**ARTIGOS / *ARTICLES***

# Susceptibility of Fruits of the 'Valência' and 'Natal' Sweet Orange Varieties to *Guignardia citricarpa* and the Influence of the Coexistence of Healthy and Symptomatic Fruits

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**ABSTRACT**

The importance of the presence of symptomatic fruits with citrus black symptoms (CBS) on the disease severity level in subsequent crop production was assessed in an orchard planted with Valência and Natal sweet orange varieties. Additionally, the period of susceptibility of the fruits of these varieties was evaluated.

Fruits were covered with paper bags at the stage of 75% fallen petals and were then exposed to natural infection at weekly intervals, from October 2000 to April 2001. This process was carried out in plants where the fruits from the previous harvest had been picked as well as in plants where fruits remained until natural drop. The evaluation of disease severity used a scale that varied from 0 (absence of symptoms) to 6 (severe symptoms). It was observed that, for the Valência and Natal varieties, conidia of *Phyllosticta citricarpa* that had formed on the lesions of fruits from the previous harvest did not significantly increase the severity of disease on the fruits of the subsequent harvest period. In this study, the protection of the fruits until 10 weeks after petal drop did not affect the number of lesions, indicating that ascospore discharges after that date were, probably, responsible for disease severity. Fruits exposed between the 20$^{th}$ and 24$^{th}$ week after 75% of the petals had fallen were symptomatic, indicating that, at this stage, the fruits were still susceptible to the pathogen.

**Additional keywords:** *Citrus sinensis*, *Phyllosticta citricarpa,* conidia, ascospores.

**RESUMO**

**Suscetibilidade de frutos das variedades de laranja 'Valência' e 'Natal' à *Guignardia citricarpa* e a influência da coexistência de frutos sadios e sintomáticos**

Em pomar de laranjeiras 'Valência' e 'Natal' avaliou-se a importância da presença de frutos sintomáticos da mancha preta citros (MPC) na severidade da doença nos frutos cítricos da safra subseqüente. Adicionalmente, avaliou-se o estádio de suscetibilidade dos frutos dessas variedades. Frutos foram protegidos com sacos de papel cristal a partir do estádio de 75% de pétalas caídas em outubro de 2000, até abril de 2001. Frutos foram expostos, em intervalos semanais, da 1ª à 24ª semana. Esse processo se deu tanto em plantas onde os frutos da safra remanescente foram previamente colhidos, como naquelas cujos frutos sintomáticos da safra remanescente permaneceram até a sua queda natural. Avaliou-se a severidade da doença usando uma escala de notas que variou de 0 (ausência de sintomas) a 6 (sintomas severos). Observou-se que para as duas variedades os conídios de *Phyllosticta citricarpa,* formados nas lesões dos frutos da safra remanescente, não provocaram incremento significativo na severidade da doença dos frutos da safra subseqüente. A proteção dos frutos até 10ª semana após a queda de pétalas não influenciou na quantidade final de lesões, indicando que as descargas de ascósporos que ocorreram a partir desse momento foram, provavelmente, responsáveis pela severidade da doença. Frutos que ficaram expostos entre a 20ª a 24ª semanas após a queda de 75% de pétalas mostraram-se sintomáticos, indicando que nesse estádio frutos encontravam-se suscetíveis ao patógeno.

**Palavras-chave adicionais:** *Citrus sinensis*, *Phyllosticta citricarpa,* conídios, ascósporos.

## INTRODUCTION

Citrus black spot (CBS) is caused by *Guignardia citricarpa* Kiely, originally described in Australia (Kiely 1948b). Its anamorphic phase corresponds to *Phyllosticta citricarpa* (McAlpine) van der Aa. Currently, CBS has been reported in several countries in Africa, Asia, Oceania, and South America (Aguilar-Vildoso *et al*., 2002). In Brazil, CBS is present in several states, and in some of them it represents the most important threat to citriculture.

Infections can originate from both ascospores and conidia. Ascospores are produced in pseudothecia formed in decomposing leaves between 40 and 180 days after they fall to the ground (Kotzé, 1981), whereas conidia are produced

in picnidia formed in dry fallen leaves, dead twigs, petioles, spines, and in infected fruits.

