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Resistance and susceptibility of mandarins and their hybrids to *Alternaria alternata*

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ABSTRACT: Caused by *Alternaria alternata*, Alternaria brown spot (ABS) is a major fungal disease of mandarins in Brazil, causing serious losses in commercial orchards of Ponkan tangerine (*Citrus reticulata* Blanco) and Murcott tangor [*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco]. The response of 31 accessions/genotypes of mandarins and their hybrids to *A. alternata*, was evaluated in São Paulo state, Brazil. The incidence and severity of the disease in fruit were evaluated in the field, using diagrammatic scale, four times during 2008 and 2009. Based on these data, the area under the disease progress curve (AUDPC) was calculated. In parallel, in vivo and in vitro inoculations with *A. alternata* were made on leaves to confirm field results. Resistance and susceptibility was evaluated in controlled experiments by counting the number of lesions per leaf using a diagrammatic scale. Most of the genotypes were symptomatic after inoculation with *A. alternata* with disease most severe on the hybrids Nova, África do Sul, Ponkan and Murcott. Cultivars like Cravo, Nules and Ortanique that have excellent fruit quality and had lower AUDPCs, whereas Fremont and Thomas were highly resistant to the fungus and did not show any symptoms in the field or in controlled inoculations. The attractiveness and pronounced flavor of Fremont and Thomas fruit make them new options for the citriculture.
Keywords: *Citrus* spp., Alternaria brown spot, fungus

Introduction

Brazil is the third largest producer of mandarin fruits, planted in around 54,000 hectares and producing 1.1 million tons (IBGE, 2010). Mandarin production is economically important for many Brazilian states, mainly São Paulo, Paraná, and Rio Grande do Sul, which are the largest producers, respectively accounting for 44, 22 and 14 % of the national production (FNP, 2009). Nevertheless, the number of economically important mandarin varieties used in the country is fairly limited as observed in the State of São Paulo, where most of the mandarin orchards are composed of Ponkan tangerine (*Citrus reticulata* Blanco), Murcott tangor [*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco], Mexerica-do-Rio (*C. deliciosa* Tenore) and Cravo tangerine (*C. reticulata* Blanco) (CEPEA, 2007).

Caused by *Alternaria alternata*, Alternaria brown spot (ABS) is currently considered the most serious fungal disease in orchards of mandarins and their hybrids. Since its outbreak in Brazil in 2001 (Goes et al., 2001), this disease has been leading to the abandonment of commercial orchards of highly susceptible varieties. It has been widely spread and become more severe in humid climate areas, where the control is very difficult (Stuart et al., 2009).

The high ABS susceptibility of the main varieties planted in the State of São Paulo (mainly Ponkan tangerine and Murcott tangor) makes their production unprofitable, since frequent applications of agrochemicals are required to control the disease. As consequence of the increased costs of production, citrus growers prefer to eradicate their mandarin orchards as evidenced by the decline of Brazilian fresh mandarin production, which has decreased from 1,270,108 tons in 2006 to 1,079,697 tons in 2008; and also by the decline of the total planted area from 60,993 ha to 54,003 ha in the same period (IBGE, 2010).

Therefore, considering the severity of the disease and the great damage caused in different Brazilian regions, basically due to the narrow range of cultivated varieties, it is necessary to select new economically important ABS-resistant genotypes. In this study, our group evaluated the susceptibility of 31 genotypes of mandarins and their hybrids to ABS.

Materials and Methods

Plant material

A total of 31 genotypes of tangerines and their hybrids (Table 1) were evaluated for susceptibility to *Alternaria alternata*, in vitro, in vivo, and in the field. The genotypes were selected from the Citrus Germplasm Bank of Centro de Citricultura 'Sylvio Moreira' – IAC, Cordeirópolis, São Paulo state, Brazil.

