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Susceptibility of citrus species to *Alternaria alternata*, the causal agent of the Alternaria brown spot

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Abstract

Alternaria alternata, the causal agent of Alternaria brown spot (ABS), causes necrosis on leaves, twigs, and fruit, reducing the productivity and quality of fruits. Tangerines and their hybrids are highly susceptible to the disease. Species, hybrids, and cultivars of *Citrus* from the germplasm bank of the Estação Experimental de Citricultura de Bebedouro, São Paulo, Brazil, were evaluated in 2004 and 2005 with respect to their resistance to *A. alternata*, both through natural infection and by inoculation. Detached leaves were also used to demonstrate susceptibility or resistance to the disease. Ten cultivars of Satsumas (*Citrus unshiu*), and 14 cultivars of Clementine mandarin (*C. clementina*) did not show any symptoms of the disease in their leaves, either through natural infection or when inoculated in the field. The Burguess SRA-412, Wallent SRA-438, Carvalhais, Ampefy SRA-459, Ananas SRA, and Macaque SRA-426 mandarin hybrids (*C. reticulata*) did not show symptoms of the disease under natural or artificial infection in the field. Some cultivars of *C. deliciosa*, *C. tangerina*, *C. erythrosa*, and *C. temple* showed symptoms of the disease, even though no previous record of their susceptibility to Alternaria brown spot had been previously reported. The hybrids Fairchild, Nova, Page, Fortune, and Sunburst were susceptible to the disease. However, Fremont mandarin (a crossing between *C. clementina* and *C. reticulata*), Encore (*C. nobilis* × *C. deliciosa*), and Fallglo (*C. reticulata* × *C. paradisi*) did not show symptoms in field, and few symptoms were verified in detached leaves. These materials are promising for the cultivation of tangerines, and will enable genetic improvement for the development of cultivars resistant to Alternaria brown spot.

Keywords: Citrus reticulata; Citrus tangerina; Resistance; Susceptibility; Hybrids

1. Introduction

Alternaria brown spot (ABS), caused by *Alternaria alternata*, is an important disease of tangerines and their hybrids, affecting leaves, twigs, and immature fruit (Pegg, 1966; Canihos et al., 1999). In addition to a decrease in productivity, fruits with Alternaria brown spot symptoms lose commercial value in the fresh fruit market. This disease was first reported affecting Emperor tangerine in 1903 in Australia (Kiely, 1964), and its pathogen was first identified as *Alternaria citri* Ellis and N. Pierce (Pegg, 1966). In 1991, the fungus was renamed as *A. alternata* (Fr.:Fr.) Keisel. pv. *citri* (Solel, 1991). Molecular phylogenetic studies suggest that the isolates of *Alternaria* from citrus plants should be designated as *Alternaria alternata* (Peever et al., 2004).

Two pathotypes of *Alternaria alternata* are currently known based on the production of host-specific toxins (HST): ACT—toxin of the tangerine pathotype, which is specific to tangerine (*Citrus reticulata* Blanco), and their hybrids; and ART—toxin of the lemon pathotype, which affects rough lemon (*C. jambhiri* Lush), and Rangpur lemon (*Citrus limonia* Osbeck) (Kohmoto et al., 1979; Nishimura and Kohmoto, 1983).

The resistance of leaves, twigs, and fruit to *A. alternata* is associated with the advance toward maturity (Pegg, 1966; Gardner et al., 1986; Solel and Kimchi, 1998; Reis et al., 2006a). According to Whiteside (1976), fruits are susceptible up until they are 4 cm in diameter, although such susceptibility might extend to 5–6 cm depending on the species (Vicent et al., 2004; Reis et al., 2006a).

The control of the ABS is mainly carried out using fungicides, a practice that is becoming increasingly difficult and costly due to the high number of applications that are necessary to ensure good quality fruit. According to Peres and

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Timmer (2005), up to 12 applications of a fungicide based on copper were necessary in the case of Murcott tangor orchard in Brazil. The use of systemic fungicides of the triazole and strobilurin group are efficient in controlling the disease, and are thus necessary to obtain healthy fruits and avoid premature fall (Timmer et al., 1998; Miles et al., 2005; Reis et al., 2006b).

The susceptibility of *Citrus* species to *A. alternata* has been studied by several researchers (Gardner et al., 1986; Solel and Kimchi, 1997; Peever et al., 2000; Vicent et al., 2004). Nearly all tangerine (*C. reticulata*) cultivars and their hybrids are susceptible to the disease, including the Minneola and Orlando tangelos (from the cross between *C. paradisi* Macfad and *C. reticulata*), the Murcott tangor (probably from the cross between *C. reticulata* and *C. sinensis* (L.) Osbeck), and the Nova, Farchild, Lee, and Sunburst hybrids (Timmer et al., 2000). However, the Satsumas (*Citrus unshiu* Mark. Marc.), and the Clementine (*Citrus clementina* Hort. ex Tan.) have proved resistant *A. alternaria* (Kohmoto et al., 1991). Other species such as *C. sinensis*, *C. limon* (L.) Burm., and *C. margarita* (Lour.) Swing. are resistant to the pathogen (Pegg, 1966; Gardner et al., 1986; Kohmoto et al., 1991).

