Australian Journal of Crop Science AJCS

AJCS 13(10):1631-1637 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.10.p1849

Winter pruning: option for management against alternaria brown spot (*Alteraria alternata* f. sp. *citri*) in Honey Murcott tangor [*Citrus reticulata* Blanco x *C. sinensis* (L.) Osbeck]

Fernando Alves de Azevedo[1*], Thiago Fernando Milaneze[1], Patrícia Marluci da Conceição[2], Camilla de Andrade Pacheco[1], Rodrigo Martinelli[1], Marinês Bastianel[1]

[1]Centro de Citricultura Sylvio Moreira, Instituto Agronômico (IAC), 13490-70, Cordeirópólis, São Paulo State, Brazil
[2]Centro de Ciências Agrárias, Universidade Federal de São Carlos (UFSCar), 13600-970, Araras, São Paulo State, Brazil

***Corresponding author: fernando@ccsm.br**

Abstract

Alternaria brown spot (ABS), caused by *Alternaria alternata* f. sp. *citri*, is the main fungal disease plaguing tangerines. The main symptoms are falling leaf and fruit, resulting in the loss of production and quality. The initial symptoms in leaves are characterized by small brown or black spots, surrounded, or not, by yellowish halos. In the fruit, dark cortical patches appear that can easily break. In this study, the winter pruning of the plants was investigated to complementary control of ABS. The experiment was conducted in 10-year-old Honey Murcott tangor plants, where two treatments were applied: pruning in winter and no pruning, using a randomized block design with ten replicates. The treatment of winter pruning was performed before the flowering of the plants (September in Brazil), where the branches were cut close to the trunk, the thicker branches were removed. The chemical control was performed in both treatments. Evaluations of disease severity in fruit were performed using a diagrammatic scale; the disease incidence and area under the disease progress curve (AUDPC) were also calculated. The fruit drop and yield (t ha^{-1}) of the plants were quantified, and the temperature and humidity inside the canopy of the plants were measured. Winter pruning had a positive effect on ABS management since it provided a decrease in incidence, severity, and AUDPC values. Fruit fall was reduced, resulting in greater productivity for Honey Murcott tangor plants. The results confirmed the positive effect of winter pruning on the management of alternaria brown spot.

Keywords: *Alternaria alternata*, fungal disease, crop management, *Citrus reticulata.*
Abbreviations: (t ha^{-1})_Alternaria brown spot, (ABS)_area under the disease progress curve, (AUDPC)_Alternaria citrus toxin, (ACT)_tonnes per hectare.

Introduction

Alternaria brown spot (ABS), caused by the fungus *Alternaria alternata* f. sp. *citri* (Fr.) Keissl, and *huanglongbing* (*Candidatus* Liberibacter spp.) are the main phytosanitary diseases impacting tangerines (*Citrus reticulata* Blanco). Alternaria brown spot was first reported by Cobb in Emperor tangerine in Australia in 1903 (Kiely, 1964). In 1974, ABS was observed in Florida, infecting fruits and branches of Dancy tangerine (Whiteside, 1976). In 1989, it was reported in Israel (Soleil, 1991) and sequentially found in South Africa (Schutte et al., 1992), Cuba (Herrera, 1992), Colombia (Castro-Caicedo et al., 1994), Turkey (Canihos et al., 1997), Spain (Vincent et al., 2000), Italy (Bella et al., 2001), Argentina (Peres et al., 2003), Peru (Marín al., 2006) and China (Wang et al., 2010).

In Brazil, the main varieties used in commercial cultivation, Ponkan tangerine and Honey Murcott tangor [*C. reticulata* Blanco x *C. sinensis* (L) Osbeck], have shown high susceptibility to *A. alternata*, which has greatly damaged fruit production and quality for citrus (Michielin et al., 2016). According to Lopes et al. (2009), the infection in the fruit can occur from the drop of the petals (anthesis), and the fruits are susceptible until four months after flowering.