Field ratings

Field ratings were made in experimental orchards in three municipalities of São Paulo state, Brazil: Capão Bonito (24°00′ S, 48°20′ W, 705 m ass), Itirapina (22°15′ S, 47°49′ W, 770 m asl), and Porto Feliz (23°12′ S, 47°31′ W, 523 m asl) (Table 1). The incidence and severity of ABS were evaluated four times between Feb. and May, 2008 and 2009. This time of the year was selected because of the availability of fruit on the plants, allowing for the evaluation of leaves and fruits. The experiment was arranged in a completely randomized block design with 31 genotypes, three plants each, and 30 fruit per

Table 1 – List with the citrus genotypes evaluated for resistance to Alternaria brown spot in three localities from São Paulo State, Brazil.

Varieties (registration number in Germoplasm Bank of Citrus)	Localities		
	Capão Bonito	Porto Feliz	Itirapina
Tangerine (*Citrus reticulata* Blanco) and hybrids			
África do Sul (557 – NC*)	X		X
Cravo (1478 – NC)	X	X	X
De Wildt (545 – NC)	X	X	
Empress (565 – NC)	X	X	X
Fremont (543 – NC)	X	X	X
Ladu × Szinkon (552 – NC)	X	X	X
Loose Jacket (515 – NC)		X	
Ponkan (172 – NC)	X	X	X
Rosehaugh Nartjee (555 – NC)	X	X	
Sul da África (529 – NC)	X	X	
Sunburst (1376 – NC)	X	X	X
Szibat (558 – NC)		X	X
Szinkon × Batangas (569 – NC)		X	X
Szinkon (564 – NC)	X	X	
Szwinkon × Szinkon-Tizon (560 – NC)	X	X	
Swatow (171 – OC)			X
Thomas (519 – NC)	X	X	
Warnuco (547 – NC)		X	X
Willowleaf mandarin (*C. deliciosa* Ten.)			
1144-12 (585 – NC)		X	
Mogi das Cruzes (606 – NC)		X	
Tardia da Sicilia (589 – NC)		X	
Clementine (*C. clementina* Hort. ex Tan.) and hybrids			
Caçula 3 (1330 – NC)	X	X	X
Caçula 4 (1318 – NC)			X
Nules (1742 – NC)	X	X	X
Tangor [*C. reticulata* × *C. sinensis* (L.) Osbeck]			
Murcott (553 – NC)	X	X	X
Murcott Irradiada (1578 – NC)	X	X	X
Ortanique (554 – NC)	X	X	X
Hansen (596 – NC)	X	X	X
Tangelo (*C. reticulata* × *C* Macfad)			
Nova (1526 – NC)	X	X	X
Page (1525 – NC)	X	X	X

*NC = new clone/OC = old clone.

plant. The severity of the symptoms on the fruit surface was determined using a specific diagrammatic scale, as described by Renaud et al. (2008). The scale ranges from '0' to '6', where '0' indicates no damage on fruit surface, and '1' to '6' correspond to 0.1, 1.0, 2.5, 5.0, 11.0 and 25.0 % the total surface, respectively. Based on the diagrammatic scale data, the area under the disease progress curve (AUDPC) was calculated by the trapezoidal integration method (Berger, 1988). The presence or absence of disease symptoms on leaves was also evaluated and the genotypes were classified as symptomatic (+) and asymptomatic (-).

In vitro evaluation

Isolated from Nova tangelo (*C. reticulata* × *C. paradisi* Macfad) infected leaves, a very aggressive *A. alternata* isolate (A1.3) was used to inoculate leaves of the 31 mandarin genotypes. This isolate was selected after aggression test in young leaves of Murcott (Azevedo et al., 2010). To produce conidial suspensions for inoculating leaves, 50 mycelial plugs (5 mm²) from five-day-old cultures on potato dextrose agar (PDA) supplemented with carbendazim fungicide (640 mg L^{-1} a. i.), were placed on a $CaCO_3$ sporulation media (30 g of $CaCO_3$; 20 g sucrose; 20 g agar L^{-1}) and covered with 2 mL of sterile distilled water and incubated for a 12-hour photoperiod for four days at 27 °C. Subsequently, 5 mL of autoclaved distilled water were added to the plates and conidia were scraped from medium surface with a sterile spatula. The conidial suspension was filtered through two layers of sterile gauze, quantified in a Neubauer counting chamber, and adjusted to 10^4 conidia mL^{-1}.

