP against major fungal diseases of grains, grapes, vegetable crops, and deciduous fruits has been well documented in field trials.[78] Preharvest applications of CYP significantly reduced gray mold development and lesion diameter on inoculated apples stored for 6 months, whereas postharvest application of CYP protected apples from gray mold for 3 months.[103] CYP was also proven to be effective on apples against isolates of *P. expansum* resistant to TBZ.[106]

The interrelationships of residues and the effectiveness of CYP in controlling green mold caused by *P. digitatum* were investigated in 'Valencia' oranges as a function of fungicide concentration, temperature, and treatment duration.[107] As found for other fungicides, the combination of CYP with heat produced higher residues, a finding depending on treatment duration, and improved control of green mold decay. In addition, CYP residues and efficacy in controlling decay generally increased by increasing fungicide dose and treatment duration. When CYP was applied to oranges at 50 °C at 50 mg/L for 30, 90, or 180 s, its residues averaged 1.36, 2.63, or 3.18 mg/kg, whereas treatments at 150 mg/L produced 1.58, 3.47, or 6 mg/kg residues, respectively.

FLU is a synthetic analogue of pyrrolnitrin belonging to the class of phenylpyrroles, developed for the chemical control of *Botrytis cinerea* in viticulture.[77,100] Kanetis et al.[108] showed that the mode of action of FLU in *P. digitatum* may be ascribed to the mitogen-activated protein kinase pathway that stimulates glycerol synthesis in isolates sensitive and moderately resistant to FLU. It inhibits spore germination, germ tube elongation, and mycelium growth of *B. cinerea* and induces morphological alterations of germ-tubes.[77,100]

FLU was found to be a valuable fungicide to control the main postharvest diseases on a wide range of fruits. When applied on mangos (*Mangifera indica* L.) as dip treatment for 30 s at 600 mg/L either at ambient temperature or at 50 °C, it effectively reduced the development of stem-end rot and soft brown rot, whereas anthracnose was better controlled by heated FLU.[109] FLU applied on pomegranate (*Punica granatum* L.) as a 30 s dip significantly reduced botrytis crown rot decay, sporulation of *B. cinerea*, and internal gray mold after long-term storage (15 weeks at 7.2 °C) when fungicide mixtures were applied at 600 mg/L and 49 °C.[110] Further studies revealed that FLU effectively reduced decay in pomegranates during 3 weeks of storage when applied at 150 mg/L and 50 °C, but was less effective against heart rot.[111] FLU residues in fruit were correlated to applied fungicide rate and treatment temperature. The application of FLU at the highest rates (600 mg/L at 20 °C or 150 mg/L at 50 °C) effectively controlled decay in pomegranates and produced 1.1−1.3 mg/kg residues on fruit, which is far less than the maximum residue limit set in the United States for pomegranates (5 mg/kg).

When applied to apples, FLU at 100 mg/L effectively controlled blue mold caused by TBZ-sensitive and -resistant isolates of *P. expansum*.[112] At 300 mg/L it completely suppressed TBZ-resistant and -sensitive strains of *P. expansum* in apples stored at 2 °C for 105 days under controlled atmosphere or standard storage at 4 °C for 42 days, whereas higher doses of FLU (450 mg/L) were required to achieve complete control of decay during the further 6 days of simulated shelf life at 20 °C.[113]

In 'Valencia' oranges, FLU applied at 500−1200 mg/L by a simulated commercial drench system before fruit ethylene degreening or by a simulated commercial packingline system before or after fruit ethylene degreening reduced the incidence of diploda stem-end rot caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maublinc.[84] FLU at 500−2000 mg/L significantly reduced green mold incidence, and its efficacy was similar to that of IMZ or TBZ at 1000 mg/L. FLU effectively reduced the incidence of green mold caused by TBZ-resistant and -sensitive *P. digitatum* isolates on 'Pineapple' oranges.[84]

