Influence of treatment time and soy lecithin co-application on residue levels and efficacy of fludioxonil in controlling Penicillium expansum and Botrytis cinerea of inoculated 'Coscia' pear [Pyrus communis L.] fruit

Mario Schirra, Salvatore d'Aquino, Quirico Migheli, Filippo Pirisi, Alberto Angioni

Influence of treatment time and soy lecithin co-application on residue levels and efficacy of fludioxonil in controlling Penicillium expansum and Botrytis cinerea of inoculated 'Coscia' pear [Pyrus communis L.] fruit

Journal:	*Food Additives and Contaminants*
Manuscript ID:	TFAC-2008-096.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	15-Jul-2008
Complete List of Authors:	Schirra, Mario; Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari D'Aquino, Salvatore; Consiglio Nazionale delle Ricerche, Istituto di Scienze delle Produzioni Alimentari Migheli, Quirico; University of Sassari, Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi and Dipartimento di Protezione delle Piante Pirisi, Filippo; University of Cagliari, Department of Toxicology Angioni, Alberto; University of Cagliari, Department of Toxicology
Methods/Techniques:	GC, Microbiology
Additives/Contaminants:	Additives general, Pesticide residues
Food Types:	Fruit

Influence of postharvest treatments with fludioxonil and soy lecithin co-application in controlling blue and grey mold and fludioxonil residues in Coscia pears

Mario Schirra[1*], Salvatore D'Aquino[1], Quirico Migheli[2], Filippo M. Pirisi[3] & Alberto Angioni[3]

[1]*C.N.R. Istituto di Scienze delle Produzioni Alimentari. Via dei Mille 48, 07100 Sassari, Italy.*

[2]*Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi* and *Dipartimento di Protezione delle Piante - Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy;*

[3]*Dipartimento di Tossicologia, Università di Cagliari. Via Ospedale 72, 09124 Cagliari, Italy.*

*Author for correspondence: Tel: 39.0783.33224; Fax: 39.0783.33959; E-mail: mario.schirra@ispa.cnr.it

Abstract

The residue levels of fludioxonil (FLU) were determined in Coscia pear following 1, 2 or 4 min dip in an aqueous mixture of FLU containing 300 or 100 mg l^{-1}, (active ingredient, a.i.) at 20 and 50°C, respectively, with or without 2% soy lecithin. The efficacy of heat treatment with water and FLU mixtures was investigated on artificially inoculated pears for the control of postharvest decay caused by blue (*Penicillium expansum* Link) and grey (*Botrytis cinerea* Pers. ex Fr.) mold. Treatment with 300 mg l^{-1} FLU at 20°C increased residues significantly when treatment time rose from 1 to 2 min; no further increase was recorded when dip time raised from 2 to 4 min. FLU residue rates were unaffected by treatment time when 300 mg l^{-1} a.i. was applied in combination with lecithin at 20°C. While treatment with 100 mg l^{-1} a.i. at 50°C for 1 and 2 min resulted in similar residue levels, significantly higher residues were detected when dip time increased from 1 to 4 min. Co-application of lecithin significantly decreased FLU residues with respect to fruit treated with FLU alone. Treatments with FLU at 20 or 50°C effectively controlled decay over 10 days of incubation. While co-application of lecithin did not affect the efficacy of FLU at 300 mg $l^{-1}$and 20°C, treatment efficacy decreased when lecithin was applied in combination with 100 mg l^{-1} FLU and 50°C for 4 min and to a greater extent when dip time was 1-2 min.

Keywords: *Pyrus communis* L, disease control, hot water dip, fludioxonil, residues, lecithin.

Running title: Decay control on pears by fludioxonil and lecithin

Introduction

The post-harvest life of summer pear cultivars is generally shorter than that of autumn and winter pears (Bell 1996). Under shelf-life conditions summer pears soften rapidly, develop storage disorders that spoil their appearance and texture and become susceptible to decay caused by various pathogens, especially blue (*Penicillium expansum*) and grey (*Botrytis cinerea*) mold. Pre- and/or postharvest treatments and cold storage are therefore needed to delay senescence and to control postharvest decay.