For inoculation, symptomless young leaves (2-3 cm length) were collected from all the 31 genotypes in the field, washed in water, and placed in Petri dishes containing a layer of filter paper and a small piece of cotton moistened with sterile distilled water to create a microenvironment of high humidity. The conidial suspension was sprayed (approximately 2 mL), with a portable hand spray, onto leaves and the material was incubated at 27 °C for a 12-hours photoperiod. Disease incidence was evaluated starting 12 hours post inoculation up to 72 hours. The number of lesions and the percentage of injured leaf area were laid out in a completely randomized design with four replicates per mandarin genotype. The evaluation was conducted in the summer of 2008 and repeated in 2009.

In vivo evaluations

Seeds collected from all the 31 mandarin genotypes were planted in plastic tubes containing a substrate for the growth of citrus. Seedlings were transferred to 4 L pots and maintained in a greenhouse. When the plants reached approximately 30 cm in height, they were pruned in order to stimulate the emergence of new shoots and young leaves. Approximately 35 days after pruning, the abaxial surfaces of young leaves (2-3 cm long) from the tip of the shoots were inoculated with a conidial suspension (10^4 conidia mL^{-1}) of *A. alternata.* The suspension was prepared as described above. Inoculation was performed with a sprayer by applying approximately 2 mL of the conidial suspension per leaf (Azevedo et al., 2010). After inoculation, the shoot tips were covered with transparent plastic bags to create a microenvironment of high humidity. Plastic bags were removed 36 h post inoculation and the symptoms were evaluated 72 h post inoculation. The number of lesions was counted and the percentage leaf area affected was estimated. Treatments were arranged in a completely randomized design with 31 genotypes and four plants per genotype.

Statistical Analysis

The average values for the variables analyzed were subjected to analysis of variance (ANOVA) and compared by Scott-Knott test ($p < 0.05$) (Scott and Knott, 1974), using the statistical program SISVAR (Ferreira, 2008).

Results and Discussion

Of the 31 genotypes tested, 29 (93.6 %) were symptomatic and some were highly susceptible, e.g., Nova, África do Sul (*Citrus reticulata* Blanco), Sul da África (*Citrus reticulata* Blanco) and Murcott (Table 2 and Figure 1). These results corroborate those obtained by Peever et al. (2000) who evaluated six hybrids of tangerines in

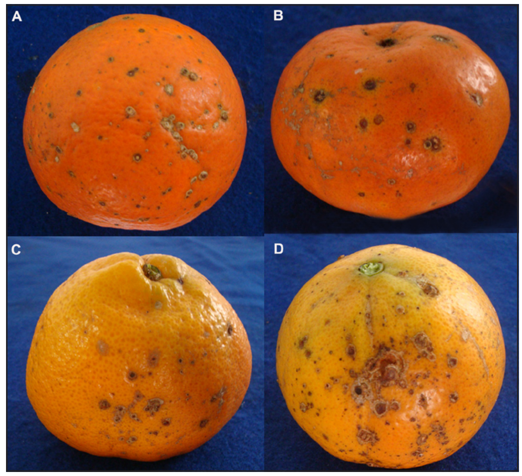

Figure 1 – Typical symptoms of Alternaria brown spot on fruit of Nova tangelo (A); África do Sul tangerine (B); Ponkan tangerine (C); Murcott tangor (D).