When applied to oranges, the lowest threshold of FLU residue capable of controlling natural decay caused by *Penicillium* spp. was ca. 0.8 mg/kg and was achieved by a 3 min dip treatment with 400 mg/L FLU at 20 °C or with 100 mg/L FLU at 50 °C.[114] When equal fungicide rates were employed, the FLU residues were notably higher (2.6−4-fold) in fruit treated at 50 °C than in fruit treated at 20 °C. Conversely, a moderate increase of FLU residues was recorded by Smilanick et al.[33] in 'Valencia' oranges when the temperature was raised from 25 to 50 °C. In addition, when FLU was applied on 'Valencia' oranges artificially inoculated with *P. digitatum* at 300 mg/L, it did not effectively control green mold and the influence of heat on FLU efficacy was small.[33] Similar results were found on 'Salustiana' oranges inoculated with *P. digitatum* before treatment with 600 mg/L and 20 °C or with 300 mg/L and 50 °C. However, the treatment durations used by Smilanick et al.[33] and Schirra et al.[95] were relatively short (30−60 s), being the same as that employed commercially in many locations in the United States, whereas much longer periods (180 s) used in previous trials[114] resulted in greater effect of heat on FLU efficacy and residue levels.

FLU at 100 mg/L and 20 °C or sy 25 mg/L and 48 °C for 2 min completely or almost completely controlled decay caused mainly by *M. laxa*, *B. cinerea*, and *Rhizopus stolonifer* in 'May Grand' nectarines, 'Glo Haven', Red Top', and 'Sun Crest' peaches, and 'Pelese' and 'Fracasso' apricots during 1 week of storage at 5 °C and effectively reduced decay after an additional week of simulated shelf life at 18 °C.[115] FLU residues were significantly correlated to fungicide rates and dip temperatures and were dependent on fruit species. Residue levels in fruit after

treatment with 100 mg/L FLU at 20 °C or with 25 mg/L FLU at 48 °C averaged approximately 0.6−2 mg/kg and showed great persistence during storage and subsequent shelf life. After FLU treatment at 200 mg/L and 20 °C, its residues in 'Venus' nectarines did not change significantly when treatment duration was increased from 1 to 3 min but increased significantly when dip time was extended to 6 min, accounting for 0.65−0.73 and 1.29 mg/kg, respectively.[115] When FLU was applied at 48 °C, its residues increased significantly (1.23, 1.82, and 2.94 mg/kg) by increasing treatment duration from 1 to 3 and 6 min, respectively. FLU treatment at 48 °C for 1 min produced residues (1.23 mg/kg) similar to those with treatment at 20 °C for 6 min (1.29 mg/kg). Investigations performed by Förster et al.[81] showed that postharvest applications of FLU were highly effective in controlling brown rot and gray mold decay of peach, nectarine, and plum caused by *M. fructicola* and *B. cinerea*, respectively. When FLU was applied up to 14−16 h after inoculation, it was significantly more effective than when applied before inoculation, demonstrating its nonsystemic activity,[81] a finding in agreement with studies on citrus fruit.[80]

FLU was also tested for efficacy against postharvest blue mold and gray mold respectively caused by *P. expansum* and *B. cinerea* on artificially inoculated 'Precoce di Fiorano', 'Coscia', and 'Spadona estiva' summer pears.[116] Results showed that relatively low residues (from 1.27 to 2.68 mg/kg, depending on cultivar and treatment type) were needed to control gray mold (90−100%) and blue mold (84−100%) over 12 or 8 days of incubation at 20 °C and were achieved with FLU treatments at 300 mg/L and 20 °C or at 100 mg/L and 50 °C for 2 min. After applications at 300 mg/L and 20 °C or 100 mg/L and 50 °C, FLU residues ranged from 1.24 to 1.33 mg/kg and from 1.63 to 2.32 mg/kg, respectively. Despite the lower residues recorded in 'Precoce di Fiorano' after FLU treatment at 60 °C for 1 min with respect to those at 50 °C for 2 min (0.98 versus 1.20 mg/kg), its efficacy in controlling gray mold was similar. However, FLU treatment at 60 °C for 1 min cannot be applied to summer pear cultivars because of the detrimental effects it has on fruit quality.

