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SHORT COMMUNICATION / *COMUNICAÇÃO*

Relative susceptibility of citrus genotypes to fruit rot caused by *Ceratocystis radicicola* in Iran

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ABSTRACT

Several citrus genotypes were evaluated for their relative susceptibility to the new lemon fruit rot pathogen, *Ceratocystis radicicola*. Wounded detached fruits were inoculated ten days before normal harvest by placing on the wound site a droplet of distilled water followed by a mycelial plug of one-week-old culture. Inoculated fruits were ranked for their relative susceptibility to the pathogen by determining disease severity based on mean lesion size. Using Duncan's multiple range test, citrus varieties were classified into three groups, as follows: most susceptible: Mandarin (cv. Clementine); moderately susceptible: Mandarin (cvs. Dancy, Ponkan, sweet lime and common sour orange) and least susceptible: Mandarin (cvs. Kinnow, Lee, Fortune and Osceola), grapefruit (cvs. Marsh and Red Blush), orange (cvs. Parson Brown, Marss Early, Salustiana, Washington Navel and Hamlin) and lemon (cv. Lisbon). Alternatively, fruit firmness was measured using a hand-held penetrometer at the time of inoculation. Disease severity was negatively correlated ($R = -0.36$, $P < 0.01$) with fruit firmness. Although this study aimed to determine the range of potential hosts for *C. radicicola*, to date the only natural host in the world is considered to be lemon.
Keywords: *Ceratocystis radicicola,* Citrus susceptibility, resistance, fruit firmness.

Ceratocystis spp. constitute important pathogens of trees as well as seeming to be saprophytic species. Among the pathogenic species, *C. fimbriata* Ellis & Halst. has been reported as the causal agent of serious dieback in other citruses as well as lemon trees in Colombia and Argentina (Mourichon, 1994; Montoya & Wingfield, 2006; van Wyk et al., 2007). Fruit rot disease of lemon caused by *Ceratocystis radicicola* (Bliss) C. Moreau (anamorph *Thielaviopsis punctulata* (Hennebert) A.E. Paulin, T.C. Harr. & McNew) and *Bacillus* sp. was reported for the first time from Iran (Mirzaee et al., 2001a, b). *C. radicicola* has previously been shown to be the causal agent of sudden death of date palm (*Phoenix dactylifera*) in the USA and South Africa (Bliss, 1941; Linde & Smit, 1999). *C. radicicola* was also isolated from *Pinus* sp. in the United Kingdom and from the rhizosphere of date palm in Iran (Alavi & Sonbolkar, 2000; Jones & Baker, 2007). Further, several citrus species including Mexican lime, sweet lime, orange, sour orange, grapefruit, rough lemon and mandarin were evaluated for their susceptibility to *C. radicicola* fruit rot. The results showed that the other citrus species were susceptible to the fungal infection whereas immature lemon fruits and seedlings were resistant (Mirzaee & Mohammadi, 2005). Further, the intact citrus fruits were not susceptible to infection by fungal spores of *C. radicicola* (Mirzaee & Mohammadi, 2005). Fruit firmness has been correlated with fruit rot incidence as well as fruit susceptibility to pathogens such as *Rhizopus* fruit rot, *Monilinia laxa* and *Botrytis cinerea* (Daubeny et al., 1980; Bassi et al., 1998; Kempler et al., 2005; Kvikliene et al., 2006; Mirzaee et al., 2007). Fruit firmness was correlated with postharvest time and fruit maturity in orange varieties (Olmo et al., 2000). The objective of this study was to determine the relative susceptibility of various citrus genotypes to *C. radicicola*, the causative agent of fruit rot of lemon in Iran, under laboratory conditions, in order to evaluate the potential risk of the disease to other cultivars.

