Universitat de Lleida
Escola Técnica Superior d'Enginyeria Agrària

AVALUACIÓ DE SISTEMES ALTERNATIUS ALS FUNGICIDES SINTÈTICS PER AL CONTROL DE LES PODRIDURES VERDA I BLAVA EN POSTCOLLITA DE CÍTRICS

Memòria presentada per
Lluís Palou Vall
per optar al grau de doctor

Directors:
Dra. Inmaculada Viñas Almenar
Dr. Josep Usall i Rodié

Lleida, gener de 2002

Capítol 1

Micoflora Epífita de los Frutos y Ambiental en Campos de Mandarino 'Clemenules' en Tarragona

L. Palou[1], J. Usall[1], J. Pons[2], I. Viñas[1]

[1] *Àrea de Postcollita, CeRTA, Centre UdL-IRTA, Lleida*
[2] *Estació Experimental de l'Ebre, IRTA, Amposta, Tarragona*

Referència: *Invest. Agr.: Prod. Prot. Veg.* 16: 257-272 (2001).

Resumen

Se caracterizó la micoflora epífita de los frutos y la población fúngica ambiental en campos de mandarino 'Clemenules' de las comarcas del Baix Ebre y Montsià (Tarragona). Durante dos campañas consecutivas se realizaron dos muestreos (el primero entre septiembre y diciembre y el segundo entre enero y marzo) en cuatro campos comerciales representativos de la zona y en un campo de producción biológica. El género fúngico mayoritariamente aislado en los dos muestreos fue *Cladosporium* (89,8% del total de colonias aisladas de la superficie de los frutos en el primer muestreo y 80,4% en el segundo; y 72,1% del total de colonias aisladas del ambiente en el primer muestreo y 32,7% en el segundo). La frecuencia del género *Penicillium* fue mayor en el segundo muestreo (8,4% y 25,9% de las colonias aisladas de la superficie de los frutos y del ambiente respectivamente) que en el primero (1,2% y 0,6% respectivamente). Lo mismo ocurrió con otros hongos patógenos como *Alternaria* y *Rhizopus*. Tanto la población fúngica total como la de *Penicillium* presentaron una variabilidad muy elevada, con interacciones significativas entre campañas, muestreos y campos. La población fúngica total se correlacionó fuerte y positivamente con la temperatura ($r > 0,9$). La población fúngica ambiental, pero no la epífita de los frutos, fue más alta en el campo de producción biológica que en los campos comerciales. Con la excepción de los géneros *Rhizopus* y *Mucor*, que se localizaron más frecuentemente en los frutos de la parte baja del árbol, no se encontraron diferencias en el nivel de población fúngica entre alturas ni caras del árbol.

Palabras clave: cítricos, clementina, enfermedades de post-cosecha, *Penicillium*

Summary

Fruit epiphyte and environmental fungal populations in 'Clemenules' mandarin orchards in Tarragona (Spain)

Fruit epiphyte and environmental fungal populations were examined in 'Clemenules' orchards in Baix Ebre and Montsià areas (Tarragona, Spain). For two consecutive seasons, sampling was conducted twice (from September to December and from January to March) in four representative commercial orchards and in an organic orchard. *Cladosporium* was the most frequent genus present (89.8% and 80.4% of colonies isolated from the fruit surface at the first and second sampling, respectively; and 72.1% and 32.7% of colonies isolated from the environment at the first and second sampling, respectively). Pathogenic fungi like *Penicillium*, *Alternaria,* or *Rhizopus* were more frequently isolated in the second sampling (8.4%

and 25.9% of *Penicillium* colonies from the fruit surface and the environment, respectively) than in the first sampling (1.2% and 0.6% of *Penicillium* colonies from the fruit surface and the environment, respectively). Both total fungal population and *Penicillium* population were found to be highly variable, with significant interactions among years, samplings and orchards. Total fungal population was highly correlated with temperature ($r > 0.9$). Environmental population, but not fruit epiphyte population, was higher in the organic orchard than in the commercial ones. With the exception of *Rhizopus* and *Mucor*, which were isolated more often from fruits located in the lower area in the tree canopy, the fungal population levels were similar in fruits located at different heights in the tree canopy.

Key words: citrus, clementine, postharvest diseases, *Penicillium*

Introducción

La mandarina clementina (*Citrus reticulata* Blanco), y concretamente el cultivar Clemenules, es el cultivo cítrico de mayor importancia económica en Tarragona. Las enfermedades de la post-recolección son una de las principales causas de las pérdidas de producción comercializable. Las infecciones en post-cosecha se producen mayoritariamente a través de heridas en la piel causadas durante la recolección y el posterior manejo de los frutos, aunque también se pueden originar en el campo por distintos agentes bióticos (insectos, pájaros, etc.) o abióticos (viento, lluvia, granizo, etc.). Las infecciones en pre-cosecha se producen cuando el agente patógeno llega a la flor o al fruto durante su desarrollo en el campo y permanece inactivo hasta que, ya en post-cosecha, las condiciones ambientales y fisiológicas del fruto permiten el desarrollo de la enfermedad (Eckert y Eaks, 1989).

El clima, especialmente la temperatura y la humedad relativa (HR) antes y durante el período de recolección, influye decisivamente en la magnitud de la incidencia de estas enfermedades pues determina la cantidad de inóculo disponible, su grado de diseminación y, en consecuencia, el nivel la población fúngica presente en los frutos en el momento de su llegada a la central citrícola (Eckert y Brown, 1986). En las condiciones climáticas mediterráneas, caracterizadas por veranos poco lluviosos, las infecciones en post-cosecha son las de mayor importancia económica, destacando sobretodo la podredumbre verde, causada por *Penicillium digitatum* (Pers.:Fr.) Sacc., y en menor medida las podredumbres azul, ácida y por *Rhizopus*, causadas respectivamente por *Penicillium italicum* Wehmer, *Geotrichum candidum* Link ex. Pers. y *Rhizopus stolonifer* (Ehrenb.) Lind. (Tuset, 1987). Las infecciones en pre-cosecha más importantes son las podredumbres negra, marrón, gris y la antracnosis, causadas respectivamente por *Alternaria citri* Ell. y Pierce (también de

forma secundaria por *Alternaria alternata* (Fr.) Keissler), *Phytophthora citrophthora* Smith y Smith, *Botrytis cinerea* Pers. ex Fr. y *Colletotrichum gloeosporioides* (Penz.) Sacc. (Tuset, 1987).

Estudiar la población fúngica para una zona productora concreta es importante porque podría determinar la presencia de hongos patógenos y su distribución, para establecer así los riesgos potenciales de pérdidas y planificar adecuadamente los métodos de control. El objetivo del presente trabajo fue determinar la población fúngica presente en la superficie de los frutos y en el ambiente en campos de mandarino Clemenules de la zona citrícola de Tarragona durante el período de recolección.

Material y métodos

Diseño experimental. Se muestreó durante dos campañas consecutivas (1995-96 y 1996-97) la micoflora epífita de la superficie de los frutos y la población fúngica ambiental en campos de mandarino Clemenules de la zona citrícola de Tarragona (comarcas del Baix Ebre y Montsià). En cada campaña se realizaron dos muestreos, el primero antes y durante la primera parte del período de recolección (de finales de septiembre a diciembre) y el segundo durante el período de recolección más tardía (de enero a principios de marzo). El primer año se seleccionaron cuatro campos comerciales que por su ubicación fuesen representativos de toda el área productora (campos BE en la zona norte, zona de Benifallet; JE y MI en la zona centro, zona de Amposta y AL en la zona sur, zona de Alcanar), y un campo de producción biológica (campo PB). El segundo año no se pudo acceder al campo JE y se sustituyó por el campo MV, de ubicación y características similares. Tampoco se pudo muestrear el campo PB. En el mapa adjunto (Fig. 1) puede observarse la situación geográfica de los campos, así como la de los tres observatorios meteorológicos (observatorios de Aldover, zona norte; Amposta, zona centro y Alcanar, zona sur) cuyos datos se utilizaron para caracterizar el clima del área productora. Se presentan los valores mensuales de precipitación y temperatura media, media de las máximas y media de las mínimas (Fig. 2), y los de HR media y mínima (Fig. 3) que se registraron en ambas campañas durante los períodos de muestreo. Para el estudio de la micoflora epífita en cada campo se eligieron aleatoriamente diez árboles de la zona central y se marcaron para poder ser identificados en posteriores muestreos. En cada árbol se consideraron dos orientaciones (cara soleada y cara sombreada) y dos alturas (baja, de la parte media de la copa hacia abajo; y alta, de la parte media de la copa hacia arriba). Se muestrearon, por tanto, cuatro zonas distintas de cada árbol. En cada una de estas zonas la unidad de muestra fue de tres mandarinas, evitándose aquellas situadas en posiciones extremas. Se muestrearon, por consiguiente, un total de 120 frutos por campo en cada uno de los muestreos.

Fig.1.
Situación geográfica de los campos de mandarino Clemenules muestreados (BE, JE, MI, AL , PB, MV) y de los observatorios meteorológicos de Aldover (OM 1), Amposta (OM 2) y Alcanar (OM 3).

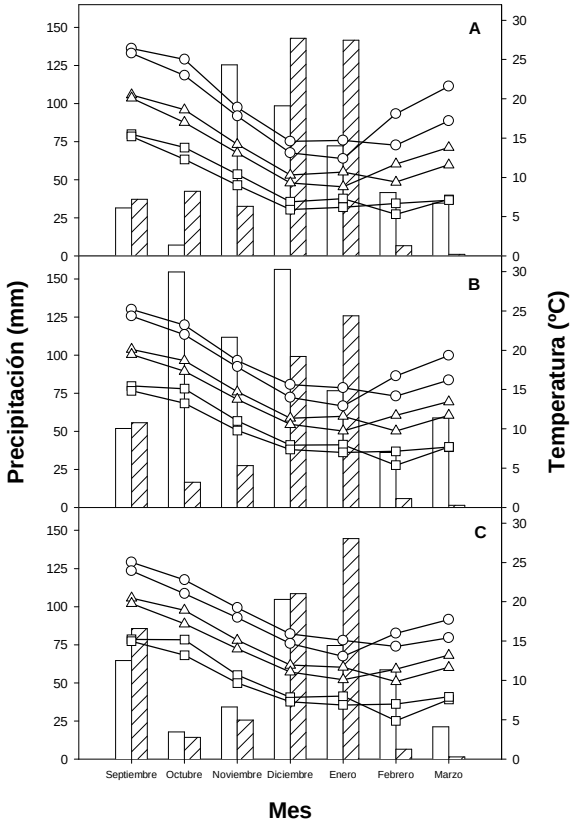

Fig. 2. Datos mensuales de la precipitación total de las campañas 1995-96 (barras blancas) y 1996-97 (barras rayadas) y de la temperatura media de las máximas (● , ○), media (▲ , Δ) y media de las mínimas (■, □) de las campañas 1995-96 (símbolos negros) y 1996-97 (símbolos blancos) en los observatorios meteorológicos de Aldover (A), Amposta (B) y Alcanar (C).

Toma de muestras, incubación y recuento. La micoflora epífita de un fruto se muestreó por impresión ('printing') de éste, sin arrancarlo del árbol, sobre el medio de cultivo de una placa Petri de 9 cm de diámetro. El medio utilizado fue patata dextrosa agar (PDA). Se utilizó una placa por fruto. La micoflora ambiental se muestreó mediante el método gravimétrico. Se utilizaron cinco placas Petri con

medio PDA por campo y muestreo. Las placas se distribuyeron entre dos filas consecutivas de árboles y se abrieron durante 1 min para que las esporas fúngicas se depositasen por gravedad sobre el medio de cultivo. En un intento de minimizar la heterogeneidad de las condiciones de muestreo, las tomas de muestra se realizaron siempre entre las 10 y las 12 h de la mañana. Las placas se incubaron en una cámara climatizada a 25ºC y 90% HR durante 7 días, transcurridos los cuales se procedió al recuento e identificación de las colonias fúngicas. La identificación de los géneros se realizó mediante observación microscópica (Webster, 1980; Ellis, 1993a, 1993b; Samson *et al*., 1995). La frecuencia de cada género fúngico se expresó como número de unidades formadoras de colonias (ufc) por placa, con la excepción de los géneros *Rhizopus* y *Mucor*, con los que, por su distinto hábito de crecimiento, se establecieron dos categorías excluyentes, presencia y ausencia, y cada placa se clasificó como perteneciente a una de las dos.

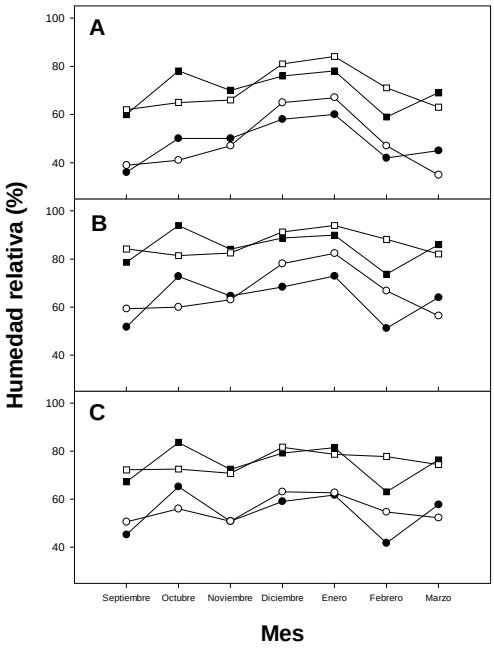

Fig. 3. Datos mensuales de la humedad relativa media (■ , □) y media de las mínimas (● , ○) de las campañas 1995-96 (símbolos negros) y 1996-97 (símbolos blancos) en los observatorios meteorológicos de Aldover (A), Amposta (B) y Alcanar (C).

Análisis estadístico. Se realizó con el paquete estadístico SAS (SAS Institute Inc., Cary, NC, EE UU). Se estudió la correlación entre cada par de variables dependientes (frecuencias de cada género fúngico) y entre la frecuencia de cada género y la frecuencia fúngica total. También se estudió la correlación de las dos variables dependientes más importantes, la frecuencia fúngica total y la frecuencia de *Penicillium*, con la temperatura, precipitación y HR medias mensuales. Para ello se determinó el coeficiente de correlación de Pearson confrontando el número de colonias total o de *Penicillium* de cada placa con el valor de las tres variables climatológicas correspondiente al campo (observatorio más cercano), mes y año en los que se muestreó esa placa.

Para las variables frecuencia fúngica total y frecuencia de *Penicillium*, se estudiaron los supuestos de normalidad y homocedasticidad de los datos. A raíz de este estudio, los datos se transformaron a la raíz cuadrada de ufc-total/placa+0,5 en el caso de la población fúngica total y al logaritmo neperiano de ufc/placa+1 en el de *Penicillium*.

(i) *Micoflora epífita de los frutos*. Los datos transformados se sometieron a un análisis de la varianza multifactorial de modelo mixto en el que los factores campaña, árbol y fruto (repetición) se consideraron aleatorios. Puesto que cada muestreo no se pudo realizar en todos los campos, el factor campo se jerarquizó al factor muestreo; el factor árbol se jerarquizó al factor campo, y el resto de factores (altura y orientación) se consideraron cruzados. Debido al gran número de factores implicados en el diseño experimental, se realizó un primer análisis con los factores campaña, muestreo y campo. En función de la significación de las interacciones se realizaron posteriores análisis por separado para cada uno de los distintos niveles de los factores. Cuando resultó procedente, se separaron medias mediante la prueba de la Mínima Diferencia Significativa Protegida de Fisher (MDS; $p = 0,05$).

(ii) *Población fúngica ambiental*. Se realizó el mismo tipo de análisis de la varianza considerando los factores campaña y placa Petri (repetición) aleatorios. El factor campo se jerarquizó al factor muestreo.

(iii) *Géneros* Rhizopus *y* Mucor. Los datos categorizados en ausencia o presencia de los hongos se contrastaron con las distintas variables explicativas mediante tablas de contingencia. La independencia entre variables se determinó mediante la prueba χ^2 ($p = 0,05$).

Resultados

Micoflora epífita de los frutos. La media (± error típico) del número total de colonias fúngicas por placa que se aislaron en el conjunto de los campos durante las dos campañas fue de 157,4 ± 2,2 ufc/placa en el primer muestreo y de 99,2 ± 2,1 ufc/placa en el segundo. En el primer muestreo, el 89,8% de las colonias aisladas correspondieron al género *Cladosporium*, el 3,7% al género *Alternaria*, el

1,9% al género *Penicillium*, el 1,2% al género *Epicoccum* y el resto a los géneros *Fusarium*, *Humicola*, *Trichoderma*, *Aspergillus* y otros géneros. En el segundo muestreo se obtuvo una distribución de géneros similar, con una clara frecuencia mayoritaria de *Cladosporium* (80,4%), un aumento de *Alternaria* (6,3%) y *Fusarium* (1,1%) y un descenso de *Aspergillus*. La principal diferencia respecto al primer muestreo fue el importante incremento de *Penicillium*, que con el 8,4% de las colonias se convirtió en el segundo género más frecuente.

El análisis de la correlación entre frecuencias de los distintos géneros fúngicos mostró una fuerte correlación positiva ($r > 0,9$) en ambos muestreos entre la población fúngica total y la de *Cladosporium* (Tabla 1). La población fúngica total también apareció correlacionada en ambos muestreos positivamente con la de *Penicillium* y negativamente con la de *Epicoccum*. Estas correlaciones fueron de baja magnitud (Tabla 1).

La población fúngica total se correlacionó positivamente con la temperatura, la precipitación y la HR medias mensuales (Tabla 2). Sin embargo, la población de *Penicillium* se correlacionó negativa y débilmente con la temperatura y la HR y no se correlacionó con la precipitación (Tabla 2).

La distribución cuantitativa tanto de la población fúngica total como de la del género *Penicillium* fue irregular y se encontraron diferencias significativas entre campañas, muestreos y campos, así como interacciones significativas entre estos factores ($p < 0,0001$ en todos los casos; análisis no presentados). Es por ello que la variabilidad entre muestreos se analizó para cada campaña y campo por separado, excepto para los campos JE y BE, que no pudieron muestrearse dos veces cada campaña (análisis no presentados). Con la excepción de la población total en el campo AL en la campaña 1995-96, la población fúngica total disminuyó significativamente ($p < 0,0001$) en el segundo muestreo respecto al primero en todos los campos las dos campañas, mientras que la población de *Penicillium* aumentó ($p < 0,0001$) (Fig. 4). Los frecuencias fúngicas más altas correspondieron al primer muestreo de la primera campaña, especialmente en los campos JE, PB y MI situados en la zona de Amposta (Fig. 4A-1). En la segunda campaña la micoflora epífita en esta zona fue inferior a la de las otras dos zonas (Fig. 4B-1). En las zonas de Benifallet (campo BE) y Alcanar (campo AL) no se produjo un contraste tan marcado entre las dos campañas. Las frecuencias más bajas correspondieron al segundo muestreo de la segunda campaña (Fig. 4B-2). En el campo de producción biológica (PB) no se detectaron unos niveles de población especialmente diferentes a los del resto de las parcelas; la población fue más alta que en los campos MI, BE y AL pero más baja que en el campo JE (Fig. 4A). En todos los campos, y con independencia de la campaña y el muestreo, *Cladosporium* fue el género más frecuente en la superficie de los frutos (Fig. 4).

Por otra parte, las variables frecuencia fúngica total y frecuencia del género *Penicillium* se analizaron en función de los factores campo, árbol, orientación y

altura para cada muestreo de cada campaña por separado. En los ocho análisis se encontraron diferencias significativas ($p < 0,0001$) entre campos y también entre árboles dentro de un mismo campo pero no entre alturas ni orientaciones (análisis no presentados).

La presencia del género *Rhizopus* en la superficie de los frutos resultó ser dependiente de la campaña, el muestreo, el campo y la altura del fruto en el árbol (Tabla 3). Su presencia fue significativamente mayor en la segunda campaña que en la primera, y en el segundo muestreo (4,72% de placas con presencia) que en el primero (2,04%). La presencia fue baja en todos los campos. Los de mayor presencia fueron MV (zona de Amposta; 5,00% de placas con presencia) y AL (zona de Alcanar; 4,17%), y los de menor JE (0,00%) y el campo de producción biológica (PB; 1,25%), ambos en la zona de Amposta. El hongo se localizó con abundancia significativamente mayor en los frutos de la parte baja del árbol (4,11% de placas con presencia) que en los de la parte alta (2,11%).

La presencia del género *Mucor* en la superficie de los frutos fue también poco generalizada, cuantitativamente similar a la del género *Rhizopus*. Resultó ser dependiente del muestreo, el campo y la altura del fruto en el árbol (Tabla 3). Fue más importante en el segundo muestreo (8,47% de placas con presencia) que en el primero (4,17%), y en los frutos de la parte baja del árbol (8,44%) que en los de la parte alta (3,33%). El campo con mayor presencia fue AL (zona de Alcanar; 8,13%) y el campo con menor presencia JE (zona de Amposta; 0,83%). La presencia en el campo de producción biológica (PB) fue similar a la de los campos comerciales (4,17%).

Tabla 1. Pares de géneros fúngicos significativamente correlacionados (p < 0,01) en el estudio de la población fúngica (ufc/placa) en campos de mandarino Clemenules en Tarragona

Muestreo 1			**Muestreo 2**		
Par de variables	**r[x]**	**p**	**Par de variables**	**r**	**p**
Superficie de frutos			Superficie de frutos		
Cladosporium-Alternaria	0,161	<0,0001	*Cladosporium-Aspergillus*	0,126	0,0007
Cladosporium-TOTAL[y]	0,941	<0,0001	*Cladosporium-Fusarium*	-0,167	<0,0001
Alternaria-TOTAL	0,146	<0,0001	*Cladosporium-Epicoccum*	-0,168	<0,0001
Penicillium-Alternaria	-0,172	<0,0001	*Cladosporium* -TOTAL	0,967	<0,0001
Penicillium-Aspergillus	0,213	<0,0001	*Penicillium*-TOTAL	0,245	<0,0001
Penicillium-Cladosporium	-0,138	<0,0001	*Alternaria-Fusarium*	-0,167	<0,0001
Penicillium-Epicoccum	-0,105	0,0006	*Alternaria-Humicola*	-0,155	<0,0001
Penicillium-TOTAL	0,196	<0,0001	*Alternaria-Epicoccum*	-0,147	<0,0001
Epicoccum-Humicola	-0,149	<0,0001	*Aspergillus*-TOTAL	0,118	0,0015
Epicoccum-TOTAL	-0,090	0,0030	*Fusarium*-TOTAL	-0,136	0,0003
			Epicoccum-TOTAL	-0,162	<0,0001
Ambiente			Ambiente		
Cladosporium-TOTAL	0,993	<0,0001	*Cladosporium-Alternaria*	0,774	<0,0001
			Cladosporium-TOTAL	0,934	<0,0001
			Penicillium-TOTAL	0,647	0,0005
			Fusarium- Humicola	0,876	<0,0001

[x] Coeficiente de correlación de Pearson.

[y] Variable frecuencia fúngica total (ufc-total/placa).

Tabla 2. Correlación de la población fúngica total y la población de *Penicillium* (ufc/placa) con las medias mensuales de temperatura (T, ºC), precipitación total (P, mm) y humedad relativa (HR, %) en campos de mandarino Clemenules en Tarragona

	Superficie de frutos		Ambiente	
Par de variables	**r[x]**	**p**	**r**	**P**
TOTAL – T	0,9320	<0,0001	0,4732	<0,0001
TOTAL – P	0,4549	<0,0001	0,5393	<0,0001
TOTAL – HR	0,1278	<0,0001	0,3012	0,0113
Penicillium – T	-0,2038	<0,0001	-0,2816	0,0182
Penicillium – P	0,0094	0,6900	-0,3539	0,0026
Penicillium – HR	-0,1483	<0,0001	-0,1694	0,1610

[x] Coeficiente de correlación de Pearson.

Población fúngica ambiental. La media (± error típico) del número total de colonias fúngicas por placa que se obtuvo al muestrear el ambiente del conjunto de los campos durante las dos campañas fue más alta en el primer muestreo (89,8 ± 14,8 ufc/placa) que en el segundo (15,9 ± 2,8 ufc/placa). En el primer muestreo el género más abundante fue *Cladosporium*, con una frecuencia relativa del 72,1%, seguido por *Fusarium* (12,9%), *Alternaria* (7,5%), *Humicola* (5,5%), y otros como *Penicillium* (0,6%) en proporciones muy inferiores. En el segundo muestreo disminuyó la frecuencia de *Cladosporium* (32,7%) y aumentó de la de otros géneros como *Penicillium* (25,9%), *Fusarium* (15,4%), *Alternaria* (10,7%), *Humicola* (6,2%) y *Botrytis* (1,9%). En ambos muestreos la presencia de *Rhizopus* y *Mucor* fue prácticamente inapreciable.

La población fúngica total se mostró fuerte y positivamente correlacionada con el género *Cladosporium* en ambos muestreos ($r > 0,9$; Tabla 1). La población de *Penicillium* se mostró significativamente correlacionada con la población total únicamente en el segundo muestreo ($r = 0,647$; Tabla 1).

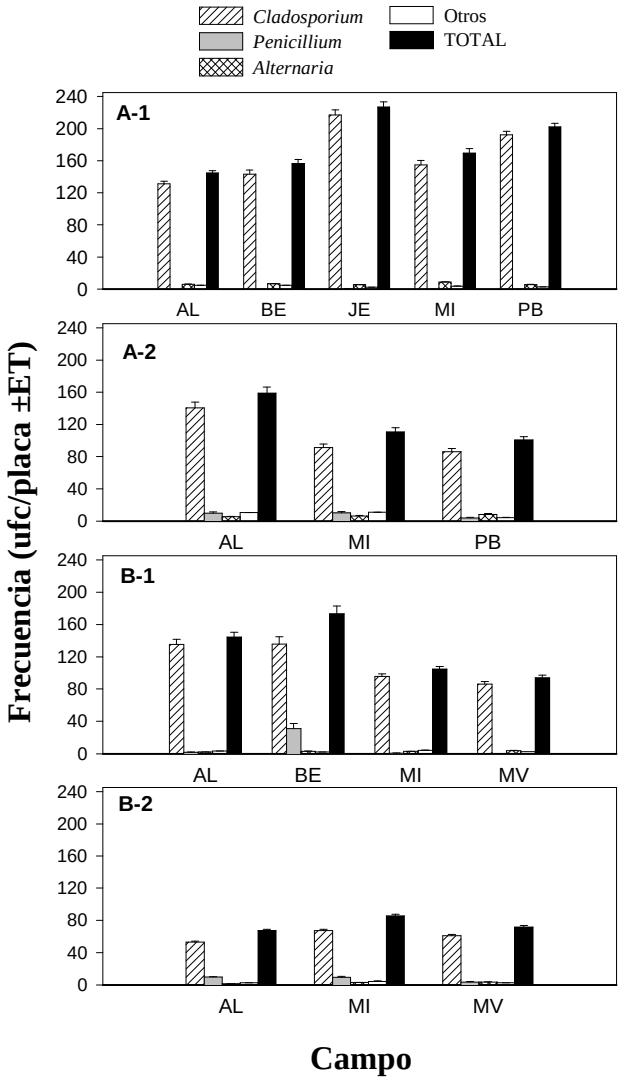

Fig. 4. Frecuencia de distintos géneros fúngicos en la superficie del fruto en campos de mandarino Clemenules de la zona citrícola de Tarragona en el primer (1) y en el segundo (2) muestreo realizado en el período de recolección de las campañas 1995-96 (A) y 1996-97 (B).

