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Integrated management approaches to control Alternaria Brown Spot (ABS) on Citrus

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Abstract

Since the excessive use of fungicides which was a non-environmentally friendly, the use of integrated management, based on agronomic practices such as pruning, spacing and minimize fungicide spray program, seem essential. Two field experiments were conducted successively at 2013/2014 and 2014/2015 in a Citrus orchard located in Beni Khalled (Cap Bon region, Tunisia) to the management of Alternaria Brown Spot. Three experimental plots (trees ordinary pruned; spaced and pruning and only treated with fungicides). All trees were treated with iprodione (March, April, June), copper sulfate (May), and mancozeb (September, October). Wider tree spacing and pruning associated with fungicide helped to reduce disease incidence on leaves and fruits. During both the spring and autumn flushes, ABS disease severity on leaves was reduced relatively for both cultivars in spring flush; the infected leaves number was reduced in wide trees spacing on Minneola plots and ordinary pruning on Fortune during autumn flush. The plot which was ordinary pruned showed an augmentation, up to 20-36%, of affected fruits and 10-13% for both cultivars with the application of tree spacing. A lower percentage (below 20%) of infected fruits recording the scale 1 and 2, was detected on trees ordinary pruned and spaced, also, on fruits, the disease severity index was lower for Minneola (10.6%) and Fortune (7.1%). The results obtained in this study demonstrated that combination of cultural practices such as pruning + spacing and fungicide spray could be an efficient integrated management of ABS in Tunisian Citrus orchards.

Keywords: *Alternaria alternata*, chemical control, citrus, pruning, spacing.

Introduction

In Tunisia, Alternaria Brown Spot (ABS) was first observed in 2008 on many citrus growing regions causing yield losses on susceptible cultivars. This disease is a limited factor some mandarin cultivars in semiarid citrus producing regions[1]. This disease was appeared on Emperor mandarin cultivar at 1903 in Australia[2]. Then, it spread to be detected in the USA[3], and now in other citrus regions worldwide[5-9]. The principal agent identified, was at first, *Alternaria citri* Ellis and Pierce[2], later *to A. alternata*[4]. ABS pathogen infection was accomplished with specific toxins to his host *Citrus reticula*[10]. ABS disease symptoms were black necrotic lesions on young leaves[11] fruits and twigs. On leaves and twigs, the lesions may develop along the veins due to the spread of the host-selective ACT-toxin[12,13], resulting in leaf drop and twig dieback. Fruit infection caused by *Alternaria* spp., vary from necrotic brownish spots to sunken lesions, reducing quality[14].

In fact, severe epidemics were detected in humid areas and semi-arid regions which were related with different environmental factors studied in Florida and Spain. Tangerine (*Citrus reticulata* Blanco) and hybrids cultivated on the major Citrus region in Tunisia revealed a severe outbreak of ABS in 2008.

The disease was first appeared at April till June when air temperatures were comprised from 15 to 27°C. All lemon and oranges cultivars were not susceptible, but Lane Late and Maltaise were less susceptible[15]. For *Alternaria* sp., the infection presents the critical factor of disease development, however, air temperature and wetness duration generated the infection process[16]. Rainfall elicited the release of conidia[1,17]. The citrus leaves infection triggered by an optimal thermal of 23 -27°C and a minimum leaf wetness of 8-12h. The lesions appeared 24 to 48h after a short infection initiation[11].

The lesions appeared continuously on all tree's organs (leaves, twigs and flower) until fruits maturity[18]. Once it penetrates the host, the pathogen invades the susceptible organs, which makes impossible to eradicate it by chemical measures and/or cultural practices alone, although, those methods were very common to reduce the ABS incidence[19]. Also, the chemical control and sprays must be used to protect susceptible citrus varieties during the critical periods of infection. Four to ten fungicide spray frequencies which depend on the climate and cultivar susceptibilities are sufficient to produce a good quality of fruits[20]. Copper products, mancozeb and chlorothalonil reported to be efficient against ABS[19, 21].

In Tunisia, little information about ABS control is available, due to the few registered active ingredients for Citrus. In view of the erratic results achieved for ABS control using only fungicides. A field experiment was undertaken to evaluate an integrated strategy combining two methods for the management of ABS in Tunisia.

