Specialty Crops Research Program Annual Report

Report Period: January 1, 2004 – December 31, 2004

Project Title: Evaluation of food additives and low-toxicity compounds as alternative chemicals to synthetic fungicides for the control of the main postharvest diseases of California stone fruits.

Principal Investigator (s): Dr. Carlos H. Crisosto; University of California, Davis; Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier, CA 93648; e-mail: carlos@uckac.edu; Tel. (559) 646-6596; Fax. (559) 646-6593

EXECUTIVE SUMMARY.

Stone fruits (peach, nectarine, plum) are major crops in California. Economic losses caused by postharvest diseases are among the most important concerns of the growers. Postharvest fruit decay has typically been controlled by application of synthetic fungicides. However, important problems associated with the massive use of these chemicals, such as the proliferation of resistant strains of the pathogens and concerns about public health and environmental contamination, have increased the need for alternatives, especially in the context of Integrated Pest Management (IPM) practices in California. In this project, we are undertaking the evaluation of a wide range of food additives and low-toxicity chemicals as potential alternatives to synthetic fungicides for the control of the most important postharvest pathogens of stone fruits. These compounds leave low or non-detectable residues on the fruit and are approved for many industrial and agricultural applications by Federal and State regulations. Many of them are registered as *Generally Recognized as Safe* (GRAS) by the EPA, or are included in the National List of substances allowed as ingredients in products labeled as organic.

Our project has three objectives that involve a sequential screening process. The goal is to set the basis for the commercial implementation of reliable and cost-effective alternative treatment(s) for the control of target postharvest diseases of stone fruit crops in California. In the first year, we performed an initial screening of 25 chemicals on 7 stone fruit cultivars against 7 pathogens (*Botrytis cinerea, Monilinia fructicola, Geotrichum candidum, Penicillium expansum, Alternaria alternata, Rhizopus stolonifer,* and *Mucor piriformis*) in *vivo* (Objective 1). Eighteen GRAS compounds were eliminated in the screening due to lack of effectiveness in controlling pathogens or damage to fruit. Six GRAS compounds [2 deoxy – D – glucose (as glucosamine), potassium carbonate, potassium sorbate, sodium carbonate, sodium sorbate, sodium benzoate] were advanced to Objective 2 for testing in 2004. Here, they were evaluated to determine the most effective solution temperature, chemical concentration, and immersion period to control decay caused by the two main stone fruit postharvest pathogens (*M. fructicola* and *G. candidum*). At ambient temperatures, all but glucosamine had activity against M. fructicola, though none

consistently distinguished itself from the others. The efficacy of the GRAS chemicals increased with solution temperature (up to 55-60°C). Hot water dips were nearly as effective at reducing disease expression, however. Damage to some fruit was observed at treatment temperatures of 60°C or greater.

OBJECTIVES.

Our project is divided into three main objectives that involve a sequential screening process. The goal is to set the basis for the commercial implementation of a reliable and cost-effective alternative chemical treatment for the control of target postharvest diseases of stone fruit crops in California.

Objective 1 (2002-2003).

Evaluate in *in vivo* primary screenings the effectiveness of a wide range of low-toxicity chemicals, mostly common food additives, for the control of the main postharvest pathogens of peach, nectarine, and plum. This objective was completed and summarized in last year's annual report.

Objective 2 (2004-2005).

Evaluate in small-scale trials (dips in aqueous solutions) the effectiveness of chemicals selected in Objective 1. Determine the most appropriate combination of solution temperature, chemical concentration, and immersion time needed to provide optimal disease control. This objective was completed this year and the results summarized in this report.

Objective 3 (2004-2005).

Evaluate in small-scale trials (dips in aqueous solutions) the effectiveness of chemicals selected in objective 2 on fruit treated then stored at low temperature. Assess decay periodically during the cold storage period, and decay and fruit quality at the end of the storage period and after a shelf life period. Study the commercial feasibility of these treatments and set the basis for commercial-scale evaluation trials. We completed some trials and will continue with this objective in the next season.

PROCEDURES AND METHODS.

Objective 2. Small scale trials.