Further studies on 'Coscia' pears revealed that FLU residues considerably increased when the fungicide was applied at 300 mg/L and 20 °C and treatment duration rose from 1 to 2 min (1.57 versus 2.68 mg/kg respectively), although no further significant increase (2.94 mg/kg) was found when dip time was extended to 4 min.[117] FLU at 100 mg/L and 50 °C for 1−2 min produced similar residues in fruit (2.25−2.32 mg/kg), whereas consistently higher residues (3.52 mg/kg) were detected when the dip time was increased from 1 to 4 min. The presence of soy lecithin significantly reduced FLU residues (2−3-fold less in most samples) with respect to fruit treated with FLU alone without affecting the efficacy of FLU when applied at 300 mg/L and 20 °C or at 100 mg/FLU and 50 °C.[117]

Investigations on oranges[95] have shown that the degradation rate of AZX, FLU, and PYR was generally slow, depending on fungicide type and such conditions as treatment duration, temperature, and the presence or not of other fungicides.

■ IMPACT OF HEAT AND FUNGICIDE TREATMENTS ON PHYSICAL CHANGES OF EPICUTICULAR WAX AND FRUIT TOLERANCE TO CHILLING INJURY

The sorption capacity of epicuticular wax is usually low for most agricultural chemicals, being affected by wax composition, permeability of cuticles, and ai diffusion through the plant cuticle.[118] Investigations on cuticular membranes isolated from pear (*Pyrus communis*) and bitter orange (*Citrus aurantium*) leaves have shown how the rate of diffusion and penetration of organic chemicals into leaves and fruits notably increase with increasing temperature.[119] Thus, when treatments are performed with heated fungicides, considerably lower ai concentrations are required to achieve comparable residue level left by standard treatments at room temperature.

Cracks or other breaks in the surface of the cuticle may have an important effect on uptake of chemicals.[119−121] Cracks are usually present in the cuticle of mature fruit,[122−128] and crack number and width increase during fruit development, depending on growing conditions[122,123,125] and climate.[124]

Studies on apples have shown that such cracks became wider and deeper during long-term storage, and these increases in cracking paralleled the increases in calcium uptake by fruit subjected to calcium chloride pressure infiltration.[126,128] Following postharvest heat treatment at 38 °C for 4 days, the cuticular cracks disappeared in apples, probably as a result of the "melting" of the wax platelets that had occurred in the cracks. Similar changes in epicuticular wax ultrastructure have been observed on other fruit species subjected to heat treatments.[129−134]

Studies on 'Tarocco' oranges showed that fruit harvested in April (late season) and treated with 1200 mg/L TBZ at room temperature contained significantly more residues (6.50 mg/kg) than fruit picked in December−March (3.40−4.62 mg/kg).[135] The higher TBZ levels in fruit harvested in April were ascribed to the presence of gaps in the epicuticular wax of the more mature fruit, which favored the penetration of the chemical. However, when treatment was performed at 50 °C, the uptake of TBZ was not significantly dependent on fruit age.[135] When TBZ was applied at 50 °C, reduced rates (200 mg/L) of fungicide were required to supply equal residue concentrations in fruit compared to that of standard treatment at 20 °C. Therefore, the enhanced mobility and penetration through the epicuticular wax and "rearrangement" of cuticular wax structure induced by heat treatment play a more important role in fungicide uptake than the presence of fractures in the cuticle. The greater persistence of TBZ in fruit treated at 50 °C with respect to fruit treated at room temperature was related to the better encapsulation and coverage of the ai by epicuticular wax, thus providing the chemical with better protection.

