Postharvest Biology and Technology 94 (2014) 74–81

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

The mechanism of differential susceptibility to alternaria black spot, caused by *Alternaria alternata*, of stem- and bottom-end tissues of persimmon fruit

Esti Biton [1], Ilana Kobiler [1], Oleg Feygenberg, Mor Yaari, Tania Kaplunov, Miriam Ackerman, Haya Friedman, Dov Prusky*

Department of Postharvest Science of Fresh Produce, The Volcani Center, Bet Dagan 50250, Israel

ARTICLE INFO

Article history:
Received 9 December 2013
Accepted 8 March 2014

Keywords:
Chlorophyll
Ethylene
Postharvest disease
Preharvest susceptibility
Respiration
Ripening

ABSTRACT

In Israel, alternaria black spot (ABS), caused by *Alternaria alternata*, is the main postharvest factor that reduces quality and impairs storability of persimmon fruit *Diospyros kaki* cv. Triumph. The fungus infects the fruit in the orchard and remains quiescent until harvest, or starts development just before harvest, following rain or high humidity. During 2–3 months of storage at 0 °C, the pathogen colonizes the fruit, eliciting ABS symptoms. Susceptibility of the fruit to *A. alternata* attack is characterized by colonization in the upper, stem-end tissue, in contrast to lack of development at the bottom end. Comparison between the physiology of the stem-end and the bottom-end tissues showed greater production of ethylene and CO_2 in the former during early stages of fruit growth, and greater cracked areas and reduced chlorophyll levels in the later stages of growth, before harvest. Increasing fruit weight by increasing irrigation in the orchard enhanced the cracked area and susceptibility to ABS during growth and at harvest. Wound inoculation enhanced ABS colonization in both ends of the fruit, but more significantly in the upper stem end. The present results suggest that the differential susceptibility to ABS during storage is caused by a differential ripening process, and possibly, by increased maturity at the stem end, leading to cracking and increased ABS development.

1. Introduction

Alternaria black spot, caused by *Alternaria alternata*, has been described as the most economically important postharvest disease of persimmon fruit cv. Triumph in all growing regions of Israel (Prusky et al., 1981a, 2001). Recently it has also been identified in persimmon-growing regions of Spain, in local cultivars such as 'Rojo brillante' (Palou et al., 2009). The primary mode of infection of persimmon fruit by *A. alternata* is either through small wounds under the sepals attached to the fruit stem and/or directly into the fruit cuticle (Prusky et al., 1981a). In most years, *A. alternata* infections remain quiescent until harvest; the disease then develops slightly during storage at 0 °C and expands further during shelf-life. However, application of the growth regulator gibberellic acid (GA_3) at 50 μg L^{-1}, intended to extend storage life and to improve fruit quality during shelf-life, might become disadvantageous: it extends the harvest season, thereby endangering the fruit remaining in the orchard by exposing it to the hazards of hailstorms and heavy rainfall, which lay the foundations for excessive decay caused by *A. alternata* in the orchard (Prusky et al., 1981b; Perez et al., 1994; Biton et al., 2013). Under conditions of heavy rain and/or high relative humidity before harvest, incidence of infection increases and already before harvest, small active infections have been observed to colonize small cracks in the tissue (Biton et al., 2013). Such conditions lead to significant increases in decay incidence during storage, and consequently, to reduction of storage periods to prevent significant losses. Interestingly, however, the increased incidence of decay before harvest occurs mainly in the upper, stem end of the fruit rather than the bottom end (Fig. 1). This differential susceptibility is maintained during storage, and most of the ABS colonization in unmarketable fruit is in the upper, stem end.

Persimmon disease development may be reduced, by growth regulators, via reduction of fruit susceptibility to fungal attack (Eshel et al., 2000), by treatments with pre- and postharvest

* Corresponding author at: Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Bet Dagan 50250, Israel. Tel.: +972 3 9683611; fax: +972 3 9683622.
E-mail address: dovprusk@agri.gov.il (D. Prusky).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.postharvbio.2014.03.008
0925-5214/

Fig. 1. Comparison between natural development of ABS symptoms on the upper and bottom ends of persimmon fruit cv. Triumph.

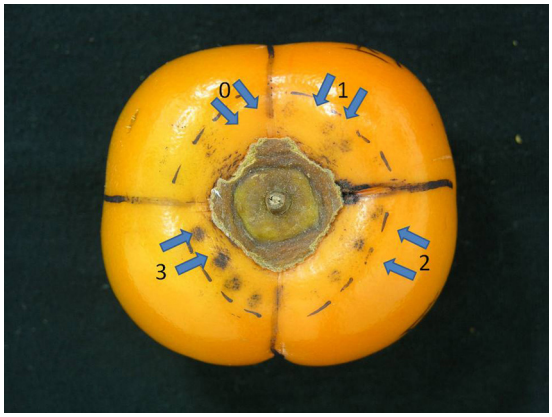

Fig. 2. Scale for symptom development of ABS evaluation, following drop inoculation of fruit. Stem end of the fruit was inoculated with 2 μL of a 10^6 spores/mL suspension, incubated at high humidity, and evaluated after 5 days at 22–23 °C. Numbers indicate the severity of *A. alternata* symptoms.

fungicides, which protect against and/or eradicate fungal infections, or by a combination of approaches during periods of high incidence of infection (Kobiler et al., 2010). However, development of optimal disease-control options requires understanding of the factors that determine fruit susceptibility and thereby contribute to fungal colonization.