Table 2 – Area under the disease progress curve (AUDPC) for the severity of Alternaria brown spot of tangerines and hybrid genotypes (Capão Bonito, Itirapina and Porto Feliz, São Paulo state, Brazil, Feb. to May 2008 and 2009).

Genotypes	AUDPC					
	Itirapina		Porto Feliz		Capão Bonito	
	2008	2009	2008	2009	2008	2009
Nova	725.12 a	180.57 b	60.12 a	24.59 c	158.19 b	144.69 b
África do Sul	301.85 b	240.30 a			18.25 c	38.75 c
Sul da África			59.40 a	82.34 b	345.69 a	178.59 a
Murcott	399.54 b	337.95 a	14.26 c	25.59 c	110.41 b	52.40 c
Murcott Irradiada	445.36 b	242.89 a	18.85 c	297.32 a	41.98 c	18.24 d
Clementina Caçula 3	170.45 c	65.48 c	19.51 b	0.97 e	78.70 b	16.06 d
Ponkan	66.63 c	125.92 b	17.60 c	15.10 c	49.22 c	56.84 c
Swzinkon			57.34 a	9.29 d	11.17 c	2.00 f
Clementina Caçula 4	353.66 b	143.43 b				
Swatow	56.58 c	47.42 c				
Warnuco	19.07 d	3.53 d				
Shekwash × Tizon	11.93 d	10.24 d	8.58 c	7.10 d		
Swzinkon × Batangas	6.95 d	7.63 d	39.44 b	3.31 e		
Mogi das Cruzes			1.32 d	5.10 e		
Loose Jacket			5.39 c	4.29 e		
1144-12			9.87 c	4.12 e		
Tardia da Sicilia			2.91 d	3.94 e		
Szibat	6.15 d	3.02 d	0.86 d	0.11 e		
Sunburst	11.58 d	17.13 d	1.02 d	0.27 e	5.80 c	7.78 e
De Wildt			2.12 d	2.74 e	6.81 c	4.28 f
Swzinkon × Swzinkon-Tizon			2.76 d	3.20 e	5.59 c	1.61 f
Rosehaugh Nartjee			38.57 b	2.10 e	5.67 c	2.07 f
Empress	6.77 d	4.84 d	4.25 d	0.98 e	7.56 c	1.09 f
Page	4.28 d	3.22 d	9.50 c	2.66 e	3.21 c	1.81 f
Ortanique	7.54 d	4.58 d	1.67 d	3.42 e	9.05 c	0.19 f
Ladu × Swzinkon	9.74 d	4.30 d	2.27 d	3.23 e	2.17 c	0.15 f
Hansen	1.50 d	4.98 d	0.79 d	1.81 e	7.69 c	0.17 f
Clementina Nules	1.12 d	5.01 d	0.15 d	1.12 e	0.30 c	1.55 f
Cravo	4.92 d	3.33 d	0.00 d	0.41 e	8.06 c	0.00 f
Thomas			0.00 d	0.00 e	0.00 c	0.00 f
Fremont	0.00 d	0.00 d	0.00 d	0.00 e	0.00 c	0.00 f
Coefficient of Variation (%)	26.11	39.14	44.28	37.71	44.26	25.15

Means followed by the same letter in column do not differ (Scott Knott, $p < 0.05$).

eight locations in Florida and found that the mandarin cultivars Minneola (*C. reticulata* Blanco × *C. paradisi* Macfad), Orlando (*C. reticulata* Blanco × *C. paradisi* Macfad), Sunburst (*C. reticulata* Blanco), Nova, Murcott, and Duncan grapefruit (*C. paradisi* Macfad) were the most susceptible and confirmed the high susceptibility of Nova tangelo to the pathogen. This high susceptibility can be explained by the Nova tangelo is a sister to the Lee, Osceola, and Robinson mandarins, all four resulting from a Clementine mandarin (*C. clementine* Hort. ex Tan.) × Orlando tangelo (*C. reticulata* Blanco × *C. paradisi* Macfad) cross, and the Orlando tangelo has a kinship with the Dancy tangerine, which this variety extremely susceptible to Alternaria brown spot (Hodgson, 1967).