Because it is a saprophytic fungus, *Alternaria alternata* survives on dead tissues, branches and dry branches of the citrus canopy interior. Their asexual spores (conidia) are produced both in leaves and branches of the plant, as well as in leaves decomposing in the soil, but are not produced in the fruit. Conidia have a thick wall and are resistant to drying and other adverse conditions (Spósito et al., 2003). The symptoms of ABS appear in the fruit 24 to 48 hours after infection, and the ACT (Alternaria Citrus Toxin) toxin produced by the fungus is responsible for the necrotic areas of the attacked tissues (Kohmoto et al., 1993). A recent study has suggested the presence of toxin receptors in susceptible genotypes (Tsuge et al., 2012).

The control of ABS is based on the use of fungicides. Depending on the climate of the region and the susceptibility of the cultivated tangerine variety, numerous applications of product to control the disease are necessary to ensure the production of fruit with sufficient quality to sell in fruit markets (Michelin et al., 2016, Cuenca et al., 2016). As the spores are produced on dead branches, the winter pruning technique could serve as a means of prevention, since it acts according to the principles of eradication and regulation, practices that reduce the initial inoculum. Winter pruning also alters the microclimate in the plants and in the orchard. These principles are not limited to one group of diseases, but they act in a way that decreases the general incidence of diseases in the next cycle of production (Laranjeira et al., 2005). Winter pruning consists of the removal of all branches with pest-damage or diseased, dead or unproductive branches and should be performed to retain the healthiest part of the branch (Carvalho et al., 2005). Thus, it is imperative to test the use of complementary cultural practices to be implemented by citrus growers that help in the fight against ABS, improving the sustainability of production. Therefore, the aim of this work was to evaluate the efficiency of winter pruning in reducing the damage caused by *Alternaria alternata* in Honey Murcott tangor fruit.

Results and discussion

Temperature and humidity

Winter pruning provided an increase in the temperature inside the canopy of Honey Murcott tangor plants in all evaluations during the two seasons (Table 1). Pruning, as performed in this test, resulted in an opening of the canopy (Figure 1 B and D), facilitating the access of the light to the inside of the tree canopy, causing a rise in temperature. Temperatures between 20 and 27 °C are considered ideal for the occurrence of new infections of *Alternaria alternata* (Timmer et al., 2003), and when winter pruning was performed, the temperature rose above the favourable range for pathogen infection in January 2012 and March 2013. Syvertsen et al. (2003) described that the higher light intensity inside the plants affects the flowering, fruiting and colouring of the fruits, being an environmental factor responsible for providing energy for photosynthesis, being one of the main factors responsible for plant growth and fixation of fruits, in addition to contributing to maintain plant health. The relative humidity of the air inside the canopy was higher in the plants without pruning in the majority of the evaluated dates, except for November 2011 and January 2012 (Table 1). High humidities favour the development of fungal diseases such as alternaria brown spot. The microclimate inside the canopy can impact several diseases. For example, several authors have observed that the severity of *Zygophiala jamaicensis* is worse in unpruned apple trees due to greater humidity in the canopy. In addition, in the pruned plants, the penetration of spray mist is facilitated (Cooley et al., 1997).

Area under the disease progress curve (Severity and Incidence)

The data from the area under the disease progress curve for alternaria brown spot show that the use of winter pruning significantly reduced the severity and incidence of the disease in the two harvests evaluated (Figure 2A and B).

The use of winter pruning positively reflected the control of alternaria brown spot caused by *A. alternata*, since the Honey Murcott tangor fruit of the pruned plants presented a lower number of lesions when compared to those without pruning. According to Martelli (2011), winter pruning for removal of dry branches is important for the reduction of the inoculum source, so it contributes to the control of ABS, since *A. alternata* is an opportunistic fungus, that is, a species that can survive saprophytically in decaying or dead material. Contrary to the drastic pruning performed in this work, Martelli (2011) proposed a light pruning, also in Honey Murcott tangor plants, removing only the dried branches from inside of the crown, without opening the crown, and did not obtain significant results in reducing the severity of ABS. Timmer et al. (1998) reported that symptomatic materials play a key role as sources of inoculum in the subsequent ABS cycles, which is why sanitation measures, such as pruning, have resulted in improved levels of disease control. The incidence data, together with the disease severity data presented as an area under the disease progress curve, confirm the effectiveness of winter pruning in reducing the inoculum source in *A. alternata* in Honey Murcott tangor orchards. The increase in incidence is related to the continuous release of *A. alternata* spores associated with pathogen development in necrotic lesions in young tissues that are produced by the plant beginning at flowering (Timmer et al., 1998). Such behaviour in the host-pathogen interaction shows that the use of winter pruning helps the management of alternaria brown spot by reducing the presence of inoculum in the orchards, but it is necessary to use it in association with complementary practices, such as the application of copper fungicides, triazoles, estrubirulins, dithiocarbamates and the use of resistant varieties, to guarantee the protection of fruit throughout its development and maturation (Martelli, 2011).