Tabla 3. Presencia de los géneros *Rhizopus* y *Mucor* en la superficie de los frutos en campos de mandarino Clemenules en Tarragona. Pruebas Chi-cuadrado de independencia entre la presencia de los hongos y las variables explicativas consideradas (p = 0,05)

Género fúngico	**Variable**	**Nivel**	**Presencia (% placas)**	χ^2	**gl**	***p***
Rhizopus	Campaña	95-96	1,77	12,259	1	0,0005
		96-97	4,64			
	Muestreo	1	2,04	10,333	1	0,0013
		2	4,72			
	Campo	AL	4,17	11,316	5	0,0455
		BE	2,92			
		JE	0,00			
		MI	2,92			
		MV	5,00			
		PB	1,25			
	Orientación	Sol	3,22	0,073	1	0,7860
		Sombra	3,00			
	Altura	Baja	4,11	5,971	1	0,0145
		Alta	2,11			
Mucor	Campaña	95-96	5,83	0,011	1	0,9148
		96-97	5,95			
	Muestreo	1	4,17	14,450	1	0,0001
		2	8,47			
	Campo	AL	8,13	16,267	5	0,0061
		BE	2,92			
		JE	0,83			
		MI	7,08			
		MV	6,25			
		PB	4,17			
	Orientación	Sol	5,67	0,160	1	0,6888
		Sombra	6,11			
	Altura	Baja	8,44	21,211	1	<0,0001
		Alta	3,33			

La población fúngica total se correlacionó positiva y débilmente con la temperatura, la precipitación y la HR ($r < 0,6$; Tabla 2). La población de *Penicillium* se correlacionó negativa y débilmente con la temperatura y la precipitación y no se correlacionó con la HR (Tabla 2).

El análisis de la varianza multifactorial de la frecuencia fúngica total y de la frecuencia de *Penicillium* indicó que, para ambas variables, las diferencias entre muestreos y entre campos fueron significativas ($p < 0,0001$; análisis no presentados). Ante la significación de las interacciones entre los factores campaña y muestreo ($p = 0,0224$) y campaña y campo ($p = 0,0002$) para la frecuencia total, se analizó la variabilidad entre muestreos de esta frecuencia para cada campaña y campo por separado, excepto para los campos JE y BE (análisis no presentados). En la campaña 1995-96, la población fúngica ambiental total disminuyó significativamente en el segundo muestreo respecto al primero tanto en el campo MI ($p = 0,0083$) como en el campo PB ($p < 0,0001$), ambos en la zona de Amposta (Fig. 5A). En la campaña 1996-97, sin embargo, no se hallaron diferencias significativas entre los dos muestreos para ningún campo ($p = 0,1300$, 0,0898 y 0,9618 para los campos AL, MI y MV respectivamente) (Fig. 5B). Para la variable frecuencia de *Penicillium*, las interacciones en el análisis multifactorial resultaron no significativas ($p = 0,1977$ para la interacción campaña por muestreo y $p = 0,9352$ para la interacción campaña por campo). En las dos campañas y en todos los campos hubo un incremento significativo de la población ambiental de *Penicillium* en el segundo muestreo respecto al primero (Fig. 5).

Por otra parte, y con el fin de caracterizar las diferencias entre zonas, las variables frecuencia fúngica total y frecuencia del género *Penicillium* se analizaron también en función del factor campo para cada muestreo de cada campaña por separado (análisis no presentados). En el primer muestreo de la primera campaña, la población fúngica total en el ambiente en el campo de producción biológica (PB, zona de Amposta) fue, con una media de 297,8 ufc/placa, significativamente mayor ($p = 0,0004$) que en el resto de los campos muestreados. Más del 99% de estas colonias correspondieron al género *Cladosporium* (Fig. 5A-1). En el segundo muestreo, la frecuencia total en este campo se redujo a tan sólo 4,6 ufc/placa (Fig. 5A-2). En el primer muestreo de la segunda campaña, como también ocurrió con la micoflora epífita, los niveles más altos de población se dieron en la zona de Benifallet (campo BE) (Fig. 5B-1). En el segundo muestreo, los niveles fueron superiores en la zona de Amposta (campos MI y MV) que en la de Alcanar (campo AL) (Fig. 5B-2). El aumento significativo de la población de *Penicillium* en el segundo muestreo de las dos campañas fue común para todos los campos, y en el campo MI el primer año (Fig. 5A-2) y en el campo AL el segundo (Fig. 5B-2), su frecuencia llegó a superar a la de *Cladosporium*.

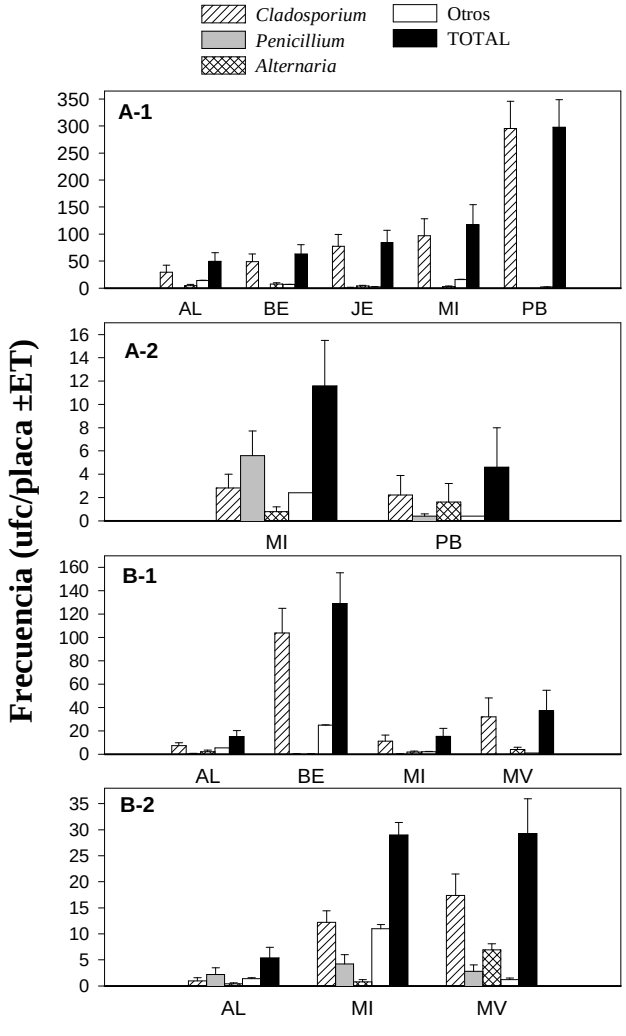

Fig. 5. Frecuencia de distintos géneros fúngicos en el ambiente de campos de mandarino Clemenules de la zona citrícola de Tarragona en el primer (1) y en el segundo (2) muestreo realizado en el período de recolección de las campañas 95-96 (A) y 96-97 (B).

Discusión

Los resultados obtenidos deben evaluarse teniendo en cuenta el método de muestreo utilizado. Con el sistema de impresión se pueden recoger esporas presentes en la superficie del fruto pero no se pueden detectar directamente infecciones latentes y quiescentes. Por otra parte, entre las esporas efectivamente recogidas puede ocurrir que debido a las distintas velocidad y características del crecimiento, la presencia de unos hongos enmascare la de otros. Además, el medio de cultivo empleado también puede favorecer el crecimiento de unos hongos sobre el de otros. Todo ello podría explicar el no aislamiento de algunos hongos como *Sclerotinia* o *Colletotrichum*, éste último presente en la mayoría de huertos de cítricos (Whiteside *et al*., 1993). Por otro lado, el método no resulta adecuado para detectar inóculo de patógenos como *Phytophthora* o *Geotrichum,* puesto que éste se encuentra básicamente en el suelo y llega sobre todo a los frutos más bajos del árbol por salpicaduras provocadas por el agua de lluvia. En este trabajo los muestreos se realizaron con los campos secos, se evitaron los frutos extremos y no se muestrearon frutos sucios.

La micoflora epífita total en los frutos de mandarino fue superior en el primer muestreo que en el segundo tanto en la campaña 1995-96 como en la 1996-97. La población fúngica ambiental total también lo fue en la campaña 1995-96. Teniendo en cuenta las correlaciones halladas entre la población total y los parámetros meteorológicos, especialmente la temperatura, esta diferencia podría atribuirse al hecho de que en la primera campaña, tanto las temperaturas como la precipitación fueron, en las tres zonas, más bajas durante el período correspondiente al segundo muestreo, de enero a febrero. Asimismo, la HR ambiental sufrió un descenso a partir de principios del mes de enero. Esta evolución de las condiciones meteorológicas parece que influyó decisivamente no sólo en la cantidad sino también en la composición de la carga fúngica. En el segundo muestreo descendió la proporción de *Cladosporium*, máximo exponente de la población fúngica total, y aumentó la de la mayoría de los otros géneros aislados, especialmente la de *Penicillium*, cuya frecuencia se correlacionó significativa y negativamente con la temperatura tanto en el ambiente como en la superficie de los frutos.

Tanto en la superficie de los frutos como en el ambiente *Cladosporium* fue el género más abundante, con independencia de la campaña, el muestreo y el campo. Aunque la especie *Cladosporium herbarum* (Pers.) Link se ha descrito como capaz de producir podredumbre en post-cosecha (Tuset, 1987), no se trata de un patógeno importante de los cítricos, ni de las hojas o los frutos en campo, ni en post-cosecha. Es un hongo saprófito mejor adaptado a la diseminación de las esporas en condiciones de sequedad que otros habitantes de la filosfera, como pueden ser *Alternaria* o *Botrytis* (Dowding, 1986). Teixidó *et al.* (1999) encontraron que *Cladosporium* era el género más frecuente en los distintos estadíos de desarrollo de manzanas 'Golden Delicious' en campos de la zona de Lleida. Trabajos propios en preparación y de Díaz y Vila (1987, 1988b, 1989) apuntan que *Cladosporium* también es uno de los géneros fúngicos de mayor frecuencia en el ambiente y en la superficie de instalaciones en centrales citrícolas españolas.

Entre los patógenos de post-cosecha con importancia económica, *Penicillium* y *Alternaria* fueron los aislados con mayor frecuencia. En un trabajo similar, Usall y Viñas (1989) encontraron que *Alternaria*, y *Cladosporium* en segundo lugar, eran los géneros de mayor presencia en la superficie de manzanas Golden Delicious muestreadas en campos de la zona de Lleida en el período previo a la recolección. *Penicillium* se halló en proporciones insignificantes, por lo que se concluyó que la contaminación de la fruta por parte de *Penicillium expansum* Link, principal hongo patógeno en frigoconservación de fruta dulce, tenía lugar en el almacén. Contrariamente, la presencia en el campo de *P. digitatum* y *P. italicum*, patógenos causantes de las mayores pérdidas en post-cosecha de cítricos, ha sido documentada por distintos autores (Barkai-Golan, 1966; Roth, 1967). Las podredumbres verde y azul, causadas por *P. digitatum* y *P. italicum* respectivamente, son también las principales enfermedades causantes de pérdidas económicas en post-cosecha de mandarinas en Tarragona. En este estudio se constató mediante observación microscópica que tanto *P. digitatum* como *P. italicum* estaban presentes tanto en placas con las que se muestreó la superficie de frutos como en placas con las que se muestreó el ambiente. Como ya se ha comentado, la frecuencia de *Penicillium* y también la de la mayoría de los otros géneros distintos de *Cladosporium* fue, tanto en la superficie de los frutos como en el ambiente, comparativamente mayor en el segundo muestreo que en el primero. Este resultado es importante puesto que a un presumible nivel de inóculo patogénico mayor debe añadirse el hecho de que los frutos llevan más tiempo maduros en el árbol, con lo cual aumenta el porcentaje de frutos afectados por distintos tipos de dermatosis. Estos frutos son extremadamente susceptibles a las infecciones fúngicas, especialmente a las de *Penicillium*, *Colletotrichum* y

Botrytis, y ello aumenta considerablemente el riesgo de una incidencia alta de podridos en post-cosecha (Tuset *et al*., 1997). *Rhizopus*, que a diferencia de *Mucor* sí puede ocasionar pérdidas severas de producto comercializable en post-cosecha, también apareció más frecuentemente en el segundo muestreo que en el primero.

En un trabajo en preparación complementario al presente, se constató la presencia generalizada en centrales citrícolas de la zona de Tarragona de cepas de *Penicillium* spp. resistentes a los fungicidas tiabendazol e imazalil. La gran mayoría de las cepas resistentes fueron de especies de *Penicillium* distintas de *P. digitatum* y *P. italicum*, aunque también se encontraron aislados de estas especies resistentes a ambos fungicidas. señalaron a *P. variabile*, *P. steckii*, y *P. velutinum* como especies de *Penicillium* con cepas resistentes al imazalil presentes en almacenes de cítricos valencianos. La importante presencia de *Penicillium* detectada en los campos de mandarino de Tarragona, especialmente cuando el período de recolección está avanzado, sugiere el campo como primera fuente de inóculo de estas cepas resistentes.

Los resultados de este estudio de las poblaciones fúngicas en campo, junto a la determinación de la carga fúngica en las centrales citrícolas, debe ser tenido en cuenta en la planificación de los medios más adecuados de lucha contra las enfermedades de la post-recolección en la zona de Tarragona. En este sentido también es importante comprobar si existen en las centrales de la zona, como ocurre en almacenes de la zona citrícola de Valencia (Díaz y Vila, 1988a), cepas fúngicas resistentes a los fungicidas de post-cosecha. Toda esta información aún cobra más importancia en el contexto de la producción integrada, en el que debe priorizarse, por un lado, el establecimiento de métodos preventivos de control y, por otro, la búsqueda de sistemas curativos sustitutivos de los fungicidas sintéticos (Usall *et al*., 1999; Palou *et al*., 1999).

Agradecimientos

Los autores agradecen la financiación de la CIRIT (Generalitat de Catalunya) y del Grup Exportador de Cítrics del Baix Ebre i Montsià, así como la colaboración de Miquel Reverter y M. Carme Cerdà.

Referencias bibliográficas

BARKAI-GOLAN R., 1966. Reinfestation of citrus fruits by pathogenic fungi in packing house. Isr. J. Agr. Res. 16, 133-138.

DÍAZ M.A., VILA R., 1987. Estudio de flora fúngica presente en cámaras frigoríficas de conservación de frutos cítricos. Alimentaria 183, 77-82.

DÍAZ M.A., VILA R., 1988a. El problema de la resistencia a los fungicidas: referencia a la situación en los almacenes españoles de comercialización de cítricos. Rev. Agroquím. Tecnol. Aliment. 28: 151-158.

DÍAZ M.A., VILA R., 1988b. Evolución de la flora fúngica durante la desverdización en almacenes españoles de comercialización de cítricos. I. Flora presente en las cámaras de desverdización. Rev. Agroquím. Tecnol. Aliment. 28, 501-508.

DÍAZ M.A., VILA R., 1989. Evolución de la flora fúngica durante la desverdización en almacenes españoles de comercialización de cítricos. II. Flora presente en la superficie de la fruta. Rev. Agroquím. Tecnol. Aliment. 29, 85-89.

DOWDING P., 1986. Water availability, the distribution of fungi and their adaptation to the environment. En: Water, fungi and plants. Ayres, P.G., Boddy, L., eds. Cambridge University Press. Cambridge, GB. pp. 305-320.

ECKERT J.W., BROWN G.E., 1986. Postharvest citrus diseases and their control. En: Fresh Citrus Fruits. Wardowski, W.F., Nagy, S., Grierson, W. eds. AVI Publising Co., Inc. Westport, CT, EE UU. pp. 315-360.

ECKERT J.W., EAKS I.L., 1989. Postharvest disorders and diseases of citrus fruits. En: The Citrus Industry. Vol. 5. Reuter, W., Calavan, E.C., Carman, G.E. eds. University of California Press. Berkeley, CA, EE UU. pp. 179-260.

ELLIS M.B., 1993a. Dematiaceous hyphomycetes. 6ª ed. CAB International. Wallingford, GB. 608 pp.

ELLIS M.B., 1993b. More dematiaceous hyphomycetes. 4ª ed. CAB International. Wallingford, GB. 507 pp.

PALOU L., USALL J., AGUILAR M.J., PONS J., VIÑAS I., 1999. Control de la podredumbre verde de los cítricos mediante baños con agua caliente y carbonatos sódicos. Levante Agrícola 348, 412-421.

ROTH G., 1967. Citrus fruit decay in South Africa caused by *Penicillium digitatum* Sacc. Phytopathol. Z. 58, 383-396.

SAMSON R.A., HOEKSTRA E.S., FRISVAD J.C., FILTENBORG O., eds., 1995. Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures. Baarn, Holanda. 322 pp.

TEIXIDÓ N., USALL J., MAGAN N., VIÑAS I., 1999. Microbial population dynamics on Golden Delicious apples from bud to harvest and effect of fungicide applications. Ann. Appl. Biol. 134, 109-116.

TUSET J.J., 1987. Podredumbres de los frutos cítricos. Conselleria d'Agricultura i Pesca. Generalitat Valenciana. Valencia. 206 pp.

TUSET J.J., HINAREJOS C., MIRA J.L., 1997. Enfermedades fúngicas de la post-recolección de los agrios actualmente en progreso. Phytoma-España 90, 69-76.

USALL J., PONS J., PALOU L., VIÑAS I., SMILANICK J.L., 1999. Alternativas a los productos químicos de síntesis en post-cosecha de cítricos en España y EE UU. Phytoma-España 110, 58-64.

USALL J., VIÑAS I., 1989. Contaminació fúngica en pre-recol.lecció en pomes destinades a frigoconservació de la comarca del Segrià. Frut 4, 250-253.

WEBSTER, J. 1980. Introduction to fungi. 2ª ed. Cambridge University Press. Cambridge, GB. 669 pp.

WHITESIDE J.O., GARNSEY S.M., TIMMER L.W., eds., 1993. Compendium of citrus diseases. 2ª ed. APS Press. St. Paul, MN, EE UU. 80 pp.

Capítol 2

Micoflora en Centrales Citrícolas de Tarragona

L. Palou[1], J. Usall[1], J. Pons[2], M.C. Cerdà[1], I. Viñas[1]

[1] *Àrea de Postcollita, CeRTA, Centre UdL-IRTA, Lleida*
[2] *Estació Experimental de l'Ebre, IRTA, Amposta, Tarragona*

Referència: *Invest. Agr.: Prod. Prot. Veg.* En premsa.

Resumen

Se caracterizó la micoflora ambiental y de la superficie de equipos e instalaciones en centrales citrícolas de Tarragona. Durante las campañas 1995-96 y 1996-97 se realizaron tres muestreos a lo largo del período de procesamiento de mandarinas en ocho centrales con un total de 18 cámaras frigoríficas. La flora fúngica, debida mayoritariamente a los géneros *Cladosporium* y *Penicillium*, fue muy elevada durante toda la campaña (medias de 25 a 50 ufc/placa). Mientras que *Cladosporium* fue el género más abundante en el primer muestreo (55-60% de las colonias aisladas), la población de *Penicillium* fue aumentando en los muestreos posteriores, especialmente en las superficies de las líneas de confección y de las cámaras frigoríficas, donde llegó a superar a la de *Cladosporium* (40-65% de las colonias aisladas). La presencia del género *Rhizopus* fue generalizada y particularmente importante (35-55%) en la superficie de los envases y líneas de confección. El 33% de las cepas del género *Penicillium* aisladas del ambiente en cinco centrales con 14 cámaras frigoríficas resultaron resistentes al tiabendazol y el 5% al imazalil. De las aisladas de las superficies resultaron resistentes el 35% y el 20% respectivamente. Se encontraron cepas tanto de *P. digitatum* como de *P. italicum* resistentes a ambos fungicidas, aunque su frecuencia fue baja en comparación con la de otras especies de *Penicillium* resistentes. Ante los altos niveles fúngicos detectados y la presencia de cepas de *Penicillium* spp. resistentes, se impone una adecuación e intensificación de las labores de higienización, especialmente en las líneas de confección.

Palabras clave: cítricos, enfermedades de post-cosecha, *Penicillium*, resistencia a fungicidas, higienización

Summary

Fungal populations in Tarragona citrus packinghouses

Fungal populations in the atmosphere and on surfaces of equipment and facilities were examined in Tarragona citrus packinghouses (Spain). In 1995-96 and 1996-97 seasons, sampling was conducted three times during the mandarin processing period in eight packinghouses with 18 cold storage rooms. Total fungal population, mostly due to the genera *Cladosporium* and *Penicillium*, was very high over the entire sampling period (average from 25 to 50 cfu/plate). While *Cladosporium* was the most frequent genus present at the first sampling (55-60% of isolated colonies), the frequency of *Penicillium* increased at the subsequent samplings, especially on the surfaces of packinglines and cold storage rooms, where it reached even higher levels than *Cladosporium* (40-65% of isolated colonies). The presence of *Rhizopus* was general and particularly high (35-55%) on surfaces of bins and packinglines. In average, 33% of the *Penicillium* spp. strains isolated from the atmosphere of five local packinghouses with 14 cold storage rooms were thiabendazole-resistant strains and 5% were imazalil-resistant strains. Resistant strains isolated from surfaces were 35% and 20%, respectively. Strains of both *P. digitatum* and *P. italicum* resistant to either thiabendazole or imazalil were found, although their frequency was lower than that of other resistant *Penicillium* spp. Before the high fungal populations detected and the presence of fungicide-resistant biotypes, effective sanitation tasks, especially in the packinglines, are required.

Key words: postharvest diseases, *Penicillium*, fungicide resistance, sanitation

Introducción

Las pérdidas económicas ocasionadas por las enfermedades de post-recolección representan uno de los principales problemas de la citricultura española y mundial (Tuset, 1987; Eckert y Eaks, 1989). Varios factores relacionados con el fruto, el patógeno, las condiciones climatológicas y las condiciones en post-cosecha determinan la incidencia y la severidad de estas enfermedades (Eckert y Eaks, 1989). Entre ellos, la cantidad y la calidad del inóculo fúngico ocupan un lugar destacado. La probabilidad de infección depende de la cantidad de inóculo presente en un punto del fruto susceptible de ser infectado. Esta relación fue demostrada para patógenos tan significativos como *Penicillium digitatum* (Pers.: Fr.) Sacc. (Wild y Eckert, 1982), *Geotrichum candidum* Link: Pers. (Baudoin y Eckert, 1982) o *Colletotrichum gloeosporioides* (Penz.) Sacc. (Brown, 1975). Según esto, y con independencia de otros factores, la cantidad

de esporas presentes en la central citrícola influye decisivamente en los niveles de pudrición, especialmente en los causados por patógenos de herida como *P. digitatum* o *P. italicum* Wehmer, causantes respectivamente de las podredumbres verde y azul, las enfermedades de post-cosecha de cítricos de mayor impacto económico tanto en España (Tuset, 1987) como en todo el mundo (Eckert y Eaks, 1989).

Los problemas derivados de altos niveles de población fúngica en las centrales se ven significativamente incrementados cuando existen cepas de los patógenos resistentes a los fungicidas sintéticos. Aunque en post-cosecha de cítricos ya se habían detectado casos de resistencia con anterioridad (Duran y Norman, 1961), fue en los años 70 cuando se puso de manifiesto en EE UU la importancia del problema de las resistencias (Houck, 1977). Desde entonces, multitud de estudios han constatado que el fenómeno de las resistencias es una de las causas principales del fracaso de muchos tratamientos fungicidas de post-cosecha. La gran mayoría de estos estudios se han referido, por su importancia económica, a *P. digitatum* y *P. italicum* frente a los fungicidas del grupo de los bencimidazoles, el ortofenilfenato sódico y el imazalil, y se han descrito casos tanto de resistencia simple como de resistencia cruzada y múltiple (Brown, 1982; Eckert, 1990; Bus *et al.*, 1991). En España, concretamente en la zona de Valencia, se estudió en la década de los 80 la presencia y características de cepas de *Penicillium* spp. resistentes a diversos fungicidas (Díaz *et al.*, 1987a, 1987b, Díaz y Vila, 1988a, 1989).

A medida que se ha ido cuestionando la aplicación de fungicidas sintéticos para el control de enfermedades en post-cosecha, por la problemática de salud y el efecto medioambiental que conllevan, y se ha ido desarrollando el concepto de producción integrada, ha ido aumentando la importancia de los métodos de control preventivos. Entre ellos pueden citarse los tratamientos culturales y/o fungicidas en campo, los tratamientos para el mantenimiento o la mejora de la resistencia intrínseca de los frutos a la infección y la correcta higienización de las centrales citrícolas (Brown, 1980; Eckert y Eaks, 1989). Identificar y cuantificar la contaminación fúngica presente en las distintas partes de la central citrícola a lo largo de la campaña de comercialización, así como estudiar la presencia de cepas resistentes, es importante para establecer riesgos potenciales de pérdidas y diseñar sistemas adecuados de control. El conocimiento de las áreas y puntos concretos del almacén donde se dan los mayores niveles de contaminación permite adecuar los programas de limpieza y desinfección y optimizar los recursos, y también puede resultar de gran interés en el diseño de nuevas centrales o en el de ampliaciones de infraestructuras ya existentes (Gardner *et al.*, 1986). La composición y abundancia de la flora fúngica presente en las centrales citrícolas depende de las condiciones locales de cada zona productora (Brown, 1990).

Los objetivos de este trabajo fueron cuantificar y caracterizar: 1) la micoflora presente en el ambiente y en la superficie de equipos e instalaciones en centrales citrícolas de la zona de Tarragona a lo largo de la campaña de comercialización de mandarinas y 2) las cepas de *Penicillium* spp. resistentes a los fungicidas tiabendazol e imazalil.