Alternaria brown spot is present in several countries where citrus are produced (Timmer et al., 1998). In Brazil, this disease was first reported in 2001 by Goes et al. (2001) affecting 'Dancy' tangerine orchards, and from 2002 was reported in several Brazilian states (Spósito et al., 2003).

With the arrival of the Alternaria brown spot in Brazil, due to the climatic conditions that are favorable to the dissemination and infection by the pathogen, the control of this disease has become difficult and costly due to the large number of necessary fungicide applications.

The objective of the present study was to assess the susceptibility of different citrus species of tangerine and their hybrids and cultivars to the Alternaria brown spot, once, the knowledge of the tangerine species and their hybrids that are resistant or tolerant to this disease is fundamental to provide alternatives for their commercial production of tangerine. In addition, the knowledge of the species or hybrids that are resistant to *A. alternata* is particularly relevant for research in genetic improvement aiming at sources of resistance to the pathogen.

2. Material and methods

2.1. List of studied citrus species and hybrids, and source of inoculum

Experiments were conducted using plants collection in the germplasm bank of the Estação Experimental de Citricultura de Bebedouro, São Paulo, Brazil. The studied species and cultivars are listed in Table 1. The species, cultivars and hybrids used in the present study are divided horticulturally according to the nomenclature of Hodgson (1967), Ortiz Marcide (1985a), and Ortiz Marcide (1986b). The analyzed genotypes were introduced in Brazil from the germplasm bank of the Instituto Valenciano de Investigaciones Agrarias (IVIA) in Spain, and from Agrumes SRA in France.

For the field inoculations, as well as in the case of detached leaves, inoculum production was conducted using an isolate of *Alternaria alternata* obtained from leaves of the Fortune mandarin in an orchard located in the Estação Experimental de Citricultura de Bebedouro. Inoculum preparation was conducted using the method adopted by Canihos et al. (1999), with modifications. Small fragments of leaf tissue showing symptoms of ABS were immersed in 50% ethanol for 30 s, followed by an immersion in a 1:3 sodium hypochlorite (HCl)/ water solution for 2 min. These fragments were rinsed with distilled and sterilized water and deposited onto a potato–dextrose–agar (PDA) medium and incubated at 25 °C in a 12/12 h photoperiod. After seven days, 5 mL of sterilized distilled water was added to the surface of the colonies, which were scraped off using a scalpel to remove the mycelium and then incubated at 20 °C in a 12/12 h photoperiod, for two to three days for the production of conidia. The conidia were collected by adding 10 mL of sterilized distilled water to each plate and scraping gently using a small brush, and conidial suspensions were filtered through two layers of cheesecloth, and the conidial concentration, was centrifuged once for 20 min at $6000 \times g$ to remove mycelia fragments. For the field inoculations, as well as in the case of detached leaves, the conidial concentration was adjusted to 1×10^4 conidia mL^{-1}, using a hemocytometer. The pathogenicity of this isolate was tested in leaves detached from the same cultivar.

2.2. Evaluation of the susceptibility of species and hybrids to natural infection and to artificial inoculation in the field

Experiments were carried out from December until February in two years, in 2004 and 2005. Plants with new foliage and maturation stage compatible with susceptibility to ABS were used to assess the susceptibility to natural infection by conidia of *A. alternata*. Fifty leaves were randomly sampled from each plant, and four plants were sampled for each species or hybrids described above.

Starting on January of 2005, new leaf measuring 2–3 cm leaves of the above mentioned species and hybrids were inoculated with the isolate from Fortune mandarin. Inoculations were carried out using a portable hand spray, and the inoculated leaves were covered with a transparent polyethylene bag to serve as a humid chamber for 16–18 h. Inoculations were always conducted in the afternoon. Assessments were performed four to five days after inoculation.

2.3. Inoculation of detached leaves

Detached leaves of the species and hybrids cited in Table 1 were inoculated in the laboratory of plant pathology of UNESP - Campus of Jaboticabal, São Paulo. Leaves 2–4 and 5–7 cm in length were placed on humid filter paper into Petri dishes and inoculated using a portable hand spray, using the same isolate described above. Control leaves were used for every species and hybrids spraying distilled water. A total of five leaves and four replicates were used for each size class. Plates were kept under ambient temperature 24–26 °C and a 12/12 h photoperiod.