Upon reaching maturity, and under favorable environmental conditions, a mass of conidia coated with a mucilaginous substance is extruded from the surface of the ostioles. The conidia can be transported from the upper to the lower plant regions through water, possibly causing new infections when they come into contact with susceptible tissues. This mechanism can disseminate conidia over short distances (Kiely, 1948a). According to Kotzé (1981), if there is coexistence between symptomatic fruits and young susceptible fruits on the same plant, it is advisable to remove the old fruits to minimize infections.

Fruits are susceptible to CBS from anthesis up to 16 weeks after the fall of the petals (Kellerman & Kotzé, 1977). This susceptibility period ranges from the fall of the petals to 4-5 weeks later, *i.e.* 17 to 21 weeks, but can extend to around 24 weeks after the fall of the petals (Kotzé, 1981; Klotz, 1978).

In Brazil, particularly in the case of semi-late maturation varieties such as 'Pêra' and late maturation varieties such as 'Valência', 'Natal' and 'Folha Murcha', it is common to observe on the same plant the coexistence of fruits in different phases of maturation and/or mature fruits of a given harvest, as well as young susceptible fruits from the next generation.

The objective in this present study was to evaluate the influence of the coexistence of symptomatic and young fruits on the incidence of *Guignardia citricarpa* in the fruits of the new harvest and to determine the critical susceptibility period of the fruits of the 'Natal' and 'Valência' sweet orange under natural infection conditions.

## MATERIAL AND METHODS

The experiments were carried out between 2000 and 2001 in orchards of 14-year-old plants of the 'Natal' and 'Valência' sweet orange (*Citrus sinensis* Osbeck), grafted onto lemon trees of the 'Volkameriano' (*Citrus volkameriana* Pasq.) rootstock, with a spacing of 8 x 5 m. The orchards were located near Mogi-Guaçu, São Paulo State, Brazil. The distance between both plots was around 800 m in a straight line.

In the experimental area, the mature fruits of the previous 2000 harvest showed 100% incidence of citrus black spot, given that, in addition to the high inoculate pressure, those fruits had been purposely prevented from receiving fungicides during the susceptibility period.

Two parallel rows were established for each variety, each containing 24 plants. All fruit was harvested before flowering from only one of the rows, whereas in the other, the mature fruits remained on the plants until their natural fall, thus allowing the coexistence of symptomatic fruits from the previous harvest and the young fruits from the subsequent harvest. Two lateral rows and three plants at each of the extremities were used as borders. The fruits of the plants in the lateral rows were all harvested, whereas the fruits of the plants at the extremities of the rows were harvested, or not harvested, prior to the flowering according to their respective treatments. The two selected rows for both varieties, as well as the lateral border rows, did not receive fungicidal treatment throughout the duration of the experiments.

On every plant of each variety, at the stage of 75% fallen petals, 100 young fruits about 0.4 cm in diameter were protected using an 18 x 10 cm white paper bag. At weekly intervals, 100 bags were removed from each of the plants in each row, a procedure that was repeated until the 24$^{th}$ week. Thus, the fruits were exposed to natural infection by ascospores and conidia and, therefore, one could evaluate the susceptibility period of the fruits, from the first week (the 75% fallen petals stage) to the 24$^{th}$ week.

The severity of the disease was evaluated starting in April 2001, using 8 to 25 fruits from each plant, corresponding to one week for each situation described previously, using a scale from 0 (absence of symptoms) to 6 (severe symptoms), according to Fagan & Goes (2000). Control fruits were not protected in either harvested or non-harvested plants. The severity of the disease in each week was fitted. Statistical comparisons were carried out based on the minimal statistical difference (MSD).

## RESULTS AND DISCUSSION

### Severity of the disease in 'Valência' and 'Natal' sweet orange

According to the obtained data, no statistically significant difference in the level of severity of CBS was detected in the orchard of the 'Valência' orange between plants with harvested and non-harvested fruits, except for those in weeks 4, 5, 6, and 9. In the plants corresponding to those weeks, the fruits from the plants that had not had their fruits removed in the previous harvest showed significantly fewer symptoms (Figure 1 A). In the case of the 'Natal' orange, there was no statistically significant difference in the severity of the disease between fruits from plants with harvested and non-harvested fruits (Figure 1 B).