Certain edible coatings have been reported as effective in preventing deteriorative changes and prolonging postharvest life when applied to fresh fruits and vegetables. Lysophosphatidylethanolamine (LPE) phospholipid, for example, which is present in egg yolk and soy lecithin, has beneficial effects on plants or parts thereof by protecting against stress-related injuries, improving keeping quality during storage and shelf-life and delaying senescence (Farag and Palta 1993; Ozgen and Palta 2003). Beneficial effects such as reducing internal breakdown of 'Granny Smith' apples (Watkins et al. 1988), delaying or preventing the formation of blemishes or micro-lesions brought about by physical and/or chemical treatments (Sardo 2004), improving turgidity, colour and flavour of fruit and vegetables and reducing fruit cracking (Rowley et al. 2004) have been observed following treatment with lecithins and/or their derivatives. However, as lecithin has no antifungal activity, co-application of a fungicide is needed to control postharvest decay.

In the pear packing-house, immersion dumping in a water mixture containing a flotation agent and disinfectants is common practice for removing fruit gently from field bins (Sugar and Basile 2005), whilst current control of storage decay is based on postharvest drench or line spray application of thiabendazole (TBZ) (Kupferman et al. 1995).

Several novel fungicides have been developed in recent years for horticultural crops to cope with the onset of fungal populations that have developed resistance to 'older' fungicides (Gullino et al. 2000). Of these, fludioxonil (FLU) is a broad-spectrum fungicide with a diverse mode of action compared to previously registered chemicals. FLU is classified as a 'reduced risk' fungicide by the Environmental Protection Agency of the United States and was registered there in 2004 for postharvest treatment of various fruit species, including pear (Förster et al. 2007).

FLU's potential for managing postharvest decay in various fruit species is well documented (Errampalli et al. 2005; Schirra et al. 2005; Zhang 2007; Kanetis et al. 2007; Förster et al. 2007). Errampalli et al. (2007) investigated its efficacy in postharvest management of blue and grey mold of pear during cold and controlled-atmosphere storage.

Previous studies on citrus fruit (Schirra et al. 2005) and stone fruits (D'Aquino et al. 2007) have shown that FLU activity increased when is applied in combination with hot water, and lower rates of active ingredient were needed with respect to standard treatment performed at ambient temperature, to achieve a comparable control of decay. Results reported herein provide information on the influence of treatment time and soy lecithin co-application on residue levels and the effectiveness of fludioxonil in Coscia pears following dip treatment in an aqueous mixture containing soy lecithin and FLU at ambient temperature or reduced rates of FLU at 50°C. The effectiveness of heat treatments with water, and fludioxonil applied separately or in combination with soy lecithin, was investigated on artificially inoculated fruits for the control of postharvest blue and gray mold.

Material and Methods

Fruit samples

Commercially mature 'Coscia' pear (*Pyrus communis* L.) fruit were hand-harvested from trees which had received standard horticultural practices, from an orchard located in the north-west Sardinia (Italy). Fruits were placed in plastic trays and delivered to the laboratory immediately after harvest. Then, fruit were selected, returned to each box and left overnight at 20°C.

Chemicals

The fungicide used was a commercial formulation of FLU (SAVIOR FLOWABLE 20, Syngenta, USA) containing 20% active ingredient (a.i.). A commercial formulation (Xedabio®) containing soy lecithin (lecithin) was obtained from CEDAX s.r.l., Italy.

Fludioxonil residues in fruit

To determine the influence of treatment time and lecithin co-application on FLU residue levels in fruit, pear samples were dipped for 1, 2 or 4 min in water mixtures containing FLU at 300 mg l^{-1} and 20°C with or without 2% lecithin or FLU at 100 mg l^{-1} and 50°C with or without 2% lecithin. Each treatment was replicated four times. After treatments, fruits were allowed to dry and stored for 14 days at 17°C and 80% relative humidity (simulated shelf-life conditions). Analyses were performed following treatment and after shelf-life.

FLU residue analysis

Chemicals

Acetone and hexane were of GC grade (Merck, Milan, Italy). Sodium chloride was analytical grade (Carlo Erba, Milan, Italy). The a.i. standard FLU (at 95%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock standard solution of the a.i. (500 mgl^{-1})

was prepared in acetone. Working standard solutions of a.i. were prepared by diluting the stock solution with the extract from untreated fruit.

Extraction procedure and FLU analysis

A total of twelve of the halves, each from different fruit from those in a replicate, were stored in a freezer at -20°C until analysis. Then, frozen samples were chopped and homogenized using a semi-industrial blender (Malavasi, Bo, Italy) and 5 g of the material was placed in a 40-ml capacity flask with 10 ml of acetone/hexane (1/1) and 6 g of NaCl. The flasks were shaken in a rotary shaker for 20 min. Subsequently, the phases were allowed to separate and the organic layer was injected into a gas chromatograph for the analysis, without any clean-up step. Residue analysis of FLU was performed by a TQ Trace Gas Chromatographer, coupled with a NPD 80 detector, a split/splitless injector, and a AS200 auto sampler (Termo Quest, Milan, Italy) as described in a previous paper (Schirra et al. 2005).