Material y métodos

Caracterización de la micoflora. Se muestreó durante dos campañas consecutivas (1995-96 y 1996-97) la flora fúngica del ambiente y de la superficie de equipos e instalaciones en centrales citrícolas de las comarcas del Baix Ebre y Montsià (Tarragona). Cada campaña se muestrearon ocho centrales, con un total de 18 cámaras frigoríficas. En cada central se muestreó la micoflora ambiental de las siguientes zonas: zona de recepción (RE), o de llegada de la fruta procedente del campo; zona de confección (CO), o área de ubicación de las líneas de confección; y cámaras frigoríficas (CA). Se muestrearon las siguientes superficies: envases (EN), normalmente palox o cajas de plástico; zona inicial (CO-1, zona de despoblación y primera selección manual de la fruta), zona central (CO-2, zona de segunda selección manual, después de la aplicación de fungicidas y ceras y del secado), y zona final (CO-3, zona de envasado de la fruta confeccionada) de las líneas de confección; y paredes (CA-P) y suelo (CA-S) de las cámaras frigoríficas. Cada campaña se realizaron tres muestreos a lo largo del período de procesamiento de mandarinas en las centrales, el primero a mediados de octubre, el segundo en los meses de noviembre-diciembre y el tercero en enero-febrero.

La micoflora ambiental de cada zona se muestreó mediante el método gravimétrico. Cinco placas Petri de 9 cm de diámetro (repeticiones) con medio patata dextrosa agar (PDA) se distribuyeron equidistantemente por la zona y se abrieron durante 2 min para que las esporas fúngicas se depositasen por gravedad sobre el medio de cultivo. En las cámaras, siempre que fue posible, se colocó una placa en

cada esquina y una en el centro. Las superficies se muestrearon mediante placas Rodac (Replicant Organism Direct Agar Contact) de 5,5 cm de diámetro con medio PDA. El medio, sobresaliendo ligeramente por encima del plástico de la placa, se ponía en contacto con la superficie realizando una ligera presión para que las esporas quedasen adheridas al mismo. Se utilizaron cinco placas (repeticiones) en los envases, dos en cada una de las tres partes de las líneas de confección, una en cada una de las cuatro paredes de las cámaras y una en el suelo de éstas. Las placas se incubaron en una cámara climatizada a 20ºC y 90% de humedad relativa (HR) durante 7 días, transcurridos los cuales se procedió al recuento e identificación de las colonias fúngicas. La identificación se hizo a nivel de género y, cuando fue necesario, se realizó mediante observación microscópica (Webster, 1980; Ellis, 1993a, 1993b; Samson *et al.*, 1995). La frecuencia de cada género fúngico se expresó como número de unidades formadoras de colonias por placa (ufc/placa), con la excepción de los géneros *Rhizopus* y *Mucor*, con los que, por su distinto hábito de crecimiento, se establecieron dos categorías excluyentes, presencia y ausencia, y cada placa se clasificó como perteneciente a una de las dos.

Determinación de cepas de *Penicillium* spp. resistentes a funguicidas. Se determinó la presencia de aislados de *Penicillium* spp. resistentes a los fungicidas tiabendazol (TBZ) e imazalil (IZ) en el ambiente y en la superficie de instalaciones e infraestructuras en centrales citrícolas de las comarcas del Baix Ebre y Montsià. Hacia finales de la campaña global de cítricos (mes de mayo), se muestrearon cinco centrales con un total de 14 cámaras frigoríficas. Para la toma de muestras, se prepararon placas Petri y Rodac con tres tipos distintos de medio de cultivo: PDA (control), PDA con 10 ppm de materia activa (m.a.) de tiabendazol (PDA+TBZ) y PDA con 1 ppm m.a. de imazalil (PDA+IZ). Estas dosis de fungicida (Brown, 1990) corresponden a las llamadas dosis de resistencia, obtenidas experimentalmente, y a las cuales se considera que únicamente las cepas resistentes son capaces de crecer. El tiabendazol (Tecto 60® WP, Tecnidex) y el imazalil (Fecundal 50® EC, Janssen) se añadieron al medio estéril a unos 40ºC en el interior de una cabina de flujo laminar. La toma de muestras y la incubación de las placas se realizaron de la forma descrita en el subapartado anterior, sólo que en este caso cada repetición estuvo constituida por tres placas, una de cada tipo. El muestreo del ambiente se realizó en las cámaras frigoríficas (CA) y en la zona de ubicación de la línea de confección, considerando ahí dos subzonas: principio de la línea o zona sucia (CO-1) y final de la línea o zona limpia (CO-2). Se utilizaron cuatro repeticiones por cámara frigorífica y por subzona. Las superficies se muestrearon en cada una de estas dos subzonas y en las paredes (CA-P) y el suelo (CA-S) de las cámaras frigoríficas. Se utilizaron tres repeticiones en cada subzona, una repetición en cada una de las cuatro paredes de las cámaras y una en el suelo. En el proceso de recuento se diferenciaron tres grupos, identificando las colonias visualmente: *P. digitatum*, *P. italicum*, y otras *Penicillium* spp.

Análisis estadístico. Se realizó con el paquete estadístico SAS (SAS Institute Inc., Cary, NC, EE UU). En el estudio de la micoflora, se calcularon las frecuencias relativas de cada género fúngico en función de la población fúngica total y se sometieron a un análisis de la varianza bifactorial (factores muestreo y zona o superficie) tomando las campañas y las centrales como repeticiones. Para las dos variables dependientes más importantes, población fúngica total y población de *Penicillium*, se estudiaron los supuestos de normalidad y homocedasticidad de los datos. A raiz de este estudio, los datos de frecuencia absoluta se transformaron al logaritmo neperiano de ufc/placa+1. Los datos transformados se sometieron al análisis de la varianza con los factores muestreo y zona (o superficie) como fijos y los factores campaña y central como repeticiones. Las separaciones de medias se realizaron mediante la prueba de la Mínima Diferencia Significativa (MDS) protegida de Fisher ($p = 0,05$).

En el caso de los géneros *Rhizopus* y *Mucor*, los datos de las dos campañas y de todas las centrales se categorizaron en ausencia o presencia de los hongos y se contrastaron con las variables explicativas muestreo y zona (o superficie) mediante tablas de contingencia. La independencia entre variables se determinó mediante la prueba de Chi-cuadrado (χ^2, $p = 0,05$).

En el estudio de las cepas resistentes, los datos, expresados como ufc/placa, se transformaron al logaritmo neperiano de ufc/placa+1 y los de cada tipo de placa (PDA, PDA+TBZ y PDA+IZ) se sometieron a un análisis de la varianza para estudiar posibles diferencias entre zonas (o superficies). La separación de medias se realizó mediante la prueba de la MDS protegida de Fisher ($p = 0,05$).

Resultados

Micoflora ambiental. La media del número total de colonias fúngicas por placa que se aislaron del ambiente en el conjunto de las centrales durante las dos campañas fue de 24,5 ufc/placa en el primer

muestreo, de 39,6 en el segundo y de 31,9 en el tercero. En el primer muestreo, el 48,4% de las colonias aisladas correspondieron al género *Cladosporium*, el 23,7% al género *Penicillium*, el 9,5% al género *Aspergillus*, el 6,6% al género *Alternaria*, el 4,1% al género *Fusarium,* el 3,4% al género *Trichoderma,* y el resto (4,3%) a géneros como *Epicoccum*, *Humicola*, *Botrytis*, *Geotrichum* y otros (datos no presentados). Aunque esta distribución fue similar para las tres zonas muestreadas, la mayor frecuencia relativa de *Cladosporium* se dió en RE, seguido de CO y de CA ($p = 0,0315$; Figura 1A). Contrariamente, la frecuencia relativa de *Penicillium* fue mayor en CA, seguido de CO y de RE ($p < 0,0001$). En el segundo muestreo (Figura 1B), el género predominante en todas las zonas pasó a ser *Penicillium*, con un 50,7% de las colonias frente a un 39,4% de colonias de *Cladosporium*. Las diferencias entre zonas no fueron significativas ni para *Penicillium* ($p = 0,3982$) ni para *Cladosporium* ($p = 0,1416$). Las proporciones de *Alternaria*, *Fusarium*, y sobre todo de *Aspergillus*, disminuyeron respecto al primer muestreo. La misma tendencia, aún más acentuada, se observó en el tercer muestreo (Figura 1C). *Penicillium*, con una media del 64,7% de las colonias fue el género más frecuente en las tres zonas, y más en CA que en RE o CO ($p = 0,0276$). *Cladosporium* ocupó el segundo lugar con el 26,6% de las colonias, y *Trichoderma* el tercero con el 3,5%. *Cladosporium* se aisló con una frecuencia significativamente mayor en CO que en RE y CA ($p = 0,0047$).

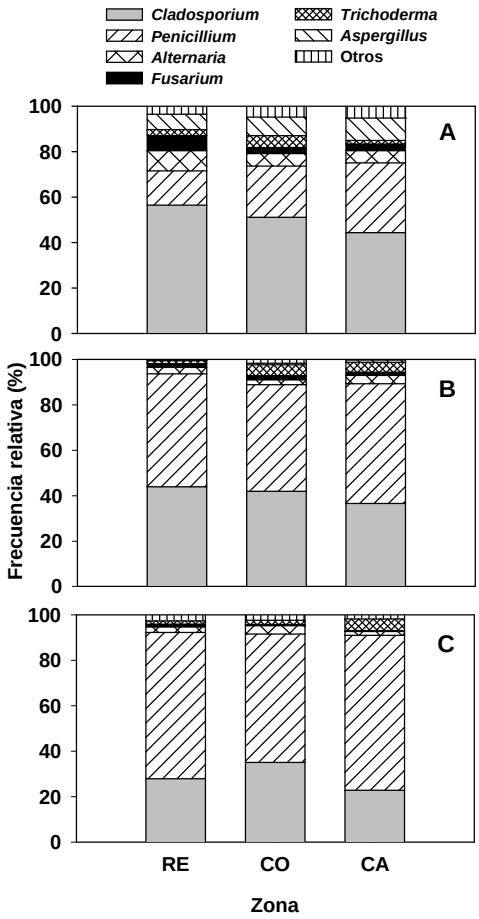

Fig. 1. Frecuencia relativa de los géneros fúngicos aislados en el primer (A), segundo (B) y tercer (C) muestreos del ambiente de la zona de recepción (RE), línea de confección (CO) y cámaras frigoríficas (CA) en centrales citrícolas de Tarragona. Datos medios de dos campañas consecutivas y ocho centrales.

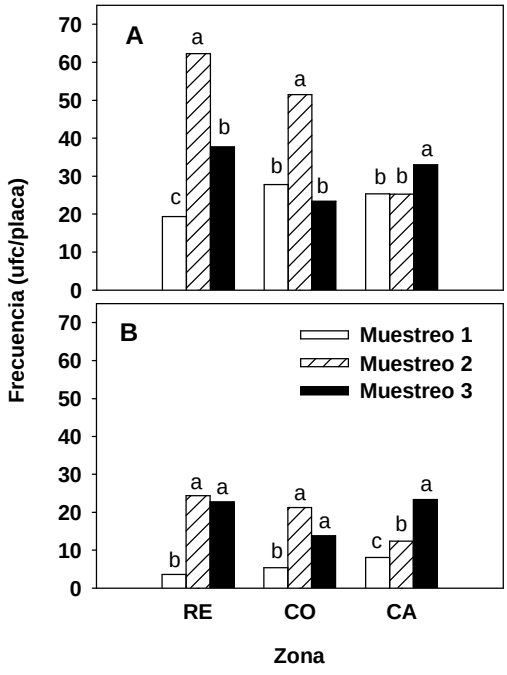

Fig. 2. Población fúngica total (A) y población de *Penicillium* (B) en el ambiente de la zona de recepción (RE), línea de confección (CO) y cámaras frigoríficas (CA) en centrales citrícolas de Tarragona. Datos medios de dos campañas y ocho centrales. Para cada zona, letras distintas indican diferencias significativas entre muestreos según la prueba de la MDS Protegida de Fisher (p = 0,05) aplicada tras el análisis de la varianza del ln(ufc/placa+1). Se presentan las medias no transformadas.

La micoflora total del ambiente resultó, en términos absolutos, significativamente mayor en el segundo muestreo que en el primero y el tercero en las zonas RE y CO, pero no en CA, donde resultó mayor en el tercer muestreo (Figura 2A). La población de *Penicillium* fue siempre mayor en los dos últimos muestreos que en el primero, y en CA fue significativamente mayor en el tercero que en el segundo (Figura 2B).

La presencia del género *Rhizopus* en el ambiente fue generalizada, aunque dependió tanto del muestreo como de la zona muestreada. Su presencia fue mayor en el segundo muestreo (16,8% de placas con presencia) y menor en la zona CA (7,5%) (Tabla 1). La presencia del género *Mucor* fue independiente de muestreos y zonas (Tabla 1).

Micoflora de las superficies. En el muestreo de las superficies, la media del número total de colonias fúngicas por placa en el conjunto de las centrales durante las dos campañas fue de 43,9 ufc/placa en el primer muestreo, de 40,2 en el segundo y de 49,2 en el tercero. En el primer muestreo, el 61,3% de las colonias aisladas correspondieron al género *Cladosporium*, el 20,1% al género *Penicillium*, el 5,9% al género *Trichoderma*, el 5,2% al género *Alternaria*, el 3,8% al género *Fusarium,* el 1,5% al género *Aspergillus,* y el resto (2,2%) a otros géneros como *Epicoccum* y *Humicola* (datos no presentados). Por superficies, *Cladosporium* fue significativamente más abundante en CO-1 y CO-3 (p = 0,0036) y *Penicillium* en EN, CA-P y CA-S (p = 0,0007; Figura 3A). En el segundo muestreo (Figura 3B), *Cladosporium* siguió siendo el género más frecuente, con el 49,9% de las colonias, especialmente en CA-S y EN (p = 0,0044), pero la frecuencia relativa de *Penicillium* aumentó en todas las superficies (37,5% de las colonias), y no se encontraron diferencias significativas entre ellas (p = 0,0664). *Fusarium* fue el tercer género más frecuente (6,3%). En el tercer muestreo (Figura 3C), y con la excepción de EN, la frecuencia de *Penicillium* superó ligeramente a la de *Cladosporium* en todas las superficies muestreadas (medias del 48,1% y 47,6% de las colonias, respectivamente). Los niveles tanto de *Penicillium* como de *Cladosporium* no fueron significativamente diferentes en las distintas superficies muestreadas (p = 0,1271 y 0,0647 respectivamente). La frecuencia relativa de los géneros restantes disminuyó hasta niveles inferiores al 1% de las colonias aisladas.

La micoflora total dependió fuertemente de la superficie muestreada y presentó un alto grado de variabilidad en los tres muestreos. Las frecuencias más elevadas se observaron en EN y CO-1 (Figura 4A). La frecuencia absoluta de *Penicillium* (Figura 4B) aumentó significativamente a partir del primer muestreo en todas las zonas excepto en EN y CA-S. El mayor incremento se dio en CO-1.

La presencia del género *Rhizopus* en las superficies no dependió del muestreo pero sí de la superficie muestreada. Se localizó con abundancia significativamente mayor en CO (50-56% de las placas) que en EN y CA (Tabla 1). La presencia de *Mucor* dependió tanto del muestreo como de la superficie muestreada. Fue más abundante en el tercer muestreo (14,4% de las placas) y en CA-S (20,2%) (Tabla 1).

Tabla 1. Presencia de los géneros *Rhizopus* y *Mucor* en el ambiente y en la superficie de equipos e instalaciones en centrales citrícolas de Tarragona. Datos medios de dos campañas consecutivas y ocho centrales. Pruebas Chi-cuadrado de independencia entre la presencia de los hongos y las variables explicativas consideradas (p = 0,05)

Género fúngico	Variable	Nivel	Presencia (% placas)	χ^2	gl	*P*
Ambiente						
Rhizopus	Muestreo	1	9,7	10,3654	2	0,0056
		2	16,8			
		3	8,3			
	Zona	RE	17,3	16,6365	2	0,0002
		CO	16,4			
		CA	7,5			
Mucor	Muestreo	1	6,8	0,4957	2	0,7805
		2	8,1			
		3	6,7			
	Zona	RE	10,3	4,2120	2	0,1217
		CO	7,7			
		CA	5,6			
Superficies						
Rhizopus	Muestreo	1	32,3	4,1916	2	0,1230
		2	29,3			
		3	38,1			
	Superficie	EN	34,7	101,6094	5	<0,0001
		CO-1	56,1			
		CO-2	54,9			
		CO-3	50,0			
		CA-P	14,6			
		CA-S	39,3			
Mucor	Muestreo	1	5,9	13,3284	2	0,0013
		2	6,7			
		3	14,4			
	Superficie	EN	4,1	31,4096	5	<0,0001
		CO-1	4,9			
		CO-	13,4			

2	
CO-3	14,1
CA-P	5,4
CA-S	20,2

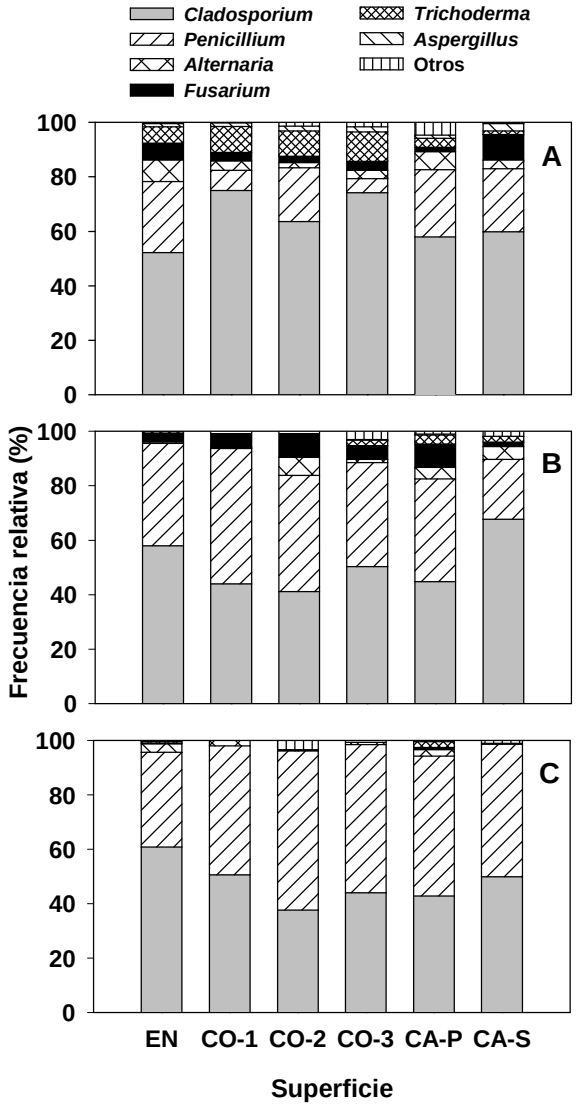

Fig. 3. Frecuencia relativa de los géneros fúngicos aislados en el primer (A), segundo (B) y tercer (C) muestreos de las superficies de envases (EN), zonas inicial (CO-1), central (CO-2) y final (CO-3) de líneas de confección, y paredes (CA-P) y suelo (CA-S) de cámaras frigoríficas en centrales citrícolas de Tarragona. Datos medios de dos campañas consecutivas y ocho centrales.

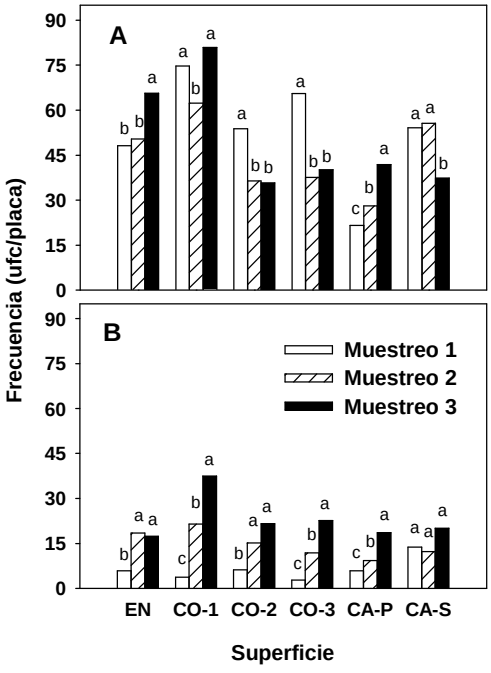

Fig. 4. Población fúngica total (A) y población de *Penicillium* (B) en las superficies de envases (EN), zonas inicial (CO-1), central (CO-2) y final (CO-3) de líneas de confección, y paredes (CA-P) y suelo (CA-S) de cámaras frigoríficas en centrales citrícolas de Tarragona. Datos medios de dos campañas y ocho centrales. Para cada superficie, letras distintas indican diferencias significativas entre muestreos según la prueba de la MDS Protegida de Fisher (p = 0,05) aplicada tras el análisis de la varianza del ln(ufc/placa+1). Se presentan las medias no transformadas.

Cepas de *Penicillium* spp. resistentes. El 33% de las cepas del género *Penicillium* aisladas del ambiente en las cinco centrales muestreadas resultaron resistentes al TBZ (Tabla 2). La frecuencia media de estas cepas fue de 4,8 ufc/placa, de las cuales 0,7 ufc/placa correspondieron a *P. digitatum* (14,6%), 1,5 ufc/placa a *P. italicum* (31,2%) y 2,6 ufc/placa a otras *Penicillium* spp. (54,2%). Las cepas resistentes al IZ fueron el 5%, con una media de 0,8 ufc/placa, de las cuales prácticamente ninguna perteneció a las especies *P. digitatum* o *P. italicum*. La frecuencia de cepas resistentes en el ambiente de la zona de confección no fue significativamente distinta de la de las cámaras frigoríficas (Tabla 2).

Tabla 2. Frecuencia de colonias del género *Penicillium* aisladas en placas Petri con medio PDA, PDA con 10 ppm de tiabendazol (PDA+TBZ) y PDA con 1 ppm de imazalil (PDA+IZ) del ambiente en las partes inicial (CO-1) y final (CO-2) de las líneas de confección y en cámaras frigoríficas (CA) en centrales citrícolas de Tarragona

			Zona			
Cepas de *Penicillium*	**Tipo placa**	**Frecuencia**	**CO-1**	**CO-2**	**CA**	**Total**
P. digitatum	PDA	ufc/placa	3,1 a	2,7 a	0,6 b	1,6
	PDA+TBZ	ufc/placa	1,6 a	1,2 a	0,2 a	0,7
		%[b]	51,6	44,4	33,3	43,7
	PDA+IZ	ufc/placa	0,0 a	0,0 a	0,1 a	≈ 0,0
		%	0,0	0,0	16,7	≈ 0,0

P. italicum	PDA	ufc/placa	12,4 a	4,6 a	9,2 a	8,9
	PDA+TBZ	ufc/placa	3,3 a	2,5 a	0,3 a	1,5
		%	26,6	54,3	3,3	16,8
	PDA+IZ	ufc/placa	0,0 a	0,0 a	0,1 a	≈ 0,0
		%	0,0	0,0	1,1	≈ 0,0
Otras *Penicillium* spp.	PDA	ufc/placa	2,9 a	7,2 a	3,3 a	4,1
	PDA+TBZ	ufc/placa	2,2 a	2,1 a	2,9 a	2,6
		%	75,9	29,2	87,9	63,4
	PDA+IZ	ufc/placa	1,0 a	0,6 a	0,8 a	0,8
		%	34,5	8,3	24,2	19,5
Total *Penicillium* spp.	PDA	ufc/placa	18,4 a	14,5 a	13,1 a	14,6
	PDA+TBZ	ufc/placa	7,1 a	5,8 a	3,4 a	4,8
		%	38,6	40,0	25,9	32,9
	PDA+IZ	ufc/placa	1,0 a	0,6 a	1,0 a	0,8
		%	5,4	4,1	7,6	5,5

[a] Dentro de cada fila, los valores seguidos por letras distintas son diferentes según la prueba de la MDS Protegida de Fisher (p = 0,05), aplicada tras el análisis de la varianza del ln(ufc/placa+1). Se presentan las medias no transformadas.

[b] Frecuencia relativa de colonias fúngicas en las placas con fungicida respecto a las placas control (PDA).

En el muestreo de las superficies, el 35% de las cepas resultaron resistentes al TBZ y el 20% al IZ (Tabla 3). La frecuencia de los aislados de *P. digitatum* y *P. italicum* resistentes a ambos fungicidas fue baja y no se encontraron diferencias significativas entre las distintas superficies muestreadas. La frecuencia de otras *Penicillium* spp. resistentes al TBZ y al IZ fue de 4 y 2,8 ufc/placa (75,5% y 90,3% del total, respectivamente) y resultó significativamente menor en las paredes de las cámaras frigoríficas que en el resto de superficies (Tabla 3).

Tabla 3. Frecuencia de colonias del género *Penicillium* aisladas en placas Rodac con medio PDA, PDA con 10 ppm de tiabendazol (PDA+TBZ) y PDA con 1 ppm de imazalil (PDA+IZ) de la superficie de las partes inicial (CO-1) y final (CO-2) de las líneas de confección y de las paredes (CA-P) y el suelo (CA-S) de cámaras frigoríficas en centrales citrícolas de Tarragona

			Superficie				
Cepas de	**Tipo placa**	**Frecuencia**	**CO-1**	**CO-2**	**CA-P**	**CA-S**	**Total centr**
P. digitatum	PDA	ufc/placa	0,2 a	0,1 a	0,7 a	0,2 a	0,4
	PDA+TBZ	ufc/placa	0,0 a	0,0 a	0,1 a	0,1 a	0,1
		%[b]	0,0	0,0	14,3	50,0	25,0

	PDA+IZ	ufc/placa	0,0 a	0,0 a	0,1 a	0,0 a	0,1
		%	0,0	0,0	14,3	0,0	25,0
P. italicum	PDA	ufc/placa	4,8 a	5,1 a	6,3 a	6,6 a	5,9
	PDA+TBZ	ufc/placa	2,4 a	0,1 a	1,1 a	1,2 a	1,2
		%	50,0	1,9	17,5	18,2	20,3
	PDA+IZ	ufc/placa	0,4 a	0,0 a	0,3 a	0,0 a	0,2
		%	8,3	0,0	4,8	0,0	3,4
Otras *Penicillium* spp.	PDA	ufc/placa	11,7 b	23,7 a	3,0 c	10,6 b	9,0
	PDA+TBZ	ufc/placa	6,8 ab	9,3 a	2,9 b	3,2 b	4,0
		%	58,1	39,2	96,7	30,2	44,4
	PDA+IZ	ufc/placa	2,9 a	5,7 a	0,5 b	3,1 a	2,8
		%	24,8	24,0	16,7	29,2	31,1
Total *Penicillium* spp.	PDA	ufc/placa	16,7 b	28,9 a	10,0 c	17,4 b	15,3
	PDA+TBZ	ufc/placa	9,2 a	9,4 a	4,1 b	4,5 b	5,3
		%	55,1	32,5	41,0	25,9	34,6
	PDA+IZ	ufc/placa	3,3 a	5,7 a	0,9 b	3,1 a	3,1
		%	19,8	19,7	9,0	17,8	20,

[a] Dentro de cada fila, los valores seguidos por letras distintas son diferentes según la prueba de la MDS Protegida de Fisher (p = 0,05), aplicada tras el análisis de la varianza del ln(ufc/placa+1). Se presentan las medias no transformadas.