Materials and methods

Study site: Two Citrus orchards located in the Technical Citrus Center in the Cap-Bon region of Tunisia with semi-arid bioclimatic stage were used. These two experimental plots (1 ha each) showed typical symptoms of ABS. Citrus trees were planted 4x5 m spacing since 1998 with Mandarin hybrids cultivars such as Minneola and Fortune. They were conducted at the same growing season consecutively (March to December) at 2013/2014 and at 2014/2015.

Meteorological databases: Environmental data (temperature and rainfall) from an automatic weather station (Station CTA, Sernum. 00000C48, iMetos 2) located in the two experimental plots.

Experimental design: The experimental trial was conducted using a randomized complete block design. Each plot (orchard) was divided into three sub-plots (cultural practices). The cultural practices were performed as follow, trees receiving an ordinary pruning, trees receiving the same pruning, but with spacing to get 8x5m, and trees receiving no pruning but treated with fungicides cited in Table-1 as negative control. Four fungicides confirmed their efficiency against ABS in field trial during 2010 to 2011 (unpublished data) was used in the assays. In total seven fungicide sprays were applied (S1 to S7); which, four treatments were performed during spring flushes from 10 March to 15 June, followed by two autumn treatments applied on 20 September 2013. Iprodione was applied three times (S1: 10 March, S2: April and S3: June at 2013/2014), with copper sulfate (S4:10 May 2013), and with mancozeb (S5:20 September and S6:07 October 2013). At the second year 2014/ 2015 only one additional treatment was applied with chlorotalonil mixed with copper sulfate (S7:15 May 2014).

The trees of the two experimental plots were pruned after harvest at 15 April (2013/2014) and at 15 March (2014/2015). Two pruning types were accomplished by removing the dead, dying, diseased, weakly attached and low-vigor branches from the tree crown and secondly a proper thinning which opens the foliage to reduce weight on heavy limbs, and retain the tree's natural shape. Each subplot contained ten trees and data were obtained from only eight, fruits were harvested randomly, from each of the four cardinal sides of the tree, with a total of fifty fruit per tree and treatment, registered at the end of the experiment.

Integrated management efficacy measurements: Disease severity on harvested fruits was evaluated at the end of growing season and rated using a scale from 0-2 as follows: 0=no lesions; 1=between 1 to 5% of lesions; 2=> 5% of lesions. Results were expressed as the percentage of lesion on fruits per category, while disease incidence was recorded as the percentage of affected fruits.

During spring and autumn flushes, assessment was performed by picking 100 shoots from 4 cardinal points of the trees per plot, on which the disease incidence on leaves was rated on a scale as follow 0 = no symptoms of leaves lesions; 1 = between 1 and 2 of leaves lesions; 2 = between 3 and 5 leaves of lesions; 3 = between 6 and 10 of leaves lesions; 4 = between 11 and 15 of leaves lesions; 5 = >15 of leaves lesions [23]. A disease severity index on leaves or fruits was estimated by calculated the percentage of affected leaves or fruits and number of lesions per leaves or fruits and number of lesions per leaves or fruits using an index[24]: $(\sum vn)/(NV)\times 100$; which v: number of fruits or leaves affected, n: rate scale 0-2 (fruits) or 1-5 (leaves), and NV: total number of leaves or fruits infested and healthy for the two varieties Fortune and Minneola.

Statistical analysis: The obtained data for each treatment were expressed by means and standard errors and analyzed according to General Linear Model using SPSS 20.0 version (SPSS Inc., Chicago, IL, USA). All the variables were analyzed with Waller-Duncan-Multiple comparison test based on a t statistic.

Table-1: Active ingredient, product name and registered concentration of fungicides applied against citrus brown spot at 2013/2014 and 2014/2015 experiments carried out in Cap Bon region, Tunisia.

Chemical Group	Active ingredient (a.i.)	Product name	Formulation	Registered concentration
Inorganic	Copper sulfate	Bouillie Bordelaise	Wet table powder	300g/100l
Dicarboximide	Iprodione	Rovral	Wet table powder	100g/100l
Dithiocarbamates	Mancozebe	Detan M45	Wet table powder	250g/100l
Chloronitrile (Phthalonitrile)	Chlorothalonil	Banco plus	Wet table powder	100g/100l

Results and discussion

Meteorological databases: ABS symptoms were observed mainly during spring and autumn and the pathogen was identified as *Alternaria alternata* (Figure-1). In fact, favorable conditions for occurred in spring (April) which is a rainfall season coinciding with the presence of susceptible shoots with 5.2mm in summer (July) and 1.2 to 1.4mm in autumn (October). Temperatures were between 17-19°C in spring and 20-24°C in autumn which are appropriate for the disease development.