Chilling injury (CI) is a major postharvest physiological disorder that develops when freshly harvested tropical and subtropical fruits are stored at temperatures below 10−12 °C but nonfreezing.[136] CI may cause significant destruction of fruit quality and a concomitant economical loss. Various methods for controlling or alleviating CI have been developed, including temperature conditioning, intermittent warming, storage under controlled or modified atmosphere, treatments with plant growth regulators, and/or other chemicals including calcium, antioxidants, free radical scavengers, and fungicides.[136] The fungicides TBZ and benomyl effectively reduced CI in grapefruit and oranges.[137,138] TBZ effectiveness in controlling CI increased when its concentration was increased from 0.04 to 2% and residues in the peel rose (from 3.2 to 48.4 mg/kg), whereas the effect of benomyl did not change with fungicide level.[138] Further studies showed that the effectiveness of these fungicides in CI control was greatly enhanced when they were used in combination with hot water.[43,135,139,140] These studies with heated and unheated TBZ mixtures were performed by using equal ai concentrations. The increased efficacy of heated chemicals may thus be ascribed to the higher residue accumulation in fruit.

On the other hand, it was shown that TBZ effectiveness in controlling CI was greatly improved by heat, and the improvement was not correlated with higher fungicide residue accumulation in the fruit.[135] In fact, whereas standard treatments with 1200 mg/L TBZ at room temperature or with 200 mg/L TBZ at 50 °C produced similar TBZ residues in 'Tarocco' oranges, treatment at 50 °C was more effective in reducing CI.[135] These results were corroborated by later studies on 'Star Ruby' grapefruit[140] and cactus pear fruit.[30] Thus far, the mode of action of TBZ in alleviating CI has been correlated with the reduced rate of peel senescence and/or inactivation of latent infections, which could weaken cell walls and make fruit more prone to CI.[137] However, studies on 'Tarocco' oranges[135] and 'Star Ruby' grapefruit[140] provided evidence that no relationships were found between CI and decay, the expression of CI being maximum when susceptibility to decay was minimum. Further studies have demonstrated that CI susceptibility of harvested citrus fruit is related to the efficiency of certain antioxidant enzyme systems in flavedo tissue[141] and that heat treatment affects the flavedo tissue's antioxidant enzyme properties and induces CI tolerance.[142,143] However, the mechanism of action of TBZ in alleviating CI stress has yet to be elucidated.

Thus far, postharvest heat treatments have proved capable of reducing rot development and enhancing fruit resistance to chilling injury in sensitive cultivars while retaining fruit quality during cold storage and shelf life.[9−11,15,16] Indeed, they are currently being applied to various horticultural crops on a commercial scale in several countries and also to meet consumer demand for "organic" crops. Despite heat therapy's beneficial effects, fungicides continue to play a key role in postharvest management of horticultural crops and cannot at present be completely replaced by other, alternative methods.

In recent years, low-risk broad-spectrum fungicides with a diverse mechanism of action compared to other approved pesticides in a crop group have become available on the market. These compounds were introduced also to cope with the problems due to strains resistant to "older" fungicides. These chemicals have proved to be very effective in decay control, and reduced rates of ai are required for complete suppression of pathogens when fungicide mixtures are applied as warm mixtures thanks to the synergistic or additive action of heat, enhanced active ingredient uptake, better encapsulation, and diffusion of the ai in the cuticular wax. During this process, the cuticle behaves as a solution-diffusion membrane: the diffusion rate increases with temperature; hence, the amount of fungicide uptake in the fruit increases. Thus, the combination of hot water and fungicides provides the opportunity of reducing the amounts of chemicals discharged in the packinghouse wastewater after treatments. Yet, as resistance to newer fungicides by disease-causing pathogens of many crops in different countries has been already reported,[144,145] the exclusive application of these materials should be avoided in sustainable management of postharvest decay. Proper strategies such as integrating fungicide application and hot water and alternate use of fungicides with different modes of action, along with effective sanitation practices within packinghouses,[144,145] may reduce the possibility of selecting fungicide-resistant populations of pathogens.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mario.schirra@ispa.cnr.it.

Funding Sources

Research supported by MiPAF, Project "Ricerche e Sperimentazioni Avanzate in Agrumicoltura e loro Applicazioni" (RAVAGRU), Paper 45.

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