A comparison of the severity of the disease between both varieties throughout the studied period indicated a decrease in frequency of the disease as the fruit protection period increased. One can therefore infer that, in addition to a higher influence of inoculate pressure starting in some specific periods of the year, it is possible that there is also an increase in the level of resistance of the fruits during their development.

In the present study, there was no statistically significant difference in the severity of the disease between the fruits from harvested plants and those from the control group until the 10$^{th}$ week, indicating that the protection of the fruits in this period did not result in a reduction in the severity of the disease for the 'Valência' and 'Natal' varieties (Figure 1 C and D, respectively). One can suppose that there

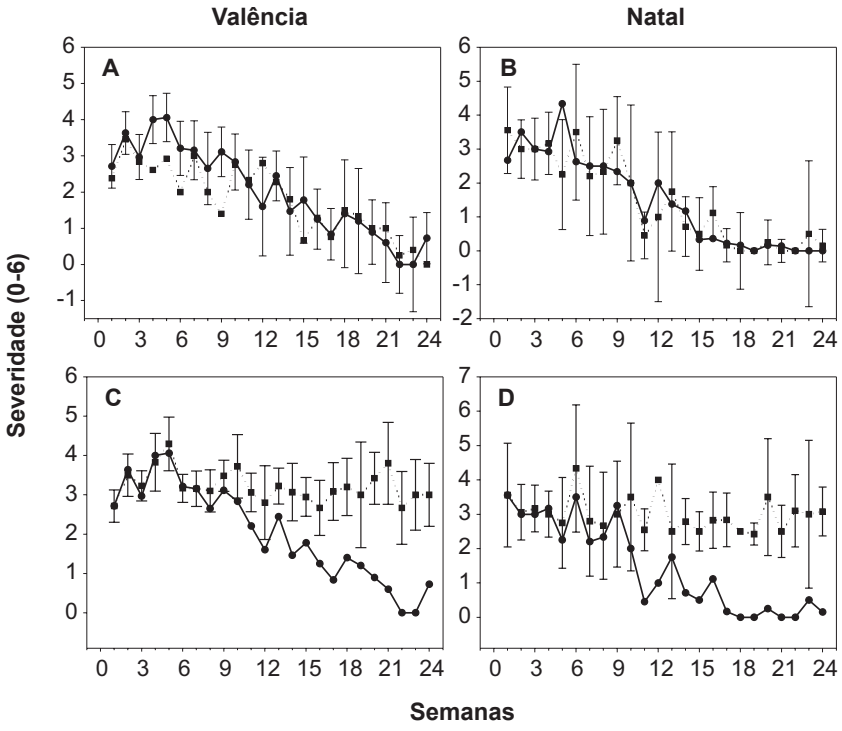


**FIG. 1 -** Severity of black spot disease on fruits of the 'Valência' and 'Natal' orange varieties for 24 weeks, from October 2000 to April 2001. Severity on fruits of the 'Valência' **A.** and 'Natal' **B.** varieties; the continuous line represents fruits from harvested plants and the dotted line represents fruits from non-harvested plants. Fruits of the 'Valência' **C.** and 'Natal' **D.** orange varieties; the continuous line represents fruits from harvested plants and the dotted line represents control fruits from harvested plants.

the discharge of ascospores during this period was not high enough to cause substantial severity levels of the disease. Therefore, the lesions found in the fruits of plants from up until the 10th protection week would have originated from ascospore discharges from the 11th week, when significant differences were detected, indicating that protection leads to a differential in the level of severity of the disease.

Moreover, fruits from the 'Valência' and 'Natal' varieties that had been exposed to natural infection during the entire duration of the experiment showed intermediate severity values, between levels 3 and 4, whereas the protected fruits, particularly from the 10th week on, showed progressively lower severity values, between levels 1 and 2, or even lower.

A comparison between the severity level of the disease corresponding to the non-harvested control plants and the protected fruits also from non-harvested plants showed no statistically significant contribution to a reduction in the severity of the disease until the 8th and 10th weeks.

In the case of the level of severity of the disease on fruits exposed to natural infection (control) from harvested and non-harvested plants, one could observe that, in most situations, there were no statistically significant differences between cases, either for the 'Valência' or the 'Natal' varieties (Figure 2 C and D).