Complementary trial

The severity of alternaria brown spot on the leaves of the seedlings introduced under the crown of the pruned plants was less than the severity of the seedlings inserted under the crown of the pruned plants (control) (Figure 3), which shows that there was a reduction in the source of *A. alternata*, which was removed from the pruned material.

In January 2013, the severity of the pruning treatments was 0.08%, increasing to 0.22% and reaching 0.18% in the beginning of March 2013. The seedlings placed inside the control plants showed a severity of 0.10%, 0.59%, 0.83% and 0.90%, respectively, on January 01, 2012, February 02 and 15 and March 03, 2013, differing statistically in the penultimate evaluation. It was verified that the pruning is an effective long-term measure, indicating a lower presence of *A. alternata* spores in the pruned plants due to the removal of the diseased material. However, it is important to emphasize that the exclusive use of pruning is insufficient to control the pathogen, making it necessary to practise an integrated disease control programme with systematic spraying with specific fungicides (Martelli, 2011).

Productive attributes

Winter pruning provided a significant reduction in the total number of fallen fruits per plant in the two-season evaluation (Figure 4A). In apple tree treatments, leaf pruning plus winter pruning (canopy opening) also resulted in a

Table 1. Temperature (°C) and humidity (%) inside the Honey Murcott tangor plants in pruning and no pruning treatments (2011/2012 and 2012/2013).

Treatements	Season 2011/2012			Season 2012/2013	
	Temperature (ºC)			Temperature (ºC)	
	Nov/11	Dec/11	Jan/12	Nov/12	Mar/13
Not pruned	32.7 b*	34.9 b	26.5 b	31.0 b	26.7 b
Winter pruned	33.5 a	36.6 a	27.1 a	32.1 a	28.0 a
CV	*3.12*	*2.71*	*1.77*	*0.56*	*1.94*
	Relative humidity (%)			Relative humidity (%)	
	nov/11	dec/11	jan/12	nov/12	mar/13
Testemunha	39.9 a	33.6 a	71.3 a	46.8 a	76.0 a
Poda	39.4 a	30.6 b	70.9 a	45.3 b	70.8 b
CV	*10.54*	*6.66*	*2.25*	*0.89*	*1.98*

* Averages followed by the same letter in the column do not differ from each other (Tukey P ≤ 005)

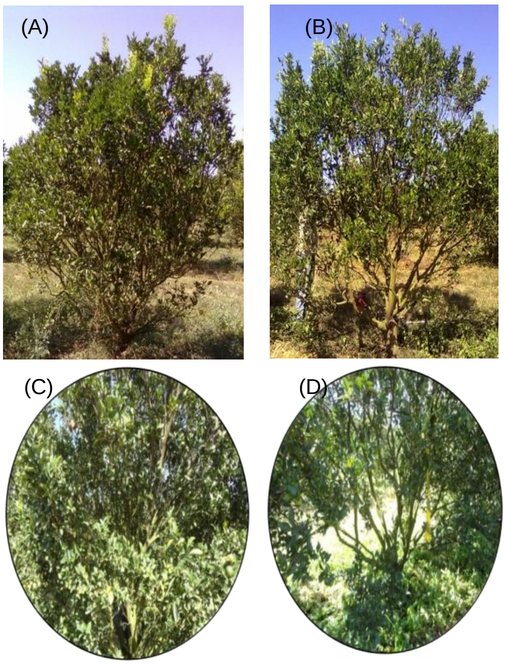

Fig 1. Plants of Honey Murcott tangor not pruned (A) and pruned (B) and view of the top of the canopy not pruned (C) and pruned (D) (2010).