Fruit. GRAS chemicals were evaluated on peach ('Flavorcrest', 'O' Henry', 'Rich Lady'), nectarine ('Spring Bright', 'Summer Fire', 'August Fire') and plum ('Casselman') at the F. Gordon Mitchell Postharvest Laboratory at the University of California, Kearney Agricultural Center. Fruit were sanitized with 100 ppm free chlorine and rinsed on a packingline (Fig. 2.), packed in commercial tray packs/boxes, then allowed to dry prior to inoculation by fungal pathogens (Fig. 3) and treatment with GRAS compounds (Fig. 4). Fruit were used after drying or stored at 1°C for up to three days prior to inoculation.

Fungal inoculum. Pure cultures of the main postharvest pathogens (*M. fructicola* and *G. candidum*) were grown on refrigerated, acidified Potato Dextrose Agar (PDA) plates at 20°C. Spores were harvested from the mycelial growth when plates were from 4 to 10 days old depending upon the growth rate of the fungal species. Spores were washed from the plates with sterile water then filtered through two layers of cheesecloth. The aqueous solutions were adjusted using a hemacytometer to a spore density of 5 X 10⁴ / ml for *M. Fructicola* and, with the exception of *G. candidum*, which was prepared at 1 X 10⁸/ ml for *Geotrichum*. Spore suspensions were applied to a single, sterile, 5mm deep by 2mm wide wound on the cheek of each fruit at the rate of 20µl. After inoculation, fruit were incubated at room temperature for 18 to 24 hours prior to the application of the treatments.

GRAS compounds. GRAS chemicals (Table 1.) were prepared as 1 M stock aqueous solutions. Dilutions were prepared as molar dilutions. Treatments were applied as 30 to 60 s dips in a 5-gallon container at room temperature. Fruit were then either rinsed in water for 5 s or unrinsed.

Temperature. Fruits were dipped into hot GRAS chemical solutions or water at the USDA Research Laboratory located in Parlier, CA. Dip temperatures of 24, 50, 55 or 60°C for 30 or 60 s durations were evaluated. Treated fruits were air dried at 20°C for 1 h after treatment and then placed in fiberboard boxes.

Evaluation. Treated fruit in boxes were incubated at 20°C and disease incidence and severity was determined after 3, 5 or 7 days. Disease incidence was assessed as the percentage of decayed fruit and disease severity as the diameter of the fungal lesion. This diameter was measured with an electronic caliper (Fig. 5). Treatment means were compared with SAS using General Linear Models (GLM).

SIGNIFICANT OBSERVATIONS.

Of the six GRAS compounds tested on stone fruit this season, five had activity against *M. fructicola*, but none consistently distinguished itself from the others. In 'Spring Bright' nectarine, potassium sorbate and sodium benzoate were more effective than the other compounds at reducing disease incidence and severity after 3 and 7 days of incubation (Table 2). In this trial, rinsing fruit with water after treatment slightly reduced the efficacy of the chemicals. All six GRAS chemicals reduced the incidence of *G.* candidum decay in 'Spring Bright' nectarines, although overall infection was low (Table 3). Sodium sorbate and potassium sorbate were more effective in this trial, reducing disease incidence to 5.0 and 7.1%, respectively, compared to 46.7% for the control. In 'Flavorcrest' peach, glucosamine was found to increase disease expression (Table 4). This phenomenon was found in 'Rich Lady' peach as well (Table 5). Glucosamine was suggested as a cost effective alternative to 2-deoxy-D-glucose, but we found it to be ineffective.

Increasing the temperature of the GRAS dip solutions did increase their efficacy. However, hot water dips were nearly as effective as dips in hot GRAS chemicals for the control of *M. fructicola*. In 'Summer Fire' nectarines, the disease severity (lesion diameter) of fruit

dipped in 55°C potassium sorbate was 7.3 mm after 3 d incubation at 20°C compared to control fruit which had lesions measuring 29.9 mm (Table 6). Nectarines dipped in 55°C potassium benzoate and 55°C water had disease severity of 23.9 and 15.1 mm, respectively. In 'O'Henry' peaches, the percentages of infected wounds in fruits treated with hot water at 55 and 60°C for 30 s were 67.8 and 40.0 %, respectively (Table 7). In contrast, in fruit treated with water at 24°C for 30 s, 90.3 % of the wounds developed decay. This level of control was not improved using solutions of potassium sorbate at 55 and 60°C. In another trial with 'Summer Fire' nectarine, hot water dips at 55°C for 60 s significantly reduced both the percentage of infected wounds and the severity of disease after 5 days of incubation (Table 8). In contrast, hot water treatment at 55°C for 30 s only reduced the disease severity. In 'Casselman' plum, hot water dips at 55 and 60°C for both 30 and 60 s significantly reduced the disease severity and the percentage of infected wounds (Table 9). Among the treatments on plums, hot water dips at 60°C for 60 s completely inhibited the disease and were significantly superior to other treatments. Similar results were obtained on 'August Fire' nectarine. However, severe skin pitting developed on fruit that had been dipped in 60°C water then stored at 0°C for 30 days (Table 10). The results of this study demonstrate that hot GRAS solutions and hot water dips have the potential to control M. fructicola on nectarine, peach and plum. However, more work is necessary to understand damage thresholds for the different species and cultivars.