Moreover, hybrids like Thomas (*C. reticulata* Blanco) and Fremont (*C. reticulata* Blanco) (Figure 2 and Table 2) were resistant, whereas Cravo (*C. reticulata* Blanco), Nules (*C. clementine* Hort. ex Tan.), Hansen [*C. reticulate* × *C. sinensis* (L.) Osbeck] and Ladu (*C. reticulata* Blanco) were less susceptible in two years of assessments. Fremont is result a Clementine mandarin × Ponkan mandarin cross (Hodgson, 1967). One possible to explanation this fact of the Fremont mandarin present more characteristics of Clementine mandarin making believe that Fremont has inherited more genes than the Clementine than Ponkan, which could explain their resistance to disease, since the Clementine mandarin is less susceptible to Alternaria brown spot than Ponkan.

Among the resistant varieties, Thomas has physical characteristics (medium size, bark smooth and adherent, average weight of 160 g, 16 seeds per fruit, juice accounting for 38 % of fruit mass), chemical qualities (13.1 °Brix, acidity 1.2 %; ratio of 10.1) and organoleptic characters (reddish-orange color, firm texture, outstanding flavor, appropriate to the foreign palate) similar to those of Murcott tangor, an important commercial cultivar in the country (Pio et al., 2005). Thus, these characteristics make Thomas a potential commercial replacement for Murcott tangor.

Figure 2 – Characteristic fruit of Fremont and Thomas, resistant to *Alternaria alternata*. The ideal time of harvest was Jun./2009 and Oct./2009, respectively.

Of the Ponkan group [Ponkan; Rosehaugh Nartjee (*C. reticulata* Blanco); África do Sul; Empress (*C. reticulata* Blanco) and De Wildt (*C. reticulata* Blanco)], all proved to be in the intermediately susceptible, whereas 1144-12 (*C. deliciosa* Ten.), Mogi das Cruzes (*C. deliciosa* Ten.) and Tardia da Sicília (*C. deliciosa* Ten.), belonging to the willow leaf mandarin group (*C. deliciosa* Ten.), the fruit symptoms in the field were almost imperceptible, and showed a high level of tolerance to the fungus (Table 2). The 1144-12 selection had no symptoms during in vitro trials whereas only Mogi das Cruzes showed lesions on the seedlings though the severity was very low compared with the other genotypes (Table 3).

The AUDPCs were much higher in Itirapina, SP in comparison with those in Capão Bonito, SP (Table 2). This can be explained by the climatic difference be-

Table 3 – Average number of lesions per leaf (ANLL) and severity (S) of Alternaria brown spot after inoculation in vitro, in vivo, and under natural infection (Cordeirópolis, São Paulo State, Brazil, means of 2008 and 2009).