Recovery assays

Samples of untreated pears were fortified with appropriate volumes of stock standard solutions to reach concentrations of 0.1, 1.5, and 2.0 mg kg^{-1}. The samples were allowed to settle for 30 min prior to extraction and then processed according to the above procedure. Average recovery from four replicates showed values of FLU ranging from 97 to 108% with a maximum coefficient of variation of 11%. All analyses were performed on 12 fruits per replicate. Residues were reported as mg kg^{-1} of the fresh weight of the fruit.

Fruit inoculation and treatments

Pears were disinfected by immersion for 1 min in diluted household bleach (1% sodium hypochlorite), rinsed with tap water by 30 s dips followed by 30 s shower and allowed to

dry at room temperature. Fruit samples were subdivided into two groups and each fruit was punctured twice equatorially at the opposite sites, and inoculated with a mixture of $1 \cdot 10^4$ conidia ml^{-1} *P. expansum* or $1 \cdot 10^5$ conidia ml^{-1} *B. cinerea* isolates, respectively. After inoculation, fruit were incubated for 20 h at 20°C before treatment. The treatments were carried out approximately 20 h after fruit inoculation. Pears were subjected to dip treatments for 1, 2 or 4 min with: (1) water at 20°C (control fruit); (2) water plus lecithin at 20°C; (3); FLU at 300 mg l^{-1} and 20°C; (4); FLU at 300 mg^{-1} and 20°C plus lecithin; (5) water at 50°C; (6) water plus lecithin at 50°C; (7) FLU at 100 mg l^{-1} and 50°C; (8) FLU at 100 mg l^{-1} and 50°C plus lecithin. Lecithin was always applied at 2% (wt vol^{-1}).. Each treatment was carried out in triplicate (three replicates of 30 fruit each) and the experiment was repeated two times. Fruits were evaluated for incidence of decay caused by blue or gray mold (percentage of fruit with any size of decay lesions of the total number of fruit) after 7 and 10 days of incubation at 20°C and 95% relative humidity.

Statistical analysis

Statistical analysis was performed by Statgraphics software (Manugistics, version 5 Professional, 2000) statistical program. Analysis of variance (ANOVA) was carried out using a unifactorial complete randomized block design. Percentages were not transformed or transformed in arcsin$\sqrt{x}$ or $\sqrt{x}$ before the ANOVA, depending on the range of variation of data (Gomez and Gomez 1984). Mean comparisons of the effects of treatments were calculated by the Fisher's least significant difference test at $P \leq 0.05$.

Results and discussion

FLU residue level in pear fruit treated for 1 min with 300 mg l^{-1} at 20°C was 1.57 mg l^{-1}. When treatment time was increased from 1 to 2 min, FLU residues significantly increased (ca 71%); no significant changes were recorded when dip time went from 2 to 4 min.

Treatment with 100 mg l^{-1} a.i. at 50°C did not significantly change residue rates when dip time was increased from 1 to 2 min but showed significantly higher values (> 98%) when dip time increased from 1 to 4 min. While co-application of 300 mg l^{-1} a.i. and lecithin at 20°C did not affect fruit FLU residue rates as a function of treatment time, treatment with 100 mg l^{-1} FLU and lecithin at 50°C increased residue levels when dip time rose from 1 to 2 min; no further increase was found when dip time went from 2 to 4 min.

In the present study the highest levels of FLU residues (3.52 mg kg^{-1}) were found in fruit treated with 100 mg l^{-1} FLU at 50°C and were below the maximum residue limit set in the USA (5.0 mg kg^{-1}) for apples and pears**.**

Trials on oranges (Schirra et al., 2005) have shown that 3-min dip treatment with 150 mg l^{-1} FLU at 50°C produced a residue level approximately 2.5-fold higher than treatment at 20°C. In apricots, FLU residue levels at 48°C were *ca.* 0.5-2-fold higher than that of treatments at 20°C, whereas in peaches and in nectarines, FLU residues after dipping at 48°C were 2.6-6.4-fold higher than those left at 20°C. The amount of fungicide deposition as a function of treatment time and temperature is thus dependent on fruit species.