Leaf wetness duration was alternated between 7-10h in spring with temperatures values registered were 15.7-17.8°C; 10 to 13h in summer (24.7 to 25.3°C) and 8 to 15h in autumn (20.6-21.5°C) (Figure-2,3). Rainfall occurred mostly in spring (58%) and autumn (52%). In total, 427.2mm of rainfall were recorded at 2013/2014 and 540.8mm at 2014/2015. Rainfall occurred in April was less than 40 mm (37.8 and 36.6 mm in 2013/2014 and 2014/2015, respectively) while it reached 44.2 mm in October 2013 and 70.2mm in October 2014 (Figure-2). Also, rainfall months were low but important in March 2013 and February 2014 with values of 139 and 132mm, respectively (Figure-3).

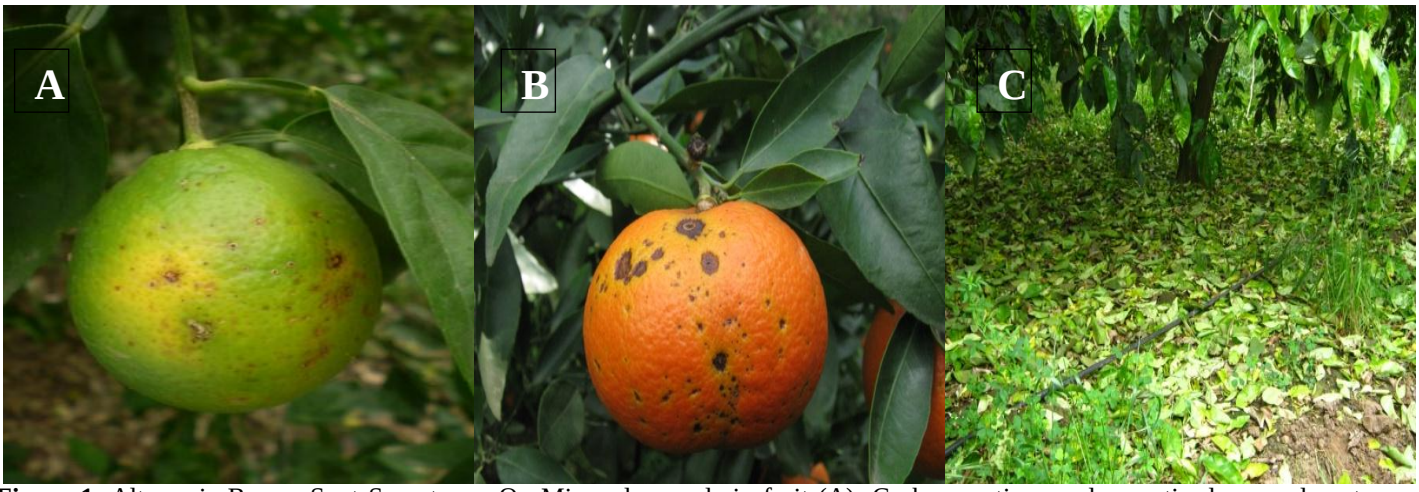

Figure-1: Alternaria Brown Spot Symptoms; On Minneola mandarin fruit (A): Corky eruptions and necrotic depressed spots on Fortune mandarin fruit, (B): necrotic spots between 1 to 10mm in diameter on Fortune mandarin leaves, (C): necrosis and severe defoliation of Citrus trees.