[b] Frecuencia relativa de colonias fúngicas en las placas con fungicida respecto a las placas control (PDA).

Discusión

Aunque la cantidad de inóculo fúngico influye decisivamente en la incidencia de enfermedades en post-cosecha de cítricos, y de fruta fresca en general, la importancia de otros factores que también afectan a esta incidencia, como son la susceptibilidad intrínseca de los frutos a la infección o las condiciones ambientales, imposibilita el establecimiento de una relación cuantitativa entre los niveles de población fúngica en las centrales y las pérdidas por podredumbres. No existe, por tanto, un criterio general que permita discernir cuáles son los límites críticos de contaminación fúngica a partir de los cuales existe un riesgo alto de que se produzca una incidencia de enfermedades inadmisible. No obstante, desde el punto de vista de la eficacia de los tratamientos de limpieza y desinfección de las centrales, sí han habido intentos de definir cuantitativamente, mediante medidas de los niveles fúngicos, que son zonas sucias y zonas limpias y también de establecer límites críticos que permitan diferenciar entre operaciones de higienización correctas u aceptables e incorrectas o inaceptables (Gardner *et al*., 1986; Sus y Viñas, 1990; Orihuel *et al*., 1996).

En el presente estudio, y desde este punto de vista, el nivel de flora fúngica presente tanto en el ambiente como en la superficie de equipos e instalaciones a lo largo de toda la campaña puede considerarse, en general, muy elevado en todas las zonas y centrales estudiadas. Por ejemplo, mientras que la frecuencia media de la población fúngica total en las superfícies no fue inferior a 40 ufc/placa en ninguno de los tres muestreos (valor equivalente a 1,7 ufc cm^{-2}, puesto que se utilizaron placas Rodac de 5,5 cm de diámetro), el valor propuesto por Orihuel *et al.* (1996) como límite crítico superior que no debe superarse para considerar aceptable la higienización de superficies es 0,7 ufc cm^{-2}. Mientras que *Cladosporium* fue el género más abundante en el primer muestreo, especialmente en la zona de recepción, la frecuencia de *Penicillium* aumentó en el segundo y tercer muestreos hasta superar incluso a la de *Cladosporium*. En un trabajo complementario al presente (Palou *et al*., 2001), en el que se caracterizó la micoflora en campos de mandarino 'Clemenules' de la zona de Tarragona, encontramos que a principios de campaña, en la época correspondiente al primer muestreo, *Cladosporium* era el género más frecuente en el ambiente y en la superficie de las mandarinas, pero que ya avanzado el período de recolección (época correspondiente al segundo y tercer muestreos), la población de *Penicillium* pasaba a ser la más abundante. En ello influían las condiciones meteorológicas. Estos resultados podrían sugerir que el origen de la contaminación fúngica en las centrales citrícolas radica mayoritariamente en la fruta proveniente del campo.

Contrariamente a la de *Cladosporium*, la población de *Penicillium* aumentó en cada muestreo respecto al anterior, especialmente en las superficies de las líneas de confección y de las cámaras frigoríficas, indicando una acumulación de esporas de este género. Ello supuso que la contaminación por *Penicillium* en zonas consideradas 'limpias' (final de la línea de confección o zona de envasado y cámaras frigoríficas) no fuera sustancialmente menor que en zonas consideradas 'sucias' (recepción, zona de primera selección manual y parte de la línea de confección anterior a la de lavado y tratamiento de los frutos). Por tanto, para cumplir el objetivo de reducir la incidencia de las enfermedades de post-cosecha en los almacenes de la zona, especialmente si se pretende trabajar en sistemas de producción integrada, deben considerarse medidas como el posibilitar una separación física efectiva entre la fruta que llega del campo y la ya confeccionada, el facilitar un manejo adecuado de la fruta desechada en las operaciones de selección y, sobre todo, el incrementar o mejorar las labores de higienización. Las ventajas derivadas de la realización de estas acciones en las centrales citrícolas, tanto para reducir la incidencia de enfermedades como para evitar la proliferación de cepas resistentes, han sido extensamente puestas de manifiesto (Hall y Bice, 1977; Bancroft *et al*., 1984; Díaz y Vila, 1988a; Eckert y Eaks, 1989). Gardner *et al.* (1986) indicaron que, al margen de otras consideraciones, una correcta higienización es mucho más eficaz y eficiente económicamente que el incremento del uso de fungicidas.

Aunque la especie *Cladosporium herbarum* (Pers.) Link se ha descrito como parásito ocasional que en algunos casos puede producir podredumbres en post-cosecha (Tuset, 1987), no se trata de un patógeno importante de los cítricos, ni en campo ni en post-cosecha. Es un hongo saprófito muy bien adaptado a la diseminación de las esporas en condiciones de sequedad (Dowding, 1986). Su presencia mayoritaria en las superficies fue constatada por Díaz y Vila (1987, 1988b) en sus recuentos de la micoflora presente en cámaras frigoríficas y de desverdización en centrales citrícolas valencianas. No obstante, y de forma similar a lo que observamos nosotros en los muestreos segundo y tercero, estos autores constataron que *Penicillium* fue el género predominante en el ambiente y que su proporción aumentó a final de campaña

respecto al principio de la misma. En estudios sobre la dinámica poblacional de microorganismos en campos de manzano de la zona de Lleida se observó que *Cladosporium* también era el género fúngico más frecuente en la superficie de la fruta (Teixidó *et al*., 1999).

Otro resultado destacable fueron los elevados niveles poblacionales de *Rhizopus* que se detectaron en los muestreos de las superficies, particularmente en envases y en líneas de confección. *Rhizopus* también fue encontrado de forma generalizada en cámaras frigoríficas y de desverdización de almacenes valencianos donde, al igual que en nuestro estudio de superfícies, sus poblaciones se mantenían más o menos constantes a lo largo de toda la temporada (Díaz y Vila, 1987, 1988b). A diferencia de *Mucor*, *Rhizopus* es un género patógeno que puede ocasionar pérdidas económicamente importantes en post-cosecha de cítricos. Su velocidad de crecimiento, su facilidad de colonización por contacto (formación de nidos) y su tolerancia a los bencimidazoles lo convierten en un género especialmente peligroso, sobre todo si el almacenamiento de los frutos se realiza a temperaturas mayores de 5ºC. Un control efectivo requiere intensas medidas profilácticas de limpieza y desinfección, especialmente de envases de campo y de las líneas de confección.

Los muestreos indicaron la presencia generalizada en los almacenes de cepas de especies de *Penicillium* resistentes al tiabendazol y al imazalil. Las cepas resistentes al imazalil se detectaron siempre con menor frecuencia que las resistentes al tiabendazol, posiblemente porque este fungicida se ha estado utilizando masivamente en la zona durante más tiempo. La gran mayoría de las cepas resistentes fueron de especies de *Penicillium* distintas de *P. digitatum* y *P. italicum*. Díaz y Vila (1989) señalaron a *P. variabile*, *P. steckii*, y *P. velutinum* como especies de *Penicillium* con cepas con resistencia cruzada al imazalil y al procloraz. No obstante, también se encontraron aislados tanto de *P. digitatum* como de *P. italicum* resistentes a ambos fungicidas. A pesar de que su frecuencia relativa fue baja, la existencia de estos biotipos resistentes es preocupante puesto que podrían proliferar fácilmente y también desarrollar resistencia múltiple. Al menos en los próximos años no se prevé una disminución de la presión de selección existente ya que presumiblemente estos fungicidas van a seguir utilizándose de forma continuada. En un estudio reciente, Holmes y Eckert (1999) pusieron de manifiesto que la proporción de aislados de *P. digitatum* y *P. italicum* con triple resistencia a los fungicidas imazalil, tiabendazol y o-fenilfenol prácticamente se duplicó en seis años en centrales citrícolas californianas debido a la utilización intensiva de estos fungicidas, tanto secuencialmente como en combinación (utilización, por ejemplo, de mezclas de imazalil y tiabendazol en las ceras).

Agradecimientos

Los autores agradecen la financiación de la CIRIT (Generalitat de Catalunya) y del Grup Exportador de Cítrics del Baix Ebre i Montsià, así como la colaboración de Jesús Ferrando, Miquel Reverter, Hector Sancho y Josep Miquel Fibla.

Referencias bibliográficas

BANCROFT M.N., GARDNER P.D., ECKERT J.W., BARITELLE J.L., 1984. Comparison of decay strategies in California lemon packinghouses. Plant Dis. 68, 24-28.

BAUDOIN A.B.A.M., ECKERT J.W., 1982. Factors influencing the susceptibility of lemons to infection by *Geotrichum candidum*. Phytopathology 72, 1592-1597.

BROWN G.E., 1975. Factors affecting postharvest development of *Colletotrichum gloeosporioides* in citrus fruits. Phytopathology 65, 404-409.

BROWN G.E., 1980. Fruit handling and decay control techniques affecting quality. En: Citrus Nutrition and Quality. Nagy S., Attaway J.A. eds. Amer. Chem. Soc. Symp. Ser. 143, pp. 193-224.

BROWN G.E., 1982. Resistance of decay fungi to benzimidazole fungicides used in Florida citrus packinghouses. Proc. Fla. State Hortic. Soc. 95, 239-242.

BROWN G.E., 1990. Quality control assessment methodology related to citrus decay control. Florida Department of Citrus, Citrus Research and Education Center, Lake Alfred, Florida, EE UU. 26 pp.

BUS V.G., BONGERS A.J., RISSE L.A., 1991. Occurrence of *Penicillium digitatum* and *Penicillium italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. Plant Dis. 75, 1098-1100.

DÍAZ M.A., VILA R., 1987. Estudio de flora fúngica presente en cámaras frigoríficas de conservación de frutos cítricos. Alimentaria 183, 77-82.

DÍAZ M.A., VILA R., 1988a. El problema de la resistencia a los fungicidas: referencia a la situación en los almacenes españoles de comercialización de cítricos. Rev. Agroquím. Tecnol. Aliment. 28, 151-158.

DÍAZ M.A., VILA R., 1988b. Evolución de la flora fúngica durante la desverdización en almacenes españoles de comercialización de cítricos. I. Flora presente en las cámaras de desverdización. Rev. Agroquím. Tecnol. Aliment. 28, 501-508.

DÍAZ M.A., VILA R., 1989. Imazalil resistant *Penicillium* isolates from Spanish citrus packinghouses. Microbiol. Alim. Nutr. 7, 191-192.

DÍAZ M.A., VILA R., HERNÁNDEZ E., 1987a. Detección de *Penicillium italicum* resistentes a OPPS, benomilo, TBZ y CGA-64251, procedentes de almacenes españoles de comercialización de frutos cítricos. Alimentaria 184, 59-70.

DÍAZ M.A., VILA R., HERNÁNDEZ E., 1987b. Resistencia de *Penicillium digitatum*, aislados en centros españoles de comercialización de cítricos, frente a los fungicidas OPPS, benomilo, TBZ y CGA-64251. Rev. Agroquím. Tecnol. Aliment. 27, 439-445.

DOWDING P., 1986. Water availability, the distribution of fungi and their adaptation to the environment. En: Water, Fungi and Plants. Ayres P.G., Boddy L. eds. Cambridge University Press, Cambridge, GB. pp. 305-320.

DURAN R., NORMAN S.M., 1961. Differencial sensitivity to biphenyl among strains of *Penicillium digitatum* Sacc. Plant Dis. Reptr. 45, 475-480.

ECKERT J.W., 1990. Impact of fungicide resistance on citrus fruit decay control. En: Managing Resistance to Agrochemicals. Green M.B., Le Baron H.M., Moberg, W.K. eds. Amer. Chem. Soc. Symp. Ser. 421, pp. 286-302.

ECKERT J.W., EAKS I.L., 1989. Postharvest disorders and diseases of citrus fruits. En: The Citrus Industry. Vol. 5. Reuter, W., Calavan, E.C., Carman, G.E. eds. University of California Press. Berkeley, CA, EE UU. pp. 179-260.

ELLIS M.B., 1993a. Dematiaceous hyphomycetes. 6ª ed. CAB International. Wallingford, GB. 608 pp.

ELLIS M.B., 1993b. More dematiaceous hyphomycetes. 4ª ed. CAB International. Wallingford, GB. 507 pp.

GARDNER P.D., ECKERT J.W., BARITELLE J.L., BANCROFT M.N., 1986. Management strategies for control of *Penicillium* decay in lemon packinghouses: economic benefits. Crop Prot. 5, 26-32.

HALL D.J., BICE J.R., 1977. Packinghouse strategies for the control of fungicide resistant molds. Proc. Fla. State Hortic. Soc. 90, 138-141.

HOLMES G.J., ECKERT J.W., 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. Phytopathology 89, 716-721.

HOUCK L.G., 1977. Problems of resistance to citrus fungicides. Proc. Int. Soc. Citriculture 1, 263-269.

ORIHUEL B., CANET J.J., BERTÓ R., 1996. Límites críticos de contaminación fúngica de superficies en una central hortofrutícola. Fruticultura Profesional 83, 114-118.

PALOU L., USALL J., PONS J., VIÑAS I., 2001. Micoflora epifita de los frutos y ambiental en campos de mandarino 'Clemenules' en Tarragona. Invest. Agr.: Prod. Prot. Veg. 16, disponible en ftp://www.inia.es

SAMSON R.A., HOEKSTRA E.S., FRISVAD J.C., FILTENBORG O., eds., 1995. Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures. Baarn, Holanda. 322 pp.

SUS V., VIÑAS I., 1990. Efecto de la desinfección sobre la contaminación fúngica de cámaras frigoríficas de fruta de pepita – Evaluación de desinfectantes en el control del *Penicillium expansum*. Microbiol. Alim. Nutr. 8, 95-102.

TEIXIDÓ N., USALL J., MAGAN N., VIÑAS I., 1999. Microbial population dynamics on Golden Delicious apples from bud to harvest and effect of fungicide applications. Ann. Appl. Biol. 134, 109-116.

TUSET J.J., 1987. Podredumbres de los frutos cítricos. Conselleria d'Agricultura i Pesca. Generalitat Valenciana. Valencia. 206 pp.

WEBSTER J., 1980. Introduction to fungi. 2ª ed. Cambridge University Press. Cambridge, GB. 669 pp.

WILD B.L., ECKERT J.W., 1982. Synergy between a benzimidazole-sensitive isolate and benzimidazole-resistant isolates of *Penicillium digitatum*. Phytopathology 72, 1329-1332.

Capítol 3

Control of Postharvest Blue and Green Molds of Oranges by Hot Water, Sodium Carbonate, and Sodium Bicarbonate

L. Palou[1], J.L. Smilanick[2], J. Usall[1], I. Viñas[1]

[1] *Àrea de Postcollita, CeRTA, Centre UdL-IRTA, Lleida*
[2] *Horticultural Crops Research Laboratory, USDA-ARS, Fresno, CA, USA*

Referència: *Plant Dis.* 85: 371-376 (2001).

Abstract

Control of citrus blue mold, caused by *Penicillium italicum*, was evaluated on artificially inoculated oranges immersed in water at up to 75ºC for 150 s; in 2 to 4% sodium carbonate (w/v) at 20 or 45ºC for 60 or 150 s; or in 1 to 4% sodium bicarbonate at room temperature for 150 s, followed by storage at 20ºC for 7 days. Hot water controlled blue mold at 50 to 55ºC, temperatures near those that injured fruit, and its effectiveness declined after 14 days of storage. Sodium carbonate and sodium bicarbonate were superior to hot water. Temperature of sodium carbonate solutions influenced effectiveness more than concentration or immersion period. Sodium carbonate applied for 150 s at 45ºC at 3 or 4% reduced decay more than 90%. Sodium bicarbonate applied at room temperature at 2 to 4% reduced blue mold by more than 50%, while 1% was ineffective. In another set of experiments, treatments of sodium bicarbonate at room temperature, sodium carbonate at 45ºC, and hot water at 45ºC reduced blue mold incidence on artificially inoculated oranges to 6, 14, and 27%, respectively, after 3 weeks of storage at 3ºC. These treatments reduced green mold incidence to 6, 1, and 12%, respectively, while incidence among controls of both molds was about 100%. When re-examined 5 weeks later, the effectiveness of all, particularly hot water, declined. As a conclusion, efficacy of hot water, sodium carbonate, and sodium bicarbonate treatments against blue mold compared to that against green mold was similar after storage at 20ºC but inferior during long-term cold storage.

Additional keywords: baking soda, citrus, cold storage, *Penicillium digitatum*, postharvest decay, soda ash

Introduction

Postharvest green mold, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., is the most economically important postharvest disease of citrus in Spain (34), California, and all production areas characterized by low summer rainfall (11). However, when citrus fruit such as Valencia oranges or Clementine mandarins are stored for long periods, usually at temperatures below 5ºC, postharvest blue mold, caused by *Penicillium italicum* Wehmer, becomes more important because it grows faster than *P. digitatum* below 10ºC (37). In Spain, several orange cultivars are often stored at about 3ºC for long periods to take advantage of seasonal changes in market prices. In California, Valencia oranges are stored at very low temperature to reduce losses of fruit stored for preparation of fresh juice. Blue mold may also predominate in fruit treated with benzimidazole fungicides, because resistance to these materials occurs more frequently in isolates of *P. italicum* than in *P. digitatum* (14). Currently, both diseases are primarily controlled by application of the fungicides imazalil, sodium ortho-phenyl phenate, and/or thiabendazole (21,30). Alternative methods are needed because the widespread use of these chemicals in commercial packinghouses has led to the proliferation of resistant strains of the pathogens (4,9). Furthermore, concerns about public health and environmental issues (8,24) have increased the need for alternatives.

Postharvest hot water treatments have been well studied for the control of postharvest decay of citrus fruits (3,19,33,35) and, in case of long-term refrigerated storage, for the improvement of fruit resistance to chilling injury (26). When combined with fungicides, hot water greatly enhances their efficacy and makes it possible to reduce their application rates (2,32,35).

Carbonic acid salts, such as sodium carbonate (Na_2CO_3, soda ash) and sodium bicarbonate ($NaHCO_3$, baking soda), are common food additives allowed with no restrictions for many applications by European and North American regulations (18,23). Sodium bicarbonate is classified as generally recognized as safe by the United States Food and Drug Administration and also proposed exempt from residue tolerances on all agricultural commodities by the United States Environmental Protection Agency. Both sodium carbonate and sodium bicarbonate were listed as approved ingredients on products labeled "organic" proposed by the United States Department of Agriculture. The antimicrobial activity of these compounds has been described in vitro (5), in meat (12), fish (6), and on leaves and fruit (29,38). Sodium bicarbonate to control postharvest green and blue molds of citrus was first described by Barger in 1928 (1) as an alternative to the use of borax. Sodium carbonate has been used to improve cleaning and also to reduce postharvest decay of lemons in California for more than 70 years (11). Both salts can be a useful tool to manage postharvest decay because they are inexpensive, readily available, and can be used with a minimal risk of injury to the fruit. Recent work shows that sodium carbonate and

sodium bicarbonate solutions, used correctly, approach the effectiveness of common synthetic fungicides used to control *P. digitatum* on lemons (30) and oranges (29). A model to predict green mold incidence among sodium carbonate treated oranges, describing the influence of sodium carbonate concentration, temperature, and immersion period, was developed and validated under commercial conditions (29). Although somewhat less effective on oranges than on lemons, the effectiveness of sodium carbonate was high enough that its commercial use on oranges is justified for the control of green mold. Recently, the influence of commercial postharvest practices on the control of green mold by sodium carbonate and sodium bicarbonate was published to facilitate their commercial adoption (31). However, little research has been focused on the control of blue mold by carbonate and bicarbonate salts.

Objectives of the present work were to: (i) determine the range of water temperatures able to control blue mold on oranges without rind injury; (ii) evaluate the efficacy of sodium carbonate and sodium bicarbonate at different concentrations, temperatures, and immersion periods; and (iii) evaluate their effectiveness against blue and green molds during long-term cold storage.

Materials and methods

Fruit. Oranges (*Citrus sinensis* (L.) Osbeck) cvs. Washington Navel, Navelate, Lanelate, and Valencia, from commercial orchards in southern Tarragona (Catalonia, Spain) or California, were selected by hand from field bins after harvest and used in the experiments before any commercial postharvest treatments were applied. The fruit were used the same day, or stored up to 2 weeks at 5ºC and 90% relative humidity (RH) before use. Before each experiment, fruit were randomized, washed with fresh water, disinfected superficially for 1 min in a diluted bleach dip (0.5% sodium hypochlorite), rinsed with water, and allowed to air dry at room temperature.

Inoculum. Several *P. italicum* isolates were obtained from decayed oranges or mandarins from Tarragona citrus packinghouses and one isolate (PIM-7) showing the highest aggressiveness in preliminary tests was used in subsequent experiments. Petri dishes of potato dextrose agar (PDA) were inoculated with this isolate and incubated at 25ºC for 7 to 10 days. A conidial suspension was prepared in Tween 80 (0.05% wt/vol) in water and diluted to 10^6 spores per milliliter after counting spores with a hemacytometer. This density is recommended for evaluation of postharvest treatments to control blue and green molds (10). About 2 h before treatments, 25 µl of this suspension was placed, using a micropipet, in a 1-by-5-by-2-mm deep wound made with a stainless steel scalpel on the equator of each fruit. The wound penetrated the albedo tissue, but not the juice sacs, and simulated frequent infection under commercial conditions. In the experiments conducted in California, *P. italicum* isolate PIM-7 or *P. digitatum* isolate M6R (obtained from J. W. Eckert, University of California, Riverside), were cultured on PDA dishes at 25ºC for 7 to 14 days. Spores were rubbed from the agar surface with a sterile glass rod after 5 ml of 0.05% (wt/vol) Triton X-100 in water were added. The spore suspension was passed through two layers of cheesecloth and diluted with water to an absorbance of 0.1 at 420 nm determined with a spectrophotometer. This density is approximately equivalent to 10^6 spores per milliliter (22). About 24 h before treatments, fruit were inoculated by immersing a stainless steel rod with a probe tip 1 mm wide and 2 mm in length into the spore suspension and wounding each fruit once on the equator. The inoculated fruit were held at room temperature.

Hot water treatments. Three stainless steel buckets each holding 24 liters of water were heated to the test temperature in a 172-liter stainless steel water tank fitted with a 9-kW electric resistance heater and thermostat. Metallic grid baskets, containing the oranges inoculated with *P. italicum* 2 to 3 h previously, were submerged in the buckets for 150 s. Three experiments, two with Navelate and one with Valencia oranges, were conducted to determine the approximate range of effective and safe temperatures. The tested temperatures were 20 (control), 35, 45, 48, 50, 53, 55, 57, 60, 65, and 75ºC (±1ºC). Another set of control fruit were inoculated but not treated. Within each experiment, each treatment was applied to four replicates of five fruits each. Treated fruit were placed in plastic cavity trays that prevented contact infections, dried in air on wooden trays at room temperature, and stored at 20ºC and 90% RH. After 7 and 14 days, the infected oranges were counted. After the first 7 days of storage, fruit were also classified into one of four categories, depending on rind appearance: (i) no rind damage, (ii) slight blemishes present, (iii) moderate blemishes present, and (iv) severe rind injury.

Sodium carbonate treatments. California-grown Valencia oranges were inoculated with *P. italicum* 24 h before treatment as previously described, placed into plastic baskets, and immersed in 22-liter tanks

containing sodium carbonate solutions (pH 11.3 to 11.5; Sigma-Aldrich, St. Louis). The treatment equipment consisted of 12 stainless steel tanks, each one individually fitted with a computer controlled electrical heater, a temperature sensor, and a mechanical agitation system. Four sodium carbonate concentrations (0 [control, water alone], 2, 3, and 4% [wt/vol]), two temperatures (20 and 45ºC), and two immersion periods (60 and 150 s) were tested. The temperature of the solutions did not change more than 0.5ºC during treatment. After treatment, the fruit were rinsed with 10 ml of deionized water per fruit at low pressure (200 kPa) in a spray 30 cm above the fruit for 5 s, placed on plastic cavity trays on wooden trays, and stored at 20ºC and 90% RH. After 7 days of storage, the incidence of blue mold was determined. Each treatment was applied to five replicates of 25 oranges each. The experiment was repeated twice with Valencia oranges.

Sodium bicarbonate treatments. Washington Navel and Navelate oranges from Tarragona were inoculated with *P. italicum* with a micropipet and, about 2 h later, immersed for 150 s in 0 (control, water alone), 1, 2, 3, or 4% (wt/vol) sodium bicarbonate (pH 8.3 to 8.6; Sigma-Aldrich) solutions at room temperature (20 ± 2ºC), using buckets and water tank as previously described. The pH of sodium bicarbonate solutions rises rapidly at high temperature; therefore, only room temperature was tested (31). Each treatment was applied to four replicates of 10 fruits each. Treated fruit were placed into plastic cavity trays and stored at 20ºC and 90% RH for 7 days, at which time the incidence of blue mold infections was determined. Valencia oranges from California were inoculated, dipped 22 to 24 h later for 150 s in 0 (control, water alone), 1, 2, 3, or 4% (wt/vol) sodium bicarbonate solutions at 20ºC (±0.5ºC), rinsed, stored at 20ºC for 7 days, and checked for decay incidence, all as were described above for the sodium carbonate treatments. Four replicates of 25 fruits each were used within each treatment, and the experiment was repeated twice.

Long-term cold storage. California-grown Lanelate oranges, inoculated with *P. italicum* or *P. digitatum* 24 h before treatment, were dipped in sodium carbonate or sodium bicarbonate solutions and rinsed as previously described, then stored at 3ºC (±1ºC) and 90% RH for 2 months. The treatments applied in the sodium carbonate experiment were: (i) water alone at 20ºC, (ii) water alone at 45ºC, and (iii) 3% (w/v) sodium carbonate at 45ºC. Each treatment was applied to six replicates of 15 oranges each. The treatments applied in the sodium bicarbonate experiment were: (i) water alone at 20ºC; and (ii) 3% (wt/vol) sodium bicarbonate at 20ºC. Each treatment was applied to five replicates of 10 fruits each. Each solution was prepared twice and each pathogen was assayed separately to avoid mixing spores. The incidence of decayed fruit was determined weekly.

Statistical analysis. Analysis of variance was applied to the arcsine of the square root of the proportion of decayed fruit using SAS software (SAS Institute Inc., Cary, NC).

