Food Microbiology 29 (2012) 56–66

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Infection capacities in the orange-pathogen relationship: Compatible (*Penicillium digitatum*) and incompatible (*Penicillium expansum*) interactions

L. Vilanova [a], I. Viñas [b], R. Torres [a], J. Usall [a], A.M. Jauset [c], N. Teixidó [a,*]

[a] IRTA, UdL-IRTA Centre, XaRTA-Postharvest, Rovira Roure 191, 25198 Lleida, Spain
[b] University of Lleida, UdL-IRTA Centre, XaRTA-Postharvest, Rovira Roure 191, 25198 Lleida, Spain
[c] University of Lleida, Department of Crop and Forest Science, Rovira Roure 191, 25198 Lleida, Spain

ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form
8 August 2011
Accepted 11 August 2011
Available online 23 August 2011

Keywords:
Blue mould
Green mould
Maturity stage
Disease resistance
Lignin
Wound healing

ABSTRACT

Penicillium digitatum and *Penicillium expansum* are the most devastating pathogens of citrus and pome fruits, respectively. Whereas *P. digitatum* is a very specific pathogen that only infects *Citrus* fruits, *P. expansum* has a broader host range but has not been reported to be infectious in *Citrus*. To determine the responses of fruits and the infection capacities of both moulds, two varieties of oranges at different maturity stages, different inoculum concentrations and two different storage temperatures were studied. In compatible interactions, no significant differences in rot dynamics among harvests were found with a 10^7 conidia mL^{-1} inoculum concentration at both temperatures tested (20 °C and 4 °C). However, at other inoculum concentrations, significant differences in rot dynamics were found, especially in immature fruits. Incompatible interactions showed that *P. expansum* could infect oranges at commercial maturity in both tested varieties. Decay incidence and severity were higher at 4 °C than at 20 °C. In addition to infection capacity studies, histochemical tests were performed to detect wound-healing compounds for both pathogens. A positive reaction for lignin was detected for both pathogens in immature oranges over a short period (48 h). In all cases, no reactions were found in control samples. Our results indicate that pathogen concentration, host maturity and storage temperature can play important roles in the defence mechanisms of fruit. Furthermore, to our knowledge, this is the first work that demonstrates that *P. expansum* can infect oranges under favourable conditions.

1. Introduction

Penicillium digitatum and *Penicillium expansum* are the most devastating pathogens of citrus and pome fruits, respectively, and are responsible for important economical losses during post-harvest handling. Whereas *P. digitatum* is a very specific pathogen that infects *Citrus* fruits and has not been shown to infect other hosts, *P. expansum* has a broad host range. To our knowledge, *P. expansum* has not been shown to cause post-harvest disease on *Citrus* fruits; thus, it is considered a non-host pathogen or an incompatible interaction. Currently, the use of synthetic fungicides constitutes the main method to control these post-harvest diseases; however, the use of chemicals is becoming increasingly restricted because of concerns about environment and health, as well as the development of fungicidal resistance in pathogens (Viñas et al., 1993). In spite of the application of fungicides and the increased implementation of new alternative strategies, green mould in *Citrus* fruits and blue mould in pome fruits continue to place high infection pressures on stored fruits worldwide. These facts justify the need and the interest for more detailed studies on host–pathogen interactions to increase our knowledge of both pathogen virulence mechanisms and host defence mechanisms. This will serve as an initial step leading to the rational design of new and safer control strategies.

In an incompatible interaction, the avirulent pathogen is recognised via the action of disease resistance (R) gene products, eliciting an accumulation of biphasic reactive oxygen species (ROS) with a low-amplitude, transient first phase, followed by a sustained phase of much higher magnitude that correlates with disease resistance (Lamb and Dixon, 1997). When this recognition occurs, the pathogen cannot infect the plant. In contrast, in a compatible interaction, virulent pathogens avoid host recognition, inducing only the transient, low-amplitude first phase of this response. The lack of the second phase is thought to play an important signalling role in the activation of plant defences. In fact, the important ROS accumulation during the second phase has been reported to

* Corresponding author. Tel.: +34 973003429; fax: +34 973238301.
E-mail address: neus.teixido@irta.cat (N. Teixidó).

0740-0020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2011.08.016

precede the hypersensitive response that often occurs during pathogen recognition, leading to an incompatible interaction (Levine et al., 1994). In spite of the important role of H_2O_2 in plant defence (Borden and Higgins, 2002; Li et al., 2005), there are few reports of the role that it might play in fruit; it has been described on apples (Castoria et al., 2003; Torres et al., 2003), mangoes (Zeng et al., 2006) and strawberries (Brown et al., 2008). Regarding citrus fruits, only lemons (Macarisin et al., 2007) and oranges (Torres et al., 2011) have been analysed to characterise the potential role of H_2O_2 during compatible and incompatible interactions.

Different functions have been postulated for ROS production in response to pathogens (Torres, 2010). One function could be to contribute to the establishment of physical barriers at the sites of infection via oxidative cross-linking of the precursor during the localised biosynthesis of lignin and suberin polymers (Huckelhoven, 2007). Early histological investigations showed that the development of resistance to infection is associated with the deposition of a material that turns red in the presence of phloroglucinol-HCl (PG-HCl) in tissues adjacent to the injuries (Baudoin and Eckert, 1985; Brown and Barmore, 1983). However, the nature of this material was not clear because some authors described it as lignin and wound gum (Baudoin and Eckert, 1985; Brown and Barmore, 1983; Stange et al., 1993). In a more recent study, cured grapefruits stored at 33 °C for 48 h excluded lignin as a component in the newly formed material, and NMR spectroscopy provided further evidence that the induced material was suberin (Lai et al., 2003).

