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Short communication

Alternaria brown spot of Minneola in Greece; evaluation of citrus species susceptibility

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Abstract

During the last three years, a new disease was observed in northwestern Greece on Minneola trees, hybrid of mandarin and grapefruit. On May small brown necrotic leaf spots surrounded by yellow halo areas of various sizes appeared and covered a major portion of the leaves with extension of necrosis into the veins. On young fruits small, slightly depressed black spots were the first symptoms, which later became 2–7 mm in diameter. Brown spots were observed on the leaves and fruits in several orchards in the same area, causing leaves and fruits to drop. In some orchards over 50% of the fruits were affected. From the fruit and leaf spots the typical small-spore species *Alternaria alternata* was isolated. Pathogenicity tests were performed by artificially inoculating fruits of Minneola, common mandarin and Clementine. The symptoms of the disease were reproduced only on fruits of Minneola hybrids by the specific strain of the fungus *Alternaria alternata* pv. *citri*. Different citrus susceptibility tests indicated that mandarins Minneola, Nova and Page were very susceptible to tested isolates while Clementine SRA and Poros Clementine were not. All lemons and lime Seedless were not susceptible. Grapefruit New Hall was not susceptible, while the Star Ruby was. Orange Lane Late, Navel Late, Oval Poros, Olinda, Navel Athos were not susceptible and only Moro showed reaction being slightly susceptible only to one isolate.

Alternaria brown spot of citrus is known worldwide as a serious disease of leaves, fruits and twigs on some mandarin hybrids or cultivars. The disease was first described in 1903 on Emperor mandarin in Australia (Whiteside, 1993), but now is also known to occur in the USA (Whiteside, 1976), Brasil and Argentina (Peres et al., 2003), Israel (Solel, 1991), Turkey (Canihos et al., 1997) and South Africa (Swart et al., 1998). In Europe, in the Mediterranean basin, the disease was reported in Spain (Vicent et al., 2000) and Italy (Bella et al., 2001). The disease was caused by a specific strain of the fungus *Alternaria alternata* pv. *citri*. There are two distinct pathotypes of this fungus according to the host specificity and toxin production. The pathotype (Aac) produces the host-specific ACT-toxin for tangerine (*Citrus reticulata*) and tangerine × grapefruit (*C. reticulata* × *Citrus paradisi*) hybrid; this is known as the tangerine pathotype and reduces yield and fruit quality. The second pathotype of the fungus produces the host-specific ACRL-toxin, that affects the rootstock cultivar Rough Lemon (*Citrus jambhiri*) and *Citrus limonia* (Kohmoto et al., 1979; Peever et al., 1998, 2002). An additional Alternaria disease, the black rot of citrus, is considered distinct from the brown spot (Peever et al., 2004).

Different citrus species are cultivated in Greece for fresh fruit or for processed products. The most severe fungal diseases of citrus in Greece are gummosis and brown rot caused by species of the

genus *Phytophthora*, mal secco caused by *Phoma tracheiphila* and post-harvest diseases by *Phytophthora* and *Penicillium* species.

During November and December 2003, a new disease was observed for the first time in northwestern Greece on 10–15 year-old Minneola trees, hybrid of mandarin and grapefruit (*C. reticulata* × *C. paradisi*). Sunken, dark brown spots were observed on the fruit surface, at the beginning of fruit ripening (according to growers observations), only on orchards with this hybrid. The spots were 2–7 mm in diameter, slightly depressed. In some orchards over 50% of the fruits were affected. The affected fruits dropped to the ground. No disease symptoms were observed on the leaves or other part of the trees.

The next years (2004, 2005) the disease was much more widespread only in northwestern Greece on Minneola hybrid. On May small brown necrotic leaf spots surrounded by yellow halo areas of various sizes appeared on Minneola trees. The spots covered a major portion of the leaves with extension of necrosis into the veins. On young fruits small, slightly depressed black spots were the first symptoms. Brown spots were observed on the leaves and fruits in several orchards in the same area causing leaves and fruits to drop. The purposes of this study were: (a) the identification of the pathogen associated with the disease of Minneola mandarins and (b) the evaluation of susceptibility of different citrus species, hybrids or cultivars, cultivated in Greece.

