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Effect of maturity stage, ripening time, harvest year and fruit characteristics on the susceptibility to *Penicillium expansum* link of apple genotypes from Queretaro, Mexico

Rocío Aurora Sandoval Chávez[a], Ramón Álvar Martínez Peniche[a,*], Sofía Arvizu Medrano[a], Lourdes Soto Muñoz[b], María del Socorro Chávaro Ortíz[a], Neus Teixidó Espasa[c], Rosario Torres Sanchis[c]

[a] *Academic Group of Microbial Food Safety, DIPA, Chemistry Department, Autonomous University of Querétaro, Cerro de las Campanas s/n Col. las Campanas, 76010 Querétaro, Qro, Mexico*
[b] *Food Technology Department, Lleida University, XaRTA-Postharvest, Agrotecnio Center, Av. Rovira Roure, 191, 25198 Lleida, Catalonia, Spain*
[c] *IRTA, XaRTA-Postharvest, Rovira Roure 191, 25198 Lleida, Catalonia, Spain*

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ABSTRACT

The aim of this study was to determine the effects of maturity stage, ripening time, year, and several quality parameters of the fruit (total soluble solids, acidity, pH, firmness, and color) on the susceptibility to *Penicillium expansum* of apple genotypes established in Querétaro, México. The accessions consisted in cultivars, creoles and low chilling hybrids obtained in Mexico. The development of the disease was determined by measuring the lesion diameter of decay (severity) on fruits inoculated with 10^4 spores mL^{-1} and incubated for 8 days at 26 °C. Data were analyzed for significant difference and for significant correlation by analysis of variance and principal component analysis (PCA), respectively. In 2011, commercial maturity apples exhibited a greater tolerance to *P. expansum* than did over-mature apples (lesion diameters of 31.3 and 34.9 mm, respectively); however, the ripening time of genotypes did not influence the development of the pathogen in either year of study (21.8, 23.2, and 25.5 mm in diameter for early-, middle-, and late-ripening genotypes, respectively, in 2012). Differences in the susceptibility of genotypes were observed in both harvest years; the most outstanding were '436', '442-9' and '441' (17.4, 17.5 and 17.6 mm, respectively) in contrast to 'Golden Delicious' (34.2 mm) in 2012; nevertheless, no correlation was observed in genotypes behavior between the two harvest years ($r = 0.11^{NS}$). Excluding total soluble solids in 2012, PCA analysis did not reveal any correlation between pathogen severity and fruit quality parameters, suggesting the possibility that other mechanisms are involved in the fruit-pathogen interaction. This study demonstrated for the first time that ripening time does not affect the susceptibility to *P. expansum* in apple fruits.

1. Introduction

Post-harvest losses of apple fruits are primarily attributable to the presence of fungal diseases (Narayanasamy, 2006), and *Penicillium expansum* is responsible for 80% of these losses (Spotts et al., 1999). This fungus has developed resistance to some chemical fungicides used for its control (Baraldi et al., 2003); therefore, regulatory agencies have established restrictions on the use of these products (Palou et al., 2009). In addition, indiscriminate use of fungicides may be harmful for the environment and for humans (Teixidó et al., 2011). For these reasons, more attention should be paid to alternative methods of fungal control, including biological control, sanitization, physical treatments and/or the application of safe substances (Janisiewicz and Korsten, 2002). However, most of these treatments are not broad-spectrum and cannot be used extensively (Janisiewicz et al., 2008).

Obtaining disease-resistant cultivars has been always important in apple breeding programs (Janick et al., 1996); however, less attention has been paid to the resistance of post-harvest fruits (Sansavini et al., 2004). Even a slight improvement in the fruit tolerance to decay could increase the efficiency of other types

* Corresponding author. Tel.: +01524421921304; fax: +01524421921304.
E-mail address: alvar@uaq.mx (R.Á.M. Peniche).

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of treatments and result in an additive or even synergistic effect (Janisiewicz et al., 2008).

Few studies concerning the evaluation of fruit diseases tolerance have been performed. Gradziel (1994) proved that peach cultivar 'Bolinha' was tolerant to *Monilinia fructicola*; Smith et al. (1996) reported that blueberry cultivars 'Centurion' and 'Southland' showed the lowest susceptibility to *Colletotrichum acutatum*; Vázquez et al. (2001) observed that kernels of pecan cultivar 'Wichita' were less affected by *Aspergillus flavus* and *A. parasiticus* than those of Mexican native pecans. As for apples, Spotts et al. (1999) detected differences in the sensitivity to some post-harvest pathogens of various commercial cultivars.

Intrinsic properties such as chemical composition, fruit firmness, peel thickness and ripening pattern, among others, may be the factors contributing the level of cultivar tolerance to the pathogen (Johnston et al., 2009). Concerning ripening time trait, a late-ripening cultivar will have a higher post-harvest accumulation and a lower post-harvest degradation of ascorbic acid, which has been identified as toxic to pathogens such as *Botrytis cinerea* in apples (Davey et al., 2007). These properties may also be modified by the environment or by the fruit maturity stage (Anttonen et al., 2006).