Various factors are known to affect fungicide uptake in fruit, including the method of application such as aqueous or wax-based mixture (see reviews by Dezman et al. 1986; Papadopoulou-Mourkidou 1991). Our study shows that when the fungicide was applied in combination with lecithin, residue levels in fruit were lower than residues left by FLU treatment applied alone, with decreases averaging from 18 to 52% at 20°C and from 51 to 67% at 50°C.

All inoculated wounds of control fruit were infected by *P. expansum* after 7 days of incubation (Table 2). *B. cinerea* infections were much less aggressive than *P. expansum*, accounting for an incidence of grey mold in untreated fruit of 43.3%. Water dip at 50°C for 1-2 min resulted in 27-31% less blue mold decay, while the 4-min dip yielded 90% less

decay than in control fruit. While the incidence of lesions caused by grey mold was unaffected by water dip at 50°C for 1 min, it was significantly reduced when dip time was 2 and 4 min. After 10 days of incubation the benefit of hot water dip for 1-2 min in controlling blue mold was lost and notably reduced when dip time was 4 min. While water dip for 1 min had a slight but significant effect against grey mold, it was more effective when fruits were submerged for 2 and 4 min. Best results in controlling decay were achieved with 300 mg l^{-1} FLU at 20°C, the effect being suppressive and long-lasting. Treatment with 100 mg l^{-1} FLU at 50°C was equally effective in controlling blue and grey mold decay, indicating the positive synergistic effects of heat treatment and FLU. These results corroborate previous findings on various fruit species (Schirra et al. 2005; D'Aquino et al. 2007; Palou et al. 2007).

The sorption ability of cuticular wax is generally low for most agricultural chemicals being affected by wax composition (Jenks and Ashworth 1999), permeability of cuticles and active ingredient diffusion through the plant cuticle (Riederer and Schreiber 1995) and increases with increasing temperature (Schönherr and Baurr 1996). For this reason, when treatments are performed with heated fungicides lower concentrations are required to supply equal residue levels and effectiveness with respect to treatments performed at ambient temperature (Schirra et al. 2000). Although reduced treatment time would be desirable so as to increase packinghouse output and shorten delays in fruit marketing, treatment duration should be long enough to produce the heat-induced beneficial effects in terms of physical changes of epicuticular wax, host defensive responses and inhibition of pathogen development (Schirra et al. 2000).

It is recognized that the co-aplication of coatings such as waxes reduces the effectiveness of certain fungicides (Dezman et al. 1986; Eckert and Eaks 1989). When imazalil (IMZ) is applied in water, for example, it controls green mold significantly better

than when it is applied in wax mixture (Brown et al. 1983). This because part of the a.i. is not available, being immobilised in the wax, and because the wax, being more viscous than water, penetrates less effectively into the wound-infection that are exploited by *P. digitatum* on the rind of citrus fruit. Accordingly, pyrimethanil was found to be more effective in aqueous suspensions than when suspended in fruit waxes (Smilanick et al. 2006). Similar results are reported by Kanetis et al. (2007), who applied the new fungicides, azoxystrobin, fludioxonil and pyrimethanil as well as imazalil and thiabendazole. Thus, to achieve a comparable effect to that in water mixture, the fungicide concentration should be increased (Eckert and Eaks 1989). Our results show that the efficacy of FLU was not compromised with co-aplication of lecithin at 300 mg l^{-1} and 20°C but significantly decreased with 100 mg l^{-1} FLU at 50°C for 4 min and to a greater extent for 1-2 min, respectively, due to the reduced levels of residues left by these treatments. Thus, to compensate for the reduced residues caused by lecithin co-treatment, higher FLU rates should be applied to achieve similar control of grey and blue mold decay.

Acknowledgements

The authors gratefully acknowledge the Syngenta Crop Protection, Italy and the CEDAX s.r.l., Italy, for kindly supplying the fungicide Fludioxonil and the soy lecithin, Xedabio® respectively. Research supported by MiPAF, 'Sviluppo delle Esportazioni di Prodotti Agroalimentari del Mezzogiorno'

.

REFERENCES

Bell RL, Quamme HA, Layne REC, Skirvin RM. 1996. Pears. In: Fruit Breeding Vol 1 Tree and Tropical Fruits. Janick J., Moore JN (Eds.). Chapter 8, p. 441-514. John Wiley & Sons Inc.