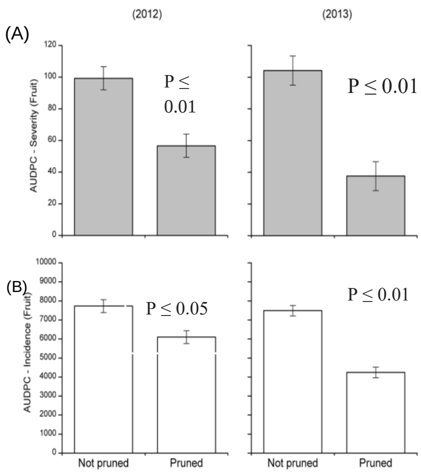

Fig 2. Area under the disease progress curve (AUDPC) for severity (A) and incidence (B) of alternaria brown spot on fruit of pruned plants and not pruned plants. The error bars represent the least significant difference (LSD; Tukey P ≤ 0.05).

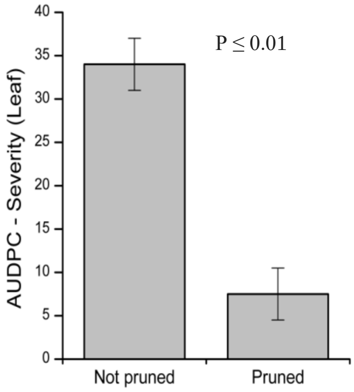

Fig 3. Area under the disease progress curve (AUDPC) for alternaria brown spot severity data on leaves of Honey Murcott tangor seedlings inserted inside the canopy of the pruned and not pruned plants (2012/2013) The error bars represent the least significant difference (LSD); Tukey P ≤ 0.05).

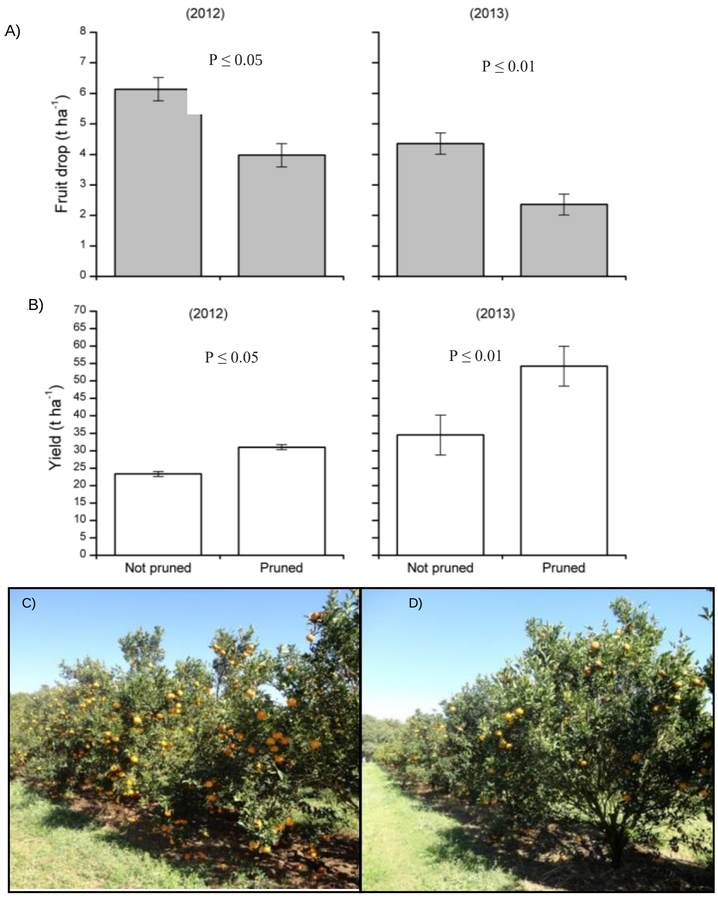

Fig.4. Fruit fall (A) and yield (B) of pruned (C) and not pruned plants (D) (2012-2013) The error bars represent the least significant difference (LSD; Tukey P ≤ 0.05).

lower incidence of apple wart (*Venturia inaequalis*), thus increasing spray efficiency (Holb, 2008).

