Postharvest Biology and Technology 56 (2010) 26–30

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Control of *Monilinia* spp. on stone fruit by curing treatments. Part II: The effect of host and *Monilinia* spp. variables on curing efficacy

C. Casals*, N. Teixidó, I. Viñas, J. Cambray, J. Usall

IRTA, Centre UdL-IRTA, XaRTA-Postharvest, 191, Rovira Roure Avenue, 25198 Lleida, Catalonia, Spain

ARTICLE INFO

Article history:
Received 16 August 2009
Accepted 18 November 2009

Keywords:
Brown rot
Fruit maturity
Nectarine
Peach
Postharvest heat treatments
Time of infection

ABSTRACT

In previous experiments, we identified that a postharvest curing treatment (50 °C for 2 h and 95–99% RH) satisfactorily controlled brown rot on several peach and nectarine varieties. In the present complementary study, the effect of fruit maturity, fruit with natural infection, time of infection and inoculum concentration on the curing efficacy was investigated. Different maturity levels affected curing efficacy. As fruit maturity increased, the efficacy of a postharvest curing treatment decreased from 95% control of brown rot (harvest mature fruit) to 65% (the most advanced mature fruit). The effect of *Monilinia fructicola* infection time prior to treatment also affected the curing efficacy. When the infection time was increased from 0 to 48 h, brown rot control decreased from 90% to 64%. A factorial experiment design was used to investigate the effect of *M. fructicola* conidial concentrations (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}) at different exposure times (1, 2, 3 and 4 h) on curing efficacy. Overall, longer curing exposure times (3 or 4 h) were required when higher conidial concentrations were applied to the wounded fruit. At the lowest *M. fructicola* conidial concentration tested (10^3 conidia mL^{-1}), 2 h of curing exposure resulted in 100% and 94% brown rot control in 'Andros' peaches and 'Flames Kid' nectarines, respectively. A high level of brown rot control was also achieved when naturally infected fruit with *Monilinia* spp. were cured. When fruit with natural inoculum were surface sterilized prior to the curing treatment, complete brown rot control resulted. This findings support our earlier demonstration that a postharvest curing treatment is an attractive non-chemical strategy for use in conventional and organic stone fruit brown rot management.

1. Introduction

Brown rot caused by *Monilinia laxa*, (Aderh et Rulh) Honey, *M. fructigena* Honey in Whetzel and *M. fructicola* (Wint.) Honey is a serious disease in stone fruit (Byrde and Willetts, 1977; De Cal et al., 2009). Although in Spain, the most important species responsible for postharvest losses in peaches and nectarines is *M. laxa* (Larena et al., 2005), *M. fructicola* is a quarantined pathogen in Europe (EPPO, 2007) and it also has been reported as affecting stone fruit in several countries including Spain (De Cal et al., 2009).

Infection by *Monilinia* spp. can take place in the field during the growing season when conditions favour disease development. Symptomless infections may occur and remain dormant in the fruit until microclimatic conditions are favourable and fruit become mature enough for disease expression (Byrde and Willetts, 1977; Fourie and Holz, 2003). These pathogens are generally wound parasites, in most cases requiring a wound in the skin to enter in order to come into direct contact with susceptible tissue and initiate infection (Spotts et al., 1998). Fruit injured during harvest or postharvest handling operations also increases the risk of infection. Generally, the level of rots in the field is low when a full season fungicide programme is used. However, when field conditions are favourable for disease development, losses during storage can be high, thereby increasing the risk of postharvest disease development. The primary problem for packinghouses is the postharvest period, as no fungicide treatments for postharvest decay of peaches and nectarines are allowed in several producing countries, including Spain. In addition, public demands to reduce pesticide use and improve environmental and human health limits the preharvest application of chemical products in the field. These concerns, combined with a lack of effective postharvest treatments against *Monilinia* spp. has stimulated research interest in developing new control methods.

In previous work (Casals et al., 2010), high temperature curing was shown to be able to control brown rot on artificially inoculated peach and nectarine fruit with *M. laxa* and *M. fructicola*, with optimal conditions of 50 °C for 2 h and 95–99% RH. Although these curing conditions effectively controlled brown rot, other factors were identified that required consideration before curing can be implemented as a commercial treatment. For example, curing

* Corresponding author. Tel.: +34 973702586/+34 973003425; fax: +34 973238301.
E-mail addresses: carla.casals@irta.cat (C. Casals), josep.usall@irta.cat (J. Usall).

0925-5214/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2009.11.009

efficacy may depend upon time of *Monilina* spp. infection before the curing treatment, fruit maturity and inoculum concentration (Stange and Eckert, 1994). Little information is available on the effect of these potentially important factors on curing efficacy in peaches or nectarines. With heat treatment applied to fruit 24 h after inoculation with *Penicillium expansum* Link., conidia sensitivity was higher than with applying heat immediately following inoculation (Karabulut et al., 2002). Moreover, in apples inoculated with low *P. expansum* inoculum concentrations, the heat treatment efficacy was much higher than in fruit inoculated with higher concentrations (Fallik et al., 1996). In relation to the host, immature stone fruit are less susceptible to brown rot infection than mature fruit (Emery et al., 2000).

The aim of this study was to evaluate the curing treatment at 50 °C for 2 h and 95–99% RH as a control measure for brown rot caused by *Monilinia* spp. on fruit: (1) with different fruit maturity levels; (2) with 0, 24 and 48 h infection times prior to treatment; (3) inoculated with different conidial concentrations; (4) with natural inoculum.