DOCUMENTATION OF ACTIVITIES.

A summary of GRAS compound trials are presented in Tables 1-10 in the appendix. A flow chart of the experimental protocol is presented in Figure 1, and photographs of protocol and results are presented in Figures 2-7 in the appendix.

Table 1. GRAS chemicals and concentrations evaluated in secondary screening trials, 2004.

Chemical	Concentrations	Advanced to
	evaluated	next stage
Deoxy-D-glucose as	1%, 2%, 3%	No. Deoxy-D-glucose was effective against
Glucosamine		most pathogens in Stage 1 trials, but its
		cost prohibits commercial use. This year
		we evaluated its chemical precursor,
		glucosamine which is considerably less
		expensive, but it had no activity in this form.
Potassium carbonate	100, 200 mM	Yes. Some control of Monilinia and
		Geotrichum at 200 mM
Potassium sorbate	100, 200 mM	Yes. One of the more effective compounds
		with some control of Monilinia and
		Geotrichum at 200 mM
Sodium benzoate	100, 200 mM	Yes. Another of the more effective
		compounds with some control of Monilinia
		and Geotrichum at 200 mM
Sodium carbonate	250, 400 mM	Yes. Some control of Monilinia and
		<i>Geotrichum</i> at 400 mM
Sodium sorbate	100, 200 mM	Yes. Some control of Monilinia and
		<i>Geotrichum</i> at 200 mM
-		

Fig. 1. Flow chart of experimental protocol.

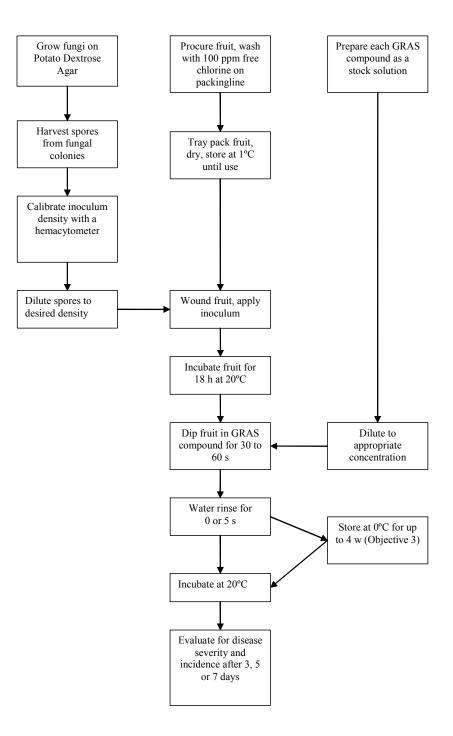


Table 2. 'Spring Bright' nectarines were wound-inoculated with *Monilinia fructicola* 24 h prior to a 60 s dip in glucosamine, potasium carbonate, potassium sorbate, sodium benzoate, sodium carbonate, sodium sorbate or water, followed by a 0 or 5 s rinse in water and then observed after 3 and 7 d incubation at 20°C for incidence and severity of decay.