Results

Hot water treatments. In the first experiment with Navelate oranges, a temperature range of 35 to 75ºC (in increments of 10ºC) was evaluated. Treatment with hot water at 65ºC controlled blue mold (Fig. 1A), but severely injured the rind surface of 100% of oranges (Table 1). Fruit treated at 75ºC were entirely discolored and soft. These fruit could not be sold commercially and had many secondary infections by contaminating saprophytic microorganisms. Below these temperatures, only 55ºC treatment controlled *P. italicum* effectively after 7 days of storage at 20ºC, but control was unsatisfactory when fruit were examined after 14 days (Fig. 1A). About 30% of the fruit treated at 55ºC had slight and moderate rind blemishes (Table 1). No rind damage was observed on oranges treated at 45ºC and lower temperatures. In the second experiment with Navelate oranges, temperatures from 50 to 60ºC were tested. All these temperatures consistently suppressed disease development after 7 days, with a 90 to 100% reduction in decay incidence compared to the control treatments (no treatment and treatment at 20ºC; Fig. 1B). After 7 days of storage, severe rind injuries were present on 30% of the fruits treated at 57ºC and on 90% of those treated at 60ºC (Table 1). In the third experiment, the effect of 150 s of hot water treatment from 45 to 55ºC on Valencia oranges was assessed. Blue mold incidence after 7 days was high among the controls (90 to 100%) and hot water effectiveness was low (Fig. 1C). Satisfactory control was obtained after treatment at 53 and 55ºC, but not after treatment at 50ºC or lower. No injuries were caused by the 53 or 55ºC treatment (Table 1). A significant increase in decay incidence occurred during the second week of storage in all three experiments (Fig. 1).

Sodium carbonate treatments. The combined effects of sodium carbonate concentration, temperature, and immersion period were evaluated on Valencia oranges. All three factors significantly

influenced the control of blue mold, although the influence of temperature was greater than those of concentration or immersion period (Table 2). Sodium carbonate improved blue mold control in comparison with water alone (Fig. 2). At room temperature, with decay incidence on the control treatment (water alone) adjusted to 100%, the incidence of blue mold at concentrations of 3 and 4% sodium carbonate was 20 to 30%. Similarly, decay incidence was 20 to 25% after treatment of the fruit for 60 s in 3 or 4% sodium carbonate at 45ºC. Lower blue mold incidence (< 10%) was obtained by treatment of the fruit for 150 s in these sodium carbonate solutions at 45ºC. The effect of the concentration was significantly dependent on the temperature (Table 2): differences in effectiveness among sodium carbonate concentrations, and especially between concentration 0 (water alone) and those treatments with sodium carbonate, were more important at 20ºC than at 45ºC (Fig. 2). The effect of the immersion period was also significantly dependent on the temperature; a treatment of 150 s enhanced the effectiveness of all the treatments compared to 60 s at 45ºC, but not at 20ºC. No visible rind injury occurred in any test.

Fig. 1. Incidence of blue mold on artificially inoculated orange cvs. **A, B,** Navelate and **C,** Valencia immersed for 150 s in water at different temperatures (control fruit were inoculated but not treated) after 7 and 14 days of storage at 20ºC and 90% relative humidity.

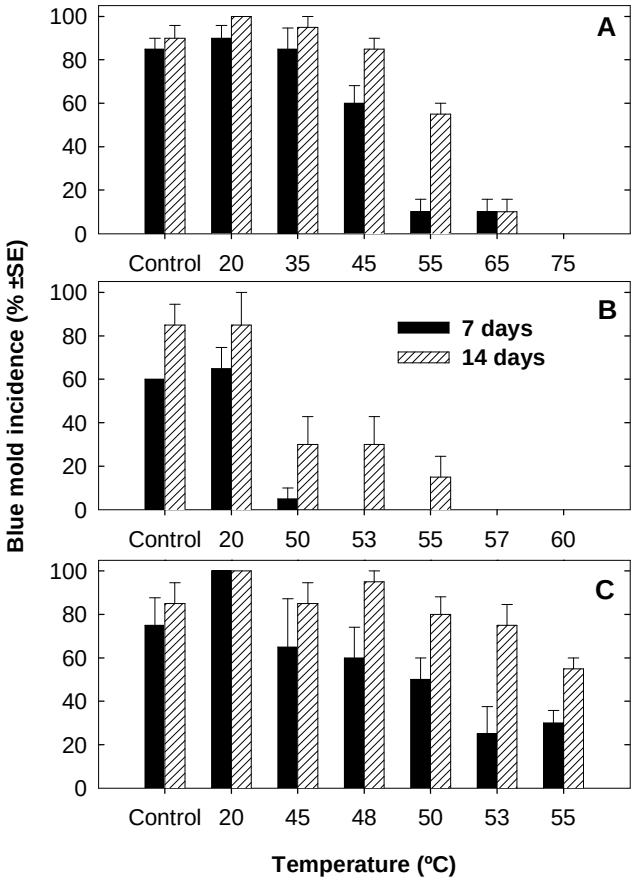

Table 1. Influence of water temperature on the appearance of Navelate (N) and Valencia (V) oranges immersed for 150 s and stored at 20ºC and 90% relative humidity for 7 days

Water temp. (ºC)	Rind injury frequency (%)[a]					
	Slight		Moderate		Severe	
	N	V	N	V	N	V
20	0	0	0	0	0	0
35	0	-	0	-	0	-
45	0	0	0	0	0	0
48	-	0	-	0	-	0
50	0	0	0	0	0	0
53	17	0	0	0	0	0
55	22	8	6	0	0	0
57	17	-	32	-	27	-
60	0	-	11	-	89	-
65	0	-	0	-	100	-
75	0	-	0	-	100	-

[a] - = temperature not assayed.

Table 2. Analysis of variance of the incidence of blue mold on artificially inoculated Valencia oranges immersed in sodium carbonate solutions and stored at 20ºC and 90% relative humidity for 7 days

Source[a]	df	MS	F	*P* > F
Experiment (E)	1	91.08	1.40	0.2384
Temperature (T)	1	22239.78	342.30	<0.0001
Concentration (C)	3	13686.77	210.66	<0.0001
Immersion period (t)	1	2846.48	43.81	<0.0001
T x C	3	3497.75	53.83	<0.0001
T x t	1	1195.06	18.39	<0.0001
C x t	3	181.77	2.80	0.0424
T x C x t	2	15.74	0.24	0.8667
Error	143	64.97		

[a] Analysis was applied to the arcsine of the square root of the proportion of decayed fruit.

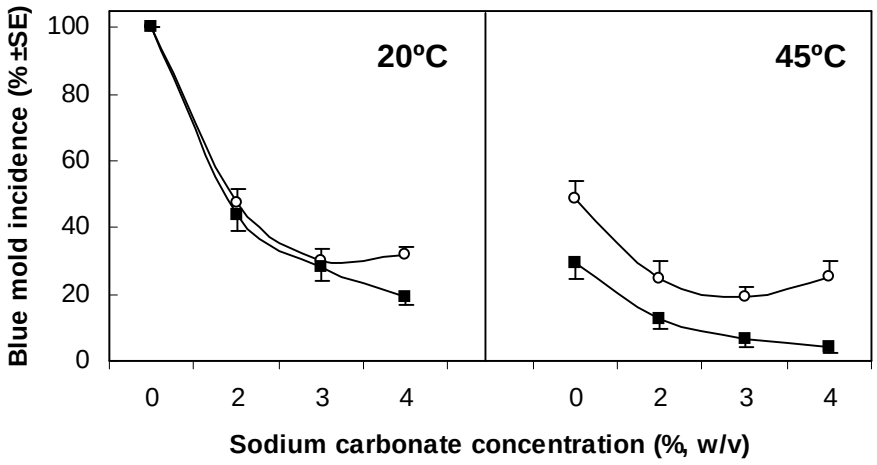

Fig. 2. Influence of solution temperature, sodium carbonate concentration, and immersion period (○ = 60 s , ■ = 150 s) on the incidence of blue mold on cv. Valencia oranges artificially inoculated 24 h before treatment, rinsed at low pressure, and stored at 20ºC and 90% relative humidity for 7 days. Data are the means of two experiments with five replicates of 25 fruit each.

Sodium bicarbonate treatments. Washington Navel and Navelate oranges inoculated 2 h before treatment and immersed for 150 s in 2, 3, and 4% sodium bicarbonate solutions at room temperature reduced blue mold incidence substantially (down to 10 to 15%, Fig. 3), while the blue mold incidence after 1% sodium bicarbonate treatment was significantly inferior on both cultivars (25 to 40%). Similar results, although the decay incidence was higher, were obtained with Valencia oranges inoculated 24 h before treatment, which was followed by a low-pressure water rinse (Fig. 3).

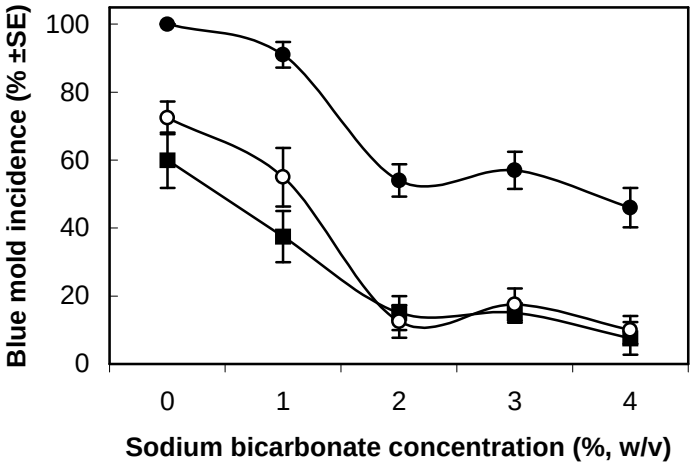

Fig. 3. Influence of sodium bicarbonate concentration on the incidence of blue mold on orange cvs. Washington Navel (○), Navelate (■), and Valencia (●) treated at room temperature for 150 s and stored at 20ºC and 90% relative humidity for 7 days. Washington Navel and Navelate oranges were inoculated 2 h before treatment and not rinsed; values are the means of four replicates of 10 fruit each. Valencia oranges were inoculated 24 h before treatment and rinsed at low pressure; values are the means of four replicates of 25 fruit each.

Long-term cold storage. Both sodium carbonate at 45ºC and sodium bicarbonate applied at room temperature significantly reduced the incidence of both green and blue molds during the 2-month storage period (Fig. 4). After 3 weeks at 3ºC, the incidence of blue and green molds on oranges treated with sodium bicarbonate, sodium carbonate or hot water at 45ºC was 6, 14, and 27%, and 6, 1, and 12% respectively, while it was 100% among the controls. Hot water at 45ºC greatly reduced decay incidence compared to treatment with water at 20ºC (control), and sodium carbonate treatment at 45ºC reduced decay incidence compared to treatment with hot water at this temperature. After 2 months of storage at 3ºC, blue mold incidence was 38% for sodium bicarbonate, 56% for sodium carbonate, and 76% for hot water. Green mold incidence was 32, 22, and 60%, respectively (Fig. 4). The incidence of blue mold was higher than the incidence of green mold, and the magnitude of the difference was smaller in sodium bicarbonate-treated fruit than in sodium carbonate- or hot water-treated fruit.

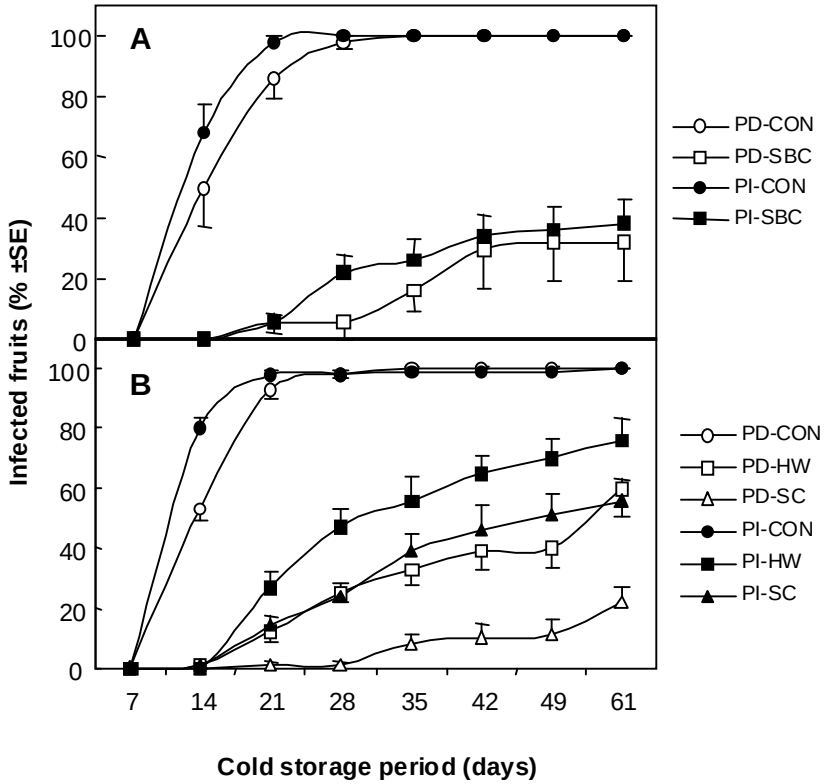

Fig. 4. Incidence of green (PD) and blue (PI) molds on artificially inoculated oranges cv. Lanelate after treatment and storage at 3ºC and 90% relative humidity for 2 months. **A,** Immersion in water (CON) or 3% sodium bicarbonate (SBC) at room

temperature; **B,** immersion in water alone at room temperature (CON), water at 45ºC (HW), or 3% sodium carbonate at 45ºC (SC).

Discussion

Control of blue mold was obtained by brief hot water treatment of Navelate and Valencia oranges without injury of apparent commercial impact, although the effective temperatures were close to those that caused injury. Control at 7 days of storage was observed with treatments between 50 and 55ºC, but after 14 days control was significantly inferior. Satisfactory control was not achieved at lower temperatures and severe rind injury occurred above 55ºC. Similarly, Barkai-Golan and Apelbaum (2) found that hot water immersion for 3 min at 36ºC did not reduced the incidence of blue mold in inoculated Shamouti oranges, and treatment at 45ºC only caused a reduction of 6% after 14 days of storage at 14ºC. Our results with blue mold are similar to those reported for green mold by other workers. Most of the research on the use of hot water to prevent postharvest citrus decay has focused on control of *P. digitatum*. Smoot and Melvin (33) and Tuset et al. (35) reported that immersion of oranges in water at 53ºC controlled green mold of artificially inoculated oranges. In Houck's tests (16) the temperature required to control green mold on Eureka lemons was 52ºC. Hot water treatment at 50 to 54ºC did not injure the rind of Fortuna mandarins, but treatment at 56 to 58ºC was injurious (27). In our work, when the incidence of the decay was low among the controls, significant control was obtained at lower temperatures than when the incidence among the controls was high. This may be related to the physiological condition of the fruit, which clearly influences the susceptibility of citrus fruit to decay. The susceptibility of Tarocco oranges to postharvest decay, chilling injury, and rind injury caused by hot water was greatly influenced by the harvest date (28). At the temperature range in which the satisfactory control of green mold occurred, the effectiveness of hot water was not very persistent. We found the same results with blue mold as with green mold in previous work (25); namely, the control of blue mold after 14 days of storage at 20ºC was significantly inferior to that after 7 days of storage. Commercial adoption of hot water treatment must be done with caution because the difference in temperature between control and severe injury was small. In general, our work and that of others indicates hot water used alone to control green and blue molds is accompanied by considerable risk of rind injury and lack of persistent control.

Significant control of blue mold was achieved by 2, 3, and 4% sodium carbonate or sodium bicarbonate solutions at room temperature. Heating sodium carbonate solutions to 45ºC improved its efficacy compared to treatment at room temperature. Heated sodium carbonate solutions were more effective than hot water alone even at lower temperatures, which is important to minimize risks of rind damage by heat. Many authors observed synergistic effects of heat and chemicals used to control postharvest decay (2,19,29,32,35). In our tests, oranges treated with carbonates were not injured. Sodium carbonate and sodium bicarbonate efficacy against blue mold was comparable to that obtained against green mold in related previous experiments (25,29-31). *P. italicum* and *P. digitatum* have similar infection processes and disease cycles; therefore, commercial application of these treatments may provide acceptable protection against both molds. Although sodium carbonate and sodium bicarbonate controlled naturally inoculated citrus fruit more effectively than artificially inoculated fruits (1,29), the present results should be confirmed in commercial-scale tests. Efficacy of sodium carbonate and sodium bicarbonate decreased when fruit susceptibility to decay was high (indicated by higher decay levels among control fruit). A high proportion of *P. italicum* and *P. digitatum* conidia remained germinable after 5 min exposure to 10% sodium carbonate or sodium bicarbonate (20); therefore, the compounds probably are not lethal. We removed *P. italicum* spores from inoculated wounds on oranges, both just after sodium carbonate or sodium bicarbonate treatment and after a 7-day storage period at 20ºC, and plated the spores on PDA dishes that were incubated at 25ºC. The spores germinated and developed colonies. The increase in decay incidence that occurred during the second-week storage period at 20ºC also indicated that the pathogen survived treatment. Therefore, the effect is primarily fungistatic and not very persistent, and is very likely due to the presence of carbonate or bicarbonate residues within the wound infection courts occupied by the fungus.

The impact of the concentration of the chemical, the immersion period, and the temperature of the solution, can influence the presence of residues and consequently may play an important role on the magnitude of control obtained. Smilanick et al. (29) developed a model describing the influence of these factors on the control of green mold on sodium carbonate-treated oranges, and validated it under

commercial conditions. Green mold incidence was significantly influenced by all three factors. The best control of green mold was obtained after treatment for 2 min at 40.6 or 43.3ºC in 4 or 6% sodium carbonate. In the present work, blue mold incidence on sodium carbonate-treated oranges was also significantly influenced by all three factors, although the influence of temperature was greater than those of concentration or immersion period. Hwang and Klotz (17) found that inhibitory activity of carbonate, bicarbonate, and other salt solutions on spores of *P. italicum* and *P. digitatum* was more dependent on temperature than on concentration. A practice that could influence the effectiveness of carbonates is the addition of emulsifiers or surfactants to their solutions. Homma et al. (15) reported that the addition of such compounds, commonly used in food and pesticide formulations, improved the sodium bicarbonate effectiveness against green mold on Satsuma mandarins. They proposed the improvement might result from less sodium bicarbonate crystallization in solution, better adhesion of sodium bicarbonate to the rind, or improved surface distribution of the sodium bicarbonate.

Sodium carbonate and sodium bicarbonate mode of action against *Penicillium* spp. on citrus fruit has not been completely elucidated. Disease development is a result of complex interactions between host, pathogen, and environment (13). Notable differences have been detected between results of in vitro and in vivo tests. In vitro activity of several carbonate salts against germinated or ungerminated spores of *P. italicum* and *P. digitatum* was higher than that of bicarbonate salts, although their efficacy was similar in vivo (17,20,31). Hwang and Klotz (17) suggested that the original toxicity of the treating solution to the spores is altered by interactions in wounds with constituents of the rind. The relatively high pH of these solutions has been proposed to be the mode of action (7), although there is evidence that high pH alone cannot explain the inhibitory action of these salts (15,17,20). The in vitro and in vivo control of green mold by sodium carbonates and bicarbonates were superior to those by potassium or ammonium carbonates and bicarbonates (20,31), which suggests the sodium cation has some role in the control of the disease. Marloth (20) found that germinated spores of both fungi were more readily killed by sodium carbonate and sodium bicarbonate than were nongerminated spores. Hwang and Klotz (17) observed that *P. italicum* spores were more sensitive to the in vitro inhibitory effect of sodium carbonate, sodium bicarbonate, and other salts than *P. digitatum* spores.

This is the first work in which citrus *Penicillium* decay on artificially inoculated fruit treated with sodium carbonate or sodium bicarbonate was periodically assessed during long refrigerated storage. Our results, especially during the first 3 weeks of storage, indicate that the treatments complemented the inhibitory action of low temperature. Control of blue mold was inferior to that of green mold, very likely because of the better adaptation of *P. italicum* to grow at low temperature.

In contrast to *P. digitatum*, *P. italicum* is readily able to spread from fruit to fruit in packed containers and create nests of decayed fruit (11). Moreover, healthy fruit can be soiled by spores of both fungi dislodged from diseased fruit (11,37). Hot water, sodium carbonate, and sodium bicarbonate are poor eradicants that do not kill spores and their inhibitory action is not very persistent; therefore, additional complementary treatments could be needed to provide protection of the fruit from reinfection after treatment. Biological control antagonists, which can persist for long periods, may accomplish this task (31). The effectiveness of sodium carbonate and sodium bicarbonate against green mold was significantly improved when these treatments were followed by the application of *Pseudomonas syringae* strain ESC10 (31). Work in our laboratory is being conducted to test the possible complementary effect of the strain CPA-2 of the antagonistic bacterium *Pantoea agglomerans* (36) and carbonate treatments against citrus postharvest green and blue molds.

Acknowledgements

We thank the Catalonian Government (CIRIT, Comissió Interdepartamental de Recerca i Tecnologia), the Grup Exportador de Cítrics del Baix Ebre-Montsià (Tarragona, Catalonia, Spain), Sunkist Growers (Lindsay, California), and the California Citrus Research Board for their financial support; and Sunkist Growers for useful suggestions and comments.

Literature cited

Barger, W. R. 1928. Sodium bi-carbonate as a citrus fruit disinfectant. Calif. Citrograph 13:164-174.

Barkai-Golan, R., and Apelbaum, A. 1991. Synergistic effects of heat and sodium o-phenyl phenate

treatments to inactivate Penicillium spores and suppress decay in citrus fruits. Trop. Sci. 31:229-233.
Barkai-Golan, R., and Phillips, D. J. 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. Plant Dis. 75:1085-1089.
Bus, V. G., Bongers, A. J., and Risse, L. A. 1991. Occurrence of Penicillium digitatum and Penicillium italicum resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. Plant Dis. 75:1098-1100.
Corral, L. G., Post, L. S., and Montville, T. J. 1988. Antimicrobial activity of sodium bicarbonate. J. Food Sci. 53:981-982.
Curran, D. M., Tepper, B. J., and Montville, T. J. 1990. Use of bicarbonates for microbial control and improved water-binding capacity in cod fillets. J. Food Sci. 55:1564-1566.
DePasquale, D. A., and Montville, T. J. 1990. Mechanism by which ammonium bicarbonate and ammonium sulfate inhibit mycotoxigenic fungi. Appl. Environ. Microbiol. 56:3711-3717.
Dezman, D. J., Nagy, S., and Brown, G. E. 1986. Postharvest fungal decay control chemicals: treatments and residues in citrus fruits. Residue Rev. 97:37-92.
Díaz, M. A., and Vila, R. 1988. El problema de la resistencia a los fungicidas: referencia a la situación en los almacenes españoles de comercialización de cítricos. Rev. Agroquím. Tecnol. Aliment. 28:151-158.
Eckert, J. W., and Brown, G. E. 1986. Evaluation of postharvest treatments for citrus fruits. Pages 92-97 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society Press, St. Paul, MN.
Eckert, J. W., and Eaks, I. L. 1989. Postharvest disorders and diseases of citrus fruits. Pages 179-260 in: The Citrus Industry. Vol. 5. W. Reuter, E. C. Calavan, and G. E. Carman, eds. Univ. Calif. Press, Berkeley.
Fletcher, D. L., Russell, S. M., Walker, J. M., and Bailey, J. S. 1993. An evaluation of a rinse procedure using sodium bicarbonate and hydrogen peroxide on the recovery of bacteria from broiler carcasses. Poultry Sci. 72:2152-2156.
Green, F. M. 1932. The infection of oranges by Penicillium. J. Pomol. Hort. Sci. 10:184-215.
Gutter, Y. 1975. Interrelationship of Penicillium digitatum and P. italicum in thiabendazole-treated oranges. Phytopathology 65:498-499.
Homma, Y., Arimoto, Y., and Misato, T. 1981. Effects of emulsifiers and surfactants on the protective values of sodium bicarbonate. J. Pestic. Sci. 6:145-153.
Houck, L. G. 1967. Hot water treatments for control of Penicillium green mold of Eureka lemons. (Abstr.) Phytopathology 57:99.
Hwang, L., and Klotz, L. J. 1938. The toxic effect of certain chemical solutions on spores of Penicillium italicum and P. digitatum. Hilgardia 12:1-38.
Lindsay, R. C. 1985. Food additives. Chapter 10 in: Food Chemistry. O. R. Fennema, ed. Marcel Decker, Inc., New York, NY.
Lurie, S. 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14: 257-269.
Marloth, R. H. 1931. The influence of hidrogen-ion concentration and of sodium bicarbonate and related substances on Penicillium italicum and P. digitatum. Phytopathology 21:169-198.
Martínez-Jávega, J. M., Cuquerella, J., and Navarro, P. 1995. Perspectiva actual de la tecnología postcosecha de frutos cítricos. Levante Agrícola 332:220-225.
Morris, S. C., and Nicholls, P. J. 1978. An evaluation of optical density to estimate fungal spore concentrations in water suspensions. Phytopathology 68:1240-1242.
Multon, J. L. 1988. Aditivos y Auxiliares de Fabricación en las Industrias Agroalimentarias. Editorial Acribia, Zaragoza, Spain.
National Research Council. 1993. Pesticides in the Diets of Infants and Children. National Academy Press, Washington, DC.
Palou, L., Usall, J., Aguilar, M.J., Pons, J., and Viñas, I. 1999. Control de la podredumbre verde de los cítricos mediante baños con agua caliente y carbonatos sódicos. Levante Agrícola 348:412-421.
Rodov, V., Ben-Yehoshua, S., Albagli, R., and Fang, D. Q. 1995. Reducing chilling injury and decay of stored citrus fruit by hot water dips. Postharvest Biol. Technol. 5:119-127.
Schirra, M., and D'hallewin, G. 1997. Storage performance of 'Fortune' mandarins following hot water dips. Postharvest Biol. Technol. 10:229-238.
Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., and Garau, V. L. 1998. Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip

treatments. J. Agric. Food Chem. 46:1177-1180.

Smilanick, J. L., Mackey, B. E., Reese, R., Usall, J., and Margosan, D. A. 1997. Influence of concentration of soda ash, temperature, and immersion period on the control of postharvest green mold of oranges. Plant Dis. 81:379-382.

Smilanick, J. L., Margosan, D. A., and Henson, D. J. 1995. Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. Plant Dis. 79:742-747.

Smilanick, J. L., Margosan, D. A., Mlikota, F., Usall, J., and Michael, I. F. 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83:139-145.

Smilanick, J. L., Michael, I. F., Mansour, M. F., Mackey, B. E., Margosan, D. A., Flores, D., and Weist, C. F. 1997. Improved control of green mold of citrus with imazalil in warm water compared with its use in wax. Plant Dis. 81:1299-1304.

Smoot, J. J., and Melvin, C. F. 1963. Hot water as a control for decay of oranges. Proc. Fla. State Hort. Soc. 76:322-327.

Tuset, J. J. 1987. Podredumbres de los Frutos Cítricos. Conselleria d'Agricultura i Pesca. Generalitat Valenciana, Valencia, Spain.