2. Materials and methods

2.1. Source of fruit

'Ryan Sun', 'Katerine', 'Andros' and 'Royal Glory' peaches (*Prunus persica* (L.) Batsch), 'Big Top' and 'Flames Kid' nectarines (*P. persica* var. *nectarine* (Ait.) Maxim.) and 'Black Diamond' plums (*P. saliciana* Lindl.) were grown in Ribera d'Ebre, Segrià and Noguera areas of Catalonia following standard cultural practices and a recommended spray programme for pest and disease control in this region, without any postharvest treatment. Fruit were selected immediately after harvest for uniformity of size and maturity and those with visual injuries or any sign of decay were discarded. Fruit that were not used at time of harvest were stored at 0 °C, until required for experimentation.

2.2. Pathogen isolates

Isolates of *M. laxa* (CPML1) and *M. fructicola* (CPMC1) used in this study were originally isolated from stone fruit and maintained in the culture collection of the Pathology Unit, Centre UdL-IRTA, Lleida, Catalonia and identified by the Department of Plant Protection, INIA, Madrid, Spain.

2.3. Inoculum production

Prior to experimentation, pathogen isolates were subcultured onto potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1%), and then incubated in the dark at 25 °C for two weeks. Low sporulation on PDA was overcome by transferring mycelium and conidia of *M. laxa* or *M. fructicola* onto healthy peaches or nectarines that were wounded prior to inoculation. Fruit were then incubated at 25 °C and 85% RH in the dark for 7–10 d (for *M. laxa*) and 5–7 d (for *M. fructicola*). Conidia were scraped from infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per litre. The conidial concentration was measured using a haemocytometer and diluted to the desired concentration. Fruit for curing treatments were wounded with a steel rod (1 mm wide and 2 mm long) and then artificially inoculated with 15 μL aliquot of conidial suspension that had been previously adjusted to the desired conidial concentration. In all experiments, curing treatments were carried out once the inoculum in the wound had dried.

Table 1
Firmness, acidity and soluble solids content of 'Ryan Sun' peaches incubated at 20 °C and 85% RH to achieve different levels of fruit maturity.

Incubation time (h)	Firmness (N)	Acidity (g m.a.[a] L^{-1})	Soluble solids (%)
0	38.2a[b]	6.68a	12.8a
24	38.2a	5.85b	12.8a
48	18.6b	5.36b	12.7a
72	12.7b	4.29c	12.7a

[a] Malic acid.
[b] Means within rows with the same letters are not significantly ($P < 0.05$) different according to LSD test.

2.4. Effect of fruit maturity on curing efficacy

In all the following experiments the curing treatment used was at 50 °C for 2 h and 95–99% RH unless otherwise stated. Temperature and RH parameters were measured using a data logger (model 177-H1, Testo, Germany) for all curing experiments.

'Ryan Sun' peaches were harvested at commercial maturity and kept at 20 °C and 85% RH for 0, 24, 48 or 72 h prior to the curing treatment, in order to achieve four sets of fruit at different maturity levels. Quality parameters of these fruit are shown in Table 1. Fruit were artificially inoculated with *M. laxa* as described above and cured. In all cases, a set of fruit was maintained at 20 °C to act as the inoculated control (no curing). All treatments were carried out with four replicates and 10 fruit per replicate. Immediately after each treatment fruit were incubated for 5 d at 20 °C and 85% RH and the number of brown rot infected fruit was recorded and expressed as incidence of brown rot (percentage).

Prior to the curing treatment the maturity of the four different fruit sets were determined by measuring standard quality parameters including fruit firmness, soluble solids and acidity. Firmness was measured on two opposite peeled sides using a penetrometer (Effegi, Milan, Italy) fitted with an 8 mm diameter flat tip. Firmness measurement was carried out on four replicates and five fruit per replicate.

Soluble solids content was determined with a digital refractometer (Atago PR-100, Tokyo, Japan), by measuring the juice refractive index and data were expressed as percentage of soluble solids. Acidity was measured by mixing 10 mL of juice with 10 mL distilled H_2O and adding three drops of phenolphthalein which was then titrated with 0.1 N NaOH. Acidity was expressed in grams of malic acid per litre of juice (g m.a. L^{-1}). Juice originated from five fruit per replicate that had been used to determine firmness and colour per four replicates.

2.5. Effect of infection time on curing efficacy

'Ryan Sun' peaches were artificially inoculated as described above and maintained for 0, 24 or 48 h at 20 °C and 85% then cured. In all cases, a set of fruit was maintained at 20 °C to act as the inoculated control (no curing). All treatments were carried out with four replicates and 10 fruit per replicate. Immediately after each treatment fruit were incubated for 5 d at 20 °C and 85% RH and the number of brown rot infected fruit was recorded and expressed as incidence of brown rot.

2.6. Effect of inoculum concentration on curing efficacy

Two experiments were carried out to investigate the effect of *M. fructicola* inoculum concentration on curing efficacy. The first experiment was carried out with 'Katerine' peaches and 'Big Top' nectarines. Fruit were artificially inoculated, as described above, with *M. fructicola* conidial suspension that were adjusted to 10^3, 10^4, 10^5 and 10^6 conidia mL^{-1} and then fruit were cured. A second experiment was also carried out with 'Andros' peaches