		De	сау	
Treatment	3 d at	: 20°C	7 d at	: 20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Chemical ^z		· · ·	- · ·	· ·
Glucosamine	19.3	96.7	59.4	100.0
K-carb	10.9	86.7	59.6	100.0
K-sorb	2.8	48.3	47.5	98.3
Na-benz	8.2	70.8	44.6	81.7
Na-carb	15.3	79.2	47.6	95.0
Na-sorb	13.8	85.8	58.2	89.3
Water	25.2	100.0	63.1	100.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	1.8	13.7	2.7	6.6
Rinse				
0 s	13.8	75.5	52.7	94.3
5 s	13.4	86.7	55.8	98.3
P-value	0.43	0.0042	< 0.0001	0.0027
LSD _{0.05}	NS	7.3	1.5	3.6
Interaction (Chemica	al*Rinse)			
Glucosamine*0 s	19.5	98.3	60.2	100.0
Glucosamine*5 s	19.1	95.0	58.5	100.0
K-carb*0 s	9.3	81.7	59.1	100.0
K-carb*5 s	12.4	91.7	60.1	100.0
K-sorb*0 s	1.6	40.0	44.1	100.0
K-sorb*5 s	3.9	56.7	50.9	96.7
Na-benz*0 s	3.6	45.0	29.1	63.3
Na-benz*5 s	12.7	96.7	60.1	100.0
Na-carb 0 s	18.7	86.7	53.3	98.3
Na-carb*5 s	11.9	91.7	41.9	91.7
Na-sorb*0 s	12.2	96.7	56.3	98.3
Na-sorb*5 s	15.4	95.0	60.1	100.0
Water*0 s	31.7	100.0	67.0	100.0
Water*5 s	18.5	100.0	59.1	100.0
P-value	<0.0001	0.0012	<0.0001	<0.0001
LSD _{0.05}	2.5	19.4	3.9	9.4

^zGlucosamine = glucosamine 1%[;] K-carb= potassium carbonate 250 mM; K-sorb=potassium sorbate 200mM; Na-benz=sodium benzoate 200 mM; Na-carb=sodium carbonate 400 mM; Na-sorb=sodium sorbate 200mM.

Table 3. 'Spring Bright' nectarines were wound-inoculated with *Geotrichum candidum* 24 h prior to a 60 s dip in glucosamine, potasium carbonate, potassium sorbate, sodium benzoate, sodium carbonate, sodium sorbate or water, followed by a 0 or 5 s rinse in water and then observed after 7 d incubation at 20°C for incidence of decay.

Treatment	
Treatment	Disease incidence (%)
Chemical ^z	
Glucosamine 1%	16.7
Potassium carbonate 250 mM	14.2
Potassium sorbate 200 mM	7.1
Sodium benzoate 200 mM	10.4
Sodium carbonate 400 mM	14.6
Sodium sorbate 200 mM	5.0
Water	46.7
<i>P</i> -value	<0.0001
LSD _{0.05}	16.2
Rinse	
0 s	19.6
5 s	13.1
<i>P</i> -value	0.14
LSD _{0.05}	NS
Interaction (Chemical*Rinse)	
P-value	0.99 ^z

^zMean separation not performed when there was no significant interaction between the main factors.

		De	сау	
Treatment	3 d at	: 20°C	7 d at	20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Chemical ^z				
Glucosamine	20.0	81.7	52.8	96.7
K-carb	6.8	60.0	32.8	86.7
K-sorb	9.4	70.0	44.8	95.0
Na-benz	10.2	81.7	42.9	93.3
Na-carb	5.6	63.3	34.2	89.3
Na-sorb	16.6	76.7	52.9	96.7
Water	15.1	65.0	42.1	83.3
P-value	<0.0001	0.27	<0.0001	0.29
LSD _{0.05}	3.8	NS	6.8	NS

Table 4. 'Flavorcrest' peaches were wound-inoculated with *Monilinia fructicola* 24 h prior to a 60 s dip in glucosamine, potasium carbonate, potassium sorbate, sodium benzoate, sodium carbonate, sodium sorbate or water and then observed after 3 and 7 d incubation at 20°C for incidence and severity of decay.

^zGlucosamine = glucosamine 1%[;] K-carb= potassium carbonate 250 mM; K-sorb=potassium sorbate 200 mM; Na-benz=sodium benzoate 200 mM; Na-carb=sodium carbonate 400mM; Na-sorb=sodium sorbate 200mM.

Table 5. 'Rich Lady' peaches were wound-inoculated with *Monilinia fructicola* 24 h prior to a 60 s dip in different concentrations and combinations of glucosamine, potassium sorbate, sodium benzoate, sodium carbonate or water, followed by a 0 or 5 s rinse in water and then evaluated after 7 d incubation at 20°C for incidence and severity of decay.