Tuset, J. J., Hinarejos, C., Mira, J. L., and Martínez-Jávega, J. M. 1996. Tratamientos térmicos a los frutos cítricos para el control de las enfermedades de la post-recolección. Levante Agrícola 337:342-347.

Viñas, I., Usall, J., Nunes, C., and Teixidó, N. 1999. Nueva cepa de la bacteria Pantoea agglomerans (Beijerinck, 1998) Gavini, Mergaert, Beji, Mielcareck, Izard, Kerstersy De Ley y su utilización como agente de control biológico de las enfermedades fúngicas de frutas. Solicitud P9900612. Oficina Española de Patentes y Marcas, Madrid, Spain.

Whiteside, J. O., Garnsey, S. M., and Timmer, L. W., eds. 1988. Compendium of Citrus Diseases. 2nd ed. American Phytopathological Society Press, St. Paul, MN.

Ziv, O., and Zitter, T. A. 1992. Effects of bicarbonate and film-forming polymers on cucurbit foliar diseases. Plant Dis. 76:513-517.

Capítol 4

Hot Water, Sodium Carbonate, and Sodium Bicarbonate for the Control of Postharvest Green and Blue Molds of Clementine Mandarins

L. Palou[1], J. Usall[1], J.A. Muñoz[1], J.L. Smilanick[2], I. Viñas[1]

[1]*Àrea de Postcollita, CeRTA, Centre UdL-IRTA, Lleida*
[2]*Horticultural Crops Research Laboratory, USDA-ARS, Fresno, CA, USA*

Referència: *Postharvest Biol. Technol.* 24: 93-96 (2002) (Research Note).

Abstract

Clementine mandarins cv. 'Clemenules', artificially inoculated with *Penicillium digitatum* or *P. italicum*, were immersed in 0, 2, or 3% (w/v) sodium carbonate (SC) solutions at 20, 45 or 50ºC for 60 or 150 s. Decay incidence was determined after 7 days of storage at 20ºC and 90% relative humidity (RH). Hot water (HW) at 45 or 50ºC did not satisfactorily control both diseases. With decay incidence for the control treatment (water alone at 20ºC) adjusted to 100%, the incidence of both green and blue molds on fruit treated with HW at 45 and 50ºC was about 80 and 60%, respectively. SC significantly enhanced decay control compared to water alone at all temperatures and for all immersion periods. Heated SC solutions were more effective than solutions at 20ºC. A 150 s dip in 3% SC at 50ºC totally controlled both green and blue molds without noticeably injuring the fruit. A low-pressure rinse of the treated fruit did not affect effectiveness of HW and SC treatments at 50ºC and avoid depositions of salt on the fruit rind. Decay incidence was in general higher on treated fruit inoculated 24 h before treatment than on fruit inoculated 2 h before treatment. SC at 50ºC significantly reduced the incidence of both green and blue molds on mandarins stored at 3.5ºC for 60 days. Both diseases were reduced by 40 to 60% on mandarins dipped for 60 or 150 s in 2 or 3% sodium bicarbonate (SBC) solutions at room temperature. The effectiveness of all HW, SC, and SBC treatments on clementines was inferior to that obtained on oranges or lemons in related previous work.

Keywords: clementine mandarin, *Citrus reticulata*, *Penicillium digitatum*, *Penicillium italicum*, hot water, sodium carbonate, sodium bicarbonate, postharvest decay

Introduction

Worldwide fresh consumption and planted surface of mandarins have been increasing during the past years. Clementine mandarin is the most economically important citrus crop in Catalonia (Spain). The most widely grown cultivar is 'Clemenules' (syn.: 'Clementina de Nules'), which is primarily exported to European markets. Postharvest diseases are among the major economic concerns related to clementine production, not only due to produce losses in the packinghouses but also to price discounts that could be applied by destiny export markets. Postharvest green mold, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mold, caused by *P. italicum* Wehmer, are the most economically important postharvest diseases of citrus in Spain (Tuset, 1987) and all production areas characterized by low summer rainfall (Eckert and Eaks, 1989). Blue mold is especially important on citrus fruit kept under cold storage (Whiteside et al., 1993). Currently, both diseases are primarily controlled by application of synthetic fungicides. Alternative methods are needed because of concerns about environmental contamination and human health risks associated with fungicide residues (National Research Council, 1993). Furthermore, the widespread use of these chemicals in commercial packinghouses has led to the proliferation of resistant strains of the pathogens (Eckert, 1990). In Spain, *Penicillium* strains resistant to thiabendazole and imazalil have been detected in packinghouses in Catalonia (Palou et al., unpublished) and Valencia (Díaz and Vila, 1988).

Hot water (HW) treatments have been well studied for the control of postharvest decay of citrus fruits (Smoot and Melvin, 1963; Barkai-Golan and Phillips, 1991) and, in case of long-term refrigerated storage, for the improvement of the resistance of the fruit, including mandarins, to chilling injury (Schirra and D'hallewin, 1997; González-Aguilar et al., 1997). When combined with fungicides, HW greatly enhances their efficacy and makes it possible to reduce their application rates (Barkai-Golan and Apelbaum, 1991; Schirra and Mulas, 1995). Recently, postharvest heat treatments (Lurie, 1998; Ferguson et al., 2000) and host-pathogen interactions modulated by heat treatments (Schirra et al., 2000) have been rewieved.

Sodium carbonate (SC, Na_2CO_3, soda ash) and sodium bicarbonate (SBC, $NaHCO_3$, baking soda) are common food additives allowed with no restrictions for many applications by European and North American regulations (Lindsay, 1985; Multon, 1988). Their antimicrobial activity has been described for a variety of fruits and vegetables. SBC to control postharvest green and blue molds of citrus was first described by Barger (1928) as an alternative to the use of borax. Heated SC solutions have been used to improve cleaning and also to reduce postharvest decay of lemons in California for more than 70 years

(Eckert and Eaks, 1989), a synergistic effect between SC and HW being showed. Solutions of both salts provided satisfactory control of green mold on lemons (Smilanick et al., 1995, 1999) and oranges (Smilanick et al., 1997, 1999; Palou et al., 1999) and blue mold on oranges (Palou et al., 2001). However, their efficacy against both diseases has not been evaluated on clementine mandarins. The aim of this work was to evaluate HW, SC, SBC, and combinations of HW and SC for the control of green and blue molds on artificially inoculated 'Clemenules' mandarins.

Materials and methods

Fruit inoculation. Clementine mandarins (*Citrus reticulata* Blanco) cv. 'Clemenules', from commercial orchards in Baix Ebre and Montsià areas (Tarragona, Catalonia, Spain), were selected from field bins and used in the experiments before any commercial postharvest treatments were applied. The fruit were used the same day, or stored up to 2 weeks at 5ºC and 90% relative humidity (RH) before use. Before each experiment, fruit were randomized, washed with fresh water, disinfected superficially for 1 min in a diluted bleach dip (0.5% sodium hypochlorite), rinsed with water, and allow to air-dry at room temperature.

Petri dishes of potato dextrose agar (PDA) were inoculated with *P. digitatum* isolate PDM-1 or *P. italicum* isolate PIM-7 and incubated at 25ºC for 7 to 10 days. A conidial suspension was prepared in Tween 80 (0.05% w/v) in water and diluted to 10^6 spores ml^{-1} after counting spores with a hemacytometer. This concentration is recommended for evaluation of postharvest treatments to control green and blue molds of citrus fruit (Eckert and Brown, 1986). Using a micropipet, 25 µl of this suspension were placed in a 1 mm x 5 mm x 2 mm deep wound made with a stainless steel scalpel on the equator of each mandarin. The wound penetrated the albedo tissue, but not the juice sacs, and simulated frequent infection under commercial conditions.

Hot water and sodium carbonate treatments. Three stainless steel buckets each holding 24 l of water or SC solutions (Sigma Chemical Co.) were heated to the test temperature in a 172-liter stainless steel water tank fitted with a 9 kW electric resistance heater and thermostat. Metallic grid baskets containing the mandarins previously inoculated with *P. digitatum* or *P. italicum* were submerged in the buckets. Fruit inoculated with each pathogen were assayed in different solutions. Treated fruit were placed in plastic cavity trays that prevented contact infections and allowed to air-dry at room temperature.

(i) *Influence of SC concentration, temperature, and immersion period.* Three SC concentrations (0, 2, and 3%, w/v), three temperatures (20, 45, and 50ºC, ±1ºC), and two immersion periods (60 and 150 s) were tested on mandarins inoculated about 2 h before with *P. digitatum* or *P. italicum*. Within each experiment, each treatment was applied to four replicates of 10 fruits each. Treated fruit were stored at 20ºC and 90% RH. The number of infected mandarins was counted after 7 days of storage. The experiment was conducted twice.

(ii) *Influence of rinsing at low pressure the treated fruit.* Mandarins inoculated about 2 h before with *P. digitatum* or *P. italicum* were immersed for 150 s in water alone at 20±1ºC (control), water alone at 50±1ºC (HW), or 2% SC (w/v) at 50±1ºC. After treatment, half of the fruit were rinsed with about 30 ml of fresh water per fruit at low pressure in a spray 50 cm above the fruit for 5 s. Once dried, both rinsed and non-rinsed fruit were held at 20ºC and 90% RH for 7 days, at which time the incidence of green or blue mold was determined. Within each treatment, both rinse and non-rinse procedures were applied to four replicates of 10 fruits each. The experiment was conducted twice.

(iii) *Influence of the period of time between fruit inoculation and treatment.* Randomized mandarins were separated into two groups. Fruit in the first group were inoculated with *P. digitatum* or *P. italicum* as previously described about 24 h before treatments were applied and kept at the environmental conditions of the laboratory. Fruit in the second group were inoculated about 2 h before treatment. Mandarins from both groups were dipped for 150 s in water alone at 20±1ºC (control), water alone at 50±1ºC (HW), or 2% SC (w/v) at 50±1ºC. Each treatment was applied to four replicates of 10 fruits each. Decay incidence was determined after 7 days of storage at 20ºC and 90% RH (7 days after the fruit were treated, not inoculated). The test was conducted twice.

(iv) *Efficacy under long-term cold storage.* Mandarins inoculated about 2 h before with *P. digitatum* or *P. italicum*, were dipped for 150 s in water alone at 20±1ºC (control), water alone at 50±1ºC (HW), or 2% SC (w/v) at 50±1ºC, let to dry, and then stored at 3.5±1ºC and 98% RH for 2 months. Each treatment was applied to four replicates of 10 fruits each. The number of decayed fruit was determined after 30 and 60 days of storage.

Sodium bicarbonate treatments. Mandarins were inoculated with *P. digitatum* or *P. italicum* as described above and, about 2 h later, immersed for 60 or 150 s in 0 (control, water alone), 2, or 3% (w/v) SBC solutions (Sigma Chemical Co.) at room temperature (20±1ºC), using the buckets and the water tank previously described. The pH of SBC solutions rises rapidly at high temperature so only room temperature was tested (Smilanick et al., 1999). Each treatment was applied to four replicates of 10 fruits each. Once dried, treated fruit were placed into plastic cavity trays and stored at 20ºC and 90% RH for 7 days, at which time decay incidence was examined. The experiment was conducted twice.

Statistical analysis. Decay incidence data were analyzed by analyses of variance of the arcsine of the square root of the proportion of decayed fruit (SAS v. 8.00; SAS Institute Inc., Cary, NC, USA). Fisher's Protected Least Significant Difference (LSD) test ($P = 0.05$) was used for means separation.

Results

Hot water and sodium carbonate treatments. (i) *SC concentration, temperature, and immersion period.* HW dips at 45 or 50ºC for 60 or 150 s did not effectively control either green or blue mold. With decay incidence on the control treatments (water alone at 20ºC, SC concentration = 0) adjusted to 100%, the incidence of both green and blue molds on fruit treated with HW at 45ºC was greater than 75%, whereas at 50ºC it was about 50 and 70% for green mold and 65 and 80% for blue mold after 150- and 60-s dips, respectively (Fig. 1). No heat damage was observed after 7 days of storage at 20ºC on mandarins treated at 45 or 50ºC.

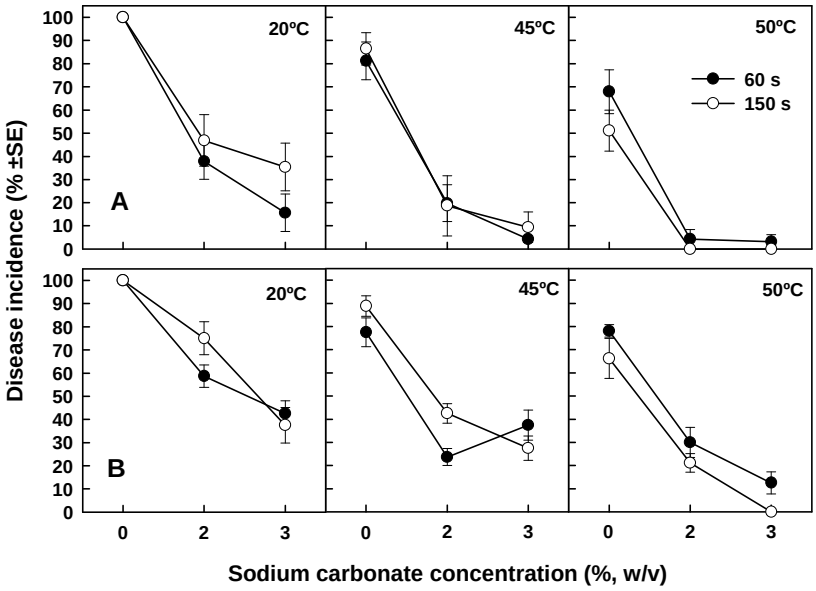

Fig. 1. Influence of temperature solution (20, 45, or 50ºC), sodium carbonate concentration (0, 2, or 3%), and immersion period (60 or 150 s) on the incidence of green (A) and blue (B) molds on artificially inoculated 'Clemenules' mandarins stored at 20ºC and 90% RH for 7 days.

Table 1. Analysis of variance of the incidence of green and blue molds on artificially inoculated 'Clemenules' mandarins immersed in sodium carbonate solutions and stored at 20ºC and 90% RH for 7 days

Source[a]	df	MS	F	*P* > F
Green mold				
Experiment (E)	1	79.71	0.15	0.7015
Concentration (C)	2	52065.94	96.41	<0.0001
Temperature (T)	2	12490.79	23.13	<0.0001
Immersion period (t)	1	13.23	0.02	0.8759
C x T	4	482.22	0.89	0.4704
C x t	2	314.51	0.58	0.5601
T x t	2	653.16	1.21	0.3018
C x T x t	4	80.85	0.15	0.9628
Error	125	540.04		
Blue mold				
Experiment (E)	1	1.94	0.02	0.9004
Concentration (C)	2	29884.20	241.46	<0.0001
Temperature (T)	2	6981.04	56.41	<0.0001
Immersion period (t)	1	434.75	3.51	0.0632
C x T	4	592.29	4.79	0.0013
C x t	2	937.22	7.57	0.0008
T x t	2	1903.87	15.38	<0.0001
C x T x t	4	81.69	0.66	0.6210
Error	125	123.76		

[a] Analysis was applied to the arcsine of the square root of the proportion of decayed fruit.

Concentration and temperature of SC solutions, but not immersion period, influenced significantly the control of both green and blue molds. The influence of concentration was greater than that of temperature (Table 1). In every test, SC treatment improved the control of both green and blue molds compared to the treatments with water alone (SC concentration = 0) (Fig. 1). With decay incidence for the control treatment (water alone at room temperature, 20±1ºC) adjusted to 100%, the incidence of green mold at concentrations of 2 or 3% SC was 5 to 20% at 45ºC and 0 to 5% at 50ºC. The incidence of blue mold was 20 to 40% and 0 to 30%, respectively (Fig. 1). While no significant interactions were observed for green mold, the effects of concentration and immersion period were significantly dependent on the temperature for blue mold (Table 1; Fig. 1B): differences in effectiveness between the control with water alone and those treatments with sodium carbonate were greater at 45 and 50ºC than at 20ºC; differences in effectiveness between concentrations of 2 and 3% SC were greater at 20 and 50ºC than at 45ºC. A treatment of 150 s enhanced the effectiveness of all concentrations compared to 60 s at 50ºC, but not at 20 or 45ºC. Likewise, for blue mold, the effect of the immersion period was dependent on the concentration (Table 1): a treatment of 150 s enhanced the effectiveness of the treatments at a concentration of 3% SC but not at concentrations of 0 or 2% SC (Fig. 1B).
No visible rind injury occurred in every test.

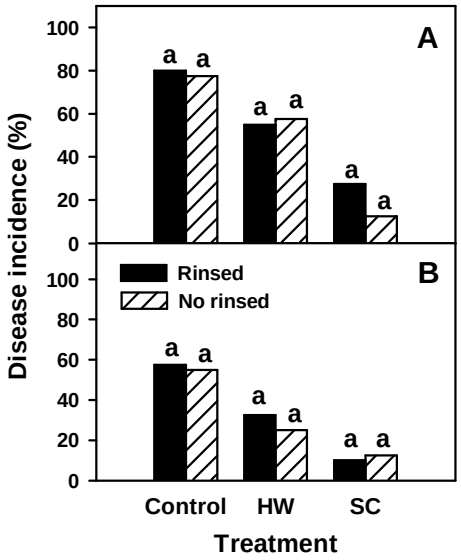

Fig. 2. Influence of rinsing at low pressure the treated fruit on the incidence of green (A) and blue (B) molds on artificially inoculated 'Clemenules' mandarins immersed for 150 s in water at 20ºC (control), water at 50ºC (HW), or 2% sodium carbonate at 50ºC (SC) and stored at 20ºC and 90% RH for 7 days. Within each treatment, columns with the same letter are not significantly different by Fisher's Protected LSD test ($P < 0.05$). Analysis of variance was performed on the arcsine-transformed data. Non-transformed means are shown.

(ii) *Low pressure rinse.* No significant differences in incidence of both green and blue molds were found between rinsed and non-rinsed mandarins treated with either HW at 50ºC or 2% SC at 50ºC and held for 7 days at 20ºC (Fig. 2). Slight deposition of SC on the skin of non-rinsed fruit (diffuse white stains) was noticed after the storage period.
(iii) *Time period between fruit inoculation and treatment.* In general, the incidence of both green and blue molds after 7 days of storage at 20ºC was higher on mandarins inoculated 24 h before treatment than on mandarins inoculated 2 h before treatment. However, the differences were only significant for control fruit (immersed in water at 20ºC) and for green mold on fruit treated with 2% SC at 50ºC (Fig. 3).

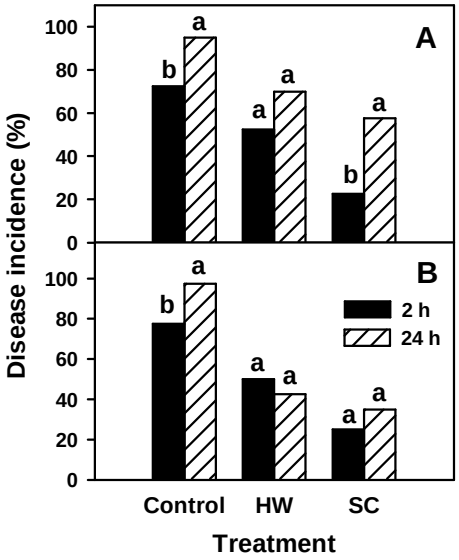

Fig. 3. Influence of the time period from inoculation of the pathogen to treatment (2 or 24 h) on the incidence of green (A) and blue (B) molds on artificially inoculated 'Clemenules' mandarins immersed for 150 s in water at 20ºC (control), water at 50ºC (HW), or 2% sodium carbonate at 50ºC (SC) and stored at 20ºC and 90% RH for 7 days. Within each treatment, columns with the same letter are not significantly different by Fisher's Protected LSD test ($P < 0.05$). Analysis of variance was performed on the arcsine-transformed data. Non-transformed means are shown.

(iv) *Long-term cold storage*. The incidence of both green and blue molds was significantly reduced by SC at 50ºC on mandarins stored at 3.5ºC for either 30 or 60 days. SC at 50ºC was more effective than HW at 50ºC in controlling both diseases. While the incidence of green and blue molds on HW-treated fruit after 30 days of storage was 42.5 and 72.5%, respectively, it was 2.5 and 15%, respectively, on SC-treated fruit (Fig. 4). No green mold reduction was observed on HW-treated fruit compared to control fruit. A significant increase in disease incidence occurred during the second month of cold storage. The incidence of blue mold (Fig. 4B) was higher than the incidence of green mold (Fig. 4A). No apparent chilling injury symptoms were observed on the fruit rind after the 60-day cold storage period.

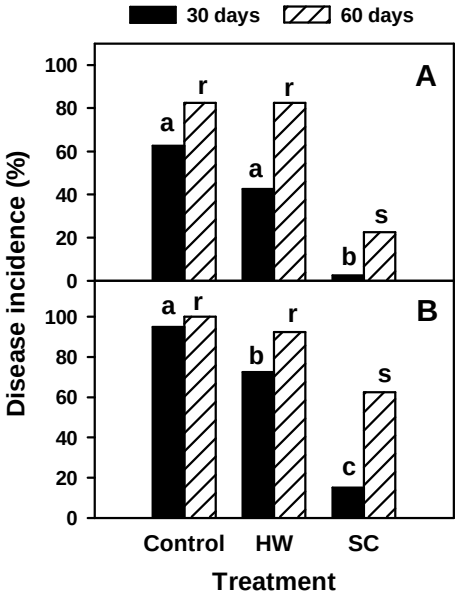

Fig. 4. Incidence of green (A) and blue (B) molds on artificially inoculated 'Clemenules' mandarins immersed for 150 s in water at 20ºC (control), water at 50ºC (HW), or 2% sodium carbonate at 50ºC (SC) after 30 and 60 days of storage at 3.5ºC and 98% RH. Within each cold storage period, columns with the same letter are not significantly different by Fisher's Protected LSD test ($P < 0.05$). Analysis of variance was performed on the arcsine-transformed data. Non-transformed means are shown.

Sodium bicarbonate treatments. While decay incidence on the control treatment (SBC concentration = 0, water alone) was 100%, the incidence of both green and blue molds after 7 days of storage at 20ºC was 40 to 60% on mandarins dipped in 2 or 3% SBC solutions at room temperature (Fig. 5). No significant differences in decay incidence were found between immersion periods of 60 and 150 s (P = 0.3528 and P = 0.7083 for green and blue molds, respectively); the effect of the immersion period was not dependent on the SBC concentration (P = 0.1427 and P = 0.3969 for green and blue molds, respectively).

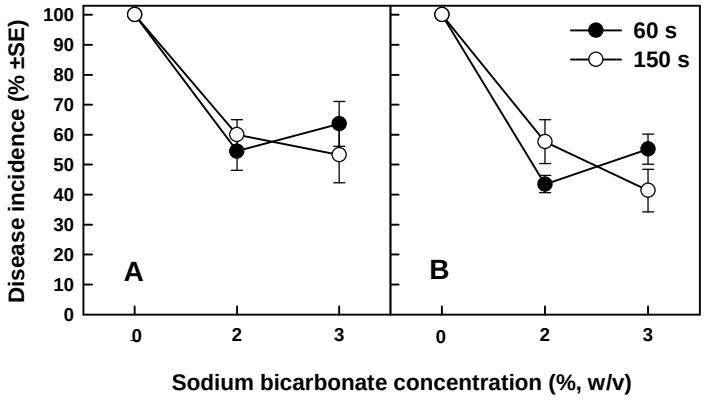

Fig. 5. Influence of sodium bicarbonate concentration (0, 2, or 3% w/v) and immersion period (60 or 150 s) on the incidence of green (A) and blue (B) molds on artificially inoculated 'Clemenules' mandarins treated at room temperature (20±1ºC) and stored at 20ºC and 90% RH for 7 days.

Discussion

Effective control of green and blue molds of clementine mandarins was not obtained by brief HW treatments at 45 or 50ºC. Similar poor effectiveness for HW at 45ºC, but considerable better effectiveness for HW at 50 to 53ºC were reported against green mold of oranges (Smoot and Melvin, 1963; Tuset et al., 1996; Usall et al., 1998), blue mold of oranges (Palou et al., 2001), green mold of lemons (Houck, 1967; Smilanick et al., 1995; Nafussi et al., 2000), or blue mold of grapefruits (Dettori et al., 1996). No heat injury to the mandarins treated at 50ºC was observed after the 7-day storage period. Schirra and D'hallewin (1997) reported that HW at 50 or 52ºC did not injure the rind of 'Fortune' mandarins whereas higher water temperatures induced rind browning.

SC significantly enhanced decay control compared to water alone at all temperatures and for all immersion periods. While moderate control of both green and blue molds was achieved by 2 or 3% SC at room temperature, heating SC solutions to 45ºC, and especially to 50ºC, improved SC effectiveness significantly. Many authors observed synergistic effects of heat and chemicals to control *Penicillium* decay on citrus fruit (Barkai-Golan and Apelbaum, 1991; Schirra and Mulas, 1995; Smilanick et al., 1995; Tuset et al., 1996). We also observed such effects for heated SC solutions in previous research (Smilanick et al., 1997, 1999; Palou et al., 1999, 2001). Hot SC solutions were more effective than HW at lower temperatures, which is important to minimize the risks of rind damage by heat. In general, commercial adoption of HW dips should be done with caution because the difference in temperature between control and severe injury is too small. In the present work, a 150 s dip in 3% SC at 50ºC controlled totally both green and blue molds of mandarins without noticeably injuring the fruit. However, these temperature and SC concentration were higher than those needed to reach effective control on oranges with a dip of 150 s, namely 2% SC and 40-45ºC (Smilanick et al., 1997; Palou et al., 2001). Although rind injuries on oranges were only associated with SC treatments at 56ºC or higher (Smilanick et al., 1999), a commercial treatment of mandarins with SC at 50ºC could probably be too risky.

In previous work with oranges, effectiveness of HW, SC, and SBC treatments in controlling blue mold was similar to effectiveness in controlling green mold (Usall et al., 1998; Palou et al., 1999, 2001). In this work, contrastingly, more severe HW or SC treatments were needed to control effectively blue mold of mandarins than to control green mold. By contrast, Hwang and Klotz (1938) observed that *P. italicum*

spores were more sensitive to the in vitro inhibitory effect of SC, SBC, and other salts than *P. digitatum* spores.

SC and SBC solutions must be rinsed off the fruit surface to prevent the deposition of salt residues on brushes and belts of packing and sorting equipment, and to prevent staining and desiccation of the fruit rind. In our tests, water rinsing by low-pressure sprays did not influence SC effectiveness significantly. Neither did it on oranges or lemons (Smilanick et al., 1999). In contrast, high-pressure water washing (about 1500 kPa) reduced the effectiveness of SC dips (Smilanick et al., 1999), presumably by removing SC residues from the fruit skin.