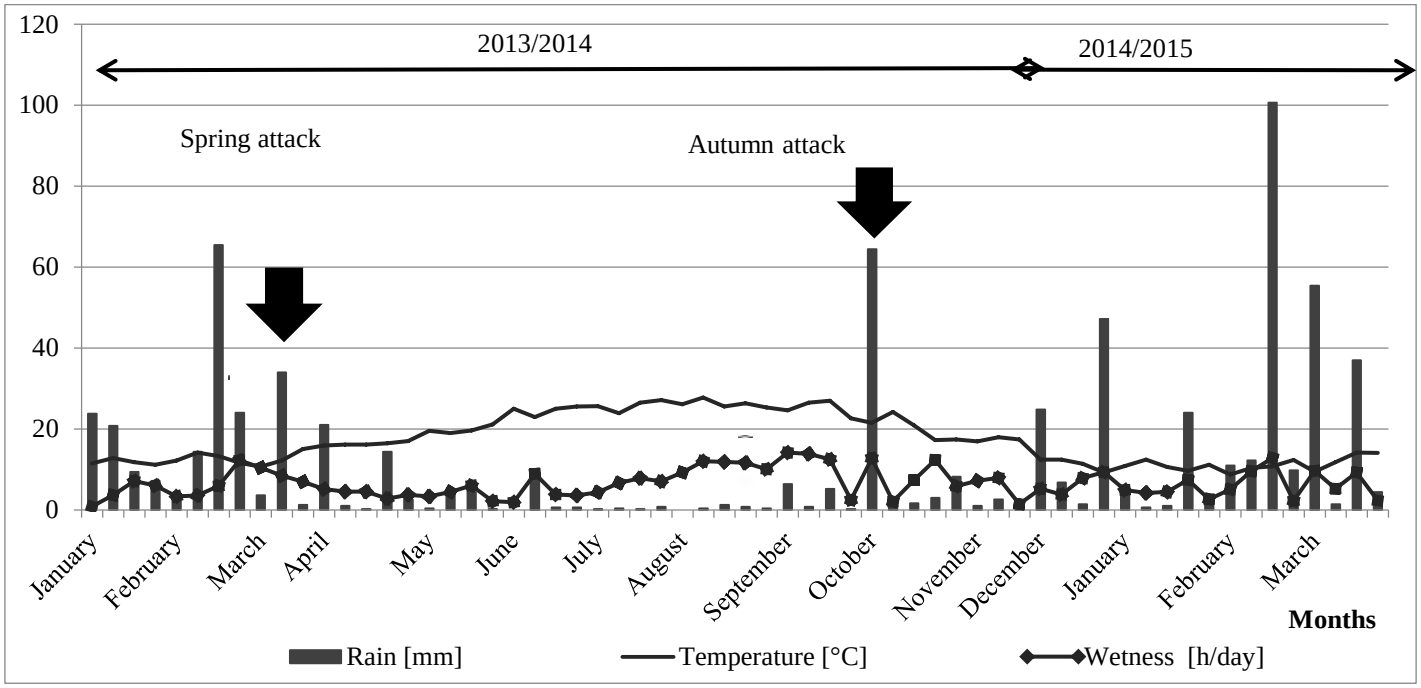

Figure-2: Daily values of rainfall (mm), temperature (°C) and average wetness duration (h) measured at two growing season 2013/2014 and 2014/2015 on Citrus orchards.

Integrated management efficacy measurements: At 2013/2014, pruning was applied after spring flush and the first fungicide application was on 15 April. However, at 2014/2015, pruning was performed one month earlier (March). For ordinary pruning on Minneola cultivar plot, early pruning showed an early spring flush and revealed a severe infection on leaves followed by defoliation and desiccation of shoots and with disease severity values of 71.5%. In the other hand, for Fortune cultivar plot, values were decreased with 49.5%. Concerning the experimental plot on both growing season with treatments including pruning and wider tree spacing, the infected shoots and symptomatic leaves per shoot were reduced for both cultivars in autumn flush with 17%. Despite the efficacy of fungicides *in vitro*, trees in the control plot, registered, in spring flush for Minneola and Fortune, the highest disease severity values with 95.6 and 89%, respectively. While, in autumn flush values were less than 30% (Table-2). The percentage of fruits affected by ABS was relatively low, but the difference in diseases classes among treatments was statistically significant ($P<0.05$). In Minneola cultivar with ordinary pruning, disease incidence and severity were relatively important about 34% in comparison with control (about 43%). Although, disease severity data, in plots pruning and wider tree spacing, was only about 10%. In this experiment, pruning and wider tree spacing were the most effective treatment with 86 and 3.5% of fruits in scale rate 0 and 2, respectively, compared to 29% in the control with scale rate 2. In Fortune cultivar, disease severity was reduced to 17.5 and 7.1% in plots with pruning alone or combined with wider tree spacing, respectively, compared with 32.8% (control). Once more, pruning combined with wider tree spacing showed efficiency on treated Fortune with the highest percentage of healthy fruits (89.3%). The control (only fungicides) gave a substantial disease control with a value of 61.7% of healthy fruits (Table-3).