	De	cay
Treatment ^z	7 d at	20°C
	Severity (mm)	Incidence (%)
Glucosamine 2%	51.1	100.0
Glucosamine 3%	53.9	100.0
K-sorb 100 mM	52.9	100.0
K-sorb 200 mM	47.8	100.0
Na-benz 100 mM	48.1	100.0
Na-benz 200 mM	48.0	100.0
K-sorb 100 mM + Na-benz 100 mM	45.3	100.0
K-sorb 100 mM + Na-carb 100 mM	49.5	100.0
Na-benz 100 mM + Na-carb 100 mM	48.0	100.0
Water (control)	49.9	100.0
<i>P</i> -value	1.7	NS
LSD _{0.05}	<0.0001	NS

^zK-carb= K-sorb=potassium sorbate; Na-benz=sodium benzoate; Na-carb=sodium carbonate.

Table 6. 'Summer Fire' nectarines were wound-inoculated with *Monilinia fructicola* 24 h prior to a 60 s dip in 24, 40, 50, or 55°C potassium sorbate, sodium benzoate or water, and then observed after 3 and 5 d incubation at 20°C for incidence and severity of decay.

		De	cay	
Treatment	3 d at	: 20°C	5 d at	20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Chemical ^z				
K-sorb	16.7	84.5	28.2	93.8
K-benz	20.8	94.8	31.0	96.0
Water	22.2	94.0	35.7	98.8
P-value	<0.0001	0.0016	<0.0001	0.034
$LSD_{0.05}$	1.4	5.8	4.7	3.7
Dip temperature				
24°C	24.5	99.5	37.4	100.0
40°C	22.0	97.9	35.3	99.4
50°C	17.7	88.9	30.6	97.3
55°C	15.4	78.3	23.2	88.0
P-value	< 0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	1.7	6.6	1.9	4.3
	mical*Dip temperat	ture)		
K-sorb*24 ^o C	21.0	<i></i> 98.4	31.0	100.0
K-sorb*40°C	22.6	98.4	35.5	98.3
K-sorb*50°C	15.9	85.7	28.5	98.4
K-sorb*55°C	7.3	55.6	17.8	78.6
K-benz*24°C	23.5	100.0	38.3	100.0
K-benz*40⁰C	20.6	96.8	33.4	100.0
K-benz*50°C	15.1	82.6	27.6	93.6
K-benz*55°C	23.9	100.0	24.6	90.2
Water*24°C	29.0	100.0	43.0	100.0
Water*40°C	22.7	98.4	37.2	100.0
Water*50°C	22.0	98.4	36.0	100.0
Water*55°C	15.1	79.4	27.1	95.2
P-value	<0.0001	<0.0001	<0.0001	0.021
LSD _{0.05}	2.9	11.5	3.4	7.4

^zK-sorb=potassium sorbate 200mM; K-benz=potassium benzoate 200 mM.

	Decay			
Treatment	5 d at	20°C	7 d at	: 20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Chemical ^z				
K-sorb	9.7	47.6	25.7	63.5
Water	12.4	52.9	29.6	65.9
P-value	0.028	0.44	0.065	0.76
LSD _{0.05}	2.4	NS	NS	NS
Dip temperature				
24°C	17.9	79.4	41.6	91.5
55°C	12.6	54.8	29.0	67.7
60°C	3.0	16.7	12.9	34.9
<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.0003
LSD _{0.05}	2.9	17.6	5.0	21.2
Interaction				
K-sorb 24°C	16.6	43.2	41.1	82.8
K-sorb 55°C	10.6	53.9	26.9	67.5
K-sorb 60°C	2.0	12.7	10.1	30.2
Water 24°C	18.6	82.6	42.2	90.3
Water 55°C	14.5	55.6	30.9	67.8
Water 60°C	4.1	20.6	15.7	39.7
P-value	0.76	0.92	0.67	0.82
LSD _{0.05}	y			

Table 7. 'Rich Lady' peaches were wound-inoculated with Monilinia fructicola 24 h prior to a 60 s dip in 24, 55, or 60°C potassium sorbate, or water, and then observed after 5 and 7 d incubation at 20°C for incidence and severity of decay.

^zK-sorb=potassium sorbate 200 mM. ^yMean separation not performed when there was no significant interaction between the main factors.