Genotypes	Inoculation in vitro		Inoculation in vivo		Natural Infection[a]
	ANLL	S	ANLL	S	
		%		%	
África do Sul	83.22 a	15.00 a	39.50 a	26.67 a	+
Page	77.62 a	14.75 a	2.84 c	2.00 c	+
Empress	63.00 a	12.13 a	16.70 b	9.10 b	+
Sul da África	55.39 b	11.25 a	7.58 c	4.47 c	+
Clementina Caçula 3	45.72 b	9.38 b	18.83 b	11.79 b	+
Clementina Caçula 4	44.45 b	9.25 b	17.50 b	10.62 b	+
Rosehaugh Nartjee	44.11 b	9.13 b	7.00 c	3.00 c	+
Murcott	34.00 b	6.88 b	27.80 a	20.05 a	+
De Wildt	25.17 c	6.63 b	3.34 c	2.01 c	+
Ponkan	23.78 c	5.00 c	14.67 b	8.34 b	+
Nova	23.56 c	4.75 c	0.97 c	0.58 c	+
Sunburst	19.78 c	4.75 c	5.84 c	2.50 c	+
Murcott Irradiada	19.00 c	4.75 c	10.03 b	2.50 c	+
Szinkon × Batangas	18.00 c	3.63 c	20.17 b	12.29 b	+
Swatow	4.00 c	1.38 c	13.50 b	7.50 b	+
Szibat	1.17 c	1.25 c	3.84 c	2.00 c	-
Mogi das Cruzes	7.17 c	3.38 c	16.67 b	9.00 b	-
Ladu × Szinkon	6.67 c	3.38 c	0.00 c	0.00 c	-
Szinkon	4.34 c	2.63 c	0.00 c	0.00 c	-
Warnuco	4.34 c	2.63 c	0.00 c	0.00 c	-
Szwinkon × Szinkon Tizon	4.00 c	1.38 c	0.00 c	0.00 c	-
Hansen	2.84 c	1.25 c	0.00 c	0.00 c	-
Clementina Nules	2.34 c	1.25 c	0.00 c	0.00 c	-
Loose Jacket	2.17 c	1.25 c	0.00 c	0.00 c	-
Tardia da Sicilia	0.84 c	1.25 c	0.00 c	0.00 c	-
1144-12	0.00 c	0.00 c	0.00 c	0.00 c	-
Ortanique	0.00 c	0.00 c	0.00 c	0.00 c	-
Cravo	0.00 c	0.00 c	0.00 c	0.00 c	-
Thomas	0.00 c	0.00 c	0.00 c	0.00 c	-
Fremont	0.00 c	0.00 c	0.00 c	0.00 c	-

Means followed by same letter in column do not differ (Scot Knott, $p < 0.05$); [a] + (symptoms) and - (no symptoms).

tween the localities and the disease epidemiology. The Itirapina region had higher precipitation (1,165.7 mm) than Capão Bonito (805.8 mm) during the evaluations, indicating the importance of moisture in the epidemiology of the disease. In addition, the assay farm Raio de Sol, Itirapina, is surrounded by Murcott orchards, which is highly susceptible to the disease and thus inoculum pressure was high.

In Porto Feliz, the assay was carried out within a commercial citrus farm which employed standard disease management in their Murcott orchard which explains the low AUDPC that may have been due to the reduced inoculum from the plants adjacent to the assay in the field and not due to temperature and precipitation effects.

There were great differences between the tangerine genotype susceptibility to *A. alternata* in the three evaluated sites. Similar results were observed by numerous studies that on resistance and susceptibility against the ACT pathotoxin in citrus. Kohmoto et al. (1991) found 28 species of citrus (tangerines, tangelos, tangors) susceptible to the tangerine pathotype; Solel and Kimchi (1997) found that Minneola, Dancy, Ellendale (*C. reticulata* × *Citrus sinensis* L. Osbeck), Murcott, Nova, Satsuma (*C. unshiu* Marcov), Orlando, and Page (*C. reticulate* × *C. paradise* Macfad) were highly susceptible; and Stuart et al. (2009) reported the susceptibility to the fungus in Daisy (*Citrus reticulata* Blanco), Temple (*Citrus reticulata* Blanco) × Dancy, and Satsuma × Murcott4 [*C. reticulata* × *C. sinensis* (L.) Osbeck].

The ACT toxin produced by *A. alternata* is specific to a particular host range, identifying some tangerines and their hybrids as susceptible. Peever et al. (2000) found a very aggressive pathogen to Minneola, Orlando, Sunburst and Nova hybrids. In this study, susceptibility to ACT toxin was observed in Nova, Sul da África, África do Sul, Ponkan and Murcott, which shows the fungal aggressiveness and the high vulnerability of these hybrids. Reis et al. (2007) also reported susceptibility to the pathogen in some genotypes of *C. deliciosa* and *C. temple*. Pegg (1966) reported the occurrence of *A. alternata* in *C. tangerine*. Souza et al. (2009) detected the susceptibility in plants derived from crosses from *C. reticulata, C. temple, C. tangerina, C. unshiu, C. sinensis and C. deliciosa*.