Brown GE, Nagy S, Maraulja M. 1983. Residues from postharvest nonrecovery spray application of imazalil to oranges and effects on green mold caused by *Penicillium digitatum*. Plant Disease 67:954-957.

D'Aquino S, Schirra M, Palma A, Tedde M, Angioni A, Garau A, Cabras P. 2007. Residue levels and storage responses of nectarines, apricots and peaches after dip treatments with fludioxonil fungicide mixtures. Journal of Agricultural and Food Chemistry 55:825-831.

Dezman DJ, Nagy S, Brown GE. 1986. Postharvest fungal decay control chemicals: Treatments and residues in citrus fruits. Residue Reviews 97:37-92.

Eckert JW, Eaks IL. 1989. Postharvest Disorders and Diseases of Citrus Fruits. In: The Citrus Industry, University California Press, Berkeley, Reuther W, Calavan EC, Carman G.E. (Eds), vol. 4. p. 179–260.

Errampalli D, Northover J, Skog L, Brubacher NR, Collucci CA. 2005. Control of blue mold (*Penicillium expansum*) by fludioxonil in apples (cv Empire) under controlled atmosphere and cold storage conditions. Pest Management Science 61:591-596.

Errampalli D, Wainman LI, Chu CL. 2007. Evaluation of fludioxonil for the control of post-harvest gray mould and blue mold in pears in cold and controlled atmosphere storages. International Journal Pest Management 53:101-109.

Farag K, Palta JP. 1993. Use of Lysophosphatidylethanolamine, a natural lipid, to retard tomato leaf and fruit senescence. Physiologia Plantarum 87:515-524.

Förster H, Driever GF, Thompson DC, Adaskaveg JE. 2007. Postharvest decay management for stone fruit crops in California using the "reduced-risk" fungicides fludioxonil and fenhexamid. Plant Disease 91:209-215.

Gomez KA, Gomez AA. 1984. Statistical procedures for agricultural research. Wiley Sons Publ. New York.

Gullino ML, Leroux P, Smith CM. 2000. Use and challenges of novel compounds for plant disease control. Crop Protection 19:1-11.

Jenks MA, Ashworth EN. 1999. Plant epicuticular waxes. Function, production and genetics. Horticultural Reviews 23:1-68.

Kanetis L, Förster H, Adaskaveg JE. 2007. Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil and pyrimethanil for managing citrus green mold. Plant Disease 91:1502-1511.

Kupferman E., Spotts R., Sugar D. 1995. Practices to reduce postharvest pear diseases. Tree Fruit Postharvest Journal 6: 18-23.

Ozgen M, Palta JP. 2003. Use of lysophosphatidylethanolamine (LPE), a natural lipid, to accelerate ripening and enhance shelf life of cranberry fruit. Acta Horticulturae 628:141-146.

Palou L, Crisosto CH, Garner D. 2007. Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of 'Wonderful' pomegranates. Postharvest Biology & Technology 43:133-142.

Papadopoulou-Mourkidou E. 1991. Postharvest-applied agrochemicals and their residues in fresh fruits and vegetables. Journal of the Association of Official Analytical Chemists 74:745-765.

Riederer M, Schreiber L. 1995. Waxes. The transport barriers of plant cuticles. In: Waxes: chemistry, molecular biology and functions; Hamilton, R.J. (Ed.). The Oily Press, Dundee, Scotland, Vol. 6, p. 131- 156.

Rowley K, Jeong SW, Cowan K, Altwies J, Trimmer M, Brar G, Ozgen M, Palta JP. 2004. Methods for treating plants and part plants. WO 2004/062364 A2.

Sardo A. 2004. Method for processing fruits and vegetables on the base of lecithin. Xeda International [FR] WO/2004/091301. Int. Appl. PCT/FR2004/000734

Schirra M, D'Aquino S, Palma A, Marceddu S, Angioni A, Cabras P, Scherm B, Migheli Q. 2005. Residue level, persistence and storage performance of citrus fruit treated with fludioxonil. Journal of Agricultural and Food Chemistry 53:6718-6724.

Schirra M, D'hallewin G, Ben-Yehoshua S, Fallik E. 2000. Host pathogen interaction modulated by heat treatment. Postharvest Biology & Technology 21:71-85.

Schönherr J, Baurr P. 1996. Effects of temperature, surfactants and other adjuvants on rates of uptake of organic compounds. In: Plant cuticles – An integrated functional approach. Kerstiens G. (Ed.). BIOS Scientific Publ. Ltd Oxford, p 135-155.