The differences in the final outcome of a plant–pathogen interaction, either susceptibility or resistance, might be due to the timing and intensity of the plant's defence responses (Tao et al., 2003). Thus, the maturity stages of fruits at harvest could be among the main factors determining the susceptibility of fruits to mechanical damage or infection during post-harvest storage (Davey et al., 2007; Torres et al., 2003). Ambient conditions may also play an important role in wound healing and resistance to infection in citrus fruits (Brown, 1975). Until now, post-harvest research has mainly focussed on different ways to control moulds (Janisiewicz and Korsten, 2002; Tripathi and Dubey, 2004; Wilson et al., 1991), while little is known about the effects of fruit maturity on mould growth.

The aim of this study was to investigate the infection capacities of the pathogens *P. digitatum* (compatible) and *P. expansum* (incompatible) in two varieties of oranges (Navelina and Valencia) at different (i) maturity stages; (ii) pathogen inoculum concentrations; and (iii) storage temperatures. A histochemical study was carried out to detect the accumulation of different compounds to define their roles in host resistance against both studied pathogens.

2. Materials and methods

2.1. Fruits

Navelina and Valencia oranges were obtained at different maturity stages from October 2008 to January 2009 (eight harvests ranging from immature to over-matured) and from March 2009 to June 2009 (seven harvests ranging from immature to over-matured), respectively, from a commercial orchard in Tortosa (Catalonia, Spain). For Navelina oranges, harvests one and two were considered as prior to commercial maturity (immature fruit), harvests three to six were considered as commercial maturity (mature fruit), and harvests seven and eight were considered as over-maturity (over-matured fruit). For Valencia oranges, harvests one and two were considered as prior to commercial maturity (immature fruit), harvests three to five were considered as commercial maturity (mature fruit), and harvests six and seven were considered as over-maturity (over-matured fruit). Oranges were used just after harvest.

2.2. Fungal cultures

P. digitatum PDM-1 and *P. expansum* CMP-1 are the most aggressive isolates from our collection capable of infecting citrus and pome fruits, respectively. They are maintained on potato dextrose agar medium (PDA; 200 mL boiled potato extract, 20 g dextrose, 20 g agar and 800 mL water) and periodically grown on wounded citrus (*P. digitatum*) or pome fruits (*P. expansum*) and then re-isolated to maintain virulence. Conidial suspensions were prepared by adding 10 mL of sterile water with 0.01% (w/v) Tween-80 over the surface of 7- to 10-day-old cultures grown on PDA and rubbing the surface of the agar with a sterile glass rod. Cells were counted in a haemocytometer and diluted to different concentrations (10^7, 10^6, 10^5 or 10^4 conidia mL^{-1}) and were then used in each infective capacity study.

2.3. Infective capacity studies

The effects of the maturity stages of oranges, inoculum concentrations and storage temperatures were assessed for both the compatible interaction (*P. digitatum*-oranges) and the incompatible interaction (*P. expansum*-oranges).

Oranges were washed thoroughly with tap water and allowed to dry before artificial inoculation. Oranges were wounded with a nail (1 mm wide, 5 mm long and 2 mm deep) and inoculated with 15 μL aqueous conidia suspensions of pathogen at four different concentrations; 10^7 and 10^6 conidia mL^{-1} are considered in this work as high inoculum concentrations, and 10^5 and 10^4 conidia mL^{-1} are considered as low inoculum concentrations. This methodology was performed individually for each pathogen. The infective capacities of each pathogen were assessed at two different storage temperatures (4 °C and 20 °C) and 85% relative humidity. As soon as visible growth started, the diameter of rot was measured along the time to obtain the development of rot dynamics for each pathogen, inoculum concentration, temperature and maturity stage. Five oranges constituted a single replicate, and each treatment was repeated four times. The experiments were performed with both orange varieties: Navelina (eight harvests) and Valencia (seven harvests).

2.4. Determination of quality parameters

Colour development, loss of firmness, soluble solids and acidity were determined to evaluate the effects of different harvest dates on fruit quality.

Colour was measured on two opposite sides of each fruit using a tri-stimulus colourimeter (Chromameter CR-200, Minolta, Japan). The mean values for the lightness (L*), red-greenness (a*) and yellow-blueness (b*) parameters were calculated for each fruit and expressed as Colour index (CI) = (1000*a)/(L*b). Firmness measurements were performed using a TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK), based on the millimetres of fruit deformation resulting from fruit responses to 2 kg of pressure on the longitudinal axis at a constant speed of 2 mm s^{-1}. Total soluble solids content (TSS) and titratable acidity (TA) were assessed in juice using a refractometer (Atago, Tokyo, Japan) and by titration of 10 mL of juice with 0.1 N NaOH and 1% phenolphthalein as an indicator. Data on maturity indexes represent the means of 20 individual fruits. Maturity index was calculated as a ratio of TSS/TA.