In this context, there are hybrids resistant to the fungus, as Thomas and Fremont, reported in this study. Kohmoto et al. (1991) also found resistance, but in King mandarins (*C. nobilis* Loureiro) and their hybrids Encore (*C. nobilis* Loureiro × *C. deliciosa* Ten.) and Kara-Kara (*C. unshiu* Marcov × *C. nobilis* Loureiro), while Souza et al. (2009) reported the possible resistance in four varieties of clementines, six varieties of tangerines, one tangelo, two tangerine hybrids, one tangor hybrid and two Satsuma hybrids.

Although the results between in vitro inoculation results (Table 3) and field observations were similar (Table 2), the number of genotypes showing disease symptoms on leaves was lower in the field assays, indicating inoculation is a more severe test of resistance. Similar results were observed by Azevedo et al. (2010), who also found high tolerance of the genotypes 1144-12, Ortanique [*C. reticulata* × *C. sinensis* (L.) Osbeck] and Cravo in seedlings (in vivo) and with detached leaves by in vitro inoculation. Vicent et al. (2003) observed resistance of Ellendale and Ortanique hybrids derived from crosses between *C. sinensis* × *C. reticulata* when they were inoculated with three isolates of *A. alternata*. Therefore, in addition to resistant genotypes Fremont and Thomas, Hansen, Nules, Cravo and Ortanique can also become new options for mandarin producers.

The results on seedlings inoculated with *A. alternata* revealed that 13 genotypes showed no disease symptoms (Table 3). Among the symptomatic genotypes in this study some were previously described as highly susceptible, such as África do Sul, Murcott, Caçulas (*C. clementina* Hort. ex Tan) and Ponkan.

Symptoms on young leaves of some of the representative genotypes after in vitro inoculation are shown (Figure 3). África do Sul, Page and Empress were highly susceptible as demonstrated in Table 3 whereas Cravo, Thomas and Fremont were highly resistant.

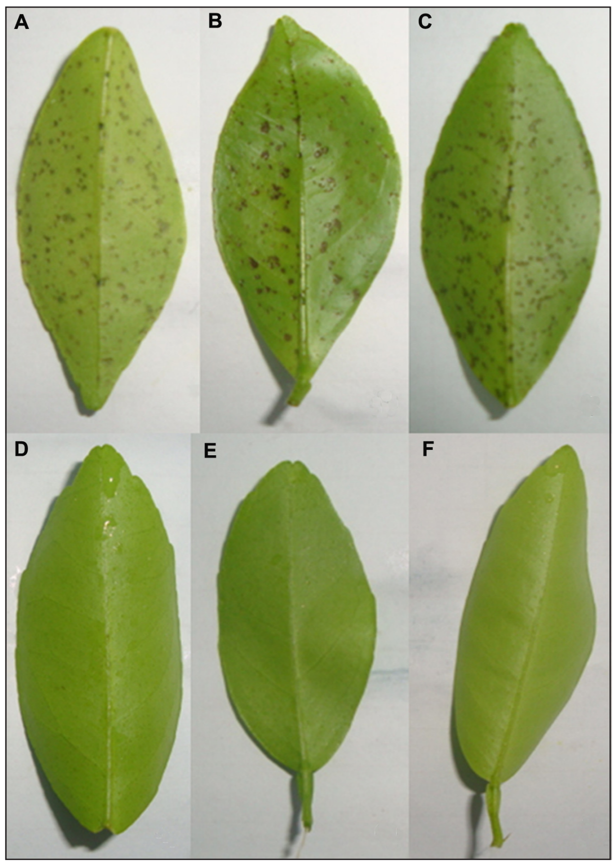

Figure 3 – Leaves of some of the tangerines and hybrids tested against *Alternaria alternata*, three days after in vitro inoculation: África do Sul tangerine (A); Page tangelo (B); Empress tangerine (C); Cravo tangerine (D); Thomas tangerine (E); Fremont tangerine (F).