When fruit inoculation was performed about 24 h before treatment, decay incidence among control or treated fruit was in general higher as compared to fruit inoculated about 2 h before treatment, although the differences were not always significant. Dettori et al. (1996) reported that earlier HW treatments effectively controlled blue mold of grapefruit, whereas treatments 48-72 h after inoculation did not. The mycelium of *P. italicum* became thinner, with reduced branching, and unable to spread into the albedo when fruit were treated with HW at 50ºC 1 h after inoculation. Marloth (1931) observed, however, that germinated spores of either *P. italicum* or *P. digitatum* were more readily killed in vitro by SC and SBC solutions than non-germinated spores.

SC treatments controlled green and blue molds significantly better than HW treatments on long-term cold-stored mandarins. Control of blue mold was inferior to that of green mold, very likely because of the better adaptation of *P. italicum* to grow at temperatures below 10ºC (Whiteside et al., 1993). Similar results were obtained in artificially inoculated oranges stored at 3ºC for 2 months (Palou et al., 2001).

Solutions of 2 or 3% SBC at room temperature failed in controlling effectively both of the molds. Similarly to what was observed in the case of HW and SC treatments, the effectiveness of SBC against both diseases was lower on mandarins than on oranges or lemons (Smilanick et al., 1999; Palou et al., 1999, 2001). Since the same fungal strains and experimental procedures were used in most of these tests, induction of some defense mechanisms by the treatments in the wound-inoculated fruit would explain decay control better than a direct action against the pathogens. All HW, SC and SBC dips are non-curative treatments which effects in vivo are primarily fungistatic and not very persistent. These effects cannot be predicted by their activity in vitro. Induction of lignin-like polymers, synthesis of phytoalexins, and biogenesis of pathogen-related proteins are mechanisms associated with postharvest heat treatments (Ferguson et al., 2000). SC or SBC mode of action has not been completely elucidated. Their inhibitory ability depends on the presence of salt residues within the wound infection courts occupied by the fungus and on interactions between this residue and constituents of the rind. These interactions presumably alter the original in vitro toxicity of the salts to the spores (Marloth, 1931; Hwang and Klotz, 1938). Likewise, the combined effect of the pathogen and HW treatment induced the build up of resistance mechanisms in the peel of wound-inoculated lemons (Nafussi et al., 2000). Apparently, the particular response of clementine mandarins to such interactions compared to the response of other citrus species resulted, as shown by our results with 'Clemenules', in not so effective defense mechanisms. Mandarins are the most fragile among citrus fruit. Harvest, transportation and handling must be extremely cautious and especial precautions are required in the packinghouses to guarantee fruit quality, extend its commercial life, and minimize postharvest losses (Grierson and Ben-Yehoshua, 1986). Within each specie, moreover, the physiological condition of the fruit can also influence the effectiveness of the treatments. Orange susceptibility to postharvest decay, chilling injury, and rind injury caused by HW dips was greatly influenced by the harvest date (Schirra et al., 1998).

The protection levels against citrus postharvest diseases provided by synthetic fungicides are, in general, difficult to achieve with most of the alternative control methods that have been evaluated to date. Therefore, considerable research is currently focused on the combination of complementary physical, chemical, and biological treatments. Hot water and sodium carbonates are, in general, unable to provide the persistence or the antisporulant action against *Penicillium* actually provided by synthetic fungicides (Smilanick et al., 1999). In previous work, we observed that control of both citrus green and blue molds of oranges was significantly improved by combining SBC treatments with the application of the antagonistic biocontrol agent *Pantoea agglomerans* CPA-2 (Usall et al., 2000; Teixidó et al., 2001). Similar approach is also being evaluated on clementine mandarins.

Acknowledgements

This work was partially supported by the European Commission Project No. 2FD97-0492, the Catalonian Government (CIRIT, Comissió Interdepartamental de Recerca i Tecnologia), the Spanish Goverment (CICYT, Comisión Interministerial de Ciencia y Tecnología), and the Grup Exportador de Cítrics del Baix Ebre-Montsià (Tarragona, Catalonia, Spain).

References

Barger, W.R., 1928. Sodium bi-carbonate as a citrus fruit disinfectant. Calif. Citrograph 13, 164-174.

Barkai-Golan, R., Apelbaum, A., 1991. Synergistic effects of heat and sodium o-phenyl phenate treatments to inactivate *Penicillium* spores and suppress decay in citrus fruits. Trop. Sci. 31, 229-233.

Barkai-Golan, R., Phillips, D.J., 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. Plant Dis. 75, 1085-1089.

Dettori, A., D'hallewin, G., Aggabio, M., Marceddu, S., Schirra, M., 1996. SEM studies on *Penicillium italicum* – 'Star Ruby' grapefruit interactions as affected by fruit hot water dipping. Proc. Int. Soc. Citriculture 2, 1158-1163.

Díaz, M.A., Vila, R., 1988. El problema de la resistencia a los fungicidas: referencia a la situación en los almacenes españoles de comercialización de cítricos. Rev. Agroquim. Tecnol. Aliment. 28, 151-158 (in Spanish).

Eckert, J.W., 1990. Impact of fungicide resistance on citrus fruit decay control. In: Managing Resistance to Agrochemicals. Green, M.B., Le Baron, H.M., Moberg, W. K. (Eds.). Amer. Chem. Soc. Symp. Ser. 421, 286-302.

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

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

Ferguson, I.B., Ben-Yehoshua, S., Mitcham E.J., McDonald, R.E., Lurie, S., 2000. Postharvest heat treatments: introduction and workshop summary. Postharvest Biol Technol. 21, 1-6.

González-Aguilar, G.A., Zacarias, L., Mulas, M., Lafuente, M.T., 1997. Temperature and duration of water dips influence chilling injury, decay and polyamine content in "Fortune" mandarins. Postharvest Biol. Technol. 12, 61-69.

Grierson, W., Ben-Yehoshua, S., 1986. Storage of citrus fruits. In: Fresh Citrus Fruits. Wardowski, W.F., Nagy, S., Grierson, W. (Eds.), AVI Publising Co., Inc. Westport, CT, USA. pp. 479-507.

Houck, L.G., 1967. Hot water treatments for control of *Penicillium* green mold of Eureka lemons. Phytopathology 57, 99 (Abstract).

Hwang, L., Klotz, L.J., 1938. The toxic effect of certain chemical solutions on spores of *Penicillium italicum* and *P. digitatum*. Hilgardia 12, 1-38.

Lindsay, R.C., 1985. Food additives. In: Food Chemistry. Fennema, O.R. (Ed.), Marcel Decker, Inc., New York, NY, USA.

Lurie, S., 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14, 257-269.

Marloth, R.H., 1931. The influence of hidrogen-ion concentration and of sodium bicarbonate and related substances on *Penicillium italicum* and *P. digitatum*. Phytopathology 21, 169-198.

Multon, J.L., 1988. Aditivos y Auxiliares de Fabricación en las Industrias Agroalimentarias. Ed. Acribia, Zaragoza, Spain (in Spanish).

Nafussi, B., Ben-Yehoshua, S., Rodov, V., Peretz, J., Ozer, B.K., D'hallewin, G., 2000. Mode of action of hot water dip in reducing decay of lemon fruit. Proc. Int. Symp. Postharvest 2000, March 26-31, Jerusalem, Israel. In press.

National Research Council, 1993. Pesticides in the Diets of Infants and Children. National Academy Press, Washington, D.C., USA.

Palou, L., Smilanick, J.L., Usall, J., Viñas, I., 2001. Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis. 85, 371-376.

Palou, L., Usall, J., Aguilar, M.J., Pons, J., and Viñas, I., 1999. Control de la podredumbre verde de los cítricos mediante baños con agua caliente y carbonatos sódicos. Levante Agrícola 348, 412-421 (in Spanish with English abstract).

Schirra, M., D'hallewin, G., 1997. Storage performance of 'Fortune' mandarins following hot water dips. Postharvest Biol. Technol. 10, 229-238.

Schirra, M., D'Hallewin, G., Ben-Yehoshua, S., Fallik, E., 2000. Host-pathogen interactions modulated by heat treatment. Postharvest Biol Technol. 21, 71-85.

Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., Garau, V.L., 1998. Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. J. Agric. Food Chem. 46, 1177-1180.

Schirra, M., Mulas, M., 1995. Improving storability of "Tarocco" oranges by postharvest hot-dip fungicide treatments. Postharvest Biol. Technol. 6, 129-138.

Smilanick, J.L., Mackey, B.E., Reese, R., Usall, J., Margosan, D.A., 1997. Influence of concentration of soda ash, temperature, and immersion period on the control of postharvest green mold of oranges. Plant Dis. 81, 379-382.

Smilanick, J.L., Margosan, D.A., Henson, D.J., 1995. Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. Plant Dis. 79, 742-747.

Smilanick, J.L., Margosan, D.A., Mlikota, F., Usall, J., Michael, I.F., 1999. Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83, 139-145.

Smoot, J.J., Melvin, C.F., 1963. Hot water as a control for decay of oranges. Proc. Fla. State Hort. Soc. 76, 322-327.

Teixidó, N., Usall, J., Palou, L., Asensio, A., Nunes, C., Viñas, I., 2001. Improving biocontrol of green and blue molds of oranges by combining *Pantoea agglomerans* (CPA-2) and sodium bicarbonate. Eur. J. Plant Pathol. In press.

Tuset, J.J., 1987. Podredumbres de los Frutos Cítricos. Conselleria d'Agricultura i Pesca. Generalitat Valenciana, Valencia, Spain (in Spanish).

Tuset, J.J., Hinarejos, C., Mira, J.L., Martínez-Jávega, J.M., 1996. Tratamientos térmicos a los frutos cítricos para el control de las enfermedades de la post-recolección. Levante Agrícola 337, 342-347 (in Spanish).

Usall, J., Palou, L., Aguilar, M.J., Viñas, I., 1998. Hot water treatments for the control of postharvest green and blue molds in oranges. Proc. COST 98, Madrid, Spain. In press.

Usall, J., Teixidó, N, Smilanick, J.L., Viñas, I., 2000. Biological control of *Penicillium digitatum* on citrus fruits with antagonistic bacterium *Pantoea agglomerans*. Proc. Int. Symp. Postharvest 2000, March 26-31, Jerusalem, Israel. In press.

Whiteside, J.O., Garnsey, S.M., Timmer, L.W. (Eds.), 1993. Compendium of Citrus Diseases. 2nd ed. American Phytopatholgical Society Press, St. Paul, MN, USA.

Capítol 5

Evaluation of Food Additives and Low-toxicity Compounds as Alternative Chemicals for the Control of *Penicillium digitatum* (Pers.: Fr.) Sacc. and *Penicillium italicum* Wehmer on Citrus Fruit

L. Palou[1], J. Usall[1], J.L. Smilanick[2], M.J. Aguilar[1], I. Viñas[1]

[1]*Àrea de Postcollita, CeRTA, Centre UdL-IRTA, Lleida*
[2]*Horticultural Crops Research Laboratory, USDA-ARS, Fresno, CA, USA*

Referència: *Pest Manag. Sci.* En premsa.

Abstract

The effectiveness of low-toxicity chemicals as possible alternatives to synthetic fungicides for the control of postharvest citrus green and blue moulds was evaluated. Chemicals, mostly common food additives, were preliminarily selected through *in vivo* primary screenings with oranges artificially inoculated with *Penicillium digitatum* or *Penicillium italicum*. Selected compounds and mixtures were tested as heated solutions in small-scale trials. Immersion of artificially inoculated oranges or lemons for 120 s in solutions at 40.6ºC and natural pH of 0.2 M potassium sorbate, 0.2 M sodium benzoate or 0.1+0.1 M mixtures of potassium sorbate with sodium benzoate, sodium propionate or sodium acetate were the most effective organic acid salts tested and reduced green mould by 70 to 80% after 7 days of storage at 20ºC. The mixtures did not significantly enhance the effectiveness of potassium sorbate or sodium benzoate alone. These solutions were as effective as sodium carbonate or calcium polysulphide treatments and, in general, they were more effective on lemons than on oranges. Satisfactory control of green and blue moulds was obtained by dipping oranges for 150 s in 24.2 mM solutions of sodium molybdate or 1.0 mM solutions of ammonium molybdate at 48 or 53ºC, but not at 20ºC. At 53ºC, however, the effectiveness of hot water was not enhanced by both molybdates. Molybdenum salts at higher concentrations were phytotoxic and stained the fruit. At nonphytotoxic concentrations, the effectiveness of these solutions was more influenced by temperature than by concentration. In general, the inhibitory effects of all compounds tested were not fungicidal but fungistatic and not very persistent. In conclusion, potassium sorbate, sodium benzoate, and ammonium molybdate, among the wide range of chemicals tested, were superior for the control of postharvest *Penicillium* decay of citrus fruit. Future investigations should focus on their compatibility with other alternative physical or biological methods.

Key words: citrus, postharvest decay, *Penicillium*, green mould, blue mould, alternative chemical control

Introduction

Postharvest green mould, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mould, caused by *Penicillium italicum* Wehmer, are the most economically important postharvest diseases of citrus in all production areas characterized by low summer rainfall.[1] Blue mould is especially important on citrus fruit kept under cold storage.[2] Currently, both diseases are primarily controlled by application of synthetic fungicides such as imazalil, thiabendazole, or sodium ortho-phenyl-phenate (SOPP).[1] Alternative methods are needed because of concerns about environmental contamination and human health risks associated with fungicide residues.[3] Furthermore, the widespread use of these compounds in commercial packinghouses has led to the proliferation of resistant strains of the pathogens.[4,5]

At present, some alternative measures to manage citrus postharvest decay have been commercially adopted or are commercially available. These measures include immersion of fruit in tanks containing sodium bicarbonate, sodium carbonate, or lime sulphur solution[6,7] and application of the registered biological control formulations Aspire (*Candida oleophila* Montrocher; Ecogen, Langhorne, PA, USA), Biosave-100, and Biosave-110 (*Pseudomonas syringe* Van Hall; EcoScience, Worchester, MA, USA). These treatments, however, cannot completely replace the application of synthetic fungicides that is required for successful export market of citrus fruit. Carbonates and lime sulphur eradicate *Penicillium* incipient infections effectively, but they are often inferior to synthetic fungicides in efficacy, particularly with late season fruit,[6,7] and they lack antisporulant activity, which is important for sanitation and cosmetic purposes.[1] Biocontrol agents have shown great variability in their efficacy and usually cannot eradicate incipient infections or prevent *Penicillium* sporulation.[8] Physical treatments such as heat treatments (curing, hot water, or hot water brushing),[9] ultraviolet light treatments,[10] cold storage, or controlled atmosphere storage have shown direct and/or indirect activity against pathogens. Extensive research is currently focused on the induction of resistance to postharvest pathogens in citrus peel tissue by several mechanisms (induction of lignin-like polymers, synthesis of phytoalexins, biogenesis of pathogen-related proteins) in response to the presence of the pathogen combined with the application of postharvest treatments like heat[11] or biocontrol agents.[12]

In recent years, many chemicals have been evaluated as alternative control methods for citrus postharvest diseases, either alone or in combination with physical or biological treatments. Some

promising ones include: potassium sorbate,[13] sodium benzoate and sodium propionate,[14] sulphur dioxide,[15] ethanol,[15,16] several essential oils and plant extracts,[17] natural terpenic compounds,[18] calcium chloride,[19] acetic, formic and propionic acids,[20] nordihydroguaiaretic acid, poly-D-lysine and poly-D-arginine,[21] jasmonate,[22] chitosan and derivatives,[23] 2-deoxy-D-glucose,[24] calcium polysulphide,[7] sodium carbonate and sodium bicarbonate.[6,25]

Unfortunately, all the alternative methods that have been evaluated to date cannot provide by themselves the protection levels that synthetic fungicides typically provide. Therefore, it is important to integrate these alternative control technologies together to develop a treatment strategy able to reach those levels without compromising the quality or cost of citrus fruit to the consumer. The objective of the present work was to evaluate the effectiveness of a range of low-toxicity chemicals, mostly common food preservatives or additives, for the control of citrus green and blue moulds of oranges and lemons. A preliminary selection of chemicals was performed by testing their effectiveness at different concentrations in primary *in vivo* screenings. Selected compounds and mixtures were tested as heated solutions in small-scale trials.

Experimental methods

Fruit. Oranges (*Citrus sinensis* (L.) Osbeck), cvs. Washington Navel, Navelate, and Valencia, from commercial orchards in southern Tarragona (Catalonia, Spain) or the San Joaquin Valley (California, USA), and lemons (*Citrus limon* (L.) Burm.) cv. Eureka, from the San Joaquin Valley, were selected from field bins after harvest and used in the experiments before any commercial postharvest treatments were applied. The fruit were used the same day, or stored up to 2 weeks at 5ºC and 90% relative humidity (RH) before use. Before each experiment, fruit were randomised, washed with fresh water, disinfected superficially for 1 min in a diluted bleach dip (5 g litre^{-1} sodium hypochlorite), rinsed with water, and allowed to air-dry at room temperature.

***In vivo* primary screenings.** Forty chemicals, mostly common food preservatives or additives allowed by European and North American regulations,[26,27] were tested at least at three concentrations of active ingredient (a.i.) (Table 1). All were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MA, USA). A sterile mother solution of each chemical was prepared by filtering the solution through a 0.22 μm membrane filter. Sterile solutions at the desired concentrations were prepared by diluting with sterile water.

Petri dishes of potato dextrose agar (PDA) were inoculated with *P. digitatum* isolate PDM-1 or *P. italicum* isolate PIM-7 and incubated at 25ºC for 7 to 10 days. Conidial suspensions were prepared in Tween 80 (0.5 g litre^{-1}) in water and diluted to 10^6 conidia per millilitre after counting spores with a hemacytometer. This density is recommended for evaluation of postharvest treatments to control green and blue moulds.[28] Twenty-five microlitres of conidial suspension of *P. digitatum* or *P. italicum* were placed, using a micropipet, in a 1 mm wide, 5 mm long, 2 mm deep wound made with a stainless steel scalpel on the equator of Tarragona-grown Washington Navel, Navelate, or Valencia oranges. The wound penetrated the albedo tissue, but not the juice sacs, and simulated frequent infection under commercial conditions.

About 2 h later, oranges were inoculated in the pathogen inoculation site with 50 μl of the chemical solution at the desired concentration. Control fruit were inoculated with 50 μl of sterile distilled water. Treated fruit were stored at 20 ± 1ºC and 90 ± 5% RH for 7 days, at which time decayed fruit were counted. For each combination of chemical, concentration, and pathogen, four replicates of two oranges each were used. Each test was repeated at least once.

Table 1. Chemicals and concentrations tested against *Penicillium digitatum* and *Penicillium italicum* in *in vivo* laboratory screenings with Washington Navel, Navelate, or Valencia oranges.

Chemical	Formula	pH[a]	Tested concentrations (g litre^{-1} a.i.)[b]
Mineral salts			

Sodium chloride	NaCl	6.6	0.5, 1.0, 2.5, 5.0, 8.0, 10.0, 12.0, 15.0, 20.0
Potassium chloride	KCl	6.7	0.5, 2.5, 5.0
Calcium chloride	$CaCl_2$	6.9	0.5, 2.5, 5.0, 10.0
Magnesium chloride	$MgCl_2 \cdot 6H_2O$	6.6	0.5, 1.0, 2.5, 5.0
Ammonium chloride	NH_4Cl	6.3	0.5, 2.5, 5.0
Sodium phosphate, dibasic	Na_2HPO_4	9.1	0.5, 2.5, 5.0, 25.0, 50.0
Sodium phosphate, monobasic	$NaH_2PO_4 \cdot 2H_2O$	4.4	5.0, 25.0, 50.0
Potassium phosphate, dibasic	K_2HPO_4	9.0	5.0, 25.0, 50.0
Potassium phosphate, monobasic	KH_2PO_4	4.5	5.0, 25.0, 50.0
Potassium phosphate, dibasic	$(NH_4)_2HPO_4$	8.2	5.0, 25.0, 50.0
Ammonium phosphate, monobasic	$NH_4H_2PO_4$	4.4	5.0, 25.0, 50.0
Sodium molybdate	Na_2MoO_4	8.1	0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0, 50.0

Table 1. Continued

Chemical	**Formula**	**pH** [a]	**Tested concentrations (g litre^{-1} a.i.)** [b]
Mineral salts			
Ammonium molybdate[c]	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	4.9[d]	0.058, 0.116, 0.291, 0.582, 0.873, 1.164, 1.746, 2.328, 2.910, 5.820
Organic acids and salts			
Sodium formate	HCOONa	7.1	0.5, 1.0, 2.5, 5.0
Potassium formate	HCOOK	7.1	0.5, 1.0, 2.5, 5.0
Calcium formate	$(HCOO)_2Ca$	6.9	0.5, 1.0, 2.5, 5.0
Sodium acetate	$CH_3COONa \cdot 4H_2O$	8.1	0.5, 0.8, 1.0, 2.5, 5.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 25.0, 50.0
Sodium diacetate	$(CH_3COO)_2HNa$	5.2	0.5, 2.5, 5.0
Potassium acetate	CH_3COOK	7.7	0.5, 0.8, 1.0, 2.5, 5.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 22.0, 25.0, 50.0
Calcium acetate	$(CH_3COO)_2Ca$	7.5	0.5, 1.0, 2.5, 5.0
Sodium propionate	$C_3H_5O_2Na$	7.8	0.5, 2.5, 5.0
Calcium propionate	$C_6H_{10}O_4Ca$	7.9	0.5, 2.5, 5.0
Sorbic acid	$C_6H_8O_2$	3.3	0.5, 2.5, 5.0
Potassium sorbate	$C_6H_7O_2K$	7.8	0.5, 1.0, 2.5, 5.0

Sodium benzoate	$C_7H_5O_2Na$	7.6	0.5, 2.5, 5.0
Potassium benzoate	$C_7H_5O_2K$	7.7	0.5, 2.5, 5.0
Citric acid	$C_6H_8O_7$	2.6	0.5, 2.5, 5.0
Sodium citrate	$C_6H_5O_7Na_3 \cdot 2H_2$	8.0	0.5, 2.5, 5.0
Potassium citrate	$C_6H_5O_7K_3 \cdot H_2O$	8.2	0.5, 2.5, 5.0
Calcium citrate		6.1	0.5, 2.5, 5.0
Sodium lactate	$C_6H_5O_7Na_3$	7.2	0.5, 2.5, 5.0

Table 1. Continued

Chemical	Formula	pH [a]	Tested concentrations (g litre^{-1} a.i.)[b]
Organic acids and salts			
Calcium lactate	$C_6H_{10}O_6Ca \cdot 5H_2$	7.1	0.5, 2.5, 5.0
L-Tartaric acid	$C_4H_6O_6$	2.7	0.5, 2.5, 5.0
Potassium L-tartrate	$C_4H_4O_6K_2 \cdot 1/2H_2$	7.2	0.5, 2.5, 5.0
L-ascorbic acid	$C_6H_8O_6$	3.1	0.5, 2.5, 5.0
Sodium L-ascorbate	$C_6H_7O_6Na$	7.3	0.5, 2.5, 5.0
Other compounds			
Sodium hydroxide	NaOH	12.8	0.5, 2.5, 5.0
Calcium hydroxide	$Ca(OH)_2$	12.2	0.5, 2.5, 5.0
L-glutamic acid	$C_5H_9NO_4$	3.5	0.5, 2.5, 5.0
Sodium L-glutamate	$C_5H_8NNaO_4 \cdot H_20$	6.8	0.5, 2.5, 5.0

[a] pH at 20ºC for a concentration of 2.5 g litre^{-1} a.i.
[b] Each concentration of each chemical was tested against each fungus at least in two screenings of four replicates of 2 fruit each.
[c] Ammonium molybdate solutions were prepared in mM a.i. Tested concentrations were: 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, and 5.00 mM.
[d] pH for a concentration of 0.291 g litre^{-1} a.i. ammonium molybdate.

Small-scale trials. Compounds selected according to the results of the *in vivo* primary screenings were assayed in small-scale trials.
(i) *Equimolar solutions of organic acid salts and mixtures. P. digitatum* isolate M6R (obtained from J. W. Eckert, University of California, Riverside) was cultured on PDA at 25ºC for 7 to 14 days. Spores were rubbed from the agar surface with a sterile glass rod after 5 ml of 0.5 g litre^{-1} Triton X-100 in water were added. The spore suspension was passed through two layers of cheesecloth and diluted with water to an absorbance of 0.1 at 420 nm determined with a spectrophotometer. This density is approximately equivalent to 10^6 spores per millilitre.[29] California-grown Valencia oranges or Eureka lemons were inoculated by immersing a stainless steel rod with a probe tip 1 mm wide and 2 mm in length into the spore suspension and wounding each fruit once on the equator.

The inoculated fruit were held at room temperature (20 ± 2ºC) for about 24 h, at which time were placed into plastic baskets and immersed for 120 s in warm solutions (40.6 ± 0.5ºC) at the natural pH of 0.2 M organic acid salt solutions or 0.1+0.1 M mixtures of two organic acid salts. Immersion in 30 g litre^{-1} sodium carbonate or 8.5 g litre^{-1} calcium polysulphide (30 g litre^{-1} lime sulphur) solutions at 40.6ºC were also performed; the efficacy of these compounds against *P. digitatum* or *P. italicum* has been established.[6,7,25] The treatment equipment consisted of twelve 22-litre stainless steel tanks, each one individually fitted with a computer-controlled electrical heater, a temperature sensor, and a mechanical

agitation system. Control fruit were inoculated but untreated. After treatment, fruit were rinsed with 10 ml of deionized water per fruit at low pressure (200 kPa) in a spray 30 cm above the fruit for 5 s, placed in plastic cavity trays in wooden trays, and stored at 20 ± 1ºC and 90 ± 5% RH. After 7 days of storage, the incidence of green mould was recorded. Two different tests were performed. In the first test, twelve previously selected organic acid salts or mixtures were assayed on oranges (each treatment was applied to four replicates of 30 fruit each) and lemons (five replicates of 25 fruit each). Based on the results of this test, six compounds or mixtures were selected and tested again in a second test (five replicates of 25 fruit each) on both oranges and lemons.

(ii) *Sodium and ammonium molybdates.* Sodium molybdate at 0, 12.1, and 24.2 mM (= 0, 2.5, and 5.0 g litre^{-1}) and ammonium molybdate at 0, 0.5, and 1.0 mM were tested at 20, 48, and 53ºC (±1ºC) for the control of green and blue moulds**.** These concentrations were selected based on results from the primary screenings. Three stainless steel buckets each holding 24 litres of sodium or ammonium molybdate solutions at the desired concentration were heated to the test temperature in a 172-litre stainless steel water tank fitted with a 9-kW electric resistance heater and thermostat. Tarragona-grown Valencia oranges were inoculated with *P. digitatum* or *P. italicum* with a micropipet as described for the primary screenings and, 2 to 3 h later, were placed in metallic grid baskets and submerged in the buckets for 150 s. Fruit inoculated with each pathogen was treated separately. Treated fruit were placed in plastic holders on corrugated cartons, allowed to air-dry at room temperature, and stored at 20 ± 1ºC and 90 ± 5% RH. Decay incidence was recorded after 7 and 14 days of storage. Each treatment was applied to four replicates of 10 fruits each. Each combination of pathogen, salt, concentration, and temperature was assayed twice.