Fruits were harvested ten days prior to their normal harvest date from a citrus collection in Jiroft region (latitude/longitude: 28/67N, 57/73E), Southeastern Iran, thoroughly washed with non-chlorinated tap water and surface-sterilized with 75% (v/v) ethanol. Inoculation sites were cleaned with sterile cotton and allowed to dry in a chamber. To obtain the optimal conditions for fruit inoculation, the following methods were tested: A) the inoculum was prepared by suspending phialoconidia of one-week-old culture of *C. radicicola* in sterile distilled water to a concentration of 5×10^5 spores/mL. Filter paper discs (5 mm diameter) were dipped in the inoculum and placed over the fruit rind; B) inoculum was prepared as described above, 100 µL suspension of phialoconidia was injected into the fruit rind and C) inoculating the fruits first by making a one-mm-deep wound through the fruit rind with a sterile 5 mm–diameter cork borer, followed by placing 10 µL distilled water in the wound site, and then filling with a 5 mm–diameter mycelial plug of a one-week-old culture grown on potato dextrose agar (PDA, Merck). Inoculated wounds were wrapped in parafilm to maintain moisture. Inoculated fruits were placed in a plastic box with a closed lid, and incubated at 25ºC for 3 days. For each cultivar, a total of 16 fruits were used, which included three independent replications (blocks) each consisting of four samples and four uninoculated controls on detached fruits. The experiment was performed as a completely randomized block design with 17 cultivars as treatments. The fungus was re-isolated periodically to confirm identity and its pathogenicity. Further, four additional fruits of each cultivar were selected for determination of fruit firmness at the time of inoculation (Biggs & Miller, 2004, 2005). Fruit firmness was measured using a hand-held penetrometer (Eijkelkamp, Netherlands) fitted with an 11-mm tip. Detached fruits were rated for disease severity by measuring length and width of each lesion three days after inoculation (Biggs & Miller, 2004). The results were analyzed using the analysis of variance (ANOVA). Mean lesion diameter data were subjected to a general linear model analysis and means were compared by Duncan's Multiple Range Test (MSTAT-C) at 1% probability level. The Spearman correlation analysis was used to determine the relationship between the disease severity and fruit firmness.

Two days after fruit inoculation, decay symptoms were observed on inoculated fruits. Control fruits showed no symptoms and no fungus was isolated from these tissues either. The causal agent was re-isolated from the inoculated fruits. Among various fruit inoculation tests, method C, which involved making a wound through the fruit rind and placing mycelial agar, was the most promising. Method A, which involved placing filter paper discs over the fruit rind, resulted in no disease symptoms. Likewise, method B, which involved the injection of phialoconidia suspension into the rind of fruits, was found to be unreliable due to the fact that the spore suspension was not distributed evenly into the flesh of a number of cultivars.

As shown in Table 1 and Figure 1, fruit firmness was negatively correlated with lesion development by *C. radicicola* on fruit rind (R = -0.36, $P < 0.01$). Fruit rot ranged from 6 cm in diameter for mandarin (*Citrus reticulata*) cv. Clementine to 0.74 cm for mandarin cv. Fortune. The former is considered the most susceptible cultivar and the latter the least. Based on Duncan's multiple range test, citrus cultivars were classified into three relative susceptibility groups: most susceptible: mandarin (cv. Clementine), moderately susceptible: mandarin (cvs. Dancy and Ponkan), sweet lime and common sour orange, and least susceptible: mandarin (cvs. Kinnow, Lee, Fortune and Osceola), grapefruit (cvs. Marsh and Red Blush), orange (cvs. Parson Brown, Marss Early, Salustiana, Washington Navel and Hamlin) and lemon (cv. Lisbon). There was no species of citrus that was more or less susceptible than the species used in this study.

