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Postharvest quality of plums in response to the occurrence of leaf scald disease

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ABSTRACT

Plum leaf scald (PLS) is considered the main barrier to expanding plum cultivation in Brazil. The disease causes water flow to be obstructed in the xylem of infected plants. Infected host plants produce fruit of lower quality; however, no information is available about the postharvest quality attributes in plum. Fruit from the 'Gulfblaze' and 'Reubennel' plant cultivars, both with and without PLS symptoms, were collected from commercial orchards. Bacterial presence was confirmed using a polymerase chain reaction, and the physicochemical, biochemical, and physiological properties were evaluated. Brown rot incidence was also determined during the ripening period. According to a molecular analysis, symptomatic plants used in the assay carried *Xylella fastidiosa* (which causes PLS), but healthy plants did not. Fruit collected from infected plants had a reduced diameter and weight in the 2015/2016 growing season. There was a greater reduction in pulp firmness and higher pectin methylesterase enzyme activity in fruit harvested from infected compared with healthy plum trees. The total soluble solids content was higher in fruit from 'Gulfblaze' and 'Reubennel' diseased compared with disease-free plants. A higher incidence of brown rot was observed in plants with leaf scald symptoms. The highest phenylalanine ammonia-lyase activity was observed in fruit from 'Gulfblaze' diseased plants. CO_2 and ethylene production was higher in the infected than healthy plants, indicating that the presence of *X. fastidiosa* can accelerate fruit ripening mechanisms.

1. Introduction

Plum is considered promising as a crop for further development in Brazil, since current supply does not meet national demand (Kist et al., 2012). However, commercial orchards have not achieved their expected productive potential due to the occurrence of plum leaf scald (PLS) disease, which is caused by the bacterium *Xylella fastidiosa*. This pathogen has a wide range of hosts and causes various diseases, including grapevine (Pierce's disease) (Hopkins, 1985), citrus (citrus variegated chlorosis; CVC) (Rossetti et al., 1990), and coffee (coffee leaf scorch; CLS) (Paradela-Filho et al., 1995). The disease is disseminated via infected propagative material and insect vectors, such as sharpshooter leafhoppers (Hemiptera: Cicadellidae, Cicadellinae) (Coletta-Filho et al., 2016).

The main symptoms of PLS occur in the leaves, only becoming visible after a long incubation period (approximately eight to nine months without hydric stress), after which the bacterial population is distributed systemically in the plum trees (Raju et al., 1982; Almeida and Nunney, 2015). Initially, the disease manifests as a slightly irregular chlorosis on the leaf edges. This chlorosis intensifies at the end of the vegetative cycle, resulting in marginal desiccation (Raju et al., 1982). However, these symptoms are not observed directly on the fruit.

Vascular diseases, such as PLS, block xylem transport and induce water stress in their hosts, increasing the resistance to water flow (Tyree and Sperry, 1989; Fletcher and Wayadande, 2002). Plum trees colonized by *X. fastidiosa* present similar symptoms to those of water deficiency, due to occlusion of the xylem bundles by bacterial cluster formation (Janissen et al., 2015). In general, tissue dehydration can lead to disturbances during the postharvest period, because the resultant increased respiration and ethylene production accelerates ripening processes. In addition, water scarcity causes a reduction in fruit diameter, weight loss, flavour changes, and decreased pulp firmness (González-Altozano and Castel, 1999; Girona et al., 2003). Physical and chemical changes have been reported during processing in citrus plants with CVC symptoms; this citrus disease affects not only the production, but also postharvest quality of these fruit (Agrios, 2005).

Plum is a highly perishable fruit (Malgarim et al., 2007). Therefore, its shelf life is reduced by any physiological or metabolic alterations

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that interfere negatively with its maturation process, compromising its commercialization and consequently affecting the prices paid to producers.

To date, no information has been found in the literature concerning how PLS interferes in the postharvest quality attributes of plums. This study therefore aimed to determine the influence of leaf scald disease on the physical, chemical, physiological, and biochemical characteristics of plum fruit.

2. Materials and methods

2.1. Plant material and experimental design

Two Japanese plum (*Prunus salicina* Lindl) cultivars ('Gulfblaze' and 'Reubennel') were selected from Sigma Agroambiental Ltd., which is located in the producing region of Paranapanema, São Paulo (SP), Brazil. 'Reubennel' was also collected from a commercial orchard located in the metropolitan region of Curitiba (Araucaria), Parana (PR), Brazil.

The treatments comprised: (1) fruit from plants with leaf scald symptoms; and (2) fruit from healthy plants. A total of 500 fruit per treatment per collection was used for each of the following cultivars: 'Gulfblaze' (SP); 'Reubennel' (PR) and 'Reubennel' (SP). Plum trees were standardized according to canopy size and fruit number, so that the relationship between fruit and leaves was the same among treatments. The environmental conditions and agricultural management were similar for each cultivar in each locality. Symptomatic trees were located at least 200 m away from healthy trees within the same field.

Plums were manually harvested in the morning, randomly selected from several plant heights at the commercial ripening stage. Fruit that presented with fungal or insect lesions or mechanical damage were discarded. Selected fruit were stored in a controlled temperature chamber at 22 °C and 85% relative humidity in darkness.

The following fruit characteristics were evaluated in the 2015/2016 growing season: diameter, fresh weight, pulp firmness, fresh weight loss, colouration, pH, titratable acidity (TA), content of total soluble solids (TSS), ascorbic acid content, pectin methylesterase (PME) and phenylalanine ammonia-lyase (PAL) enzyme activity, CO_2 and ethylene production, and brown rot incidence. In the 2016/17 growing season, further analyses were conducted to measure diameter, fresh weight, fresh weight loss, pulp firmness, TA, and TSS content for 'Reubennel' (PR) fruit, which were stored under the same conditions as fruit analysed in the previous growing season.