Smilanick JL, Mansour MF, Gabler FM, Goodwine WR. 2006. The effectiveness of pyrimethanil to inhibit germination of *Penicillium digitatum* and to control citrus green mold after harvest. Postharvest Biology & Technology 42:75-85.

Smilanick JL, Michael IF, Mansour MF, Mackey BE, Margosan DA, Flores D, Weist CF. 1997. Improved control of green mold of citrus with imazalil in warm water compared with its use in wax, Plant Disease 81:1299–1304

Sugar D., Basile S. 2005. Effects of flotation solutions on sodium *o*-phenyl phenate injury to pears and on incidence of postharvest decay. Postharvest Biology & Technology 37: 122-128.

Zhang J. 2007. The potential of a new fungicide fludioxonil for stem-end-rot and green mold control on Florida citrus fruit. Postharvest Biology & Technology 46:262-270.

Watkins CB, Barman JE, Hopkirk G. 1988. Effects of lecithin, calcium, and antioxidant formulations on superficial scald and internal breakdown of 'Granny Smith' apples. New Zealand of Experimental Agriculture 196:55-61.

Table 1. Residues of Fludioxonil in 'Coscia' pears following 1, 2 or 4 min dip treatments with FLU-based commercially available fungicide mixture, without or with 2 % lecithin and after 14 days at 17°C.

Treatments	Treatment time (min)	FLU residues[x] (mg kg^{-1} on a whole fruit basis)	
		At time 0	after shelf-life
300 mg l^{-1} FLU, 20°C	1	1.57 d	1.30 b
	2	2.68 b	1.70 b
	4	2.93 b	2.50 a
300 mg l^{-1} FLU + Lecithin, 20°C	1	1.28 e	1.18 c
	2	1.39 de	1.17 c
	4	1.41 de	1.18 c
100 mg l^{-1} FLU, 50°C	1	2.25 c	1.75 b
	2	2.32 c	2.01 b
	4	3.52 a	2.70 a
100 mg l^{-1} FLU + Lecithin, 50°C	1	0.71 f	0.56 d
	2	1.13 e	0.76 d
	4	1.16 e	1.01 c

[x]The fungicide concentration refers to active ingredient.

[y]In each column grouping means, means followed by a common letter are not significant different by Fisher's least significant difference (LSD) procedure, $P \leq 0.05$.

Table 2. Influence of postharvest dip treatments on the incidence of blue mold and gray mold caused by *Penicillium expansum* and *Botrytis cinerea* of inoculated 'Coscia' pears after selected incubation period.

Treatments[x]	Dip time (min)	*P. expansum* (%)		*B. cinerea (%)*	
		Incubation period (days)[y]			
		7	10	7	10
Water, 20°C	1	100 a	100 a	43.3 a	100 a
	2	100 a	100 a	37.2 b	100 a
	4	100 a	100 a	37.2 b	100 a
300 mg l^{-1} FLU, 20°C	1	0.0 d	0.0 g	0.0 e	0.0 e
	2	0.0 d	0.0 g	0.0 e	0.0 e
	4	0.0 d	0.0 g	0.0 e	0.0 e
300 mg l^{-1} FLU + 2% Lecithin, 20°C	1	0.6 d	2.8 ef	0.0 e	0.0 e
	2	0.0 d	1.7 fg	0.0 e	0.0 e
	4	0.0 d	0.0 g	0.0 e	0.0 e
Water, 50°C	1	73.3 b	100 a	42.8 a	97.2 b
	2	68.9 b	100 a	26.7 c	36.1 c
	4	10.0 c	72.0 b	21.1 d	17.8 d
100 mg l^{-1} FLU, 50°C	1	0.6 d	1.1 fg	0.0 e	0.0 e
	2	0.0 d	0.0 g	0.0 e	0.0 e
	4	0.0 d	0.0 g	0.0 e	0.0 e
100 mg l^{-1} FLU + 2% Lecithin, 50°C	1	1.1 d	10.6 c	0.0 e	0.0 e
	2	0.0 d	8.9 cd	0.0 e	0.0 e
	4	0.6 d	2.8 ef	0.0 e	0.0 e

[x]The fungicide concentration refers to active ingredient.

[y]Each value is the mean of two experiments (three replicates of 30 fruit each). In each column grouping means, means followed by a common letter are not significant different by Fisher's least significant difference (LSD) procedure, $P \leq 0.05$.