Citrus fruits tested in this study represent a unique collection of cultivars from the same location in order to eliminate any variability due to environmental factors as well as soil texture on fruit quality. The results showed that the potential host range of *C. radicicola* is broader than that observed under natural infection conditions. The disease has been observed and reported on lemon, a natural host for *C. radicicola*, whereas under laboratory conditions, all citrus genotypes were found to be susceptible. Many genotypes investigated in this study were more susceptible to fruit rot than lemon. On the other hand, in spite of the existence of very susceptible genotypes, lemon fruit damage only occurs during the harvest as well as when branch thorns come into direct contact with fruits in southern Iran. This is the first study

TABLE 1 - Disease severity as a function of mean lesion diameter and fruit firmness in various citrus genotypes inoculated with *Ceratocystis radicicola*[a]

Genotype	Species	Lesion diameter (cm)	Fruit firmness (Kg/cm^2)[b]
Clementine	Mandarin	6.00[a]	0.66
Dancy	Mandarin	4.23[b]	1.00
Ponkan	Mandarin	4.13[b]	0.75
Local	Sweet lime	3.90[b]	1.83
Common	Sour orange	3.86[b]	2.92
Osceola	Mandarin	2.60[c]	0.75
Marsh	Grapefruit	2.33[c]	2.08
Kinnow	Mandarin	2.22[cd]	2.25
Lee	Mandarin	2.17[cde]	0.92
Hamlin	Orange	1.97[cde]	2.27
Red Blush	Grapefruit	1.82[cdef]	1.83
Washington Navel	Orange	1.72[def]	2.08
Salustiana	Orange	1.51[efg]	2.75
Marss Early	Orange	1.44[efg]	2.25
Parson Brown	Orange	1.33[fg]	2.58
Lisbon	Lemon	1.33[fg]	2.42
Fortune	Mandarin	0.74[g]	2.18

[a]Data represent the mean of 12 observations from three experiments each consisting of four replicates per cultivar. Different letters denote significant differences among means according to the Duncan's Multiple Range Test. Standard errors (SE) and coefficient of variation (CV) = 0.195, 12.7 and 0.106, 13.2 for lesion size and fruit firmness trials, respectively.

[b]Fruit firmness was tested on four different uninoculated fruits.

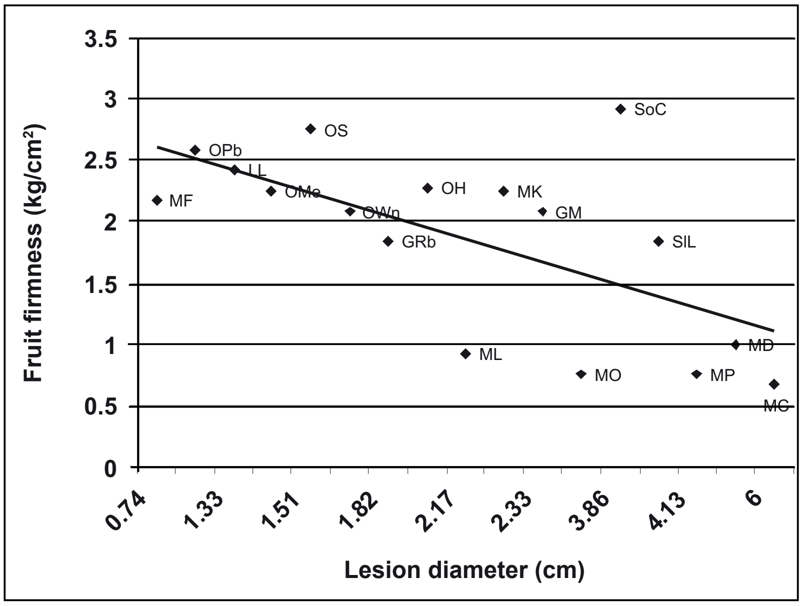

FIGURE 1- The correlation between lesion size and fruit firmness in inoculated citrus cultivars. Abbreviations: MF, ML, MK, MO, MP, MD and MC: Mandarin cvs. Fortune, Lee, Kinnow, Osceola, Ponkan and Clementine; OPb, OS, OMe, OWn, OH: Orange cvs. Parson Brown, Salvestiana, Marss Early, Washington Navel, Hamline; GM and GRb: Grapefruit cvs. Marsh, Red Blush; LL: Lemon cv. Lisbon; SlL: Sweet lime cv. Local; SoC: Common sour orange.

of citrus fruit rot on susceptible varieties in Iran. Therefore, a thorough investigation of its natural prevalence on other citrus cultivars, as well as fruit rot etiology and life cycle of the causative pathogen, are needed.