Greater fruit drop was observed when the fruit were still green (data not shown), which is associated with the greater aggressiveness of the pathogen in young fruit (beginning of flowering). As the fruit develops, it gains not only in size but also in protection from fungus penetration. By increasing its protection, a decrease in fruit drop occurs, even with increasing severity over time. According to Lopes et al. (2009), the infection in the fruit can occur from the drop of the petals until their development, being the most susceptible until four months after the flowering.

According to Laranjeira et al. (2005), winter pruning can alter the microclimate of the orchard, reducing the incidence of diseases in the next production cycle. As the causative agent of ABS, *Alternaria alternata* has the characteristic of being able to settle in places with high humidity. The alteration of the microclimate inside the canopy is an alternative for the decrease of inoculum sources and, consequently, the number of fruits presenting symptoms, thus ensuring the retention of fruit. This is in agreement with the results obtained in this work, where winter pruning acted according to the principles of eradication and regulation, which are prevention practices that reduce the initial inoculum.

The productivity of pruned plants was higher than those not pruned in both crops (Figure 4 B, C and D). This superiority in the production is a reflection of a greater retention of fruit in pruned plants when compared with the control. The productive efficiency was higher in the second harvest compared to the first, presenting 0.67 kg fruit m^{-3} of canopy in the treatment with pruning. The retention of fruit in the pruned plants was approximately 19% higher than in the control.

According to Bevington (1980), one of the causes responsible for the lower productivity in closed plants (without pruning) is the auto shading of the trees, often caused by the use of the planting densification technique. Thus, the need for an appropriate vegetative control of the crown is essential, and pruning is widely used for this purpose.

By increasing the access of the light inside the crown of pruned plants, together with the evaluations of fallen fruit, severity and incidence of the disease, it was possible to prove the efficiency of the use of winter pruning/cleaning in reducing the inoculum source of *A. alternata*. This reduction of the inoculum present in the orchard causes a lower number of fruits with the presence of the pathogen, that is, fewer fruits will be lost (greater retention of fruit), thus increasing the productive parameters of pruned plants.

In Brazil, the main application of manual pruning by Brazilian citrus growers has been an auxiliary measure in pest and disease management programmes, especially those that develop in the interior of the canopy, such as rubella (Rossetti, 1995), melanose (Bassanezi, 2001; Gravena, 2005), variegated chlorosis of citrus (Lopes, 1999), black spot, *Guinardia citricarpa* (Kiely) and cankers such as *Xanthomonas axonopodis* pv. citri (Valterin) (Carvalho et al., 2005). Copper has been the basis of the control programme of *A. alternata* f. sp. *citri* in both Florida and Brazil, mainly because of its low cost and effectiveness (Colturato et al., 2009).

Honey Murcott tangor canopy pruning is not a widely used practice and can be incorporated into orchard management to facilitate culturing, aiding in disease control. With the present work, it is possible to introduce the pruning into the integrated management practices of citrus diseases, since this contributes to a greater efficiency of fungicide application, protecting several cultures against conidia infection (Amponsah et al., 2012) and reducing the source of inoculum of *Alternaria alternata*, which is of extreme importance for the integrated management of diseases.

Materials and Methods

Experimental design and plant materials

The trial was carried out between 2011 and 2013 in São Paulo State, Brazil, with 10-year-old Honey Murcott tangor plants grafted on rangpur lime (*C. limonia* Osbeck). Trees were planted with a spacing of 7 × 4 meters. Two treatments were conducted:

(i) winter pruning: canopy opening, with removal of dead, sick and crooked branches (Figure 1A and C);

(ii) without pruning (Figure 1B and D);

Each plot consisted of thirty plants distributed in three rows, with four plants being considered useful for evaluation purposes. The experimental design was a randomized complete block design with ten replications.