Brown spot infected fruits and leaves were collected from Minneola trees at different fields in northwestern Greece during the last three years. Isolations were made on potato dextrose agar (PDA) medium at 25 °C.

From the margins of fruit and leaf spots a typical small-spore *Alternaria* species was isolated. Twenty-five *Alternaria* isolates from Minneola mandarins were obtained. Single conidial isolates were selected from each isolate for pathogenicity tests. All isolates were stored on V8 and PDA medium at 22 °C. The morphological characteristics of the fungus were close to these of *Alternaria alternata*.

Pathogenicity tests were performed by artificially inoculating fruits of Minneola hybrid and also fruits of common mandarin (*C. reticulata*) and Clementine (*C. reticulata* × *C. aurantifolia*). The inoculum consisted of 3 week-old isolates grown on V8 medium (50 ml V8, 7.5 g agar, 500 ml H_2O, pH 5.5) at 25 °C and 12 h dark fluorescent light. The surface of the plates was covered with sterile distilled water (SDW), scraping the conidia with a glassy bar. Then the suspension was stained through a cheesecloth, and finally a suspension of 6×10^5 conidia ml^{-1} was used. The suspension was sprayed onto the fruits detached from the trees, which were incubated in polyethylene bag humid chambers at 25 °C in the dark. The control was sprayed with SDW. Two representative *Alternaria* isolates, A2 and A3, two monoconidial strains $A4_4$ and $A9_3$, and seven fruits of each different mandarin, were used. The experiments were repeated twice.

The symptoms of the disease were reproduced only on fruits of Minneola hybrids. They started from the second day of inoculations and increased over the next days (Table 1). Similar results were obtained from the second experiment. The isolates were host specific. Koch's postulates were satisfied by reisolation of the fungus from symptomatic tissue. The disease was caused by the specific strain of the fungus *Alternaria alternata* pv. *citri*.

The susceptibility of 18 citrus hybrids or cultivars (Table 2) was determined by using leaves and fruits collected from the fields of the Citrus Germplasm Bank of the Arboricultural Station of Poros (Ministry of Rural Development and Food), in June. The highly virulent monoconidial strains $A4_4$ and $A9_3$ were grown in 9 cm Petri dishes containing V8 medium for 13 days at 25 °C and 12 h dark fluorescent light. Conidial suspensions (10^5 conidia ml^{-1}) were prepared as above. Two drops (40 μl each) were placed on the lower surface of each leaf. For fruit inoculation, four filter

Table 1. Pathogenicity tests of *Alternaria alternata* pv. *citri* on different mandarins

Treatmentss	Mandarins		
	Minneola	Clementine	Common mandarin
Control	1[a]	1	1
Isolate A2	5	1	1
Isolate A3	5	1	1
Single spore Isolate $A4_4$	5	1	1
Single spore Isolate $A9_3$	5	1	1

[a]Disease index (average of seven replications), 1: healthy fruit, 5: 100% infected fruit.

Table 2. Susceptibility of different citrus to artificial inoculations by *Alternaria alternata* pv. *citri*