If one assumes that the distribution of ascospores has been similarly uniform over the experimental areas, an additional effect of the conidia would have involved the production of fruits with an increased level of severity in plants that were not harvested before flowering. The absence of a statistically significant difference between both situations indicates that this additional source of inoculum, resulting from asymptomatic fruits, although biologically important, was not sufficient to cause significant increases in the severity of the disease.

In South Africa, the critical susceptibility period of the fruits varies between 17 and 21 weeks (Kiely, 1948a; Kotzé, 1981). According to Klotz (1978), this long period of fruit susceptibility might last up to the 24th week.

In practice, there is consensus among some technicians and citrus growers that the susceptibility stage of the fruits lasts until the period when they reach approximately the size of a ping-pong ball, *i.e.* 3.8 cm in diameter. However, the fruits of the 'Valência' and 'Natal' varieties were susceptible, regardless of whether they were protected or not, from harvested or non-harvested plants, until at least the 24th week after the fall of 75% of the petals, when they were 5 to 6 cm in diameter. Given that, up to this point, the fruits that were exposed to natural infection were susceptible to the pathogen, the need to extend the period for their protection becomes clear, particularly when the fruits are for fresh marketable.

According to Kotzé (1981), the formation and release of ascospores is conditioned by the alternation between wetness and drying of leaves in decomposition, between 40 and 180 days after their fall. In addition to rain, the moistening of these leaves also occurs from dew, which, therefore, could be sufficient for the development, maturation, and dissemination of ascospores (Kiely, 1948a). A positive relationship between leaf moistening and ascospore release in *G. citricarpa* in an orchard of the 'Natal' orange variety has been verified in Brazil by Reis *et al.* (2006).

In the present study, the severity levels of the disease were shown to be higher starting from the 10th week after the fall of the petals. Rainfall was more frequent and in higher intensity during this period, as has been recorded in the orchard

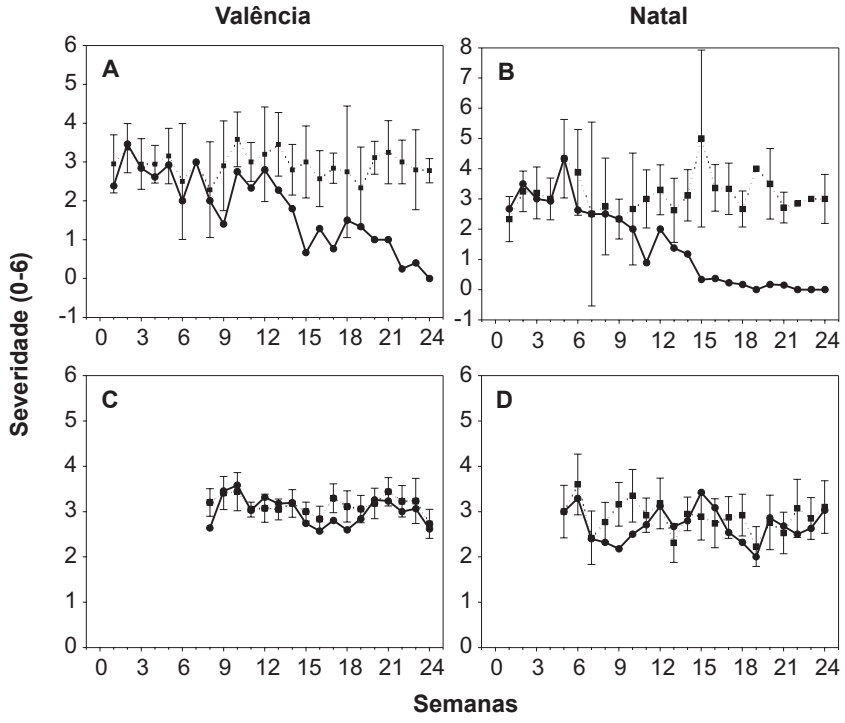


**FIG. 2 -** Severity of black spot disease on fruits of the 'Valência' and 'Natal' orange varieties for 24 weeks, from October 2000 to April 2001. Severity on fruits of the 'Valência' **A.** and 'Natal' **B.** varieties; the continuous line represents fruits from non-harvested plants and the dotted line represents fruits from non-harvested plants. Fruits of the 'Valência' **C.** and 'Natal' **D.** orange varieties; the continuous line represents fruits from the control plant in non-harvested areas and the dotted line represents control fruits from the control harvested plants.

of the 'Natal' orange variety, where rains occurred in the order of 100 mm, 240 mm and 70 mm during the 3rd, 7th, and 8th weeks, respectively. The same situation has been observed by Reis *et al.* (2006) as a high correlation between the occurrence of rain and the severity of CBS in the 'Natal' and 'Valência' varieties.