The treatment of winter pruning was performed before the flowering of the Honey Murcott tangor plants in September 2011 to promote an intense opening of the canopy of the plants (Figure 1 B-D). The branches were cut close to the trunk, the thicker branches were removed with the use of saw, and thin branches were removed with scissors. All the cut material was removed from the experimental area and burned. Subsequent to these operations, copper paste (copper oxychloride) was applied in the region of the cut to prevent the entry of pathogens. In 2012, also before the flowering of the plants, pruning maintenance was carried out, which consisted of the removal of thin branches.

In this experiment, eight fungicide sprays were utilized to control alternaria brown spot, with spraying initiated after anthesis (September) and continuing until April. Copper fungicides (copper oxychloride), triazoles (tebuconazole) and estrubirulines (pyraclostrobin) were rotated throughout the experimental area.

Evaluations

Temperature and humidity

The temperature and humidity inside the canopy of the plants were evaluated using digital hygrometers, which were installed approximately 1.5 metres above the soil on two plants in each plot. The evaluations were carried out in January, November and December in the 2011/2012 and 2012/2013 seasons.

Severity, incidence and area under disease progress curve (AUDPC)

The severity of the symptoms of alternaria brown spot, due to the infection of the *A. alternata* fungus, was quantified using a specific diagrammatic scale (Renaud et al., 2008), analysing 200 fruits per plot. This scale ranged from '0' to '6'. That is, '0' indicated there was no lesion on the surface of the fruit, and '1' to '6' corresponded, respectively, to injured areas of 0.1%; 1%; 2.5%; 5%; 11% and 25% (severity). In parallel, the incidence of the disease was also obtained via determining the percentage of fruit affected by *Alternaria alternata*.

Using the disease severity and incidence data, the area under the disease progress curve (AUDPC), which is expressed by plotting the proportion of disease in percent, was calculated by the formula described by Shaner and Finney (1977):

AACPD = Σn-1 [(Yi + 1 + Yi) / 2] * [(Ti + 1-Ti)],

Where; n is the number of observations/evaluations, Yi is the proportion of disease in the ith observation/evaluation, and Ti is the time in days in the ith observation/evaluation.

The evaluations were initiated in October 2011 (28/10/2011, 25/11/2011, 19/12/2011 and 21/03/2012) in the first season and in November 2012 (30/11/2012, 11/01/2013, 01/02/2013 and 01/03/2013) in the second.

Complementary test

Honey Murcott tangor seedlings were inserted into the canopy of the plants with and without pruning during the 2012/2013 season. The objective of this study was to verify the reduction of *A. alternata* inoculum source promoted by pruning in the previous harvest, by means of evaluations carried out on new shoots of the seedlings.

Three branches were evaluated per seedling, and each seedling represented one plot. The pots containing the seedlings were placed near the trunk of the trees. Ten seedlings were used for each treatment. The pots of the seedlings were introduced in the field on January 04, 2013, under the canopy of the plants and were evaluated on January 18, February 02 and 15, and March 01, 2013.

To evaluate the lesions of *Alternaria alternata* in the leaves of Honey Murcott tangor plants, a diagrammatic scale developed by Martelli et al. (2016) was used. This scale presented symptom levels in ten properly illustrated notes, where "0" represents a symptom-free leaf and grades "1" to "9" represent 0.3, 3.5, 8, 15, 34, 61, 80, 90 and 97 percent of leaf area covered by spots caused by *Alternaria alternata*.

Yield attributes

The number of fallen fruits was evaluated in the plots through the collection and counting of the number of fruits that had fallen from the plants due to the occurrence of alternaria brown spot. Fruits that had fallen as a result of pests and/or other physical injuries (cracks, damage from agricultural implements, etc.) were discarded. These evaluations were carried out monthly, from September until the harvest, and later added to the calculation of the loss of productivity.