Citrus	Treatments					
	Control		Strain 4_4		Strain 9_3	
	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves
Mandarins						
Nova	1[a]	1	5 a	2.6 ab	5 a	2.4 b
Page	1	1	5 a	2 abc	5 a	2 bc
Minneola	1	1	5 a	3 a	5 a	3.8 a
SRA (Clementine)	1	1	1 c		1 d	1 c
Clementine Poros	1	1	1 c	1 c	1 d	1 c
Oranges						
Moro	1	1	1 a	1 c	2 c	1 c
Lane Late	1	1	1 a	1 c	1 d	1 c
Navel Late	1	1	1 a	1 c	1 d	1 c
Oval Poros	1	1	1 a	1 c	1 d	1 c
Olinda	1	1	1 a	1 c	1 d	1 c
Navel Athos	1	1	1 a	1 c	1 d	1 c
Lemons						
S. Bonanca	1	1	1 a	1 c	1 d	1 c
Adamopoulos	1	1	1 a	1 c	1 d	1 c
Zambetaki	1	1	1 a	1 c	1 d	1 c
Opsimo Argos	1	1	1 a	1 c	1 d	1 c
Grapefruits						
New Hall	1	1	1 a	1 c	1 d	1 c
Star Ruby	1	1	2.85 b	1 c	3.28 b	1 c
Limes						
Seedless	1	1	2.43 b	1 c	3.14 b	1 c

[a]Disease index (average of seven replications for the fruits and five for the leaves) 1: no infection, 5: 100% infection.
Numbers followed by the same letters within the column do not differ significantly according to LSD test at $P \leq 0.05$.

paper squares (0.5×0.5 cm) were dipped in the inoculum suspension and placed on the rind of each fruit. Instead of conidial suspension SDW was used for the controls (Solel and Kimchi, 1997; Vicent et al., 2004). Seven fruits and five leaves were used for each strain and each different citrus. The inoculated fruits and leaves were incubated in humid boxes at 25–27 °C in the dark for 4 days. The severity of the disease was recorded as % positive inoculations on each fruit, using a scale 1–5 where 1 represented no infection and 5 represented 100% infection. The experiments were repeated twice. Data for two experiments were analyzed by ANOVA using the LSD test at $P \leq 0.05$.

The results of this study indicated that the mandarins Minneola, Nova and Page, the lime Seedless and the grapefruit Star Ruby were susceptible to artificial inoculations with Greek isolates $A4_4$ and $A9_3$, while all the tested oranges (except for the orange Moro that showed an intermediate reaction), lemons and the grapefruit New Hall were resistant (Table 2). The data report the results of the first experiment, since the results of the second experiment analyzed in the same way, were similar to the first. Under natural conditions in Greece brown spot disease was so far only recorded on Minneola mandarins. According to Solel and Kimchi (1997), in artificial inoculations the host range of *A. alternata* pv. *citri* is broader than that under natural infection conditions.

Our results, with tested citrus, agree with those from Spain and Israel with some differences. The oranges Lane Late and Navel Late were resistant in inoculations with our strains while they were slightly susceptible in the Spanish experiments. Peever et al. (2002) determined the quantitative differences in virulence among isolates from different citrus growing areas of the world, and found statistically significant differences. Although the host range varied in each country there were similarities such as the disease symptoms and basic host range (Solel and Kimchi, 1997; Vicent et al., 2004).

According to Solel (1991) brown spot disease is due to a citrus pathotype called *A. alternata* pv. *citri*, that causes brown spot of Minneola, clearly distinct from other strains of *A. alternata* (previously *A. citri*) which cause other types of diseases on citrus. This disease has also been reported as Alternaria-induced brown spot disease of Dancy tangerines (*C. reticulata*) in Florida, Emperor mandarin (*C. reticulata*) in Australia and certain other citrus cultivars (Whiteside, 1976, 1993). Isolates causing black rot of citrus are considered biologically distinct from the brown spot pathogens because they are unable to cause this disease on leaves or fruits and do not produce host-specific toxins. According to Peever et al. (2004) there appears to be no basis for considering black rot isolates as a distinct taxon but the small-spore species of *Alternaria* from citrus grouped into a single phylogenetic species. It seems that most small-spore species of *Alternaria* associated with citrus or other hosts can cause black rot but not brown rot.

Minneola, which has been mainly grown in northwestern Greece during the last few years, has already been progressively eliminated in these areas, owing to the severe yield losses (low yield and unacceptable blemished fruits) due to infections by *Alternaria* since the disease incidence increased.

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