We hypothesize that, although infection by the postharvest pathogen *A. alternata* might occur by direct penetration and via wounds, activation of quiescent infection is strongly dependent on physiological changes that occur in the tissue during fruit growth. The report of Nakano et al. (2003) showing that signaling for ethylene production was initiated from the calyx soon after fruit set and the specific stem-end susceptibility observed in stored fruit, led us to examine the possibility of a relationship between ethylene production during fruit growth and increased susceptibility to ABS. Our objective in the present study was to characterize the factors, including ethylene and CO_2 production and differential ripening of specific tissue, that lead to the increased susceptibility of the stem-end tissue of persimmon fruit to ABS symptoms before harvest.

2. Materials and methods

2.1. Pathogen, host and commercial treatments

All the experiments were carried out in persimmon (*Diospyros kaki* Thunb. cv. Triumph) fruit in 10- to 15-year-old orchards in the center of Israel, the country's main persimmon-growing region. The experiments were carried out during two or three consecutive years (according to the experiment), in orchards whose fruit usually showed high natural incidence of black spot infection during storage.

A. alternata was cultured for 1–2 weeks on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, USA) at 25 °C. The isolate used was obtained from infected persimmon fruit in Israel. Spores were harvested by adding 3–4 mL of sterile, deionized water (diH_2O) to the Petri dish. The spores were then rubbed with a sterile glass rod to free them from the PDA medium, and the spore suspension was passed through two layers of cheesecloth. The suspension was diluted with water to obtain the spore concentrations needed, as determined with a haemocytometer.

To determine the differential susceptibility of the fruit tissues to *A. alternata* colonization, the stem and bottom ends of harvested fruit were inoculated by placing 2 μL drops over the intact and/or wounded tissue, at different time points during fruit development after fruit set, and at harvest. For the upper, stem end of the fruit, twenty-four 2 μL drops, each containing 10^6 spores mL^{-1}, were placed 5 mm apart and 5–10 mm outside the stem scar of the fruit. The bottom ends of different fruit were inoculated in a similar manner at points around a circle with a radius of about 15 mm at the center of the bottom end. In some cases, fruit were wound-inoculated following 2 mm deep pricking with a needle, by placing a spore-suspension drop on each wound; at the stem end, the fruit were pricked at points 5 mm apart and 5–10 mm outside the stem scar; at the bottom end they were pricked in the same locations as for the intact-fruit inoculation, i.e., about 15 mm from the center. Ten to fifteen fruit were inoculated in each treatment, at each harvest time. The fruit were stored for 5 days in plastic containers at 21–23 °C under high humidity, pending assessment. Symptoms were detected by direct visual observation and were assessed according to a 0–3 scale on which 0 represented no symptoms and 3 indicated development of 3- to 4-mm black spots (Fig. 2). In some experiments, infection was assessed as 1 or 0, representing presence or absence of symptoms, respectively (Eshel et al., 2000). All the results presented were obtained in direct inoculation trials.

2.2. Preharvest treatments of persimmon trees

2.2.1. Growth regulators

The growth regulator Gibberellin 3 (Giberellon containing gibberellic acid technical at 40 g L^{-1}, with at least 90% of GA_3; Fine Agrochemicals Ltd, Whittington, Worcs, UK) was applied according to commercial practice to all the treatments 10–14 days before harvest (Ben-Arie et al., 1996), and the gibberellic acid biosynthesis inhibitor, Paclobutrazol (CULTAR 25 SC; Syngenta, Junction City, USA), was applied within 20–30 days after fruit set (dafs) (Ben-Arie et al., 1996). The GA_3- and Paclobutrazol-treated fruit were sampled from commercially treated orchards, unless specifically indicated.

2.2.2. Irrigation levels

To examine the relationship between fruit size and crack development, irrigation was applied at several levels. Commercial irrigation comprises water application via two irrigation lines that provide 27.2 L h^{-1} per tree, for an average total irrigation level of 500 L dunam^{-1} per year (1 dunam = 1000 m^2). The treatments were applied to one single row of trees at 0.5, 1.5, or 2.0 times the commercial irrigation level, via one, two or four irrigation lines, respectively. The experiments used a randomized block design with four replications, each comprising one row of 15 trees. All the trees in this experiment were treated with GA_3. At various stages after fruit set and at harvest, about 40 fruit were sampled from each row (i.e., a row being a replicate).