Fruit firmness has been demonstrated to be an important contributing factor for resistance (Daubeny et al., 1980; Bassi et al., 1998; Biggs & Miller 2004; Kvikliene et al., 2006; Blazec et al., 2007). On the other hand, fruit firmness was correlated with postharvest time and fruit maturity in orange varieties (Olmo et al., 2000). This characteristic suggests that fruit maturity is related to mechanical properties and fruit quality (Ladaniya 2008). Therefore, it is recommended that fruit firmness and its correlation to disease severity be evaluated. Negative correlations were also observed between fruit firmness in apple cultivars and *Botryosphaeria* spp., ideotypes of apple and storage diseases and red raspberry and *Rhizopus* fruit rot (Daubeny et al., 1980; Biggs & Miller, 2004; Blazec et al., 2007).

In this study, fruit firmness had a negative correlation with susceptibility of citrus genotypes to *C. radicicola*. We observed unexpected results in the case of fruit firmness correlating with susceptibility of mandarin cvs. Osceola, Lee and common sour orange (Figure 1). These discrepancies show that the interaction between the fungus and the citrus host is much more complex than was thought and, in addition to fruit firmness, other factors may play a role in disease development and host resistance. Expression of defense-related genes, pathogenesis-related proteins, phytoalexins, flavonoids and other secondary metabolites induced citrus fruit protection against fungal infections (Arcas et al., 2000; Ortuno et al., 2008). For instance, Ortuno et al. (2008) have suggested that ethylene may be considered as a possible marker for susceptibility of citrus fruits to *Alternaria alternata* pv. *citri*. Fortune, which is the most susceptible variety, produced more ethylene during growth than the less susceptible *C. lemon* and *C. paradisi*. Thus, further investigation is needed to unravel the mechanism(s) underlying the interaction between citrus fruits and *C. radicicola*.

C. radicicola is known to be a soil-borne pathogen (Wingfield et al., 1993). Poor sanitary conditions, fruit damage during harvest and uncut branches touching the soil surface in citrus groves may contribute to fungal infection and spread. Further, citrus and date palm trees, which are natural hosts of the pathogen, are planted together in Jiroft region (Mirzaee & Mohammadi, 2005). Under laboratory conditions, disease progress caused by *C. radicicola* on Mandarin fruit (cv. Dancy) was significantly greater than that caused by *Alternaria* sp. (Mirzaee & Mohammadi, 2005). Therefore, *C. radicicola* should be considered a serious threat to the citrus industry in the region and neighboring countries where citrus and date palm trees are planted together (Mirzaee & Mohammadi, 2005). Furthermore, *C. radicicola* may become a threatening pathogen in the US and South Africa, where it was originally reported.

There are several sanitary measures for managing citrus fruit rot disease. These include pruning citrus tree branches touching the soil surface, avoiding fruit damage, separating fruits that had contacted the soil from the rest and preventing the fruits from touching the soil. These measures are also advised for the control of *Phytophthora* fruit rot, *Botrytis* fruit rot and citrus sour rot (Whiteside et al., 1988). In order to alleviate disease severity due to fungal fruit rot, and to increase fruit firmness, it is highly recommended that fruit damage be prevented during harvest. Loss of citrus fruit firmness has been correlated with the degradation of the cell wall (Olmo et al., 2000). It is well known that calcium has a major effect on enhancing fruit firmness, cell wall structure and reducing the incidence of physiological disorders and decay (Salcedo et al., 2000; Malakouti & Tabatabaee, 2001; Kader & Rolle, 2004). High nitrogen content and excess water supply to plants, on the other hand, are often associated with reducing fruit firmness and increasing susceptibility to mechanical damage and decay (Kader & Rolle, 2004). Therefore, it would also be possible to use fertilizers to boost plant nutrition, aiming at enhancing fruit resistance to mechanical injuries.

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