For the calculation of productivity, all the fruit from the plot were harvested and weighed to obtain the production per plant and tons of fruits per hectare. The productive efficiency (kg of fruit per m^3 of canopy) was also estimated. To calculate the productive efficiency, the volume of the crown of the plants was determined using the formula proposed by Mendel (1956): V = 2/3 π R2 H, where V represents the volume, R the radius of the crown, and H is the height of the plant. For this, the height and radius of the evaluated plants were measured.

Statistical analysis

The mean values obtained in the evaluations were submitted to analysis of variance (F test, $p \leq 0.05$) and later compared by a Tukey test ($p \leq 0.05$). All tests were performed using the software R (v 3.4.3) (R Development Core Team 2016).

Conclusions

The application of winter pruning reduces the severity and incidence of symptoms of alternaria brown spot, caused by the fungus *Alternaria alternata*, on Honey Murcott tangor fruit. This technique also led to a decrease in fruit drop and an increase in plant productivity.

Acknowledgments

We thank the National Council for Scientific and Technological Development - CNPq (process: 472463/2009-8), and the São Paulo Research Foundation - FAPESP (process: 2006/55585-0; 2008/55118-8) for financial support and for granted scholarships to FA Azevedo (process: CNPq 313813/2017-5).

References

Amponsah NT, Jones E, Ridgway HJ, Jaspers M (2012) Evaluation of fungicides for the management of *Botryosphaeria* dieback diseases of grapevines. Pest Manag Sci. 68 (5): 676-683.

Bevington KR (1980) Response of Valencia orange trees in Australia to hedging and topping. P Am Soc Hort Sci. 93: 65-66.

Bassanezi RB (2001) Aspectos da leprose dos citros. Fitopatol Bras. 26(1): 246-247.

Bella P, Guarino CR, Catara A (2001) Severe infections of *Alternaria* spp on a mandarin hybrid. J Plant Pathol. 83: 231.

Canihos Y, Erkilic A, TImmer LW (1997) First reporter of Alternaria Brown Spot of Minneola tangelo in Turkey. Plant Dis. 81(10): 1214.

Carvalho JEB, Neves CSVJ, Menegucci JLP, Silva JAA (2005) Práticas Culturais. In: Mattos JR D, De Negri J, Pio RM, Pompeu JR J (Eds) Citros: Instituto Agronômico e Fundag. 16: 451- 475.

Castro-Caicedo BL, Leguizamon C, Lopez RJA (1994) La mancha foliar de los cítricos em la zona cafetera, Avances Técnicos Cenicafé. 198: 26.

Colturato AB, Paulossi T, Venâncio WS, Furtado EL (2009) Efficiency and cost of chemical control of alternaria brown spot. Summa Phytopathology. 35(3): 210-215.

Cooley DR, Gamble JW, Wesley R (1997) Summer pruning as a method for reducing flyspeck disease on apple fruit. Plant Dis. 81(10): 1123-1126.

Cuenca J, Aleza P, Garcia-Lor A, Ollitrault P, Navarro L (2016) Fine mapping for identification of citrus alternaria brown spot candidate resistance genes and development of new snp markers for marker-assisted selection. Front Plant Sc. 7: 1948

Gravena S (2005) Manual Prático de Manejo Ecológico de Pragas de Citros. Jaboticabal: Gravena. 1-372.

Herrera L (1992) La mancha parda de los cítricos en Cuba. Levante Agrícola Valência 31(229): 49-50.

Holb IJ (2008) Timing of first and final sprays against apple scab combined with leaf removal and pruning in organic apple production. Crop Prot. 27(3-5): 814-822.

Kiely TB (1964) Brown spot of Emperror mandarin. Agric Gaz. 2: 854-856.

Kohmoto K, Itoh Y, Shimomura N, Kondoh Y, Otani H, Kodama M, Nishimura S, Nakatsuka S (1993) Isolation and biological activities of two host-specific toxins from the tangerine pathotype of *Alternaria alternata*. Phytopathology. 83: 495-502.

Laranjeira FF, Feichtenberger E, Bassanezi RB, Spósito MB (2005) Manejo integrado de doenças dos citros In: Mattos Jr D De Negri JD Pio RM Pompeu Jr J (Eds) Citros. Campinas: Instituto Agronômico e Fundag. 21: 631- 652.