Statistical analysis. Depending on the experiment, one-, two-, or three-way analyses of variance were applied to the arcsine of the square root of the proportion of decayed fruit using SAS software (SAS Institute Inc., Cary, NC, USA). When appropriate, means were separated by Fischer's Protected Least Significant Difference test (LSD, $P = 0.05$).

Results

***In vivo* primary screenings.** Among the chemicals tested in this set of experiments (Table 1), only the following compounds reduced green or blue mould by more than 50% compared to the control treatments: calcium lactate, sodium lactate, sodium propionate, sodium acetate, potassium acetate, sodium benzoate, potassium benzoate, potassium sorbate, sodium molybdate, and ammonium molybdate (data not shown).

With the exception of dibasic sodium phosphate, phosphates at concentrations from 5.0 to 50.0 g litre^{-1} favoured the development of the pathogens. Decay incidence on oranges treated with dibasic sodium phosphate was not different than on control fruit. In order to determine the more suitable concentrations to be tested in the small-scale trials, most of these compounds were repeatedly assayed at different concentrations. Phytotoxicity (dark greenish blemishes on the rind area surrounding the inoculation site) was noticed on fruit treated with concentrations of sodium molybdate higher than 24.2 mM (5.0 g litre^{-1}). Ammonium molybdate was phytotoxic at very low concentrations (1.5 mM or higher). The highest nonphytotoxic concentrations of these compounds were assayed in the small-scale trials.

Small-scale trials. (i) *Equimolar solutions of organic acid salts and mixtures.* In the first test with selected organic acid salts and mixtures, the incidence of green mould (means of the tests with oranges and lemons) was significantly lower on fruit treated with potassium benzoate, sodium benzoate, potassium sorbate, potassium sorbate + sodium propionate, potassium sorbate + sodium acetate, and potassium sorbate + sodium benzoate. These compounds or mixtures reduced green mould incidence by more than 75% and their effectiveness was similar to that of sodium carbonate or calcium polysulphide (data not shown). A second test with these selected salts and mixtures was performed with both oranges and lemons. In a three-way analysis of variance of data from both tests, significant differences in decay incidence were found among treatments; the effect of the treatment was dependent on the fruit specie (Table 2). Joint results from both tests (Table 3) showed that, in general, organic acid salts and mixtures controlled green mould more effectively on lemons than on oranges. While no significant differences among treatments were observed on lemons (about 15 to 25% decay incidence), sodium benzoate, potassium sorbate, and the mixtures (20 to 30% decay incidence) were superior to potassium benzoate (about 46% decay incidence) on oranges. On oranges, further, the mixtures potassium sorbate + sodium

propionate and potassium sorbate + sodium acetate were as effective as sodium carbonate and superior to calcium polysulphide (Table 3). No visible rind injury occurred in any test.

(ii) *Sodium and ammonium molybdates*. The combined effect of concentration and temperature of solutions of sodium molybdate or ammonium molybdate were evaluated on Valencia oranges. For both compounds, the influence of temperature was greater than that of concentration. When compared to the control treatment (water alone, concentration = 0), solutions at 20ºC of sodium molybdate at nonphytotoxic concentrations effectively controlled blue mould, but not green mould, after 7 days of storage at 20ºC (Fig. 1). Both diseases were significantly controlled by sodium molybdate applied at 48ºC. At 53ºC, water alone was as effective as sodium molybdate solutions. After 7 days of storage at 20ºC, no significant differences were observed between sodium molybdate concentrations of 12.1 and 24.2 mM at every temperature (Fig. 1).

Table 2. Analysis of variance of the incidence of green mould on artificially inoculated Valencia oranges and Eureka lemons

Source[a]	**df**	**MS**	**F**	***P* > F**
Experiment (E)	1	157.756	3.91	0.0500
Fruit (F)	1	929.172	23.05	<0.0001
Treatment (T)	8	7609.930	188.80	<0.0001
E x F	1	14.681	0.36	0.5472
E x T	8	65.660	1.63	0.1223
F x T	8	202.142	5.02	<0.0001
E x F x T	7	235.233	5.84	<0.0001
Error	143	40.307		

[a] Analysis was applied to the arcsine of the square root of the proportion of decayed fruit.

Table 3. Green mould incidence on artificially inoculated Valencia oranges and Eureka lemons immersed for 120 s in solutions of equimolar organic acids or mixtures at 40.6ºC and at natural pH, rinsed with 10 ml of deionized water per fruit, and stored for 7 days at 20ºC

Treatment	a.i. conc. (M)	pH (at 20ºC)	Green mould incidence (%)[a]	
			Oranges	Lemons
Control	NA[b]	NA	99.1 a	100.0 a
K benzoate	0.2	7.6	45.7 b	17.2 b
Na benzoate	0.2	7.7	29.1 cd	18.4 b
K sorbate	0.2	7.9	28.6	22.0 b
K sorbate + Na	0.1+0.1	8.3	27.2	19.2 b
K sorbate + Na	0.1+0.1	8.3	22.0 de	24.8 b
K sorbate + Na acetate	0.1+0.1	7.0	20.9 de	16.4 b
Na carbonate	0.28	11.3	19.8 e	22.4 b
Ca polysulphide	8.5[c]	10.6	33.8 c	19.6 b

[a] Values within columns followed by unlike letters are different by Fisher's Protected LSD test (P = 0.05) applied after an analysis of variance of the arcsine of the square root of the proportion of decayed fruit. Non-transformed data are shown. For oranges, values are the means of two experiments, one with four replicates of 30 fruit each and the other with five replicates of 25 fruit each; for lemons, values are the means of two experiments with five replicates of 25 fruits each.

[b] NA = not applicable.

[c] Active ingredient concentration (g litre^{-1}) in a 30 g litre^{-1} lime sulphur solution.

When compared to the control treatment (water alone, concentration = 0), nonphytotoxic concentrations of ammonium molybdate significantly controlled green mould only at 48ºC (with concentrations of 0.5 and 1.0 mM ammonium molybdate, green mould incidence was 10 and 0%, respectively, after 7 days of storage at 20ºC; Fig. 2A). Blue mould was significantly controlled only by a 1.0 mM solution of ammonium molybdate at 48ºC (Fig. 2B). At 53ºC, sodium molybdate did not enhance the effectiveness of hot water. Water alone at 53ºC reduced the incidence of both green and blue moulds by more than 95% (Fig. 2).

In every test, decay incidence after 14 days of storage was higher than after 7 days (Fig. 1, 2). No visible rind injuries were caused by any treatment.

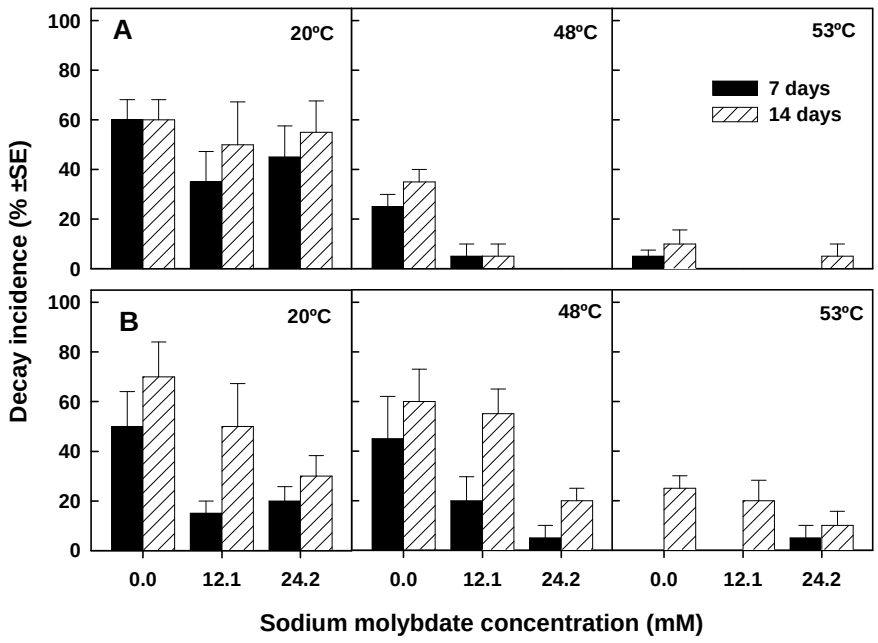

Fig. 1. Incidence of green (A) and blue moulds (B) on artificially

inoculated Valencia oranges immersed for 150 s in sodium molybdate solutions at 20, 48, or 53ºC (±1ºC) and stored at 20ºC and 90% RH for 7 or 14 days. Values are the means of two experiments. Each treatment was applied to four replicates of 10 oranges each.

Discussion and conclusions

Most of the chemicals tested during the selection process showed no *in vivo* inhibitory activity against either green or blue mould. Some compounds, such as most of the phosphates, even increased decay incidence among artificially inoculated oranges. Presumably, these chemicals provided additional nutrients and/or enhanced environmental conditions for the development of the pathogens. The most effective treatments in reducing both green and blue moulds were organic acid salts and their mixtures and sodium and ammonium molybdates. Improved effectiveness of chemical treatments to control citrus postharvest decay (synthetic fungicides as well as alternative chemicals) by heating the solutions as compared to solutions at room temperature has been repeatedly reported.[7,15,25,30,31] We chose temperatures ranging from 40 to 53ºC because synergistic effects between these temperatures and alternative chemicals such as sodium carbonate or lime sulphur had been previously observed; lower temperatures did not contributed to control ability and heat was phytotoxic at higher temperatures.[1,6,7,25]

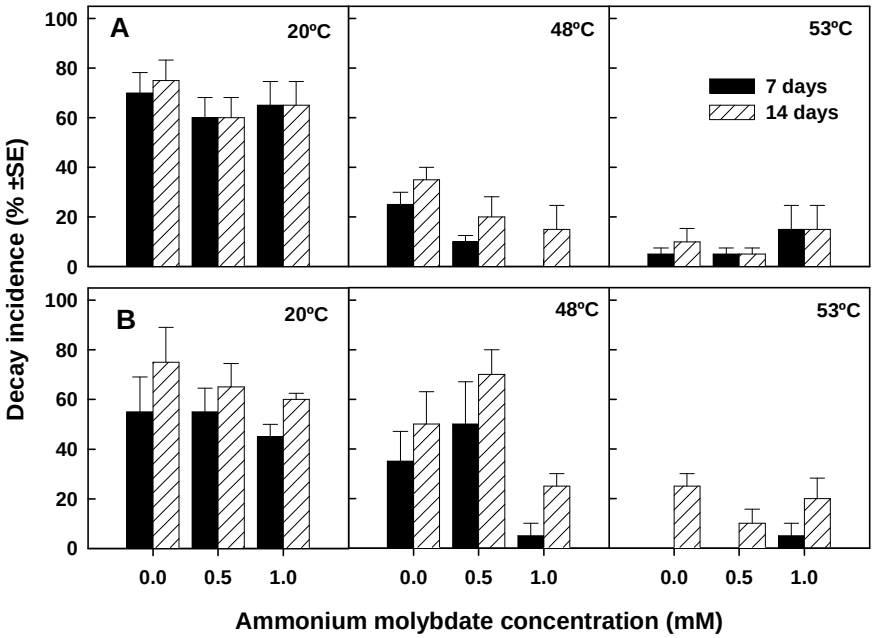

Fig. 2. Incidence of green (A) and blue (B) moulds on artificially inoculated Valencia oranges immersed for 150 s in ammonium molybdate solutions at 20, 48, or 53ºC (±1ºC) and stored at 20ºC and 90% RH for 7 or 14 days. Values are the means of two experiments. Each treatment was applied to four replicates of 10 oranges each.

In this work, the treatments with organic acid salts and mixtures were, in general, comparatively more effective in controlling green mould on lemons than on oranges. The reason was not a difference in fruit susceptibility to green mould because decay incidence among untreated control fruit was, in every test, high and similar among oranges and lemons. Disease development is a result of complex interactions between host, pathogen, and environment. The inhibitory ability of the salts depends on the presence of residues within the wound infection courts occupied by the fungus and on interactions between this residue and constituents of the rind. Apparently, the nature of such interactions would be different in oranges and lemons as a consequence of different albedo characteristics or presence of different constituents in the rind (for instance, the pH of the albedo is lower in lemons than in oranges). We observed in other work that the effectiveness of hot water, sodium carbonate and sodium bicarbonate

against green and blue moulds was lower on mandarins than on oranges (Palou L, unpublished). Furthermore, these interactions presumably alter the original toxicity of the salts to the pathogen and, therefore, their control ability cannot be predicted by their activity *in vitro*. In experiments with carbonates, notable differences were detected between results of *in vitro* and *in vivo* tests.[6,32] Wisniewski *et al.*[21] found no correlation between the inhibitory activities *in vitro* and *in vivo* of different compounds tested against *P. digitatum* and *Botrytis cinerea*. For these reasons, our primary screenings were performed *in vivo* and we did not preliminarily assess the toxicity *in vitro* of the substances.

Sodium benzoate, potassium sorbate, and its mixtures were the most effective of the tested organic acid salts. These compounds are common processed food preservatives with a broad-spectrum activity against moulds and yeasts that are generally considered safe and accepted worldwide for use in foods. The use of potassium sorbate to control citrus postharvest decay was first described by Smoot and McCornack in 1978.[13] Solutions at 20 g litre^{-1} were claimed to be effective in controlling benzimidazole-resistant strains of *P. digitatum* when used alone[13] or in combination with the synthetic fungicide thiabendazole.[33] However, Wild[34] reported no benefit from the use of such mixture and observed that potassium sorbate was only 20% as effective as the fungicide sodium ortho-phenyl-phenate in reducing green mould. This author observed that the control ability of potassium sorbate was increased by heating the solution to temperatures higher than 40ºC. Hall[14] reported similar effectiveness for potassium sorbate, sodium benzoate and sodium propionate in controlling green mould of oranges. In our tests, sodium propionate was inferior to the other two salts. Potassium sorbate at 0.15 and 0.20 g litre^{-1} prevented the growth in PDA medium of *P. digitatum* and *P. italicum*, respectively.[35] Potassium sorbate at 20 g litre^{-1} heated to 50ºC effectively controlled sour rot, caused by *Geotrichum citri-aurantii*, on lemons.[36] In general, although potassium sorbate treatments have not been widely adopted for use by the citrus industry because more effective postharvest treatments are still available, their value to control benzimidazole-resistant strains of *Penicillium* has been demonstrated.[1,37] In our experiments, although their effectiveness was usually slightly higher, the mixtures did not provide a significant benefit compared to potassium sorbate or sodium benzoate alone. When the incubation period of treated fruit was prolonged to 14 days at 20ºC, we recorded an increase of decay incidence compared to 7 days (data not shown). Therefore, the effects *in vivo* of potassium sorbate, sodium benzoate, mixtures and, in general, all organic acid salts were not fungicide but primarily fungistatic and not very persistent. This response is similar to the hot water, sodium carbonate, and sodium bicarbonate treatment.[6,25] The effectiveness of organic acids is pH dependent because the undissociated form of the acid is primarily responsible for the antimicrobial activity.[38] Therefore, the pK_a of the acid and the pH of the environment where it resides are important. The pH of albedo tissue, where infections by *P. digitatum* and *P. italicum* begin, is 5 to 5.5 on oranges and lemons, and most organic acid salts are effectively inhibitory at this pH and lower. The pK_a of potassium sorbate and sodium benzoate are 4.8 and 4.2, respectively. We presume, once the near neutral pH solutions of the salts were applied, they became more active within the relatively low pH of the wounds in the albedo tissue.

Among the inorganic salts we tested, only sodium and ammonium molybdates controlled green and blue moulds effectively. Both chemicals are fertilizers that have been used as sources of molybdenum for foliar or soil applications.[39] Moreover, sodium molybdate has a role on the microbial fixation of nitrogen and its application improved the yield of leguminous crops.[40] In contrast to sodium molybdate, some inhibitory activity of ammonium molybdate against pathogenic fungi has been reported.[41] Ammonium molybdate was effective at considerably lower concentrations than sodium molybdate, although it was also phytotoxic at lower concentrations. In other work,[42] our group found that 5 mM solutions of ammonium molybdate at room temperature were effective in controlling postharvest decay caused on apple by the pathogens *Penicillium expansum*, *B. cinerea*, and *Rhizopus stolonifer*. When combined, this compound improved the efficacy of the biocontrol agent *Candida sake* CPA-1 against these fungi.[43] Furthermore, it also inhibited the *in vitro* spore germination of *P. expansum* and *B. cinerea* more effectively than sodium molybdate or other molybdenum salts. An interference with the metabolic processes of phosphorylation/dephosphorylation by inhibiting the enzymatic activity of acid phosphatases has been suggested as a possible mechanism of action.[42] In our primary screenings, such concentration of 5 mM ammonium molybdate completely controlled both green or blue moulds on all cultivars of oranges tested, but this concentration injured and stained the rind. In the small-scale trials, we did not obtain satisfactory control of green or blue moulds with 1 mM solutions of ammonium molybdate at room temperature but we did with solutions heated to 48ºC. The feasibility of ammonium molybdate as a preservative for fresh fruit was supported by studies quantifying its acute oral toxicity performed by the

Centre d'Investigació i Desenvolupament Aplicat (Barcelona, Catalonia, Spain). Its LD_{50} is 1,714.3 mg per kg of live weight of Sprague Dawley rat; at this concentration no mortality or alterations in tested animals were observed (data not shown).

In conclusion, potassium sorbate, sodium benzoate, and ammonium molybdate were, among the wide range of chemicals tested, the most promising compounds to control postharvest *Penicillium* decay of citrus fruit. Thus, they are two more alternatives to be included in a decay control program when synthetic fungicides will be definitively prohibited. Future investigations should focus on their compatibility with other alternative physical or biological methods.

Acknowledgements

We thank the Catalonian Government (CIRIT, Comissió Interdepartamental de Recerca i Tecnologia), the Grup Exportador de Cítrics del Baix Ebre-Montsià (Tarragona, Catalonia, Spain), and the California Citrus Research Board for their financial support.

References

1. Eckert JW and Eaks IL, Postharvest disorders and diseases of citrus fruits, in The Citrus Industry Vol 5, ed by Reuter W, Calavan EC and Carman GE, Univ. of California Press, Berkeley, CA, USA, pp 179-260 (1989).
2. Whiteside JO, Garnsey SM and Timmer LW, Eds, Compendium of Citrus Diseases, 2nd ed, APS Press, St Paul, MN, USA (1993).
3. National Research Council, Pesticides in the Diets of Infants and Children, National Academy Press, Washington DC, USA (1993).
4. Eckert JW, Impact of fungicide resistance on citrus fruit decay control, in Managing Resistance to Agrochemicals, ed by Green MB, Le Baron HM and Moberg WK, ACS Symp Ser 421:286-302 (1990).
5. Holmes GJ and Eckert JW, Sensitivity of Penicillium digitatum and P. italicum to postharvest citrus fungicides in California. Phytopathology 89:716-721 (1999).
6. Smilanick JL, Margosan DA, Mlikota F, Usall J and Michael IF, Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis 83:139-145 (1999).
7. Smilanick JL and Sorenson D, Control of postharvest decay of citrus fruit with calcium polysulphide. Postharvest Biol Technol 21:157-168 (2001).
8. Eckert JW, Past and present in the post-harvest treatment of citrus fruits to control decay during storage and marketing, in Proc Int Symp Post-harvest Treatment of Citrus Fruits to Control Decay during Storage and Marketing, October 13, Acireale, Italy, pp 129-135 (1997).
9. Ferguson IB, Ben-Yehoshua S, Mitcham EJ, McDonald RE and Lurie S, Postharvest heat treatments: introduction and workshop summary. Postharvest Biol Technol 21:1-6 (2000).
10. Chalutz E, Droby S, Wilson CL and Wisniewski ME, UV-induced resistance to postharvest diseases of citrus fruit. J Phytochem Photobiol 15:367-374 (1992).
11. Schirra M, D'Hallewin G, Ben-Yehoshua S and Fallik E, Host-pathogen interactions modulated by heat treatment. Postharvest Biol Technol 21:71-85 (2000).
12. Droby S, Porat R, Vinocur V, Cohen L, Weiss B and Daus A, Induction of resistance to Penicillium decay in citrus fruit by the yeast biocontrol agent Candida oleophila. Proc Int Symp Postharvest 2000, March 26-31, Jerusalem, Israel (In press).
13. Smoot JJ and McCornack AA, The use of potassium sorbate for citrus decay control. Proc Fla State Hort Soc 91:119-122 (1978).
14. Hall DJ, Comparative activity of selected food preservatives as citrus postharvest fungicides. Proc Fla State Hort Soc 101:184-187 (1988).
15. Smilanick JL, Margosan DA and Henson DJ, Evaluation of heated solutions of sulphur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. Plant Dis 79:742-747 (1995).
16. Brown GE and Baraka MA, Effect of washing sequence and heated solutions to degreened Hamlin oranges on Diplodia stem-end rot, fruit colour and phytotoxicity. Proc Int Soc Citriculture 2:1164-1170 (1996).

17. Wilson CL, Solar JM, El-Ghaouth A and Wisniewski ME, Rapid evaluation of plant extracts and essential oils for antifungal activity against Botrytis cinerea. Plant Dis 81:204-210 (1997).
18. Bompeix G and Cholodowski-Faivre D, Fungicides and natural plant products combined with hot dip treatments in water, in Proc Int Symp Post-harvest Treatment of Citrus Fruits to Control Decay during Storage and Marketing, October 13, Acireale, Italy, pp 153-164 (1997).
19. Droby S, Wisniewski ME, Cohen L, Weiss B, Touitou D, Eilam Y, Chalutz E, Influence of CaCl2 on Penicillium digitatum, grapefruit peel tissue, and biocontrol activity of Pichia guilliermondii. Phytopathology 87:310-315 (1997).
20. Sholberg PL, Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay. Plant Dis 82:689-693 (1998).
21. Wisniewski ME, Droby S, El-Ghaouth A and Wilson CL, The use of food additives to control postharvest decay and enhance biocontrol activity of yeast antagonists, in Proc Int Congress Plant Pathol, August 9-16, Edinburgh, Scotland, Abstract 5.2.61 (1998).
22. Droby S, Porat R, Cohen L, Weiss B, Shapiro B, Philosoph-Hadas S and Meir S, Suppressing green mold decay in grapefruit with postharvest jasmonate application. J Amer Soc Hort Sci 124:184-188 (1999).
23. El-Ghaouth A, Smilanick JL and Wilson CL, Enhancement of the performance of Candida saitoana by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest Biol Technol 19:103-110 (2000).
24. El-Ghaouth A, Smilanick JL and Wilson CL, Wisniewski M and Wilson CL, Improved control of apple and citrus fruit decay with a combination of Candida saitoana and 2-Deoxy-D-Glucose. Plant Dis 84:249-253 (2000).
25. Palou L, Smilanick JL, Usall J and Viñas I, Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis 85:371-376 (2001).
26. EPCD, European Parliament and Council Directive (95/2/EC), Food Additives other than Colours and Sweeteners, 20 February 1995, OJ L61, 18.03.1995, pp 1 (1995).
27. EAFUS, "Everything" Added to Food in the United States: a Food Additive Database, On-line, US Food and Drug Administration, Center for Food Safety and Applied Nutrition, http://vm.cfsan.fda.gov/~dms/eafus.html (2001).
28. Eckert JW and Brown GE, Evaluation of postharvest treatments for citrus fruits, in Methods for Evaluating Pesticides for Control of Plant Pathogens, ed by Hickey KD, APS Press, St Paul, MN, USA, pp 92-97 (1986).
29. Morris SC and Nicholls PJ, An evaluation of optical density to estimate fungal spore concentrations in water suspensions. Phytopathology 68:1240-1242 (1978).
30. Barkai-Golan R and Apelbaum A, Synergistic effects of heat and sodium o-phenyl phenate treatments to inactivate Penicillium spores and suppress decay in citrus fruits. Trop Sci 31:229-233 (1991).
31. Schirra M and Mulas M, Improving storability of "Tarocco" oranges by postharvest hot-dip fungicide treatments. Postharvest Biol Technol 6: 129-138 (1995).
32. Hwang L, Klotz LJ, The toxic effect of certain chemical solutions on spores of Penicillium italicum and P. digitatum. Hilgardia 12:1-38 (1938).
33. Nelson PM, Wheeler RW and McDonald PD, Potassium sorbate in combination with benzimidazoles reduces resistant Penicillium digitatum decay in citrus. Proc Int Soc Citriculture 2:820-823 (1983).
34. Wild BL, Fungicidal activity of potassium sorbate against Penicillium digitatum as affected by thiabendazole and dip temperature. Scientia Hortic 32:41-47 (1987).
35. Matamoros-León B, Argaiz A and López-Malo A, Individual and combined effects of vanillin and potassium sorbate on Penicillium digitatum, Penicillium glabrum, and Penicillium italicum growth. J Food Prot 62:540-542 (1999).
36. Kitagawa H and Kawada K, Effect of sorbic acid and potassium sorbate on the control of sour rot of citrus fruits. Proc Fla State Hort Soc 97:133-135 (1984).
37. Bancroft MN, Gardner PD, Eckert JW and Baritelle JL, Comparison of decay strategies in California lemon packinghouses. Plant Dis 68:24-28 (1984).
38. Davidson PM, Chemical preservatives and natural antimicrobial compounds, in Food Microbiology, Fundamentals and Frontiers, ed by Doyle MP, Beuchat LR and Montville TJ, ASM Press, Washington DC, USA, pp 520-556 (1997).
39. Mondy NI and Munshi CB, Effect of soil and foliar application of molybdenum on the lycoalkaloid and nitrate concentration of potatoes. J Agric Food Chem 41:256-258 (1993).

40. Ranganayaki S and Mohan C, Effect of sodium molybdate on microbial fixation of nitrogen Azotobacter chroococcum. Z Allg Mikrobiol 21:607-610 (1981).
41. Singh RS and Khanna RN, Effect of certain inorganic chemicals on growth and spore germination of Alternaria tenuis Auct., the fungus causing core rot of mandarin oranges in India. Mycopath Mycol Appl 37:89-96 (1969).
42. Nunes C, Usall J, Teixidó N, Ochoa de Eribe X and Viñas I, Control of postharvest decay of apples by preharvest and postharvest application of ammonium molybdate. Pest Manag Sci (In press).
43. Nunes C, Usall J, Teixidó N and Viñas I, Improvement of Candida sake biocontrol activity against postharvest decay by the addition of ammonium molybdate. J Appl Microbiol (In press).