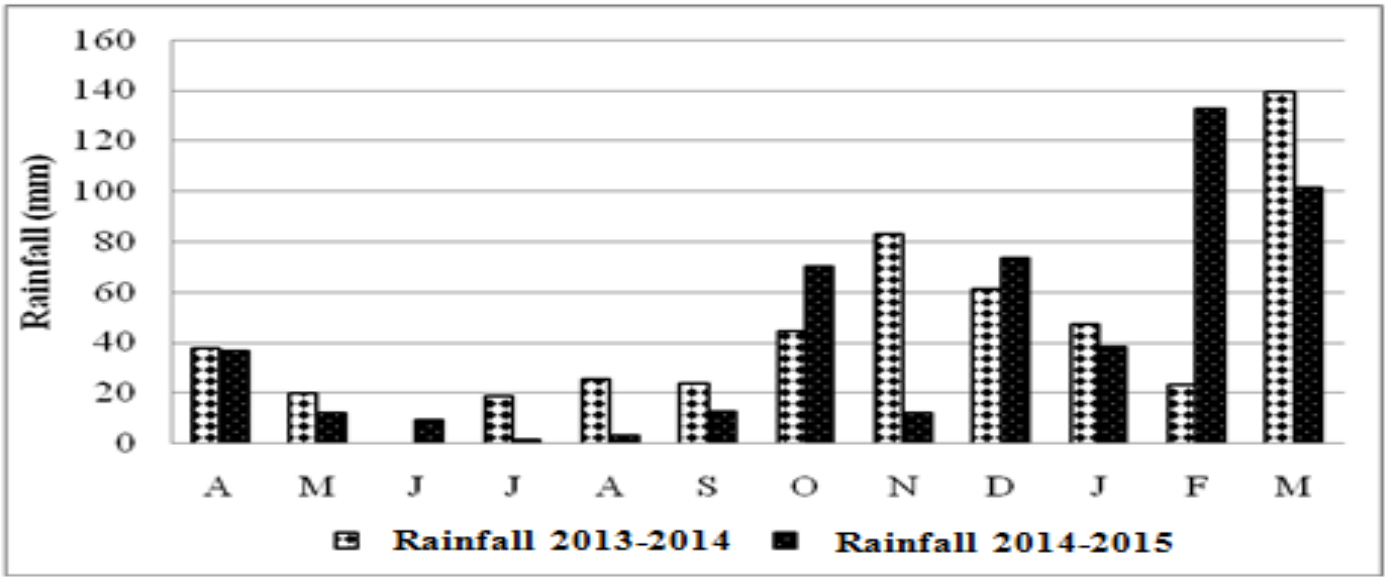

Figure-3: Monthly rainfall (mm) values at two growing season 2013/2014 and 2014/2015 on Citrus orchards; 12 months: A: April, M: March, J: June, J: July, A: August, S: September, O: October, N: November, D: December 2013/2014 and J: January, F: February, M: March 2014/2015.

Table-2: Disease severity of *Alternaria alternata* at the end of the two experiments registered on Minneola and Fortune leaves (%) at spring and autumn flushes; Means± standard error of 100 shoots per tree (8 trees per plot).

	Minneola		Fortune	
	Spring flush[A]	Autumn flush[A]	Spring flush[A]	Autumn flush[A]
Ordinary Pruning	71.50±0.07b[B]	24.80±0.09b	49.50±0.08b	16.40±0.09b
Pruning +spacing	54.10±0.10c	16.00±0.06c	47.10±0.07b	13.20±0.04c
Control	95.60±0.070a	64.20±.0.13a	89.00±0.080a	59.30±0.10a

[A]Disease severity rated on scale 0-5 count as individual lesion per infected leaf and rated as percentage of affected leave on shoot.
[B]± Standard error; according to Waller Duncan's Test, values followed by same letters weren't significantly different at $P\leq 0.05$.

Table-3: Disease incidence, severity index and percentage of infested fruits on on Minneola and Fortune fruits at the end of the two experiments; Means ± standard error of 50 fruits per tree (8 trees per plot), evaluated at December 2014.