Lopes EB, Albuquerque IC, Araújo E (2009) Mancha-marrom-de-alternaria: uma grave doença nos pomares de tangerina da Paraíba. Tecnol Ciên Agropec. 3(3): 23-27.

Lopes JRS (1999) Estudos com vetores de *Xylella fastidiosa* e implicações no manejo da clorose variegada dos citros. Laranja. 20(2): 329-344.

Marín JE, Fernández HS, Peres NA, Andrew M, Peever TL, Timmer LW (2006) First report of Alternaria Brown spot of citrus caused by *Alternaria alternata* in Peru. Phytopathology. 90(5): 686.

Martelli IB (2011) Práticas culturais no manejo de mancha marrom de alternária. (Dissertação de Mestrado). 41p.

Martelli IB, Pacheco CA, Bastianel, M, Schinor EH, Conceição PM, Azevedo FA (2016) Diagrammatic scale for assessing foliar symptoms of alternaria brown spot in citrus. Agron Sc Biotech. 2: 56-61.

Michielin THV, Cristofani-Yaly M, Schinor EH, Azevedo FA, BAstianel M (2016) Reação de híbridos de citros à inoculação com *Alternaria alternata*. Summa Phytopathology. 42(4): 313-320

Mendel K (1956) Rootstock-scion relationships in Shamouti trees on light soil. Ktavim. 6: 35-60.

Peres NA, Agostini JP, Timmer LW (2003) Outbreakers of alternaria brown spot of citrus in Brazil and Argentine Plant Dis. 81(6): 750 2003.

Renaud MAS, Amorin L, Lourenço AS, Spósito MB (2008) Escala Diagramática para avaliação da Mancha Marrom de Alternária de citros. Summa Phytophatol. 34(3): 270-271.

Rossetti VA (1995) Leprose dos citros no Brasil In: Oliveira CAL, Donadio LC. Leprose dos Citros. Jaboticabal: FUNEP. 1-12.

Schutte GC, Lesar KH, Pelser PT, Swart SH (1992) The use of tebuconazole for the control of *Alternaria alternata* on “Minneola” tangelos and its potencial to control postharvest decay when applied as a preharvest spray. In: International Citros Congres. 3: 1070-1074.

Shaner G, Finney RE (1977) The effects of nitrogen fertilization on the expression of slow mild wing in knox wheat. Phytopathology. 67: 1051-1055.

Spósito MB, Feichtenberger E, Pio RM, Castro JL, Renaud MSA (2003) Ocorrência de mancha-marrom de Alternária em diferentes genótipos de citros nos Estados de Minas Gerais São Paulo e Rio Grande do Sul. Fitopatol Bras. 28: 231.

Timmer LW, Peever TL, Solel Z, Akimitsu K (2003) Alternaria diseases of citrus-novel pathosystems. Phytopathology. 42: 3-16.

Timmer LW, Solel Z, Gottwald TR, Ibañez AM, Zitko SE (1998) Environmental factors affecting production release and field populations of conidia of *Alternaria alternata* the cause of brown spot of citrus. Phytopathology. 88(11): 1218-1223.

Syvertsen JP, Goñi C, Otero A (2003) Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. Tree Physiol. 23(13): 899-906.

Soleil Z (1991) Alternaria brown spot on Minneola tangelos in Israel. Plant Pathol. 40:145-147.

Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M, Akagi Y (2012) Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. FEMS Microbiol Rev. 37(1): 44–66.

Vincent A, Armengol J, Sales R, Garcia-Jimenez J (2000) First report of alternaria brown spot of citrus in Spain. Plant Dis. 84(9): 1044.

Wang XF, Li ZA, Tang KZ, Zhou CY, Yi L (2010) First report of Alternaria brown spot of citrus caused by *Alternaria alternata* in Yunnan province China. Plant Dis. 94(3): 375.

Whiteside JO (1976) A newly recorded Alternaria induced Brown Spot disease on Dancy tangerines in Florida. Plant Dis. Rep. 60: 326-329.