Lesions on detached leaves (in vitro) were more severe than on leaves in plants (in vivo) (Table 3), similar to results obtained by Souza et al. (2009), where Daisy tangerine and Temple tangor × Daisy tangerine and Satsuma × Murcott4 hybrids had more severe symptoms in detached leaves than those on the plant or fruits. This can be explained by the fact that detached leaves have physiological changes that may affect their resistance level, making them more susceptible to *A. alternata* and other microorganisms, since detached seedling leaf loses its capacity to respond to the pathogen. As they reach physiological maturity, the seedling leaves become resistant to the pathogen, since the leaf cell lignification increases as they mature and acts as a barrier to prevent the fungal colonization (Baudoin and Eckert, 1985); the occurrence of antimicrobial secondary metabolite synthesis (phytoalexins) results in fungal and bacterial activity suppression (Broetto et al., 1999), the plant pre-existing compounds (phytoanticipins) act as antimicrobial compounds (Van Etten et al., 1994); oil glands become prominent (Pegg, 1966; Reis et al., 2006), and can occur the joint action between essential oils and herbal compounds, lowering the pathogen survival chance through its cell lyses (Chutia et al., 2009; Matasyoh et al., 2007). It is worth noting that the fungus is more likely to proliferate under controlled conditions (in vitro) by offering the best environment to the pathogen.

As Dancy mandarin is a parent of most hybrids and tangelos that are susceptible to Alternaria brown spot, some authors speculated that the susceptibility to this disease would be genetically inherited tangerine. Kohmoto et al. (1991) based in susceptibility of citrus varieties and hybrids of these suggested that susceptibility was inherited from the parent Dancy mandarin, as a dominant feature, and therefore the resistance of Alternaria brown spot recessive. To support the hypothesis that the resistance to *A. alternata* is controlled by a single recessive allele inherited from his female parent, reciprocal crosses between the resistant variety Clementine (ss) and susceptible Clementine × Minneola tangelo (Ss) show that the inheritance of the recessive allele was Clementine. A segregation ratio 1:1(resistant:susceptible), expected by the authors, was obtained from the backcross Clementine × Clementine × Minneola. However, when used as female parental Clementine was a distortion a 3:1 segregation ratio (resistant susceptible), suggesting the influence of cytoplasmic genes on offspring resistance (Dalkilic et al., 2005).

There was a good correlation between the results obtained in vitro and in vivo and both confirmed the results obtained in the field. Thus, some genotypes of the mandarin group have a higher tolerance to *A. alternata* and may, in the near future, be new options for citrus plantings.

As mentioned earlier, Thomas and Fremont proved to be resistant to *A. alternata* to both detached and attached leaves and fruits. Similarly, Reis et al. (2007) found genotypes such as Fremont, Encore and Fallglo {[(*C. reticulata* Blanco × (*C. paradisi* Macf. × *C. reticulata* Blanco)] × (*C. reticulata* × *C. sinensis* L. Osbeck)}, were resistant when subjected to in vivo and in vitro inoculation. Varietal resistance confirmation, as well attractive fruits with a pronounced flavor, raises the agronomic potential of Thomas and Fremont as options for the citrus market to replace Ponkan and Murcott. Pio et al. (2005) showed how promising these cultivars can be by describing the export potential of Thomas; fruit characteristics suitable to foreigner consumers, and Fremont with its peel and strong orange color fruit characteristics suitable for the fresh fruit market. However, Thomas and Fremont tangerines were resistant to *A. alternata*, whereas Cravo tangerine, Nules clementine and Ortanique tangor showed low susceptibility in the three regions.

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