Treatments		Infested fruit[A] (%)			Incidence[B] (%)	Severity[C] (%)
		0	1	2		
Minneola	Ordinary pruning	63.50±0.09b[D]	24.00±0.06a	12.40±0.05b	36.50±0.09b	32.90±0.08b
	Pruning+spacing	86.80±0.04a	9.60±0.06c	3.50±0.04c	13.20±0.04c	10.60±0.04c
	Control	53.30±0.08c	17.70±0.05b	29.00±0.07a	46.70±0.08a	40.90±0.08a
Fortune	Ordinary pruning	79.10±0.08b	12.90±0.04b	8.10±0.04b	20.80±0.08b	17.50±.0.10b
	Pruning+spacing	89.30±0.04a	8.50±0.06c	2.10±0.04c	10.70±0.04c	7.10±0.06c
	Control	61.70±0.09c	24.00±0.06a	14.30±0.05a	38.10±0.09a	32.80±0.06a

[A] Incidence rated as 0, 1 and 2. [B] Severity was rated using a 0–2 scale, based on percentage of affected fruit and as individual lesion as follows: 0= no lesions; 1= 1–5 lesions; 2= <5 lesions. [C] The results were expressed as percentage of fruits per category. Scale 0 provides the highest income, scale 1 fruits can be sold in the local market, scale 2 form unmarketable fruit. [D]± Standard error; according to Waller Duncan's Test, values followed by same letters weren't significantly different at P≤0.05

Discussion: The effect of environmental factors on citrus ABS in field were not yet investigated in Tunisia. ABS infection favorable conditions in this study, occurred in spring (April) which is a rainfall season coinciding with the presence of susceptible shoots. However, *Alternaria* spp., are considered as flexible pathogens that can grow under high rainfall conditions and relatively arid areas[25]. It was revealed that *A. alternata* conidial sporulation was the highest on mature leaves moistened at 100% relative humidity for 24h[18]. It was concluded that the lack of rain during the summer seems to limit the presence of the canopy wetness necessary for infection[21]. Additionally, it was reported that ABS infections occurred mainly in spring and in autumn[17]. The intensive utilization of site-specific fungicides causes the development of fungicide resistance[19,26].

Despite numerous chemical treatments applied to ensure fruits coverage at the infection critical period, the number of affected fruits was very high. In fact, most of the fruits have shown symptoms of the disease and so many fruits fell into the highest disease class, values comprised between 22.1% in August and 33.2% in December for the cultivar Minneola. Although, a slight decrease of asymptomatic fruits from 36% in August to 29% in December 2011 were noticed.

The onset and progress of the disease symptoms were related to the climatic condition and the phonological stage of the tree (leaf and fruit), which also explain some variability between the cultivars. Field experiment carried out during several seasons, demonstrated the effectiveness of some fungicides to control ABS by increasing the number of asymptomatic fruits, except the number of sprays was very high to provide good outcomes[19]. This work presented cultural practices in orchards, as new approaches to control ABS of citrus in Tunisia, which improve ventilation and prevent the growth of lush foliage. In this investigation, the treatment (ordinary pruning and pruning + spacing) proved their effectiveness to reduce the incidence and severity of ABS on cultivars Minneola and Fortune in this investigation. Indeed, it have demonstrated that Fortune subjected only to pruning increased infected leaves and twigs up to 15-30%, also, a high percentage of infected fruits (> 60%) in all treatments (only pruning, intercropping and no pruning + thinning) were noted[27]. Even with this increased number of sprays, control of ABS is often incomplete.

The possible mechanisms action of hexanoic acid-primed Fortune mandarin defense to *A. alternata* was demonstrated[28].

The origin of resistance and susceptibility of citrus cultivars to ABS were poorly investigated have been poorly investigated. The characterization of ABS resistance in 235 citrus population hybrids, which, only 30% showing disease symptoms on detached leaves, and 70% were asymptomatic[29]. Cultural practices can reduce ABS severity index in Citrus orchard. However, several fungicides sprays are also recommended for ABS control.

Conclusion

The collected data confirms the difficulties of ABS management because of the pathogen germination speed and infection that creates an obstacle to ensure a proper fruits coverage, because of the short incubation period lesions appearance and secondly because of the curative fungicides. The efficacy of ABS control program depends on synchronization between the fungicide sprays and period of infection.

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