2.2. Detection of Xylella fastidiosa in plum trees

In order to detect the bacterium (*X. fastidiosa*) in symptomatic and asymptomatic plants, DNA was extracted from the petioles using a Biopur Mini Spin Planta Extraction Kit (Mobius Life Sciences, Pinhais, PR, Brazil), according to the manufacturer's instructions. The extracted DNA samples were amplified using a pair of specific primers, RST31 and RST33 (Minsavage et al., 1994). The amplification was performed in a PTC-100 thermal cycler (Research Inc., Watertown, MA, USA), programmed with the following conditions: one initial cycle of 94 °C for 5 min; 32 denaturation cycles at 94 °C for 40 s; annealing at 50 °C for 40 s; an extension at 72 °C for 59 s; followed by one stabilization cycle at 72 °C, indefinitely. Polymerase chain reaction (PCR) products were separated by agarose gel electrophoresis (1.5%) with a Tris/borate/EDTA (TBE) buffer (89 mM Tris, 89 mM boric acid, and 2 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0). The amplified fragments were visualized under ultraviolet light, and documented using the Eagle Eye II system (Stratagene, LaJolla, CA, USA).

2.3. Physical and chemical analyses

Transversal and longitudinal diameter (mm) was determined using a digital calliper (Vonder PPV 1506). Mean fresh fruit weight (g) was measured using an analytical balance (Balmak MP5) when fruit was harvested. Fresh weight loss (%) during ripening was estimated according to the formula: $FWL = \left[\frac{IFW - FWS}{IFW}\right] \times 100$,where: FWL = fresh weight loss during ripening (%), IFW = initial fresh weight of the sample, and FWS = fresh weight of the sample at the evaluated ripening periods.

A digital penetrometer (TR-Turoni) with a tip diameter of 8 mm was used to measure pulp firmness from two readings taken from opposite sides of each fruit's equatorial region. The results were expressed in Newton (N) as the average of the two readings.

Skin colour was determined using a colorimeter (Model CR-300; Minolta Co., Ltd, Osaka, Japan) that had previously been calibrated on a white surface. For this evaluation, two readings were taken from opposite sides of the equatorial region of the fruit, evaluating three colour parameters: L* (luminosity), a* (colouration in the red to green region), and b* (colouration in the yellow to blue region). The results were expressed as hue angle (hue °) ($h° = tan{-}1(b*/a*)$) and in chroma (C*) ($C = (a*2 + b*2)1/2$) (Minolta, 1994).

The pH of the pure juice was determined using a digital potentiometer (MS Tecnopon mPA-210, Piracicaba, Brazil), standardized buffer solutions of pH 4.0 and 7.0. TA was determined using 0.1 N NaOH solution to titrate 10 mL of fruit juice diluted in 90 mL of distilled water, until a pH of 8.1 was achieved. Values were expressed as % of malic acid equivalent (Instituto Adolfo Lutz, 1985). The TSS content was determined using a digital benchtop refractometer (Palette PR-101; Atago Co Ltd., Tokyo, Japan), with results expressed as %. Ascorbic acid (AA) content was determined by reducing the 2,6-dichlorophenol indolfenol-sodium indicator (DCFI) with ascorbic acid, using titration (AOAC, 1997). All analyses were performed on the first, third, fifth, and seventh days after harvest.

2.4. Biochemical analyses

PME (EC 3.1.1.11) activity was determined from the carboxylic acid groups released by the action of this enzyme, using titration (Kertesz, 1951). The enzymatic activity measurement results were expressed in nmol of hydrolysed esters per gramme of pulp per unit of time (nmol g^{-1} min^{-1}).

PAL (EC 4.3.1.5) activity was determined using the conversion rate of L-phenylalanine to trans-Cinnamic acid at 290 nm, according to a methodology adapted by Peixoto, 1999. The result of this enzymatic activity was expressed in mmol min^{-1}. All analyses were performed on the first, third, fifth, and seventh days after harvest.

2.5. Physiological analyses

Freshly harvested plums from the 'Gulfblaze' (SP) and 'Reubennel' (SP) cultivars were placed in 0.59 L hermetically sealed glass jars, and maintained at 25 °C for 1 h. A headspace volume of 1 mL was removed from the glass jars with a syringe (Gastight; Hamilton, Nevada, USA) via a rubber and injected into a gas chromatograph (Thermo Finnigan Trace GC 2000, Thermo Fisher Scientific, Waltham, MA, USA) coupled to flame ionization detector and equipped with two Porapak N columns (1.8 m and 4 m in length for ethylene and CO_2 samples, respectively). The carrier gases used in these analyses were hydrogen (27 mL min^{-1} constant flow) and nitrogen (30 mL min^{-1} constant flow) for quantifying CO_2 concentration and determining ethylene, respectively. Temperatures were maintained in the apparatus at 140 °C (column), 120 °C (injector), 250 °C (detector), and 350 °C (methanator). Standard CO_2 and ethylene gases were used to elaborate the standard curves. CO_2 and ethylene quantifications were determined from the difference between the initial gas concentration inside the containers at the time of closure and after 1 h, expressed as mg kg^{-1} s^{-1} for CO_2 and as ng kg^{-1} s^{-1} for ethylene (C_2H_4). Evaluations were performed on the