In the situations where the severity of the disease was compared in relation to the presence or absence of symptomatic fruits from the previous harvest, either for the Valência or the Natal varieties, there were indications that the ascospores exceed the importance of conidia. According to Kotzé (1981), once the disease has reached epidemic proportions, the importance of conidia is obfuscated by the importance of ascospores. In addition, according to Cobb (1904) and Darnell-Smith (1919), cited by Kiely (1948b), conidia do not germinate promptly, requiring special conditions for this process to begin. Germination can occur in up to 12 hours when the recently released conidia are stimulated with extracts from orange peels. After three days of age, the germination of conidia is either hindered or unviable.

Kiely (1948b) and McOnie (1965) concluded that the distribution of the symptoms of the disease produced by conidia was random along the vertical axis of the plant, indicating that the conidia did not contribute to the increment in the severity of the disease in the fruits located in the lowest regions of the plant. Such an observation demonstrates, therefore, that the conidia, if they were of great importance, would differentially affect the fruits in the lower parts of the plants, which would show symptoms of higher magnitude. McOnie (1965) reported that this relative uniformity in the vertical distribution of symptomatic fruits in a tree indicates that the conidia originating from the dead leaves are not relevant to the severity of the disease, given that the fruits located in areas subject to splashes falling from those leaves did not show significant increments in the level of severity.

Studies on citrus black spot have suggested different behaviors may result when control of the disease is temporarily suspended after a number of successive years of control. McOnie (1965) reports on the case of an orchard in South African conditions, where the control of *G. citricarpa* was carried out using the appropriate doses for 10 consecutive years. It was recorded that, in the following year without any such control, the disease occurred as severely as in the years preceding the treatment. In this location, the levels of pathogen remained as high as those recorded in the years before treatment. However, it has been observed that under Brazilian conditions, and in the cases of scab and melanose, non-application of cupric fungicide after several years of repeated treatment is followed by a low frequency of the disease in the subsequent untreated year, contrary to what is observed in relation to black spot. This fact can be related to the shorter susceptibility period of the fruits to the causal agents of scab and melanose, as well as to the quantity and efficacy of each of the types of inoculate produced by the respective pathogens.

In respect to ascospores, Kiely (1948b) demonstrated that they show a germination behavior that is the opposite of that shown by conidia. Ascospores, when produced under natural conditions, show 0% germination after 24 h, reaching 100% after 96 hours. However, in the case of conidia, even after receiving stimuli from the addition of citric acid at 0.3%, at 25°C, the germination fell from 80% to approximately 17% after 96 h of incubation. This gradual increase in viability of ascospores over time apparently increases the possibilities of the fungus in the sense of allowing a higher rate of infection.

This author also reports that, in infected orchards, the simple removal of symptomatic fruits prior to presence of new fruits was not sufficient for the control of the disease, and specific pulverizations were required.

Spósito (2004) studied the incidence of fruits affected by CBS and the intensity of the disease on fruits of the 'Valência' variety during two consecutive harvests. Evaluations were carried out in the absence and in the presence of decomposing leaves, as well as in the presence or absence of symptomatic fruits from the previous harvest. In one of the areas, the results were consistent with the present study, whereas in the other area, the removal of fruits and symptomatic fruits caused a reduction only in the intensity of the disease in relation to the controls. However, the disease was not suppressed. In orchards where the disease has reached epidemic levels, the conidia produced in lesions of the fruits from the previous harvest are an additional source of inoculate, yet are not able to make a difference in relation to ascospores with respect to the severity of the disease on fruits from the subsequent harvest.

In the present study, it is emphasized that, given the use of benomyl in previous years, it is possible that the effect of this fungicide might have reduced the formations of picnidia on the various plant organs, thus affecting the formation of conidia, given that this fungicide inhibits the formation of such structures (Whiteside, 1977). In addition, the good vegetative state of the plants, particularly in relation to dry branches, might also be an additional factor to minimize the importance of conidia.

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