The objective of this study was to determine the effects of maturity stage, ripening time, year and quality parameters on the fruit susceptibility of different apple genotypes obtained in Querétaro, México to *P. expansum* Link.

2. Materials and methods

2.1. Biological material and experimental site

A total of 28 early-, middle-, and late-ripening apple genotypes including cultivars, creoles and low-chilling hybrids obtained in México and introduced into Queretaro region in 2001 by top-grafting on 25 years old trees of 'Golden Delicious' on 'MM-111' were used. The evaluation program for 18 genotypes was achieved in 2011 and for 23 in 2012 with 13 assessed in the two years. In addition, 10 genotypes were evaluated in commercial mature and over-mature phase in 2011 (Table 1).

These genotypes are found in a germplasm bank established in the Community "El Suspiro" in Cadereyta, Querétaro, México, located at 20°51′NL, 99°35′WL, and an elevation of 2495 m.a.s.l. Annual mean temperature and precipitation are 13.1 °C and 812.5 mm, respectively (SMN, 2014). According to the Köppen climate classification, the climate of this region is Cb (w2) (*i*′) gw″, which corresponds to a humid temperate (García, 1988). Table 2 shows monthly average temperature and monthly total precipitation for the two years during which this study was conducted.

The CFNL2016 strain of *P. expansum* Link was used as the source of inoculum; it was selected because of its high virulence (Sánchez et al., 2008).

2.2. Preparation of spore suspension and inoculation of fruit

Conidial suspension was prepared by adding 10 mL of Tween-80 (0.05%) solution over the surface of eight-day-old cultures grown on potato dextrose agar (PDA) at 25 °C (Cermeño and Torres, 1998). The resulting suspension was filtered and cultured in PDA supplemented with rose bengal (300 ppm) and ampicillin (100 ppm). After 48 h of incubation at 25 °C, a count of viable spores was performed, and the concentration was adjusted to 10^4 spores mL^{-1} (Sandoval et al., 2011).

For *in vivo* assays, the apples were disinfected with 3% sodium hypochlorite for 30 s and then rinsed with distilled water and dried in a laminar flow hood for 1 h. Subsequently, in the equatorial region of the fruit's surface, three equidistant wounds were made (3 mm in diameter) with a nail, and 10 μL of the *P. expansum* spore suspension was deposited in each. Finally, fruits were individually incubated at 26 °C, and lesion diameters were measured after 8 days

Table 1
Origin, fruit color, ripening time, and year of study of apple genotypes used in this work.

Genotype	Origin	Color	Ripening time*	MS[1]	Year of study	
					2011	2012
'Rayada'	Creole from La Esperanza, Querétaro	Striped red	Early	X	X	X
403	Unknown	Blushed	Early			X
418	'Anna' × CLR9T10	Yellow-blushed	Early		X	
424	'Anna' × 'Princesa'^{R}	Yellow-blushed	Early	X	X	X
441	'Anna' × 'Gala'	Yellow-blushed	Early			X
442-9	'Anna' × CLR9T10	Yellow-blushed	Early			X
449	'Anna' × CLR9T10	Striped red	Early	X	X	X
SM6	Unknown	Striped red	Early			X
SMA	Unknown	Red-blushed	Early			X
SMG	Unknown	Yellow	Early		X	X
'Golden Delicious'	'Golden Reinette' × 'Grimes Golden'	Yellow	Middle	X	X	X
'Red Delicious'	Selected in Madison, USA	Striped red	Middle	X	X	X
411	'Anna × 'Gala'	Red	Middle			X
428	'Anna' × 'Gala'^{R}	Blushed	Middle	X	X	X
429A	'Anna' × CLR9T10	Solid red	Middle			X
429B	'Anna' × CLR9T10	Red	Middle			X
436	'Anna' × 'Gala'^{R}	Yellow-blushed	Middle		X	X
468	'Anna' × 'Liberty'^{R}	Red	Middle	X	X	X
488	Unknown	Red	Middle	X	X	
SM1	Unknown	Solid red	Middle		X	
SM2	Unknown	Solid red	Middle		X	
SM3	Unknown	Red	Middle		X	X
SMB	Unknown	Striped red	Middle	X	X	
SME	Unknown	Yellow-blushed	Middle		X	X
Criolla	Unknown	Blushed	Middle			X
'Lourdes'	Unknown	Red and green-blushed	Late		X	X
419	'Golden 650' × 'Gala'	Red striped and blushed	Late	X	X	X
443	Einsheimer' × 'Princesa'	Red-blushed	Late			X

* Ripening time: (a) early, in July; (b) middle, in August; (c) late, in September.
[1] MS = genotypes evaluated in 2011 at commercial and over-mature stage.