	Decay			
Treatment	3 d at	: 20°C	5 d at	20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Water temperate	ure			
24°C	37.4	100.0	57.5	100.0
55°C	12.3	71.4	25.1	86.9
60°C	5.3	35.7	8.4	44.7
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	1.4	11.7	1.4	8.4
Dip duration				
30 s	22.4	88.5	37.5	94.8
60 s	14.3	49.6	23.1	59.5
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	1.1	9.6	1.1	6.9
Interaction (Wat	er temperature*Dip	duration)		
24°C*30 s	37.2	100.0	58.1	100.0
24°C*60 s	37.5	100.0	56.9	100.0
55°C*30 s	20.1	96.4	38.8	100.0
55°C*60 s	4.5	46.4	11.3	73.8
60°C*30 s	9.8	69.1	15.6	84.5
60°C*60 s	0.8	2.4	1.2	4.8
P-value	<0.0001	0.0001	<0.0001	<0.0001
LSD _{0.05}	2.0	16.6	2.0	11.9

Table 8. 'Summer Fire' nectarines were wound-inoculated with *Monilinia fructicola* 24 h prior to a 30 or 60 s dip in 24, 55 or 60°C water, and then observed after 3 and 5 d incubation at 20°C for incidence and severity of decay.

		cay		
Treatment	3 d at	: 20°C	5 d at	: 20°C
	Severity (mm)	Incidence (%)	Severity (mm)	Incidence (%)
Water temperat	ture			
24°C	12.8	91.3	15.1	99.3
55°C	3.9	37.3	4.9	43.3
60°C	0.8	9.3	1.9	9.3
65°C	0.0	0.0	0.0 ^z	0.0 ^z
70°C	0.0	0.0	0.0 ^y	0.0 ^y
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	0.8	5.6	1.4	4.8
Dip duration				
30 s	4.3	34.7	8.7	37.6
60 s	2.7	20.5	7.8	23.2
<i>P</i> -value	<0.0001	<0.0001	0.0036	<0.0001
LSD _{0.05}	0.5	3.6	1.1	3.0
Interaction				
24°C 30 s	13.1	93.3	15.5	98.7
24°C 60 s	12.5	89.3	14.7	100.0
55°C 30 s	7.0	62.7	8.5	70.7
55°C 60 s	0.9	12.0	1.3	16.0
60°C 30 s	1.5	17.3	2.0	18.7
60°C 60 s	0.1	1.3	0.0	0.0
65°C 30 s	0.0	0.0	0.0 ^z	0.0 ^z
65°C 60 s	0.0	0.0	0.0 ^z	0.0 ^z
70°C 30 s	0.0	0.0	0.0 ^y	0.0 ^y
70°C 60 s	0.0	0.0	0.0 ^y	0.0 ^y
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD _{0.05}	1.1	8.0	3.9	6.8

Table 9. 'Casselman' plums were wound-inoculated with *Monilinia fructicola* 24 h prior to a 30 or 60 s dip in 24, 55, 60, 65 or 70°C water, and then observed after 3 and 5 d incubation at 20°C for incidence and severity of decay.

^zSlight to moderate damage on skin. ^yModerate to severe damage on skin.

Table 10. 'August Fire' nectarines were wound-inoculated with *Geotrichum candidum* or *Monilinia fructicola* 24 h prior to a 60 s dip in 24, 55 or 60°C water, stored for 30 days at 0°C, ripened for 5 d at 20°C, and then evaluated for incidence and severity of decay and incidence of skin pitting.

Pathogen	Dee	cay	Ditting (%)
Treatment	Severity (mm)	Incidence (%)	- Pitting (%)
Geotrichum			
24°C	2.0	30.7	42.9
55°C	1.0	16.0	19.1
60°C	0.3	4.0	88.9
P-value	0.0004	0.16	0.0074
$LSD_{0.05}$	0.8	NS	34.9
Monilinia			
24°C	22.1	73.3	36.5
55°C	13.2	58.7	11.1
60°C	6.4	28.0	85.7
P-value	<0.0001	0.0004	0.014
LSD _{0.05}	3.7	12.8	42.8

 Fruit were sanitized with 100 p to use in trials. 	opm free chlorine on a packingline prior	3. After wounding fruit to a uniform size and depth, a drop of inoculum was placed on the wound site. Infection was then allowed to establish during 18 h incubation at 20°C.
 Eighteen hours after inoculation, chemical treatments were applied. Fruit were then incubated up to 7 d at 20°C. 	 Decay lesion size was measured, recorded and analyzed statistically. 	 Nectarines inoculated with <i>M. fructicola</i>, dipped for 60s in (left to right) 24, 55 and 60°C water, then incubated for 5 d at 20°C.

Figs 2-7. Inoculation, treatment, and evaluation of GRAS chemical compounds.

